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Developing Climate Resilient Grain and Forage Legumes

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About the Editors

Uday C Jha graduated from Visva-Bharati University, Santiniketan, India. He is working in the area of grain legume breeding, genetics, and genomics for abiotic stress tolerance since 2010 at the Indian Institute of Pulses Research, Kanpur, ICAR, India. He has more than 32 peered-reviewed international publications and 1 edited book. He has attended and delivered oral and poster presentation in various national and international conferences. He is associated in developing six chickpea varieties. Dr. Jha was awarded with ISPRD fellowship in 2016 by the Indian Society of Pulses & Research Development (ISPRD) and Young Scientist Award by IJBSM society in 2017 at Jaipur, India. He has also received the Chickpea Team Award in 2020 from IIPR, Kanpur. He is serving as editorial board member of the journal *BMC Plant Biology*.

Harsh Nayyar is currently a professor in the Department of Botany, Panjab University, Chandigarh. He graduated from Punjab Agricultural University, India, and worked at the Indian Council of Agricultural Research and H.P. Agricultural University, Palampur, India, prior to his present position. His research program aims to study the responses of various food legumes such as chickpea, lentil, mungbean, and urdbean to abiotic stresses like heat, drought, and cold as well as their combination. He is developing convenient and easy screening methods to identify stress-tolerant legumes and also investigating the mechanisms at various organizational levels underlying stress tolerance. He has published about 140 research papers in peer-reviewed high impact factor research journals.

Shiv Kumar Agrawal holds a Ph.D. in plant breeding from the GB Pant University of Agriculture and Technology, Pantnagar, India. His area of expertise includes crop improvement through resistance breeding, widening the genetic base through pre-breeding, and development of genetic and genomic resources in food legumes. During the 30 years of his professional career, he, in collaboration with national partners, has contributed to the development of 59 lentil, 5 mung bean, 2 urdbean, 1 rice, and 3 grass pea varieties and published 196 peer-reviewed journal articles, 72 book chapters, 9 books, 7 technical bulletins, and 2 training manuals. He is a fellow of professional societies including the International Legumes Society, the

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1 Improving Chickpea Genetic Gain Under Rising Drought and Heat Stress Using Breeding Approaches and Modern Technologies

Uday C Jha, Harsh Nayyar, Rintu Jha, Prasant Kumar Singh, Girish Prasad Dixit, Yogesh Kumar, Biswajit Mondal, Avinash Kumar Srivastava, Eric J. B. von Wettberg, Pronob J. Paul, Ajaz A. Lone, Srinivasan Samineni, Sailesh K. Tripathi, and Kadambot H. M. Siddique

Abstract

Increasing grain legume production, particularly for chickpea, will provide essential "plant-based dietary protein" and other micronutrients under the changing global climate. Drought and terminal heat stress limit plant growth and

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negatively affect various phenological events, causing severe yield losses. Among various strategies for improving stress tolerance, the judicious utilization of available genetic variation in the chickpea gene pool could minimize the adverse effects of drought and heat stress, sustaining chickpea yields. In addition, advancements in chickpea genomic resources, from molecular markers, namely, SSR, SNP, and INDELs and tools for association genetics, RNA-seq, to the availability of chickpea genome sequences and efforts of global chickpea germplasm resequencing allow us to identify loci and haplotypes contributing to drought and heat tolerance across the whole genome. Thus, molecular markers have enabled the successful transfer of drought-tolerant traits to elite chickpea cultivars using marker-assisted and haplotype-based breeding approaches. Likewise, the role of drought- and heat-responsive proteins and metabolites could significantly improve our understanding of the molecular mechanisms of drought and heat tolerance in chickpea via proteomics and metabolomics. Moreover, emerging novel breeding technologies (e.g., genomic selection, speed breeding, and genome editing) could enhance the necessary genetic gain to feed the increasing global population under an abruptly changing global climate.

Keywords

Chickpea · Drought · Heat · Molecular marker · |Genetic variability

1.1 Introduction

Chickpea is a highly nutritious grain legume crop that contributes to global food security, providing essential amino acids and micronutrients to the global human population (Jukanti et al. 2012). Chickpea ameliorates soil nitrogen content by fixing atmospheric N_2 through symbiotically efficient rhizobacteria (Graham and Vance 2003), enriching soil nitrogen for subsequent crops in the rotation (Marques et al. 2020a). Global climate change and shifting production practices frequently expose chickpea to terminal drought and heat stress, causing severe yield losses (Jha et al. 2014a). Efficient utilization of chickpea germplasm resources, including landraces, wild relatives, and improved breeding lines, could sustain chickpea yields under drought and heat stress. Despite the genetic complexity of drought and heat tolerance mechanisms, various genomic resources—including draft genome sequences, resequencing panels, and segregating populations—have been developed in the last decade to elucidate the genetic determinants governing drought and heat tolerance (Thudi et al. 2014; Jha et al. 2018a, 2021a; Paul et al. 2018b; Jain et al. 2013; Varshney et al. 2013a, 2019). Furthermore, molecular markers have enabled the successful transfer of drought-tolerant traits to elite chickpea cultivars using a marker-assisted breeding approach (Roorkiwal et al. 2020). Thus, haplotype-based breeding could introduce desired allelic combinations to improve drought and heat stress tolerance in chickpea. Functional genomics could be used to pinpoint drought and heat-tolerant candidate gene(s) and their putative functions. Likewise, proteomics and metabolomics could greatly improve our current knowledge on various proteins and metabolites produced in response to drought and heat stress (Khan et al. 2019a, b). The knowledge to be gained from these approaches can be quickly harnessed due to the power of novel breeding technologies including genomic selection, haplotype-based breeding, speed breeding, and genome editing (Meuwissen et al. 2001; Hickey et al. 2019; Samineni et al. 2019; Bohra et al. 2020; Badhan et al. 2021). These approaches combined with knowledge on the genetic basis of stress tolerance traits should improve crop breeding to help meet the challenge of feeding the growing global population under climate change.

1.2 Impacts and Anomalies Associated with Drought Stress in Chickpea

Drought stress alone causes up to 50% yield losses in chickpea (Varshney et al. 2014). Chickpea frequently faces terminal drought stress as it is mostly grown under rainfed conditions in semiarid and arid regions (Gaur et al. 2012). Drought stress can occur at any stage of the plant growth cycle, with terminal drought the most common across the major production areas in South Asia, East Africa, and Mediterranean climates. Key physiological and biochemical, and developmental processes—photosynthesis, RuBisCo activity, carbohydrate synthesis, TCA cycle, respiration, and root growth—are seriously affected under drought stress, restricting plant growth (Yordanov et al. 2003; Parry et al. 2002; Guo et al. 2018). Drought stress during germination and vegetative growth decreases stomatal conductance, chlorophyll synthesis, plant vigor, and biomass accumulation (Krishnamurthy et al. 2013a, b; Purushothaman et al. 2015, 2016; Pang et al. 2017b). Drought stress during reproductive growth significantly affects anther formation, pollen formation, pollen fertility, pollen tube germination, and fertilization (Leport et al. 1999), resulting in improper pod formation, seed development, and seed filling, and ultimately significant yield losses. Moreover, drought stress affects nitrogen fixation by decreasing nitrogenase activity due to reduced expression of the *nifK* gene (Serraj et al.1999; Nasr Esfahani et al. 2014).

1.3 Impact of Heat Stress on the Reproductive Process in Chickpea

Rising global temperatures are challenging chickpea growth and development processes, leading to profound yield losses of at least 10–15%, or 53–330 kg/ha (Kalra et al. 2008; Upadhyaya et al. 2011; Kaushal et al. 2013; Jha et al. 2014b, 2017). Heat stress affects plant growth processes during vegetative, reproductive, and maturity phases (Devasirvatham et al. 2015; Bhandari et al. 2020). Heat stress hastens plant phenological events, decreasing biomass accumulation and grain filling, and ultimately causing yield losses (Kaushal et al. 2013). The reproductive stage is the most vulnerable to heat stress (Jagadish et al. 2021) because it severely impairs all reproductive processes (Devasirvatham et al. 2013; Jha et al. 2017). Increased atmospheric temperatures beyond normal during anthesis affect anther formation, the pollination process, including pollen germination and pollen tube formation, fertilization, and pod formation (Kaushal et al. 2013; Devasirvatham et al. 2013, 2015; Bhandari et al. 2020). Consequently, heat exposure inhibits proper seed development, resulting in shriveled seed and thus severe yield losses (Devasirvatham et al. 2013, 2015). Furthermore, nodule development and biological nitrogen fixation processes are impeded under heat stress, negatively impacting plant growth.

1.4 Genetic Resources for Drought and Heat Stress Tolerance

Harnessing genetic variability from various wild gene pools, landraces, and improved breeding lines could be the most eco-friendly and economic approach for developing abiotic stress tolerance, including drought and heat stress tolerance.

Several promising chickpea genotypes conferring drought tolerance have been identified based on various phenological, physiological and yield-related traits (Kumar and Rao 1996; Krishnamurthy et al. 2003; Shah et al. 2020). Drought stress can be mitigated by hastening plant phenological processes before the onset of terminal drought stress. The advantage of early phenology to tackle drought stress can be harnessed by transferring this trait into high-yielding yet drought-sensitive elite chickpea genotypes, for example, ICC96029, ICC96030, ICCV2, ACC316, and ACC317 (Kumar and Rao 1996; Kumar and Abbo 2001; Krishnamurthy et al. 2003; Canci and Toker 2009a) (see Table 1.1). However, early phenology can come with a yield penalty. Root system architecture—an important parameter manifesting plasticity under various environmental stresses, including drought—could be harnessed to develop drought tolerance in plants. Numerous studies have identified chickpea genotypes harboring improved root traits under drought stress. For example, ICC495 and ICC8261, with high root biomass and rooting depth (Krishnamurthy et al. 2003; Kashiwagi et al. 2008) have been judiciously used in chickpea breeding programs to develop drought-tolerant genotypes (Varshney et al. 2014). Targeting root traits for improving drought tolerance, Khandal et al. (2020) recently manipulated cytokinin levels to increase lateral root development and root biomass, attributing to drought tolerance in chickpea. Several promising chickpea lines exhibit improved photosynthesis, stomatal conductance, chlorophyll fluorescence, and other physiological traits under water stress (Toker et al. 2007; Makonya et al. 2020; Shah et al. 2020).

Several biochemicals, including various sugars, osmolytes, antioxidants, and other biochemicals, are produced in response to drought stress. Based on these parameters, MCC 544, MCC 696 and MCC 693 (Mafekheri et al. 2010), ILC482 (Mafakheri et al. 2011), Bakhar-2011 (Farooq et al. 2018), and D0091-10, K010-10, and D0085-10 (Shah et al. 2020) have been identified as potentially stress tolerant and useful as parents for crosses. In terms of yield and yield-related traits, ICC 14778, ICCV 10 (Ramamoorthy et al. 2016), ICC8950 (Awasthi et al. 2013),

Table 1.1 List of chickpea genotypes contributing to heat and drought stress tolerance

Stress	Resistance source	Origin/reported from	Tested field/laboratory/greenhouse	Trait used for selection imparting stress tolerance	References
Drought stress	ICC 96029	India	Field	Earliness	Kumar and Rao (1996)
	ICCV 2	India	Field	Earliness	Kumar and Abbo (2001)
	ICC 4958	India	Field	Root and root-related traits	Kashiwagi et al. (2008), Krishnamurthy et al. (2003), Kashiwagi et al. (2005)
	C. anatolicum, C. microphyllum, C. songaricum, C. oxydon, C. montbretii	Turkey	Field	Low wilting under water stress	Toker et al. (2007)
	ICC 8261	India	Field	Root and root-related traits	Gaur et al. (2008)
	ACC316, ACC317	Turkey	Field	Earliness	Canci and Toker (2009a)
	ILC482	Iran	Field	Osmolyte regulation	Mafakheri et al. (2011)
	ICC 7571	India	Field	High harvest index	Kashiwagi et al. (2013)
	ICC 14778, ICCV 10	India	Field	Grain yield trait	Ramamoorthy et al. (2016)
	ICC8950	India	Laboratory	High antioxidant defense capacity, and high seed yield	Awasthi et al. (2017)
	ICC 16374B, ICC 15510, ICC 9586, ICC 867	India	Field	Deeper root for acquiring water	Chen et al. (2017)

(continued)

Table 1.1 (continued)

Stress	Resistance source	Origin/reported from	Tested field/ laboratory/ greenhouse	Trait used for selection imparting stress tolerance	References
	ICC 14778, ICCV 10	India	Field	Root growth	Ramamoorthy et al. (2017)
	Neelam	Australia	Greenhouse	Improved seed yield	Pang et al. (2017a)
	Bakhar-2011	Australia	Laboratory	Increased proline, trehalose content	Farooq et al. (2018)
	Acc#7	University of Cape Town, South Africa	Greenhouse	Increased shoot biomass, *Pn*, *F*v/*F*m, and RWC; smaller root diameter	Makonya et al. (2020)
	D0091–10, K010–10, D0085–10, K005–10, D0078–10, 08AG016, 08AG004, D0080–10, 09AG002, K002–10, D0099–10	Pakistan	Growth chamber and field	Cell membrane stability, proline content, glycine betaine, nitrate reductase	Shah et al. (2020)
	CH55/09	Pakistan	Field	Stable yield	Arif et al. (2021)
	C. reticulatum (Kalkan_64, Savur_63)	University of California	Laboratory	Metabolic pathways, viz., phenylpropanoid metabolism	Moenga et al. (2020)
Heat stress	ILC482, Annegiri, ICCV10		Laboratory	Cell membrane	Srinivasan et al. (1996)
	ICCV88512 and ICCV88513	India	Field	Grain yield	Dua (2001)
	ACC316and ACC317		Field	Earliness	Canci and Toker (2009a)
	ICC1205	India	Field and laboratory	High pollen viability	Devasirvatham et al. (2010)
	ICC4958, ICC4991, ICC6279, ICC6874, ICC7441, ICC8950	India	Field	Earliness, seed yield/plant	Krishnamurthy et al. (2011)

	ICC14346	India	Field	Earliness	Upadhyaya et al. (2011)
	ICC14778	India	Field	Cooler canopy	Zaman-Allah et al. (2011a, b)
	ICCV92944	India	Field	Earliness	Gaur et al. (2012)
	ICC15614	India	Field and laboratory	High pollen viability	Devasirvatham et al. (2012, 2013), Kaushal et al. (2013)
	BG 256	India	Laboratory	Seed yield	Jumrani and Bhatia (2014)
	ICC8950	India	Laboratory	Physiological, biochemical and yield traits	Awasthi et al. (2014)
	Katila, Vaibhav, Avrodhi	India	Field	Increased grain yield	Jha and Shil (2015)
	RVG 203, JAKI 9218, JG 130, ICCV0 7118, ICC1356	India	Field	Increased grain yield	Jha et al. (2015, 2018b, c, 2019a, b)
	ICC 14778, ICC 15618	India	Field	Improved yield	Varshney et al. (2019)
	Cicer turcicum	Antalya, Turkey	Screenhouse	High pod set	Toker et al. (2021)

FLIP03-145C, ILC 3182, and ILC 588 (Hamwieh and Imtiaz 2015), ICC 7571 (Kashiwagi et al. 2013), Neelam (Pang et al. 2017a), and CH55/099 (Arif et al. 2021) were identified as promising genotypes conferring drought tolerance; these genotypes could be used as donors for transferring yield and yield-related traits into elite yet drought-sensitive chickpea cultivars.

Heat stress is emerging as a serious abiotic stress in chickpea, especially during the reproductive stage (Jha et al. 2014a). Several chickpea genotypes have been identified that exhibit heat tolerance based on various parameters (Gaur et al. 2012; Jha et al. 2018b; Bhandari et al. 2020). Heat-tolerant lines can be selected based on yield parameters using various selection indices (e.g., heat tolerance index, tolerance index, heat susceptibility index) by growing genotypes under timely and late-sown conditions. Using this screening method, several potential heat-tolerant chickpea lines (e.g., RVG 203, RSG 888, JAKI 9218, GNG 469, IPC 06-11) have been reported (Jha et al. 2018b). Likewise, ICCV92944, RVG203, JAKI9218, ICC1356, and JG130 showed improved yield performance under heat stress (Gaur et al. 2012; Jha et al. 2015, 2018b, c). Within Ethiopian germplasm, DZ-Cr-0034 was identified as heat tolerant in multisite trials in Ethiopia and India (Getahun et al. 2021). Assessing yield and yield-related traits of breeding lines at multiple locations will provide further impetus for selecting heat-tolerant lines under a range of target environments. In this context, JAKI 9218 showed promising results under heat stress (Jha et al. 2019b).

Earliness is an important heat escape mechanism. ACC316, ACC317 (Canci and Toker 2009a), and ICCV92944 (Gaur et al. 2012) were identified for their earliness to escape heat stress. Likewise, ILC482, Annegiri, ICCV10, ICC8950, and ICC14778 were identified as promising genotypes in terms of various physiological traits contributing to heat tolerance (Srinivasan et al. 1996; Zaman-Allah et al. 2011a, b; Awasthi et al. 2014). Screening for pollen and pollen-related traits under heat stress at the reproductive phase could be important for selecting heat-tolerant chickpea lines. Based on these traits, ICCV92944, ICC15614, ICC1205, and ICC07110 were identified as heat-tolerant in both the field and a growth chamber (Kaushal et al. 2013; Awasthi et al. 2014; Devasirvatham et al. 2015; Bhandari et al. 2020) (see Table 1.1). Significant genetic variation for various phenological, morphophysiological, and yield-related traits was found in the ICC15614 × ICC4567, and DCP92-3 × ICCV92944 mapping populations under heat stress (Paul et al. 2018a; Jha et al. 2021a, b).

1.5 Crop Wild Relatives (CWRs): A Hidden Reservoir of Drought and Heat Tolerance in Chickpea

Judicious exploitation of CWRs in crop breeding programs could broaden the genetic base of improved breeding lines for sustaining grain yield and plant adaptation under challenging environments (Coyne et al. 2020; von Wettberg et al. 2018). Likewise, various CWR accessions of chickpea are being incorporated into breeding programs for developing abiotic stress tolerance, including drought and heat tolerance. An evaluation of various chickpea CWRs—*C. anatolicum, C. microphyllum, C. montbretti, C. oxydon,* and *C. songaricum*—under water stress revealed their potential for conferring drought tolerance (Toker et al. 2007). Similarly, an assessment of various annual *Cicer* species for drought and heat stress tolerance identified better adaptation of four *C. reticulatum* accessions and one *C. pinnatifidum* accession under drought and heat stress (Canci and Toker 2009b); these *Cicer species* could be used to introgress "adaptive traits" contributing to drought and heat stress tolerance into elite chickpea cultivars. To date, only *C. reticulatum* and some *C. echinospermum* are compatible with cultivated chickpea (e.g., Kahraman et al. 2017). However, with an increased understanding of the nature of genome organization in the genus (Varshney et al. 2021), it may be feasible to cross more distantly related *Cicer* species, such as *C. pinnatifidum*.

Recent studies harnessing new collections of *C. reticulatum* and *C. echinospermum* from southeastern Turkey (von Wettberg et al. 2018; Coyne et al. 2020) have identified new sources of drought and heat tolerance (e.g., Talip et al. 2018; von Wettberg et al. 2018; Marques et al. 2020b; Getahun et al. 2021). Initial analyses of these collections identified multiple populations from lower elevation, more exposed sites with greater drought tolerance (e.g., von Wettberg et al. 2018). Some higher elevation sites showed greater cold tolerance (Mir et al. 2021), but interestingly, a lower elevation, low water availability site was among the most cold-tolerant parents. This suggests that wild populations may exhibit interesting patterns of cross-tolerance among abiotic and biotic stresses (von Wettberg et al. 2014).

To harness variation beyond *C. reticulatum* and *C. echinospermum*, which have far narrower environmental distributions than other *Cicer* species (Coyne et al. 2020), it makes sense to continue looking at other wild taxa. Recently, Toker et al. (2021) reported *C. turcicum* as a species exhibiting better reproductive function under heat stress. Ongoing work is assessing the potential to cross this species with cultivated chickpea (Toker, pers. comm). Besides CWRs, "adaptive loci" of landraces conferring plant phenotypic plasticity and adaptation under various hostile environments could be harnessed to develop climate-resilient genotypes. Considering this, Varshney et al. (2019) identified ICC 14778 and ICC 15618 as two chickpea genotypes possessing gene(s) that contribute to chickpea adaptation in hot environments.

1.6 Genomic Resources for Drought and Heat Tolerance

During the last decade, unprecedented advances in developing chickpea genomics resources have enabled the mapping of various breeding importance traits, including drought and heat stress (Thudi et al. 2014; Jha 2018; Jha et al. 2020).

Understanding the genetic basis of traits has improved with the advent of SSR markers. Several drought-related traits QTLs were identified on different linkage groups by employing SSR markers (Rehman et al. 2011; Varshney et al. 2014; Hamwieh et al. 2013). Comprehensive phenotyping of various drought-relevant traits in two biparental mapping populations evaluated at multiple locations underpinned a QTL hotspot on CaLG04 (Varshney et al. 2014). Subsequently, the availability of SNP markers developed through genotyping-by-sequencing (GBS) technology-enabled partitioning of this QTL hotspot into *QTL-hotspot_a* (harboring 15 genes) and *QTL-hotspot_b* (harboring11 genes) (Kale et al. 2015). Furthermore, Jaganathan et al. (2015) refined this QTL-hotspot genomic region to 14 cM, harboring several novel SNPs in the ICC1882 × ICC4958 mapping population using GBS technology. To identify drought tolerance QTLs, Sivasakthi et al. (2018) elucidated 13 M-QTLs related to plant vigor (rather than drought tolerance *per se*) on LG4 coinciding with the QTL hotspot and one M-QTL contributing to stomatal conductance on LG3 under drought stress. The availability of the chickpea genome sequence assisted in pinpointing the candidate gene(s) underlying these drought stress QTLs (Jaganathan et al. 2015; Kale et al. 2015; Srivastava et al. 2016).

Jha et al.(2019a) shed light on the genetic control of heat tolerance in chickpea using a diallel analysis based on phenological and yield parameters assessed under heat stress. The authors reported the presence of both additive and nonadditive gene action controlling heat tolerance. Thus, the availability of molecular markers, especially SSRs and SNPs, further improves our understanding of the genetics of heat tolerance (Thudi et al. 2014; Jha et al. 2019c, 2021a, b).

Assaying the F_2-derived DCP92-3 × ICCV92944 mapping population using SSR markers identified one QTL controlling primary branch number and one QTL linked to chlorophyll content under heat stress (Jha et al. 2019c). Paul et al. (2018b) mapped yield-related QTLs (pod number per plant, seed number per plant, biomass, and percentage pod set) on CaLG05 and CaLG06 chromosomes under heat stress by genotyping the ICC15614 × ICC4567 mapping population using GBS technology. Likewise, deploying the GBS-derived 788 SNP markers in the DCP92-3 × ICCV92944 mapping population identified 37 major QTLs related to various physiological and yield-related traits evaluated under heat stress (Jha et al. 2021b) (see Table 1.2). The authors also elucidated 28 candidate genes related to HSPs underlying the identified QTLs. Moreover, a large set of global germplasm offers great opportunity to capture significant marker-trait associations for heat-tolerant traits across the whole genome using genome-wide association mapping (Thudi et al. 2014; Varshney et al. 2019). Based on this approach, several MTAs for various phenological, physiological, and yield-related traits evaluated under heat stress have been uncovered in chickpea (Thudi et al. 2014; Jha et al. 2017, 2018a, 2021a; Varshney et al. 2019).

Using 81 SSR markers assayed in 71 chickpea genotypes, several significant MTAs related to physiological traits, namely, membrane stability index and chlorophyll content were identified under heat stress (Jha et al. 2018a). Likewise, GWAS performed on a set of 182 chickpea germplasm using 120 SSR markers elucidated a plethora of significant MTAs for various phenological, physiological, and yield and yield-related traits phenotyped under heat stress (Jha et al. 2021a) (see Table 1.2). Apart from this, various root traits contributing to higher water use efficiency and the QTL hotspot from ICC4958 to elite chickpea cultivars, such as JG11, ICCV10, RSG888, Pusa 372, Pusa 362, and JAKI9218, have been transferred using

Table 1.2 List of QTLs related to drought and heat-stress tolerance in chickpea

Stress	Mapping approach	Population name and size	QTLs/ MTA	Marker type	No. markers used	LG group	References
Drought	Biparental	ILC588 × ILC3279, RILs (155)	15 QTLs	SSR	97	LG1, LG3, LG4, LG7, LG8	Rehman et al. (2011)
	Biparental	ILC588 9 × ILC3279, RILs (181)	93 QTLs	SSR		LG3, LG4	Hamwieh et al. (2013)
	Biparental	JG 11 × ICC4958, BC	QTL-hotspot	SSR, AFLP		–	Varshney et al. (2013b)
	Biparental	ICC 4958 × ICC1882, RILs (264)	QTL-hotspot	SSR	551	CaLG04	Varshney et al. (2014)
		ICC 283 × ICC 8261, RILs (288)	312 significant MTAs	DArT, SNP	1072 DArTs +651 + 113 + 36 SSRs	CaLG04	Thudi et al. (2014)
	Biparental	ICC 4958 × ICC 1882, RILs (232)	164 main-effect QTLs	SNP, CAPS, dCAPS,	743 SNPs +232 SSRs+21 DArTs	CaLG04	Jaganathan et al. (2015)
			QTL-hotspot	SSR	7 EST-SSRs, 4 genic molecular markers		
	Biparental	ICC 4958 × ICC 1882, RILs (232)	QTL-hotspot_a (15 genes)	SNP	53,223 SNPs	CaLG04	Kale et al. (2015)
			QTL-hotspot_b (11 genes)			CaLG08, CaLG07	
	Biparental	ICC 4958 × ICC 1882	3 candidate genes	SNP		CaLG01, CaLG04	Singh et al. (2016)

(continued)

Table 1.2 (continued)

Stress	Mapping approach	Population name and size	QTLs/ MTA	Marker type	No. markers used	LG group	References
	Biparental	ICC 4958 × ICC 17163	6 candidate genes	SNP		CaLG2, CaLG3, CaLG4, CaLG5, CaLG6, CaLG7	Srivastava et al. (2016)
	Biparental	CC 4958 × ICC 1882, RILs (232)	21 major QTLs	SNP	241 SSRs, 1007 SSRs and 1557 SNPs	CaLG01, CaLG03, CaLG04, CaLG05, Ca LG06, CaLG07, Ca LG08	Sivasakthi et al. (2018)
	Super GWAS		38 significant MTAs	SNP	1,44,000	Ca3, Ca4, Ca5, Ca6	Li et al. (2018)
Heat	Biparental	ICC 4567 × ICC 15614, RILs (292)	4 QTLs	SNP	271		Paul et al. (2018b)
	GWAS	71 genotypes		SSR	81		Jha et al. (2018a)
	Biparental	DCP92–3 × ICCV92944, F2 (206)	2 QTLs	SSR	78		Jha et al. (2019c)
	GWAS			SNP			Varshney et al. (2019)
	GWAS	182 genotypes	24 + 14 MTAs under normal condition; 17 + 34 MTAs in heat-stressed environment	SSR	120 SSRs	LG1 to LG8	Jha et al. (2021a)
	Biparental	DCP92–3 × ICCV92944	77 QTLs	SNP	788	LG1-LG8	Jha et al. (2021b)

marker-assisted backcrossing approach to improve drought tolerance in chickpea (Varshney et al. 2013b; Roorkiwal et al. 2020; Bharadwaj et al. 2020).

1.7 Functional Genomics Approach for Uncovering Candidate Genes for Drought and Heat Tolerance

In the last decade, advances in functional genomics approaches, especially RNA-seq and earlier related technologies, have been used to identify several candidate gene (s) and their relevant function contributing to drought and heat stress in chickpea (Badhan et al. 2018; Mahdavi Mashaki et al. 2018). Earlier work using microarray technology identified several thousand genes expressed in response to drought stress (Mantri et al. 2007; Varshney et al. 2009). Varshney et al. (2009) reported 20,162 ESTs responding to drought stress. Likewise, Deokar et al. (2011) obtained 3062 unigenes responding to drought stress in a suppression subtraction hybridization study. Subsequently, high throughput RNA-seq technology assisted in unveiling a plethora of transcription factors (TFs), including WRKY, NAC, MYB, AP2-EREBP, and bHLH, controlling drought stress tolerance (Badhan et al. 2018; Mahdavi Mashaki et al. 2018; Kumar et al. 2019). Myriad of DEGs has been investigated using RNA-seq studies in chickpea to explore the role of various differentially expressed genes (DEGs) in response to drought stress (Kumar et al. 2019; Bhaskarla et al. 2020; Sagar et al. 2021). Recently, the roles of phospholipases D genes (Sagar et al. 2021) and oxylipin biosynthesis genes (Bhaskarla et al. 2020) contributing to drought stress response were elucidated in a functional genomic study (see Table 1.3). Agarwal et al. (2016) uncovered five HSP 90 genes contributing to heat stress tolerance based on the results of an RNA-seq study on reproductive tissues treated with heat stress. Thus, transcriptomic studies have facilitated the deciphering of candidate gene(s) and complex network of gene (s) and their functional role in attributing plant adaptation to drought and heat stress in chickpea.

1.8 Proteome and Metabolome Dynamics for Resolving Drought and Heat Tolerance in Chickpea

Proteomics could enhance our understanding of drought stress tolerance by providing insight into the proteins produced in response to drought stress (Parankusam et al. 2017). Drought-responsive participatory proteins obtained under drought stress include cellular proteins involved in glycolysis, protein synthesis, TCA cycle, signal transduction, protecting cells from reactive oxygen species (ROS)-related damage, heat shock proteins, and cellular osmotic adjustment (Gupta et al. 2020; Vessal et al. 2020). Vessal et al. (2020) reported the participatory activity of RuBisCo, ATP synthase, carbonic anhydrase, L-ascorbate peroxidase, auxin-binding protein contributing to drought stress tolerance in chickpea. Similarly, a comparative proteomics analysis revealed 75 proteins—related to oxidative stress

Table 1.3 List of differentially expressed gene(s) under drought and heat stress identified by functional genomic approaches

Stress	Tissue used	Transcriptomic technique used	Differentially expressed gene (s) with putative function	References
Drought	Leaves and flowers		Repression of aquaporin-like membrane channel protein; inhibition of auxin-repressed proteins	Mantri et al. (2007)
	Roots	SuperSAGE	7532 unitags and 880 unitags involved in ROS scavenging activity under water stress	Molina et al. (2008)
	Roots	ESTs	20,162 ESTs, dehydrin DHN3, late embryogenesis abundant (LEA) genes coexpressed under drought stress	Varshney et al. (2009)
	Shoots and roots	Suppression subtractive hybridization	3062 unigenes; upregulation of myoinositol-1-phosphate synthase (MIPS) and pyrroline-5-carboxylate synthetase (P5CS) genes helps in drought stress tolerance; regulation of Myb, ERF-2, NAC, bZIP, HD-ZP, etc. could participate in drought tolerance	Deokar et al. (2011)
	Roots	ESTs	44,639 tentative unique sequences involved in regulating various stress-responsive TFs and HSPs under drought stress	Hiremath et al. (2011)
	Roots	RNA-seq	4053 and 1330 regulate TFs (bHLH, AP2-EREBP and MYB HB, WRKY and NAC) under water stress	Garg et al. (2016)
	Roots and shoots	RNA-seq	–	Srivastava et al. (2016)
	Leaves from shoots at apical meristem stage	RNA-seq	1562 genes, 2592 genes related to controlling expression of MYB-related protein, ethylene response under water stress	Badhan et al. (2018)
	Roots and shoots	RNA-seq	261and 169 genes, TFs (bHLH, leucine-rich repeat), aldo/ keto reductase, potassium channel, chlorophyll A-B binding protein, inositol polyphosphate-related phosphatase	Mahdavi Mashaki et al. (2018)
	–	RNA-seq	1624 genes including regulation of mannitol dehydrogenase, serine hydroxymethyl-transferase 4-like, cytochrome P450 81E8-like and galactinol-	Kumar et al. (2019)

(continued)

Table 1.3 (continued)

Stress	Tissue used	Transcriptomic technique used	Differentially expressed gene (s) with putative function	References
			sucrose galactosyltransferase-like, AP2- EREBP, bHLH, bZIP, C3H, MYB, NAC, WRKY TFs under water-scarce environment	
	Roots	RNA-seq	Upregulation of stress-responsive transcription factors, kinases, ROS signaling and scavenging, transporters, root nodulation, and oxylipin biosynthesis genes	Bhaskarla et al. (2020)
	Roots	qRT-PCR	*Ca_06899, Ca_18090, Ca_22941, Ca_04337, Ca_04069, Ca_04233, Ca_12660, Ca_16379, Ca_16946,* and *Ca_21186*	Singh et al. (2016)
	Leaves Seedling	RNA-seq SqRT-PCR	Genes involved in phenylpropanoid biosynthesis pathway were upregulated in tolerant genotype *WRKY, DREB2A*, and *CarNAC3* genes	Moenga et al. (2020) Borhani et al. (2020)
	–	qRT-PCR	Phospholipases D genes differentially expressed in response to drought stress	Sagar et al. (2021)
Heat	Flowers, shoots, roots	RNA-seq	Five HSP 90 genes	Agarwal et al. (2016)

tolerance, HSPs, cellular metabolism, ROS metabolism—involved in drought stress tolerance in chickpea (Gupta et al. 2020).

A comparative proteomics analysis revealed that proline and sucrose biosynthesis-related proteins, glutamine synthetase, and cytosolic fructose-bisphosphate aldolase were involved in drought stress tolerance in *C. reticulatum* than *C. arietinum* (Cevik et al. 2019). A proteomics analysis of reproductive tissue identified 154 proteins differentially expressed in two contrasting heat-tolerant genotypes under heat stress; the actively participating proteins included acetyl-CoA carboxylase, pyrroline-5-carboxylate synthase (P5CS), ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), phenylalanine ammonia-lyase (PAL) 2, ATP synthase, glycosyltransferase, sucrose synthase, and late embryogenesis abundant (LEA) proteins (Parankusam et al. 2017). Further study is needed to decipher the role of various proteins related to drought- and heat-sensing and signal transduction pathways contributing to drought and heat tolerance in chickpea.

Likewise, metabolomics profiling could be harnessed to obtain insights into the various metabolites generated in response to drought and heat stress, allowing plants to survive under these stresses (Khan et al. 2019a, b). Metabolomic analysis of two contrasting chickpea genotypes under water stress revealed the accumulation of various metabolites (e.g., choline, phenylalanine, gamma-aminobutyric acid, alanine, phenylalanine, tyrosine, glucosamine, guanine, and aspartic acid) attributed to drought stress adaptation (Khan et al. 2019a). Khan et al. (2019b) reported the accumulation of various metabolites (proline, L-arginine, L-histidine, L-isoleucine, and tryptophan) in the leaves of chickpea genotypes treated with plant growth-promoting rhizobacteria (PGPR) and various plant growth regulators (PGRs) under drought stress. The PGPR- and PGR-treated plants also had enhanced riboflavin accumulation, L-asparagine, aspartate, and glycerol in leaves. The same research group also witnessed the increased accumulation of malonate, 5-oxo-L-proline, and trans-cinnamate in genotypes treated with PGPR and PGR, which was attributed to drought tolerance (Khan et al. 2019c). Hence, these drought- and heat-responsive metabolites allow chickpea to maintain growth and development and ultimately survive in drought- and heat-stress environments.

1.9 Emerging Modern Breeding Tools for Accelerating Genetic Gain in Chickpea

Traditional breeding approaches have significantly improved chickpea yield but these approaches alone will not meet the rising demand for chickpea due to the growing human population (Langridge and Fleury 2011). The augmentation of several novel breeding tools, such as genomic selection, could predict the genetic merit/phenotypic performance/breeding value of various untested progenies developed in crossing programs using various prediction/statistical models, thus reducing the breeding cycle and assisting in the selection of superior progenies with improved genetic gain (Meuwissen et al. 2001). Such an approach has been used to predict genetic gain in chickpea under drought stress (Li et al. 2018). Speed breeding/rapid generation has been recently introduced to grow three to four crop generations per year in chickpea, thus allowing faster development of new cultivars (Hickey et al. 2019; Samineni et al. 2020). Likewise, genome editing tools could be used to manipulate nucleotide sequences (addition/deletion) at targeted locations without the intervention of transgene (Nasti and Voytas 2021). This technology has been used to develop drought-tolerant chickpea by editing *4-coumarate ligase* (*4CL*) and *Reveille 7* (*RVE7*) genes attributed to water stress tolerance (Badhan et al. 2021). In addition, the multiparent advanced generation intercross (MAGIC) population scheme was developed at ICRISAT, India, to broaden the genetic base by capturing high allelic diversity and increasing resilience to various abiotic stresses, including drought and heat stress. Potential diverse founder parents, including ICC 4958, JG 130, ICCV 10, JAKI 9218, JG 130, JG 16, ICCV 97105, and ICCV 00108 were included in the crossing programme for this scheme (Samineni et al. 2017). The recombinant inbred lines from these parents could map drought- and heat-tolerant QTLs with higher resolution and improve chickpea genetic gain under harsh environments. A similar hybrid-nested association mapping and backcross introgression approach has been used to harness variation in recent crop wild relative collections (von Wettberg et al. 2018), crossing a diverse panel of wild parents into elite cultivated lines.

1.10 Scope of High Throughput Phenotyping for Capturing the Precise Phenotypic Response of Drought and Heat Stress in Plants

As drought and heat stress are governed by multiple gene(s)/QTLs and greatly influenced by G × E × M interactions, measuring their accurate response remains challenging (Hein et al. 2021; Smith et al. 2021). The evolution of various phenomics facilities in the last decade has dramatically advanced our understanding, measuring the drought and heat stress response both spatially and temporally. Advances in nondestructive methods, including sensor-based technologies, image-based platforms, unmanned aerial vehicles, and drone-based technologies, have assisted in measuring drought and heat stress responses at a large scale under target environments in the field (Houle et al. 2010; Fiorani and Schurr 2013; Furbank and Tester 2011; Großkinsky et al. 2015; Hein et al. 2021). Among image-based techniques, Red–Green–Blue images can measure plant area and color, time of day of flowering, yield, and yield parameters (Sadeghi-Tehran et al. 2017; Xiong et al. 2017). Likewise, infrared is used to assess plant temperature, and SPAD meters, hyperspectral radiometers, and field spectroradiometers are used to assess photosynthesis efficiency (Peng et al. 2017). Moreover, emerging deep learning and machine learning approaches could be used to measure drought and heat stress responses to develop better drought- and heat-tolerant chickpea lines.

1.11 Conclusion and Future Perspective

With global climate change, frequent episodes of drought and heat stress are becoming a major concern, exacerbating chickpea yield losses. A thorough screening of chickpea germplasm is urgently needed in the target environment to increase chickpea resilience to drought and heat stress. As drought and heat stress sometimes occur concurrently, the selection of traits conferring combined drought tolerance should be targeted and transferred into elite chickpea lines for increased resilience to drought and heat tolerance. Concomitantly, studies should focus on pre-breeding activities and exploration of landraces with inherent drought and heat tolerance capability (Rani et al. 2020). Marker-assisted breeding schemes allow us to transfer the genomic regions conferring drought and heat tolerance into elite chickpea cultivars, thus improving the genetic gain of elite chickpea cultivars (Roorkiwal et al. 2020).

Efforts to resequence global chickpea germplasm and a pangenomics approach could underpin the novel causal variants attributing drought and heat tolerance in chickpea (Varshney et al. 2021). Likewise, genomic selection will assist in predicting superior progenies conferring drought and heat tolerance based on the prediction model and thus reduce breeding cycles. In parallel, MAGIC and NAM populations are being developed to increase resilience and broaden the genetic base. In addition, rapid generation advancement and genome editing technology could be used to design a more climate-resilient chickpea to sustain global food security.

References

Agarwal G, Garg V, Kudapa H, Doddamani D, Pazhamala LT, Khan AW, Thudi M, Lee SH, Varshney RK (2016) Genomewide dissection of AP2/ERF and HSP90 gene families in five legumes and expression profiles in chickpea and pigeonpea. Plant Biotechnol J 14:1563–1577

Arif A, Parveen N, Waheed MQ, Atif RM, Waqar I, Shah TM (2021) A comparative study for assessing the drought-tolerance of chickpea under varying natural growth environments. Front Plant Sci 11:2228

Awasthi S, Peto R, Read S, Clark S, Pande V, Bundy D, DEVTA (Deworming and Enhanced Vitamin A) team (2013) Vitamin A supplementation every 6 months with retinol in 1 million pre-school children in north India: DEVTA, a cluster-randomised trial. Lancet 381(9876): 1469–1477

Awasthi R, Kaushal N, Vadez V, Turner NC, Berger J, Siddique KH, Nayyar H (2014) Individual and combined effects of transient drought and heat stress on carbon assimilation and seed filling in chickpea. Func Plant Biol 41:1148–1167

Awasthi JP, Saha B, Regon P, Sahoo S, Chowra U, Pradhan A et al (2017) Morpho-physiological analysis of tolerance to aluminum toxicity in rice varieties of North East India. PLoS One 12(4): e0176357. https://doi.org/10.1371/journal.pone.0176357

Badhan S, Kole P, Ball A, Mantri N (2018) RNA sequencing of leaf tissues from two contrasting chickpea genotypes reveals mechanisms for drought tolerance. Plant Physiol Biochem 129:295–304

Badhan S, Ball AS, Mantri N (2021) First Report of CRISPR/Cas9 Mediated DNA-Free Editing of 4CL and RVE7 Genes in Chickpea Protoplasts. Int J Mol Sci 22:396

Bhandari K, Sita K, Sehgal A, Bhardwaj A, Gaur P, Kumar S, Singh S, Siddique KH, Prasad PV, Jha U, Nayyar H (2020) Differential heat sensitivity of two cool-season legumes, chickpea and lentil, at the reproductive stage, is associated with responses in pollen function, photosynthetic ability and oxidative damage. J Agron Crop Sci 206:734–758

Bharadwaj C, Tripathi S, Soren KR, Thudi M, Singh RK, Sheoran S et al (2020) Introgression of “QTL-hotspot” region enhances drought tolerance and grain yield in three elite chickpea cultivars. Plant Genome. https://doi.org/10.1002/tpg2.20076

Bhaskarla V, Zinta G, Ford R, Jain M, Varshney RK, Mantri N (2020) Comparative root transcriptomics provide insights into drought adaptation strategies in chickpea (*Cicer arietinum* L.). Int J Mol Sci 21:1781

Bohra A, Chand Jha U, Godwin ID, Kumar Varshney R (2020) Genomic interventions for sustainable agriculture. Plant Biotechnol J 18:2388–2405

Borhani S, Vessal S, Bagheri A, Shokouhifar F (2020) Differential gene expression pattern of drought responsive transcription factors in chickpea: an expressional analysis. J Plant Growth Regul 39:1211–1220

Canci H, Toker C (2009a) Evaluation of yield criteria for drought and heat resistance in chickpea (*Cicer arietinum* L.). J Agron Crop Sci 19:47–54

Canci H, Toker C (2009b) Evaluation of annual wild *Cicer* species for drought and heat resistance under field conditions. Genet Resour Crop Evol 56:1. https://doi.org/10.1007/s10722-008-9335-9

Cevik S, Akpinar G, Yildizli A, Kasap M, Karaosmanoğlu K, Ünyayar S (2019) Comparative physiological and leaf proteome analysis between drought-tolerant chickpea *Cicer reticulatum* and drought-sensitive chickpea *C. arietinum*. J Biosci 44:1–3

Chen Y, Ghanem ME, Siddique KHM (2017) Characterising root trait variability in chickpea (Cicer arietinum L.) germplasm. J Exp Bot 68:1987–1999

Coyne CJ, Kumar S, von Wettberg EJ, Marques E, Berger JD, Redden RJ, Ellis TN, Brus J, Zablatzká L, Smýkal P (2020) Potential and limits of exploitation of crop wild relatives for pea, lentil, and chickpea improvement. Legume Sci 2:e36

Deokar AA, Kondawar V, Jain PK, Karuppayil M, Raju NL, Vadez V, Varshney RK, Srinivasan R (2011) Comparative analysis of expressed sequence tags (ESTs) between drought-tolerant and -susceptible genotypes of chickpea under terminal drought stress. BMC Plant Biol 11:70

Devasirvatham V, Tan DKY, Trethowan RM, Gaur PM, Mallikarjuna N (2010) Impact of high temperature on the reproductive stage of chickpea. In: Food security from sustainable agriculture. Proceedings of the 15th Australian Society of Agronomy Conference, 15–18 November 2010, Lincoln, New Zealand

Devasirvatham V, Gaur P, Mallikarjuna N, Raju TN, Trethowan RM, Tan DKY (2012) Effect of high temperature on the reproductive development of chickpea genotypes under controlled environments. Funct Plant Biol 39:1009–1018

Devasirvatham V, Gaur P, Mallikarjuna N, Raju TN, Trethowan RM, Tan DKY (2013) Reproductive biology of chickpea response to heat stress in the field is associated with the performance in controlled environments. Field Crops Res 142:9–19

Devasirvatham V, Gaur PM, Raju TN, Trethowan RM, Tan DKY (2015) Field response of chickpea (*Cicer arietinum* L.) to high temperature. Field Crops Res 172:59–71

Dua RP (2001) Genotypic variations for low and high temperature tolerance in gram (*Cicer arietinum*). Indian J Agric Sci 71:561–566

Farooq M, Ullah A, Lee DJ, Alghamdi SS, Siddique KH (2018) Desi chickpea genotypes tolerate drought stress better than kabuli types by modulating germination metabolism, trehalose accumulation, and carbon assimilation. Plant Physiol Biochem 126:47–54

Fiorani F, Schurr U (2013) Future scenarios for plant phenotyping. Annu Rev Plant Biol 64:267–291

Furbank RT, Tester M (2011) Phenomics—technologies to relieve the phenotyping bottleneck. Trends Plant Sci 16:635–644

Garg R, Shankar R, Thakkar B, Kudapa H, Krishnamurthy L, Mantri N, Varshney RK, Bhatia S, Jain M (2016) Transcriptome analyses reveal genotype- and developmental stage-specific molecular responses to drought and salinity stresses in chickpea. Sci Rep 6:19228

Gaur PM, Krishnamurty L, Kashiwagi J (2008) Improvement drought-avoidance root traits in chickpea (*Cicer arietinum* L.)-current status of research at ICRISAT. Plant Prod Sci 1:3–11

Gaur PM, Jukanti AK, Varshney RK (2012) Impact of genomic technologies on chickpea breeding strategies. Agronomy 2:199–221

Getahun T, Negash K, Chang PL, von Wettberg E, Carrasquilla-Garcia N, Gaur PM, Fikre A, Haileslassie T, Cook D, Tesfaye K (2021) Screening of heat-tolerant Ethiopian chickpea accessions: assessment of phenological and agromorphological traits and genomic relationships. Agrosyst Geosci Environ 4(3):e20211

Graham PH, Vance CP (2003) Legumes: importance and constraints to greater use. Plant Physiol 131:872–877

Großkinsky DK, Svensgaard J, Christensen S, Roitsch T (2015) Plant phenomics and the need for physiological phenotyping across scales to narrow the genotype-to-phenotype knowledge gap. J Exp Bot 66:5429–5440

Guo R, Shi L, Jiao Y, Li M, Zhong X, Gu F, Liu Q, Xia X, Li H (2018) Metabolic responses to drought stress in the tissues of drought-tolerant and drought-sensitive wheat genotype seedlings. AoB Plants 10(2):ply016

Gupta S, Mishra SK, Misra S, Pandey V, Agrawal L, Nautiyal CS, Chauhan PS (2020) Revealing the complexity of protein abundance in chickpea root under drought-stress using a comparative proteomics approach. Plant Physiol Biochem 151:88–102

Hamwieh A, Imtiaz M (2015) Identifying water-responsive and drought-tolerant chickpea genotypes. Crop Pasture Sci 66:1003–1011

Hamwieh A, Imtiaz M, Malhotra RS (2013) Multi-environment QTL analyses for drought-related traits in a recombinant inbred population of chickpea (*Cicer arietinum* L.). Theor Appl Genet 126:1025–1038

Hein NT, Ciampitti IA, Jagadish SV (2021) Bottlenecks and opportunities in field-based high-throughput phenotyping for heat and drought stress. J Expt Bot. 2021 Jan 20

Hickey LT, Hafeez AN, Robinson H, Jackson SA, Leal-Bertioli SC, Tester M, Gao C, Godwin ID, Hayes BJ, Wulff BB (2019) Breeding crops to feed 10 billion. Nat Biotechnol 37:744–754

Hiremath PJ, Farmer A, Cannon SB, Woodward J, Kudapa H, Tuteja R, Kumar A, Bhanuprakash A, Mulaosmanovic B, Gujaria N, Krishnamurthy L, Gaur PM, Kavikishor PB, Shah T, Srinivasan R, Lohse M, Xiao Y, Town CD, Cook DR, May GD, Varshney RK (2011) Large-scale transcriptome analysis in chickpea (*Cicer arietinum* L.), an orphan legume crop of the semi-arid tropics of Asia and Africa. Plant Biotechnol J 9:922–931

Houle D, Govindaraju DR, Omholt S (2010) Phenomics: the next challenge. Nat Rev Genet 11: 855–866

Jagadish SVK, Way DA, Sharkey TD (2021) Plant heat stress: concepts directing future research. Plant Cell Environ 44(7):1992–2005. https://doi.org/10.1111/pce.14050

Jaganathan D, Thudi M, Kale S, Azam S, Roorkiwal M, Gaur PM, Kishor PB, Nguyen H, Sutton T, Varshney RK (2015) Genotyping-by-sequencing based intra-specific genetic map refines a “QTL-hotspot” region for drought tolerance in chickpea. Mol Gen Genomics 290:559–571

Jain M, Misra G, Patel RK, Priya P, Jhanwar S, Khan AW, Shah N, Singh VK, Garg R, Jeena G, Yadav M, Kant C, Sharma P, Yadav G, Bhatia S, Tyagi AK, Chattopadhyay D (2013) A draft genome sequence of the pulse crop chickpea (*Cicer arietinum* L.). Plant J 74:715–729

Jha UC (2018) Current advances in chickpea genomics: applications and future perspectives. Plant Cell Rep 37:947–965

Jha UC, Shil S (2015) Association analysis of yield contributing traits of chickpea genotypes under high temperature condition. Trends Biosci 8:2335–2341

Jha UC, Chaturvedi SK, Bohra A, Basu PS, Khan MS, Barh D (2014a) Abiotic stresses, constraints and improvement strategies in chickpea. Plant Breed 133:163–178

Jha UC, Bohra A, Singh NP (2014b) Heat stress in crop plants: its nature, impacts and integrated breeding strategies to improve heat tolerance. Plant Breed 133:679–701

Jha UC, Basu PS, Singh DK (2015) Genetic variation and diversity analysis of chickpea genotypes based on quantitative traits under high temperature stress. Int J Bio-Resour Stress Manag 6:700–706

Jha UC, Bohra A, Jha R, Parida S (2017) Integrated’ omics’ approaches to sustain major global grain legume productivity under heat stress. Plant Breed 136:437–459

Jha UC, Jha R, Bohra A, Parida SK, Kole PC, Thakro V, Singh D, Singh NP (2018a) Population structure and association analysis of heat stress relevant traits in chickpea (*Cicer arietinum* L.). 3. Biotech 8:43

Jha UC, Jha R, Singh NP, Shil S, Kole PC (2018b) Heat tolerance indices and their role in selection of heat stress tolerant chickpea (*Cicer arietinum* L.) genotypes. Indian J Agric Sci 88:260–267

Jha UC, Kole PC, Singh NP (2018c) Genetic variability and marker trait association analysis of various phenological and yield related traits for heat tolerance in chickpea (*Cicer arietinum* L.). Int J Bioresour Stress Manag 9:345–352

Jha UC, Kole PC, Singh NP (2019a) Nature of gene action and combining ability analysis of yield and yield related traits in chickpea (*Cicer arietinum* L.) under heat stress. Indian J Agric Sci 89: 500–508

Jha UC, Kole PC, Singh NP, Sandip S, Hemant K (2019b) GGE bi-plot analysis for grain yield in chickpea (*Cicer arietinum*) under normal and heat stress conditions. Indian J Agric Sci 89:721–725

Jha UC, Kole PC, Singh NP (2019c) QTL mapping for heat stress tolerance in chickpea (*Cicer arietinum* L.). Legume Res. https://doi.org/10.18805/LR-4121

Jha UC, Bohra A, Nayyar H (2020) Advances in “omics” approaches to tackle drought stress in grain legumes. Plant Breed 139:1–27

Jha UC, Jha R, Thakro V, Kumar A, Gupta S, Nayyar H, Basu P, Parida SK, Singh NP (2021a) Discerning molecular diversity and association mapping for phenological, physiological and yield traits under high temperature stress in chickpea (*Cicer arietinum* L.). J Genet 100:1–5

Jha UC, Palakurthi R, Nayyar H, Jha R, Valluri VK, Bajaj P, Chitikineni A, Singh NP, Varshney RK, Thudi M (2021b) Major QTLs and potential candidate genes for heat stress tolerance identified in chickpea (*Cicer arietinum* L.). Front Plant Sci 12:1241

Jukanti AK, Gaur PM, Gowda CL, Chibbar RN (2012) Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. Br J Nutr 1:S11–S26

Jumrani K, Bhatia VS (2014) Impact of elevated temperatures on growth and yield of chickpea (*Cicer arietinum* L.). Field Crop Res 164:90–97

Kahraman A, Pandey A, Khan MK, Lindsay D, Moenga S, Vance L, Bergmann EM, Carrasquilla-Garcia N, Chang PL, Shin MG, von Wettberg EJ, Tar'an B, Cook DR, Penmetsa RV (2017) Distinct subgroups of Cicer echinospermum are associated with hybrid sterility and hybrid breakdown in interspecific crosses with cultivated chickpea. Crop Sci. https://doi.org/10.2135/cropsci2017.06.0335

Kale SM, Jaganathan D, Ruperao P, Chen C, Punna R, Kudapa H, Thudi M, Roorkiwal M, Katta MA, Doddamani D, Garg V, Kishor PB, Gaur PM, Nguyen HT, Batley J, Edwards D, Sutton T, Varshney RK (2015) Prioritization of candidate genes in "QTL-hotspot" region for drought tolerance in chickpea (*Cicer arietinum* L.). Sci Rep 5(15296)

Kalra N, Chakraborty D, Sharma A, Rai HK, Jolly M, Chander S, Kumar PR, Bhadraray S, Barman D, Mittal RB, Lal M, Sehgal M (2008) Effect of temperature on yield of some winter crops in northwest India. Curr Sci 94:82–88

Kashiwagi J, Krishnamurthy L, Upadhyaya HD, Krishna H, Chandra S, Vadez V, Serraj R (2005) Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea. Euphytica 146(3):213–222

Kashiwagi J, Krishnamurty L, Gaur PM, Chandra S, Upadhyaya HD (2008) Estimation of gene effects of the drought avoidance root characteristics in chickpea (*Cicer arietinum* L.). Field Crops Res 105:64–69

Kashiwagi J, Krishnamurthy L, Gaur PM, Upadhyaya HD, Varshney RK, Tobita S (2013) Traits of relevance to improve yield under terminal drought stress in chickpea (*C. arietinum* L.). Field Crops Res 145:80–95

Kaushal N, Awasthi R, Gupta K, Gaur PM, Siddique KHM, Nayyar H (2013) Heat-stress- induced reproductive failures in chickpea (*Cicer arietinum*) are associated with impaired sucrose metabolism in leaves and anthers. Funct Plant Biol 40:1334–1349

Khan N, Bano A, Rahman MA, Rathinasabapathi B, Babar MA (2019a) UPLC-HRMS-based untargeted metabolic profiling reveals changes in chickpea (*Cicer arietinum*) metabolome following long-term drought stress. Plant Cell Environ 42:115–132

Khan N, Bano A, Rahman MA, Guo J, Kang Z, Babar MA (2019b) Comparative physiological and metabolic analysis reveals a complex mechanism involved in drought tolerance in chickpea (*Cicer arietinum* L.) induced by PGPR and PGRs. Sci Rep 9:1–9

Khan N, Bano A, Babar MA (2019c) Metabolic and physiological changes induced by plant growth regulators and plant growth promoting rhizobacteria and their impact on drought tolerance in *Cicer arietinum* L. PLoS One 14:e0213040

Khandal H, Gupta SK, Dwivedi V, Mandal D, Sharma NK, Vishwakarma NK, Pal L, Choudhary M, Francis A, Malakar P, Singh NP (2020) Root-specific expression of chickpea cytokinin oxidase/dehydrogenase 6 leads to enhanced root growth, drought tolerance and yield without compromising nodulation. Plant Biotechnol J 18:2225

Krishnamurthy L, Kashiwagi J, Upadhyaya HD, Serraj R (2003) Genetic diversity of drought avoidance root traits in the mini-core germplasm collection of chickpea. Int Chickpea Pigeonpea Newslett 10:21–24

Krishnamurthy L, Gaur PM, Basu PS, Chaturvedi SK, Tripathi S, Vadez V, Rathore A, Varshney RK, Gowda CLL (2011) Large genetic variation for heat tolerance in the reference collection of chickpea (*Cicer arietinum* L.) germplasm. Plant Genet Res 9:59–69

Krishnamurthy L, Kashiwagi J, Upadhyaya HD, Gowda CLL, Gaur PM, Singh S et al (2013a) Partitioning coefficient—a trait that contributes to drought tolerance in chickpea. Field Crops Res 149:354–365

Krishnamurthy L, Kashiwagi J, Tobita S, Ito O, Upadhyaya HD, Gowda CLL, Gaur PM, Sheshshayee MS, Singh S, Vadez V, Varshney RK (2013b) Variation in carbon isotope discrimination and its relationship with harvest index in the reference collection of chickpea germ-plasm. Funct Plant Biol 40:1350–1361

Kumar J, Abbo S (2001) Genetics of flowering time in chickpea and its bearing on productivity in semi-arid environments. Adv Agron 72:107–138

Kumar J, Rao BV (1996) Super early chickpea developed at ICRISAT Asia center. Int Chickpea Pigeonpea Newslett 3:17–18

Kumar M, Chauhan AS, Kumar M, Yusuf MA, Sanyal A, Chauhan PS (2019) Transcriptome sequencing of chickpea (*Cicer arietinum* L.) genotypes for identification of drought-responsive genes under drought stress condition. Plant Mol Biol Rep 17:186–203

Langridge P, Fleury D (2011) Making the most of' omics' for crop breeding. Trends Biotechnol 29: 33–40

Leport L, Turner NC, French RJ, Barr MD, Duda R, Davies SL, Siddique KHM (1999) Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. Eur J Agron 11:279–291

Li Y, Ruperao P, Batley J, Edwards D, Khan T, Colmer TD, Pang J, Siddique KH, Sutton T (2018) Investigating drought tolerance in chickpea using genomewide association mapping and genomic selection based on whole-genome resequencing data. Front Plant Sci 9:190

Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y (2010) Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust J Crop Sci 4:580–585

Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y (2011) Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (*Cicer arietinum*) cultivars. Aust J Crop Sci 5:1255–1260

Mahdavi Mashaki K, Garg V, NasrollahnezhadGhomi AA, Kudapa H, Chitikineni A, Zaynali Nezhad K et al (2018) RNA-Seq analysis revealed genes associated with drought stress response in kabuli chickpea (*Cicer arietinum* L.). PLoS One 13:e0199774

Makonya GM, Ogola JB, Muasya AM, Crespo O, Maseko S, Valentine AJ, Ottosen CO, Rosenqvist E, Chimphango SB (2020) Intermittent moisture supply induces drought priming responses in some heat-tolerant chickpea genotypes. Crop Sci 60:2527–2542

Mantri NL, Ford R, Coram TE, Pang EC (2007) Transcriptional proling of chickpea genes differentially regulated in response to high-salinity, cold and drought. BMC Genome 8:303

Marques E, Kur A, Bueno E, von Wettberg E (2020a) Defining and improving the rotational and intercropping value of a crop using a plant–soil feedbacks approach. Crop Sci 60(5):2195–2203

Marques E, Krieg CP, Dacosta-Calheiros E, Bueno E, Sessa E, Penmetsa RV, von Wettberg EJ (2020b) The impact of domestication on aboveground and belowground trait responses to nitrogen fertilization in wild and cultivated genotypes of chickpea (*Cicer* sp.). Front Genet. https://doi.org/10.3389/fgene.2020.576338

Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genomewide dense marker maps. Genetics 157:1819–1829

Mir AH, Bhat MA, Dar SA, Sofi PA, Bhat NA, Mir RR (2021) Assessment of cold tolerance in chickpea (Cicer spp.) grown under cold/freezing weather conditions of North-Western Himalayas of Jammu and Kashmir, India. Physiol Mol Biol Plants 27(5):1105–1118

Moenga SM, Gai Y, Carrasquilla-Garcia N, Perilla-Henao LM, Cook DR (2020) Gene co-expression analysis reveals transcriptome divergence between wild and cultivated chickpea under drought stress. Plant J 104:1195–1214

Molina C, Rotter B, Horres R, Udupa SM, Besser B, Bellarmino L, Baum M, Matsumura H, Terauchi R, Kahl G, Winter P (2008) SuperSAGE: the drought stress-responsive transcriptome of chickpea roots. BMC Genomics 9:553

Nasr Esfahani M, Sulieman S, Schulze J, Yamaguchi-Shinozaki K, Shinozaki K, Tran LS (2014) Mechanisms of physiological adjustment of N_2 fixation in *Cicer arietinum* L. (chickpea) during early stages of water deficit: single or multi-factor controls. Plant J 79:964–980

Nasti RA, Voytas DF (2021) Attaining the promise of plant gene editing at scale. Proc Nat Acad Sci 118(22)

Pang J, Turner NC, Du YL, Colmer TD, Siddique KHM (2017a) Pattern of water use and seed yield under terminal drought in chickpea genotypes. Front Plant Sci 8:1375

Pang J, Turner NC, Khan T, Du YL, Xiong JL, Colmer TD et al (2017b) Response of chickpea (*Cicer arietinum* L.) to terminal drought: leaf stomatal conductance, pod abscisic acid concentration, and seed set. J Exp Bot 68:1973–1985

Parankusam S, Bhatnagar-Mathur P, Sharma KK (2017) Heat responsive proteome changes reveal molecular mechanisms underlying heat tolerance in chickpea. Environ Exp Bot 141:132–144

Parry MA, Andralojc PJ, Khan S, Lea PJ, Keys AJ (2002) Rubisco activity: effects of drought stress. Ann Bot 89:833–839

Paul PJ, Samineni S, Sajja SB, Rathore A, Das RR, Khan AW, Chaturvedi SK et al (2018a) Capturing genetic variability and selection of traits for heat tolerance in a chickpea recombinant inbred line (RIL) population under field conditions. Euphytica 214:27

Paul PJ, Samineni S, Thudi M, Sajja SB, Rathore A, Das RR, Khan AW, Chaturvedi SK, Lavanya GR, Varshney RK, Gaur PM (2018b) Molecular mapping of QTLs for heat tolerance in chickpea. Int J Mol Sci 19:E2166

Peng Y, Zeng A, Zhu T, Fang S, Gong Y, Tao Y, Zhou Y, Liu K (2017) Using remotely sensed spectral reflectance to indicate leaf photosynthetic efficiency derived from active fluorescence measurements. J Appl Remote Sens 11:026034

Purushothaman R, Thudi M, Krishnamurthy L, Upadhaya HD, Kashiwagi J, Gowda CLL, Varshney RK (2015) Association of mid-reproductive stage canopy depression with the molecular markers and grain yield of chickpea (*Cicer arietinum* L.) germplasm under terminal drought. Field Crops Res 174:1–11

Purushothaman R, Krishnamurthy L, Upadhyaya HD, Vadez V, Varshney RK (2016) Shoot traits and their relevance in terminal drought tolerance of chickpea (*Cicer arietinum* L.). Field Crop Res 197:10–27

Ramamoorthy P, Krishnamurthy L, Upadhyaya HD, Vadez V, Varshney RK (2016) Shoot traits and their relevance in terminal drought tolerance of chickpea (*Cicer arietinum* L.). Field Crop Res 197:10–27. https://doi.org/10.1016/j.fcr.2016.07.016

Ramamoorthy P, Krishnamurthy L, Upadhyaya HD, Vadez V, Varshney RK (2017) Root traits confer grain yield advantages under terminal drought in chickpea (*Cicer arietinum* L.). Field Crop Res 201:146–161. https://doi.org/10.1016/j.fcr.2016.11.004

Rani A, Devi P, Jha UC, Sharma KD, Siddique KH, Nayyar H (2020) Developing climate-resilient chickpea involving physiological and molecular approaches with a focus on temperature and drought stresses. Front Plant Sci 10:1759

Rehman AU, Malhotra RS, Bett K, Taran B, Bueckert R, Warkentin TD (2011) Mapping QTL associated with traits affecting grain yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. Crop Sci 51:450–463

Roorkiwal M, Bharadwaj C, Barmukh R, Dixit GP, Thudi M, Gaur PM, Chaturvedi SK, Fikre A, Hamwieh A, Kumar S, Sachdeva S (2020) Integrating genomics for chickpea improvement: achievements and opportunities. Theor Appl Genet 133:1703–1720

Sadeghi-Tehran P, Sabermanesh K, Virlet N, Hawkesford MJ (2017) Automated method to determine two critical growth stages of wheat: heading and flowering. Front Plant Sci 8:252

Sagar S, Biswas DK, Chandrasekar R, Singh A (2021) Genomewide identification, structure analysis and expression profiling of phospholipases D under hormone and abiotic stress treatment in chickpea (*Cicer arietinum*). Int J Biol Macromol 169:264–273

Samineni S, Thudi M, Sajja S, Varshney RK, Gaur PM (2017) Impact of genomics on Chickpea breeding. In: The chickpea genome. Compendium of plant genomes book series (CPG). Springer, pp 125–134

Samineni S, Sen M, Sajja SB, Gaur PM (2020) Rapid generation advance (RGA) in chickpea to produce up to seven generations per year and enable speed breeding. Crop J 8:164–169. https://doi.org/10.1016/j.cj.2019.08.003

Serraj R, Sinclair TR, Purcell LC (1999) Symbiotic N_2 fixation response to drought. J Exp Bot 50: 143–155

Shah TM, Imran M, Atta BM, Ashraf MY, Hameed A, Waqar I, Shafiq M, Hussain K, Naveed M, Aslam M, Maqbool MA (2020) Selection and screening of drought tolerant high yielding chickpea genotypes based on physio-biochemical indices and multi-environmental yield trials. BMC Plant Biol 20:1–6

Singh VK, Khan AW, Jaganathan D, Thudi M, Roorkiwal M, Takagi H, Garg V, Kumar V, Chitikineni A, Gaur PM, Sutton T, Terauchi R, Varshney RK (2016) QTL-seq for rapid identification of candidate genes for 100-seed weight and root/total plant dry weight ratio under rainfed conditions in chickpea. Plant Biotechnol J 14:2110–2119

Sivasakthi K, Thudi M, Tharanya M, Kale SM, Kholová J, Halime MH, Jaganathan D, Baddam R, Thirunalasundari T, Gaur PM, Varshney RK, Vadez V (2018) Plant vigour QTLs co-map with an earlier reported QTL hotspot for drought tolerance while water saving QTLs map in other regions of the chickpea genome. BMC Plant Biol 18:29

Smith DT, Potgieter AB, Chapman SC (2021) Scaling up high-throughput phenotyping for abiotic stress selection in the field. Theor Appl Genet 134:1845–1866

Srinivasan A, Takeda H, Senboku T (1996) Heat tolerance in food legumes as evaluated by cell membrane thermo stability and chlorophyll fluorescence techniques. Euphytica 88:35–45

Srivastava R, Bajaj D, Malik A, Singh M, Parida SK (2016) Transcriptome landscape of perennial wild *Cicer microphyllum* uncovers functionally relevant molecular tags regulating agronomic traits in chickpea. Sci Rep 6:1–17

Talip M, Adak A, Kahraman A, Berger J, Sari D, Sari H, Penmetsa RV, von Wettberg EJ, Cook DR, Toker C (2018) Agro-morphological traits of *Cicer reticulatum* Ladizinsky in comparison to *C. echinospermum* P.H. Davis in terms of improvement. Genet Resour Crop Evol. https://doi.org/10.1007/s10722-017-0587-0

Thudi M, Upadhyaya HD, Rathore A, Gaur PM, Krishnamurthy L, Roorkiwal M, Nayak SN, Chaturvedi SK, Basu PS, Gangarao NV, Fikre A, Kimurto P, Sharma PC, Sheshashayee MS, Tobita S, Kashiwagi J, Ito O, Killian A, Varshney RK (2014) Genetic dissection of drought and heat tolerance in chickpea through genomewide and candidate gene-based association mapping approaches. PLoS One 9:e96758

Toker C, Canci H, Yildirim T (2007) Evaluation of perennial wild Cicer species for drought resistance. Genet Resour Crop Evol 54:1781–1786

Toker C, Berger J, Eker T, Sari D, Sari H, Gokturk RS, Kahraman A, Aydin B, von Wettberg EJ (2021) Cicer turcicum: a new Cicer species and its potential to improve chickpea. Front Plant Sci 12:662891. https://doi.org/10.3389/fpls.2021.662891

Upadhyaya H, Dronavalli D, Gowda C, Singh S (2011) Identification and evaluation of chickpea germplasm for tolerance to heat stress. Crop Sci 51:2079–2094

Varshney RK, Hiremath PJ, Lekha P, Kashiwagi J, Balaji J, Deokar AA, Vadez V, Xiao Y, Srinivasan R, Gaur PM, Siddique KH (2009) A comprehensive resource of drought- and salinity-responsive ESTs for gene discovery and marker development in chickpea (*Cicer arietinum* L.). BMC Genome 10:1–8

Varshney RK, Song C, Saxena RK, Azam S, Yu S, Sharpe AG, Cannon S, Baek J, Rosen BD, Tar'an B, Millan T, Zhang X, Ramsay LD, Iwata A, Wang Y, Nelson W, Farmer AD, Gaur PM, Soderlund C, Penmetsa RV, Xu C, Bharti AK, He W, Winter P, Zhao S, Hane JK, Carrasquilla-Garcia N, Condie JA, Upadhyaya HD, Luo MC, Thudi M, Gowda CL, Singh NP, Lichtenzveig J, Gali KK, Rubio J, Nadarajan N, Dolezel J, Bansal KC, Xu X, Edwards D, Zhang G, Kahl G, Gil J, Singh KB, Datta SK, Jackson SA, Wang J, Cook DR (2013a) Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nat Biotechnol 31:240–246

Varshney RK, Gaur PM, Chamarthi SK, Krishnamurthy L, Tripathi S, Kashiwagi J, Samineni S, Singh VK, Thudi M, Jaganathan D (2013b) Fast-track introgression of 'QTL-hotspot' for root traits and other drought tolerance traits in JG 11, an elite and leading variety of chickpea. Plant Genome. https://doi.org/10.3835/plantgenome2013.07.0022

Varshney RK, Thudi M, Nayak SN, Gaur PM, Kashiwagi J, Krishnamurthy L, Jaganathan D, Koppolu J, Bohra A, Tripathi S, Rathore A, Jukanti AK, Jayalakshmi V, Vemula A, Singh SJ, Yasin M, Sheshshayee MS, Viswanatha KP (2014) Genetic dissection of drought tolerance in chickpea (*Cicer arietinum* L.). Theor Appl Genet 127:445–462

Varshney RK, Thudi M, Roorkiwal M, He W, Upadhyaya H, Yang W, Bajaj P, Curby P, Rathore A, Jian J, Doddamani D, Khan AW, Garg V, Chitikineni A, Xu D, Gaur PM, Singh NP, Chaturvedi SK, Krishnamurth L, Fikre A, Kimurto P, Sheshashayee MS, Bharadwaj C, Wang J, Lee SH, Edwards D, Kavikishor PB, Nguyen HT, Siddique KHM, Sutton T, Penmetsa RV, von Wettberg EJ, Vigouroux Y, Liu X, Xun X (2019) Resequencing of 429 chickpea accessions from 45 countries provides insights into genome diversity, domestication and agronomic traits. Nat Genet 51(5):857

Varshney RK, Roorkiwal M, Sun S, Bajaj P, Chitikineni A, Thudi M, Singh NP, Du A, Khan AW, Wang Y, Garg V, Upadhyaya HD, Fan G, Cowling W, Crossa J, Gentzbittel L, Voss-Fels K, Valluri V, Sinha P, Singh VK, Ben C, Rathore A, Ramu P, Singh MK, Tar'an B, Bharadwaj C, Yasin M, Pithia MS, Singh S, Soren KR, Kudapa H, Jarquin D, Hickey L, Dixit GP, Hamwieh A, Kumar S, Deokar S, Chaturvedi SK, Howard R, Edwards D, Lyons E, Kumar A, Vigouroux Y, Hayes B, von Wettberg EJ, Datta SK, Yang H, Nguyen H, Wang J, KHM S, Mohapatra T, Bennetzen JL, Xun X, Xin L (2021) A chickpea genetic variation map based on the sequencing of 3,366 genomes. Nature 599:622–627

Vessal S, Arefian M, Siddique KH (2020) Proteomic responses to progressive dehydration stress in leaves of chickpea seedlings. BMC Genome 21:1–5

von Wettberg EJ, Ray-Mukherjee J, Adesky ND, Sistla S (2014) The evolutionary ecology and genetics SRS traits: revisiting Chapin, Autumn, and Pugnaire 1993. 2014. Chapter 9. In: Rajakaruna N, Boyd RS, Harris TB (eds) Plant ecology and evolution in harsh environments. Nova Publishers

von Wettberg EJ, Chang PL, Greenspan A, Carrasquila-Garcia N, Basdemir F, Moenga S, Bedada G, Dacosta-Calheiros E, Moriuchi KS, Balcha L, Mamo B, Singh V, Cordeiro MA, Vance L, Bergmann E, Warschefsky EJ, Marques E, Dinegde KN, Sani SG, Getahun T, Yilmaz MA, Cacmak A, Rose J, Migneault A, Krieg CP, Saylak S, Temel H, Noujdina NV, Friesen ML, Siler E, Linday D, Akhmetov Z, Ozelik H, Kholova J, Can C, Caur P, Yildirim M, Sharma H, Vadez V, Tesfaye K, Woldemedhin AF, Tar'an B, Ayodogan A, Bekun B, Penmetsa RV, Berger J, Kahraman A, Nuzhdin SV, Cook DR (2018) Ecology and community genomics of an important crop wild relative as a prelude to agricultural innovation. Nat Commun 9(1):1–13. https://doi.org/10.1038/s41467-018-02867-z

Xiong X, Duan L, Liu L, Tu H, Yang P, Wu D, Chen G, Xiong L, Yang W, Liu Q (2017) Panicle-SEG: a robust image segmentation method for rice panicles in the field based on deep learning and superpixel optimization. Plant Methods 13:104

Yordanov I, Velikova V, Tsonev T (2003) Plant responses to drought and stress. Bulg J Plant Physiol (Special Issue):187–206

Zaman-Allah M, Jenkinson DM, Vadez V (2011a) A conservative pattern of water use, rather than deep or profuse rooting, is critical for the terminal drought tolerance of chickpea. J Exp Bot 62: 4239–4252

Zaman-Allah M, Jenkinson DM, Vadez V (2011b) Chickpea genotypes contrasting for seed yield under terminal drought stress in the field differ for traits related to the control of water use. Funct Plant Biol 38:270–281

Breeding Chickpea for Climate Resilience: An Overview

2

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Abstract

Chickpea (*Cicer arietinum* L.) is an important food legume that plays a critical role in ensuring the food and nutritional security of resource-poor smallholder farmers. However, chickpea production is seriously constrained by various climate-induced stresses, such as drought, heat, cold, salinity, diseases and insect pests. These factors often affect the crop independently or in combinations to severely impact chickpea yields. Intensive breeding efforts made during the past few years have led to the development of elite chickpea cultivars with tolerance to key abiotic and biotic stresses; still, there is enough scope to improve productivity. Furthermore, enhancement of genetic gains in chickpea breeding requires integration of rapid generation advancement, genomics-assisted breeding, high-throughput and precise phenotyping and application of novel breeding approaches to harness the favourable alleles in the modern cultivars tolerant to single or multiple stresses leading to the accelerated replacement of climate-vulnerable cultivars with superior varieties that can sustain production under changing climates.

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Chickpea · Abiotic stress · Drought · Heat · Cold · Salinity · Genomics · High-throughput phenotyping · Quantitative trait loci · Marker-assisted breeding

2.1 Introduction

Chickpea (*Cicer arietinum* L.; $2n = 2x = 16$) is the second most important food legume after the common bean and is cultivated in more than 50 countries across the continents except Antarctica. It is a diploid cool season legume grown on an area of 13.71 mha with an annual production of 14.24 mt globally (FAOSTAT 2019). India accounts for 69.7% of the global production with a production of 9.93 mt from an area of 9.54 mha. Other major chickpea producing countries include Turkey, Russian Federation, Myanmar, Pakistan, Ethiopia, the USA, Australia, etc. Apart from being an important source of dietary protein (~18–24%), chickpea is highly valued for its rich nutritional qualities (carbohydrate, fat, fibre, minerals, vitamins and essential amino acids such as lysine, methionine, threonine, valine, isoleucine and leucine, β-carotene, calcium, magnesium, phosphorus and potassium) and health benefits (Jukanti et al. 2012; Thudi et al. 2014a). It also plays an integral part in cropping systems by fixing atmospheric nitrogen, breaking disease cycles and improving crop yields, thereby contributing significantly to agricultural sustainability.

Despite continuous breeding efforts in chickpea, the enhancement in productivity in the past decades was marginal and hence, the global chickpea productivity is low (1.03 tha^{-1}). This is largely due to the occurrence of several biotic and abiotic stresses, the severity of which varied across regions and seasons in the chickpea growing ecologies. Among abiotic stresses, drought, heat, cold and salinity are the most important factors limiting crop yield to a considerable extent. Since chickpea crop is predominantly grown on resource-poor soils under rainfed conditions, drought and extreme temperature stresses can account for yield losses up to 50% and 15–20%, respectively (Kaloki et al. 2019). This situation is further exacerbated by changes in climatic conditions, namely, higher intensity and frequency of heat waves, cold waves/spells and erratic rainfall distribution pattern leading to drought spells in the arid and semi-arid regions. Added to this, the prevalence of unpredictable weather conditions is projected to influence the dynamics of pests/pathogens (Atlin et al. 2017) leading to the incidence and emergence of new pests and pathogens. This necessitates the development of suitable varieties tolerant to multiple stresses to enhance and sustain the production and productivity of crops.

Crop improvement has made remarkable contributions towards achieving self-sufficiency in chickpea (Dixit et al. 2019). However, addressing various climate-related challenges requires the development of 'climate-resilient cultivars' through a combination of breeding approaches by connecting genotypes with phenotypes to realize the fullest potential of genotype in a given environment. Advances in chickpea genomics and the availability of high-throughput phenotyping have provided new concepts and tools to make more precise and efficient crop breeding procedures (Varshney et al. 2018). For instance, reference genome assembly, re-sequencing germplasm lines, sequence-based markers, high-density genetic maps help to identify alleles and haplotypes associated with key traits such as yield components, abiotic stress tolerance or disease resistance (Roorkiwal et al. 2020). This chapter highlights various climate-induced stresses affecting chickpea production, adaptation mechanisms and provides an insight into key climate-resilient traits. It furnishes an overview of conventional and advanced breeding efforts made for developing stress-tolerant chickpea cultivars. Also, we discuss the strategies to enhance genetic gains, through integrated breeding approaches involving rapid generation advancement, genomic technologies, high-throughput phenotyping (HTP) to speed up the breeding of stress-tolerant chickpea varieties for sustainable production.

2.2 Chickpea Growing Environments

Chickpea is widely grown as a rabi crop in the post-rainy season on residual soil moisture with very less or without rainfall during the crop season. For example, in the arid and semiarid regions of south and southeast Asia, it is grown as *rabi* crop after the rainy season. In Mediterranean environments (such as southern Australia), it is grown as a rainfed crop during the spring season on stored soil moisture from winter and early spring rainfall. In both these environments, the crop suffers from moisture shortage during critical reproductive stages including pod filling and seed development (Siddique et al. 2000). The current trend of growing chickpea in warmer, short-season environments of central and southern India has increased the chances of exposure of crop to moisture and high-temperature stresses at the reproductive stage resulting in severe yield losses (Gaur et al. 2014b). Due to changing climate, the frequency of heat waves has increased in different parts of the world. High day and night temperatures (>30/16 °C) during the reproductive stage have become a major limiting factor on winter-sown chickpea in Mediterranean in-season rainfall areas, south Asia and spring-sown regions (Berger et al. 2011). On the other hand, cold and freezing temperatures (−1.5°–15 °C) greatly affect the seedling stage of winter-sown chickpea in the Mediterranean region, tropical highlands and autumn-sown crops in temperate regions (Singh 1993). The chickpea grown in Canada, southern Australia and parts of northern India are most affected by chilling temperatures (<15 °C) at flowering, resulting in considerable yield loss (Clarke and Siddique 2004; Berger et al. 2011), while winter grown chickpea in Western Asia North Africa and Europe are constrained by freezing temperatures (−10 °C) (Toker et al. 2007; Kanouni et al. 2015).

2.3 Climate-Induced Stresses Affecting Chickpea Production

2.3.1 Drought Stress

Erratic precipitation and an increase in temperature due to climate change increase the frequency of drought and temperature extremes (Gaur et al. 2014a). Drought stress at various stages of the crop cycle can reduce chickpea yield up to 40–50% (Ahmad et al. 2005; Sabaghpour et al. 2006; Thudi et al. 2014a). It affects all the stages of chickpea growth and development beginning from the emergence and proper establishment resulting in poor plant stands, stunted growth and reduced biomass accumulation (Siddique et al. 1999). In subtropical (South Asia and north-eastern Australia) and Mediterranean (such as southern Australia) regions, in addition to early drought, chickpea also experiences terminal drought during the reproductive phase that affects anthesis and pollination and causes malfunction of reproductive organs especially pollen-germination, viability, fertility and pollen tube growth, and even dysfunction of stigma and style, thereby limiting seed development (Leport et al. 1998, 1999; Sita et al. 2017; Pang et al. 2017). The extent of terminal drought stress varies due to various factors such as the amount of rainfall in the previous season, atmospheric evaporative demand and soil characteristics, such as soil type, depth, structure and texture (Gaur et al. 2008a). Drought often occurs in combination with a high temperature in short-season tropical environments, which can aggravate plant injury during critical stages, mainly in the reproductive stage. Moisture stress results in loss of membrane integrity and causes irreversible cell damage by impeding lipid biosynthesis and reducing the membrane lipid; causing negative effects on plant growth and development (Vieira da Silva et al. 1974; Pham-Thi et al. 1987). It also affects important biochemical and physiological processes including photosynthesis, CO_2 availability, cell growth, respiration, stomatal conductance, etc. (Mansfield and Atkinson 1990; Chaves 1991; Chaves et al. 2009; Pinheiro and Chaves 2011). Several studies have reported the effect of drought stress on different traits of chickpea including maturity, root traits, carbon isotope discrimination, shoot biomass (Kashiwagi et al. 2005; Krishnamurthy et al. 2010; Upadhyaya et al. 2012; Krishnamurthy et al. 2013a; Purushothaman et al. 2016) and morphological (Sabaghpour et al. 2006), physiological (Turner et al. 2007), biochemical (Gunes et al. 2006) and molecular traits (Mantri et al. 2007; Thudi et al. 2014a). Drought stress during the pod filling stage increases ABA content that may affect pod set resulting in pod abscission and ultimately causing yield losses. Moisture deficit can also lead to accelerated root nodule senescence (Ashraf and Iram 2005) and collapse of symbiotic N_2 fixation processes in chickpea, resulting in serious yield losses (Wery et al. 1993).

2.3.2 Heat Stress

High temperature stress, alone and in combination with drought, is becoming another major constraint to chickpea production. It is estimated that drought and heat stresses may cause up to 50% yield losses (Gaur et al. 2012a). Depending on the emissions scenario, global mean temperatures are projected to rise by 2 °C during the twenty-first century leading to variation in rainfall, frequent heat waves and fewer cold temperature extremes. This would impact chickpea production and yield reduction up to 19% (Kadiyala et al. 2016). It is estimated that for every rise of 1 °C above the optimum temperature, the yield losses could be up to 10–15%. Similarly, Kalra et al. (2008) observed a decrease in chickpea yield of 53 kg ha^{-1} with a 1 °C rise in seasonal temperature. Chickpea is a cool season crop and exposure to high temperatures (30–35°) for a few days at the flowering stage can lead to substantial yield loss (Summerfield and Wein 1980; Saxena et al. 1988). Exposure to hot and dry conditions (>30 °C) reduces the reproductive phase and causes yield loss in chickpea (Summerfield et al. 1984). Heat stress affects all stages of chickpea growth and development including germination, vegetative and reproductive phases, key physiological and biochemical traits and cellular mechanisms (Rani et al. 2020). Elevated temperatures at sowing impair mobilization of cotyledon reserves required for embryo growth; thereby affecting seed germination, seedling growth and establishment (Kaushal et al. 2011, 2013). Exposure of chickpea genotypes to 40/30 °C, decreases plant biomass at maturity (Kumar et al. 2013). High temperature damages both structural and functional activity of chlorophyll and reduces the chlorophyll content and hence, photosynthetic process (Xu et al. 1995). Heat stress (35/16 °C for 10 days) disrupts chloroplasts, damages chlorophyll and induces leaf senescence in chickpea (Wang et al. 2006). Higher temperature (>40 °C) disrupts photosystem I and II (Baker 1991) and also affects respiration (Kurets and Popov 1988), membrane composition and its stability (Levitt 1969), nitrogen fixation (Black et al. 1978), water relation (McDonald and Paulsen 1997) and transpiration efficiency (Singh et al. 1982). The effect of heat stress is more pronounced in the reproductive phase than that during the vegetative stage, ultimately resulting in loss of seed yield (Sita et al. 2017). High temperature stress at the reproductive phase causes reduced flower number, increased flower abortion, pollen sterility, poor pollen germination, pollen tube growth, reduced fertilization and stigma receptivity, ovary abnormalities, lower remobilization of photosynthates to seeds, reduced pod number importantly the filled pods and ultimately reduced seed number, weight and yield (Wang et al. 2006; Basu et al. 2009; Devasirvatham et al. 2012, 2013; Kaushal et al. 2013).

2.3.3 Cold Stress

Chickpea encounters chilling temperature (<15 °C) in northern India and southern Australia and freezing temperature (−10 °C) in Western Asia, North Africa and Europe. The chilling and freezing injuries have damaging effects on plant growth and development. Chilling temperature at germination causes poor crop establishment, reduced seedling vigour, resulting in poor vegetative growth (Singh et al. 1997). Lower temperature also increases susceptibility to soil-borne pathogens; thereby restricting plant growth and dry matter production (Wery et al. 1993). Chilling temperature causes chlorosis, whole plant necrosis and plant death in sensitive genotypes. Cold stress decreases membrane stability, alters proteins and lipid composition and affects respiration and photosynthesis (Croser et al. 2003). It damages photosystem II and lowers the stability of chloroplast membranes and thereby affecting photosynthesis. Low temperature stress aggravates chilling injury by increasing activities of reactive oxygen species (ROS) (Prasad et al. 1994). Under freezing temperature, there will be ice formation in plant tissues resulting in dehydration (Yadav 2010). The reproductive stage is most sensitive to cold stress (Sharma and Nayyar 2014), It causes dropping of flower buds, reduced pollen viability and stigma receptivity, impaired pollen tube growth, aborted pods, poor seed set and lower seed yield (Croser et al. 2003; Berger et al. 2004; Kumar et al. 2010). In flowers, cold stress is correlated to an increase in ABA content, which affects sugar translocation in flowers and eventually leads to flower abortion (Thakur et al. 2010). Chilling temperature (<15 °C) leads to reduced anther dehiscence, lower pollen load on stigma, flower abortion and decline in pods per plant and seeds per pod in chickpea (Berger et al. 2004). It is observed that cold stress has a more pronounced effect on pollen function than on pistil function (Clarke and Siddique 2004).

2.3.4 Salinity Stress

The estimated yield loss of chickpea due to soil salinity is 8–10% (Flowers et al. 2010). In chickpea, it is reported that salinity affects germination (Khalid et al. 2001) and vegetative growth (Maliro et al. 2008), inhibits photosynthesis, energy and lipid metabolism (Parida and Das 2005) and affects the reproductive process by hampering flower and pod formation (Vadez et al. 2007, 2012a, b; Samineni et al. 2011; Turner et al. 2013; Khan et al. 2017). Growth under saline stress condition is reduced under increased concentrations of Na+ and/or Cl− in the shoots. Reduction in growth is related to an inability of chickpea plants to prevent high concentrations of saline ions (Na+ and Cl−) from reaching the leaves. Genotypes differ in their ability to retain water. High shoot water content is associated with resistance of genotypes to saline conditions. The reduction in grain yield is mainly because of the sensitivity of genotypes during the reproductive period. Salinity also impairs nodulation; thereby affecting nitrogen fixation. Chickpea plants produce smaller and fewer number of nodules in saline soils (Babber, et al. 2000) than those produced in the salt stress-free soils. In addition, NaCl promotes senescence of chickpea nodules (Sheokand, et al. 1995), contributing to a decline in the number and mass of functional nodules and thus N_2-fixation under saline conditions (Flowers et al. 2010).

Climate change predictions project warmer and more frequent hot days and nights, uneven rainfall distribution pattern leading to more frequent droughts or high precipitation (Foyer et al. 2016). Changes in these climatic factors may cause the varying intensity of diseases and insect pests leading to risks of crop failure. Varying climatic conditions affect geographical distribution of host and pathogens, changes host–pathogen interactions, development, virulence and infection rates of pathogens (Pande et al. 2010). It has been observed that changing scenario of climate with the rise in temperature and erratic rainfall has resulted in a drastic shift of chickpea diseases in major chickpea growing regions (Sharma and Pande 2013). Diseases like dry root rot of chickpea, which were previously less important, are becoming more prevalent and gaining importance in typically tropical humid areas, under the changing climates when the crop is exposed to high temperature and moisture stress (Sharma et al. 2016). Also, drought-prone areas in central and south India are prone to diseases such as collar rot (*Sclerotium rolfsii*), black root rot (*Fusarium solani*) and *Ascochyta* blight (Sinha et al. 2019). On the other hand, prolonged moisture condition may lead to the occurrence of potential diseases like anthracnose, collar rot, wet root rot and stunt diseases in chickpea (Pande et al. 2010). Recently, chickpea rust (caused by *Uromyces ciceris-arietini*) is becoming prevalent at later stages of crop under moderate warm weather conditions.

2.4 Adaptation Mechanisms and Genetic Architecture of Key Climate-Resilient Traits

2.4.1 Drought Tolerance

Drought tolerance is a highly complex trait and plants are known to acclimatize under drought conditions through different mechanisms like drought escape, avoidance and tolerance (Gaur et al. 2008a). Various researchers reported different constitutive traits to understand the mechanism of drought tolerance in chickpea. Early phenology (early flowering, podding and maturity) in chickpea has been identified as the most important terminal drought escape mechanism (Subbarao et al. 1995; Kumar and Abbo 2001). High initial growth and early vigour are associated with early flowering in chickpea (Sabaghpour et al. 2003). Though genotypes with shorter vegetative periods (early-maturing varieties) may have yield penalty (Basu and Singh 2003), they help to stabilize yield under terminal drought conditions. A large variability for time to flowering has been documented in chickpea germplasm (Gaur et al. 2018), which enables the development of chickpea cultivars with the desired maturity. In chickpea, flowering time is reported to be controlled by one or a few major genes (Gumber and Sarvjeet 1996; Or et al. 1999; Kumar and van Rheenen 2000; Hegde 2010; Gaur et al. 2015). Till now, four non-allelic genes have been identified to control flowering time in chickpea. These are *efl-1, efl-2/ppd, efl-3 and efl-4* from ICCV 2/ICCV 96029, ICC 5810, BGD 132 and ICC 16641, respectively (Kumar and van Rheenen 2000; Or et al. 1999; Hegde 2010; Gaur et al. 2015). Consequently, the genomic regions/QTLs corresponding to four early flowering genes and linked molecular markers were identified by Mallikarjuna et al. (2017). These discoveries would be useful in breeding for short duration varieties in chickpea by incorporating one or more early flowering genes in the desired background through appropriate breeding strategies (Gaur et al. 2015).

Drought avoidance can be achieved by moisture uptake by the roots from deeper layers, by osmotic adjustment and by reducing water loss (stomata conductance or by a reduction in leaf area) (Gaur et al. 2008a). Root architecture traits help plants to maintain a high-water potential or turgor pressure under drought stress (Turner 2003) and contribute positively to grain yield by acquiring soil moisture by deep penetration of root, adequate root density and by reducing water loss or by osmotic adjustment (Turner and Jones 1980; Turner 1986). Efficient and high-throughput drought phenotyping techniques have been suggested for screening root traits of a large number of accessions in chickpea (Kashiwagi et al. 2005, 2006a; Krishnamurthy et al. 2010; Upadhyaya et al. 2012). Considerable progress has been made in understanding the role of various root traits (root biomass, root length, root length density (RLD), total root dry weight (RDW), root diameter, root volume, root surface area and deep root dry weight) in controlling plant water status and adaptation of chickpea to drought stress (Krishnamurthy et al. 2003; Kashiwagi et al. 2006a, 2015; Gaur et al. 2008a; Purushothaman et al. 2015). Also, a wide variability for root length density (RLD), root dry weight (RDW), rooting depth (RDp) and root to total plant weight ratio (R/T) has been reported in mini-core collection and wild species of chickpea (Kashiwagi et al. 2005). Gaur et al. (2008a) reported that under drought stress conditions, higher RLD and maximum root depth (RDp) in shallow soils could assist in increasing seed yield in chickpea. Later, Ramamoorthy et al. (2017) gave evidence about the positive association of RLD and grain yield under drought stress. Furthermore, Kashiwagi et al. (2008) studied the genetic effects of root and shoot traits using two different crosses, namely, ICC283 × ICC8261 and ICC4958 × ICC1882 and suggested that additive gene action and additive × additive gene interactions control RLD and root dry weight (RDW). Chandra et al. (2004) identified a major QTL explaining one-third of the variation for root length and root biomass (Table 2.1). Varshney et al. (2014) used two drought mapping populations (ICC 4958 × ICC 1882 and ICC 283 × ICC 8261) to phenotype 20 drought component traits. The QTL analysis along with extensive marker data identified several stable, consistent and robust main-effect QTLs for 13 out of 20 drought tolerance traits explaining phenotypic variation ranging from 10–58.20%. This study also identified a genomic region (named as '*QTL-hotspot*') spanning ~29 cM on linkage group 4 of the genetic map of the cross ICC 4958 × ICC 1882. This region was found to harbour main effect QTLs for 12 drought component traits. This genomic region and the linked markers were targeted for marker-assisted introgression to enhance drought tolerance of popular varieties of chickpea, which will be discussed later in greater detail. The *QTL-hotspot* region was refined to 14 cM (~3 Mb on the physical map) using a Genotype by Sequencing (GBS) derived high-density map by Jaganathan et al. (2015). Furthermore, this genomic region was fine mapped into two sub-regions '*QTL-hotspot a* and *QTL-hotspot b*' spanning less than 400 kb (Kale et al. 2015) (Table 2.1). Das et al. (2015) mapped QTL for 100 seed weight on chromosome 1 using an intra-specific mapping population of the cross ICC 7184 × ICC 15061. By employing QTL-Seq strategy, this QTL was further refined from 1.37 Mb to 35 kb region containing six candidate genes. Using a similar approach, Singh et al. (2016) refined the already mapped region for the

Table 2.1 Summary of QTLs identified for various abiotic stress tolerance traits in chickpea

Abiotic stress	Trait	Cross	Markers	QTL	Linkage group	%PVE	Candidate genes identified	Reference
Drought	Shoot dry weight, Root dry weight and Root length	Annigeri × ICC 4958	14 SSR markers	QTL-linked markers	–	56.2% (SDW), 33.1% (RDW), 33.4% (RL)	–	Chandra et al. (2004)
	Traits Affecting Grain Yield under Terminal Drought Stress	ILC 588 × ILC 3279	97 SSRs	15 QTLs significantly associated with various traits affecting drought tolerance	LG 1 and 3	Up to 52%	–	Rehman et al. (2011)
	Drought-related Traits	ILC 588 × ILC 3279	77 SSRs	93 significant QTLs for 12 drought-related traits	LG 1, 2, 3, 4, 5, 7 and 9	Up to 24.2%	–	Hamwieh et al. (2013)
	Drought escape Drought avoidance and Drought tolerance traits	ICC 4958 × ICC 1882 ICC 283 × ICC 8261	241 (214 SSRs, 6 GMMs and 21 DArT loci) 168 (151 SSRs, 10 GMMs and 7 DArT loci)	• 45 robust main effect QTLs and 973 epistatic QTLs for various component traits of drought tolerance • 9 QTL clusters including g a genomic region 'QTL-hotspot' on CaLG04 harbouring severalQTLs for drought tolerance-related traits.	LG 01, 03, 04, 05, 06, 08	Up to 58.20%	–	Varshney et al. (2014)

(continued)

Table 2.1 (continued)

Abiotic stress	Trait	Cross	Markers	QTL	Linkage group	%PVE	Candidate genes identified	Reference
	Refining 'QTL-hotspot' region harbouring drought tolerance traits	ICC 4958 × ICC 1882	828 GBS based SNPs	The QTL-hotspot region was refined to 14 cM (~3 Mb on the physical map)	CaLG04	Up to 67.71%	286 genes	Jaganathan et al. (2015)
	Refinement of 'QTL-hotspot' region	ICC 4958 × ICC 1882	1610 bins	Identified 71 major QTLs and delineates the 'QTL-hotspot' region from ca. 3 Mb to two QTL regions viz. 'QTL-hotspot_a' of 139.22 Kb and "QTL-hotspot_b" of 153.36 Kb sizes	CaLG04	Up to 59.83%	15 genes in 'QTL-hotspot_a' region and 11 genes in 'QTL-hotspot_b' region	Kale et al. (2015)
	Plant vigour and Canopy conductance traits	ICC 4958 × ICC 1882	Low density (241 SSRs), High density (1007 SSR + SNP), Ultra-high density (1557 SNPs)	13 major QTLs for plant vigour traits	CaLG04	53%	–	Sivasakthi et al. (2018)
				One major QTL for canopy conductance	CaLG03	10%	–	
Heat	Heat Tolerance	ICC 4567 × ICC 15614	271 GBS based SNPs	QTLs for number of filled pods per plot, total number of seeds per plot, grain yield per plot, visual score and % pod setting	CaLG05 and CaLG06	Cumulative PVE >50%	25 genes	Paul et al. (2018b)

Cold	Vernalization response	ICC 4958 × PI 489777	1291 (SSRs and DArT loci)	A major QTL for Vernalization response	LG 03	Up to 54.9%	84 genes	Samineni et al. (2016)
	Cold tolerance	ICC 4958 × PI 489777	747 SNPs	Three significant QTLs for cold tolerance	LG 1B, 3 and 8	Up to 48.4%	–	Mugabe et al. (2019)
Salinity	Salt tolerance traits	ICC 6263 × ICC 1431	75 SSRs	Minor QTLs related to yield, seed size and shoot biomass under salt stress	LG 1, 2, 3 and 7	<10%	–	Samineni (2010)
	Salinity-related traits	ICCV 2 × JG 62	135 markers	Multiple QTLs for days to flowering, shoot dry weight, seed yield, pod number, seed number, yield ratio, yield difference and 100-seed weight	LG 3, 4, 5, 6 and 7	Up to 21.4%	–	Vadez et al. (2012b)
	Salinity tolerance	ICCV 2 × JG 11	56 markers (28 SSRs and 28 SNPs)	Two key genomic regions that harboured QTLs for different salinity tolerance associated traits	CaLG05 and CaLG07	Up to 66%	48 genes	Pushpavalli et al. (2015)
	Salinity tolerance	ICCV 10 × DCP 92–3	1856 Axiom®CicerSNP array-based SNPs	28 QTLs for various salinity tolerance component traits	CaLG03 and CaLG06	Up to 28.40%	138 genes	Soren et al. (2020)

100SDW and total dry root weight to total plant dry weight ratio (RTR) to ~1 Mb region within the *QTL hotspot* region on CaLG04.

Water-use efficiency (WUE) is another key physiological trait and it is an important component of yield under drought. It can be measured by carbon isotope discrimination (D13C) technique in chickpea (Kashiwagi et al. 2006b). Carbon isotope discrimination (D13C) has been proposed as an integrator of plant behaviour influencing transpiration efficiency. Using reference collection of chickpea germplasm, Krishnamurthy et al. (2013a) reported the existence of a large variation in D13C and its effect on grain yield through harvest index. Furthermore, Varshney et al. (2014) reported that minor QTLs showing interaction play a significant role in controlling transpiration efficiency in a RIL population of the cross ICC 4958 × ICC 1882. Another key trait, namely, partitioning coefficient is an important contributor of grain yield under terminal drought. A large variation for partitioning coefficient in chickpea reference collection was reported by Krishnamurthy et al. (2013b) and established its association with grain yield under drought conditions. In addition, the importance of several other physiological parameters related to drought tolerance, such as photochemical efficiency of PII system, RWC, SPAD chlorophyll content, cell membrane integrity and stomatal conductance have also been studied in chickpea (Pouresmael et al. 2013).

2.4.2 Heat Tolerance

Plants use different adaptive strategies to escape, avoid and tolerate heat stress. Early phenology (flowering and maturity) helps plants to escape heat stress. Although flower bud initiation in chickpea is highly regulated by temperature, early phenology is considered as an escape mechanism against heat stress (Toker et al. 2007), particularly in Mediterranean spring-sown environments and short winter conditions of southern India (Berger et al. 2011). Upadhyaya et al. (2011) found large variations for heat tolerance among early maturing chickpea lines. Leaves play a vital role in avoiding heat stress by changing orientation, reducing transpiration rate and leaf reflectance (Wery et al. 1993). Reflecting light, reduction of non-photosynthetic energy intercepted by the canopy and reduced transpiration are important physiological components of heat avoidance.

Heat tolerance is linked to membrane thermostability, stomatal conductance, alteration of membrane lipid composition, chlorophyll fluorescence (CFL), canopy temperature depression (CTD), accumulation of heat shock proteins and specific solutes (proline and glycine), particularly in pollen (Blum 1988). Pollen viability and pod set under high temperature are considered as key traits for the selection of heat-tolerant genotypes in chickpea (Devasirvatham et al. 2012, 2013). A simple and efficient field screening method for heat tolerance at the reproductive stage has been developed at ICRISAT by delayed sowing, which enables crop to expose its reproductive phase to high temperature stress (>35 °C). Accordingly, the number of filled pods per plant in late-sown crop was suggested as a selection criterion for reproductive-stage heat tolerance (Krishnamurthy et al. 2011a). Recent studies have suggested using various heat stress tolerance indices (such as mean productivity, geometric mean productivity, yield index, tolerance index, stress susceptibility index and superiority measure) to identify chickpea genotypes based on grain yield under normal and heat stress conditions (Jha et al. 2018). Association between DArT (Diversity Arrays Technology) markers and traits targeted for high temperature tolerance (total number of pods, filled pods, number of seeds and grain yield) have been reported under heat-stressed and non-stressed conditions and genomic regions linked with heat tolerance have been identified (Devasirvatham 2012). Thudi et al. (2014b) reported significant marker-trait associations for 100-seed weight under drought and heat stress. In another study, a wide variation for heat tolerance traits (number of filled pods, total number of seeds, harvest index, per cent pod set and grain yield) was observed under heat-stress environments in a RIL population of the cross ICC 4567 (heat sensitive) × ICC 15614 (heat tolerant) by Paul et al. (2018a). Furthermore, QTLs for the number of filled pods per plant, total number of seeds per plant, grain yield and percent pod set were identified (Paul et al. 2018b) (Table 2.1). These QTLs and linked markers will enable introgression breeding and assist in understanding the mechanism of heat tolerance.

2.4.3 Cold Tolerance

Previous studies describe low temperature stress affecting all the stages of crop development beginning from germination to maturity in chickpea (Croser et al. 2003). Therefore, it is suggested that phenology should be taken into consideration for assessing cold tolerance. Cold tolerance in chickpea is reported to be controlled by at least five sets of genes with cold tolerance being dominant over susceptibility (Malhotra and Singh 1990). Furthermore, both additive and non-additive gene (additive × additive and dominance × dominance) interactions were found to govern cold tolerance in chickpea (Malhotra and Singh 1991). Chickpea wild relatives have been suggested as important sources of cold tolerance (Mugabe et al. 2019). Samineni et al. (2016) used an interspecific recombinant inbred line (RIL) population and mapped vernalization response in chickpea on Linkage Group 3 (LG3). Recently, Mugabe et al. (2019) mapped QTLs associated with cold tolerance on LGs 1B, 3 and 8 in chickpea. The QTL on LG 3 (CT Ca-3.1) linked to cold tolerance was the same as the QTL mapped by Samineni et al. (2016) on LG 3 governing vernalization response (Table 2.1). These genomic regions provide a basis towards understanding cold tolerance and introgression into cultivated chickpea. Considering pollen as a key adaptive trait in manipulating the chilling tolerance (Clarke et al. 2004), pollen viability and its relationship with pod set, seed development and yield are suggested as an indirect trait for cold tolerance in chickpea (Kaloki et al. 2019).

2.4.4 Salt Tolerance

Chickpea is salt-sensitive crop and salinity tolerance has been studied at different developmental stages starting from germination, vegetative stage and reproductive phase (Kaashyap et al. 2017). Genetic variation in chickpea genotypes based on shoot biomass and seed yield under saline stress was documented by several researchers (Vadez et al. 2007, Krishnamurthy et al. 2011b; Turner et al. 2013). Vadez et al. (2012a) observed a greater number of flowers in chickpea tolerant lines under salt stress at sowing and flowering and emphasized the importance of constitutive (higher number of flowers) and adaptive traits (higher number of seeds under salt stress) for salinity tolerance in chickpea. Salinity tolerance is reported to be a physiological and biochemical trait governed by polygenes with complex underlying genetic and physiological control (Kaashyap et al. 2017). Several studies focussed on mapping QTLs conferring salinity tolerance in chickpea. Samineni et al. (2010) identified a set of minor QTLs (explaining less than 10% phenotypic variation) related to yield, seed size and shoot biomass under salt stress using a RIL population (ICC6263 × ICC1431). Later, Vadez et al. (2012b) identified multiple QTLs for salinity tolerance on LG 3 and 6 using a RIL population (JG62 × ICCV2) including a major QTL for in seed number on LG 6. Furthermore, Pushpavalli et al. (2015) identified two key genomic regions for salinity tolerant traits on LG 5 and 7 in a RIL population (ICCV2 × JG11). This study also revealed 48 putative candidate genes within these genomic regions. Recently, Soren et al. (2020) identified two genomic regions that harbour QTLs for salinity tolerance on CaLG03 and major QTLs for yield and salinity tolerance on CaLG06 (Table 2.1). Identification of multiple QTLs/ genes in these studies suggests the complex nature of the salinity tolerance in chickpea.

2.4.5 Resistance to Emerging Diseases Under Climate Change

Disease such as dry root rot (DRR) has an increasing impact on chickpea yield under changing climatic conditions, especially under warmer environments. Inheritance of dry root rot resistance is not clear so far. It is reported to be controlled by a single dominant gene (Rao and Haware 1987; Talekar et al. 2017). However, Reddy (2016), through generation mean analysis in three chickpea crosses, reported that genetics of dry root rot is determined by more than one gene with the predominance of additive type of gene action with non-significant dominant gene effects. By using bulk segregant analysis approach, Talekar et al. (2017) reported two markers (ICCM0299 and ICCM0120b) co-segregating with dry root rot resistance gene. However, these markers could not be validated as they did not detect polymorphism among moderately resistant and susceptible genotypes (Reddy 2016). These findings necessitate for refinement of disease screening techniques, and confirmation of resistant sources under both controlled environment and field conditions in order to facilitate inheritance studies and to identify the genomic regions/gene (s) controlling DRR resistance in chickpea. In a recent study, Karadi et al. (2021)

identified a minor QTL (qDRR-8; PVE = 6.70%) on CaLG08 in a RIL population derived from the cross BG 212 (susceptible) x ICCV 08305 (moderately resistant). Similarly, for chickpea rust, a major QTL flanked by markers TA18 and TA180 was identified on LG 7 and proposed that a single dominant gene (proposed as *Uca1/uca1*) controlled rust resistance in adult plants (Madrid et al. 2008).

2.4.6 Breeding Efforts to Address Climate-Induced Stresses in Chickpea

Drought tolerance: In chickpea, a range of traits have been targeted for breeding for drought tolerance. These include early phenology (drought escape), root traits (drought avoidance), carbon isotope discrimination, partitioning rate, shoot biomass and grain yield under drought stress conditions (Thudi et al. 2014a). Early maturity helps to escape from these stresses and is a key trait for the adaptation of chickpea to semi-arid regions. Efforts for developing short duration lines in chickpea have started in the 1980s (Kumar et al. 1985). Thereafter, chickpea breeding programs around the world have placed high emphasis on the development of early maturing varieties (having high yield, disease resistance, drought tolerance, heat tolerance, etc.) for increasing adaptation of chickpea to terminal drought stress conditions (Gaur et al. 2008b). As a result of the efforts of researchers across the National Agricultural Research System (NARS) and The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), several early maturing chickpea cultivars (e.g. ICCV 2, ICCC 37, JG 11, JGK 1, JAKI 9218 and KAK 2) well adapted to semi-arid tropics have been developed in India. Some of these short duration varieties (e.g. JG 11 and JAKI 9218) are highly preferred by farmers in southern India and their adaption has led to a tremendous increase in area and productivity (Gaur et al. 2018). Further advancements have been made in breeding for earliness by developing two super early desi chickpea lines (ICCV 96029 and ICCV 96030) which mature in 75–80 days in southern India (Gaur et al. 2012b).

In most cases, breeding programs expose the breeding material under terminal drought by growing the crop under rainfed conditions or under rainout shelters and use grain yield under drought stress as selection criteria to enhance drought avoidance/tolerance (Gaur et al. 2018). Several phenotyping techniques have been developed for screening large number of chickpea accessions to identify drought tolerance traits (Upadhyaya et al. 2012). For instance, the use of polyvinyl chloride pipe-based high-throughput drought phenotyping has been successfully applied for screening large chickpea germplasm collections, advanced breeding lines and mapping populations (Kashiwagi et al. 2005, 2006a; Krishnamurthy et al. 2010; Upadhyaya et al. 2012). Based on simple field-based screening techniques and yield performance under non-stress and water stress conditions, several chickpea genotypes have been identified (Singh et al. 1997; Canci and Toker 2009). Also, several studies documented a considerable amount of genetic variability for various morphophysiological and yield-associated parameters under contrasting water regimes in the field conditions (Krishnamurthy et al. 2010; Pang et al. 2017).

Furthermore, Krishnamurthy et al. (2010) used a drought tolerance index and reported considerable genetic variability for constituent traits under drought condition among the mini-core collection comprising 211 accessions. It is reported that under drought conditions, partitioning coefficient (p) contributed significantly towards grain yield in a chickpea reference collection consisting of 280 accessions (Krishnamurthy et al. 2013a). In another study, Krishnamurthy et al. (2013b) reported the existence of a large variation in carbon isotope discrimination (CID) in chickpea reference collection and found positive associations of CID with harvest index under terminal drought conditions.

Extensive research has been made in understanding the role of various root traits in conferring drought stress tolerance in chickpea (Kashiwagi et al. 2006a, 2015). Also, a considerable amount of genetic variability for root length density (RLD) has been identified in mini-core collection and wild species of chickpea (Kashiwagi et al. 2005). Based on root characters, the genotypes ICC 4958 (having higher root length density) and ICC 8261 (having prolific and deep root system) have been identified as drought-tolerant lines (Saxena et al. 1993; Kashiwagi et al. 2005). The genotypes with prolific root system such as ICC 4958 has been extensively used as drought-resistant donor parent for transferring important drought adaptive root traits, but the selection of breeding lines was based invariably on higher grain yield under drought stress conditions rather than on root traits (Gaur et al. 2018). By now the breeding community has understood that the traits targeted for enhancing yield under water-stress condition should have a high heritability, strong association with yield, large genetic variability and a lack of yield penalties under favourable conditions (Thudi et al. 2014a).

Heat stress tolerance: In chickpea, a simple field screening technique for heat tolerance at the reproductive stage has been developed. It involves delayed sowing to coincide the reproductive stage of the crop to high temperatures (>35 °C). Accordingly, the number of filled pods per plant in delayed sown crop has been identified as a selection criterion for reproductive stage heat tolerance. This method was highly effective in identifying heat tolerant germplasm in India. It has been suggested to grow segregating populations (F_4 or F_5) under late-sown conditions for selecting heat-tolerant plants based on the number of filled pods per plant and subsequently, to develop single plant progenies from the selected heat-tolerant plants and evaluate further for grain yield and other desired traits (such as resistance to major diseases, seed traits, etc.) under normal and high-temperature stress conditions (Gaur et al. 2018).

Several studies reported the existence of large genetic variation for reproductive stage heat tolerance in chickpea (Krishnamurthy et al. 2011a; Upadhyaya et al. 2011). Also, several heat-tolerant genotypes including landraces (ICC 1205, ICC 1356, ICC 4958, ICC 6279, ICC 15614, ICC 5597, ICC 5829, ICC 6121, ICC 7410, ICC 11916, ICC 13124, ICC 14284, ICC 14368 and ICC 14653), breeding lines (ICCV 07104, ICCV 07105, ICCV 07108, ICCV 07109, ICCV 07110, ICCV 07115, ICCV 07117, ICCV 07118 and ICCV 98902) and cultivars (JG 14, IPC 2006-77, JG 16, GG 2, JG 130, JGK 2 and KAK 2) have been identified. Jha et al. (2018) used several heat tolerance indices and identified the cultivars such as RVG

203, RSG 888, GNG 469, IPC 06-11 and JAKI 9218 having moderate to high tolerance to heat stress. In addition, Krishnamurthy et al. (2011a) reported the lines ICC 3362, ICC 12155 and ICC 6874 as heat-tolerant. Furthermore, a heat-tolerant breeding line (ICCV 92944) has been released for cultivation in India (as JG 14) and several other countries. JG 14 is a highly popular variety in India and performing well under late-sown conditions (Gaur et al. 2018).

Cold tolerance: Breeding for low-temperature stress tolerance aims to develop cultivars adapted to cold temperature both at seedling and reproductive stages. Screening of chickpea germplasm has identified cold-tolerant lines from the cultivated (Singh et al. 1995) and wild sources (Robertson et al. 1995). Several breeding lines (ICCV 88501, ICCV 88502, ICCV 88503, ICCV 88506, ICCV 88509, ICCV 88510 and ICCV 88516) that set pods at cold temperature have been developed (Srinivasan et al. 1998). Furthermore, ICAR-Indian Agricultural Research Institute has developed cold-tolerant genotypes such as BGD 112 green, BG 1100, BG 1101, PUSA 1103, BGD 1005, PUSA 1108, DG 5025, DG 5027, DG 5028, DG 5036 and DG 5042 (Gaur et al. 2007). Pollen selection method was applied to assess cold tolerance in the chill-tolerant line (ICCV 88516) and chill-sensitive varieties (Amethyst and Tyson) and developed popular chilling tolerant cultivars Sonali and Rupali (Clarke et al. 2004). Recently, Mir et al. (2019) identified cold-tolerant accessions among wild (Ortan-066, Cudi 1-022, Bari-3-106D, Bari-2-072) and cultivated (ICC 13090 and AGBL-G-170004) chickpea.

Salinity tolerance: Several studies assessed genetic variability among chickpea accessions and subsequently identified some genotypes with low to high salinity tolerance (Lauter and Munns 1986; Serraj et al. 2004; Maliro et al. 2004, 2008; Turner et al. 2013). Similarly, Vadez et al. (2007) identified a large genetic variation within mini-core collection consisting of 263 chickpea accessions and found a positive relationship between seed yield obtained under salinity and non-salinity stress conditions. Maliro et al. (2004) identified the breeding line ICCV 96836 (Genesis 836) as salinity-tolerant line based on pot trials. In India, a desi variety Karnal Chana 1 (CSG 8963) has been released for cultivation in saline soils with ECe up to 6 dSm^{-1} (Gaur et al. 2007).

Dry root rot: For emerging diseases like dry root rot, developing varieties with a high level of resistance has been challenging due to the lack of specific resistance to DRR in chickpea. However, efforts have been made to identify resistance sources from germplasm and breeding lines/cultivars using different screening techniques. Jayalakshmi et al. (2008) found four chickpea cultivars (GCP- 101, GBM-2, GBM-6 and ICCV-10) tolerant to DRR. Similarly, several chickpea breeding lines (e.g. ICCV 05530, ICCV 08305, ICCV 05529, ICCV 05532, ICCV 07117 and ICCV 07112) showing a moderate level of resistance to DRR has been reported (Sharma et al. 2016). Based on the sick pot experiment, six genotypes (K 850, KAK 2, H 208, BG 14-4, BG 12119 and ICC 14395) were identified as highly resistant to DRR (Desai et al. 2017). For chickpea rust, a germplasm line, that is, RIL58-ILC72/Cr5 having resistance to both rust and *Ascochyta blight* has been developed by Rubio et al. (2006).

2.4.7 Accelerating Genetic Gain in Chickpea Breeding for Stress-Prone Marginal Environments

Efforts were made in the past to improve yield and other traits by using conventional breeding methods in chickpea. As a result, hundreds of improved chickpea cultivars were developed for various traits. However, the rate of genetic gains using the conventional approaches is not sufficient to bridge the gap with growing demands (Varshney et al. 2019). Also, most of the obsolete cultivars were developed in a climate different than today's, placing farmers at risk. To reduce this risk, breeding efficiency and the rate of genetic gains need to be improved with short breeding cycles, selection intensity, selection accuracy supported by modern genomics technology and precise phenotyping (Atlin et al. 2017).

Reducing breeding cycle time by half will double the rate of genetic gain if the selection accuracy and intensity are constant (Atlin et al. 2017). In the case of chickpea, most breeding programmes generally take 7–9 years after hybridization to develop homozygous lines with only one generation is taken per year under field conditions. Subsequent testing of homozygous lines for their performance and stability would further delay the variety development process. In this scenario, rapid generation advancement (recently developed 'speed breeding') that allows growing more generations per year will be highly advantageous in shortening breeding cycles and increasing genetic gain. Recently, a rapid generation advance protocol developed in chickpea with up to seven generations per year (Samineni et al. 2019). This study induced early flowering by extended photoperiod under glasshouse conditions and the immature seeds were germinated to reduce the generation cycle time. In another study, 4–6 generations per year in chickpea were produced under speed breeding conditions with extended photoperiod and controlled temperature (Watson et al. 2018; Ghosh et al. 2018). These results have significant implications for accelerating the rate of genetic gain and cultivar development in chickpea.

2.5 Genomic Approaches

To expedite the breeding cycles, large-scale genomic resources have been developed in chickpea in the past decade (Varshney et al. 2013a). These resources enhanced better understanding as well as simplifying complex traits (Varshney et al. 2015). Furthermore, a range of molecular markers (mainly simple sequence repeats and Single Nucleotide Polymorphisms), mapping populations (bi-parental, multi-parent and natural populations) were employed for mapping of several abiotic (Varshney et al. 2014; Paul et al. 2018b) and biotic (Sabbavarapu et al. 2013; Garg et al. 2018) stress resistance genes and QTLs in chickpea. As a result, several improved chickpea lines developed with enhanced resistance/tolerance to biotic or abiotic stresses using marker-assisted backcrossing (Roorkiwal et al. 2020). In recent years, sequencing efforts motivated largely by advancements in next-generation sequencing (NGS) technologies have resulted in the availability of chickpea reference genome sequence

(Varshney et al. 2013b) and re-sequencing of germplasm and breeding lines in chickpea (Thudi et al. 2016a, b). This post-genome sequencing era in chickpea also witnessed a paradigm shift towards sequencing-based markers (such as SNPs, DArT) for genotyping the large breeding populations and diverse germplasm panels. The revolution in NGS technologies has enabled the application of genotyping by sequencing (GBS), diversity array technology (DArT)-seq, restriction-site associated DNA sequencing (RAD-seq), skim sequencing and whole-genome re-sequencing (WGRS) approaches for developing high-density genome/haplotype maps, mapping QTLs, refining the QTL regions, identifying candidate genes and markers linked to key traits. Recently, a cost-effective, high-throughput SNP genotyping platform, that is, 'Axiom® CicerSNP Array' with >50 K SNPs with uniform genome coverage has been designed and validated. This high precision array is being used for characterizing germplasm lines, trait mapping and molecular breeding in chickpea (Roorkiwal et al. 2018). With a rapid increase in cost-effective NGS technologies and high-throughput phenotyping platforms (HTP), novel breeding methods such as genomic selection (GS) has been applied for targeting complex traits controlled by polygenes with relatively small genetic effects. By combining genotyping and phenotyping data with six statistical GS models, high prediction accuracies (up to 0.91) were reported for yield and associated traits in chickpea (Roorkiwal et al. 2016). In a recent study, GWAS results were incorporated into GS models to increase the prediction accuracies for complex traits like drought (Li et al. 2018). Thus, integrating modern genomic technologies with breeding methods holds great promise to accelerate the rate of genetic gain in chickpea.

2.6 High-Throughput Phenotyping Techniques

Accurate and targeted phenotyping is critical for accelerating breeding strategies for higher yield and biotic and abiotic stress tolerance. High-throughput phenotyping contributes to improve genetic gain by increasing selection accuracy by increasing heritability (H), improved stress phenotyping helping to identify the genetic variation more efficiently and making the decision support systems more robust. Recognizing the importance of phenotyping, the field of high-throughput phenotyping is rapidly evolving in recent years and led to the development and use of state-of-the-art phenotyping facilities and precise phenotyping protocols for recording various traits. These are largely based on sensors (such as multispectral, hyperspectral, fluorescence and thermal sensors) and imagery tools, basically designed to capture the characteristic signature of the reflectance returning from the interaction between natural electromagnetic spectrum and plant cellular components (Maphosa et al. 2020). Plethora of HTP platforms along with their salient features are summarized by Varshney et al. (2018).

To support the development of climate-resilient varieties, HTP approaches that can quantitatively measure key traits on a large number of accessions are available. For instance, effective assessment of flowering time in chickpea is highly feasible by HTP with aerial multispectral imagery and RGB imagery as done in other crops

(Guo et al. 2015; Wu et al. 2019). Rapid assessment of early vigour can be done by HTP using sensors or multispectral imagery (Nguyen et al. 2018). For phenotyping root traits, advanced image-based root phenotyping methods such as GROWSCREEN-Rhizo, magnetic resonance imaging (MRI-for phenotyping in 4D), X-ray computed tomography (CT) and positron emission tomography (PET) can be promising for screening chickpea germplasm (Tracy et al. 2020). Canopy-related traits can be captured using a 3D scanning technique on LeasyScan platform (Vadez et al. 2015). Canopy temperature, an important adaptive trait for terminal drought and heat tolerance can be measured by infrared thermometers, aerial thermal and RGB imagery (Zhang et al. 2019). Pollen fertility is a key trait for abiotic stress tolerance and digital microscopy RGB image can be used for counting stained viable pollen (Tello et al. 2018). Photosynthetic activities of crop plants can be automatically and rapidly captured by chlorophyll fluorescence imagery under abiotic stressed condition (Zarco-Tejada et al. 2009). Similarly, airborne multispectral imagery has been used to rapidly assess yield potential in chickpea (Quirós et al. 2019). Furthermore, rapid advancements in machine learning offer powerful HTP tools in near future to improve the accuracy of phenotyping and also facilitate breeders in decision making. Thus, integration of genomics with high-throughput phenotyping techniques and application of advanced breeding techniques (Fig. 2.1) could help to develop superior lines with enhanced stress tolerance and resilience to climate change.

2.6.1 Advanced Breeding Techniques to Combat Climate-Induced Stresses in Chickpea

Novel breeding populations, such as nested association mapping (NAM) and multi-parent advanced generation intercross (MAGIC) populations are being developed in chickpea to generate new breeding material with enhanced genetic diversity for high-resolution mapping of target traits (Roorkiwal et al. 2020; Gaur et al. 2018). A NAM population is being developed at ICRISAT with ICC 4958 as a common female parent. A total of 14 different crosses were made to generate F_1s and these F_1s are in advanced generations (Pandey et al. 2016). At ICRISAT a MAGIC population in desi chickpea has been developed involving a drought-tolerant genotype (ICC 4958), five popular varieties from India (ICCV 10, JAKI 9218, JG 11, JG 130 and JG 16) and two varieties from Africa (ICCV 97105 and ICCV 00108). The progenies derived from the MAGIC population serve as a valuable genetic resource for trait mapping, gene discovery and provide superior breeding lines. At ICRISAT, MAGIC approach has been found promising in developing several lines with enhanced tolerance to drought and heat stresses (Gaur et al. 2018). Another novel approach for creating novel genetic variation and identification of useful allelic variants, that is, TILLING (Targeting Induced Local Lesions IN Genomes) population was developed from chickpea accession ICC 4958 at ICRISAT through chemical mutagenesis using ethyl methane sulphonate (EMS) (Gaur et al. 2014c). This TILLING population comprising hundreds of M_2 lines is currently being used for fine mapping using

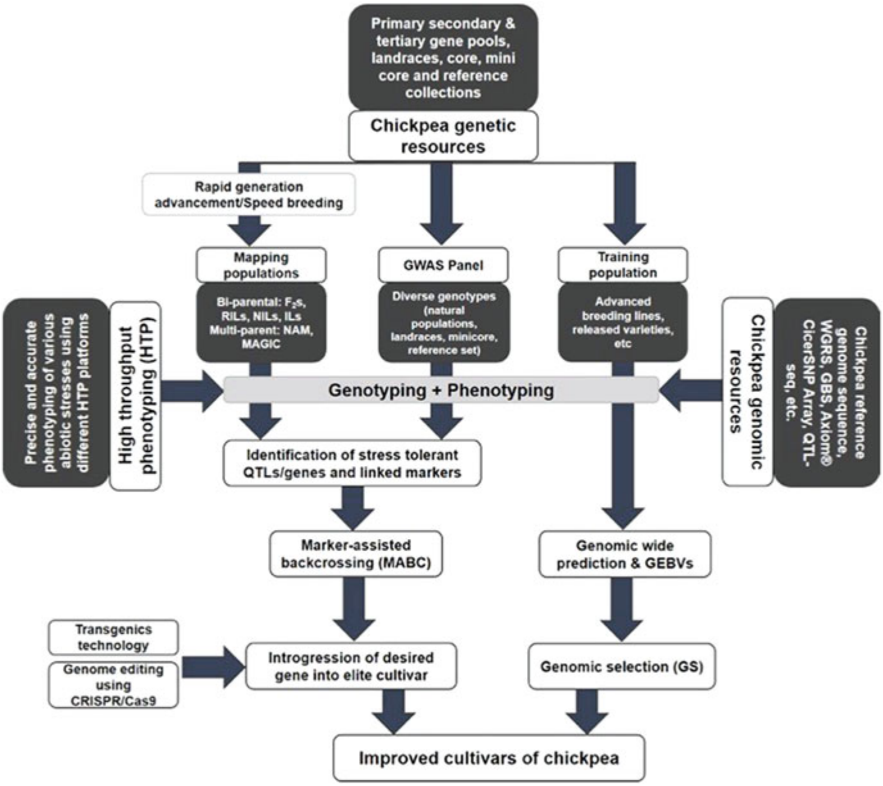

Fig. 2.1 Integration of genomics, high-throughput phenotyping and advanced breeding techniques to develop superior chickpea lines and cultivars with climate resilience

the MutMap approach and allele mining for various agronomically important traits. Transgenics approach offers great promise to overcome sexual reproduction barriers for introducing specific genes/traits into chickpea. Transgenics approach is being deployed for developing helicoverpa resistance in chickpea (Ganguly et al. 2014). Recently, Das et al. (2021) developed transgenic chickpea lines harbouring rd29A driven *AtDREB1a* gene, which are better adapted to water deficit by modifying important physiological traits. Furthermore, emerging technology such as Genome editing (GE) has shown great promise in its ability to precisely edit the genomes of living organisms and the GE tool especially CRISPR/Cas9 has already confirmed its application in several plants. In chickpea also, GE technology has been applied by utilizing CRISPR/Cas9 DNA-free gene editing of *4CL* and *RVE7* genes associated with drought tolerance (Badhan et al. 2021).

2.6.2 Development of Stress-Tolerant Chickpea Varieties Using Genomics Assisted Breeding (GAB)

The advances in genomics involving modern sequencing tools and multi-omics approaches have led to the identification of QTLs and putative candidate genes for drought and heat tolerance and these genes could be introgressed into leading chickpea varieties for abiotic stress tolerance. In chickpea, the deployment of genomic technologies in breeding programmes has yielded several molecular breeding products ready for testing and release. By employing marker-assisted backcross breeding (MABB), Varshney et al. (2013c) introgressed 'QTL-hotspot' containing root traits and other drought tolerance traits into leading chickpea variety, JG 11. Recently, the collaborative efforts of ICRISAT, ICAR-IARI and ICAR-IIPR has led to introgression of the genomic region containing 'QTL-hotspot' from ICC 4958 that contains QTLs for key drought-tolerance related traits into three elite Indian chickpea cultivars, that is, Pusa 372, Pusa 362 and DCP 92-3 (Bharadwaj et al. 2021). Pusa 10,216 is the first MABB variety with enhanced drought tolerance developed in a record time (4 years) and released for cultivation in India. This variety was developed by introgressing drought-tolerant genes from ICC 4958 into Pusa 372 using the MABB approach. This variety has increased seed size and exhibits a yield improvement of 11% over the recipient parent under moisture stress in the central zone of India. In addition, several introgression lines with enhanced drought tolerance and disease resistance developed using the MABB approach are at various stages of testing and release (Roorkiwal et al. 2020). These demonstrate the potential of genomics application in breeding programs to replace old and climate-vulnerable chickpea cultivars with improved stress-tolerant cultivars in semi-arid regions.

2.7 Summary

Current trends of unpredictable global climate change have resulted in the frequent occurrence of climate-related extreme weather events such as rainfall, drought, heat, cold, etc. and are limiting the productivity of various crop plants including chickpea. Also, the emergence of chickpea diseases such as dry root rot is generally found to have an increasing impact in changing climates. All these stresses affect both physiological and biochemical traits at different stages of growth and development. Drought, extreme temperatures and salinity stress, generally, have negative effects on the reproductive stage. Conventional breeding techniques have played a major role in improving and stabilizing chickpea yields in semi-arid regions. However, the current rate of improvement in productivity is not sufficient to meet future demands, particularly with changing climate and population explosion. Advances in chickpea genomics have provided breeders with optimum genomic resources together with modern tools and technologies, especially rapid generation advancement/speed breeding, sequence-based high-density genotyping, accurate and high-throughput phenotyping, marker-assisted breeding, genomic selection and novel approaches such as transgenics technology and genome editing tools to harness the superior and favourable alleles in the modern cultivars.

References

Ahmad F, Gaur PM, Croser J (2005) Chickpea (*Cicer arietinum* L.). Gen Resour Chromosome Eng Crop Improv -Grain Legumes 1:185–214

Ashraf M, Iram AT (2005) Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. Flora 200:535–546

Atlin GN, Cairns JE, Das B (2017) Rapid breeding and varietal replacement are critical to adaptation of cropping systems in the developing world to climate change. Global Food Secur 12C:31–37

Babber S, Sheokand S, Malik S (2000) Nodule structure and functioning in chickpea (*Cicer arietinum*) as affected by salt. Biol Plant 43:269–273

Badhan S, Ball AS, Mantri N (2021) First report of CRISPR/Cas9 mediated DNA-free editing of 4CL and RVE7 genes in chickpea protoplasts. Int J Mol Sci 22(1):396

Baker NR (1991) Possible role of photosystem II in environmental perturbations of photosynthesis. Physiol Plant 81:563–570

Basu PS, Singh DN (2003) Physiology and abiotic stress in chickpea. In: Ali M, Kumar S, Singh NB (eds) Chickpea research in India. Indian Institute of Pulses Research, Kanpur, India, pp 137–167

Basu PS, Ali M and Chaturvedi SK (2009) Terminal heat stress adversely affects chickpea productivity in Northern India- Strategies to improve thermo tolerance in the crop under climate change. ISPRS Archives XXXVIII-8/W3 Workshop Proceedings: Impact of Climate Change on Agriculture, 189—193. 23-25 February, New Delhi, India

Berger JD, Turner NC, Siddique KHM, Knights EJ, Brinsmead RB, Mock I et al (2004) Genotype by environment studies across Australia reveals the importance of phenology for chickpea (*Cicer arietinum* L.) improvement. Aust J Agric Res 55:1071–1084

Berger JD, Milroy SP, Turner NC et al (2011) Chickpea evolution has selected for contrasting phenological mechanisms among different habitats. Euphytica 180:1–15

Bharadwaj C, Tripathi S, Soren KR et al (2021) Introgression of "QTL-hotspot" region enhances drought tolerance and grain yield in three elite chickpea cultivars. Plant Genome:e20076. https://doi.org/10.1002/tpg2.20076

Black CC, Brown RH, Moore RC (1978) Plant photosynthesis. In: Dobereiner J, Burns RM, Hollander A (eds) limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York, pp 95–110

Blum A (1988) Plant breeding for stress environments. CRC Press, Boca Raton, FL

Canci H, Toker C (2009) Evaluation of yield criteria for drought and heat resistance in chickpea (*Cicer arietinum* L.). J Agron Crop Sci 19:47–54

Chandra S, Buhariwalla HK, Kashiwagi J, Harikrishna S, Sridevi KR, Krishnamurthy L, Serraj R and Crouch JH (2004) Identifying QTL-linked markers in marker-deficient crops. In: 4th International Crop Science Congress, 26 Sep–1 Oct 2004, Brisbane, Australia

Chaves MM (1991) Effects of water deficits on carbon assimilation. J Exp Bot 42:1–16

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot 103:551–560

Clarke HJ, Siddique KHM (2004) Response of chickpea genotypes to low temperature stress during reproductive development. Field Crop Res 90:323–334

Clarke H, Khan TN, Siddique KHM (2004) Pollen selection for chilling tolerance at hybridisation leads to improved chickpea cultivars. Euphytica 139:65–74

Croser JS, Clarke HJ, Siddique KHM, Khan TN (2003) Low temperature stress: implications for chickpea (*Cicer arietinum* L.) improvement. Crit Rev Plant Sci 22:185–219

Das A, Basu PS, Kumar M et al (2021) Transgenic chickpea (*Cicer arietinum* L.) harbouring AtDREB1a are physiologically better adapted to water deficit. BMC Plant Biol 21:39

Das S, Upadhyaya HD, Bajaj D et al (2015) Deploying QTL-seq for rapid delineation of a potential candidate gene underlying major trait-associated QTL in chickpea. DNA Res 22(3):193–203

Desai PB, Patil BS, Vijayakumar AG, Basavarajappa MP, Subramanya AE, Bharadwaj C, Kulkarni VR (2017) Dry root rot resistance in chickpea: Studies on genetic and molecular variations. Vegetos (Special) 30(1):20–25

Devasirvatham V (2012) The basis of chickpea heat tolerance under semi-arid environments. PhD thesis. University of Sydney

Devasirvatham V, Gaur P, Mallikarjuna N, Raju TN, Trethowan RM, Tan DKY (2012) Effect of high temperature on the reproductive development of chickpea genotypes under controlled environments. Funct Plant Biol 39:1009–1018

Devasirvatham V, Gaur P, Mallikarjuna N, Raju TN, Trethowan RM, Tan DKY (2013) Reproductive biology of chickpea response to heat stress in the field is associated with the performance in controlled environments. Field Crops Res 142:9–19

Dixit GP, Srivastava AK, Singh NP (2019) Marching towards self-sufficiency in chickpea. Curr Sci 116:239–242

FAOSTAT, (2019) http://www.fao.org/faostat/en/#data/QC. Accessed on 4th, April 2021

Flowers TJ, Gaur PM, Gowda CL, Krishnamurthy L, Samineni S, Siddique KH, Turner NC, Vadez V, Varshney RK, Colmer TD (2010) Salt sensitivity in chickpea. Plant Cell Environ 33:490–509

Foyer CH, Lam HM, Nguyen HT, Siddique KHM, Varshney RK, Colmer TD et al (2016) Neglecting legumes has compromised human health and sustainable food production. Nat. Plants 2:16112. https://doi.org/10.1038/nplants.2016.112

Ganguly M, Molla KA, Karmakar S, Datta K, Datta SK (2014) Development of pod borer-resistant 660 transgenic chickpea using a pod-specific and a constitutive promoter-driven fused cry1Ab/Ac661 gene. Theor Appl Genet 127:2555–2565

Garg T, Mallikarjuna BP, Thudi M et al (2018) Identification of QTLs for resistance to Fusarium wilt and Ascochyta blight in a recombinant inbred population of chickpea (*Cicer arietinum* L.). Euphytica 214(3):1–11

Gaur PM, Gowda CLL, Knights EJ, Warkentin T, Acikgoz N, Yadav SS, Kumar J (2007) Breeding achievements. In: Yadav SS, Redden RJ, Chen W, Sharma B (eds) Chickpea breeding and management. CABI, UK, pp 391–416

Gaur PM, Krishnamurthy L, Kashiwagi L (2008a) Improving drought-avoidance root traits in chickpea (*Cicer arietinum* L.)—current status of research at ICRISAT. Plant Prod Sci 1:3–11. https://doi.org/10.1626/pps.11.3

Gaur PM, Kumar J, Gowda CLL, et al. (2008b) Breeding chickpea for early phenology: perspectives, progress and prospects. In: Proceedings of the Fourth International Food Legumes Research Conference, 18–22 Oct 2005, New Delhi, India

Gaur PM, Jukanti AK, Samineni S et al (2012a) Climate change and heat stress tolerance in chickpea. In: Tuteja N, Gill SS (eds) Climate change and plant abiotic stress tolerance. Wiley Blackwell, Weinheim, Germany, pp 839–855. ISBN 978-3-527-33491-9

Gaur PM, Jukanti AK, Varshney RK (2012b) Impact of genomic technologies on chickpea breeding strategies. Agronomy 2:199–221

Gaur PM, Samineni S, Varshney RK (2014a) Drought and heat tolerance in chickpea. Legume Perspetives 3:15–17

Gaur PM, Jukanti AK, Srinivasan S (2014b) Climate change and heat stress tolerance in chickpea. In: Tuteja N, Gill SS (eds) Climate change and plant abiotic stress tolerance. Wiley VCH, Weinheim, Germany, pp 839–855

Gaur PM, Thudi M, Srinivasan S, Varshney RK (2014c) Advances in chickpea genomics. In: Gupta S, Nadarajan N, Gupta DS (eds) Legumes in the Omic Era. Springer, New York, pp 73–94

Gaur PM, Samineni S, Tripathi S, Varshney RK, Gowda CLL (2015) Allelic relationships of flowering time genes in chickpea. Euphytica 203:295–308

Gaur PM, Samineni S, Thudi M et al (2018) Integrated breeding approaches for improving drought and heat adaptation in chickpea (*Cicer arietinum* L.). Plant Breed 138(4):389–400. ISSN 01799541

Ghosh S, Watson A, Gonzalez-Navarro OE et al (2018) Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. Nat Protoc 13:2944–2963

Gumber RK, Sarvjeet S (1996) Genetics of flowering time in chickpea: a preliminary report. Crop Improv 23:295–296

Gunes A, Cicek N, Inal A, Alpaslan M, Eraslan F, Guneri E et al (2006) Genotypic response of chickpea (*Cicer arietinum* L.) cultivars to drought stress implemented at pre-and post-anthesis stages and its relations with nutrient uptake and efficiency. Plant Soil Environ 52:368–376

Guo W, Fukatsu T, Ninomiya S (2015) Automated characterization of flowering dynamics in rice using field-acquired time-series RGB images. Plant Methods 11(1):7

Hamwieh A, Imtiaz M, Malhotra RS (2013) Multi-environment QTL analyses for drought-related traits in a recombinant inbred population of chickpea (*Cicer arientinum* L.). Theor Appl Genet 126:1025–1038

Hegde VS (2010) Genetics of flowering time in chickpea in a semiarid environment. Plant Breed 129:683–687

Jaganathan D, Thudi M, Kale S et al (2015) Genotyping-by-sequencing based intra-specific genetic map refines a "QTL-hotspot" region for drought tolerance in chickpea. Mol Gen Genomics 290(2):559–571

Jayalakshmi SK, Usharani S, Benagi MDD (2008) Sources of resistance to dry root rot of chickpea caused by *Rhizoctonia bataticola*. Agric Sci Dig 28:147–148

Jha UC, Jha R, Singh NP, Shil S, Kole PC (2018) Heat tolerance indices and their role in selection of heat stress tolerant chickpea (*Cicer arietinum* L.) genotypes. Indian J Agric Sci 88:260–270

Jukanti AK, Gaur PM, Gowda CLL, Chibbar RN (2012) Chickpea: nutritional properties and its benefits. Br J Nutr 108:S11–S26

Kaashyap M, Ford R, Bohra A, Kuvalekar A, Mantri N (2017) Improving salt tolerance of chickpea using modern genomics tools and molecular breeding. Curr Genomics 18(6):557–567. https://doi.org/10.2174/1389202918666170705155252

Kadiyala MDM, Kumara Charyulu D, Nedumaran S, Moses Shyam D, Gumma MK, Bantilan MCS (2016) Agronomic management options for sustaining chickpea yield under climate change scenario. J Agrometeorol 18:41–47

Kale SM, Jaganathan D, Ruperao P, Chen C et al (2015) Prioritization of candidate genes in "QTL-hotspot" region for drought tolerance in chickpea (*Cicer arietinum* L.). Sci Rep 5:15296

Kaloki P, Devasirvatham V, Tan DK (2019) Chickpea abiotic stresses: combating drought, heat and cold. In: Abiotic and biotic stress in plants. IntechOpen, London, UK. https://doi.org/10.5772/intechopen.83404

Kalra N, Chakraborty D, Sharma A et al (2008) Effect of temperature on yield of some winter crops in northwest India. Curr Sci 94:82–88

Kanouni H, Farayedi Y, Saeid A, Sabaghpour SH (2015) Stability analyses for seed yield of chickpea (*Cicer arietinum* L.) genotypes in the western cold zone of Iran. J Agric Sci 7(5):219

Karadi S, Samineni S, Sajja S, Sharma M et al (2021) Molecular mapping of dry root rot resistance genes in chickpea (*Cicer arietinum* L.). Euphytica 217:123

Kashiwagi J, Krishnamurthy L, Upadhyaya HD, Krishna H, Chandra S, Vadez V et al (2005) Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). Euphytica 146:213–222

Kashiwagi J, Krishnamurthy L, Crouch JH, Serraj R (2006a) Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. Field Crops Res 95:171–181

Kashiwagi J, Krishnamurthy L, Singh S, Gaur PM, Upadhyaya HD, Panwar JDS et al (2006b) Relationships between transpiration efficiency and carbon isotope discrimination in chickpea (*Cicer arietinum* L.). J SAT Agric Res 2:1–3

Kashiwagi J, Krishnamurty L, Gaur PM, Chandra S, Upadhyaya HD (2008) Estimation of gene effects of the drought avoidance root characteristics in chickpea (*C. arietinum* L.). Field Crops Res 105:64–69

Kashiwagi J, Krishnamurthy L, Purushothaman R, Upadhyaya HD, Gaur PM, Gowda CLL et al (2015) Scope for improvement of yield under drought through the root traits in chickpea (*Cicer arietinum* L.). Field Crops Res 170:47–54

Kaushal N, Gupta K, Bhandhari K, Kumar S, Thakur P, Nayyar H (2011) Proline induces heat tolerance in chickpea (*Cicer arietinum* L.) plants by protecting vital enzymes of carbon and antioxidative metabolism. Plant Physiol Mol Biol 17:203–213

Kaushal N, Awasthi R, Gupta K, Gaur PM, Siddique KHM, Nayyar H (2013) Heat-stress-induced reproductive failures in chickpea (*Cicer arietinum*) are associated with impaired sucrose metabolism in leaves and anthers. Funct Plant Biol 40:1334–1349

Khalid MN, Iqbal HF, Tahir A, Ahmad AN (2001) Germination potential of chickpeas (*Cicer arietinum*) under saline condition. Pak J Biol Sci 4:395–396

Khan HA, Siddique KHM, Colmer TD (2017) Vegetative and reproductive growth of salt-stressed chickpea are carbon-limited: sucrose infusion at the reproductive stage improves salt tolerance. J Exp Bot 68:2001–2011

Krishnamurthy L, Kashiwagi J, Upadhyaya HD, Serraj R (2003) Genetic diversity of drought avoidance root traits in the mini-core germplasm collection of chickpea. Int. Chickpea Pigeonpea Newslett 10:21–24

Krishnamurthy L, Kashiwagi J, Gaur PM, Upadhyaya HD, Vadez V (2010) Sources of tolerance to terminal drought in the chickpea (*Cicer arietinum* L.) minicore germplasm. Field Crops Res 119:322–330

Krishnamurthy L, Gaur PM, Basu PS, Chaturvedi SK, Tripathi S, Vadez V, Varshney RK, Gowda CLL (2011a) Large genetic variation for heat tolerance in the reference collection of chickpea (*Cicer arietinum* L.) germplasm. Plant Genet Res 9:59–69

Krishnamurthy L, Turner NC, Gaur PM, Upadhyaya HD, Varshney RK, Siddique KHM, Vadez V (2011b) Consistent variation across years in salinity resistance in a diverse range of chickpea (*Cicer arietinum* L.) genotypes. J Agron Crop Sci 197:214–227

Krishnamurthy L, Kashiwagi J, Tobita S et al (2013a) Variation in carbon isotope discrimination and its relationship with harvest index in the reference collection of chickpea germplasm. Funct Plant Biol 40:1350–1361

Krishnamurthy L, Kashiwagi J, Upadhyaya HD, Gowda CLL, Gaur PM, Singh S, Purushothaman R, Varshney RK (2013b) Partitioning coefficient – a trait that contributes to drought tolerance in chickpea. Field Crop Res 149:354–365

Kumar J, Haware MP, Smithson JB (1985) Registration of four short-duration, fusarium wilt-resistant kabuli (Garbanzo) chickpea germplasms. Crop Sci 25:576–577

Kumar J, Abbo S (2001) Genetics of flowering time in chickpea, its bearing on productivity in semi-arid environments. Adv Agron 72:107–138

Kumar J, van Rheenen HA (2000) A major gene for time of flowering in chickpea. J Hered 91:67–68

Kumar S, Nayyar H, Bhanwara RK, Upadhyaya HD (2010) Chilling stress effects on reproductive biology of chickpea. J SAT Agric Res 8:1–14

Kumar S, Thakur P, Kaushal N, Malik JA, Gaur P, Nayyar H (2013) Effect of varying high temperatures during reproductive growth on reproductive function, oxidative stress and seed yield in chickpea genotypes differing in heat sensitivity. Arch Agron Soil Sci 59:823–843

Kurets VK, Popov EG (1988) Evaluating the requirements of a genotype in respect of environmental conditions. In: Diagnostika urtoichivosti rastenii stressovym vozdeistviyam. USSR, Leningrad, pp 222–227

Lauter DJ, Munns DN (1986) Salt resistance of chickpea genotypes in solutions salinized with NaCl or Na2SO4. Plant Soil 95:271–279

Leport L, Turner NC, French RJ, Tennant D, Thomson BD, Siddique KHM (1998) Water relations, gas exchange and growth of cool-season grain legumes in a Mediterranean-type environment. Eur J Agron 9:295–303

Leport L, Turner NC, French RJ, Barr MD, Duda R, Davies SL et al (1999) Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. Eur J Agron 11:279–291

Levitt J (1969) Growth and survival of plants at extreme of temperature—a unified concept. Symp Soc Exp Biol 23:395–448

Li B, Zhang N, Wang YG, George AW, Reverter A, Li Y (2018) Genomic prediction of breeding values using a subset of SNPs identified by three machine learning methods. Front Genet 9:237

Madrid E, Rubiales D, Moral A, Moreno MT, Millan T, Gil J, Rubio J (2008) Mechanism and molecular markers associated with rust resistance in a chickpea interspecific cross (*Cicer arietinum* × *Cicer reticulatum*). Eur J Plant Pathol 121:43–53

Malhotra RS, Singh KB (1990) The inheritance of cold tolerance in chickpea. J Gene Plant Breed 44:227–230

Malhotra RS, Singh KB (1991) Gene action for cold tolerance in chickpea. Theor Appl Genet 82: 598–601

Maliro MFA, McNeil D, Kollmorgen J, Pittock C and Redden B (2004) Screening chickpea (*Cicer arietinum* L.) and wild relatives germplasm from diverse sources for salt tolerance. New directions for a diverse planet. In: Proceedings of the 4th International Crop Science Congress, Brisbane, Australia (September 26–October 1)

Maliro MFA, McNeil D, Redden B, Kollmorgen JF, Pittock C (2008) Sampling strategies and screening of chickpea (*Cicer arietinum* L.) germplasm for salt tolerance. Genet Resour Crop Evol 55:53–63

Mallikarjuna BP, Samineni S, Thudi M et al (2017) Molecular Mapping of Flowering Time Major Genes and QTLs in Chickpea (*Cicer arietinum* L.). Front Plant Sci 8(1140)

Mansfield TJ, Atkinson CJ (1990) Stomatal behavior in water stressed plants. In: Alscher RG, Cumming JR (eds) Stress responses in plants: adaptation and acclimation mechanisms. Wiley-Liss, New York, pp 241–264

Mantri NL, Ford R, Coram TE, Pang EC (2007) Transcriptional profiling of chickpea genes differentially regulated in response to high-salinity, cold and drought. BMC Genomics 8:1–14

Maphosa L, Richards MF, Norton SL, Nguyen GN (2020) Breeding for abiotic stress adaptation in chickpea (*Cicer arietinum* L.): a comprehensive review. Crop Breed Genet Genom 2(4): e200015

McDonald GK, Paulsen GM (1997) High temperature effects on photosynthesis and water relations of grain legumes. Plant Soil 196:47–58

Mir AH, Bhat MA, Fayaz H, Dar SA, Maqbool S, Bhat NA, Thudi M, Mir RR (2019) Assessment of cold tolerance in chickpea accessions in North-Western Himalayas of Jammu and Kashmir. India J Pharmacogn Phytochem 8(4):2268–2274

Mugabe D, Coyne CJ, Piaskowski J, Zheng P, Ma Y, Landry E et al (2019) Quantitative trait loci for cold tolerance in chickpea. Crop Sci 59:573–582

Nguyen GN, Norton SL, Rosewarne GM, James LE, Slater AT (2018) Automated phenotyping for early vigour of field pea seedlings in controlled environment by colour imaging technology. PLoS One 13(11):e0207788

Or E, Hovav R, Abbo S (1999) A major gene for flowering time in chickpea. Crop Sci 39:315–322

Pande S, Desai S, Sharma M (2010) Impact of climate change on rainfed crop diseases: current status and future research needs. In: Lead papers. National Symposium on Climate Change and Rainfed Agriculture, 2010 Feb. 18–20. Indian Society of Dryland Agriculture, Central Research Institute for Dryland Agriculture, Hyderabad, pp 55–59

Pandey MK, Roorkiwal M, Singh VK et al (2016) Emerging genomic tools for legume breeding: current status and future prospects. Front Plant Sci 7:455

Pang J, Turner NC, Du YL, Colmer TD, Siddique KHM (2017) Pattern of water use and seed yield under terminal drought in chickpea genotypes. Front Plant Sci 8:1–14

Parida SK, Das AB (2005) Salt tolerance and salinity effects on plants. Ecotoxicol Environ Saf 60: 324–349

Paul PJ, Samineni S, Sajja SB, Rathore A, Das RR, Chaturvedi SK et al (2018a) Capturing genetic variability and selection of traits for heat tolerance in a chickpea recombinant inbred line (RIL) population under field conditions. Euphytica 214:27

Paul PJ, Samineni S, Thudi M, Sajja SB, Rathore A, Das RR et al (2018b) Molecular mapping of QTLs for heat tolerance in chickpea. Int J Mol Sci 19:2166

Pham-Thi AT, Borrel-Flood C, Vieira da Silva J, Justin AM, Mazliak P (1987) Effects of drought on [1-14C]-oleic and [1-14C]-linoleic acid desaturation in cotton leaves. Physiol Plant 69:147–150

Pinheiro C, Chaves MM (2011) Photosynthesis and drought: can we make metabolic connections from available data? J Exp Bot 62:869–882

Pouresmael M, Khavari-Nejad RA, Mozafari J, Najafi F, Moradi F (2013) Efficiency of screening criteria for drought tolerance in chickpea. Arch Agron Soil Sci 59:1675–1693

Prasad TK, Anderson MD, Martin BA, Steward CR (1994) Evidence for chilling induced oxidative stress maize seedling and a regulatory role for hydrogen peroxide. Plant Cell 6:65–75

Purushothaman R, Thudi M, Krishnamurthy L, Upadhyaya HD, Kashiwagi J, Gowda CLL et al (2015) Association of mid-reproductive stage canopy temperature depression with the molecular markers and grain yields of chickpea (*Cicer arietinum* L.) germplasm under terminal drought. Field Crops Res 174:1–11

Purushothaman R, Krishnamurthy L, Upadhyaya HD, Vadez V, Varshney RK (2016) Shoot traits and their relevance in terminal drought tolerance of chickpea (*Cicer arietinum* L.). Field Crop Res 197:10–27

Pushpavalli R, Krishnamurthy L, Thudi M, Gaur PM, Rao MV, Siddique KH et al (2015) Two key genomic regions harbour QTLs for salinity tolerance in ICCV 2 x JG 11 derived chickpea (*Cicer arietinum* L.) recombinant inbred lines. BMC Plant Biol 15:124

Quirós JJ, McGee RJ, Vandemark GJ, Romanelli T, Sankaran S (2019) Field phenotyping using multispectral imaging in pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.). Eng Agric Environ Food 12(4):404–413

Ramamoorthy P, Lakshmanan K, Upadhyaya HD, Vadez V, Varshney RK (2017) Root traits confer grain yield advantages under terminal drought in chickpea (*Cicer arietinum* L.). Field Crops Res 201:146–161

Rani A, Devi P, Jha UC, Sharma KD, Siddique KHM, Nayyar H (2020) Developing climate-resilient chickpea involving physiological and molecular approaches with a focus on temperature and drought stresses. Front Plant Sci 10:1759

Rao A, Haware MP (1987) Inheritance of dry root rot (*Rhizoctonia bataticola*) resistance in chickpea (*Cicer arietinum* L.). Plant Breed 98:349–352

Reddy AT (2016) Identification of resistant sources, inheritance and biochemical basis of resistance to dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. in chickpea (*Cicer arietinum* L.). PhD Thesis. University of Agricultural Sciences GKVK, Bengaluru

Rehman AU, Malhotra RS, Bett K, Taran B, Bueckert R, Warkentin TD (2011) Mapping QTL associated with traits affecting grain yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. Crop Sci 51:450–463

Robertson LD, Singh KB, Ocampo B (1995) A catalog of annual wild Cicer species. ICARDA, Aleppo, Syria

Roorkiwal M, Rathore A, Das RR et al (2016) Genome enabled prediction models for yield related traits in chickpea. Front Plant Sci 7:1666

Roorkiwal M, Jain A, Kale SM, Doddamani D, Chitikineni A, Thudi M, Varshney RK (2018) Development and evaluation of highdensity Axiom ®CicerSNP Array for high-resolution genetic mapping and breeding applications in chickpea. Plant Biotechnol J 16:890–901

Roorkiwal M, Bharadwaj C, Barmukh R et al (2020) Integrating genomics for chickpea improvement: achievements and opportunities. Theor Appl Genet (TSI) 133(5):1703–1720. ISSN 0040-5752

Rubio J, Moreno MT, Moral A, Rubiales D, Gil J (2006) Registration of RIL58-ILC72/Cr5, a chickpea germplasm line with rust and ascochyta blight resistance. Crop Sci 46:2331–2332

Sabaghpour SH, Kumar J, Rao TN (2003) Inheritance of growth vigour and its association with other characters in chickpea. Plant Breed 122:542–544

Sabaghpour SH, Mahmodi AA, Saeed A, Kamel M, Malhotra RS (2006) Study on chickpea drought tolerance lines under dryland condition of Iran. Indian J Crop Sci 1:70–73

Sabbavarapu MM, Sharma M, Chamarthi SK et al (2013) Molecular mapping of QTLs for resistance to Fusarium wilt (race 1) and Ascochyta blight in chickpea (*Cicer arietinum* L.). Euphytica 193:121–133

Samineni S (2010) Physiology, genetics and molecular mapping of salt tolerance in chickpea. The University of Western Australia Australia

Samineni S, Siddique KHM, Gaur PM, Colmer TD (2011) Salt sensitivity of the vegetative and reproductive stages in chickpea (*Cicer arietinum* L.) podding is a particularly sensitive stage. Environ Exp Bot 71:260–268

Samineni S, Kamatam S, Thudi M, Varshney RK, Gaur PM (2016) Vernalization response in chickpea is controlled by a major QTL. Euphytica 207:453–461

Samineni S, Sen M, Sajja SB, Gaur PM (2019) Rapid generation advance (RGA) in chickpea to produce up to seven generations per year and enable speed breeding. Crop J. https://doi.org/10.1016/j.cj.2019.08.003

Saxena MC, Saxena NP, Mohammed AK (1988) High temperate stress. In: Summerfield RJ (ed) World crops: cool-season food legumes. Kluwer Academic Publisher, Dordrecht, the Netherlands, pp 845–856

Saxena NP, Krishnamurthy L, Johansen C (1993) Registration of a drought-resistant chickpea germplasm. Crop Sci 33:1424–1424

Serraj R, Krishnamurthy L, Upadhyaya HD (2004) Screening chickpea mini-core germplasm for tolerance to soil salinity. Int Chickpea Pigeonpea Newslett 11:29–32

Sharma M, Pande S (2013) Unravelling effects of temperature and soil moisture stress response on development of dry root rot [*Rhizoctonia bataticola* (Taub.)] Butler in Chickpea. Am J Plant Sci 4:584–589

Sharma M, Ghosh R, Pande S (2016) Dry root rot [*Rhizoctonia bataticola* (Taub.) Butler] an emerging disease of chickpea–where do we stand? Arch Phytopath Plant Prot 48:1–16

Sharma KD, Nayyar H (2014) Cold stress alters transcription in meiotic anthers of cold tolerant chickpea (*Cicer arietinum* L.). BMC Res Notes 7:1–13

Sheokand S, Dhandi S, Swaraj K (1995) Studies on nodule functioning and hydrogen peroxide scavenging enzymes under salt stress in chickpea nodules. Plant Physiol Biochem 33:561–566

Siddique KHM, Loss SP, Regan KL, Jettner RL (1999) Adaptation and seed yield of cool season grain legumes in Mediterranean environments of south-western Australia. Aust J Agric Res 50: 375–388

Siddique KHM, Brinsmead RB, Knight R, Knights EJ, Paul JG, Rose IA (2000) Adaptation of chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.) to Australia. In: Knight R (ed) Linking research and marketing opportunities for pulses in the 21st century. Kluwer, Dordrecht, The Netherlands, pp 289–303

Singh KB (1993) Problems and prospects of stress resistance breeding in chickpea. In: Singh KB, Saxena MC (eds) Breeding for stress tolerance in cool-seasons food legumes. John Wiley & Sons, Ltd., Chichester, UK, pp 17–37

Singh DP, Rawson HM, Turner NC (1982) Effect of radiation, temperature and humidity on photosynthesis, transpiration and water use efficiency of chickpea (*Cicer arietinum* L.). Ind Plant Physiol 25:32–39

Singh KB, Malhotra RS, Saxena MC (1995) Additional sources of tolerance to cold in cultivated and wild Cicer species. Crop Sci 35:1491–1497

Singh KB, Omar M, Saxena MC, Johansen C (1997) Screening for drought resistance in spring chickpea in the Mediterranean Region. J Agron Crop Sci 178(4):227–235

Singh VK, Khan AW, Jaganathan D, Thudi M et al (2016) QTL-seq for rapid identification of candidate genes for 100-seed weight and root/total plant dry weight ratio under rainfed conditions in chickpea. Plant Biotechnol J 14(11):2110–2119

Sinha R, Irulappan V, Mohan-Raju B, Suganthi A, Senthil-Kumar M (2019) Impact of drought stress on simultaneously occurring pathogen infection in field-grown chickpea. Sci Rep 9(1): 5577

Sita K, Sehgal A, HanumanthaRao B, Nair RM, Vara Prasad PV, Kumar S et al (2017) Food legumes and rising temperatures: effects, adaptive functional mechanisms specific to reproductive growth stage and strategies to improve heat tolerance. Front Plant Sci 8:1–30

Sivasakthi K, Thudi M, Tharanya M, Kale SM, Kholová J, Halime MH et al (2018) Plant vigour QTLs co-map with an earlier reported QTL hotspot for drought tolerance while water saving QTLs map in other regions of the chickpea genome. Plant Biol 18:29

Soren KR, Madugula P, Kumar N, Barmukh R et al (2020) Genetic dissection and identification of candidate genes for salinity tolerance using Axiom®CicerSNP array in chickpea. Int J Mol Sci 21(14):5058

Srinivasan A, Johansen C, Saxena NP (1998) Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L.): characterization of stress and genetic variation in pod set. Field Crop Res 57:181–193

Subbarao GV, Johansen C, Slinkard AE, Rao RCN, Saxena NP, Chauhan YS (1995) Strategies for improving drought resistance in grain legumes. Crit Rev Plant Sci 14:469–523

Summerfield RJ, Wein HC (1980) Effects of photoperiod and air temperature on growth and yield of economic legumes. In: Summerfield RJ, Bunting AH (eds), Advances in legumes science. Volume1 of the Proceedings of the International Legumes Conference, pp. 17—36. Kew, UK

Summerfield RJ, Hadley Roberts EH, Minchin FR, Awsthorne S (1984) Sensitivity of chickpea (*Cicer arietinum* L.) to hot temperatures during the reproductive period. Exp Agr 20:77–93

Talekar SC, Lohithaswa HC, Viswanatha KP (2017) Identification of resistant sources and DNA markers linked to genomic region conferring dry root rot resistance in chickpea (*Cicer arietinum* L.). Pl Breed 136:161–166

Tello J, Montemayor MI, Forneck A, Ibáñez J (2018) A new image-based tool for the high throughput phenotyping of pollen viability: evaluation of inter- and intra-cultivar diversity in grapevine. Plant Methods 14(1):3

Thakur P, Kumar S, Malik JA et al (2010) Cold stress effects on reproductive development in grain crops: an overview. Environ Exp Bot 67(3):429–443

Thudi M, Gaur PM, Krishnamurthy L, Mir RR et al (2014a) Genomics-assisted breeding for drought tolerance in chickpea. Funct Plant Biol 41(11):1178–1190

Thudi M, Upadhyaya HD, Rathore A, Gaur PM, Krishnamurthy L, Roorkiwal M et al (2014b) Genetic dissection of drought and heat tolerance in chickpea through genome-wide and candidate gene-based association mapping approaches. PLoS One 9:e96758

Thudi M, Chitikineni A, Liu X, He W, Roorkiwal M et al (2016a) Recent breeding programs enhanced genetic diversity in both desi and kabuli varieties of chickpea (*Cicer arietinum* L.). Scientific Rep 6:38636

Thudi M, Khan AW, Kumar V, Gaur PM, Katta AVSK, Garg V, Roorkiwal M, Samineni S, Varshney RK (2016b) Whole genome re-sequencing reveals genome wide variations among parental lines of mapping populations in chickpea (*Cicer arietinum*). BMC Plant Biol 16(Suppl. 1):10

Toker C, Lluch C, Tejera NA, Serraj R, Siddique KHM (2007) Abiotic stress. In: Yadav SS, Redden R, Chen W, Sharma B (eds) Chickpea breeding and management. CAB International, UK, pp 474–496

Tracy SR, Nagel KA, Postma JA, Fassbender H, Wasson A, Watt M (2020) Crop improvement from phenotyping roots: highlights reveal expanding opportunities. Trends Plant Sci 25(1): 105–118

Turner NC, Jones MM (1980) Turgor maintenance by osmotic adjustment. In: Turner NC, Kramer PJ (eds) Adaptation of plants to water and high temperature stress. John Wiley & Sons, New York, pp 87–103

Turner NC (1986) Crop water deficit: a decade of progress. Adv Agron 39:1–51

Turner NC (2003) Drought resistance: a comparison of two research frameworks. In: Saxena NP (ed) Management of agricultural drought – agronomic and genetic options. Oxford & IBH Publishing Co., Pvt. Lid, New Delhi, India, pp 103–122

Turner NC, Abbo S, Berger JD, Chaturvedi SK, French RJ, Ludwig C et al (2007) Osmotic adjustment in chickpea (*Cicer arietinum* L.) results in no yield benefit under terminal drought. J Exp Bot 58:187–194

Turner NC, Colmer TD, Quealy J, Pushpavalli R, Krishnamurthy L, Kaur J, Singh G, Siddique KHM, Vadez V (2013) Salinity tolerance and ion accumulation in chickpea (*Cicer arietinum* L.) subjected to salt stress. Plant Soil 365:347–361

Upadhyaya HD, Dronavalli N, Gowda CLL, Singh S (2011) Identification and evaluation of chickpea germplasm for tolerance to heat stress. Crop Sci 51:2079–2094

Upadhyaya HD, Kashiwagi J, Varshney RK, Gaur PM, Saxena KB, Krishnamurthy L et al (2012) Phenotyping chickpeas and pigeonpeas for adaptation to drought. Front Physiol 3:1–10

Vadez V, Krishnamurthy L, Serraj R, Gaur PM, Upadhyaya HD, Hoisington DA, Varshney RK, Turner NC, Siddique KHM (2007) Large variation in salinity tolerance in chickpea is explained by differences in sensitivity at the reproductive stage. Field Crop Res 104:123–129

Vadez V, Rashmi M, Mithila K, Muralidharan R et al (2012a) Large number of flowers and tertiary branches, and higher reproductive success increase yields under salt stress in chickpea. Eur J Agron 41:42–51

Vadez V, Krishnamurthy L, Thudi M, Anuradha C, Colmer T, Turner N, Siddique K, Gaur PM, Varshney RK (2012b) Assessment of ICCV 2 × JG 62 chickpea progenies shows sensitivity of reproduction to salt stress and reveals QTL for seed yield and yield components. Mol Breed 30: 9–21

Vadez V, Kholová J, Hummel G, Zhokhavets U, Gupta SK, Hash CT (2015) LeasyScan: a novel concept combining 3D imaging and lysimetry for high-throughput phenotyping of traits controlling plant water budget. J Exp Bot 66:5581–5593

Varshney RK, Murali Mohan S, Gaur PM et al (2013a) Achievements and prospects of genomics-assisted breeding in three legume crops of the semi-arid tropics. Biotechnol Adv 31:1120–1134

Varshney RK, Song C, Saxena RK, Azam S et al (2013b) Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nat Biotechnol 31:240–246

Varshney RK, Gaur PM, Chamarthi SK, Krishnamurthy L, Tripathi S et al (2013c) Fast-track introgression of “QTL-hotspot” for root traits and other drought tolerance traits in JG 11, an elite and leading variety of chickpea. Plant Genome 6:1–9

Varshney RK, Thudi M, Nayak SN, Gaur PM et al (2014) Genetic dissection of drought tolerance in chickpea (*Cicer arietinum* L.). Theor Appl Genet 127:445–462

Varshney RK, Kudapa H, Pazhamala L, Chitikineni A, Thudi M, Bohra A, Gaur PM, Janila P, Fikre A, Kimurto P, Ellis N (2015) Translational genomics in agriculture: some examples in grain legumes. Crit Rev Plant Sci 34:169–194

Varshney RK, Thudi M, Pandey MK, Tardieu F et al (2018) Accelerating Genetic gains in legumes for the development of prosperous smallholder agriculture: integrating genomics, phenotyping, systems modelling and agronomy. J Exp Bot 69(13):3293–3312

Varshney RK, Pandey MK, Bohra A et al (2019) Toward the sequence-based breeding in legumes in the post-genome sequencing era. Theor Appl Genet 132:797–816

Vieira da Silva J, Naylor AW, Kramer J (1974) Some ultrastructural and enzymatic effects of water stress in cotton (*Gossypium* L.) leaves. Proc Natl Acad Sci U S A 71:3243–3247

Wang J, Gan YT, Clarke F, McDonald CL (2006) Response of chickpea yield to high temperature stress during reproductive development. Crop Sci 46:2171–2178

Watson A, Ghosh S, Williams MJ, Cuddy WS et al (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nat Plants 4:23–29

Wery J, Turc O, Lecoeur J (1993) Mechanism of resistance to cold, heat and drought in cool-season legumes, with special reference to chickpea and pea. In: Food legumes, UK, Wiley, pp 271–291

Wu G, Miller ND, de Leon N, Kaeppler SM, Spalding EP (2019) Predicting Zea mays flowering time, yield, and kernel dimensions by analyzing aerial images. Front Plant Sci 10:1251

Xu Q, Paulsen AQ, Guikema JA, Paulsen GM (1995) Functional and ultrastructural injury to photosynthesis in wheat by high temperature during maturation. Environ Exp Bot 35:43–54

Yadav SK (2010) Cold stress tolerance mechanisms in plants. A review. Agron Sustain Dev 30(3): 515–527

Zarco-Tejada PJ, Berni JA, Suárez L, Sepulcre-Cantó G, Morales F, Miller JR (2009) Imaging chlorophyll fluorescence with an airborne narrow-band multispectral camera for vegetation stress detection. Remote Sens Environ 113(6):1262–1275

Zhang L, Niu Y, Zhang H, Han W, Li G, Tang J et al (2019) Maize canopy temperature extracted from UAV thermal and RGB imagery and its application in water stress monitoring. Front Plant Sci 10:1270

3 Reduction of Phytic Acid and Enhancement of Bioavailable Micronutrients in Common Beans (*Phaseolus vulgaris* L.) in Changing Climatic Scenario

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Abstract

Climate change impacts on food security will involve negative impacts on crop yields and potentially on the nutritional quality of staple crops. Despite efforts to increase global food availability, a key requirement for food and nutrition security, the global burden of malnutrition and micronutrient deficiencies remains alarming and closely linked to climate changes, particularly in low-income communities. Among the pulses, the common bean is the most important grain legume for human diets worldwide. However, common bean contains high levels of phytates that may play a role in preventing certain human diseases and are an important source of P in early plant growth. However, phytates are considered as one of the major antinutritional compounds in legume seeds (esp. Iron and zinc) and meet the criterion of a chelating agent, as they form complexes with numerous divalent and trivalent metal cations and can bind to one or more phosphate

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groups of a single phytate molecule or bridge two or more phytate molecules. The studies have revealed that IP5 and IP6 bind zinc and iron sufficiently strongly to inhibit both iron and zinc absorption. The development of low phytic acid (lpa) grain crops is considered an important goal in plant breeding programs aimed at improving mineral cation availability as well as at developing environment-friendly and sustainable production. However, even though phytic acid is an antinutrient, it has various other properties of preservation, antioxidation, and treating health ailments, therefore any reduction in the amount of phytates must be measured and must take into account the form of the phytates being modified. It is imperative to know in more detail the impacts of climate change on food security and undernourishment and its potential implications for nutritional outcomes.

Keywords

Common bean · Phytic acid · Fe and Zn · LPA mutants · Climate change

3.1 Introduction

The trilemma of malnutrition, namely, undernutrition, hidden hunger (micronutrient deficiencies), and overweight, are rapidly emerging as commonalities around the world. Among the malnutrition, micronutrient deficiencies in human populations are one of the greatest health concerns given the fact that the iron deficiency causing anemia and zinc deficiency causing stunted growth is more prevalent to affect a larger number of pregnant women, young children, and those affected by illness belonging to poor farmers in all parts of the world. Based on the most recent data available, UNICEF estimates that at least 340 million children under five suffer from micronutrient deficiencies (UNICEF 2019). There are approx. 2 billion people in the world who suffer from micronutrient deficiencies. Approximately 17.3% of the world's population is at high risk of inadequate zinc (Wessels and Brown 2012), while 30% population are anemic because of iron deficiency (WHO 2013), which causes a major public health concern worldwide. Among the different multisectoral approaches, the use of nutritionally rich crops is one of the better options to combat malnutrition. One of its probable remedies is in realizing the potential of enhancing the bioavailability of micronutrients through biofortification to further strengthen or enrich their micronutrient contents. In this direction, improved agricultural tools, techniques, and approaches could be handy in enabling desired quality enrichment in food crops through the reduction in antinutrient levels.

Plants absorb and use inorganic phosphorus (Pi) from the soil (Raboy and Dickinson 1984), which is responsible for many cellular functions. Typically, plants take up Pi in the form of phosphoric acid (H_3PO_4), translocated to the developing seed and then synthesized to become the storage form of P which is called phytic acid (Raboy 2002). Phytates are considered to be the primary form of inositol and phosphate in most seeds (Raboy 2009), which occurs mostly in the cytoplasm and plastids. However, the amount and distribution of phytic acid in different parts of the seed can be quite variable depending upon the species, with the most striking differences found in cereal grains. A significant and large amount (80%) of phytic acid is stored in the aleurone and bran (maternal teguments) and only a limited amount accumulates in the embryo in the case of barley, wheat, and rice (Sparvoli and Cominelli 2015), although in maize seeds, 80% of phytate accumulates in the embryo and scutellum (O'Dell et al. 1972). In the case of common bean, more than 95% of seed phytic acid is accumulated in the cotyledons (Ariza-Nieto et al. 2007) while as in the model species *Arabidopsis*, it is mostly stored in the embryo (Otegui et al. 2002). Phytate is a key component of numerous developmental and signaling processes and plays a crucial to deoxyribonucleic acid (DNA) repair, in chromatin remodeling, in ribonucleic acid (RNA) editing, and in the regulation of gene expression (Raboy 2009). Also, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) structures are held together with phosphorus bonds, which are important for the synthesis of proteins (Raboy et al. 2001). At the same time, phytates are thought to have an active role as antioxidant and anticancer agents and have other benevolent effects on health as well. Although phytic acid does show some beneficial effects, studies indicated that when it is digested, the structure of the phytate molecule (six reactive phosphate groups) acts as a chelating agent to mineral cations forming salts that are non-digestible, which essentially removes the mineral bioavailability from the seeds (especially Fe and Zn) by forming the more complex phytate molecule (Erdman 1979; Maenz et al. 1999), thereby inhibiting the absorption of these minerals (Raboy 2000). At physiological pH values, the formation of insoluble metal cation–phytate complexes is regarded as the major reason for poor mineral availability because these complexes are essentially non-absorbable from the gastrointestinal tract. Furthermore, phytic acid impedes the activity of enzymes, which are necessary for protein degradation in the small intestine and stomach (Kies et al. 2006). Phytate has probably the greatest effect on iron (Mendoza et al. 2001) and Zn^{2+} (Lopez et al. 2002) status, as there are a large variety of plants with high phytate content, which limits the absorption of these essential minerals. Furthermore, there is a considerable amount of evidence to support the fact that dietary phytate has a negative effect on the bioavailability of dietary zinc in humans, and more often they have a strong effect on infants and pregnant and lactating women, due to consumption of large portions of cereal-based foods (Al Hasan et al. 2016). The higher the amount of phytate present in a particular food crop, the less zinc accumulation would take place in the digestive tract (Hefferon 2019). Basically, the phytic acid is not broken down in the intestinal tract of monogastric animals because they possess little to no phytase activity in their digestive tracts, which is why phytic acid is not efficiently utilized (Brinch-Pedersen et al. 2002). The phytate is a negatively charged structure, which predominantly binds with positively charged metal ions such as zinc, iron, magnesium, and calcium to make complexes and turn down the bioavailability of these ions through lower absorption rates (Samtiya et al. 2020). In addition to it, livestock is fed primarily with grain-based feed, the excreted phytate contributes to environmental P pollution by being washed into surface waters (Lott et al. 2000). Phytates also interact with basic protein residues and can inhibit digestive enzymes such as pepsin, pancreatin, and amylase (Agostini and Ida 2006). The limitations that have been shown in the uptake of minerals especially Iron (Fe) and Zinc (Zn) is a serious concern and therefore, the reduction of phytate concentration in crop plants especially in cereals and pulses is of dire importance as it would serve to enhance the dietary nutrition and also to increase the metabolic energy available to ruminant and non-ruminant livestock by increasing P bioavailability (Shu 2009). A lot of efforts have been made in numerous directions to reduce its antinutritional power (Murgia et al. 2012; Petry et al. 2015). However, according to several authors, the development of either *lpa* (low phytic acid) or biofortified varieties is the most sustainable approach in different species (Dwivedi et al. 2012).

Food protection necessitates a nutritious and healthy food supply. Climate change will cause an increase in temperature and heat waves that is predicted to become more intense, frequent, and longer lasting (Lake et al. 2012), which is expected to cause 95,000 deaths (WHO 2014). As a result, the high temperature will as well have a definite impact on food security by increasing the incidence of drought and crop failure as the majority of farmers rely on rainfall for crop cultivation. Predicted 550 ppm of atmospheric CO_2 in 2050 will reduce protein, iron, and zinc concentration of staple food crops by 3–17% and affect a significant extent, population that relies on vegetal sources for nutrients, mostly rural population in South and East Asia and Sub Saharan Africa. An additional 175 million people will be zinc deficient, 122 million will be protein deficient, and 1.4 billion women of childbearing age and children under the age of five will have a higher than 20% anemia prevalence and lose >4% of their dietary iron. (Smith and Myers 2018). Such smallholder farming communities are extremely at high risk to negative impacts of climate change on their livelihoods and nutritional status, including through reductions in yields and/or nutritional quality of staple crops they both consume and trade (Lloyd et al. 2011; Challinor et al. 2007).

3.2 Impact of Normal Phytate Crops on Humans, Animals, and Environment

3.2.1 Phytic Acid Synthesis and Structure

Phytic acid (PA; myo-inositol [MI] hexaphosphoric acid or IP6) is an important plant constituent of phosphorus which is produced by a series of enzymatic reactions in a fairly complex biosynthetic pathway that converts glucose into myo-inositol and then into myoinositol hexakisphosphate (Raboy 2003). The intermediates (IP3, IP4a, and IP5) formed are less phosphorylated along the way to IP6 (Fig. 3.1) and have similar properties to this final product but are less likely to bind iron and act as antinutrients (Petry et al. 2010). Phytic acid is a major form of phosphorus (P) storage in seeds (up to 85% of total P) and in other plant organs, such as pollen, roots, tubers, and turions (Cominelli et al. 2020).

Among the genes known to control phytate accumulation are those of the de novo phytic acid pathway and a pathway for recycling of phytates. The first type of

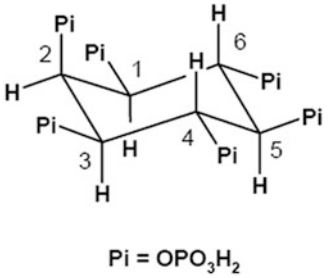

Fig. 3.1 Structure of phytic acid (InsP6) (Chen et al. 2004)

synthesis can be further divided into two parts: the initial part of the pathway produces myo-inositol (Ins(3)P1) which is a cyclic alcohol derivative from glucose 6-P, and the latter part that phosphorylates myoinositol into InsP6. The second pathway for recycling of phytates can be achieved by several alternative means through intermediates such as PtdIns, Ins(3,4,6)P3, Ins(1,4,5)P3, and various InsP4 or InsP5 analogs until InsP6 is finally reached (Raboy 2003). The first step in this pathway [the conversion of glucose 6-P to Ins(3)P] is carried out by myo-inositol (3) P1 synthase (MIPS), considered to be the most important enzyme in the process (Loewus and Murthy 2000; Raboy 2003). After MIPS production, inositol monophosphatase (IMP) and myo-inositol kinase (MIK) are involved in catalyzing the removal or addition of the phosphate group, and various inositol kinases (IPK1, IPK2, and ITPKs) are responsible for further phosphorylation (Raboy and Bowen 2006). Fileppi et al. (2009) has cloned many of the genes in the common bean phytic acid pathway, such as one MIPS and several IPKs, evaluating them through synteny analysis as well as their probable locations in the genome.

3.3 Methods of Assessment of Low Phytic Acid

3.3.1 Phytic Acid Reduction Through Physical Processes

3.3.1.1 Dephytinization and Nutrition

In legumes, phytic acid is found in the cotyledon layer and can be removed prior to consumption (Nissar et al. 2017). However, the removal of phytic acid increases the bioavailability of many cations and thus the nutritional value of the meal. The digestive enzyme phytase can unlock the phosphorus stored as phytic acid, but in the absence of phytase, it can impede the absorption of other minerals like iron, zinc, magnesium, and calcium by binding to them due to chelating property (Hallberg et al. 1989; Reddy et al. 1996; Bohn et al. 2004; Phillippy 2006). This results in highly insoluble salts that are poorly absorbed by the gastrointestinal tract leading to lower bioavailability of minerals.

3.3.1.2 Milling and Soaking

Milling is the most commonly used method to remove phytic acid from grains but also has major disadvantages as it also removes major parts of minerals and dietary fibers. (Gupta et al. 2015). The cereal soaking such as pearl millet with endogenous or exogenous phytase increased in vitro solubility of iron and zinc by 2–23% (Lestienne et al. 2005). The soaking and cooking together has shown much more effective to reduce phytic acid than only soaking for a short duration (Vidal-Valverde et al. 1994). Two varieties of pearl millets were used for evaluating their nutrients, anti-nutrients, and mineral bioavailability after milling them into whole flour, bran-rich segment, and semi-refined flour (Suma and Urooj 2014), and the results of nutrient composition have revealed that the contents of phytate and oxalate were found to be low in semi-refined flour when compared with whole flour. Soaking of grains and beans is quite an effective method for reduction of phytic acid as well as consequent increase in mineral bioavailability (Perlas and Gibson 2002; Coulibaly et al. 2011; Shi et al. 2017). The phytases are present in the grains, so the complete submergence of grains in water for a certain amount of time period results in the activation of endogenous phytases. The soaking at a temperature between 45 °C and 65 °C and pH value between 5 and 6 results in hydrolysis of phytate and significant removal of phytic acid content from grains (Greiner and Konietzny 2006). Similarly, the phytic acid concentration in chickpea was decreased to 47.45 from 55.71% in another study when the soaking time was increased from 2 to 12 h (Ertaş and Türker 2014). However, this treatment also has certain disadvantages as during this treatment there occurs loss of minerals and water-extractable proteins.

3.3.1.3 Fermentation

A process in which carbohydrates are oxidized to release energy in the absence of an external electron acceptor is known as fermentation and it improves the bioavailability of minerals in food grains by lowering the content of anti-nutrients such as phytic acid, polyphenols, and tannins of cereals (Simwaka et al. 2017). The phytic acid is present in cereals in the form of complexes with metal cations, namely, iron, zinc, calcium, and proteins. The fermentation of millet grain for 12 and 24 h could reduce the food inhibitors like phytic acid and tannins (Coulibaly et al. 2011). Natural fermentation can achieve a large reduction in phytic acid in rice flour by the action of microbial as well as grain phytases. Phytases reduce the hexa form of phytic acid (IP6, myo-inositol 1,2,3,4,5,6- hexakisphosphate) into lower forms (IP5, IP4, IP3, IP2, IP1, and myo-inositol) (Ragon et al. 2008). It is worthwhile to mention that the lower forms of phytic acid have a lower binding capacity for metals like iron and zinc (Agte et al. 1997). It has been reported that there is 88.3% reduction in phytate content when germinated pearl millet sprouts were fermented with mixed pure cultures of *Saccharomyces diasticus*, *S. cerevisiae*, *Lactobacillus brevis*, and *L. fermentum* at 30 °C for 72 h (Kaur et al. 2011). It is reported that malting of millet reduces 23.9% phytic acid after 72 h and 45.3% after 96 h (Makokha et al. 2002; Coulibaly et al. 2011). The greatest reduction of phytic acid phosphorus has been found in rye while smallest decrease was found in maize (Poiana et al. 2009).

Additionally, autoclave and microwave treatments decreased phytic acid content as they also increased total mineral content and HCl-extractability of minerals in whole wheat bread. To check the effect of fermentation on anti-nutritional factors, maize flour was fermented with a consortium of lactic acid bacteria by standard method with 12-h intervals (Ogodo et al. 2019), the results revealed that with increasing fermentation period, significant ($p < 0.05$) reductions in antinutrients, including tannin, polyphenol, phytate, and trypsin inhibitor activity. Fermentation has been reported to increase the protein content in chickpea by 13% and decrease the content of phytic acid by 45% (Valdez-González et al. 2018). Similarly, the effects of fermentation on sorghum at 0, 72, and 120 h by Adeyemo et al. (2016) revealed the significant reduction of trypsin inhibitor (69%); protease inhibitor (30%); phytate (60%); and tannin (72%) at 120 h with *L. plantarum* used as a starter culture.

3.3.2 Phytic Acid Reduction Through LPA Mutants

Phytic acid is considered as one of the major antinutritional compounds in cereal and legume seeds. The *low phytic acid* (*lpa*) or "low-phytate" seed trait can provide numerous potential benefits which include enhanced "global" bioavailability of minerals (iron, zinc, calcium, magnesium) for both humans and non-ruminant animals; enhanced phosphorus (P) management contributing to enhanced sustainability in non-ruminant (poultry, swine, and fish) production; reduced environmental impact due to reduced waste P in non-ruminant production and enhancement of quality of animal products, which ultimately would lead to the sustainability of agricultural production, (Raboy 2020). A number of *lpa* mutants have been or are being used in breeding programs after testing under field conditions (Raboy 2002; Liu et al. 2006). This process involves the creation of mutants by kicking out genes involved in phytic acid biosynthesis pathway (Fig. 3.2). The *lpa* mutations can be grouped into three classes depending on the affected step of the biosynthetic pathway or mode of transport: (i) those mutations involved the first steps of the pathway commonly indicated as "supply pathway"; (from glucose 6-P to *myo*-inositol 3-phosphate), (ii) mutations dismaying the end of the pathway (from *myo*-inositol 3-phosphate to InsP6); and (iii) those mutations affecting tissue compartmentation of InsP6 and/or its transport and storage to the vacuole (MRP transporter) (Sparvoli and Cominelli 2015). The mutants ascribed to the first and the third classes are generally characterized by decreased InsP6 levels accompanied by a molar equivalent increase in inorganic Pi (Table 3.1). The DNA sequence of the two LP mutant alleles (*lpa1* and *lpa2*) are known, and perfect molecular markers have been constructed to detect their presence which breeders can use to make selections (Bilyeu et al. 2008) for crop improvement in soybean. From a mutagenized population of common bean, Campion et al. (2009) isolated a homozygous *lpa* mutant line (*lpa*-280-10) which showed a 90% reduction of phytic acid, a 25% reduction of raffinosaccharides, and a much higher amount of free or weakly bound iron cations in the seed compared to wild type. They used Phytic acid phosphate (PAP) fractions for determination of PAP concentration employing a modification approach of the

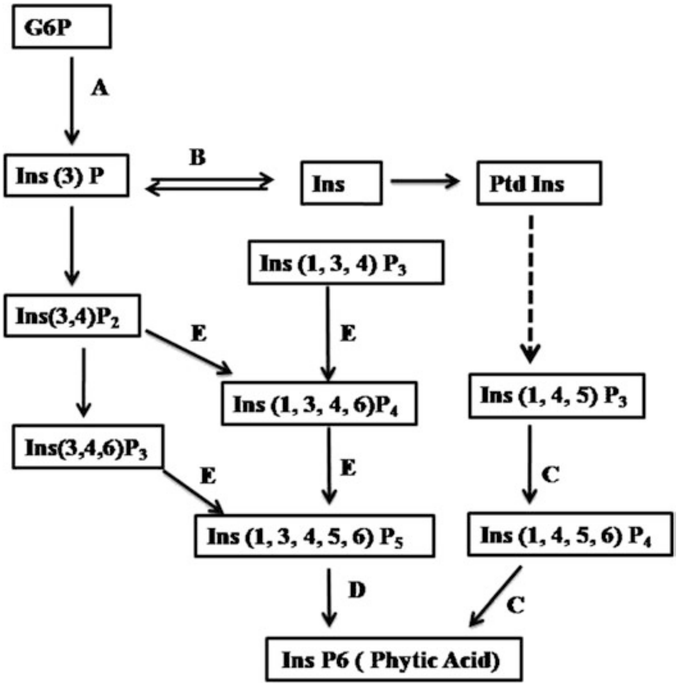

Fig. 3.2 Phytic acid biosynthetic pathway in plants

Table 3.1 Classification of *lpa* mutations on the basis phytic acid synthesis and its functions

Class	Gene function	Effects on the pathway
Type-1	MIPS MIK IMP	Decrease in phytic acid accompanied by a molar increase in free phosphate
Type-2	2PGK IPK2 ITPK IPKI	Decrease in phytic acid accompanied by a low increase in free phosphate and increased content of lower inositol phosphates (InsPs)
Type-3	MRP Putative Sulfate Transporter	Decrease in phytic acid accompanied by a molar increase in free phosphate and/or decrease in phytic acid in specific seed tissues

Sparvoli and Cominelli (2015)

ferric precipitation method (Raboy 1997), as described by Pilu et al. (2003). Furthermore, genetic analysis showed that the *lpa* character is due to a recessive mutation that segregates in a monogenic, Mendelian fashion. The bean line *lpa*-280-10 showed a better germination response than the wild type using varying aging or stress conditions. In tropical adapted maize line (P16), the *lpa1-1* gene was introgressed which resulted in a high percentage of reduction of phytic acid

(66%). The two mutants of maize (*lpa1-1*, *lpa2*) have been mapped onto the maize chromosome map (Raboy 2000). The *lpa1-1* mutants had 1.1 mg/g total inositol P (23% of total P) and 3.1 mg/g Pi (66% of total P) compared to the normal maize of 3.4 mg/g total inositol P (76% of total P) and 0.3 mg/g Pi (7% of total P) (Raboy et al. 2001). Genetically, *lpa1-1* trait was monogenically recessive in nature. The segregation ratios of low phytic plants analyzed by Oltmans et al. (2005) determined that the recessive alleles exhibit duplicate dominant epistasis making it necessary for both recessive alleles to be homozygous before a plant can express the LP trait. Bilyeu et al. (2008) revealed that the DNA sequence of the two LP mutant alleles (*lpa1* and *lpa2*) is known and perfect molecular markers have been constructed to detect their presence which breeders can use to make selections.

A, B, C, D, and E are the genes involved in the phytic acid biosynthesis pathway. **A**.MIPS(myo-inositol 3-phosphate synthase) **B.** IMP(inositol monophosphatase) **C.** IPK2(inositol1,4,5-tris phosphate kinase) **D.** IPK1(inositol 1,3,4,5,6-pentakis phosphate 2-kinase) **E.** ITP5/ 6 K(inositol 1,3,4-trisphosphate 5/6-kinase). G6P:glucose 6-phosphate, Ins:myo-inositol, PtdIns:phosphatidyl inositol (Suzuki et al. 2007).

In Pusa Basmati rice, the TILLING population was developed by Random mutations using Ethyl methanesulfonate (EMS) chemical mutagen agents for generation of low phytate mutant as well as high endogenous phytase (Shukla and Singh 2012). Similarly, RNAi technology has been used to reduce maize phytic acid by silencing MRP4 ATP-binding cassette (ABC) transporter (Shi et al. 2007; Gupta et al. 2011).

3.3.3 SNP and High-Resolution Melt Curve Analysis

The knowledge gained from the analysis of plant genes is beneficial to all aspects of plant research, including crop improvement and the new methods and tools are continually being developed to facilitate rapid and accurate mapping, sequencing, and analyzing of genes. Single-nucleotide polymorphisms (SNPs) are defined as single base pair positions in genomic DNA (detecting a single base change, insertion or deletion of many bases), which occur in all coding and noncoding regions, and are at a higher density in the genome than microsatellites (Batley et al. 2003). Marker-assisted selection (MAS) that entails the use of SNP markers is the most useful tool for the selection of introgression of recessive traits, as the trait is difficult to identify by conventional breeding (Taheri et al. 2017). The SNP markers are highly stable, biallelic, and occur abundantly in genomes and have relatively low mutation rates and are relatively easy to detect. Although developing SNPs detection assays involves a high cost (Batley et al. 2003; Kim and Misra 2007), the advancement in technology leading to specific low-density SNP chips and other affordable technologies, such as HRM, means that SNPs are being used more in plant studies (Gupta et al. 2001; Rafalski 2002; Barker and Edwards 2009). High-resolution melt analysis (HRM) is one of the innovative approaches for the simultaneous detection and differentiation of PCR products after PCR amplification based on their melt profiles in the presence of an appropriate dye or probe and melt analysis can be performed in one closed tube reaction with no post-PCR analyses required (Montgomery et al. 2007). The PCR product has a distinct melting temperature (Tm) that depends on which of the two primers is responsible for the amplification, and genotypes can be determined by inspection of a melting curve in real time. The melting curve is due to the plotting of fluorescence as a function of time as the thermal cycler heats through the dissociation temperature of the product (Ririe et al. 1997). The data of fluorescence is converted into melting curves by plotting the derivative of fluorescence with respect to temperature (dF/dt) over the temperature range in degrees Celsius. Based on the different shapes of the melting curves, the software clusters the test population into different genotype groups. The unique shape and position of the melting curve depends on the amplicon's length, sequence, GC content, and complementarity of DNA strands (Reed et al. 2007). The homozygous genotypes can be distinguished by the shift in melting curves due to the difference in melting temperature (Tm) between the genotypes, while heterozygotes are distinguished by the altered curve shape, not by Tm (Wittwer et al. 2003; Graham et al. 2005; Park et al. 2009). HRM is the most inexpensive, simple, and rapid of these technologies for detecting SNPs (Gundry et al. 2003; Wittwer et al. 2003). The melting process in HRM analysis is faster, and data analysis can be performed automatically in a few minutes (Vossen et al. 2009) and the PCR products can be analyzed without gels and hazardous chemicals, such as ethidium bromide (EB). Furthermore, in HRM analysis, the sensitivity is very high; even a single base-pair difference can be detected (Reed and Wittwer 2004). Because of these benefits and facts, HRM has expanded considerably in many investigations including the detection of SNP mutations (Margraf et al. 2006; Wu et al. 2008), identification of transgenic plants (Akiyama et al. 2009), varietal identification (Mackay et al. 2008), RNA editing (Chateigner-Boutin and Small 2007), and food traceability (Jaakola et al. 2010).

The *lpa-1* gene was first developed and mapped onto chromosome 1 of the distal region in maize (Raboy et al. 2001) and later sequenced (5149 bp) (Shi et al. 2007). The low phytic acid *lpa* in common beans is a monogenic recessive controlled trait (Campion et al. 2009). The co-dominant *lpa* SNP marker is able to detect homozygous dominant, homozygous recessive, and heterozygous genotypes using RT-PCR high-resolution melt (HRM) analysis and differentiating genotypes based on fluorescence change of PCR amplicons saturated with a double strand (Tan et al. 2016). A homozygous *lpa* mutant line (*lpa*-280-10) showed 90% reduction of phytic acid and 25% reduction of raffinosaccharides from a mutagenized population of common bean and was further revealed that the *lpa* character is due to a recessive mutation which segregates in monogenic Mendelian fashion (Campion et al. 2009). Utterly the previously proclaimed soybean *lpa* mutants (LR 33, M156, and M733) had a PA-P reduction of about 50–70% (Wilcox et al. 2000; Hitz et al. 2002).

The three types of low phytic acid mutation, namely, *lpa 1* and *lpa 2* (Raboy et al. 2001; Shi et al. 2003) and *lpa 3* (Shi et al. 2005) have already been identified and characterized in maize. The *lpa 1* type mutants usually have a decreased PAP content, accompanied by mole equivalent increase of Pi (Shukla et al. 2004; Pilu et al. 2005); *lpa 2* type of mutation also results in the reduction of PAP content, but is complemented by an increase of both Pi and lower inositol phosphates and the Pi level in *lpa1* mutants is usually higher than in *lpa2* due to involvement of phosphate kinase gene (Raboy et al. 2001); and *lpa 3* involving the *myo*-inositol kinase (MIK) gene is characterized by increased *myo*-inositol levels and a lack of significant amounts of *myo*-inositol phosphate intermediates in seeds (Shi et al. 2005). Similar rice LPA mutant lines have been identified with SNP markers developed from similar amino acid changes which result in lower phytic acid content (Kim et al. 2008; Xu et al. 2009). The resultant rice mutant *Lpa* N15-186 from mutant allele of the rice myo-inositol kinase (OsMIK) gene is due to a single recessive gene with a single base pair change from the wild type (C to T transition) in the first exon of the gene resulting in a nonsense mutation. This mutant allele shows a 75% reduction in phytic acid P and was mapped to chromosome 3, showing a similar phenotype to maize *lpa3* mutant (Kim et al. 2008).

PA mainly accumulates in rice bran, and chelates mineral cations, and therefore considerable efforts have been focused on the development of low PA (LPA) rice cultivars. Pertaining to this, genetic and molecular analyses of *OsLpa*1 (a major PA biosynthesis Gene) in Sanggol was performed which is a low PA mutant variety developed via chemical mutagenesis of Ilpum rice cultivar (Kishor et al. 2019). The study of Genetic segregation and sequencing analyses has revealed that a recessive allele, *lpa1-3*, at the *OsLpa*1 locus (Os02g0819400) was responsible for a significant reduction in seed PA content in Sanggol, and the *lpa1-3* gene harbored a point mutation (C623T) in the fourth exon of the predicted coding region, resulting in threonine (Thr) to isoleucine (Ile) amino acid substitution at position 208 (Thr208Ile). Two dominant markers each specific to wild type (*LPA1*) and mutant (*lpa1-1*) allele were developed and validated across seven F_2 populations in maize kernel conferring low phytic acid which behaved in a co-dominant fashion in joint segregation using co-dominant CAPS marker analysis (Abhijith et al. 2020). The study revealed that across populations, segregants with *lpa1-1/lpa1-1* (1.77 mg/g) and *lpa2-1/lpa2-1* (1.85 mg/g) possessed significantly lower phytic acid compared to *LPA1/LPA1* (2.58 mg/g) and *LPA2/LPA2* (2.53 mg/g), respectively. The inorganic phosphorus was nonetheless higher in recessive homozygotes (*lpa1-1/lpa1-1*: 0.77 mg/g, *lpa2-1/lpa2-1*: 0.53 mg/g) than the dominant homozygotes (*LPA1/LPA1*: 0.33 mg/g, *LPA2/LPA2*: 0.19 mg/g) and in general, homozygous segregants of *lpa1-1* and *lpa2-1* showed 31% and 27% reduction of phytic acid, respectively.

3.4 Climatic Change and Its Impact on Fe-Zn in Beans

Climate change represents a threat to food security, particularly resulting from ongoing and anticipated negative impacts on agricultural productivity (yields/hectare) (Kang et al. 2009; Rosenzweig et al. 2014; Challinor et al. 2014). Although a number of studies have found negative effects on major staple crop yields (Zhao et al. 2016, 2017), there have been fewer studies on the influence of climate change stresses (e.g., increasing CO_2, heat, drought) on staple crop grain quality parameters

(DaMatta et al. 2010; Zhu et al. 2018). Common beans are the most important grain legume supporting food security and human nutrition globally, responsible for almost 15% of daily calories and 36% of daily protein in some countries in Africa and the Americas (Schmutz et al. 2014). Under potential climate change-induced drought stress scenarios, both nutritional quality and growing area for biofortified beans will be reduced in Africa, according to Hummel et al. (2018). They discovered that under future climate-scenario-relevant drought stress conditions, iron levels in common bean grains are reduced. In contrast, protein, zinc, lead, and phytic acid increase in the beans under such drought stress conditions. In addition to drought-induced reductions in the levels of iron, results demonstrated that the drought-stressed common bean varieties also display increases in the levels of the antinutritional compounds phytic acid and lead, however, and Hart et al. (2015) have shown that they reduce iron and zinc bioavailability and uptake from dietary common bean, but the physiological basis for increases in antinutritional compounds like phytic acid and lead during drought stress is unknown.

The increases in phytic acid are of particular concern as it is considered the main anti-nutritional compound in legumes. The increased phytic acid accumulation in legumes is most likely due to its role in reducing oxidative stress in dry conditions (Irvine and Schell 2001; Kido et al. 2013a, b). Indeed, in response to drought stress, phytic acid is known to accumulate in legume seeds (e.g., chickpeas) (Joshi-Saha and Reddy 2015). It should be noted however that phytic acid in field peas is reduced under elevated CO_2 levels (Myers, et al. 2014). If a similar response occurs in common beans, then the increase in phytic acid levels we observe could be counteracted. Growth trials combining changes in climate and CO_2 simultaneously will be needed to assess interactions between possibly competing effects. According to our findings, ongoing efforts to grow biofortified bean varieties will need to ensure that such varieties retain elevated iron and zinc levels as well as low levels of antinutritional compounds (e.g., phytic acid, lead, and specific inhibitory polyphenols) under drought and other environmental stresses. To avoid unintended consequences, our results highlight that it is critically important that biofortified crop varieties (including under abiotic stresses) do not accumulate anti-nutritionals (e.g., phytic acid, lead, arsenic, polyphenols) 71. Hummel et al. (2018) revealed that there will be a reduction in the nutritional quality of a typical bean serving if the common bean varieties have been cultivated under the levels of drought stress predicted for southeastern Africa to 2050 and beyond.

References

Abhijith KP, Muthusamy V, Chhabra R, Dosad S, Bhatt V, Chand G, Jaiswal SK, Zunjare RU, Vasudev S, Yadava DK, Hossain F (2020) Development and validation of breeder-friendly gene-based markers for lpa1-1 and lpa2-1 genes conferring low phytic acid in maize kernel. 3. Biotech 10:1–2

Adeyemo SM, Onilude AA, Olugbogi DO (2016) Reduction of antinutritional factors of sorghum by lactic acid bacteria isolated from Abacha—an African fermented staple. Front Sci 6:25–30

Agostini JS, Ida EI (2006) Effect of sunflower germination conditions for reducing phytate content and enhancing phytase and acid phospatase activities. Ciências Agrárias 27:61–70. in Portuguese (English abstract)

Agte VV, Gokhale MK, Chiplonkar SA (1997) Effect of natural fermentation on in vitro zinc bioavailability in cereal-legume mixture. Int J Food Sci Tech 31:29–23

Akiyama H, Nakamura F, Yamada C, Nakamura K, Nakajima O, Kawakami, . . .Teshima, R. (2009) A screening method for the detection of the 35S promoter and the nopaline synthase terminator in genetically modified organisms in a real-time multiplex polymerase chain reaction using high-resolution melting-curve analysis. Biol Pharm Bull 32:1824–1829

Al Hasan SM, Hassan M, Saha S, Islam M, Billah M, Islam S (2016) Dietary phytate intake inhibits the bioavailability of iron and calcium in the diets of pregnant women in rural Bangladesh: a cross-sectional study. BMC Nutr 2:24

Ariza-Nieto M, Blair MW, Welch RM, Glahn RP (2007) Screening of iron bioavailability patterns in eight bean (*Phaseolus vulgaris* L.) genotypes using the Caco-2 cell in vitro model. J Agric Food Chem 55:7950–7956

Barker GLA, Edwards KJ (2009) A genome-wide analysis of single nucleotide polymorphism diversity in the world's major cereal crops. Plant Biotechnol J 7:318–325

Batley J, Barker G, O'Sullivan H, Edwards KJ, Edwards D (2003) Mining for single nucleotide polymorphisms and insertions/deletions in maize expressed sequence tag data. Plant Physiol 132:84–91

Bilyeu KD, Zeng P, Coello P, Zhang ZJ, Krishnan HB, Bailey A, Beuselinck PR, Polacco JC (2008) Quantitative conversion of phytate to inorganic phosphorus in soybean seeds expressing a bacterial phytase. Plant Physiol 146:468–477

Bohn T, Davidsson L, Walczyk T, Hurrell RF (2004) Phytic acid added to white-wheat bread inhibits fractional apparent magnesium absorption in humans. Am J Clin Nutri 79:418–423

Brinch-Pedersen H, Sørensen LD, Holm PB (2002) Engineering crop plants: getting a handle on phosphate. Trends Plant Sci 7:118–125

Campion B, Sparvoli F, Doria E, Tagliabue G, Galasso I, Fileppi M, Bollini R, Nielsen E (2009) Isolation and characterisation of an lpa (low phytic acid) mutant in common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 118:1211–1221

Challinor A, Wheeler T, Garforth C, Craufurd P, Kassam A (2007) Assessing the vulnerability of food crop systems in Africa to climate change. Clim Chang 83:381–399

Challinor AJ, Watson J, Lobell DB, Howden SM, Smith DR, Chhetri N (2014) A meta-analysis of crop yield under climate change and adaptation. Nat Clim Chang 4:287–291

Chateigner-Boutin AL, Small I (2007) A rapid high throughput method for the detection and quantification of RNA editing based on high resolution melting of amplicons. Nucleic Acids Res 35:e114

Chen CX, Wang ZL, Yang DE, Ye CJ, Zhao YB, Jin DM, Weng ML, Wang B (2004) Molecular tagging and genetic mapping of the disease resistance gene RppQ to southern corn rust. Theor Appl Genet 108:945–950

Cominelli E, Pilu R, Sparvoli F (2020) Phytic acid and transporters: what can we learn from low phytic acid mutants. Plan Theory 9:69

Coulibaly A, Kouakou B, Chen J (2011) Phytic acid in cereal grains: Healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. Am J plant Nutr Fert Technol 1:1–22

DaMatta FM, Grandis A, Arenque BC, Buckeridge MS (2010) Impacts of climate changes on crop physiology and food quality. Food Res Int 43:1814–1823

Dwivedi SL, Sahrawat KL, Rai KN, Blair MW, Andersson MS, Pfeiffer W (2012) 3 Nutritionally enhanced staple food crops. Plant Breed Rev 36:169

Erdman JW (1979) Oilseed phytates: nutritional implications. J Am Oil Chem Soc 56:736–741

Ertaş N, Türker S (2014) Bulgur processes increase nutrition value: possible role in in-vitro protein digestability, phytic acid, trypsin inhibitor activity and mineral bioavailability. J Food Sci Technol 51:1401–1405

Fileppi M, Galasso I, Tagliabue G, Daminati MG, Campion B, Doria E, Sparvoli F (2009) Characterization of structural genes involved in phytic acid biosynthesis in common bean (*Phaseolus vulgaris* L.). Mol Breed 25:453–470

Graham R, Liew M, Meadows C, Lyon E, Wittwer CT (2005) Distinguishing different DNA heterozygotes by high-resolution melting. Clin Chem 51:1295–1298

Greiner R, Konietzny U (2006) Phytase for food application. Food Technol Biotechnol 44:125–140

Gundry CN, Vandersteen JG, Reed GH, Pryor RJ, Chen J, Wittwer CT (2003) Amplicon melting analysis with labeled primers: a closed-tube method for differentiating homozygotes and heterozygotes. Clin Chem 49:396–406

Gupta PK, Roy JK, Prasad M (2001) Single nucleotide polymorphisms: a new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. Curr Sci 80:524–535

Gupta RK, Singh NK, Sharma S, Shukla KP, Singh V (2011) Role of MicroRNA in crop plant improvement. OIJB 1:14–24

Gupta RK, Gangoliya SS, Singh NK (2015) Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. J Food Sci Technol 52:676–684

Hallberg L, Brune M, Rossander L (1989) Iron-absorption in man ascorbic-acid and dose-dependent inhibition by phytate. Am J Clin Nutr 49:140–144

Hart JJ, Elad Tako E, Kochian LV, Glahn RP (2015) Identification of black bean (*Phaseolus vulgaris* L.) polyphenols that inhibit and promote iron uptake by Caco-2 cells. J Agri Food Chem 63:5950–5956

Hefferon K (2019) Biotechnological approaches for generating zinc-enriched crops to combat malnutrition. Nutrients 11:253

Hitz WD, Carlson TJ, Kerr PS, Sebastian SA (2002) Biochemical and molecular characterisation of a mutation that confers a decreased raffinosaccharide and phytic acid phenotype on soybean seeds. Plant Physiol 128:650–660

Hummel M, Talsma EF, Van der Honing A, Gama AC, Van Vugt D, Brouwer ID et al (2018) Sensory and cultural acceptability tradeoffs with nutritional content of biofortified orange-fleshed sweetpotato varieties among households with children in Malawi. PLoS One 13: e0204754

Irvine RF, Schell MJ (2001) Back in the water: the return of the inositol phosphates. Nat Rev Mol Cell Biol 2:327

Jaakola L, Suokas M, Häggman H (2010) Novel approaches based on DNA barcoding and high-resolution melting of amplicons for authenticity analyses of berry species. Food Chem 123:494–500

Joshi-Saha A, Reddy KS (2015) Repeat length variation in the 5′UTR of myo-inositol monophosphatase gene is related to phytic acid content and contributes to drought tolerance in chickpea (Cicer arietinum L.). J Expt Bot 66:5683–5690

Kang Y, Khan S, Ma X (2009) Climate change impacts on crop yield, crop water productivity and food security—a review. Progress Nat Sci 19:1665–1674

Kaur KD, Jha A, Sabikhi L, Singh AK (2011) Significance of coarse cereals in health and nutrition: a review. J Food Sci Tech 51:1429–1441

Kido EA, Neto JR, Silva RL, Belarmino LC, Neto JP, Soares-Cavalcanti NM, Pandolfi V, Silva MD, Nepomuceno AL, Benko-Iseppon AM (2013a) Expression dynamics and genome distribution of osmoprotectants in soybean: identifying important components to face abiotic stress. BMC Bioinformatics 14:1–1

Kido EA, Neto JRF, Silva RL, Belarmino LC, Neto JPB, Soares-Cavalcanti NM, Pandolfi V, Silva MD, Nepomuceno AL, Benko-Iseppon AM (2013b) Expression dynamics and genome distribution of osmoprotectants in soybean: identifying important components to face abiotic stress. BMC Bioinformatics 14:S7

Kies AK, De Jonge LH, Kemme PA, Jongbloed AW (2006) Interaction between protein, phytate, and microbial phytase. In vitro studies. J Agric Food Chem 54:1753–1758

Kim S, Misra A (2007) SNP genotyping: technologies and biomedical applications. Annu Rev Biomed Eng 9:289–320

Kim SI, Andaya CB, Newman JW, Goyal SS, Tai TH (2008) Isolation and characterisation of a low phytic acid rice mutant reveals a mutation in the rice orthologue of maize MIK. Theor Appl Genet 117:1291–1301

Kishor DS, Lee C, Lee D, Venkatesh J, Seo J, Chin JH, Jin Z, Hong SK, Ham JK, Koh HJ (2019) Novel allelic variant of *Lpa1* gene associated with a significant reduction in seed phytic acid content in rice (*Oryza sativa* L.). PLoS One 14(3):e0209636

Lake IR, Hooper L, Abdelhamid A, Bentham G, Boxall ABA, Draper A, Fairweather-Tait S, Hulme M, Hunter PR, Nichols G, Waldron KW (2012) Climate change and food security: health impacts in developed countries. Environ Health Perspect 120:1520–1526

Lestienne I, Caporiccio B, Besançon P, Rochette I, Trèche S (2005) Relative contribution of phytates, fibers, and tannins to low iron and zinc in vitro solubility in pearl millet (Pennisetum glaucum) flour and grain fractions. J Agric Food Chem 53:8342–8348

Liu ZH, Wang HY, Wang XE, Zhang GP, Chen PD, Liu DJ (2006) Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron, and zinc contents in wheat (Triticum aestivum L.). J Cereal Sci 44(2):212–219. https://doi.org/10.1016/j.jcs.2006.06.001

Lloyd SJ, Kovats RS, Chalabi Z (2011) Climate change, crop yields, and undernutrition: development of a model to quantify the impact of climate scenarios on child undernutrition. Environ Health Perspect 119:1817–1823

Loewus FA, Murthy PPN (2000) Myo-inositol metabolism in plants. Plant Sci 150:1–19

Lopez HW, Leenhardt F, Coudray C, Remesy C (2002) Minerals and phytic acid interactions: is it a real problem for human nutrition? Int J Food Sci Technol 37:727–739

Lott JN, Ockenden I, Raboy V, Batten GD Phytic acid and phosphorus in crop seeds and fruits: a global estimate. Seed Sci Res 2000 10:11–33

Mackay JF, Wright CD, Bonfiglioli RG (2008) A new approach to varietal identification in plants by microsatellite high resolution melting analysis: application to the verification of grapevine and olive cultivars. Plant Methods 4:8

Maenz DD, Engele-Schaan CM, Newkirk RW, Classen HL (1999) The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal. Anim Feed Sci Technol 81:177–192

Makokha AO, Oniango RK, Njoroge SM, Kamar OK (2002) Effect of traditional fermentation and malting on phytic acid and mineral availability from sorghum (Sorghum bicolor) and funger millet (Eleusine caracana) grain varieties grown in Kenya. Food Nutr Bull 23:241–245

Margraf RL, Mao R, Wittwer CT (2006) Masking selected sequence variation by incorporating mismatches into melting analysis probes. Hum Mutat 27:269–278

Mendoza C, Viteri FE, Lönnerdal B, Raboy V, Young KA, Brown KH (2001) Absorption of iron from unmodified maize and genetically altered, low-phytate maize fortified with ferrous sulfate or sodium iron EDTA. Am J Clin Nutr 73:80–85

Montgomery J, Wittwer CT, Palais R, Zhou L (2007) Simultaneous mutation scanning and genotyping by high-resolution DNA melting analysis. Nat Protoc 2:59–66

Murgia I, Arosio P, Tarantino D, Soave C (2012) Biofortification for combating 'hidden hunger' for iron. Trends Plant Sci 17:47–55

Myers SS, Zanobetti A, Kloog I, Huybers P, Leakey AD, Bloom AJ, Carlisle E, Dietterich LH, Fitzgerald G, Hasegawa T, Holbrook NM, Nelson RL, Ottman MJ, Raboy V, Sakai H, Sartor KA, Schwartz J, Seneweera S, Tausz M, Usui Y (2014) Increasing CO_2 threatens human nutrition. Nature 510:139–142

Nissar J, Ahad T, Naik HR, Hussain SZ (2017) A review phytic acid: as antinutrient or nutraceutical. J Pharmacogn Phytochem 6:1554–1560

O'Dell BL, De Boland AR, Koirtyohann SR (1972) Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. J Agric Food Chem 20:718–723

Ogodo AC, Agwaranze DI, Aliba NV, Kalu AC, Nwaneri CB (2019) Fermentation by lactic acid bacteria consortium and its effect on anti-nutritional factors in maize flour. J Biol Sci 19:17–23

Oltmans SE, Fehr WR, Welke GA, Raboy V, Peterson KL (2005) Agronomic and seed traits of soybean lines with low-phytatephosphorus. Crop Sci 45:593–598. (8) (PDF) Agronomic Performance of Low Phytic Acid Wheat. Available from: https://www.researchgate.net/publication/228473890_Agronomic_Performance_of_Low_Phytic_Acid_Wheat. Accessed 2 Apr 2022

Otegui M, Capp R, Staehelin L (2002) Developing seeds of *Arabidopsis* store different minerals in two types of vacuoles and in the endoplasmic reticulum. Plant Cell 14:1311–1327

Park S-W, An S-J, Yang H-B, Kwon J-K, Kang B-C (2009) Optimization of high resolution melting analysis and discovery of single nucleotide polymorphism in *Capsicum*. Hort Environ Biotechnol 50:31–39

Perlas LA, Gibson RS (2002) Use of soaking to enhance the bioavailability of iron and zinc from rice-based complementary foods used in the Philippines. J Sci Food Agric 82:1115–1121

Petry N, Egli I, Zeder C, Walczyk T, Hurrell R (2010) Polyphenols and phytic acid contribute to the low iron bioavailability from common beans in young women. J Nutr 22:1977–1982

Petry N, Boy E, Wirth JP, Hurrell RF (2015) Review: the potential of the common bean (*Phaseolus vulgaris*) as a vehicle for iron biofortification. nutrients 7:1144–1173

Phillippy BQ (2006) Transport of calcium across Caco-2 cells in the presence of inositol hexakisphosphate. Nutr Res 26:146–149

Pilu R, Panzeri D, Gavazzi G, Rasmussen SK, Consonni G, Nielsen E (2003) Phenotypic, genetic and molecular characterization of a maize low phytic acid mutant (*lpa241*). Theor Appl Genet 107:980–987

Pilu R, Landoni M, Cassani E, Doria E, Nielsen E (2005) The maize *lpa241* mutation causes a remarkable variability of expression and some pleiotropic effects. Crop Sci 45:2096–2105

Poiana MA, Alexa E, Bragea M (2009) Studies concerning the phosphorus bioavailability improvement of some cereals used in nourishment. Roumanian Biotechnol Lett 14:4467–4473

Raboy V (1997) The genetics of seed storage phosphorus pathways. In: Lynch JP, Deikman J (eds) Phosphorus in plant biology: regulatory roles in molecular, cellular, organismic, and ecosystem processes. American Society of Plant Physiologists, Rockville, MD, pp 192–203

Raboy V (2000) Low-phytic-acid grains. Food Nutr Bull 21:423–427

Raboy V (2002) Progress in breeding low phytate crops. In: Symposium: plant breeding: a new tool for fighting micronutrient malnutrition, experimental biology 2001. Orlando, Florida

Raboy V (2003) myo-Inositol-1,2,3,4,5,6-hexakisphosphate. Phytochemistry 64:1033–1043

Raboy V (2009) Approaches and challenges to engineering seed phytate and total phosphorus. Plant Sci 177:281–296

Raboy V (2020) *Low phytic acid* Crops: Observations Based On Four Decades of Research. Plan Theory 9:140

Raboy V, Dickinson DB (1984) Effect of phosphorus and zinc nutrition on soybean seed phytic acid and zinc. Plant Physiol 75:1094–1098

Raboy V, Bowen D (2006) Genetics of inositol polyphosphates. In: Lahiri Majumder A, Biswas BB (eds) Biology of inositols and phosphoinositides, pp 71–102

Raboy V, Gerbasi PF, Young KA, Stoneberg SD, Pickett SG, Bauman AT, Murthy PP, Sheridan WF, Ertl DS (2000) Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. Plant Physiol 124:355–368

Raboy V, Gerbasi PF, Young KA, Dooch JA, Cook A (2001) Genetics and breeding of seed phosphorus and phytic acid. J Plant Physiol 158:489–497. https://doi.org/10.1078/0176-1617-00361

Rafalski JA (2002) Novel genetic mapping tools in plants:SNPs and LD-based approaches. Plant Sci 162:329–333

Ragon M, Aumelas A, Chemardin P, Santiago S, Moulin G, Boze H (2008) Complete hydrolysis of myo-inositol hexakisphosphate by a novel phytase from Debaryomyces castellii CBS 2923. Appl Microbiol Biotechnol 78:47–53

Reddy MB, Hurrell RF, Juillerat MA, Cook JD (1996) The influence of different protein sources on phytate inhibition of nonheme-iron absorption in humans. A J Clin Nutr 63:203–207

Reed GH, Wittwer CT (2004) Sensitivity and specificity of single-nucleotide polymorphism scanning by high-resolution melting analysis. Clin Chem 50:1748–1754

Reed GH, Kent JO, Wittwer CT (2007) High-resolution DNA melting analysis for simple and efficient molecular diagnostics. Pharmacogenomics 8:597–608

Ririe KM, Rasmussen RP, Wittwer CT (1997) Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. Anal Biochem 245:154–160

Rosenzweig C, Elliott J, Deryng D, Ruane AC, Müller C, Arneth A, Boote KJ, Folberth C, Glotter M, Khabarov N, Neumann K (2014) Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. Proc Nat Acad Sci 111:3268–3273

Samtiya M, Aluko RE, Dhewa T (2020) Plant food anti-nutritional factors and their reduction strategies: an overview. Food Prod Process Nutr 2(6):14

Schmutz J, McClean PE, Mamidi S, Wu GA, Cannon SB, Grimwood J, Jenkins J, Shu S, Song Q, Chavarro C, Torres-Torres M et al (2014) A reference genome for common bean and genome-wide analysis of dual domestications. Nat Genet 46:707–713

Shi JR, Wang HY, Wu YS, Hazebroek J, Meeley RB, Ertl DS (2003) The maize low-phytic acid mutant *lpa2* is caused by mutation in an inositol phosphate kinase gene. Plant Physiol 131:507–515

Shi J, Wang H, Hazebroek J, Ertl DS, Harp T (2005) The maize low-phytic acid 3 encodes a myo-inositol kinase that plays a role in phytic acid biosynthesis in developing seeds. Plant J 42: 708–719

Shi J, Wang H, Schellin K, Li B, Faller M, Stoop JM, Meeley RB, Ertl DS, Ranch JP, Glassman K (2007) Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. Nat Biotechnol 25:930–937

Shi L, Mu K, Arntfield SD, Nickerson MT (2017) Changes in levels of enzyme inhibitors during soaking and cooking for pulses available in Canada. J Food Sci Technol 54(4):1014–1022

Shu QY (2009) Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome, pp 316–318

Shukla A, Singh NK (2012) Development and characterization of Indian Indam rice TILLING population and identification of mutants having low phytic acid content by endogenous phytase activity determination. Proc World Congress Biotechnol, Hyderabad:4–6

Shukla S, VanToai TT, Pratt RC (2004) Expression and nucleotide sequence of an INS (3) P1 synthase gene associated with low-phytate kernels in maize (*Zea mays* L.). J Agric Food Chem 52:4565–4570

Simwaka JE, Chamba MV, Huiming Z, Masamba KG, Luo Y (2017) Effect of fermentation on physicochemical and antinutritional factors of complementary foods from millet, sorghum, pumpkin and amaranth seed flours. Int Food Res J 24(5)

Smith MR, Myers SS (2018) Impact of anthropogenic CO_2 emissions on global human nutrition. Nat Clim Chang 8:834–839. https://doi.org/10.1038/s41558-018-0253-3

Sparvoli F, Cominelli E (2015) Seed biofortification and phytic acid reduction: a conflict of interest for the plant. Plan Theory 4:728–755

Suma PF, Urooj A (2014) Nutrients, antinutrients & bioaccessible mineral content (invitro) of pearl millet as influenced by milling. J Food Sci Technol 51:756–761

Suzuki M, Tanaka K, Kuwano M, Yoshida KT (2007) Expression pattern of inositol phosphate-related enzymes in rice (*Oryza sativa* L.): implications for the phytic acid biosynthetic pathway. Gene 405:55–64

Taheri S, Lee Abdullah T, Jain SM et al (2017) TILLING, high-resolution melting (HRM), and next-generation sequencing (NGS) techniques in plant mutation breeding. Mol Breed 37:40. https://doi.org/10.1007/s11032-017-0643-7

Tan W, Proudfoot C, Lillico SG, Bruce CA, White l. (2016) Gene targeting, genome editing: from Dolly to editors. Transgenic Res 2016(25):273–287

UNICEF (2019) UNICEF estimates, 2019 based on Black *et al*, 2013 and Stevens *et al.* (2015). See Methodology on p. 178 for more information

Valdez-González FJ, Gutiérrez-Dorado R, García-Ulloa M, Cuevas- Rodríguez BL, Rodríguez-González H (2018) Effect of fermented, hardened, and dehulled of chickpea (*Cicer arietinum*) meals in digestibility and antinutrients in diets for tilapia (*Oreochromis niloticus*). Spanish J Agric Res 16(1):e0605

Vidal-Valverde C, Frias J, Estrella I, Gorospe MJ, Ruiz R, Bacon J (1994) Effect of processing on some antinutritional factors of lentils. J Agric Food Chem 42:2291–2295

Vossen RHAM, Aten E, Roos A, den Dunnen JT (2009) High resolution melting analysis (HRMA)-more than justsequence variant screening. Hum Mutat 30:860–866

Wessels KR, Brown KH (2012) Estimating the global prevalence of Zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. PLoS One 7: E50568

WHO (2013) Micronutrient deficiencies. Retrieved May, 2018

WHO (2014) World Health Statistics. WHO Library Cataloguing-in-Publication Data

Wilcox JR, Premachandra GS, Young KA, Raboy V (2000) Isolation of high seed inorganic P, low-phytate soybean mutants. Crop Sci 40:1601–1605

Wittwer CT, Reed GH, Gundry CN, Vandersteen JG, Pryor RJ (2003) High-resolution genotyping by amplicon melting analysis using LCGreen. Clini Chem 49:853–860

Wu SB, Wirthensohn MG, Hunt P, Gibson JP, Sedgley M (2008) High resolution melting analysis of almond SNPs derived from ESTs. Theor Appl Genet 118:1–4

Xu XH, Zhao HJ, Liu QL, Frank T, Engel KH, An G, Shu QY (2009) Mutations of the multi-drug resistance-associated protein ABC transporter gene 5 result in reduction of phytic acid in rice seeds. Theor Appl Genet 119:75–83

Zhao C, Piao S, Wang X, Huang Y, Ciais P, Elliott J, Huang M, Janssens IA, Li T, Lian X, Liu Y (2016) Plausible rice yield losses under future climate warming. Nat Plants 3:1–5

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, Durand JL (2017) Temperature increase reduces global yields of major crops in four independent estimates. Proc Nat Acad Sci 114:9326–9331

Zhu C, Kobayashi K, Loladze I, Zhu J, Jiang Q, Xu X, Liu G, Seneweera S, Ebi KL, Drewnowski A, Fukagawa NK (2018) Carbon dioxide (CO_2) levels this century will alter the protein, micronutrients, and vitamin content of rice grains with potential health consequences for the poorest rice-dependent countries. Sci Adv 4:eaaq1012

Developing Climate-Resilient Cowpea (*Vigna unguiculata* [L.]Walp.) Through Genomics-Assisted Breeding Approaches

4

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Abstract

Cowpea (*Vigna unguiculata* [L].Walp) is an important multifunctional crop of the tropics and subtropics where it constitutes the main source of protein and minerals of the vegetarian diet. It is primarily grown on marginal lands with low inputs, thereby being exposed to various abiotic and biotic stresses, leading to substantial yield losses. The changing climate due to global warming is expected to further increase the yield loss in near future. The drastic change in climate has compelled the plant breeders to develop climate-resilient cowpea, which can withstand abiotic stresses along with new emerging insect pests and pathogens. Unlocking the repository of genetic diversity of cowpea and its wild relatives and their efficient utilization in climate-resilient cowpea pre-breeding programs is imperative now. Recent advances in genomics along with high-throughput phenotyping and genotyping platforms have been exploited toward identifying underlying genes/QTLs for climate change relevant traits in cowpea. Genomics-assisted breeding approaches such as marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genomic selection (GS) have proven helpful in developing climate-resilient cowpea. Moreover, genome editing tools can further accelerate the improvement of cowpea for climate change adaptive traits. This chapter discusses the progress and prospects of various genomics-assisted breeding approaches in improving climate resilience and developing better-adapted cowpea.

Keywords

Cowpea · Genetic resource · Molecular marker · Climate resilience · QTL

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4.1 Introduction

Cowpea, *Vigna unguiculata* [L.] Walp., is an important food legume and a versatile crop cultivated between 35° N and 30° S of the equator, covering Asia and Oceania, the Middle East, southern Europe, Africa, southern United States, and central and south America. It belongs to the family Fabaceae, tribe Phaseoleae, genus *Vigna* and section Catjang (Verdcourt 1970) and the chromosome number is $2n = 2x = 22$. Padulosi and Ng (1997) reported Southern Africa as the center of origin for wild cowpea. The highest genetic diversity along with primitive forms of wild *V. unguiculata* is concentrated in the region from Namibia to Mozambique and Zambia to Cape Agulhas. *V. unguiculata*, *V. stenophylla*, *V. dekindtiana*, and *V. tenuis* are the four subspecies of which *V. dekindtiana* is the cultivated species while others are wild relatives. Cultivated cowpea is categorized into four groups, namely, *Biflora*, *Sesquipedalis*, *Textilis*, and *Unguiculata* (Ng and Marechal 1985). This crop is emerging as an important fodder crop grown during summer and rainy season in sole and mixed cropping systems as it is high in protein, vitamins, and minerals which makes it a key crop for food and nutritional security. The annual global production of cowpea is over seven million tonnes from an area of 14.5 million hectares (Singh 2014). As per one estimate, about 6.5 lakh hectare area is under different forms of cowpea and in India cowpea fodder share is 3 lakh ha. *V. unguiculata* var. *spontanea* (formerly var. *dekindtiana*) is believed to be the progenitor of cowpea and it has been found in all low-lying areas of sub-Saharan Africa (SSA) (Boukar et al. 2013).

Cowpea is a drought-tolerant annual crop grown in conditions where moisture deficiency has less effect on seed formation. It grows with less rainfall and under more adverse conditions than the haricot bean. Like other leguminous crops, it is also grown for improving soil fertility (Tesema and Eshetayehu 2006). Cowpea withstands heat better than most other legumes. Cowpea can be grown in a wide variety of soils but yields better on well-drained soils with medium fertility (Arnon 1972). It provides nutritious fodder for livestock and is used as feed, forage, hay, and silage for livestock, and green manure or maintaining the productivity of soils. The young leaves, green pods, and green seeds are used as vegetables and dry seeds are used in various food preparations (Singh et al. 2003). It is also grown as livestock feed, green manure, and as an environment-friendly crop (water-efficient and low carbon footprint food).

Fodder cowpea is a legume genetically tolerant to drought than other fodder legumes and provides nutritionally rich seasonal fodder to sustain livestock productivity. In rain-deficit regions and relatively infertile soils, cowpea is widely cultivated as a fodder crop. Most of the livestock owners grow fodder cowpea as an intercrop with other crops and it forms an integral component of crop livestock farming system (Singh and Tarawali 2011). Samireddypalle et al. (2017) have stressed for multidimensional improvement of cowpea for mixed crop livestock systems in Africa. It is a fast-growing leguminous fodder of Kharif season yielding 90–120 qt fodder/acre. It can be grown as a sole crop as well as a mixed crop with sorghum, maize, pearl millet, or other grasses and is usually ready for feeding after 2 months of growth.

It is the most important source of nutrition, food, and fodder in West Africa (Singh 2007) with about 23–35% protein in its grains, 62% soluble carbohydrates, and small amounts of other nutrients (Steele 1972), and relatively free from anti-nutritive factors (Kay 1979). The crude protein content in the leaves ranges from 22% to 30% on a dry weight basis (in comparison to alfalfa has 18–20% protein) and from 13% to 17% in the haulms with high digestibility and low fiber level. Controlled sheep feeding experiments have shown that feeding 200–400 g per day of haulms as a supplement to a basal diet of sorghum stover had 100% higher average live weight gain compared to animals fed with sorghum fodder alone (Singh et al. 2003). Cowpea is an important source of qualitative nourishment to the urban and rural poor who cannot afford protein food such as fish, meat, and milk products. It is estimated that cowpea supplies 40% of the daily protein requirement to most people in Nigeria. Cowpea is also cultivated for their leafy vegetables which are a good source of minerals including iron, calcium, phosphorus, and rice (Kay 1979), green pods for consumption, and green manure when plowed into the soil (Singh et al. 1990). Cowpea haulms contain 15% protein and constitute a valuable source of fodder. Owing to its excellent quality, this important pulse crop is predominantly grown in many parts of the tropics, especially in Africa and Asia (Tripathi et al. 2019).

When intercropped with cereals, it compensates for the loss of nitrogen absorbed by cereals through nitrogen fixation. It is also a good cover crop that limits soil erosion (Onwueme and Sinha 1991). Cowpea has the unique ability to fix nitrogen even in very poor soil (PH range 4.5–9.0, organic matter 85%). Due to its shade tolerance, it is compatible as an intercrop with a number of cereals and root crops as well as with cotton, sugarcane, and several plantation crops. Coupled with these attributes, its quick growth and rapid ground cover have made cowpea an essential component of subsistence agriculture in marginal lands and drier regions of the tropics, where rainfall is scanty and soil is sandy with little organic matter.

4.2 Impact of Climate Change on Cowpea Production

Global warming and global climate change have affected water distribution, daily temperatures, salinity patterns, and nutrient availability in soil, which have greatly affected crop growth and caused more than 50% of worldwide losses in the yields of major crops every year (Gusta 2012; Verma and Deepti 2016). Since global food demand is expected to approximately double by the 2050s (Tilman et al. 2011), increasing crop production to meet rising demands against the threats of global warming and global climate change remains a challenge in agriculture. Cowpea is an important multipurpose leguminous crop that has been a part of the human diet since ancient times. Besides being an excellent source of nutrition, cowpea is resilient to abiotic stresses induced due to climate change. It is superior to other competitive legumes with respect to both nutritional and agronomical performance. Considering the adverse impact of abiotic and biotic stresses on cowpea production in India and to ensure food and nutritional security to the millions of the poor residing in the

Table 4.1 Production and yield losses due to fungal pathogens and associated diseases in cowpea

Disease	Causal fungi	Distribution	Yield loss
1. Seed and seedling diseases			
Seed decay and seedling mortality	*Pythium aphanidermatum, Rhizoctonia solani, Phytophthora* spp., *Pythium ultimum*	Widespread. South Africa.	Locally and seasonally damaging and up to 75% incidence in Nigeria. Locally important
2. Stem, collar, and root diseases			
Anthracnose	*Colletotrichum lindemuthianum, Colletotrichum dematium*	Widespread. Recorded from east and west Africa and Brazil.	Locally important and losses up to 50% in Nigeria.
3. Foliar diseases			
Cercospora leaf spot	*Cercospora cruenta*	Widespread.	Major. Yield losses of 20–40%.
Septoria leaf spot	*Septoria ignae, Septoria ignicola*	Widespread. Reported from east and west Africa, Brazil, and India.	Probably important in savannahs of Africa
Ascochyta blight leaf spot	*Ascochyta phaseolorum, Ascochyta boltshauseri*	Widespread in Africa and Central America. Reported recently from India.	Major. Causes severe losses under cooler conditions.
Web blight	*Rhizoctonia solani*	Worldwide.	Major. Devastating under hot, wet conditions.
4. Pod diseases			
Scab (also a foliar and stem disease)	*Sphaceloma* sp.	Widespread in tropical Africa.	Major. very damaging pathogen in savannah areas.
Brown blotch Locally important in the African savannahs.	*Colletotrichum capsici, Colletotrichum truncatum*	Recognized only from Nigeria, Upper olta, Cameroon, Kenya and Zambia.	Locally important in the African savannahs.

Source: Aveling (1999)

developing world, it is imperative to search for new genotypes under changing climate. The global cowpea collections still have considerable genetic diversity to mine within and the diverse germplasm stored in genebanks are a vital source of desirable traits. Adequate phenotyping for discovering useful genes that may aid in the cowpea improvement program is essential.

Cowpea suffers from a wide range of natural enemies that attack the crop at all stages of growth *viz*. insect pests, bacterial diseases, fungi, viruses, and nematodes (Table 4.1 and 4.2). Among these, insects cause devastating losses in cowpea yields followed by weevils; other post-harvest pests can destroy a granary full of cowpeas

Table 4.2 Production and yield losses due to insect pests in cowpea

Stress	Causal organism	Damages caused	References
Insect pests			
Aphids	*Aphis craccivora*	Attack seedlings and devastating after drought growing points of the host plant, including tips, flowers, and developing bean pods. Feeds on plant sap thus causing malformation, stunting, and even drying up of the parts. Yield losses due to the aphids were estimated between 13 to 100%.	Abdalla (1992), Pathak (1988), Blackman and Eastop (1984), Shoyinka et al. (1997)
Foliage beetle	*Ootecha* spp.	Foliage feeder on cowpea seedlings Defoliate and dead cowpea seedling occurs when the population of this beetle is high.	Singh and van Emden (1979)
	Megalurothrips sjostedti	Flower buds	
Blister beetle	*Mylabris* spp.	Flower feeder, the average percent damage of cowpea flower was estimated as 37–60%. the average percent of damage on cowpea leaves was determined approximately as 13.5%	Durairaj (2000), Maheshwari (1986)
Pod borer	*Marucavitrata* Fabricius	The larval stage is the damaging stage, attacking flower buds, flowers, and young pods. Up to 80% cowpea yield losses are recorded	Dannon (2011)
Weevils	*Callosobruchus maculatus*	Attack seeds and bore holes	
Flower thrips	*Megalurothrips sjostedti* Trybom	Damages are related to adult ovipositing in the plant tissues especially on the flower buds. This pest causes 20–100% yield losses under severe infestation.	Hall (2003) Karungi et al. (2000)
Pod-sucking bugs	*Clavigralla tomentosicollis* stal. *Anoplocnemis curvipes* Fabricus	Post-flowering insect species of cowpea are in cowpea fields only at the podding stage. Females of this pod-bug lay eggs on different parts of the cowpea plant (young leaves, pods, and	Singh and Jackai (1985) Koona et al. (2001) Oiewalw and Bamaiyi (2013)

(continued)

Table 4.2 (continued)

Stress	Causal organism	Damages caused	References
		tender branches), and nymphs complete development within 12 days on the crop. The total life cycle takes 25–30 days. Yield losses vary from 30 to 90%.	
Green leafhopper	*Empoasca kerri, E. dolichi*, *E. kraemeri*	*Empoasca* spp. infests cowpeas at the seedling stage. They attack the leaves. When infestation is very severe, up to 60% of the yield may be lost (when the plants are attacked between the 25th and 55th days after germination just before flowering.	de Moraes et al. (1980)
cowpea seed moth	*Cydiaptychora* Meyrick	The first instar larvae enter the pod and feed on the seeds remaining inside the pod. Maximum percent infestation of pods and damaged grain is about 56%.	Singh and Allen (1979), Ausha (2013)
Gram pod borer	*Helicoverpa armigera* Hubner	In the early stage of the plant, larvae feed on leaves and cause defoliation also later stages of larvae enter the pods and feed on seeds. There is no accurate information about the damage rate of these pests on cowpea.	Wubneh (2016)
Cowpea weevil	*Callosobruchus maculatus*	A storage pest of global importance and *C. chinensis*(L.) is a minor cowpea storage pest. Losses of up to 30–90% have been recorded on stored cowpea in the absence of insect pest control measures. Severe infestation can lead to grain losses of about 50–60% within 6 months in storage, but losses can be over 90%.	Caswel (1981), Gomez (2004), Oluwafemi (2012)
Bruchid beetle	*Acanthoscelides obtectus* Say and *Bruchidius atrolineatus*	Symptom of damages by these beetles is same to *C. maculates*. About 80–100% of the grains can be destroyed by three species of bruchids in a period of two or three months after the storage.	Koona and Bouda (2006) NDoutoume-Ndong and Rojas-Rousse (2008)

within 2 or 3 months. Annual losses are caused by Bacterial blight (*Xanthomonas vignicola*) and Aphid-borne mosaic virus (CabMV) amounting to yield losses (10–100%). Also crop failure occurs due to the activities of a wide range of insect pests that attack cowpea crops in the field at different growth stages (Singh and Van Emden 1979). These pests can severely reduce the yield of cowpea plants or the stored grain. Losses of up to 30–90% have been recorded on stored cowpea in the absence of insect pest control measures (Gomez 2004).

Cowpea is highly vulnerable to drought. During a devastating drought, aphids attack seedlings growing points of the host plant, including tips, flowers, and developing bean pods. They feed on plant sap, causing malformation, stunting, and even drying up of the parts, and cause severe yield losses. Resistance to drought is confounded by root health and vigor and with resistance to soil-borne root rot pathogens. Early maturing cowpea varieties that escape terminal drought have been developed (Fatokun et al. 2012). Cowpea yield can also be affected considerably by heat in sensitive varieties. When the night temperature reaches about 35 °C, cowpea flowers abort due to poor pollen development, which can result in poor seed and pod sets (Hall 1993).

4.3 Harnessing Genetic Variability to Mitigate Negative Effects of Climate Change

It is well known that wild relatives provide important sources of genetic variation for crop improvement. However, their exploitation is limited by different sexual incongruity and linkage drag (Wang et al. 2017). Many pests and diseases affect cowpea, resulting in partial to total crop loss. Management practices such as crop rotation, maintenance of soil fertility, and chemical applications can alleviate pests and diseases, but improved genetic resistance is imperative to sustainable intensification of production. Fortunately, resistance to many pests (aphids, root-knot nematodes, foliar thrips, and *Striga*) and diseases (*Fusarium* wilt, bacterial blight, *Macrophomina* disease, and viruses) have been identified in diverse cowpea accessions. *Vigna vexillata* is one such wild cowpea relative with resistance to pod sucking bugs and bruchids (Birch et al. 1986). A strong incompatibility barrier, however, exists between cowpea and *V. vexillata* (Fatokun 2002) which has prevented transferring the desirable genes from the latter to cultivated cowpea through conventional breeding. Some wild cowpea relatives are cross-compatible with cultivated types but have hardly been used in developing improved varieties. Cowpeas recover well from early-season drought, but drought during flowering and pod-filling has lasting negative impacts on yield and quality even after re-watering. Drought tolerance is a complex trait, but many components are understood. Early maturation and other traits, like photoperiod-insensitivity, confer drought tolerance because they give farmers the flexibility to plant when rain is most likely to coincide with flowering. Extra-early maturation (45–50 days after planting) identified in a landrace has recently been introgressed into a medium maturing Ghanaian variety.

Cowpea's tremendous genetic diversity is captured in germplasm collections worldwide, and new sources of alleles are continually being identified and genetically characterized. The era of big data has arrived for cowpea breeding, along with the need to archive, query, and share data. Institutes, like IITA, and other international collaborations are using the Breeding Management System to record and share cowpea breeding data in a unified format. Continued characterization of cowpea diversity and knowledge sharing are critical to fully harnessing available genetic diversity and maximizing cowpea genetic gain for food security. Although genomic resources for cowpea still lag behind as compared with similar crops, a number of cowpea genetic linkage maps and QTLs associated with desirable traits such as resistance/tolerance to *Striga,* drought, *Macrophomina, Fusarium* wilt, bacterial blight, root-knot nematodes, aphids, and foliar thrips have been reported. Several national and international cowpea breeding programs are exploiting the developed genomics resources to some extent to implement molecular breeding for abiotic and biotic traits, especially by MABC, MARS, and GWAS to accelerate cowpea improvement. Further, the construction of introgression libraries (ILs) and advanced backcross QTL (AB-QTL) analysis has been suggested for transferring superior alleles from wild species to cultivated lines. With an initiative of the Global Crop Diversity Trust project in cowpea, the use of wild cowpea accessions in cowpea breeding program to introgress genes for drought tolerance into cultivated cowpea lines was initiated. In the future, the AB-QTL approach may be employed to introgress genes for drought tolerance in cowpea improvement efforts.

4.4 High-Throughput Precision Phenotyping Platforms for Climate Change Relevant Traits

High-throughput precision phenotyping has gathered major attention in recent years leading to the development of several new protocols for recording various plant traits of interest. Phenotyping of plants for breeding and for precision agriculture has different requirements due to different sizes of the plots and fields, differing purposes, and the urgency of the action required after phenotyping. Plant phenotyping is still the bottleneck as the development of techniques for the precise and accurate recording of important agronomical traits and crop monitoring are lagging behind. Further advances in phenotyping techniques are therefore required for improving the selection efficiency of the breeding programs, accelerating genetic gains and for automated monitoring of plant health status to reduce qualitative and quantitative losses during crop production. Cost efficiency must be considered for the phenotyping techniques to be accepted by both breeders and farmers. High-throughput field phenotyping is currently mainly done for monitoring crops for fertilizer requirement and weed detection in crop cultivation. Future detection of pathogens and pests holds great promise to revolutionize precision agriculture. The method of phenotyping may also differ based on the crop, trait, developmental stages, and the resources available. Development of phenotyping tools and methods for both proximal and remote sensing accelerates screening and selection of germplasm. HTPP can enable screening of a larger number of samples with higher accuracy and reduced costs, thereby improving the selection intensity and accuracy. HTPP can also enable the evaluation of traits that are otherwise invisible to the naked eye or are correlated with the trait of interest. This broadens the genetic variation in the breeding material as germplasm with such traits could then be retained in the breeding programs.

In the cowpea breeding trials, it is often necessary to evaluate plants for multiple traits such as morphology, phenology, adaptation to abiotic stresses, host resistance to pathogens and pests, and overall health. Hence, it is beneficial in a phenotyping platform to have the possibility to evaluate multiple traits in parallel. This approach reduces costs, saves time, and reduces possible errors caused by multiple rounds of phenotyping done by different people on the same plots. Low-cost phenotyping handheld sensors are useful for the evaluation of a few hundred plots. However, for a large breeding nursery, it is less feasible to use handheld sensors due to the time and labor required and the errors associated with temporal variation in measurements. Thus, major challenges for field phenotyping in plant breeding are measuring thousands of plots at multiple environments while considering the resources available, time required for the measurements, quality of the acquired data, and data analysis. In this aspect, high-throughput phenotyping provides possibilities for increasing selection intensity, improving selection accuracy, and improving the decision support system. Any imprecise phenotyping protocols, especially in small or finite breeding populations, will lower genetic gains because of the very low frequency of favorable alleles. HTPP data can be integrated with the genotypic data to further improve genetic gain. Rutkoski et al. (2016) integrated HTPP and genotypic data for building genomic prediction models to predict grain yield in wheat.

Current plant phenotyping technologies to characterize agriculturally relevant traits have been primarily developed. A study focuses on a diversity panel of 188 cowpea (*Vigna unguiculata*) genotypes to identify which traits are sufficient to differentiate genotypes even when comparing plants whose harvesting date differs up to 14 days. More recently, Burridge et al. (2017) developed an integrated low-cost and high-throughput visual, manual (shovelomics) and image-based (DIRT: digital imaging of root traits, an automated image analysis software) phenotyping technique for in situ field and laboratory evaluation of root phenes in cowpea. The method was used for quantitative evaluation of root architectural traits, and identification and selection of useful root phenotypes. It was found that plants with steep and profuse root systems were better adapted to drought conditions while those with shallow and dense root systems were tolerant to low phosphorus and *Striga* infestation.

4.5 Genomic Resources Available in Cowpea: Linkage Maps and High-Density Genotyping Assay

The narrow genetic diversity in cowpea has always been a major constraint to their improvement for adaptive traits. However, the existing genetic diversity still provides an opportunity for developing climate-resilient cultivars. Earlier studies on genetic diversity in cowpea are based on morphological and physiological traits (Stoilova and Pereira 2013). The more recent developments in molecular genetics enabled the use of different markers for diversity analysis, including early days of allozymes (Panella and Gepts 1992; Vaillancourt et al. 1993; Pasquet 1999) to restriction fragment length polymorphism (RFLP; Fatokun et al. 1993), random amplified polymorphic DNA (RAPD; Ba et al. 2004, Patil et al. 2013), amplified fragment length polymorphism (AFLP; Fang et al. 2007), chloroplast microsatellite primer pairs (cpSSRs; Monteiro et al. 2020), simple sequence repeats (SSRs; Asare et al. 2010; Sarr et al. 2021), and DArTSeq SNP (Ketema et al. 2020). Single-nucleotide polymorphisms (SNP) are the markers of choice for genetic mapping and diversity screening due to their abundance throughout the genome and their amenability for high-throughput genotyping (Mammadov et al. 2012). Thus SNPs were extensively utilized in genetic diversity analysis in cowpea (Carvalho et al. 2017; Nkhoma et al. 2020). The aforementioned markers will help in the accurate assessment of the genetic variability present in the germplasm resources, which can be utilized for the development of climate-resilient cultivars.

Availability of extensive genomic information in public domain represents a significant step forward for cowpea research. The genetic map supported by molecular markers and QTLs associated with climate-resilient traits assist the plant breeders to develop new cultivars for climate resilience. Further, the consensus genetic map in cowpea accelerated the development of genomic resources in cowpea. A consensus map containing EST-derived SNP markers also have been developed in cowpea (Lucas et al. 2011). The availability of high-density SNP markers across the cowpea genome makes it possible to perform genome-wide association analysis. Muchero et al. (2009a, b, c) developed 1536-SNP Golden Gate genotyping array and validated these markers in six RIL mapping populations. In addition to this, the information on 29,728 "unigene" consensus sequences, which were derived from 183,118 ESTs, are available in software HarvEST:Cowpea (http://harvest.ucr.edu). The SNP and candidate genes associated with flowering time in Korean cowpea germplasm have been already identified (Seo et al. 2020). Muñoz-Amatriaín et al. (2017) developed the BAC physical map, whole-genome shot gun short reads, minimal tilling path BACs, and more than a million SNPs from 36 diverse accessions. These genomic resources do not cover complete genome sequence of cowpea but support the assessment of genetic diversity, QTL mapping, association mapping, and map-based cloning for climate-resilient traits in cowpea. The integration of genomics-assisted approaches is depicted in Fig. 4.1.

4.6 Marker Trait Association for Climate-Resilient Traits

4.6.1 Bi -Parental QTL Mapping

The advances in cowpea molecular breeding enable the construction of genetic linkage map and identification of quantitative trait loci associated with some climate-resilient traits. In cowpea, the improved consensus genetic map is

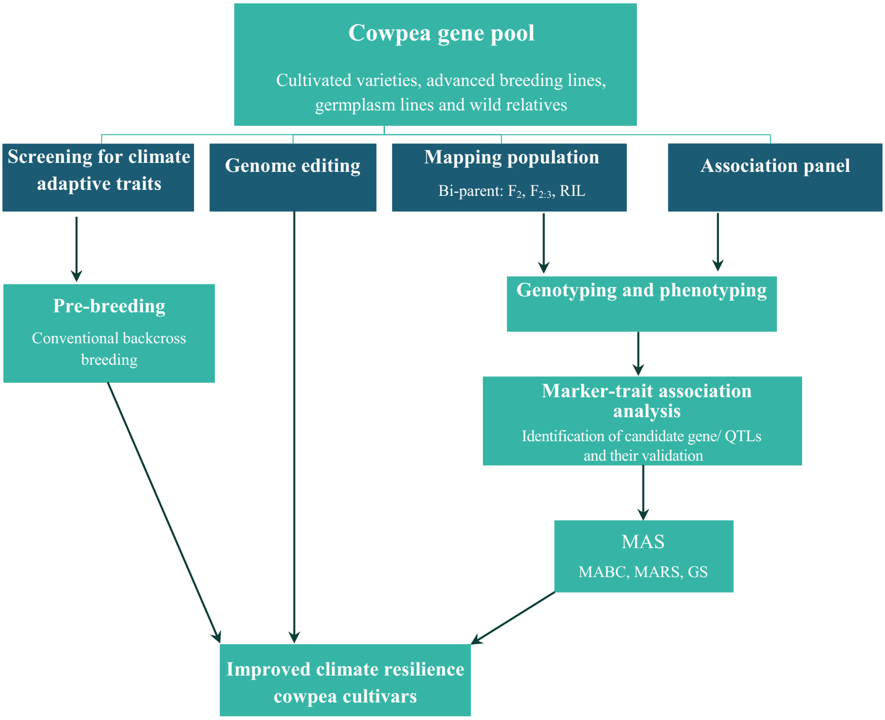

Fig. 4.1 Integration of genomic approaches for improving climate resilience in cowpea

extensively utilized for the identification of QTLs for climate-resilient traits. The sources for climate-resilient traits are continued to be identified in cowpea, and new genotyping platforms are also available to identify the marker trait association. Linkage mapping provides a framework for the identification of quantitative trait loci, diversity analysis, association mapping, map-based cloning, and molecular breeding (Lucas et al. 2011). Furthermore, marker-assisted selection is a promising strategy, which facilitates the selection for climate resilience traits and incorporates those traits into high-yielding cultivars. The main prerequisite of marker-assisted selection is the availability of markers that are tightly linked to targeting traits which are difficult to assess manually. In this direction, several linkage maps were used to identify QTLs for climate resilience traits in cowpea (Table 4.3). Flowering time is the major adaptive and domesticated trait in cowpea, which is mainly dependent on temperature and photoperiod for its expression. QTLs identified for this trait will provide strong foundation for the development of cultivars adapted to particular agro-ecological zone. In this direction, few studies have been conducted to identify QTLs for days to flowering in cowpea. Andargie et al. (2013) identified the five QTLs for time of flower opening and three QTLs days to flower in RILs. Ubi et al. (2000) reported two major QTLs for days to flowering using RAPD markers in RILs population derived from interspecific cross. High-density SNP linkage map also has

Table 4.3 List of the mapped QTLs for climate resilience traits in cowpea

Trait	Type of population	Population size	Type of marker	No. of QTL (s) identified	Range of Phenotypic variation explained (%)	Reference
Heat tolerance	RIL	141	SNP	5	11.5–18.1	Lucas et al. (2013)
	Two RIL	113 and 136	SNP	3	6.2–77.3	Pottorff et al. (2014a)
Root knot nematode	RIL & F2:3	87–170	SNP	1	52–83	Huynh et al. (2016)
	F2 & F2:3	119–137	SNP	2	33–73.3	Ndeve et al. (2019)
Drought tolerance	Association panel	60	SNP	65	10–53	Nkhoma et al. (2020)
	MAGIC	305	SNP	5–35	10.84–42.17	Ravelombola et al. (2021)
Salt tolerance at germination stage	Association panel	116	SNP	3	12.24–14.99	Ravelombola et al. (2018)
Salt tolerance at seedling stage	Association panel	155	SNP	7	6.45–13.35	Ravelombola et al. (2018)
Root architecture traits	Association panel	189	SNP	11	–	Burridge et al. (2016)
Cowpea bacterial blight resistance	Association panel	249	SNP	4	2.5–15.8	Shi et al. (2016)
	RIL	113	SNP	3	9.72–22.1	Agbicodo et al. (2010)
Cowpea golden mosaic virus	F2	286	AFLP	3	–	Rodrigues et al. (2012)
Striga resistance	F2	150	AFLP	3–6	–	Ouedraogo et al. (2001)
	F2	286	SCAR	2	–	Ouedraogo et al. (2012)
	F2	62	AFLP/ SCAR	4/1	–	Boukar et al. (2004)

Macrophomina resistance	RIL	108	SNP	9	6.1–40	Muchero et al. (2011)
Seedling drought-induced senescence	RIL	127	AFLP	10	4.7–24.2	Muchero et al. (2009a, b, c)
Seedling drought-induced maturity	RIL	127	AFLP	2	11.7–14.4	Muchero et al. (2009a, b, c)
Foliar thrips	RIL	127	AFLP	3	9.1–32.1	Muchero et al. (2010)
	Two RIL	114 and 160	SNP	3	9.1–31.5	Lucas et al. (2012)
Fusarium wilt resistance (Fot race 3)	RIL	90	SNP	1	27.8	Pottorff et al. (2012)
Fusarium wilt resistance (Fot race 4)	Three RIL	90–162	SNP	3	18.9–46.5	Pottorff et al. (2014b)
Brown blotch resistance	F2	200	AS-PCR	4	–	Ohlson et al. (2018)
Cowpea flower bud thrips	F2	150	SNP	3	6.5–24.5	Sobda et al. (2017)
Cowpea aphid resistance	RIL	92	SNP	2	13.3–65.7	Huynh et al. (2015)
	F2 & RIL	169 and 92	SSR & SNP	1	–	Kusi et al. (2018)
	Association panel	338	SNP	2	–	Qin et al. (2017)

been developed for days to flowering using RILs population in cowpea (Angira et al. 2020). Heat stress during flowering affects the seed yield and seed number in cowpea. For heat stress, Angira (2016) identified two QTLs in RILs using SNP markers. The lack of information about the availability of markers associated with drought tolerance is the major constraint for developing the cultivar for drought tolerance. However, few studies have been carried out to understand the genetic basis of drought tolerance and identify the genomic regions associated with it. Muchero et al. (2009a, b, c) used the cowpea AFLP linkage map to identify QTLs for seedling drought tolerance and maturity in the recombinant inbred (RIL) population. AFLP technique offers several advantages over other marker techniques since it will not require prior genome sequence information. Salt stress in cowpea significantly reduces growth by affecting its physiological processes. Thus developing the cultivar resistant to salinity is the cost-effective and reasonable approach in cowpea. However, no studies have been reported on QTL mapping for salt tolerance using bi-parental population. The lack of genetic information underlying salt tolerance in cowpea limits the development of breeding strategy in improving the cultivar for salt tolerance.

Cowpea production is affected by various biotic stresses. Although chemical methods are available to control the pests and diseases in cowpea, it is not an economically feasible option at the farmer level. However, resistance to major pests and diseases has been identified, which can be properly utilized to increase cowpea yield through molecular breeding approach. For bacterial blight, Agbicodo et al. (2010) identified three QTLs using SNP markers. This study provides a basis for molecular screening of a large number of genotypes, as well as the development of high-yielding cowpea cultivars combined with bacterial blight resistance. Essem et al. (2019) identified the SSR markers linked to *Striga* resistance. The markers associated with *Striga* resistance will facilitate the marker-assisted development of cultivars resistant to *Striga*. The fungus *Macrophomina phaseolina* will cause significant yield loss in cowpea under high temperature and drought. However, limited sources of resistance hinder the cultivar development for this biotic stress. Muchero et al. (2011) identified the strong sources of resistance and also constructed the gene-derived single-nucleotide polymorphism (SNPs) and amplified fragment length polymorphism (AFLPs) linkage map to identify QTLs for *Macrophomina phaseolina*. To attain an easy and durable solution for insect damage control, host plant resistance is the best approach. However, breeding a cultivar through conventional approach is not a feasible option since it is a complex trait. Molecular markers linked with insect resistance genes in cowpea were reported in different studies. Omo-Ikerodah et al. (2008) identified five QTLs for flower bud thrips using AFLP and microsatellite markers. Muchero et al. (2009a, b, c) identified three QTLs for *Thripstabaci* and *Frankliniellaschultzei*: Thr-1, Thr-2, and Thr-3, on linkage groups 5 and 7 respectively. Huynh et al. (2015) used cowpea linkage map of SNP markers to identify QTLs associated with aphid resistance. These markers will provide basis for genetic characterization and molecular marker development for major pest resistance in cowpea.

4.6.2 Genome-Wide Association Studies

Genome-wide association study (GWAS) is a robust and powerful tool for mapping of the traits with complex phenotype. Bi-parental QTL mapping has revealed marker-trait association for many climate-resilient traits in cowpea (Discussed in the previous section). However, low resolution power of QTL mapping limits its use in retrieving polygenic complex traits. GWAS provides greater resolution power over QTL mapping, and therefore permits the dissection of complex climate resilience traits like drought and salt tolerance (Gupta et al. 2005). During the past two decades, with the advances in next-generation sequencing (NGS) approaches, the genomic resources of cowpea have been enriched with high-density SNP maps (Boukar et al. 2016). GWAS performs statistical associations between genotyping and phenotyping data for the trait of interest in a diverse set of germplasms. GWAS coupled with resequencing of several germplasm lines can be used for precise mapping of QTLs (Varshney et al. 2017). Like other legumes such as chickpea and pigeon pea, GWAS has also been applied in cowpea for climate-resilient traits mapping (Table 4.3). Muchero et al. (2009a, b, c) mapped the QTL for drought response phenotypes using Kruskal-Wallis and multiple QTL models. Qin et al. (2017) performed GWAS for aphid resistance using three models in 338 cowpea accessions from USDA cowpea core collection. The study identified strong association of two SNP markers with aphid resistance. Shi et al. (2016) conducted GWAS for cowpea bacterial blight using 1031 SNPs in 249 USDA germplasm accessions. The results identified four SNPs that explained 2.05–15.8% of the phenotypic variation for cowpea bacterial blight. Burridge et al. (2016) performed association analysis between root architecture phenes and SNP-based genotypic data in a diverse panel of 189 cowpea entries. The study revealed significant association of 11 QTLs with root architecture traits scored manually, while 21 QTLs were identified from DIRT image analysis based phenotyping of root architecture traits. The results also suggested that the selection for root architecture traits in breeding programs can be used to improve cowpea production in multiple stresses. Ravelombola et al. (2018) conducted GWAS for salt tolerance at germination and seedling stages using 116 and 155 cowpea accessions, respectively. A total of 1049 SNPs were employed in association analysis, and of them, three and seven SNPs were found to be highly associated with salt tolerance at germination and seedling stages, respectively. Nkhoma et al. (2020) using association mapping identified 65 SNPs with 10–53% control of variation for drought and yield-related traits. Nested association mapping (NAM) and Multi-parent advance generation inter-cross (MAGIC) populations are the special type of mapping populations. NAM covers both historical and recent recombination events, and therefore minimizes the markers density in GWAS and shows high statistical and resolution powers in association mapping (McMullen et al. 2009). MAGIC shuffles the genetic background of the diverse parental lines through several rounds of genetic recombination, and therefore increases the frequency of rare alleles in the population (Huang et al. 2015). Huynh et al. (2018) developed the first MAGIC population in cowpea using eight diverse founder parents carrying the genes for various biotic, abiotic, and seed quality traits.

Ravelombola et al. (2021) conducted GWAS for drought tolerance indices in cowpea MAGIC population (305 F_8 RILs) using 51,128 SNPs. The study identified 15 SNPs for plant growth habits, while 5, 35, 5, and 18 SNPs were associated with drought tolerance indices for flowering time, grain yield, 100-seed weight, and maturity, respectively.

The knowledge on marker-trait association provides a tool for cloning of genes imparting major role in climate resilience. This will also help the researchers to design the tools to combine multiple climate resilience traits into a single cultivar adapted to the global changing climate. The cowpea genetic maps and quantitative trait loci associated with climate resilience traits such as tolerance to drought, salt, heat, resistance to bacterial blight, golden mosaic virus, *Striga*, *Macrophomina*, *Fusarium* wilt, flower bud thrips, and aphid are available. These markers identified in the above studies can be applied as a selection tool in MABC, MARS, and GS for developing cowpea genotypes that can withstand biotic and abiotic constraints.

4.7 Marker-Assisted Selection for Climate-Resilient Traits

Marker-assisted selection (MAS) helps the plant breeders to select plants with target trait (s) using the molecular markers associated with them omitting the need of costly and laborious phenotyping. MABC, MARS, and genomic selection are the three important strategies where molecular markers are being used for improving climate resilience in crops.

4.7.1 Marker-Assisted Backcrossing (MABC)

Marker-assisted backcrossing (MABC) involving foreground, background, and phenotypic selection in segregating backcross and selfed progenies has been widely used in many crops to introgress desired traits into elite high-yielding and most popular varieties (Varshney et al. 2018). It has several advantages over conventional backcross breeding such as rejection of unfavorable progenies during backcrossing, reduction of phenotyping cost of the target trait that is difficult to visual screening, and reduction of breeding cycles to achieve desired level of recurrent parent genome recovery, thus leading to substantial saving of time and resources (Mehta et al. 2020). In legumes, MABC has been used to improve climate resilience traits such as drought tolerance (Varshney et al. 2013), tolerance to mosaic virus (Parhe et al. 2017) and rust in soybean (Khanh et al. 2013), resistance to anthracnose and *Ascochyta* blight in lentil (Tar'an et al. 2003). The advances in genomics of cowpea have accelerated the development of genomic resources and trait-associated markers for climate resilience traits. However, utilization of trait-associated markers in improving the climate resilience of cowpea has just started, and very few studies have demonstrated the use of MABC in cowpea. Batieno et al. (2016) improved the drought tolerance, root-knot nematode and *Striga* weed resistance of cowpea landrace Moussa Local using three cycles of MABC. Two drought-tolerant lines, IT93K-503-1(root-knot resistant) and IT97K-499-35 (*Striga* resistant), were used as donor parents. Gene-linked foreground SNPs and EST-derived background SNPs were employed in genotyping of plants in backcross and selfed generations to select plants with targeted alleles and maximum recovery of Moussa Local. Salifou et al. (2016) applied MABC to transfer *Striga*-resistant gene (*Rsg1*) from IT93K-693-2 into three susceptible but popular cowpea varieties, namely, TN5-78, KVx30-309-6G, and IT90K-372-1-2. Selection of R*sg1* gene in segregating generations was carried out using SSR_1 microsatellite marker. Dinesh et al. (2016) mapped *qtlblb-1* QTL for bacterial blight resistance in cowpea and transferred the same from V16 cultivar into a popular and high yielding variety C-152 using two generations of MABC. MABC was also used to transfer cowpea mosaic virus resistance from V-57817 to C-152 variety using MA15 linked SSR foreground marker (Dinesh et al. 2018). The study reported 84-09-93.18% recovery of C-152 in BC_3F_3 progenies using 32 SSR background markers. Besides the above studies, At Eduardo Mondlane University (EMU), Mozambique, MABC has been used to improve nematode resistance and drought tolerance in T85F-3139 using INIA-41 as donor (see Boukar et al. 2016). At ISRA, Senegal, cowpea varieties resistant to multiple diseases (Aphid, *Macrophomina* root rot, and *Striga*) are being developed applying MABC (see Boukar et al. 2016). The above studies indicate the successfulness of MABC in developing climate-resilient cowpea varieties that can cope up with future climatic conditions.

4.7.2 Marker-Assisted Recurrent Selection (MARS)

MARS is a method to accumulate large number of QTLs within a population using subset of markers significantly associated with target traits (Bernardo 2008; Chamarthi et al. 2011; Boukar et al. 2016). Using MARS breeder is able to increase favorable alleles with additive effects in the individual within the population. This involves the mapping in the early F2 or F3 generation using genotyping and phenotyping and then followed by recombination of selected individuals based on marker index only for three generations to develop the population with a high frequency of favorable alleles (Tester and Langridge 2010; Lande and Thompson 1990). In cowpea, MARS was used to develop drought-tolerant cowpea varieties at IITA Nigeria. Two parents IT84S-2246-4 and IT98K-1111-1 were crossed and QTL mapping was done using 102 SNPs and 7 QTLs were identified for drought tolerance, grain yield, and staygreen. The favorable alleles were fixed for these QTLs in one hundred and seventy-seven lines. Further MARS was applied for grain quality traits, large seed, and heat tolerance traits in the cross between CB27 and IT97K-499-35 at Eduardo Mondlane University, Mozambique. At ISRA, Senegal, the cross between IT93K-503-1 and Mouride was made and MARS was applied to the progeny for drought tolerance, resistance to nematodes, and *Striga*. MARS lines with the highest molecular scores for target QTLs derived from cross Suvita2 × IT97K-499-35 (Burkina Faso) following two cycles of recombinant crosses performed using 164 SNPs polymorphic between the two parents were tested in the 2014 main season at INERA, Burkina Faso, for grain yield, drought tolerance, *Striga* resistance, and *Macrophomina* resistance. Two cycles of recombinant crosses were performed using 164 SNPs polymorphic between the two parents (Boukar et al. 2016; Chamarthi et al. 2019).

4.7.3 Genomic Selection

MABC and MARS use the molecular markers known to be associated with QTLs having a major effect on phenotypes, but its application is limited for complex quantitative traits that are governed by a large number of minor QTLs (Zhou et al. 2014). The reason behind this is the estimation of allelic effects of minor QTLs through association mapping and linkage mapping is often biased. The best solution for utilizing these minor QTLs is through genomic selection (GS). GS estimates the genetic worth of individuals based on a large set of markers distributed across the whole genome of individuals. The process of genomic selection includes the development of prediction models based on the information obtained from genotyping and phenotyping of the training population. This data is used to derive genomic estimated breeding values (GEBV) for all the individuals in the breeding population from the genome-wide markers (Meuwissen et al. 2001). The GEBV of individual lines allows us to select an individual to be used in breeding programs for crossing and next-generation advancement.

Cowpea being a primarily self-pollinated crop has generally been bred through pedigree or single seed descent methods. GS can be combined along with traditional breeding methods in several ways. One design is to incorporate GEBV during the early pedigree line development process to eliminate undesirable lines in an initial generation, thus saving resources and time that would otherwise be needed to carry lines to complete fixation. Further, GS can be applied during later generations of line fixation or after complete fixation to make a positive selection of genotypes to carry forward into yield trials. Based on GEBVs parents can also be selected for crossing programs to derive superior recombinants. GS has been used with modification in wheat and other small grain crops for recurrent selection (Poland et al. 2012; Rutkoski et al. 2014; Spindel et al. 2015). In this process, individual lines derived from bi-parental cross are selected based on GEBV, crossed and used directly for future rounds of selection (Bassi et al. 2016; Heslot et al. 2015). GS has been utilized for improving genetic gain in legumes such as pigeon pea, soybean, lentil, and alfalfa. The availability of cost-effective high-throughput genotyping platforms and genomic resources in cowpea provides excellent opportunities for GS in cowpea (Huynh et al. 2018). Olatoye et al. (2019) analyzed the MAGIC population of cowpea for determining the epistatic architecture of flowering time, maturity, and seed size for genomic-enabled breeding (GEB) using parametric, semi-parametric, and non-parametric GS models. The results showed that parametric and semi-parametric GS models outperformed over non-parametric GS model, while using known quantitative trait nucleotide(s) as fixed effects improved prediction accuracy when traits were controlled by large effect loci. Ravelombola et al. (2021) conducted

GS for plant growth habit under drought stress and drought tolerance indices for maturity, flowering time, grain yield, and 100-seed weight using ridge regression best linear unbiased predictor model (rrBLUP) in cowpea MAGIC population of 249 genotypes. The GS model revealed low to moderate genomic prediction accuracy across the traits. Thus, GS has the potential in cowpea breeding programs to select individuals with high GEBV for improving genetic gain under climate change.

4.8 Genome Editing for Climate Resilience Adaptive Traits

Genome editing is an important technique to accelerate the progression of varietal development programs. Alteration at single gene level has been successfully demonstrated by using gene-editing tools like Zinc Finger Nucleases (ZFNs), TAL effector proteins(TALENs), and CRISPR(clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR associated protein 9). All these tools work on the same principle of introducing double-strand breaks at a specific site followed by the onset of DNA repair mechanism. However, CRISPR/Cas9 technology has been widely accepted by researchers around the world because of its ease of use and user-friendly nature. The first successful application of genome editing in cowpea was communicated by a group of Chinese researchers in 2019, where they have efficiently disrupted the symbiosis receptor-like kinase (*VuSYMRK*) gene (Ji et al. 2019). Three single-guide RNA (sgRNAs) targeting different sites of exon 2 of this particular gene was transformed into cotyledonary nodes using *Agrobacterium rhizogenes* mediated hairy root transformation. The mutants showed complete blockage of nodule formation with disruption of both alleles and achieved 67% mutagenic efficiency in the transformants. The authors suggested the success of this technology in the aforementioned study opens the door for future application of CRISPR/Cas in important agronomic traits in cowpea. However, cowpea is still recalcitrant to transformation with 1% transformation efficiency in cotyledonary node-based explants under stringent selection (Bett et al. 2019). Hence, the development of a standard protocol for stable transformation in cowpea is the need of the hour. Of late, Che et al. (2021) reported a highly efficient transformation system in cowpea using embryonic axis explants isolated from imbibed mature seeds of IT86D-1010 genotype. The removal of the shoot apical meristem from the explants triggered multiple shoot organogenesis from the cotyledonary node. *Agrobacterium*-mediated transformation and use of spcN as a selectable marker led to effective transgenic selection, recovery, and transgenic plant regeneration without chimera formation. The transformation protocol was tested in cotyledonary nodes of nine cowpea genotypes that showed transformation frequencies ranging from 4% to 37%. The developed protocol was also applied to study its efficiency in genome editing of IT86D-1010 genotype using CRISPR/Cas technology. Cowpea meiosis gene *Vu-SPO11-1* that impairs homologous recombination, was knocked out (d'Erfurth et al. 2009) using sgRNA driven by U6 promoter. A total of 35 T_0 transgenics were obtained and deep sequencing result identified 2 biallelic mutants that were completely infertile with no pod development. Furthermore, the study also demonstrated stable inheritance of edited alleles in the succeeding generation. Cowpea has a longer generation time of about 14 weeks or more, depending on the cultivar, so in order to study the efficiency of gene editing vectors as well as target guides, we require a transient assay to rapidly test gene-editing vector constructs and gene expression. Juranić et al. (2020) demonstrated a detached leaf transient system in cowpea where they have agroinfiltrated vector constructs carrying *SPO11-1*, *REC8*, and *OSD1* genes which resulted in wilting and necrosis of leaves. Finally, they demonstrated the utility of this transient assay for pre-testing of CRISPR construct by a stable transformation of SPO*11-1* vector construct. The transgenic cowpea plants showed impaired meiosis and completely sterile T_0 plants.

The above-cited studies suggest CRISPR technology holds immense potential to alter candidate genes involved in various environmental stresses in cowpea. Cowpea transgenics have been developed for various insect pests like *Maruca*-resistant cowpea cultivar for Nigerian agricultural production (Ba et al. 2018) suggesting a positive attitude and acceptance toward cowpea transgenics. This opens an avenue for the implementation of highly potential CRISPR technology to expedite the genetic improvement in cowpea for better adaptation to changing climate.

4.9 Conclusion

Cowpea provides a major proportion of protein and minerals to millions of people in resource-scare countries and is equally important as nutritious fodder for livestock. Global warming has resulted in unpredicted climatic events such as erratic rainfall and extreme temperatures as well as outbreak of major insect pests and diseases, and even the occurrence of novel pathogens, thereby challenging food security. In developing climate-resilient cowpea, breeders need to broaden the adaptability of varieties over variable environments along with increased yield stability to minimize the impact of climate change. Available genetic variability in cultivated and wild relatives of cowpea can be exploited to introgress resistance genes/QTLs into high-yielding cowpea varieties. Mapping of resistance genes/QTLs followed by the use of advanced genomic tools such as MABB, MARS, and GS can accelerate the pace of cowpea breeding for climate resilience traits.

References

Abdalla, M.E. (1992). Resistance in cowpea (Vignaunguiculata (L.) Walp.)to a California biotype of the cowpea aphid (Aphis craccivora Koch), inheritance and mechanisms . Ph D dissertation University of California

Agbicodo EM, Fatokun CA, Bandyopadhyay R (2010) Identification of markers associated with bacterial blight resistance loci in cowpea [*Vigna unguiculata* (L.) Walp.]. Euphytica 175:215–226. https://doi.org/10.1007/s10681-010-0164-5

Andargie M, Pasquet RS, Muluvi GM, Timko MP (2013) Quantitative trait loci analysis of flowering time related traits identified in recombinant inbred lines of cowpea (*Vigna unguiculata*). Genome 56(5):289–294

Angira B (2016) Genetic and physiological studies of heat tolerance in cowpea. (PhD thesis). Texas A&M University

Angira B, Zhang Y, Scheuring CF, Zhang Y, Masor L, Coleman JR, Liu YH, Singh BB, Zhang HB, Hays DB, Zhang M (2020) Quantitative trait loci influencing days to flowering and plant height in cowpea, *Vigna unguiculata* (L.) Walp. Mol Gen Genomics 295:1187–1195

Arnon I (1972) Field crop production in tropical Africa. Leonard Hill Book Company, London

Asare AT, Gowda BS, Galyuon IK, Aboagye LL, Takrama JF, Timko MP (2010) Assessment of the genetic diversity in cowpea (Vignaunguiculata L. Walp.) germplasm from Ghana using simple sequence repeat markers. Plant Genetic Resources 8(2):142–150

Ausha C (2013) Varietal screening, loss estimation and management of cowpea pests (MSc thesis). Dharwad University of Agricultural Sciences, Karnatak University, India. 84 pp.

Aveling, T, 1999. Cowpea pathology research. (Also available at www.ap.ac.za/academic/microbio/plant/pr-colwpea.html)

Ba FS, Pasquet RS, Gepts P (2004) Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. Genet Resour Crop Evol 51(5):539–550

Ba MN, Huesing JE, Tamò M, Higgins TJ, Pittendrigh BR, Murdock LL (2018) An assessment of the risk of Bt-cowpea to non-target organisms in West Africa. J Pest Sci 91(4):1165–1179

Bassi FM, Bentley AR, Charmet G, Ortiz R, Crossa J (2016) Breeding schemes for the implementation of genomic selection in wheat (Triticum spp.). Plant Sci 242:23–36

Batieno BJ, Danquah E, Tignegre JB, Huynh BL, Drabo I, Close TJ, Ofori K, Roberts P, Ouedraogo TJ (2016) Application of marker-assisted backcrossing to improve cowpea (Vignaunguiculata L. Walp) for drought tolerance. J Plant Breed Crop Sci 8(12):273–286

Bernardo R (2008) Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Sci 48:1649–1664

Bett B, Gollasch S, Moore A, Harding R, Higgins TJ (2019) An improved transformation system for cowpea (Vignaunguiculata L. Walp) via sonication and a kanamycin-geneticin selection regime. Front Plant Sci 10:219

Birch ANE, Fellows LE, Evans SV, Dhoerty K (1986) Para- aminophenylalanine in Vigna. Possible taxonomic and ecological significance as a seed defence against bruchids. Phytochemistry 25: 2745–2749

Blackman R, Eastop VF (1984) Aphids on the world crops. Wiley Inter Science, New York. 400 pp

Boukar O, Kong L, Singh BB, Murdock L, Ohm HW (2004) AFLP and AFLP-derived SCAR markers associated with Striga gesnerioides resistance in cowpea. Crop Sci 44(4):1259–1264

Boukar O, Bhattacharjee R, Fatokun C, Kumar PL, Gueye B (2013) Cowpea. In: Singh M, Upadhyaya HD, Bisht IS (eds) Genetic and genomic resources of grain legume improvement, pp 137–155

Boukar O, Fatokun CA, Huynh BL, Roberts PA, Close TJ (2016) Genomic tools in cowpea breeding programs: status and perspectives. Front Plant Sci 7:757

Burridge J, Jochua CN, Bucksch A, Lynch JP (2016) Legume shovelomics: high—throughput phenotyping of commonbean (*Phaseolus vulgaris* L.) and cowpea (*Vignaunguiculatasubsp, unguiculata*) root architecture in the field. Field Crops Res 192:21–32. https://doi.org/10.1016/j.fcr.2016.04.008

Burridge JD, Schneider HM, Huynh BL (2017) Genome-wide association mapping and agronomic impact of cowpea root architecture. Theor Appl Genet 130:419–431. https://doi.org/10.1007/s00122-016-2823-y

Carvalho M, Muñoz-Amatriaín M, Castro I, Lino-Neto T, Matos M, Egea-Cortines M, Rosa E, Close T, Carnide V (2017) Genetic diversity and structure of Iberian Peninsula cowpeas compared to world-wide cowpea accessions using high density SNP markers. BMC Genomics 18(1):1–9

Caswel GH (1981) Damage to stored cowpea in the northern part of Nigeria. Samaru J Agric Res 5: 4–5

Chamarthi SK, Kumar A, Vuong T, Blair MW, Gaur PM, Nguyen HT, Varshney RK (2011) Trait mapping and molecular breeding in legumes: concepts and examples in soybean, common bean and chickpea. In: Pratap A, Kumar J (eds) Biology and breeding of food legumes. CABI International, Oxfordshire, UK, pp 296–313

Chamarthi SK, Belko N, Togola A, Fatokun CA, Boukar O (2019) Genomics-assisted breeding for drought tolerance in cowpea. In: Genomics assisted breeding of crops for abiotic stress tolerance, vol II. Springer, Cham, pp 187–209

Che P, Chang S, Simon MK, Zhang Z, Shaharyar A, Ourada J et al (2021) Developing a rapid and highly efficient cowpea regeneration, transformation and genome editing system using embryonic axis explants. Plant J 106(3):817–830

Dannon EA (2011) PhD thesis, Wageningen University, Netherlands, 188 pp.

de Moraes GJ, Oliveira CAV, De Albuquerque MM, Salviano LMC, De Possidio PL (1980) The appropriate time to control the green leafhopper on cowpea crops, ComunicadoTecnico, EMBRAPA (CPATSA), 2 pp.

d'Erfurth I, Jolivet S, Froger N, Catrice O, Novatchkova M, Mercier R (2009) Turning meiosis into mitosis. PLoS Biol 7(6):e1000124

Dinesh HB, Lohithaswa HC, Viswanatha KP, Singh P, Manjunatha L, Ambika DS (2018) Genetic analysis and marker assisted backcrossing for transfer of mosaic virus resistance in cowpea [Vignaunguiculata (L.) Walp.]. Legum Res 41:663–668

Dinesh HB, Lohithaswa HC, Viswanatha KP, Singh P, Rao AM (2016) Identification and marker-assisted introgression of QTL conferring resistance to bacterial leaf blight in cowpea (*Vigna unguiculata* (L.) Walp.). Plant Breed 135(4):506–512

Durairaj C (2000) A note on the host preference by two species of blister beetle in pulse crops. Madras Agric J 87(4/6):355–356

Essem F, Ohlson EW, Asare AT, Timko MP (2019) Genetic markers linked to *Strigagesnerioides* resistance for the improvement of Ghanaian cowpea (*Vigna unguiculata)* cultivars. Plant Breed 138(5):599–604

Fang J, Chao CCT, Roberts PA, Ehlers JD (2007) Genetic diversity of cowpea [*Vignaunguiculata* (L.) Walp.] in four West African and USA breeding programs as determined by AFLP analysis. Genet Resour Crop Evol 54(6):1197–1209

Fatokun CA (2002) Breeding cowpea for resistance to insect pests: attempted crosses between cowpea and Vignavexillata. In: Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4–8 September 2000

Fatokun CA, Danesh D, Young ND, Stewart EL (1993) Molecular taxonomic relationships in the genus Vigna based on RFLP analysis. Theor Appl Genet 86(1):97–104

Fatokun CA, Boukar O, Kamara A, Coulibaly O, Alene A, Boahen S et al (2012) Enhancing cowpea productivity and production in drought- prone areas of Sub-Saharan Africa. In: Abate T (ed) Four seasons of learning and engaging smallholder farmers: progress of phase 1. International Crops Research Institute for the Semi-Arid Tropics, Nairobi, pp 81–112

Gomez C (2004) Cowpea: post-harvest operations. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. 71 pp.

Gupta PK, Rustgi S, Kulwal PL (2005) Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Mol Biol 57(4):461–485

Gusta L (2012) Abiotic stresses and agricultural sustainability. J Crop Improv 26:415–427

Hall AE (1993) Physiology and breeding for heat tolerance in cowpea, and comparison with other crops. In: Kuo CG (ed) Proc. International Symposium on Adaptation of Food Crops to Temperature and Water Stress. August 13–18, 1992. Publ. No. 93-410. Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp 271–284

Hall AE, Cisse N, Thiaw S, Elawad HO, Ehlers JD, Ismail AM, Fery RL, Roberts PA, Kitch LW, Murdock LL, Boukar O (2003) Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. Field Crop Res 82:103–134

Heslot N, Jannink J-L, Sorrells ME (2015) Perspectives for genomic selection applications and research in plants. Crop Sci 55:1–12

Huang BE, Verbyla KL, Verbyla AP, Raghavan C, Singh VK, Gaur P, Leung H, Varshney RK, Cavanagh CR (2015) MAGIC populations in crops: current status and future prospects. Theor Appl Genet 128(6):999–1017

Huynh BL, Ehlers JD, Ndeve A (2015) Genetic mapping and legume synteny of aphid resistance in African cowpea (*Vignaunguiculata* L. Walp) grown in California. Mol Breed 35:36. https://doi.org/10.1007/s11032-015-0254-0

Huynh BL, Matthews WC, Ehlers JD (2016) A major QTL corresponding to the *Rk* locus for resistance to root-knot nematodes in cowpea (*Vignaunguiculata* L. Walp.). Theor Appl Genet 129:87–95. https://doi.org/10.1007/s00122-015-2611-0

Huynh BL et al (2018) A multi-parent advanced generation inter-cross MAGIC population for genetic analysis and improvement of cowpea (*Vigna unguiculata* L. Walp.). Plant J 93:1129–1142

Ji J, Zhang C, Sun Z, Wang L, Duanmu D, Fan Q (2019) Genome editing in cowpea Vignaunguiculata using CRISPR-Cas9. Int J Mol Sci 20(10):2471

Juranić M, Nagahatenna DS, Salinas-Gamboa R, Hand ML, Sánchez-León N, Leong WH, Koltunow AM (2020) A detached leaf assay for testing transient gene expression and gene editing in cowpea (Vignaunguiculata [L.] Walp.). Plant Methods 16(1):1–17

Karungi J, Adipala E, Kyamanywa S, Ogenga-Latigo MW, Oyobo N, Jackai LEN (2000) Pest management in cowpeas. Part 2. Integrating planting time, plant density and insecticide application for management of cowpea field insect pests in Uganda. Crop Prot 19(4):237–245

Kay DE (1979) Food legumes. Tropical Development and Research Institute, London

Ketema S, Tesfaye B, Keneni G, Amsalu Fenta B, Assefa E, Greliche N, Machuka E, Yao N (2020) DArTSeq SNP-based markers revealed high genetic diversity and structured population in Ethiopian cowpea [Vignaunguiculata (L.) Walp] germplasms. PLoS One 15(10):e0239122

Khanh, T.D., Anh, T.Q., Buu, B.C. and Xuan, T.D., 2013. Applying molecular breeding to improve soybean rust resistance in Vietnamese elite soybean

Koona P, Bouda H (2006) Biological activity of Pachypodanthiumstaudtii (Annonaceae) against the bean beetle, Acanthoscelidesobtectus (Say) (Coleoptera: Bruchidae). J Appl Sci Res 2(12): 1129–1131

Koona P, Osisanya EO, Jackai LEN, Tamo M, Tonye J, Ngeve JM (2001) Interaction between pod age and position on damage to cowpea Vignaunguiculata by hemipteran pod sucking bugs. Bull Entomol Res 91(6):453–459

Kusi F, Padi FK, Obeng-Ofori D, Asante SK, Agyare RY, Sugri I, Timko MP, Koebner R, Huynh BL, Santos JR, Close TJ (2018) A novel aphid resistance locus in cowpea identified by combining SSR and SNP markers. Plant Breed 137(2):203–209

Lande R, Thompson R (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756. Legume Research, 42(4) 2019: 437-446 Print ISSN:0250-5371 / Online ISSN:0976-0571 Cowpea genetic resources and its utilization: Indian perspective – A review Kuldeep Tripathi, P.G. Gore, S.P. Ahlawat, V. Tyagi, D.P. Semwal, N.K. Gautam*, J.C. Rana1 and A. Kumar

Lucas MR, Diop NN, Wanamaker S, Ehlers JD, Roberts PA, Close TJ (2011) Cowpea–soybean synteny clarified through an improved genetic map. Plant Genome 4(3):1–11

Lucas MR, Ehlers JD, Roberts PA, Close TJ (2012) Markers for quantitative resistance to foliar thrips in cowpea. Crop Sci 52:2075–2081. https://doi.org/10.2135/cropsci2011.12.0684

Lucas MR, Ehlers JD, Huynh BL (2013) Markers for breeding heat-tolerant cowpea. Mol Breed 31: 529–536. https://doi.org/10.1007/s11032-012-9810-z

Maheshwari UK (1986) Biological control of major agricultural pulse pest, Mylabrispustulata—a new approach. Indian J Entomol 48:381–387

Mammadov J, Aggarwal R, Buyyarapu R, Kumpatla S (2012) SNP markers and their impact on plant breeding. Int J Plant Genomics 2012

McMullen MD, Kresovich S, Villeda HS, Bradbury P, Li H, Sun Q, Flint-Garcia S, Thornsberry J, Acharya C, Bottoms C, Brown P (2009) Genetic properties of the maize nested association mapping population. Science 325(5941):737–740

Mehta BK, Muthusamy V, Zunjare RU, Baveja A, Chauhan HS, Chhabra R, Singh AK, Hossain F (2020) Biofortification of sweet corn hybrids for provitamin-A, lysine and tryptophan using molecular breeding. J Cereal Sci 96:103093. https://doi.org/10.1016/j.jcs.2020.103093

Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 257:1819–1829

Monteiro E, Castro I, Carvalho M, Martín JP, Rosa E, Carnide V (2020) Iberian Peninsula cowpea diversity: chloroplast, microsatellite and morpho-agronomic variability. Syst Biodivers 19(2): 121–134

Muchero W, Diop NN, Bhat PR, Fenton RD, Wanamaker S, Pottorff M, Hearne S, Cisse N, Fatokun C, Ehlers JD, Roberts PA (2009a) A consensus genetic map of cowpea [*Vigna unguiculata* (L) Walp.] and synteny based on EST-derived SNPs. Proc Natl Acad Sci 106(43):18159–18164

Muchero W, Ehlers JD, Close TJ, Roberts PA (2009b) Mapping QTL for drought stress-induced premature senescence and maturity in cowpea [Vignaunguiculata (L.) Walp.]. Theor Appl Genet 118(5):849–863

Muchero N, Diop N, Bhat PR (2009c) A consensus genetic map of cowpea [*Vignaunguiculata*(-L) walp.] and synteny based on EST-derived SNPs. Proc Natl Acad Sci 106(43):18159–18164

Muchero W, Ehlers JD, Roberts PA (2010) QTL analysis for resistance to foliar damage caused by T*hripstabaci* and *Frankliniellaschultzei* (Thysanoptera: Thripidae) feeding in cowpea [*Vigna unguiculata* (L.) Walp.]. Mol Breed 25(1):47–56

Muchero W, Ehlers JD, Close TJ (2011) Genic SNP markers and legume synteny reveal candidate genes underlying QTL for *Macrophomina phaseolina* resistance and maturity in cowpea [*Vigna unguiculata* (L) Walp.]. BMC Genomics 12:8. https://doi.org/10.1186/1471-2164-12-8

Muñoz-Amatriaín M, Mirebrahim H, Xu P, Wanamaker SI, Luo M, Alhakami H, Alpert M, Atokple I, Batieno BJ, Boukar O, Bozdag S (2017) Genome resources for climate-resilient cowpea, an essential crop for food security. Plant J 89(5):1042–1054

Ndeve AD, Santos JR, Matthews WC, Huynh BL, Guo YN, Lo S, Muñoz-Amatriaín M, Roberts PA (2019) A novel root-knot nematode resistance QTL on chromosome Vu01 in Cowpea. G3: Genes Genomes Genetics 9(4):1199–1209

NDoutoume-Ndong A, Rojas-Rousse D (2008) Rôle de l'intensitélumineuse sur les capacitésparasitairesd'Epelmusorientalis Crawford etd'Epelmusvuilleti Crawford, parasitoïdes des Bruchidaeravageurs de graines de niébé (VignaunguiculataWalp.). Biotechnol Agron Soc Environ 12(1):3–8. [Role of light intensity on the parasitic capacities of Epelmusorientalis Crawford and Epelmusvuilleti Crawford, parasitoids of Bruchidae pests of cowpea seeds (VignaunguiculataWalp.). Biotechnology, Agronomy, Society and Environment]

Ng NQ, Marechal R (1985) Cowpea taxonomy, origin and germplasm. In: Singh SR, Rachie KO (eds) Cowpea research, production and utilization. John Wiley and Sons, Chichester, pp 11–12

Nkhoma N, Shimelis H, Laing MD, Shayanowako A, Mathew I (2020) Assessing the genetic diversity of cowpea [Vignaunguiculata (L.) Walp.] germplasm collections using phenotypic traits and SNP markers. BMC Genet 21(1):1–16

Ohlson EW, Thio GI, Sawadogo M (2018) Quantitative trait loci analysis of brown blotch resistance in cowpea variety KN1. Mol Breed 38:110. https://doi.org/10.1007/s11032-018-0867-1

Oiewalw RO, Bamaiyi LJ (2013) Management of cowpea insect pests. Scholars Acad J Biosci 1(5): 217–226

Olatoye MO, Hu Z, Aikpokpodion PO (2019) Epistasis detection and modeling for genomic selection in cowpea (*Vigna unguiculata* L. Walp). Front Genet 10:677

Oluwafemi AR (2012) Comparative effects of three plant powders and pirimiphos-methyl against the infestation of Callosobruchusmaculatus (F.)(Coleoptera: Bruchidae) in cowpea seeds. SOAJ Entomol Stud 1:108–117

Omo-Ikerodah EE, Fawole I, Fatokun CA (2008) Genetic mapping of quantitative trait loci (QTLs) with effects on resistance to flower bud thrips (*Megalurothrips sjostedti*) identified in recombinant inbred lines of cowpea (*Vigna unguiculata* (L.) Walp). Afr J Biotechnol 7:263–270

Onwueme IC, Sinha TD (1991) Field crop production in tropical Africa. Principles and practice. CTA, Wageningen, Netherlands, pp 324–336

Ouedraogo J, Maheshwari V, Berner D, St Pierre C-A, Belzile F, Timko M (2001) Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by Striga gesnerioides. Theor Appl Genet 102:1029–1036

Ouedraogo JT, Ouedraogo M, Gowda BS, Timko MP (2012) Development of sequence characterized amplified region (SCAR) markers linked to race-specific resistance to Striga gesnerioides in cowpea (*Vigna unguiculata* L.). Afr J Biotechnol 11:12555–12562

Padulosi S, Ng NQ (1997) Origin, taxonomy and morphology of *Vigna unguiculata* (I) Walp. In: Singh BB, Mohanraj DR, Dahiell KE, Jackai LEN (eds) Advances in cowpea research. A co-Publication of IITA/JIRCAS, IITA, Ibadan, Nigeria, pp 1–12

Panella L, Gepts P (1992) Genetic relationships within *Vigna unguiculata* (L.) Walp. based on isozyme analyses. Genet Resour Crop Evol 39:71–88

Parhe SD, Chimote VP, Deshmukh MP, Chandra K, Akash M (2017) Marker-assisted pyramiding of four QTL/genes for Asian rust (*Phakopsorapachyrhizi*) resistance in soybean. J Crop Improv 31(5):689–711

Pasquet RS (1999) Genetic relationships among subspecies of *Vigna unguiculata* (L.) Walp. based on allozyme variation. Theor Appl Genet 98(6–7):1104–1119

Pathak RS (1988) Genetics of resistance to aphid in cowpea. Crop Sci 28:474–476

Patil DM, Sawardekar SV, Gokhale NB, Bhave SG, Sawant SS, Sawantdesai SA, Lipne KA, Sabale SN, Joshi SN (2013) Genetic diversity analysis in cowpea [*Vignaunguiculata* (L.) Walp.] by using RAPD markers. Int J Innov Biotechnol Biochem 1(1):15–23

Poland JA, Endelman J, Dawson J, Rutkoski J, Wu S, Manes Y, Dreisigacker S, Crossa J, Sánchez-Villeda H, Sorrells M, Jannink JL (2012) Genomic selection in wheat breeding using genotyping-by-sequencing. Plant Genome 5(3):103–113

Pottorff M, Wanamaker S, Ma YQ, Ehlers JD, Roberts PA, Close TJ (2012) Genetic and physical mapping of candidate genes for resistance to *Fusarium oxysporumf*.sp. *tracheiphilum* race 3 in cowpea [*Vigna unguiculata* (L.)Walp]. PLoS ONE 7:e41600. https://doi.org/10.1371/journal.pone.0041600

Pottorff MO, Roberts PA, Close TJ, Lonardi S, Wanamaker S, Ehlers JD (2014a) Identification of candidate genes and molecular markers for heat-induced brown discoloration of seed coats in cowpea [*Vignaunguiculata* (L.) Walp]. BMC Genomics 15(1):328

Pottorff MO, Li G, Ehlers JD (2014b) Genetic mapping, synteny, and physical location of two loci for *Fusarium oxysporum* f. sp. *tracheiphilum* race 4 resistance in cowpea [*Vigna unguiculata* (L.) Walp]. Mol Breed 33:779–791. https://doi.org/10.1007/s11032-013-9991-0

Qin J, Shi A, Mou B (2017) Association mapping of aphid resistance in USDA cowpea (*Vignaunguiculata* L. Walp.) core collection using SNPs. Euphytica 213:36. https://doi.org/10.1007/s10681-016-1830-z

Ravelombola W, Shi A, Weng Y (2018) Association analysis of salt tolerance in cowpea (*Vignaunguiculata* (L.) Walp) at germination and seedling stages. Theor Appl Genet 131:79–91. https://doi.org/10.1007/s00122-017-2987-0

Ravelombola W, Shi A, Huynh BL (2021) Loci discovery, network-guided approach, and genomic prediction for drought tolerance index in a multi-parent advanced generation intercross (MAGIC) cowpea population. Hortic Res 8:24. https://doi.org/10.1038/s41438-021-00462-w

Rodrigues MA, Santos CAF, Santana JRF (2012) Mapping of AFLP loci linked to tolerance to cowpea golden mosaic virus. Genet Mol Res 11(4):gmr1819. https://doi.org/10.4238/2012.August.17.12

Rutkoski JE, Poland JA, Singh RP, Huerta-Espino J, Bhavani S, Barbier H et al (2014) Genomic selection for quantitative adult plant stem rust resistance in wheat. Plant Genome 127:1441–1448

Rutkoski J, Poland J, Mondal S, Autrique E, Pérez LG, Crossa J, Reynolds M, Singh R (2016) Canopy temperature and vegetation indices from high-throughput phenotyping improve accuracy of pedigree and genomic selection for grain yield in wheat. G3: Genes Genomes Genet 6(9):2799–2808. https://doi.org/10.1534/g3.116.032888

Salifou M, Tignegre JBLS, Tongoona P, Offei S, Ofori K, Danquah E (2016) Introgression of Striga resistance gene into farmers' preferred cowpea varieties in Niger. Int J Plant Breed Genet 3(6): 233–240

Samireddypalle A, Boukar O, Grings E, Fatokun C, Kodukula P, Devulapalli R et al (2017) Cowpea and groundnut haulms fodder trading and its lessons for multidimensional cowpea improvement for mixed crop livestock systems in West Africa. Front Plant Sci 8:30. https://doi.org/10.3389/fpls.2017.00030

Sarr A, Bodian A, Gbedevi KM, Ndir KN, Ajewole OO, Gueye B, Foncéka D, Diop EA, Diop BM, Cissé N, Diouf D (2021) Genetic diversity and population structure analyses of wild relatives and cultivated Cowpea (Vignaunguiculata (L.) Walp.) from Senegal using simple sequence repeat markers. Plant Mol Biol Report 39(1):112–124

Seo E, Kim K, Kang R, Kim G, Park A, Kim WJ, Sun H, Ha BK (2020) Genome-wide association study for flowering time in Korean Cowpea Germplasm. Plant Breed Biotechnol 8(4):413–425

Shi A, Buckley B, Mou B (2016) Association analysis of cowpea bacterial blight resistance in USDA cowpea germplasm. Euphytica 208:143–155. https://doi.org/10.1007/s10681-015-1610-1

Shoyinka SA, Thottappilly G, Adebayo GG, Anno-Nyako FO (1997) Survey on cowpea virus incidence and distribution in Nigeria. Int J Pest Manage 43(2):127–132

Singh BB (2007) Potential and constraits of improved cowpea varieties in increasing the productivity of cowpea cereal system in dry savanna of west Africa. In: Majiwa P, Odera M, Muchiri N, Omanya G, Werehire P (eds) A plan to apply technology in the improvement of cowpea productivity and utilization for the benefit of farmers and consumers in Africa: proceeding of cowpea stakeholders workshop. African Agricultural Technology Foundation, Nairobi Kenya, pp 14–26

Singh BB (2014) Cowpea: the food legume of the 21st century. Crop Science Society of America, Madison, WI

Singh, S.R. and Allen, D.J. (1979). Cowpea pest and diseases. Manual Series No. 2, International Institute of Tropical Agriculture, Ibadan

Singh SR, Jackai LEN (1985) Insect pests of cowpeas in Africa: their life cycle, economic importance and potential for control. In: Singh SR, Rachie KO (eds) Cowpea research, production and utilization. John Wiley and Sons, London, pp 217–231

Singh BB, Tarawali SA (2011) Cowpea and its improvement: key to sustainable mixed crop/livestock farming systems in West Africa. Agrofor Syst 2:34–41

Singh SR, Van Emden HF (1979) Insect pests of grain legumes. Ann Rev Entomol 24(1):255–278

Singh KJ, Singh OP, Banafer RN (1990) Evaluation of cowpea varieties against aphid, (Aphis craccivora Koch.) infestation. J Aphidol 4:86–88

Singh BB, Ajeigbe HA, Tarawali SA, Fernandez-Rivera S, Musa A (2003) Improving the production and utilization of cowpea as food and fodder. Field Crops Res 84:169–177

Sobda G, Boukar O, Tongoona PB, Ayertey J, Offei KS (2017) Quantitative trait loci (QTL) for cowpea resistance to flower bud thrips (*Megalurothripssjostedti*Trybom). Int J Plant Breed Genet 4(6):292–299

Spindel J, Begum H, Akdemir D, Virk P, Collard B, Redona E et al (2015) Genomic selection and association mapping in rice (Oryza sativa): effect of trait genetic architecture, training population composition, marker number and statistical model on accuracy of rice genomic selection in elite, tropical rice breeding lines. PLoS Genet 11:e1004982

Steele WM (1972) Cowpeas in Nigeria. Ph. D. Dissertation. University of Reading, UK

Stoilova T, Pereira G (2013) Assessment of the genetic diversity in a germplasm collection of cowpea (Vignaunguiculata (L.) Walp.) using morphological traits. Afr J Agric Res 8(2): 208–215

Tar'an B, Buchwaldt L, Tullu A, Banniza S, Warkentin TD, Vandenberg A (2003) Using molecular markers to pyramid genes for resistance to *ascochyta* blight and anthracnose in lentil (*Lens culinaris*Medik). Euphytica 134(2):223–230

Tesema T, Eshetayehu T (2006) Collection, conservation, characterization and sustainable utilization of Grain Legumes in Ethiopia. In: Proceedings of the workshop on food and forage legumes 22–26 September 2003, Addis Ababa, Ethiopia, pp 15–22

Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. Science 327:818–822

Tilman D, Balzer C, Hill J, Befort BL (2011) Global food demand and the sustainable intensification of agriculture. Proc Natl Acad Sci U S A 108:20260–20264

Tripathi K, Gore PG, Ahlawat SP, Tyagi V, Semwal DP, Gautam NK, Rana JC, Kumar A (2019) Cowpea genetic resources and its utilization: Indian perspective—a review. Legum Res 42 (3):439–448. https://doi.org/10.18805/LR-4146

Ubi BE, Mignouna H, Thottappilly G (2000) Construction of a genetic linkage map and QTL analysis using a recombinant inbred population derived from an intersubspecific cross of Cowpea: *Vignaunguiculta* (L.) Walp. Breed Sci 50(3):161–172

Vaillancourt RE, Weeden NF, Barnard J (1993) Isozyme diversity in the cowpea species complex. Crop Sci 33(3):606–613

Varshney RK, Gaur PM, Chamarthi SK, Krishnamurthy L, Tripathi S, Kashiwagi J, Samineni S, Singh VK, Thudi M, Jaganathan D (2013) Fast-track introgression of "*QTL-hotspot*" for root traits and other drought tolerance traits in JG11, an elite and leading variety of chickpea. Plant Genome 6:1–9

Varshney RK, Saxena RK, Upadhyaya HD (2017) Whole-genome resequencing of 292 pigeonpea accessions identifies genomic regions associated with domestication and agronomic traits. Nat Genet 49:1082–1088

Varshney RK, Singh VK, Kumar A, Powell W, Sorrells ME (2018) Can genomics deliver climate-change ready crops? Curr Opin Plant Biol 45:205–211

Verdcourt B (1970) Studies in the Lepminosue-Papilionoideae for the Flora of Tropical East Africa. IV Kew Bull 24:507–569

Verma AK, Deepti S (2016) Abiotic stress and crop improvement: current scenario. Adv Plants Agric Research 4:345–346

Wang C, Hu S, Gardner C, Lubberstedt T (2017) Emerging avenues for utilization of exotic germplasm. Trends Plant Sci 22:624–637

Wubneh WY (2016) Biological control of chickpea pod borer, HelicoverpaarmigeraHubner (Lepidoptera: Noctuidae): a global concern. World Science News 45(2):92–110

Zhou Q, Fu D, Mason A, Zeng Y, Zhao C, Huang Y (2014) In silico integration of quantitative trait loci for seed yield and yield-related traits in *Brassica napus*. Mol Breed 33:881–894. https://doi.org/10.1007/s11032-013-0002-2

Fenugreek, A Legume Spice and Multiuse Crop Adapted to a Changing Climate

5

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Abstract

Fenugreek (*Trigonella foenum-graecum*), known as methi in much of South Asia, is a widely used spice and vegetable crop. Fenugreek is a multiuse legume crop grown in dry and semiarid regions of the developing world. It is an annual, dicotyledonous, self-pollinated plant belonging to the family Fabaceae. It is a diploid with $2n = 16$ and is estimated to have 685 Mbp of genome size. The genus *Trigonella L.* includes about 135 species worldwide and is native to South-Eastern Europe and West Asia. Most widely known as a spice in Europe, it is also widely used medicinally and as a green vegetable or sprout and as a forage crop. Surprisingly it does not yet have a published genome, and refocusing attention on its uses may stimulate much-needed research on this underutilized crop. Key questions a genome will help address are trade-offs in performance among the various uses of fenugreek and improved understanding of its unique secondary metabolite profile.

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Keywords

Spice domestication · Forage crop · Food security · Nutrition

5.1 History, Domestication, Geographic Distribution, and Production Practices

The plant species *Trigonella foenum-graecum* (L, *Fabaceae*) otherwise known as fenugreek, is one of the oldest spices and medicinal plants in the world. Fenugreek is an annual herbaceous legume crop originating from the Mediterranean and western Asia. The crop is grown in many countries, specifically in warm temperate and tropical regions of Europe, North and East Africa, South Asia, China, Latin America, Australia, and North America (Fauquet and Nesterova 2013; Mikić 2015; Chaudhary et al. 2018). India is currently the largest producer of fenugreek; however, it is also produced in several other regions of the world (e.g., Acharya et al. 2007; Żuk-Gołaszewska and Wierzbowska 2017). The key fenugreek producing countries are India, Morocco, Egypt, Ethiopia, and Turkey (Malhotra 2011), and the major importing countries are UAE, Yemen, Japan, South Africa, United Kingdom, the United States, Egypt, Bangladesh, Saudi Arabia, France, Germany, Malaysia, Netherlands, and Nepal (Malhotra 2011). India is the largest producer (nearly over 115 million tonnes produced in 2011 on 93.6 million hectares) and exporter (21.8 million tonnes to other nations), with Rajasthan being the leading state in India for production (Vidyashankar 2014). In the 2017–2018 cropping season in Ethiopia, over 600,000 smallholder households grew fenugreek on 32,587 ha of land with a total production of 43,637 t where 55% is used for home consumption; 12% as saved seeds for planting, and over 30% for sales in the local market (http://www.csa.gov.et/survey-report/category/359-eth-agss-2017). Fenugreek is a sufficiently minor spice for which accurate international trade and consumption figures are not available. Recent international production estimates are provided by Zandi et al. (2017).

The genus *Trigonella* includes about 135 species of herbaceous plants, although further work is needed to verify species boundaries and relationships (Acharya et al. 2006) and some accounts estimate closer to 250 species (e.g., Malhotra 2011). Fenugreek plants have trifoliate and pinnate leaves, can grow erect or bushy reaching heights between 30 and 60 cm (Chaudhary et al. 2018). Flowers can be either white or yellow, depending on the variety, and are indeterminate. The initiation of flowering occurs between 30 and 40 days post planting. After 60–80 days, plants produce long pointed pods containing green or yellow seeds that undergo dehiscence or shattering (Basu et al. 2009). Fenugreek is a self-pollinated diploid ($2n = 16$) with a rate of cross pollination ranging between 2.1 and 7.0%.

While fenugreek is most commonly grown in warm climates such as in India and the Mediterranean region, its ability to adapt to different climates has allowed it to be cultivated in a variety of agroecologies. Overall trends for cultivation have been established, however, fenugreek prefers well-drained, loamy soil with a slightly alkaline pH of around 8–8.5 (Acharya et al. 2007), but it has also been reported to tolerate sandy gravel-rich soils (Petropoulos 2002). In India where it is grown as a winter annual, seeds are sown in early October or November and are spaced 20–30 cm apart (Acharya et al. 2007). Studies performed in western Canada and the United States observed that fenugreek performed best when seeded in spring in late April to early May with similar spacing (Acharya et al. 2007; Islam et al. 2017). Temperate climates that experience mild winters, cool summers, and lack extreme temperatures have been described as optimal for plant growth (Petropoulos 2002). Growing fenugreek in temperate climates with short growing seasons could prove difficult as the crop is frost sensitive and can only tolerate up to 10–15 °C nighttime temperatures (Islam et al. 2017). With climate change, challenges from aspects of a warming climate may grow, such as drought and heat stress and emerging pests. In Ethiopia, fenugreek is grown on waterlogged vertisols dominated highlands toward the end of the rainy season (Late September) and can grow on residual moisture. Some farmers produce fenugreek using irrigation during dry seasons in cool highlands. Fenugreek can be grown as a sole crop, double crop, and intercropped with other crops. Fenugreek plays an important role in crop rotation and improves soil fertility through nitrogen fixation. The crop is used to control parasitic weeds on fava bean (*Orobanche* spp.) through intercropping where root exudates inhibit seed germination of the parasitic weed (Fernández-Aparicio et al. 2008).

5.2 Genetic Diversity and Crop Improvements

Many countries in Europe, East and Southern Africa, West Asia, South East Asia, North Africa, and Australia have fenugreek accessions in their genebanks and research institutions (Zandi et al. 2017). There are 1074 registered varieties globally, which is much less than genebank holds as most accessions are not registered. For example, there are three registered US varieties reported by Zandi et al. (2017), but the USDA repository has 192 accessions. The largest germplasm collections are kept in India followed by Ethiopia, Canada, and Oman. Studies on 207 fenugreek accessions from 13 countries showed high variability to days to flowering (98–147 days) and yield (0.6–2.5 t/ha) where the highest yielding accessions are from countries in West Asia, North Africa, and India indicating availability the diversity needed to develop cultivars suitable for different agroecologies (Malhotra 2011). However, we hope to see more work in the future to develop genetic resources such as mapping populations, core collections, and so on, which have been effectively used by multiple groups in many other crops. When genetic resources are developed and shared internationally, they can allow us to dissect the genetic basis of traits across multiple environments. These genetic resources are truly critical and are most powerful when shared broadly by research communities and empowered by a genome.

To harness the diversity of these collections, a few previous studies have documented high genetic diversity for agronomic and yield traits as well as seed color in fenugreek (McCormick et al. 2009a; Fikreselassie et al. 2012). There are also findings on the existence of genetic diversity for resistance to *Cercospora* leaf spot (*Cercospora traversiana*), powdery mildew (*Erysiphe polygoni*), drought, and frost (Prasad et al. 2014). The genetic diversity among varieties and germplasm accessions has been characterized through the development of molecular markers (RAPD and AFLP markers) as well as transcriptome sequencing. For instance (Kumar et al. 2012) used AFLP and RAPD analysis to assess the genetic diversity and polymorphic levels among five Indian varieties. RAPD markers revealed high levels of polymorphism and genetic diversity (Nei's distance = 23.8%) while ALFP analysis showed lower levels of polymorphism and genetic diversity (Nei's distance = 2.1%). A more recent study by Amiriyan et al. (2019) used SRAP (sequence-related amplified polymorphism) markers to assess diversity in a collection of Iranian accessions. Studies using more recently developed sequencing approaches, such as genotyping by sequencing or whole-genome resequencing are just now being performed, as described later. Such studies in more widely grown legumes, such as chickpea (von Wettberg et al. 2018) and pigeon pea (Varshney et al. 2017) are useful in harnessing diversity held by genebanks and guiding future collection and crossing efforts.

Although there is limited information on the genetics of fenugreek relative to more widely grown legume crops, a couple of studies have gone as far as to characterize genes using next-generation sequencing technology. For example, Vaidya et al. (2013) used whole transcriptome sequencing to identify genes involved in diosgenin biosynthesis in GMV-1, an Indian variety known for producing high levels of diosgenin, while three more recent studies have expanded transcriptomic resources for fenugreek (Ciura et al. 2017, 2018; Shubha and Tomar 2019; Zhou et al. 2019). More recently El-Wahab et al. (2020) produced the first ddRAD dataset of which we are aware for fenugreek, characterizing over 112 genotypes from Egypt with over 38,000 SNPs. In the absence of a genome, homology to *Trifolium* was used to perform a partial GWAS (genome-wide association study). Despite extensive homology in legume genomes, the lack of micro-synteny between *Trigonella* and *Trifolium* hinders the use of linkage to map the genetic basis of traits.

However, a complete genome sequence is not yet available in the public domain in a published form. We find this surprising, as the fenugreek genome is relatively small, at just 685 Mb, about 1.5 times larger than the model legumes, *Medicago truncatula* and *Lotus japonica* (Bauchet et al. 2020). Technically, it is far easier than large legume genomes such as peas, lentils, or groundnuts. Harnessing the power of genomic techniques in fenugreek will be of vital importance for improving and expanding its use as both a leafy green and forage crop in new areas around the world. As shown by recent efforts to develop RADseq markers in fenugreek (El-Wahab et al. 2020), a genome would be extremely useful for mapping SNPs to chromosomal positions. This would facilitate GWAS and QTL mapping, as well as help detangle some of the fascinating complexity of fenugreek secondary chemistry, which we will discuss further in Sect. 5.5.

Mutational breeding has been an important tool for fenugreek breeding. For example, several cultivars have been developed for Western Canada using this approach (Basu et al. 2009). However, the development of a reference genome and the application of gene editing approaches may prove more directed in its potential to directly modify either the complex secondary chemistry or domestication traits like pod dehiscence and seed size, as recent examples at neodomestication have shown in several perennial crops (Van Tassel et al. 2020).

Despite its potential, fenugreek improvement for seed and forage is limited in the world but many countries (India, Canada, Ethiopia, Tunisia, Turkey, Russia, Australia, Romania, France, UK, Greece, Libya, Slovenia, Hungary, Yemen, Syria, Spain, and Egypt) have released cultivars for production (Malhotra 2011). One of the cultivars developed for forage production in temperate North American climates is cv. *Tristar,* which is specifically suited for conditions in Western Canada. This cultivar was developed for its ability to maintain consistently high yields year after year in the temperate climate of Western Canada (Acharya et al. 2007). While this variety produces high yields when the conditions are ideal, it takes around 120 days to reach full maturity, while some locations in Western Canada only experience about 100 days without frost (Acharya et al. 2006). Four more cultivars (AC Amber, CDC Quatro, CDC Canagreen, and CDC Canafen) have been developed and released for Canadian conditions (Thomas et al. 2011).

In Australia, four accessions (A150118, A150147, A150265, and A150292) are recommended for commercial release through selections (McCormick et al. 2009b). In the United States, recent studies have been looking at introducing it as a forage crop in semiarid regions of the country (Islam et al. 2017). In India, 16 cultivars with varying maturity and adaptation areas were released for seed, leaf, and multipurpose uses (Verma et al. 2020). Besides variability for seed and leaf yields, variability in diosgenin content is reported among 13 promising fenugreek genotypes in India (Giridhar et al. 2016). Recently the Bishoftu variety has been released in Ethiopia (Bekele et al. 2020).

The productivity and production of fenugreek are generally lowered by low-yielding landraces, poor production practices, biotic (diseases and insect pests), and abiotic factors like drought and frost. The major biotic stressors are powdery mildew (*Erysiphe polygoni)*, *Cercospora* leaf spot (*Cercospora traversiana*), and wilt/root rot (*Fusarium* spp., *Rhizoctonia* spp.) (Acharya et al. 2007; Malhotra 2011). Bacterial diseases (*Pseudomonas syringae* pv. syringae and *Xanthomonas alfalfa*), viral, and nematodes are reported in affecting fenugreek (Acharya et al. 2007). Insect pests like thrips, aphids, *Lygus* bugs, and pod borers have also been reported to affect fenugreek.

A long-term question for breeding fenugreek is the extent of trade-offs among different uses, on which we elaborate in the following sections. For example, does breeding for forage yield impact the concentration of medicinal secondary metabolites, or alter the flavor of the spice? These questions will all benefit from a greater range of genomic tools, which have not yet been developed. In the face of climate change, these trade-offs among uses may also interact with climatic tolerances. These complex interactions need further investigation as well.

5.3 Uses of Fenugreek as a Leafy Vegetable

Fenugreek (*T. foenum-graecum),* also referred to as methi in Hindi and several other South Asian languages, is commonly used as leafy greens and sprouts in South Asia and wherever the South Asian diaspora has traveled. The fresh leaves are used in salads and cooked with winter vegetables (Wani and Kumar 2018; Basu and Srichamroen 2010). Fenugreek microgreens, known as Samudra methi, are parts of the cuisine in Maharashtra, India. A different species, *T. corniculate*, is grown in Pakistan but used in both Pakistani and Indian dishes, known as kasuri methi. In addition to the leaves being consumed as a raw/fresh or cooked green vegetable, they are also commonly dried to flavor dishes, such as curries, or added to flour in baking.

The leaves of fenugreek contain macro- and micronutrients, vitamins and are considered a good source of potassium, calcium, iron, Zinc, β-carotene, Phosphorus, fiber, antioxidants, and protein (Chaudhary et al. 2018). In Ethiopia, heavy metals like lead and cadmium were found with varying levels of concentration in seeds collected from different parts of the country (Verma et al. 2020). Different authors reported varying values for nutrient profiles of fenugreek seeds and leaves. The nutritional profile of leaves is protein (25%), starch (25.9%), fiber (12.9%), ash (10.8%), lipids (6.5%), and gum (4.3%) (Wani and Kumar 2018). Leaves are also rich in thiamine, riboflavin, ascorbic acid, and nicotinic acid (Wani and Kumar 2018). Different values are reported (Table 5.1) by Wani and Kumar (2016). Leaves can be stored in the refrigerator, or in dried forms, or blanched for 5 min (Wani and Kumar 2018).

5.4 Fenugreek Seeds for Flavor and Spice

Fenugreek seeds are consumed in different countries around the world for a wide variety of purposes including stews with rice in Iran, flavoring cheese in Switzerland, syrup and bitter rum in Germany, mixed seed powder with flour for making flatbread in Egypt, curries and dyes in India), roasted grain as coffee substitute (in Africa), controlling insects in grain storages, and as an ingredient for perfume industries. Fenugreek seeds are rich in carbohydrate (45–60%), lipid (6–10%), protein (20–30%), and also alkaloid (2–3%), fatty acid, vitamins like A, B, and C. However, the most common use of fenugreek is as a spice. The seeds are

Table 5.1 Nutrient profile of fenugreek seeds and leaves (g/100 g) (Adopted from [29])

Nutrients	Seed	Leaf
Carbohydrate	42.3	
Gum	20.9	
Ash	3.38	
Fiber	50	48
Fat	7.9	1
Protein	25.4	4.4
Moisture	7.5	86

either ground into a powder or used whole and are known to have a distinct odor, which has been described as strong, spicy, sweet, and bitter (Wani and Kumar 2018). The seeds or spice is used for making pickles, curry powders, and paste in Indian cuisine (Malhotra 2011). In Ethiopia, besides its wide uses as a spice crop, fenugreek is added to many traditional food preparations, drinks, and baby food. In a number of cuisines from around the world, seeds or powder are added to bread. The raw seeds themselves have been described as having a maple flavor, and extracts are used in flavoring artificial maple syrup (41, Petropoulos 2002).

5.5 Medicinal Roles of Fenugreek Bioactive Compounds

Many studies have shown that fenugreek plays many roles in treating diabetics, obesity, sexual function, ulcer, analgesic, and are also antioxidant and anti-inflammatory roles (Yao et al. 2020). Seeds of fenugreek are more commonly consumed for their nutritional and medicinal benefits; the ability to consume the leaves and sprouts for this same purpose elevates the versatility of the plant as a whole. Extracts from fenugreek shoots demonstrate elevated antimicrobial, antibiotic, and DNA protective properties compared to seeds, suggesting that the leaves are a richer source of antioxidants (Zandi et al. 2017; Wani and Kumar 2018). Some traditional medicines and pharmaceutical uses of seeds and leaves are reviewed by Chaudhary et al. (2018).

Seeds and leaves as well as sprouts have antioxidant properties with health benefits and are the plant parts most often used as traditional medicines (Yao et al. 2020; Omezzine et al. 2014). Fenugreek has a long and rich tradition of use as a medicine for a variety of treatments. For example, fenugreek leaves and seed powders have been used to treat diabetes and indigestion (e.g., Basu and Srichamroen 2010). There is also evidence to suggest that specific components of fenugreek have multiple other nutraceutical properties (Malhotra 2011; Zandi et al. 2017; Yao et al. 2020). The major biologically active compounds with pharmacological effects are phenolics (gallic acid, protocatechuic acid, catechin, gentisic acid, chlorogenic acid vanillic acid), alkaloids, and flavonoids (Chaudhary et al. 2018; Yao et al. 2020). Likely these all have activities, but more work is needed to truly clarify their method of action and interactions.

5.5.1 Antidiabetic Activity

The primary active compounds that have been identified to have an effect are steroidal sapogenins (diosgenin), dietary fibers (galactomannans), and 4-hydroxyisoleucine. Diosgenin is a steroidal sapogenin that is found in the young leaves and mature seeds of fenugreek (Chaudhary et al. 2018) and it has been suggested to have antidiabetic, hypocholesteremic, and anti-inflammatory effects (Kassaian et al. 2009; Uemura et al. 2010). In studies using Streptozotocin-induced diabetic rats, blood glucose levels were significantly decreased compared to the control after a dose of diosgenin was administered orally, in one case for 30 days and another for 45 days (Uemura et al. 2011). Diosgenin has also been found to promote adipocyte differentiation and decrease inflammation in adipose tissues in obese diabetic rats (Srichamroen et al. 2009). Adipocyte differentiation increases the number of smaller adipocytes and prevents adipocyte hypertrophy, and larger adipocytes have been associated with lowered insulin sensitivity and hyperglycemia (Srichamroen et al. 2009). Diosgenin was also identified as the biologically active compound mediating these responses (Srichamroen et al. 2008). Shtriker et al. (2018) found that a diet containing 2% powdered fenugreek seed resulted in improved blood glucose levels in obese diabetic rats through the promotion of adipocyte differentiation and decreased inflammation of adipose tissues.

Dietary fibers, specifically the soluble fiber galactomannan can reduce the blood sugar level in people with type II diabetes by delaying gastric emptying of carbohydrates, and inhibiting digestive enzymes (Gaddam et al. 2015). This is particularly beneficial in managing blood glucose levels for type II diabetics. In addition to having an antihyperglycemic effect, viscous fibers are also thought to play a role in lowering serum cholesterol.

Galactomannans are soluble dietary fibers that makeup 45–60% of fenugreek seeds (Srichamroen et al. 2008). Recent studies have evaluated the response that these specific fibers have on glucose absorption and cholesterol levels. Srichamroen et al. (2008, 2009) used extracted galactomannan from fenugreek seeds to assess in vitro small intestine glucose absorption in lean and obese rats and observed a decrease in glucose uptake as galactomannan concentrations increased. It also lowered plasma triglyceride and cholesterol levels in rats fed a diet with supplemented galactomannan extract for 3 weeks (Gaddam et al. 2015). Other studies looked at the impact of fenugreek fiber on blood glucose and serum insulin levels after being added to a diet for a period of time; galactomannans were found to lower fasting glucose levels and improve glucose tolerance in mice and increase serum insulin levels and decrease fasting plasma glucose levels in prediabetic patients (Meghwal and Goswami 2012). In addition, galactomannans have been found to lower low-density lipoprotein cholesterol (LDLc) in prediabetic patients that consumed a diet supplemented with defatted fenugreek fiber for 3 years (Meghwal and Goswami 2012). Trigonelline, Flavone C-glycosides, and water extracts of fenugreek seeds are known to have antidiabetic activities.

5.5.2 Roles in Managing Obesity

Obesity is becoming a major problem in many countries and fenugreek can play a role in addressing the problem. Obesity drives a number of other afflictions, particularly diabetes but also susceptibility to COVID and other contagious diseases, heart problems, and fertility issues (e.g., Zheng et al. 2020). In studies on model animal and volunteer people, dietary fiber (galactomannan), Trigonelline, and extract of fenugreek seeds showed anti-obesity activity with different mechanisms of action (Gaddam et al. 2015). The anti-glycemic effects of fenugreek may be driven by saponins, which are converted to sapogenins in the digestive tract, as well as by fiber in the seed (Basch et al. 2003). While more research is needed to identify the specific mechanisms, these results suggest these viscous dietary fibers from fenugreek seeds have an effective role in reducing hyperglycemia, improving glucose tolerance, and lowering blood cholesterol levels.

5.5.3 Fenugreek in Ayurvedic, Tibetan, and Chinese Medicine

Fenugreek has long been used in a number of Asian medical systems (Basch et al. 2003). Although some of these uses are covered earlier, it is also worth examining how fenugreek is understood and valued in these ancient knowledge systems. In Ayurvedic thinking, fenugreek is hot, and can therefore be used to treat ailments of a cold disposition (Ninivaggi 2010). Similarly, in Traditional Chinese medicine fenugreek has a warming function, being used to treat Yang deficiency (Maciocia 2015). Its traditional uses include impotence and low libido, helping the kidneys, and being used for abdominal pain and discomfort (Maciocia 2015).

5.6 Fenugreek as a Forage and Feed Crop

While fenugreek is used commonly as a spice or herb, the name *foenum-graecum* translates as “Greek hay”, which suggests that the crop was used as a forage crop in its past, such as in ancient Greece (Petropoulos 2002). Although it isn’t currently popular as a forage crop, it is still grown to feed cattle in some areas of the world and is being explored in others. Countries in the eastern Mediterranean region (Greece and Turkey), and India still grow fenugreek as green fodder or hay for livestock. In Ethiopia, straw is valuable animal feed. As noted in our discussion of crop varieties, cultivars well-adapted to temperate regions such as the Canadian high plains are available and growing in utilization (e.g., Acharya et al. 2007). Other perennial crops require land to be dedicated to one crop for many seasons, whereas an annual crop can serve as a rotation in between crops and allow for an improvement in soil quality (Marques et al. 2020). In a study comparing the nutrient content and yield of fenugreek as forage to that of alfalfa, one of the most commonly used forage crops, Mir et al. (1997) found that fenugreek was comparable to early-bloom alfalfa in all stages of its growth. Like other legume crops, fenugreek is able to fix atmospheric nitrogen, but it is also non-bloating and contains high levels of protein and diosgenin (Mir et al. 1997; Islam et al. 2017). Diosgenin, a steroidal sapogenin, has been found to promote animal growth (Basu et al. 2009) among other benefits. These qualities have created an interest from researchers to pursue fenugreek as a forage crop for beef and dairy in North America and Latin America, however, research is still in its preliminary stages and needs to address a range of questions. In Nebraska, 3 years field trial showed that the average seed and straw yield of 1.7–4.9 t/ha, respectively. Fenugreek is also being used in poultry (Hamid 2018) and fish feed formations because of its high protein content (Roohi et al. 2017). Work on the medicinal properties of fenugreek in livestock is much needed, as its impacts on animals, particularly ruminants, may differ from humans. Evidence suggesting that fenugreek improves the functional fatty acid profile of fenugreek is encouraging in this regard (Sureshkumar et al. 2018) but further work is needed. Similarly, its strong flavor has impacts on meat and dairy quality that need investigation.

As mentioned previously, Basu et al. (2009) used chemical mutation breeding to try and develop a cultivar with determinate growth that would reach full maturity before 100 days while still producing high yields. While they were able to produce determinate lines, yields decreased with later generations. However, they were able to show that mutagenesis was capable of producing lines with traits that were uncommon in current accessions, which suggests that targeted breeding will be useful in future studies looking to improve varieties for use as forage, as well as improving its medicinal or flavor profile.

Fenugreek may have potential in pastures in some challenging agroecologies and restoration plantings. One of us (EBvW) has observed the widespread occurrence of *Trigonella* in saline and gypsum-rich marginal lands in Tunisia and Portugal. These lands, in coastal saline zones (*Sebka* in Tunisian Arabic) and near the edge of the Sahara, are often grazed by livestock, although historically they were not intentionally planted or maintained as pastures and they are too abiotically challenging for other forages, such as *Trifolium* or *Medicago*. Fenugreek or its wild relatives could be used in these settings to improve the grazing values of these lands and to help restore plant diversity to these habitats that have been degraded by overgrazing.

As we noted in the medicinal section earlier, the rich secondary chemistry likely has significant impacts on both animal health and meat and dairy quality and sensory characteristics. We are aware of unpublished reports in Vermont that cows pastured on fenugreek generate off-tasting milk (Heather Darby, personal communication).

5.7 Negative Effects of Fenugreek

In many studies, it has been found that consumption of fenugreek by humans and animals has few negative effects (Yao et al. 2020; Shah and Mir 2004; Srinivasan 2006). Some of the negative effects of fenugreek consumption are an unpleasant smell on the skin; allergy, diarrhea, flatulence, and dizziness (Yao et al. 2020). More importantly, it does have teratogenic effects as well as antifertility and abortifacient effects (Srinivasan 2006; Ouzir et al. 2016). Fenugreek is not usually advised for women during pregnancy since it has potential side effects of stimulating the uterus (Ouzir et al. 2016) and a range of negative impacts on pups of rats that were fed fenugreek in pregnancy (Oufquir et al. 2020). Saponins and alkaloids in fenugreek seeds are anti-nutritional factors but if defatted, they can be consumed by people who cannot tolerate fat in their diet (Bruce-Keller et al. 2020). Heath workers who provide fenugreek as a galactagogue to breastfeeding have noted some side effects in India (Shawahna et al. 2018). The side effects reported were diarrhea and intestinal discomfort. Sevrin et al. (2019) and Kandhare et al. (2019) further examine the side effects of fenugreek in human and animal models.

There are reports of allergic reactions in humans to fenugreek (Kandhare et al. 2019; Fæste et al. 2010; Patil et al. 1997; Claver et al. 2019). Although generally rare, particularly in South Asia and sub-Saharan Africa, allergic reactions can be severe and include anaphylaxis (Kandhare et al. 2019). Most worryingly, there are reports of cross-reactivity with peanuts (groundnuts) where allergic reactions are more common (Claver et al. 2019). The basis of this cross-reactivity needs more investigation, particularly as groundnut allergies increase.

5.8 Response to Climate Change

Climate change will lead to a number of predicted impacts in fenugreek production regions, including elevated temperature and CO_2 having direct impacts, and indirect effects such as increased drought or flooding in some regions, shifted seasonal timing or patterns of precipitation, or the emergence of new pathogens. For many more widely produced crops, efforts to directly measure or model impacts of expected climatic shifts on production are underway (e.g., Saadi et al. 2015; Sivakumar et al. 2005; Ramirez-Villegas et al. 2013; Kozlov et al. 2019), and similar efforts would be welcome for fenugreek. Hozzein et al. (2020) looked at the impacts of elevated CO_2 on fenugreek seed quality, finding that elevated CO_2 improved antioxidant and anti-lipid peroxidase activities of fenugreek seeds. Although the elevated levels were intended as a seed treatment rather than a simulation of expected future CO_2 levels, they are consistent with a body of work in other species on changes in secondary chemistry due to elevated CO_2. Abou-Shleel (2014) planted fenugreek at three dates in Egypt, simulating the differing end-of-season conditions as might be expected with a generally warming climate. Results indicated the highest yield and greatest composition of biologically active compounds with earlier winter planting dates, suggesting that end-of-season heat stress limited productions and leads to losses. Future breeding efforts will likely need to tackle these challenges. Although not specifically about climate change, rather pollution, Xalxo and Keshavkant (2018) examined the response of fenugreek to lead (Pb) and simulated acid rain, finding that fenugreek has moderate tolerance of both (despite lowered yield) through several metabolic responses. As these anthropogenic impacts co-occur with climate change and remediation of polluted land will be essential for food production, understanding these impacts is also critical as part of a strategy of addressing future climatic challenges.

5.9 The Need for Domestication Models for Spices

Over the past decades, there has been growing interest in understanding the genetic basis of the domestication of crops. Work on domestication can both clarify the rich human-associated histories of different crops and provide insight into traits we may want to improve to facilitate further cultivation. Much of the research on crop domestication has focused on staple annual crops, such as cereals, grain legumes, and vegetables. Most of our understanding of domestication focuses either on traits that are essential for cultivation, such as dehiscence of fruits (Ogutcen et al. 2018), loss of dormancy (e.g., Smýkal et al. 2014), reduction in toxicity (Ku et al. 2020), or on inadvertent consequences of domestication such as population genetic bottlenecks (e.g., Warschefsky et al. 2014; Smýkal et al. 2018). In most cultivated legumes, four traits are thought to have been most strongly selected: non-shattering pods and loss of seed dormancy, both of which are essential for ease of cultivation, and an increase in seed size and loss of defensive compounds that both improve the value of these crops (Ku et al. 2020; Ogutcen et al. 2018). However, what is meant by domestication can vary across a spectrum of traits, among taxonomic context and usage by particular researchers. In some cases, particularly cereals and grain legumes, it often refers to traits that both reduce the crop's potential to grow naturally (loss of dispersal and dormancy) but make it a more stable food crop. In other taxa, such as trees domestication may instead be used to indicate an effort simply to cultivate a species instead of wild harvesting it (e.g., Lee et al. 2019) for an example from Sandalwood, a wild-harvested tree with multifaceted uses, not unlike fenugreek, and Carrillo-Galván et al. (2020) for an underutilized and understudied traditional spice crop from Mexico). In many forages, such as the most widely grown legume forages like alfalfa (Muller et al. 2003) and clovers (Trifolium, e.g., Annicchiarico and Carelli 2014), there are long histories of cultivation, as well as shifts in a range of traits (palatability, cyanogenesis as well as biomass, disease resistance, phenology) and population genetic variation consistent with human selection and movement, but not a change in some domestication syndrome traits such as seed dormancy and pod indehiscence. Consistent with viewing domestication as a spectrum, these forages are less domesticated than grain legumes like peas or lentils in the sense that they are less dependent on human cultivators. They are far more likely to persist in areas near where they have been planted as volunteers and potentially weeds. Despite only partial domestication, however, these forages have been remarkedly improved as forages and fodders by modern breeding (reviewed comprehensively by Annicchiarico et al. (2015), with lessons for minor multiuse forages like fenugreek. Yet, further domestication of traits, such as seed hardness and indehiscence, would further improve them for human use (Kissing Kucek et al. 2020).

Despite the growing interest in crop domestication, crops with multiple uses such as fenugreek can make excellent study systems because different selection pressures may have been applied by human cultivators. These different uses have inherent trade-offs. For example, as a spice and herbal medicine, the loss of defensive chemistry was likely not a target of human domestication selection in fenugreek as it was in peas or beans. By increasing seed size, human selection may reduce the flavor and medicinal properties. Furthermore, considering the genetic basis of traits that have been a part of domestication in other taxa can lead to improvements in fenugreek. Many spice crops, forage species, and medicinal plants are more difficult to cultivate due to low emergence and seed dehiscence. In some other forage legumes, such as hairy vetch (*Vicia villosa*), efforts are underway to neo-domesticate the species to improve cultivation and reduce the extent to which it forms a problematic volunteer weed problem for subsequent crops (Kissing Kucek et al. 2020). These same traits would improve fenugreek cultivation (although fenugreek has less dormancy than *Vicia villosa* in our experience). We suspect that domestication selection has improved its lack of seed dormancy in fenugreek although we are not aware of studies showing this in relation to wild *Trigonella*. Investigation of domestication syndrome traits in minor, multiple-facet crops like fenugreek, however, may help clarify the extent to which shared pathways may be involved in these traits, or guide efforts to select other forages or spices for improvement. In particular, the impacts of domestication on secondary chemistry require further investigation.

5.10 Directions for Future Research

Fenugreek is a multiuse legume with broad cultural appeal. It has a long cultural history in many contexts, from the Mediterranean to South Asia, East Africa Highlands, and cool temperate climates like the Canadian high plains. As a minor crop, it would benefit from more statistics on its production, exports, and consumption. When a complete genome sequence is available, the potential for marker-assisted and genomic selection for medicinal properties, different uses, and different agroecologies will increase greatly. However, we think that the genome will be more useful as a research tool if coupled with other research tools. Although controversial in some regions for breeding, gene editing is a powerful research tool. The function of many compounds produced by fenugreek is simply poorly known. Gene editing coupled with a complete genome would provide a legume research model with greater diversity in secondary chemistry than other legume research models such as *Lotus japonicus* or *Medicago truncatula* (von Wettberg et al. 2019). We look forward to a published genome in the coming years, as well as other resources such as mapping populations and mutants. With an understanding of the genetic basis of traits that contribute to the multiple uses of fenugreek, we hope that breeding for its varied uses as a spice, medicinal crop, and for age can be met, with the development of either specialized or multiple-use varieties.

A complete genome for fenugreek will also facilitate comparative work with other legumes, as well as facilitate identification of genes for traits within fenugreek and comparative work with wild *Trigonella* to understand the complexities of the domestication of a multiuse legume. As shown by a RAD-seq study (El-Wahab et al. 2020), performing GWAS in fenugreek is inhibited by the lack of a reference genome. Comparative genomics with other grain, forage, or potentially legumes with nonfood uses based on their unique chemistry such as *Derris* and *Tephrosia* (both used traditionally as sources of rotenone) or *Indigofera* (a source of indigo dye). Comparative work is essential to determining the basis of genetic trade-offs that may exist between different uses of fenugreek (forage, secondary chemistry, etc.). Comparative work also allows any development of fenugreek to assist with improvement or neodomestication of other legumes, such as forages, spices, and so on. Finally, comparative work with peanuts (*Arachis*) can allow insight into the potential causes of allergen cross-reactivity. As allergies to peanuts/groundnuts have increased, understanding the basis of these is critical.

More research is needed on the resilience of fenugreek to the expected consequences of climate change. Will yields be consistent? Will its production areas need to shift? How will phenology change? Is there sufficient drought tolerance, heat tolerance, and other traits in the cultivated gene pool? Answers to these questions are needed to maintain this underappreciated but important crop.

As a medicinal herb with ancient uses, there is likely more value in traditional knowledge than perhaps widely accepted by the scientific community. The warming properties attributed to fenugreek in Ayurvedic and Traditional Chinese medicine appear to focus on its pungent flavor but may also suggest further properties warranting investigation. Studies of interactive effects of fenugreek with other foods or medicines are also needed. Among the potential health benefits, the antidiabetic properties may be particularly important, given the growing prevalence of type II diabetes globally.

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References

Abou-Shleel SM (2014) Effect of air temperature on growth, yield and active ingredients of fenugreek (*Trigonella foenum-graecum*). Nat Sci 12:50–54

Acharya SN, Thomas JE, Basu SK (2006) Fenugreek: an “old world” crop for the “new world”. Biodiversity 7:27–30

Acharya SN, Blade S, Mir Z, Moyer JR (2007) Tristar fenugreek. Can J Plant Sci 87:901–903

Amiriyan M, Shojaeiyan A, Yadollahi A, Maleki M, Bahari Z (2019) Genetic diversity analysis and population structure of some Iranian Fenugreek (*Trigonella foenum-graecum* L.) landraces using SRAP markers. Mol Biol Res Commun 8:181

Annicchiarico P, Carelli M (2014) Origin of Ladino white clover as inferred from patterns of molecular and morphophysiological diversity. Crop Sci 54:2696–2706

Annicchiarico P, Barrett B, Brummer EC, Julier B, Marshall AH (2015) Achievements and challenges in improving temperate perennial forage legumes. Crit Rev Plant Sci 34:327–380

Basu TK, Srichamroen A (2010) Health benefits of fenugreek (*Trigonella foenum-graecum* leguminosse). In: Bioactive foods in promoting health. Academic Press, pp 425–435

Basu SK, Acharya SN, Bandara MS, Friebel D, Thomas JE (2009) Effects of genotype and environment on seed and forage yield in fenugreek ('*Trigonella foenum-graecum* L.') grown in western Canada. Austr J Crop Sci:3:305

Bauchet G, Bett K, Cameron C, Campbell JD, Cannon EKS, Cannon SB, Carlson JW, Chan A, Cleary A, Close TJ, Cook DR, Cooksey AM, Coyne C, Dash S, Dickstein R, Farmer AD, Fernáandez-Baca D, Hokin S, Jones ES, Kang Y, Monteros MJ, Muñoz-Amatriain M, Mysore K, Pislariu CI, Richards C, Shi A, Town C, Udvardi M, von Wettberg EJ, Young ND, Zhao P (2020) The future of legume genetic data resources: challenges, opportunities, and priorities. Legume Sci. https://doi.org/10.1002/leg3.16

Bekele D, Lulseged T, Yadeta D, Kifelew H, Getu A, Demis F, Asmamaw B (2020) Release of fenugreek (*Trigonella foenum-graecum* L.) variety Bishoftu. Afr J Agric Res 16:1726–1729

Bruce-Keller AJ, Richard AJ, Fernandez-Kim SO, Ribnicky DM, Salbaum JM, Newman S, Carmouche R, Stephens JM (2020) Fenugreek counters the effects of high fat diet on gut microbiota in mice: links to metabolic benefit. Sci Rep 10:1245. https://doi.org/10.1038/s41598-020-58005-7

Carrillo-Galván G, Bye R, Eguiarte LE, Cristians S, Pérez-López P, Vergara-Silva F, Luna-Cavazos M (2020) Domestication of aromatic medicinal plants in Mexico: Agastache (Lamiaceae)—an ethnobotanical, morpho-physiological, and phytochemical analysis. J Ethnobiol Ethnomed 16:1–6

Chaudhary S, Chaudhary PS, Chikara SK, Sharma MC, Iriti M (2018) Review on fenugreek (Trigonella foenum-graecum L.) and its important secondary metabolite diosgenin. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 46(1):22–31

Ciura J, Szeliga M, Grzesik M, Tyrka M (2017) Next-generation sequencing of representational difference analysis products for identification of genes involved in diosgenin biosynthesis in fenugreek (Trigonella foenum-graecum). Planta 245:977–991

Ciura J, Szeliga M, Grzesik M, Tyrka M (2018) Changes in fenugreek transcriptome induced by methyl jasmonate and steroid precursors revealed by RNA-Seq. Genomics 110:267–276

Claver A, Morales M, Elduque C, Navarro B, Botey E, Fernandez MC, Pineda F, Cistero-Bahima A (2019) Fenugreek: a hidden allergen to consider in peanut allergic patients. J Allergy Clin Immunol 143:AB241

El-Wahab A, Mustafa MH, Aljabri M, Sarhan MS, Osman G, Wang S, Mabrouk M, El-Shabrawi HM, Gabr AM, El-Haliem A, Ahmed M (2020) High-density SNP-based association mapping of seed traits in fenugreek reveals homology with clover. Genes 11:893

Fæste CK, Christians U, Egaas E, Jonscher KR (2010) Characterization of potential allergens in fenugreek (*Trigonella foenum-graecum*) using patient sera and MS-based proteomic analysis. J Proteome 73:1321–1333

Fauquet-Alekhine-Pavlovskaya E, Nesterova IM (2013) Fenugreek in the ecological areas of Belarus and France. Int J Agric Biosyst Eng 7:758–762

Fernández-Aparicio M, Emeran AA, Rubiales D (2008) Control of Orobanche crenata in legumes intercropped with fenugreek (*Trigonella foenum-graecum*). Crop Prot 27:653–659

Fikreselassie M, Habtamu Z, Nigussie A (2012) Genetic variability of Ethiopian fenugreek (*Trigonellafoenum graecum* L.) landraces. J Plant Breed Crop Sci 4:39–48

Gaddam A, Galla C, Thummisetti S, Marikanty RK, Palanisamy UD, Rao PV (2015) Role of Fenugreek in the prevention of type 2 diabetes mellitus in prediabetes. J Diabetes Metab Disord 14:74

Giridhar K, Kumari SS, Rajani A, Sarada C, Naidu L (2016) Identification of potential genotypes of fenugreek in rainfed vertisols for yield and diosgenin content. Indian J Agric Res 50(4). https://doi.org/10.18805/ijare.v0iOF.8603

Hamid HHA (2018) Effect of feeding of adding fenugreek oil on the performance and blood serum profile of broiler chicks (Doctoral dissertation, Sudan University of Science & Technology)

Hozzein WN, Saleh AM, Habeeb TH, Wadaan MA, AbdElgawad H (2020) CO_2 treatment improves the hypocholesterolemic and antioxidant properties of fenugreek seeds. Food Chem 308:125661

Islam MA, Obour AK, Rule DC, Bandara M, Acharya SN (2017) Forage and seed production potential, nutritive value, and fatty acid profile of fenugreek. Crop Sci 57:1764–1172. https://doi.org/10.2135/cropsci2016.08.0685

Kandhare AD, Thakurdesai PA, Wangikar P, Bodhankar SL (2019) A systematic literature review of fenugreek seed toxicity by using ToxRTool: evidence from preclinical and clinical studies. Heliyon 5:e01536. https://doi.org/10.1016/j.heliyon.201

Kassaian N, Azadbakht L, Forghani B, Amini M (2009) Effect of fenugreek seeds on blood glucose and lipid profiles in type 2 diabetic patients. Int J Vitamin Nutr Res 79:34–39

Kissing Kucek L, Riday H, Rufener BP, Burke AN, Eagen SS, Ehlke N, Krogman S, Mirsky SB, Reberg-Horton C, Ryan MR, Wayman S (2020) Pod Dehiscence in Hairy Vetch (Vicia villosa Roth). Front Plant Sci 11:82

Kozlov K, Singh A, Berger J, Bishop-von Wettberg E, Kahraman A, Aydogan A, Cook D, Nuzhdin S, Samsonova M (2019) Non-linear regression models for time to flowering in wild chickpea combine genetic and climatic factors. BMC Plant Biol 19:1–4

Ku YS, Contador CA, Ng MS, Yu J, Chung G, Lam HM (2020) The effects of domestication on secondary metabolite composition in legumes. Front Genet 2020:11

Kumar V, Srivastava N, Singh A, Vyas MK, Gupta S, Katudia K, Vaidya K, Chaudhary S, Ghosh A, Chikara SK (2012) Genetic diversity and identification of variety-specific AFLP markers in fenugreek (Trigonella foenum-graecum). Afr J Biotechnol 11:4323–4329

Lee DJ, Burridge AJ, Page T, Huth JR, Thompson N (2019) Domestication of northern sandalwood (Santalum lanceolatum, Santalaceae) for Indigenous forestry on the Cape York Peninsula. Aust For 82(Suppl. 1):14–22

Maciocia G (2015) Foundations of Chinese medicine: a comprehensive text for acupuncturists and herbalist. Elsevier Health Science

Malhotra SK (2011) Fenugreek (*Trigonellafoenum-graecum* L.). In: Singh RJ (ed) Genetic resources, chromosome engineering, and crop improvement: medicinal plants. CRC Press, pp 801–846

Marques E, Kur A, Bueno E, von Wettberg E (2020) Defining and improving the rotational and intercropping value of a crop using a plant–soil feedbacks approach. Crop Sci 60:2195–2203

McCormick KM, Norton RM, Eagles HA (2009a) Phenotypic variation within a fenugreek (*Trigonellafoenum-graecum* L.) germplasm collection. I. Description of the collection. Genet Resour Crop Evol 56:639–649

McCormick KM, Norton RM, Eagles HA (2009b) Phenotypic variation within a fenugreek (*Trigonellafoenum-graecum* L.) germplasm collection. II. Cultivar selection based on traits associated with seed yield. Genet Resour Crop Evol 56:651–661

Meghwal, Goswami (2012) A Review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. J Food Process Technol 3(9). https://doi.org/10.4172/2157-7110.1000181

Mikić M (2015) Brief but alarming reminder about the need for reintroducing 'Greek hay' (*Trigonellafoenum graecum* L.) in Mediterranean agricultures. Genet Resour Crop Evol 62: 951–958

Mir Z, Acharya SN, Mir PS, Taylor WG, Zaman MS, Mears GJ, Goonewardene LA (1997) Nutrient composition, in vitro gas production and digestibility of fenugreek (*Trigonella foenum-graecum*) and alfalfa forages. Can J Anim Sci 77:119–124

Muller MH, Prosperi JM, Santoni S, Ronfort J (2003) Inferences from mitochondrial DNA patterns on the domestication history of alfalfa (Medicago sativa). Mol Ecol 12:2187–2199

Ninivaggi FJ (2010) Ayurveda: a comprehensive guide to traditional Indian medicine for the West. Rowman & Littlefield, New York, USA

Ogutcen E, Pandey A, Khan MK, Marques E, Penmetsa RV, Kahraman A, Von Wettberg EJ (2018) Pod shattering: a homologous series of variation underlying domestication and an avenue for crop improvement. Agronomy 8:137

Omezzine F, Daami-Remadi M, Ladhari A, Haouala R (2014) Variation in phytochemical content and antifungal activity of *Trigonella foenum-graecum* L. with plant developmental stage and ploidy level. South Afr J Bot 92:120–125

Oufquir S, Ait Laaradia M, El Gabbas Z, Bezza K, Laadraoui J, Aboufatima R, Sokar Z, Chait A (2020) *Trigonella foenum-graecum* L. sprouted seed extract: its chemical HPLC analysis, abortive effect, and neurodevelopmental toxicity on mice. Evid-Based Complem Altern Med 2020. https://doi.org/10.1155/2020/1615794

Ouzir M, El Bairi K, Amzazi S (2016) Toxicological properties of fenugreek (Trigonella foenum graecum). Food Chem Toxicol 96:145–154

Patil SP, Niphadkar PV, Bapat MM (1997) Allergy to fenugreek (*Trigonella foenum graecum*). Ann Allergy Asthma Immunol 78(3):297–300

Petropoulos GA (2002) Fenugreek: the genus Trigonella medicinal and aromatic plants—industrial profiles, vol 11. CRC Press. https://doi.org/10.1017/CBO9781107415324.004

Prasad R, Acharya S, Erickson S, Thomas J (2014) Identification of Cercospora leaf spot resistance among fenugreek accessions and characterization of the pathogen. Austr J Crop Sci 8:822–830

Ramirez-Villegas J, Jarvis A, Läderach P (2013) Empirical approaches for assessing impacts of climate change on agriculture: the EcoCrop model and a case study with grain sorghum. Agric For Meteorol 170:67–78

Roohi Z, Imanpoor MR, Jafari V, Taghizadeh V (2017) The use of fenugreek seed meal in fish diets: growth performance, haematological and biochemical parameters, survival and stress resistance of common carp (*Cyprinus carpio* L.). Aquaculture Res 48:1209–1215

Saadi S, Todorovic M, Tanasijevic L, Pereira LS, Pizzigalli C, Lionello P (2015) Climate change and Mediterranean agriculture: impacts on winter wheat and tomato crop evapotranspiration, irrigation requirements and yield. Agric Water Manag 147:103–115

Sevrin T, Alexandre-Gouabau MC, Castellano B, Aguesse A, Ouguerram K, Ngyuen P, Darmaun D, Boquien CY (2019) Impact of fenugreek on milk production in rodent models of lactation challenge. Nutrients 11:2571

Shah MA, Mir PS (2004) Effect of dietary fenugreek seed on dairy cow performance and milk characteristics. Can J Anim Sci 84:725–729

Shawahna R, Qiblawi S, Ghanayem H (2018) Which benefits and harms of using fenugreek as a galactogogue need to be discussed during clinical consultations? A Delphi study among breastfeeding women, gynecologists, pediatricians, family physicians, lactation consultants, and pharmacists. Evid Based Complement Alternat Med. https://doi.org/10.1155/2018/2418673

Shtriker MG, Hahn M, Taieb E, Nyska A, Moallem U, Tirosh O, Madar Z (2018) Fenugreek galactomannan and citrus pectin improve several parameters associated with glucose metabolism and modulate gut microbiota in mice. Nutrition 46:134–142

Shubha R, Tomar RS (2019) Transcriptome sequencing, De novo assembly from three developmental growth stages in fenugreek (*Trigonella foenum-graecum* L.). J Pharmacogn Phytochem 8:1779–1794

Sivakumar MV, Das HP, Brunini O (2005) Impacts of present and future climate variability and change on agriculture and forestry in the arid and semi-arid tropics. In: Increasing climate variability and change. Springer, Dordrecht, pp 31–72

Smýkal P, Vernoud V, Blair MW, Soukup A, Thompson RD (2014) The role of the testa during development and in establishment of dormancy of the legume seed. Front Plant Sci 5:351

Smýkal P, Nelson MN, Berger JD, Von Wettberg EJ (2018) The impact of genetic changes during crop domestication. Agronomy 8:11

Srichamroen A, Field CJ, Thomson AB, Basu TK (2008) The modifying effects of galactomannan from Canadian-grown fenugreek (*Trigonella foenum-graecum* L.) on the glycemic and lipidemic status in rats. J Clin Biochem Nutr 43:167–174

Srichamroen A, Thomson AB, Field CJ, Basu TK (2009) In vitro intestinal glucose uptake is inhibited by galactomannan from Canadian fenugreek seed (*Trigonella foenum graecum* L.) in genetically lean and obese rats. Nutr Res 29:49–54

Srinivasan K (2006) Fenugreek (*Trigonella foenum-graecum*): a review of health beneficial physiological effects. Food Rev Int 22:203–224

Sureshkumar D, Begum S, Johannah NM, Maliakel B, Krishnakumar IM (2018) Toxicological evaluation of a saponin-rich standardized extract of fenugreek seeds (FenuSMART®): acute, sub-chronic and genotoxicity studies. Toxicol Reps 5:1060–1068

Thomas JE, Bandara M, Driedger D, Lee EL (2011) Fenugreek in western Canada. Am J Plant Sci Biotech 5:32–44

Uemura T, Hirai S, Mizoguchi N, Goto T, Lee JY, Taketani K, Nakano Y, Shono J, Hoshino S, Tsuge N, Narukami T (2010) Diosgenin present in fenugreek improves glucose metabolism by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues. Mol Nutr Food Res 54:1596–1608

Uemura T, Goto T, Kang MS, Mizoguchi N, Hirai S, Lee JY, Nakano Y, Shono J, Hoshino S, Taketani K, Tsuge N (2011) Diosgenin, the main aglycon of fenugreek, inhibits LXRα activity in HepG2 cells and decreases plasma and hepatic triglycerides in obese diabetic mice. J Nutr 141:17–23

Vaidya K, Ghosh A, Kumar V, Chaudhary S, Srivastava N, Katudia K, Tiwari T, Chikara SK (2013) De novo transcriptome sequencing in *Trigonellafoenum graecum* L. to identify genes involved in the biosynthesis of diosgenin. Plant Genome 6(2)

Van Tassel DL, Tesdell O, Schlautman B, Rubin MJ, DeHaan LR, Crews TE, Streit Krug A (2020) New food crop domestication in the age of gene editing: genetic, agronomic and cultural change remain co-evolutionarily entangled. Front Plant Sci 11:789

Varshney RK, Saxena RK, Upadhyaya HD, Khan A, Yu O, Kim C, Rathore A, Seon D, Kim J, An S, Kumar V, Anauradha G, Yamini K, Zhang W, Muniswamy Z, Kim B, Penmetsa RV, von Wettberg EJ, Datta SK (2017) Whole genome re-sequencing of 292 pigeonpea cultivars, landraces and wild species accessions provides targets for domestication and genomic regions associated with agronomic traits for crop improvement. Nat Genet 49:1082. https://doi.org/10.1038/ng.3872

Verma N, Verma N, Goyal M, Rawat I (2020) Chapter 30: *Trigonellafoenum-graecum* L. -Fenugreek. In: Medicinal plants in india importance and cultivation. Jaya Publishing House, New Delhi, India

Vidyashankar GK (2014) Fenugreek: an analysis from trade and commerce perspective. Am J Soc Issues Human 16270(10)

von Wettberg EJ, Chang PL, Greenspan A, Carrasquila-Garcia N, Basdemir F, Moenga S, Bedada G, Dacosta-Calheiros E, Moriuchi KS, Balcha L, Mamo B, Singh V, Cordeiro MA, Vance L, Bergmann E, Warschefsky EJ, Marques E, Dinegde KN, Sani SG, Getahun T, Yilmaz MA, Cacmak A, Rose J, Migneault A, Krieg CP, Saylak S, Temel H, Noujdina NV, Friesen ML, Siler E, Linday D, Akhmetov Z, Ozelik H, Kholova J, Can C, Caur P, Yildirim M, Sharma H, Vadez V, Tesfaye K, Woldemedhin AF, Tar'an B, Ayodogan A, Bekun B, Penmetsa RV, Berger J, Kahraman A, Nuzhdin SV, Cook DR (2018) Ecology and community genomics of an important crop wild relative as a prelude to agricultural innovation. Nat Commun 9. https://doi.org/10.1038/s41467-018-02867-z

von Wettberg EJ, Ray-Mukherjee J, Moriuchi K, Porter SS (2019) Medicago truncatula as an ecological, evolutionary, and forage legume model: new directions forward. The Model Legume Medicago truncatula 27:31–40

Wani SA, Kumar P (2016) Development and parameter optimization of health promising extrudate based on fenugreek oat and pea. Food Biosci 14:34–40

Wani SA, Kumar P (2018) Fenugreek: a review on its nutraceutical properties and utilization in various food products. J Saudi Soc Agric Sci. https://doi.org/10.1016/j.jssas.2016.01.007

Warschefsky E, Penmetsa RV, Cook DR, Von Wettberg EJ (2014) Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. Am J Bot 101:1791–1800

Xalxo R, Keshavkant S (2018) Hydrolytic enzymes mediated lipid-DNA catabolism and altered gene expression of antioxidants under combined application of lead and simulated acid rain in Fenugreek (*Trigonella foenum graecum* L.) seedlings. Ecotoxicology 27:1404–1413

Yao D, Zhang B, Zhu J, Zhang Q, Hu Y, Wang S, Wang Y, Cao H, Xiao J (2020) Advances on application of fenugreek seeds as functional foods: pharmacology, clinical application, products, patents and market. Crit Rev Food Sci Nutr 60:2342–2352

Zandi P, Basu SK, Cetzal-Ix W, Kordrostami M, Chalaras SK, Khatibai LB (2017) Fenugreek (*Trigonellafoenum-graecum* L.): an important medicinal and aromatic crop. In: El-Shemy HA (ed) Active ingredients from aromatic and medicinal plants. Cairo University, Giza, Egypt, pp 207–224

Zheng KI, Gao F, Wang XB, Sun QF, Pan KH, Wang TY, Ma HL, Chen YP, Liu WY, George J, Zheng MH (2020) Obesity as a risk factor for greater severity of COVID-19 in patients with metabolic associated fatty liver disease. Metabolism 108:154244

Zhou C, Li X, Zhou Z, Li C, Zhang Y (2019) Comparative transcriptome analysis identifies genes involved in diosgenin biosynthesis in *Trigonella foenum-graecum* L. Molecules 24:140

Żuk-Gołaszewska K, Wierzbowska J (2017) Fenugreek: productivity, nutritional value and uses. J Elem 22:1067–1080

Grass Pea an Inherent Abiotic Stress-Tolerant Legume: Current Status and Future Scope Under Changing Environment

6

Kuldeep Tripathi, Padmavati G. Gore, K. R. Ramya, and Ashutosh Sarker

Abstract

The alarming changes in the climate and global warming have led to a severe effect on agriculture productivity in the long run. Abiotic factors such as heat, drought, salinity, and other stresses pose an increased threat to the existing agriculture. Pulses are the third most widely grown crops globally after cereals and oilseeds, which have their inherent quality of being tolerant to various abiotic and biotic stresses. Grass pea (*Lathyrus sativus* L.) is a climate-smart leguminous crop that requires minimal inputs for cultivation and shows excellent tolerance to the various abiotic stresses like heat, drought, waterlogging, cold, soil erosion, and salinity by different morphological adaptations like rolled leaves, reduced leaf surface, increased root length, increasing soil fertility by nitrogen fixation, having high water use efficiency, and several other inbuilt antioxidant and enzyme mechanisms in the plant. The present chapter emphasized the inherent quality of the grass pea species, tolerant to various abiotic stresses and its multifaceted uses like food, fodder, pharmaceutical, and industrial sectors. The superior nutritional value like high protein, folic acid, and source of L-homoarginine makes it a cheap and wonderful recruit for being included in the agriculture cropping systems under climate changing regime. The sequencing

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of grass pea genome will open an era of functional genomics to understand, identify, and unravel the mechanism of abiotic stress tolerance.

Keywords

Grass pea · Abiotic stress · Genetic resource · Climate resilience

6.1 Introduction

Agriculture depends on the climatic conditions of a specific geographical region. As a result, any changes in climatic conditions directly impact plant growth development and the ultimate products. Ecosystem resilience and food security are currently a concern and attention around the world. Agriculture is severely affected by global warming, and food security is in jeopardy across the globe. Globally, 25% of the total land area is degraded. The earth's temperature is anticipated to rise rapidly, with an average increase of 1.8–4 °C by twenty-first century. As a result, heat stress plays a significant role in plant growth and crop yield. Like this, several other abiotic and biotic factors disrupt the plant–environment balance, significantly impacting crop productivity and food security. Fabaceae is one of the largest genera of food yielding plants. Legumes are more vulnerable to abiotic stresses than cereals (Dita et al. 2006). Matching a crop's phenology to its environment, particularly avoiding drought and heat, is an important aspect of enhancing adaptation and increasing crop yields, and has been a primary emphasis in breeding for local and broad adaptation of all cool-season food legumes around the world (Materne et al. 2011; Khan et al. 2010). Overdependence on a few basic crops and underutilization of other crop plants, and ongoing climate change and soil degradation jeopardize the goal of sustainable agriculture. Among grain legumes, grass pea is the climate-resilient crop with multiple uses in the pharmaceutical, industrial sectors besides being food and fodder. It requires minimal input for cultivation, can grow in any soil from volcanic soils to heavy clays, and adapts to a range of climates. Grass pea is tagged as Future Smart Food (FSF) of South Asia due to its high potential for food, nutrition security, and adaptability (Li and Siddique 2018). It is tolerant to drought, excess moisture, cold or frost tolerance, and fixes atmospheric nitrogen into the soil. Grass pea has a remarkable ability to endure environmental changes and has proven to be a successful stress resilience crop material for study. Understanding stress adaptation in grass pea could open up new avenues for addressing the looming global food crisis. However, grass pea has an ambivalent reputation. It contains a toxin element (β-ODAP) in its plant parts that impedes its acceptance as human food. Efforts are underway at national and global levels to reduce/free toxin content in developing new varieties. Once the appropriate genomic tools are entirely in place, exploring its intrinsic climate-smart features to transfer into other crops will become much more viable.

6.2 History and Importance of the Crop

Lathyrus sativus L. ($2n = 14$), commonly called Grass pea, Khesari, Kalai, Chickling vetch, or Sabberi is an annual pulse crop that belongs to the family Fabaceae, subfamily Papilionoideae, tribe Vicieae. It is a distributed and has been cultivated all over the world since neolithic period. *Lathyrus* species have been discovered as gathered or cultivated goods in archaeological excavations in Turkey and Iraq. Seeds dating back to 2500 BC have been found in India's oldest excavations (Kislev 1989) and the Balkans as early as 8000 BC. Later, these crops expanded to the neighboring Mediterranean and Irano-Turanian regions. They spread to the northern hemisphere's temperate regions, eventually extending into Tropical East Africa and South America (Kupicha 1983). More than 160 annual and perennial species (Plitmann et al. 1995; Chtourou-Ghorbel et al. 2001; Ramya et al. 2022) and subspecies (Allkin et al. 1986) make up the genus *Lathyrus*, which is divided into 15 divisions based on morphological features. The different species and their economic uses are given in Fig. 6.1. It is considered an orphan or underutilized crop as it has received relatively little attention in national or international priorities. Grass pea was selected as one of the priority crops in the Kew's Millennium Seed Bank and the Global Crop Diversity Trust (GCDT) project "Adapting Agriculture to Climate Change" (Dempewolf et al. 2014) and one of the listed crops by ITPGR FA (International Treaty on Plant Genetic Resources for Food and Agriculture).

Grass pea is a herbaceous climbing annual, with a strong taproot system. Its rootlets are covered with tiny, cylindrical, branched nodules, usually densely clustered. The flowers are axillary, solitary, which are blue, white, pink, and variously colored. It has diadelphous (9 + 1) stamens with vexillary stamens, an ovary is sessile with style is upturned and the stigma papillate. Pods are oblong terminated

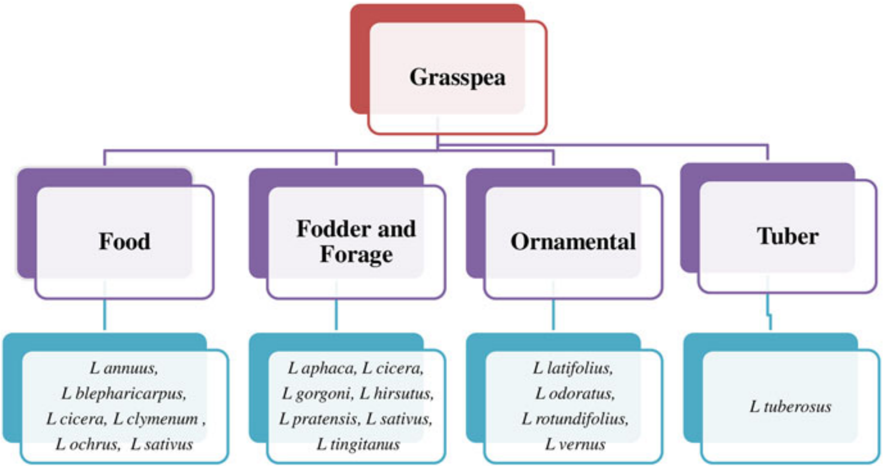

Fig. 6.1 Different species of *Lathyrus* genus and its economic uses. Source: Rizvi et al. (2016) and Tyagi et al. (2017)

with beak and enclose 3–6 seeds per pod. Seed coat color varies from creamy white, gray, brown, black with a mottled, or smooth surface appearance. The grass pea is currently acknowledged as a versatile crop and one of the future's climate-smart crops, and it has achieved the status of a multipurpose crop.

The protein content of seeds is about 26–31.6% (Tamburino et al. 2012; Barpete et al. 2012). Apart from protein, it is a fair source of iron, potassium, magnesium, folic acid, etc. Folic acid content is high in grass pea compared to all other pulses, which will have its role in erythropoiesis (red blood cells) and nucleic acid and protein synthesis and is needed for tissues undergoing proliferation and differentiation. Therefore, it is essential in preventing congenital disabilities (Tamburino et al. 2012). Grass pea is the only known source of L-homoarginine, which helps to cure cardiovascular diseases and treat hypoxia—Alzheimer's disease and long-term potentiation of memory-related neurons (Bell 2003). The presence of phenol phytochemicals in their roots accounts for their radical scavenging activity. Grass pea can be cultivated in the unproductive marginal lands or along hill slopes and during droughts to prevent soil erosion (Tamburino et al. 2012). It could be used as an effective lead phytoextracting species in phytoremediation and rhizofiltration systems (Brunet et al. 2008). Metalloproteases isolated from the dry grass pea seeds are helpful in the biotechnology, food, medical, and pharmaceutical industries (Ramakrishna et al. 2010). The different proportions of grass pea protein isolates and glycerol were used to combine *Lepidium perfoliatum* seed gum to form biodegradable composite biopolymer films (Ebrahimi et al. 2016). The grass pea metabolite β–ODAP (β-N-oxalyl-L-α,β-diaminopropionic acid), which is used as a haemostatic agent following surgery, has also been patented by a Chinese company (Lan et al. 2013). The multiple uses of grass pea are given in Fig. 6.2. Thereby, multifaceted uses of this crop and tolerance of other abiotic stresses would make grass pea a better choice for future sustainable agriculture.

6.3 Abiotic Stress and Grass Pea

After cereals and oilseeds, legumes are the third most widely planted group of crops globally (Tripathi et al. 2020). These crops are essential sources of food, feed, and fodder in various agricultural systems. They are also widely farmed in the semiarid tropics (Popelka et al. 2004). Climate change increases the irregularity and unpredictability of abiotic stress on a global scale, necessitating the development of adaptation mechanisms that target crops to specific environments (Beebe et al. 2011). Abiotic stresses like high temperature, drought, and salt all impact the growth of legumes at different phases of development (Suzuki et al. 2014) and reduce the yield of the plants by more than 50% (Rodriguez et al. 2006). Abiotic stresses influence crop and forage productivity (Boyer 1982; Rao et al. 2013) and the differential distribution of species of plants over the environments (Chaves et al. 2003). The effects of climate change and high temperature in legumes are given in Fig. 6.3. Unfavorable environmental circumstances affect the production of legumes, just as they do many other crops. Researchers have clarified the many

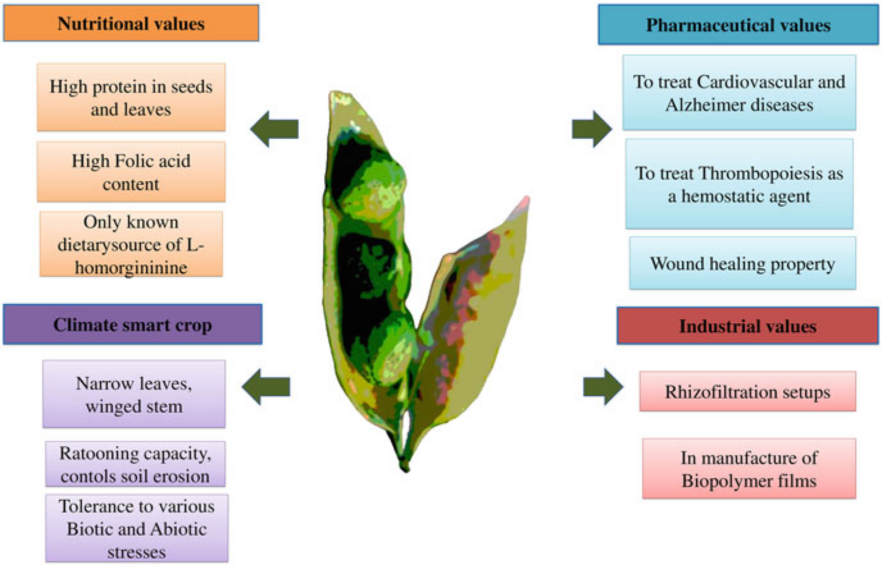

Fig. 6.2 Multiple uses of grass pea

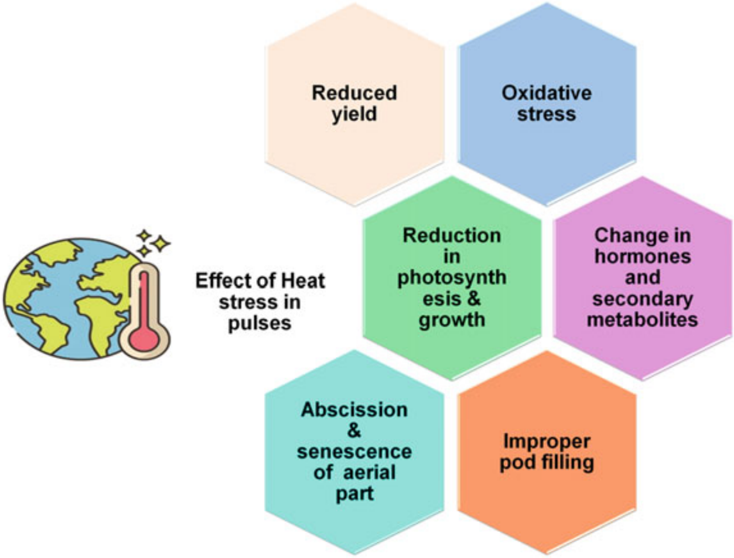

Fig. 6.3 Effects of climate change and heat stress in legumes

physiological and molecular components behind abiotic stress responses in a wide range of species, both model and cultivated crops, over the last decade (Araujo et al. 2015). Stress tolerance-related traits should be identified through breeding. The genetic basis of these critical traits must be elucidated (as significant responsible genes or associated Quantitative Trait Loci, QTLs) using molecular biology and genomics methodologies (Mir et al. 2012). Abiotic stress tolerance mechanism in grass pea is still unexplored (Jiang et al. 2013; Piwowarczyk et al. 2016).

6.3.1 Morphological Adaptations

Grass pea has evolved various morphological drought tolerance qualities as adaptive mechanisms, including narrow leaves, winged stems, and a deep and broad root system compared to other legumes. As a result, the performance of grass peas under drought stress is tightly linked to root system development and distribution (Lambein et al. 2019). The multiple abiotic stress tolerance of grass pea is given in Fig. 6.4. Extended root depth and high root biomass are identified as important traits for extracting soil moisture efficiently (Blum 2011). Moreover, early maturity is an important trait that can avoid drought at terminal stages. To breed for drought tolerance, green leaf area and stomatal openings can be reduced to keep water status higher for photosynthesis maintenance and decrease the transpiration rate, respectively (Cullis and Kunert 2017). Grass pea also has a tendency to reduce its number of flowers and pods under stress, which helps in concentrating its resources in surviving pods (Gusmao et al. 2012). Grass pea also curls or rolls its leaves to reduce water loss through transpiration (Jiang et al. 2013). Leaf curling or rolling was reported as one of the most common responses to water deficit stress in plants (Kadioglu and Terzi 2007). Under drought stress, moderate leaf curling or rolling

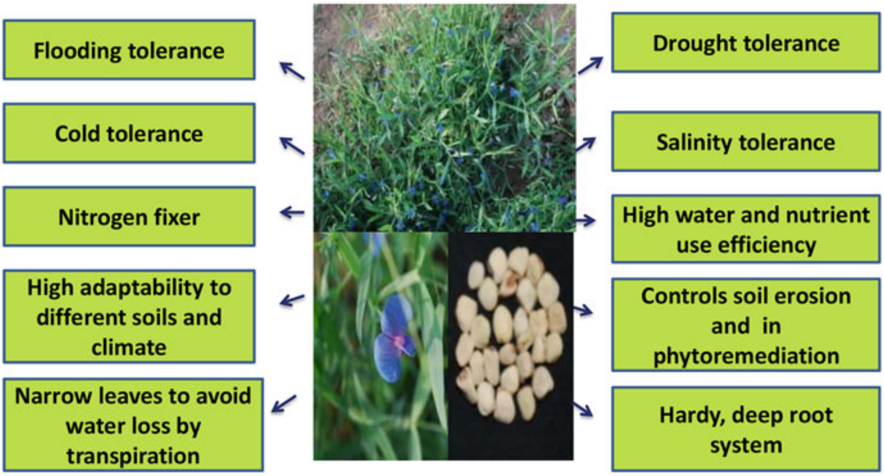

Fig. 6.4 Multiple abiotic stress-tolerant grass pea

improves photosynthetic efficiency, promotes dry-matter buildup, increases yield, lowers the effect of solar radiation on leaves, and lowers leaf transpiration.

Grass pea is already a climate-smart crop. Being resilient to extreme conditions is expected to thrive and produce a crop even if temperatures rise as projected and climatic conditions worsen. One would also hope that it could be brought into more widespread use under such conditions and become a staple legume in, for example, Northern Europe. Even if it were not a crop already, it is also a valuable model for obtaining climate-smart genes because of its resilience. The barrier to such acceptance is due to the presence of β–ODAP, and hence it should be the target trait for breeding programs to develop low or null β–ODAP varieties or cultivars. Examining its inherent climate-smart traits for transfer into other less climate-smart crops will become much more feasible once the appropriate genomic tools are entirely in place. According to Campbell (1997), many of the grass pea features that render it a climate-smart crop are outlined in the following sections.

6.3.2 Flowering Time

Flowering time is a major determinant trait of the time to maturity of any crop plant, depending on the environment and other input factors. Selection for early flowering genotypes has been essential for avoiding terminal abiotic stresses like high temperature and drought in both winter and spring-sown crops (Materne et al. 2011). Genes including Late Flowering (LF), Early Flowering 1 (ELF1), High Response to Photoperiod (HR), and Short Vegetative Phase (SVP) are needed to be targeted to develop varieties that are adaptable to different environments (Sarkar et al. 2019).

6.3.3 Root Characters

Deep taproot and profuse lateral roots of the plant help in greater absorption of water and other essential nutrients. It also helps in mobilizing phosphorous, which is the key nutrient that plays an essential role in pulse productivity. Grass pea has a robust penetrating root system that allows the crop to survive during drought conditions (Campbell et al. 1994) and helps control soil erosion (Tamburino et al. 2012).

6.4 Heat Tolerance

In the present era of climate change, temperature is one of the most important factors impacting legume yield and quality. Heat stress from extreme temperatures and heat waves (multiple days of hot weather in a row) have increased markedly in some locations in the last three decades and are virtually sure to continue during the twenty-first century (Olsson et al. 2014). High temperature alters the cycling of nitrogen and carbon in soils, partly due to impacts on soil microbiota (Souza et al. 2017). High-temperature stress causes problems in plants at all levels of the organization, wreaking havoc on the plant's vegetative and reproductive growth stages (Hamidou et al. 2013). Cool-season annual crop species are more sensitive to hot weather than warm-season annual crops (Hall 1992). High temperatures can wreak photosynthesis, respiration, water relations, membrane stability, and modulate hormone and secondary metabolite levels. In response to heat stress, the vegetative stage exhibits a variety of morphological symptoms, including scorching and sun burning of leaves, twigs, branches, and stems, senescence of leaves followed by abscission, inhibition of shoot and root growth, and discoloration of fruits, all of which can reduce yield significantly (Bita and Gerats 2013). The reproductive growth stage is more susceptible, resulting in a significant loss in yield potential due to the depletion of buds, flowers, fruits, pods, and seeds (Kaushal et al. 2016). Although grass pea has a specific tolerance limit to heat, being a cool-season crop, it is vulnerable to the influence of high temperature. Scientists are working to comprehend the complex reactions that lead to heat tolerance in such challenging environments. The combination of varying degrees of temperature, time, and plant genotype results in a dramatic breakdown of cellular functions. Genes OsSIZ and heat shock proteins of bentgrass or cotton GhHSP26 are targeted to increase crop growth under drought and thermal stresses (Mishra et al. 2017).

6.5 Frost or Cold Tolerance

The varied cool season legumes, including chickpea, lentil, faba bean and field pea were found more prone to injuries by chilling and freezing temperatures, particularly at the flowering, early pod formation and seed filling stages. For many countries, cold or frost tolerance has been an essential feature for improving crop adaptation (Materne et al. 2011). Grass pea is found to have cold and frost tolerance than the other cool-season legumes. Genes including SCOF-1 (Kim et al. 2011) in sweet potatoes, CRLK-1 (Yang et al. 2010) in arabidopsis are overexpressed during low temperatures and confers cold tolerance. Similarly, genes or genomic regions can be identified in legumes for inducing cold tolerance. In a preliminary study, some accessions of *L. sativus* were reported to be more cold-tolerant in Jordan (Hopkinson 1975) and Iraq (Kernick 1976) than local germplasm. However, no large-scale systematic screening was carried out to search genotypes with frost or cold tolerance.

6.6 Drought Tolerance

Drought is a kind of water stress brought on by a lack of rain and insufficient irrigation. Drought affects over 60% of all crop production (Iqbal et al. 2013). The United Nations Convention to Combat Desertification report stated that drought affects about 1.9 billion hectares of land and 1.5 billion people globally and desertification land degradation. Drought stress reduces total biomass, pod number, seed number, seed weight and quality, and seed output per plant in legumes (Pagano 2014). Drought will alter the plant sucrose and sugar levels (Gálvez et al. 2005) that will affect the photosynthesis rate, further reducing the symbiotic nitrogen fixation, and nodule formation in the legume crops (Valentine et al. 2018). Although grass pea is a drought-tolerant crop, genes identified in the other model crops can be explored for the grass pea and legumes. The hardy root system of grass pea facilitates the crop to cultivate under drought conditions compared to many other pulse crops. Negere and Mariam (1994) reported that this crop could survive under prolonged drought conditions at maturity and grain-filling stage. Drought tolerant trait in grass pea leads to adaptation in the arid regions of Asia, including India, Bangladesh, Nepal, Pakistan, China, and the Middle East and North Africa (MENA).

6.7 Flooding or Waterlogging Tolerance

Flooding/waterlogging is a primary abiotic factor that causes loss in the productivity of the pulse crops by affecting different stages including germination, seedling emergence, root and shoot growth, and plant density (Toker and Mutlu 2011). This will decrease the nutrient uptake of the plants. Grass pea shows a good level of tolerance to waterlogging or flooding. Negere and Mariam (1994) reported that the crop could tolerate rainstorms in its early growth stage and extended water stress during the grain filling and terminal stage. This is due to a very hardy root system that allows the crop to nurture under harsher conditions than other leguminous crops. Being tolerant to waterlogging stress, grass pea has gained its lost area in southeast Asia where the seeds are broadcasted into the standing rice crop. It also has been reported from coastal regions of West Bengal, India, bordering with Bangladesh. Due to the onset of frequent cyclones in these coastal areas, growing other crops is not profitable for farmers due to flooding and salt intrusion. Farmers grow grass pea as insurance crops to take higher returns in these affected areas. Large-scale grass pea cultivation in the coastal district of Purba Medinipore, West Bengal, India, is depicted in Fig. 6.5.

6.8 Salinity Tolerance

Salinity is a key abiotic factor limiting agricultural crop germination, vigor, and yield, particularly in dry and semiarid climates (Latef et al. 2014). Salinity is almost present in all countries under all climatic and geographic conditions induced from both manmade and natural causes. It leaves the land unproductive over the years, and it will be aggravated even more in the future due to climate changes. Globally 1128 mha of land is a saline-affected area (Mandal et al. 2018). The impacts of salinity include water stress, nutritional problems, oxidative stress, changes in metabolic processes, membrane disruption, ion toxicity, reduced cell division and development, and reduced nodule formation (Munns and Tester 2008; Shanker and Venkateswarlu 2011; Djanaguiraman and Prasad 2013). Grass pea has a great potential to be used as a source of multiple stress-tolerant genes for crop improvement with appropriate breeding approaches (Hao et al. 2017), and it has tolerance to

Fig. 6.5 Large-scale grass pea cultivation in rice fallow of coastal district, Purba Medinipur, West Bengal, India

salinity like sweet sorghum (Ding et al. 2018). Increased phenolic compounds and peroxidase activities elevated the antioxidant enzymes in the root cells, which might be the critical factor behind the salinity tolerance in grass pea (Jiang et al. 2013; Piwowarczyk et al. 2016).

6.9 Antioxidant Activity

The higher content of phenols and flavonoids and lower abundance of parabanic acid in the grass pea show its antioxidant potential. Both nonenzymatic and enzymatic components, as well as osmoprotectants, have been documented to activate antioxidant defence, and metabolic changes such as ABA-responsive stomatal closure, have been observed in grass pea (Jiang et al. 2013). One of the strategies for scavenging hydroxyl radicals has been proposed as an increase in polyamines content in the grass pea (Xiong et al. 2002). Jiang et al. (2013) reported that grass pea has stronger defense responses as compared to garden pea, with upregulated expression of genes encoding antioxidant enzymes like ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX). Moreover, alteration of mitochondrial calcium (Van Moorhem et al. 2010) and ß-ODAP also plays a crucial role in imparting resilience to multivariate stress conditions through reduction of cytosolic reactive oxygen species (ROS) (Fikre et al. 2006; Jiao et al. 2011; Rao 2011).

6.10 Water Use Efficiency

Drought, flooding, submergence, and salinity tolerance are linked to this trait, and some of the genes are identified to control all four abiotic stresses (Karaba et al. 2007). The PsbS gene from the *Nicotiana benthamiana* is the recent target to get this gene into grass pea to increase the water use efficiency (Głowacka et al. 2018). The deep-rooted system of the grass pea renders the plants in increased absorption of water.

6.11 Nitrogen Fixation

Legumes help improve soil composition and structure and microbial community activity, which benefits future crops (Kumar et al. 2018). Therefore, as an efficient nitrogen fixer with up to 60–124 kg/ha in dry conditions (Schulz et al. 1999), it meets its nitrogen requirements and positively benefits subsequent crops. Grass pea has the immense potential to grow as a rice-fallow pulse crop in northern India. Out of 11.6 mha fallow areas in India, at least 0.5 mha can be brought under grass pea cultivation to boost income for farmers as a second crop and improve land productivity (Tripathi et al. 2021). The climate-smart focus here is to increase grass pea use, particularly in crop rotations or as a rice-fallow crop, or to transfer its natural ability to fix atmospheric nitrogen to other plants, such as cereals, so minimizing carbon loss from soils (Sarkar et al. 2019).

6.12 Future Perspectives

Despite the tag of neurolathyrism associated with grass pea, it can be considered a neolithic crop with an optimistic future. The development of grass pea as a safe crop for human consumption will necessitate an ODAP content of zero or very low ($<0.1\%$). Several improved varieties of food legumes with abiotic stress tolerance have been developed using biotechnological approaches to resistance breeding. These approaches have no substitutes, and they will remain the mainstay in the future. Modern techniques like genomics will be helpful to study legume responses to abiotic stresses. Proteomics and metabolomics are proving to be excellent methods for uncovering previously unknown plant pathways. There is a lot of interest in using these approaches to understand better crop stress responses (Pandey et al. 2016). However, successful application of "omics" to abiotic constraints needs knowledge of stress responses at the molecular level, including gene expression to protein or metabolite and its phenotypic effects. These cutting-edge methods will aid our efforts to identify candidate genes and pathways that are responsible for important features in the β-ODAP metabolism. It will be extremely useful in crop breeding efforts. All accessible genetic resources in global and regional gene banks must be comprehensively characterized. The genetic control of economic traits should be clarified, and a dense linkage map along with genomic data for *Lathyrus* species should be created. Following targets to be accomplished for making grass pea from marginalized to a mainstream crop.

1. Phenotypically and genotypically well-characterized germplasm.
2. Genetic enhancement using crop wild relatives (CWR).
3. Iterative core set and trait-specific reference sets for major abiotic stresses.
4. Biochemical profiling of entire germplasm.
5. Whole-genome sequence of dominant grass pea variety.
6. Genomic resources in the form of SNP markers and candidate genes for important traits.
7. Markers/genes for critical traits for genomics assisted breeding.
8. Superior recombinants/pre-breeding lines for marker-assisted introgression of potential traits into desirable genetic backgrounds.

References

Allkin R, Macfarfarlane T, White R, Bisby F, Adey M (1986) Names and synonyms species and subspecies in the Vicieae: Issue 3. Vicieae Database project. Experimental Taxonomic Information Products Publication (7)

Araujo SS et al (2015) Abiotic stress responses in legumes: strategies used to cope with environmental challenges. Crit Rev Plant Sci 34(1–3):237–280

Barpete S, Dhingra M, Parmar D, Sairkar P, Sharma N (2012) Intraspecific genetic variation in eleven accessions of Grass Pea using seed protein profile. Sci Secure J Biotechnol 1:21–27

Beebe S, Ramirez J, Jarvis A, Rao IM, Mosquera G, Bueno G, Blair M (2011) Genetic improvement of common beans and the challenges of climate change. In: Yadav SS, Redden RJ, Hatfield JL, Lotze-Campen H, Hall AE (eds) Crop adaptation to climate change, 1st edn. Wiley, New York, pp 356–369

Bell EA (2003) Nonprotein amino acids of plants: significance in medicine, nutrition, and agriculture. J Agric Food Chem 51(10):2854–2865

Bita C, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci 4:273

Blum A (2011) Drought resistance and its improvement. In: Plant breeding for water-limited environments. Springer, New York, NY, pp 53–152

Boyer JS (1982) Plant productivity and environment. Science 218:443–448

Brunet J, Repellin A, Varrault G, Terryn N, Zuily-Fodil Y (2008) Lead accumulation in the roots of grass pea (*Lathyrus sativus* L.): a novel plant for phytoremediation systems? C R Biol 331(11): 859–864

Campbell CG (1997) Grass pea, *Lathyrus sativus* L, vol 18. Bioversity International

Campbell C, Mehra R, Agrawal S, Chen Y, Moneim AA, Khawaja H, Yadov C, Tay J, Araya W (1994) Current status and future strategy in breeding grasspea (*Lathyrus sativus*). In: Expanding the production and use of cool season food legumes. Springer, pp 617–630

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought- from genes to the whole plant. Funct Plant Biol 30:239–264

Chtourou-Ghorbel N, Lauga B, Combes D, Marrakchi M (2001) Comparative genetic diversity studies in the genus Lathyrus using RFLP and RAPD markers. Lathyrus Lathyrism Newslett 2(2):62–68

Cullis C, Kunert KJ (2017) Unlocking the potential of orphan legumes. J Exp Bot 68(8):1895–1903

Dempewolf H, Eastwood RJ, Guarino L, Khoury CK, Müller JV, Toll J (2014) Adapting agriculture to climate change: a global initiative to collect, conserve, and use crop wild relatives. Agroecol Sustain Food Syst 38(4):369–377

Ding T, Yang Z, Wei X, Yuan F, Yin S et al (2018) Evaluation of salt-tolerant germplasm and screening of the salt-tolerance traits of sweet sorghum in the germination stage. Funct Plant Biol 45:1073–1081. https://doi.org/10.1071/FP18009

Dita MA et al (2006) Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. Euphytica 147(1):1–24

Djanaguiraman M, Prasad V (2013) PV Effects of salinity on ion transport, water relations and oxidative damage. In: Ecophysiology and responses of plants under salt stress. Springer, New York, NY, pp 89–114

Ebrahimi SE, Koocheki A, Milani E, Mohebbi M (2016) Interactions between Lepidium perfoliatum seed gum–Grass pea (*Lathyrus sativus*) protein isolate in composite biodegradable film. Food Hydrocoll 54:302–314

Fikre A, Lambein F, Gheysen G (2006) A life-saving food plant producing more neurotoxin under environmental stress. Commun Agric Appl Biol Sci 71(1):79–82

Gálvez L, González EM, Arrese-Igor C (2005) Evidence for carbon flux shortage and strong carbon/nitrogen interactions in pea nodules at early stages of water stress. J Exp Bot 56(419): 2551–2561

Głowacka K et al (2018) Photosystem II Subunit S overexpression increases the efficiency of water use in a field-grown crop. Nat Commun 9(1):1–9

Gusmao M et al (2012) Water deficit during the reproductive period of grass pea (*Lathyrussativus* L.) reduced grain yield but maintained seed size. J Agron Crop Sci 198(6):430–441

Hall AE (1992) Breeding for heat tolerance. Plant Breed Rev 10:129–168. https://doi.org/10.1016/j.eja.2004.07.005

Hamidou F, Halilou O, Vadez V (2013) Assessment of groundnut under combined heat and drought stress. J Agron Crop Sci 199:1–11. https://doi.org/10.1111/j.1439-037x.2012.00518.x

Hao X et al (2017) An RNA sequencing transcriptome analysis of Grasspea (*Lathyrussativus* L.) and development of SSR and KASP markers. Front Plant Sci 8:1873

Hopkinson D (1975) Technical report on the development of crop husbandry in the dry farming areas around Karak. Jordan 169/518. FAO, Rome

Iqbal M et al (2013) Inducing drought tolerance in upland cotton (*Gossypium hirsutum* L.), accomplishments and future prospects. World Appl Sci J 21(7):1062–1069

Jiang J, Su M, Chen Y, Gao N, Jiao C, Sun Z, Li F, Wang C (2013) Correlation of drought resistance in grass pea (*Lathyrussativus*) with reactive oxygen species scavenging and osmotic adjustment. Biologia 68:231–240

Jiao C-J et al (2011) β-ODAP accumulation could be related to low levels of superoxide anion and hydrogen peroxide in *Lathyrussativus* L. Food Chem Toxicol 49(3):556–562

Kadioglu A, Terzi R (2007) A dehydration avoidance mechanism: leaf rolling. Bot Rev 73(4): 290–302

Karaba A et al (2007) Improvement of water use efficiency in rice by expression of HARDY, an Arabidopsis drought and salt tolerance gene. Proc Natl Acad Sci 104(39):15270–15275

Kaushal N et al (2016) Food crops face rising temperatures: an overview of responses, adaptive mechanisms, and approaches to improve heat tolerance. Cogent Food Agric 2(1):1134380

Kernick MD (1976) Rainfed forage crops in northern Iraq 1: agronomy and performance of promising rainfed forage crops at field crop and livestock stations and farmer's cooperatives. Tech Rep IRQ 71/542. Mimeo

Khan HR et al (2010) Faba bean breeding for drought-affected environments: a physiological and agronomic perspective. Field Crops Res 115(3):279–286

Kim Y-H et al (2011) SCOF-1-expressing transgenic sweet potato plants show enhanced tolerance to low-temperature stress. Plant Physiol Biochem 49(12):1436–1441

Kislev ME (1989) Origins of the cultivation of *Lathyrussativus* and *L. cicera* (Fabaceae). Econ Bot 43(2):262–270

Kumar S et al (2018) Role of legumes in soil carbon sequestration. In: Legumes for soil health and sustainable management. Springer, Singapore, pp 109–138

Kupicha F (1983) The infrageneric structure of Lathyrus. Notes RBG Edinburgh 41:209–244

Lambein F et al (2019) Grass pea (*Lathyrussativus* L.): orphan crop, nutraceutical or just plain food? Planta 250(3):821–838

Lan G, Chen P, Sun Q, Fang S (2013) Methods for treating hemorrhagic conditions. Google Patents

Latef AA, Abdel H, Chaoxing H (2014) Does inoculation with Glomusmosseae improve salt tolerance in pepper plants? J Plant Growth Regul 33(3):644–653

Li X, Siddidque KHM (eds) (2018) Future smart food—rediscovering hidden treasures of neglected and underutilized species for zero Hunger in Asia. FAO, Bangkok

Mandal S et al (2018) Current status of research, technology response and policy needs of salt-affected soils in India—a review. J Indian Soc Coast Agric Res 36(2):40–53

Materne M et al (2011) Breeding for improvement of cool season food legumes. In: Pratap A, Kumar J (eds) Biology and breeding of food legumes. CABI, pp 49–62

Mir RR, Zaman-Allah M, Sreenivasulu N, Trethowan R, Varshney RK (2012) Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. Theor Appl Genet 125:625–645

Mishra N et al (2017) Overexpression of the rice SUMO E3 ligase gene OsSIZ1 in cotton enhances drought and heat tolerance, and substantially improves fiber yields in the field under reduced irrigation and rainfed conditions. Plant Cell Physiol 58(4):735–746

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Negere A, Mariam SW (1994) An overview of grass pea (Lathyrus sativus) production in Ethiopia. In: Abegaz BM, Haimanot RT, Palmer VS, Spencer PS (eds) Nutrition, neurotoxins and lathyrism: the ODAP challenge. Third World Medical Research Foundation, New York, pp 67–72

Olsson L et al (2014) Cross-chapter box on heat stress and heat waves. Climate change 2014: Impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. In: Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, pp 109–111

Pagano MC (2014) Drought stress and mycorrhizal plant. Use of microbes for the alleviation of soil stresses, vol 1. Springer, New York, NY, pp 97–110

Pandey MK, Roorkiwal M, Singh VK, Ramalingam A, Kudapa H, Thudi M et al (2016) Emerging genomic tools for legume breeding: current status and future prospects. Front Plant Sci 7:455. https://doi.org/10.3389/fpls.2016.00455

Piwowarczyk B, Tokarz K, Kamińska I (2016) Responses of grass pea seedlings to salinity stress in in vitro culture conditions. Plant Cell Tissue Organ Culture (PCTOC) 124(2):227–240

Plitmann U, Gabay R, Cohen O (1995) Innovations in the tribe Vicieae (Fabaceae) from Israel. Israel J Plant Sci 43(3):249–258

Popelka JC, Terryn N, Higgins TJV (2004) Gene technology for grain legumes: can it contribute to the food challenge in developing countries? Plant Sci 167:195–206. https://doi.org/10.1016/j.plantsci.2004.03.027

Ramakrishna V, Rajasekhar S, Reddy LS (2010) Identification and purification of metalloprotease from dry grass pea (*Lathyrus sativus* L.) seeds. Appl Biochem Biotechnol 160(1):63–71

Ramya KR, Tripathi K, Pandey A et al (2022) Rediscovering the potential of multifaceted orphan legume grasspea-a sustainable resource with high nutritional values. Front Nutr 8. https://doi.org/10.3389/fnut.2021.826208

Rao SLN (2011) A look at the brighter facets of β-N-oxalyl-L-α, β-diaminopropionic acid, homoarginine and the grass pea. Food Chem Toxicol 49(3):620–622

Rao IM, Beebe SE, Polania J, Ricaurte J, Cajiao C, Garcia R, Rivera M (2013) Can tepary bean be a model for improvement of drought resistance in common bean? Afr Crop Sci J 21:265–281

Rizvi AH, Sarker A, Dogra A (2016) Enhancing grass pea (Lathyrus sativus L.) production in problematic soils of South Asia for nutritional security. Indian J Genet Plant Breed 76:583–592. https://doi.org/10.5958/0975-6906.2016.00074.2

Rodríguez M, Canales E, Borroto CJ, Carmona E, Lopez J, Pujol M et al (2006) Identification of genes induced upon water-deficit stress in a drought-tolerant rice cultivar. J Plant Physiol 163: 577–584. https://doi.org/10.1016/j.jplph.2005.07.005

Sarkar A et al (2019) Grass Pea: remodeling an ancient insurance crop for climate resilience. In: Genomic designing of climate-smart pulse crops. Springer, Cham, pp 425–469

Schulz S, Keatinge J, Wells G (1999) Productivity and residual effects of legumes in rice-based cropping systems in a warm-temperate environment: I. Legume biomass production and N fixation. Field Crops Res 61(1):23–35

Shanker A, Venkateswarlu B (eds) (2011) Abiotic stress in plants: mechanisms and adaptations. BoD–Books on Demand

Souza RC et al (2017) Responses of soil extracellular enzyme activities to experimental warming and CO_2 enrichment at the alpine treeline. Plant Soil 416(1):527–537

Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R (2014) Abiotic and biotic stress combinations. New Phytol 203:32–43. https://doi.org/10.1111/nph.12797

Tamburino R, Guida V, Pacifico S, Rocco M, Zarelli A, Parente A, Di Maro A (2012) Nutritional values and radical scavenging capacities of grass pea ('*Lathyrus sativus*' L.) seeds in Valle Agricola district, Italy. Aust. J Crop Sci 6(1):149–156

Toker C, Mutlu N (2011) 16 Breeding for abiotic stresses. In: Biology and breeding of food legumes, p 241

Tripathi K, Gore PG, Singh M et al (2020) Legume genetic resources: status and opportunities for sustainability. Legume crops-prospects, production and uses. Intech Open, pp 1–16

Tripathi K, Pamarthi RK, Gowthami R, Gore PG, Gayacharan C, Barpete S et al (2021) Deciphering morpho-taxonomic variability in Lathyrus species. Ind J Plant Genet Resour 34:279–289. https://doi.org/10.5958/0976-1926.2021.00027.9

Tyagi R, Pandey A, Agrawal A et al (2017) Regional expert consultation on underutilized crops for food and nutritional security in Asia and the pacific thematic, strategic papers and country status reports. Asia-Pacific Association for Agricultural Research Institutions (APAARI), Bangkok

Valentine AJ, Benedito VA, Kang Y (2018) Legume nitrogen fixation and soil abiotic stress: from physiology to genomics and beyond. Ann Plant Rev Online:207–248

Van Moorhem M et al (2010) l-β-ODAP alters mitochondrial Ca2+ handling as an early event in excitotoxicity. Cell Calcium 47(3):287–296

Xiong LM, Schumaker KS, Zhu JK (2002) Cell signaling during cold, drought, and salt stress. Plant Cell 14:S165–S183

Yang T et al (2010) A calcium/calmodulin-regulated member of the receptor-like kinase family confers cold tolerance in plants. J Biol Chem 285(10):7119–7126

Breeding Groundnut Cultivars for Resilience to Climate Change Effects

7

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Abstract

The major effects of climate change such as variation in annual rainfall, drought, high temperature, and elevated CO_2 affect the morphology, physiology, pod yield, and quality of groundnut. Genetic variability in groundnut is reported for tolerance to drought and heat stress as well as for other key traits such as photosynthetic rate, daily growth partitioned to pods, and seed-filling duration for pods that are important traits for climate change adaptation. Development of climate-resilient groundnut cultivars is an environmentally sustainable approach that requires understanding the responses and coping mechanisms under climate change scenarios and its genetics. Modeled projections showed that by 2050, −34 to 43% change in groundnut yields will occur across various regions in India due to climate change, indicating yield gains in some regions while losses in others. Tolerance to drought and heat is a complex trait, and the co-occurrence of these two stresses can exaggerate the consequences on yield and quality in the rainfed agro-ecologies where groundnut is largely grown. This chapter reviews various climatic factors, namely, drought, heat stress, and elevated CO_2 affecting groundnut productivity and quality under the changing climate scenario, and genetic interventions to breed improved groundnut cultivars with resilience to climate change effects.

Keywords

Groundnut · Abiotic stress · Heat stress · Drought stress · Climate resilience · QTL

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7.1 Introduction

Groundnut or peanut (*Arachis hypogaea* L.) is an oil, food, fodder, and feed crop of the world. It is the third most important oilseed crop after soybean and rapeseed and cultivated in 29.59 million hectares with a total production of 48.75 million metric tons (FAO 2019). Major groundnut growing countries are China followed by India, Nigeria, and United States. It is cultivated in about 113 countries, but major cultivation is confined to tropical and subtropical regions of the world. Comparison of groundnut area and production over the last decade reveal slight increase; however, the productivity was stable mainly due to increased use of genetically superior cultivars that are better adapted to a particular agro-ecological region and adaptation of better cultural management practices (FAO 2019) (Fig. 7.1).

Groundnuts are a good source of oil and protein and are rich in fiber, minerals, vitamins, and other bioactive compounds (resveratrol, tocopherol, arginine, etc.) with health benefits, hence often referred to as functional food (Arya et al. 2016; Variath and Janila 2017). The nutrient composition of groundnut kernels consists of crude fat 31–46%, crude fiber 1.4–3.9%, protein 20.7–25.3%, carbohydrates 21–37%, ash 1.2–2.3%, and moisture 4.9–6.8% (Alhassan 2018). Several health benefits of groundnut are documented such as the reduced risk of cardiovascular diseases (Guasch-Ferré et al. 2017), obesity, diabetes (Jackson and Hu 2014), and cancer (Nieuwenhuis and van den Brandt 2019). Groundnut and groundnut-based food products such as Ready-to-Use Therapeutic Food (RUTF) and Ready-to-Use Supplementary Food (RUSF) are widely used to overcome malnutrition in several developing and underdeveloped countries (UNICEF 2007).

Globally, about 60% of harvested groundnut goes to oil mills for oil extraction and the remaining 40% is used for consumption and seed purposes (Janila et al. 2013). The priority of edible oil for a healthy human diet has increased the world's remarkable demand for groundnut production. Owing to the changing climatic scenario and the exponential rise in population (9.7 billion by 2050), the agriculture

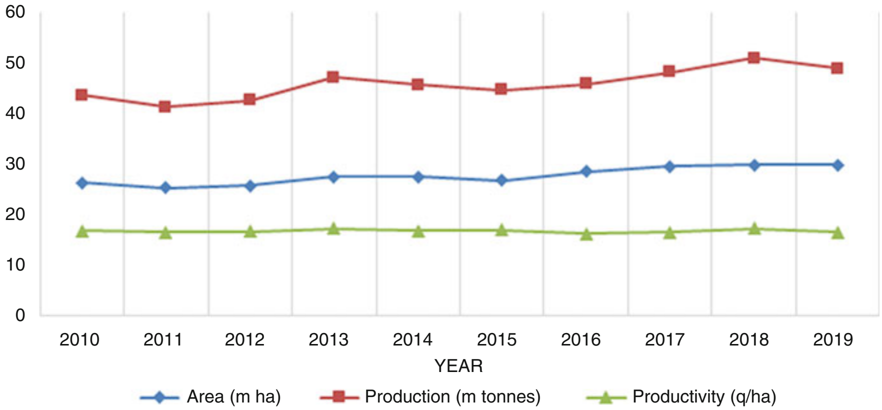

Fig. 7.1 World area, production and productivity of groundnut over the last decade (FAO 2019)

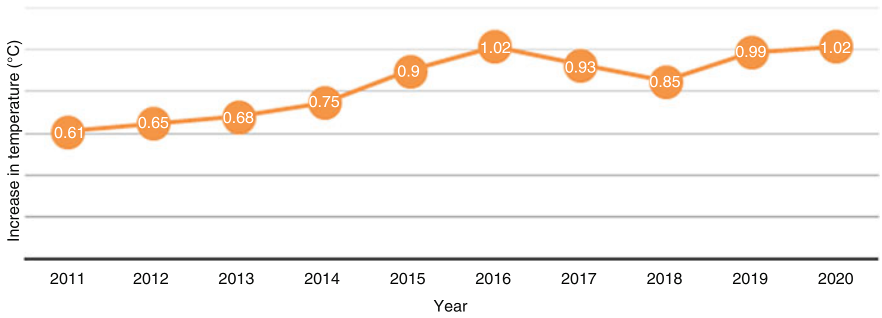

Fig. 7.2 The trend of temperature change in the last decade in the world (https://climate.nasa.gov)

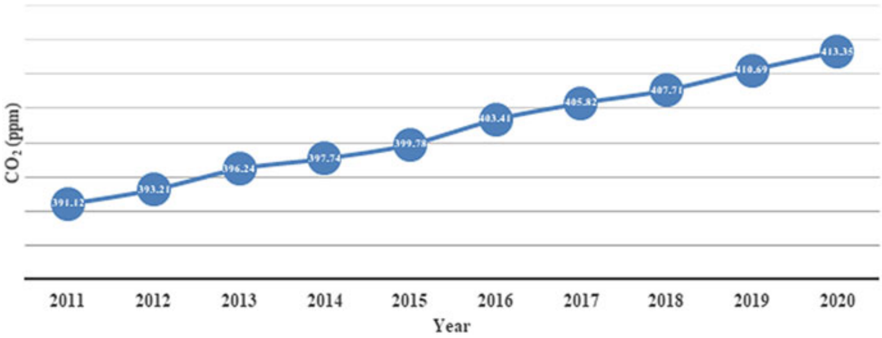

Fig. 7.3 The trend of carbon dioxide (CO_2) change in the last decade in the world (https://climate.nasa.gov)

sector would be tremendously affected (Cole et al. 2018). Globally, the three major impacts of climate change on agriculture are the shift in the hydrological cycle, rising temperatures (heat stress), and increasing levels of atmospheric carbon dioxide (CO_2) (IPCC 2014). The changes in the hydrological cycle can lead to intense floods and droughts in many agricultural regions and cause major damage by destroying the entire crop. Over the next 30–50 years, the average temperature will increase by at least 1 °C and the CO_2 concentration will rise to about 450 parts per million by volume (IPCC 2014). It is estimated that even a small temperature change could reduce crop productivity in dry tropical regions. Due to climate change, there could be a decrease in 3–5% yield for most of the cereals (rice, maize, wheat, sorghum, and barley) and legumes (soybean and cowpea) in Asia, Africa, and Latin-American countries (Lobell et al. 2008). In groundnut, modeled projection by 2050 showed a yield change of −34% to 43% across the growing regions in India owing to climate change scenarios (Kadiyala et al. 2021). A global increasing trend of temperature and carbon dioxide (CO_2) was observed in the last decade (Figs. 7.2 and 7.3).

Groundnut can be cultivated across various regimes of temperature, rainfall, and on a variety of soils. In Asia and Africa, groundnut is largely cultivated by marginal farmers on resource-depleted farms. The use of old cultivars, poor crop management practices, poor soil fertility, pests and diseases, unreliable rainfall, and drought are major constraints in groundnut production affecting the smallholder farmers. As most of the groundnut cultivation is restricted to the tropical regions where the climate factors such as temperature and drought are already at a critical level for groundnut production, any further change in conditions can potentially alter yield and deteriorate the quality of the produce. Heat stress and drought are the major abiotic stresses limiting groundnut production (Prasad et al. 2010). Modeled projections shows that by 2050, climate change under an optimistic scenario will result in −2.3 to 43.2% change in groundnut yields across various regions in India when climate alone was factored in. But the change in groundnut yields ranged from −0.9 to 16.2% when economic (population and income) and market variables (elasticities, trade, etc.) were also considered. Similarly, under the pessimistic climate change scenario, the percentage change in groundnut yields would be −33.7 to 3.4% with only the climate factored in and −11.2 to 4.3% with the additional economic and market variables included (Kadiyala et al. 2021). Improving genetic traits by increasing maximum leaf photosynthesis rate, partitioning daily growth to pods, and seed filling duration can contribute to climate resilience. The chapter reviews the effect of drought, heat stress, and elevated CO_2 on groundnut productivity and quality under the changing climate scenario, responses to these changing factors, and genetic interventions to breed climate-resilient groundnut cultivars.

7.2 Drought

Rainfall is an important climatic factor for groundnut production as 70% of groundnut crop is cultivated under rainfed areas (FAO 2002). Groundnut can be grown in areas with rainfall ranging from 200 to 1000 mm (Prasad et al. 2010) and is considered as a moderately drought-tolerant crop, but changes in rainfall patterns affect its growth and development (Chakraborty et al. 2015a). Groundnut yield was drastically reduced under moisture stress conditions due to delay in flower bud formation, reduction in the number of flowers and pods, and extended growth period (Dai et al. 2019).

Based on the crop growth period of groundnut during which drought occurs, drought is classified into early-season, mid-season, and end-of-season drought (Nigam et al. 2005). The characteristics of these three types of drought along with their effects on the crop are given in Table 7.1.

Various methods based on field, pot, rainout shelters, chamber houses, rhizotrons, lysimeter, and in vitro conditions are used for drought screening in groundnut. The most common method used in field screening is the empirical approach that involves evaluating genotypes under water-stress and well-watered conditions. Water-stress condition is stimulated in the field by withholding irrigation for a particular period and the field screening is generally conducted in the post-rainy season to avoid the confounding effects of rainfall. Genotypes with high productivity combined with minimum yield penalty under stress (resilience) are selected. Wilt scoring is also

Table 7.1 Types of drought and their effects on groundnut crop

Type of drought	Stage	Effect on groundnut	References
Early season drought (from sowing to the flowering stage)	20–25 DAE	Poor germination and seedling establishment in groundnut	Farooq et al. (2009)
Mid-season drought (pegging, pod, and seed development)	40–80 DAE	Restricts the penetration of peg into the soil and also reduces the formation of pods and seeds	Haro et al. (2010)
End-season drought	80 DAE	Affects seed development and predisposed the pods to aflatoxin contamination	Arunyanark et al. (2009)

DAE Days after emergence

used as a surrogate trait to score genotypes for drought adaptation. The empirical approach is often combined with the trait-based/physiological screening approach. Field screening is the best method to identify drought-tolerant genotypes. However, the lack of control on climate and other variables can often lead to biased selections. Growth chambers, where all the environmental factors are controlled, can be used but screening of a large number of genotypes is resource intensive.

The rain-out shelters can be used to screen the genotypes during the rainy season. These are artificial structures that are very useful for the induction of drought by excluding natural precipitation in the experimental plots. These are harbored with rain sensors and cover the crop during rains. Quantifying root length and a better understanding of the root ecosystem using special scanners called rhizotrons helps in identifying drought-tolerant genotypes (Mohamed et al. 2017). Evaluating plant response at early stages can be possible through in vitro screening with polyethylene glycol (PEG), a chemical desiccator that acts by mimicking to drought stress by lowering water potential by creating osmotic stress in the root zones of plants (Ahmad et al. 2020). Lysimeters, which are (polyvinyl chloride) PVC tubes filled with soil, can be used to measure the plant water use efficiency (WUE). It acts as a bridge between field and laboratory-based screening and measures precise WUE data on water consumption concerning yield.

7.2.1 Genetic and Physiological Mechanism for Drought Tolerance in Groundnut

Drought tolerance is a complex trait. The agronomic traits related to drought tolerance are dry weight, harvest index (HI), seed weight, canopy conductance, carbon discrimination ratio, and root traits (Shanker et al. 2014; Li et al. 2014), whereas physiological traits are transpiration (T), transpiration efficiency (TE), specific leaf area (SLA), relative water content (RWC), membrane stability, and SPAD chlorophyll meter reading (SCMR) (Painawadee et al. 2009; Thakur et al. 2013; Pandey et al. 2014; Mahantesh et al. 2018; Devi et al. 2019; Oppong-Sekyere et al. 2019). The biochemical compounds associated with drought tolerance are chlorophyll content, abscisic acid (ABA), superoxide dismutase (SOD) activity, and proline content (Furlan et al. 2020; Zhu et al. 2016).

Various studies have been taken up to examine the effect of drought on the yield and quality of groundnut and also the influence of water stress on various physiological and biochemical traits. The ICRISAT groundnut cultivar ICGS-1 can recover moderately from mid-season drought (Nigam et al. 1991). An investigation was taken up with 184 accessions of mini-core collection of groundnut at ICRISAT and identified 18 superior accessions with relatively high values for SLA, RWC, and SCMR (Upadhyaya et al. 2011). Assessment of 20 groundnut genotypes with lysimeter under severe, medium, and mild stress revealed that pod yield decreased at the rate of 70%, 55%, and 35%, respectively (Ratnakumar and Vadez 2011). Screening of eight groundnut genotypes for drought tolerance using SCMR, canopy temperature (CT), canopy temperature depression (CTD), and normalized difference vegetation index (NDVI) expressed Tifguard and Tifrunner as the best genotypes for drought tolerance mechanism (Luis et al. 2016).

Genetic variability was reported among groundnut genotypes (Thakur et al. 2013; Kakeeto et al. 2020) for root traits, SLA, and SCMR, and the genotypes B-4, Baidehi, ICGV 98089, ICGV 99171, ICGV 00440, and ICGV 97100 were identified as drought tolerant with high pod yield under stress (Thakur et al. 2013). Studies reported high heritability for dry biomass weight, SCMR, plant height, days to flowering, days to maturity, seed yield under water-deficient condition (Girdthai et al. 2012; Oppong-Sekyere et al. 2019), and low to moderate broad-sense heritability (0.2–0.5) for root and drought-tolerant traits (Painawadee et al. 2009). Inheritance studies showed that SLA and HI are controlled by additive genes (Nigam et al. 2001; Suriharn et al. 2005).

Drought studies revealed that oil content is not influenced by moisture stress (Sharma and Singh 1987) and early or late season drought does not affect oil, protein, and mineral content of kernels (Conkerton et al. 1989). Stress during maturation reduces the Oleic/Linoleic (O/L) ratio by lowering the percentage of oleic acid (18:1) and increasing linoleic acid (18:2) (Hashim et al. 1993; Chakraborty et al. 2015a). Under drought stress, there is an alteration in the oil/protein ratio in legumes due to the accumulation of proteins and carbohydrates faster than oil in the pods (Kambiranda et al. 2011). Glucose, fructose, and sucrose content decreased significantly in the leaf tissue and in the kernels, whereas the sugar alcohols like inositol, mannitol, and trehalose increased in leaf and kernels under stress. Water stress leads to more aflatoxin contamination of mature kernels compared to the crop grown under irrigated conditions (Pettit et al. 1971; Wilson and Stansell 1983; Sanders et al. 1993).

There is a dramatic decrease in chlorophyll content under moisture stress conditions compared to well-watered conditions in groundnut (Sun et al. 2010). A study using drought-tolerant (TAG 24) and drought-susceptible (JL 24) cultivars under controlled and water-deficit regime created with 20% PEG 6000 revealed that seven metabolic pathways such as starch and sucrose metabolism, galactose metabolism, fructose and mannose metabolism, propanoate metabolism, pentose and glucuronate interconversion, biosynthesis of unsaturated fatty acids and amino sugar and nucleotide sugar metabolism are significantly affected under water-deficient condition (Gundaraniya et al. 2020).

7.2.2 Breeding for Drought Tolerance

Drought tolerance is a complex trait controlled by polygenes and significantly influenced by genotype × environment (G × E) interaction effects (Pasupuleti et al. 2013). Most common methods for drought breeding rely on screening of advanced genotypes under field conditions. Studies showed the influence of genotype, environment, and G X E interactions on traits such as SLA, WUE (Basu 2004), haulm yield, pod yield and HI (Hamidou et al. 2013), and WUE, SLA, and SCMR (Lal et al. 2019). Selection based on drought tolerance efficiency (DTE), stress tolerance index (STI), drought susceptibility index (DSI) for pod yield per plant, and percentage change of performance in yield traits identified ICGV 07262, ICGV 07219, and ICGV 07268 with high pod yield, high DTE and low DSI (Arunachalam and Kannan 2013). Terminal drought showed a significant decrease in pod yield (Junjittakarn et al. 2014); hence, it is desirable to select the genotypes with the least yield penalty under drought stress, and some genotypes, ICGV 06319 and ICGV 92206, showed high pod yield with low transpiration under both well-watered and stress conditions (Bacharou Falke et al. 2019). GGE, AMMI, and simultaneous selection index (SSI) under drought stress could be useful to select stable performing high pod yielders in breeding programs (Lal et al. 2019).

7.2.3 Genomic Tools for Drought Tolerance in Groundnut

The complex nature of drought tolerance and resource-intensive field screening methods necessitates genomic tools for selection. QTLs have been reported for surrogate traits of drought by various researchers. A genetic map was constructed using a Recombinant inbred line (RIL) population (TAG 24 × ICGV 86031) composed of 318 F_8/F_9 plants with 1145 SSR markers identified 2–5 QTLs for TE, SLA, and SCMR with phenotypic variation (PV) of 3.5–14.1% (Varshney et al. 2009) (Table 7.2). Genetic analysis of groundnut with 191 SSR loci using TAG 24 × ICGV 86031 population identified 8 epistatic QTLs with 1.7–8.34% PV using QTLNetwork (Ravi et al. 2011). The influence of several minor effect QTLs suggests the utility of genomic selection (GS) or marker-assisted recurrent selection (MARS) instead of marker-assisted back-cross (MABC) breeding for the deployment of drought QTLs because the transfer of multiple QTLs is difficult with MABC.

Gautami et al. (2012) evaluated three RIL populations ICGS 76 × CSMG 84-1 (RIL-2) and ICGS 44 × ICGS 76 (RIL-3) and a reference map of TAG 24 × ICGV 86031 (RIL-1) with 119, 82, and 191 SSR loci to identify 153 main effect QTLs (M-QTL) and 25 epistatic QTLs (EQTL) associated with 25 drought-tolerance related traits. Extensive phenotypic data of 20 drought-tolerant traits for 8 years

Table 7.2 The QTLs for drought tolerance and its related traits in groundnut

QTLs for drought and its related surrogate traits	Number of QTLs	Phenotypic variation explained (PVE%)	Major QTLs	Parents used	Reference
T, TE, SCMR, SLA	38	2.90–17.60	6	TAG 24 × ICGV 86031	Varshney et al. (2009)
T, TE, SCMR, SLA, DW, TDM, Carbon discrimination ratio, Canopy conductance	105	3.28–33.36	8 EQTLs	TAG 24 × ICGV 86031	Ravi et al. (2011)
T, TE, SCMR, LA, TDW, LDW	178	1.70–40.10	153 (MQTLs) and 25 (EQTLs)	ICGC 76 × CSMG 84-1; ICGS 44 × ICGS 76 + genetic map of TAG 24 × ICGV 86031	Gautami et al. (2012)
SLA, SCMR	12	8.50–31.20	8	K30076 × K30097	Leal-Bertioli et al. (2016)
20 traits including agronomic, pod, seed, and yield components	13	10.40–20.10	13	Fleur 11 × (*A. ipaensis* × *A. duranensis*)	Fonceka et al. (2012)
DFF, HW, TDM, HI, CC, LN, NB, PW, TE, S%, SDW, LA, SW, TR, SCMR, WUE	20	10.0–33.9	19	TAG 24 × ICGV 86031	Pandey et al. (2020)

MQTLs—major effect QTLs; EQTLs—epistatic QTLs; T—transpiration; TE—transpiration efficiency; SLA—specific leaf area; SCMR—soil plant analytical development (SPAD) chlorophyll meter reading; DW—dry weight; TDM—total dry matter, leaf area, TDW—total dry weight; LDW—leaf dry weight; DFF—days to 50% flowering; HW—Haulm weight; HI—Harvest Index; CC—canopy conductance; LN—leaf number; NB—number of branches; PW—pod weight; S%—shelling %; SDW—shoot dry weight; SW—seed weight; TR—transpiration rate; WUE—water use efficiency

using RIL (TAG 24 × ICGV 86031) population was used for conducting genome-wide QTL analysis to reveal 19 main effects QTLs with PV 10.0–33.9%. This study also identified candidate genes such as malate dehydrogenases, glycosyl hydrolases, MADS-box transcription factor, myeloblastosis (MYB), basic helix-loop-helix (bHLH), NAM, ATAF, and CUC (NAC) and microtubule-associated proteins controlling drought QTLs (Pandey et al. 2020). Drought screening analysis with BC_2F_3 from a cross (Sen That × L18) showed three polymorphic markers

TC4E10, TC4D02, and TC1A02 under water-deficient conditions for wilted level and recovery level of plants and these markers could be linked to drought tolerance (Cuc et al. 2021).

The developments in genomics technologies and the availability of genomic resources in groundnut have facilitated the deployment of genomic tools to understand the molecular mechanisms of drought tolerance. Differentially expressed genes under moisture-deficit conditions in comparison with the well-watered condition in groundnut were reported which can be used as gene-specific markers to identify drought-tolerant genotypes. Differential gene expression analysis of groundnut revealed 43 differentially expressed transcripts under water stress, of which 16 transcripts indicated that 12 genes were completely suppressed due to prolonged water stress, 2 (PTRD—7 and PTRD—8) were upregulated and 2 (PTRD—19 and PTRD—20) were downregulated (Jain et al. 2001).

Transcriptomic analysis of two groundnut genotypes NH5 (drought tolerant) and FH18 (drought-sensitive) showed that NH5 is more stable because of maintenance of osmotic potential in the cells, elimination of reactive oxygen species, and faster stomatal closure through a salicylic acid pathway, all of which are essential for drought-tolerance (Jiang et al. 2021). Comparative expression analysis using C76-16 (C-76) and Valencia-C (Val-C) which are best and poor performers under water-deficient conditions, respectively, showed 4508 differentially expressed genes (DEGs), of which 514 are single-nucleotide polymorphisms (SNPs) and 1554 genes are encoding transcription factors (TFs). The genes controlling ABA and sucrose metabolic pathways play an important role in triggering tolerance mechanism in C-76 and interestingly one SNP coding F-box protein and other SNP coding lipid transfer protein showed polymorphism between the two contrasting genotypes and further validation of these SNPs would help in the screening for drought tolerance at early stages (Bhogireddy et al. 2020).

7.2.4 Short Duration Groundnut Cultivars and Drought Escape Mechanism

To escape from terminal drought or end-of-season drought, early maturity is one of the key traits in groundnut; however, the yield potential of early maturing cultivars is lower than medium and late maturing cultivars. Therefore, when comparing pod yield of cultivars across the maturity durations, it is desirable to compare pod yield per day. To a greater extent, the pod development duration contributes to the total maturity duration of a genotype. Early maturity is a complex trait. Chico, a short-duration cultivar, matures in about 85 days is extensively used in groundnut hybridization programs to develop short-duration commercial cultivars. Cumulative Thermal Time (CTT), measures the heat units accumulated over a period of time during crop season, is useful to measure maturity rather than days after planting as CTT is season neutral. CTT is a very simple method to predict the early maturity in groundnut genotypes (Rao et al. 1992). Groundnut variety ICGV 91114 derived from ICGV 86055 × ICGV 86533 developed at ICRISAT is a popular early maturing variety that copes up with mid-season drought to some extent and escapes end-season drought.

7.3 Heat Stress Tolerance

Temperature, an important environmental factor, determines the growth and development of crop plants. According to the report by Intergovernmental Panel on Climate Change (IPCC), the global mean temperature will increase by 0.3 °C in every 10 years, reaching approximately 1 °C and 3 °C by 2025 and 2100, respectively (Wahid et al. 2007). Under the changing climate scenario, heat stress is considered as a serious threat to crop production and a major challenge in attaining food security.

As groundnut is mostly grown in tropical and sub-tropical regions of the world, the optimal growth temperature is 25–35 °C, and any further rise in temperature above 35 °C will effect pollen viability, fruit set, and crop yield (Chakraborty et al. 2018). In India, an increase in average air temperature of 2–3 °C is predicted to reduce groundnut yields by 23–26% (Akbar et al. 2017). The high temperatures of about 38 °C lead to a reduction in dry matter accumulation, flower production, the proportion of pegs forming pods, and seed mass (Craufurd et al. 2003). High temperature affects the rate of development and duration of various developmental stages in plants by altering the pattern of plant development such as delayed germination, enzyme inactivation in chloroplasts and mitochondria, loss of membrane integrity, loss of viability, increased membrane lipid fluidity, inhibition of protein synthesis, denaturation and protein aggregation, protein degradation, and also affects the organization of cell membranes (Howarth 2005).

Partitioning of photosynthates in pods, affects the pod filling, and consequently, the pod yield is one of the important processes affected by high-temperature stress in groundnut (Akbar et al. 2017). Heat stress influences seed filling by changing the concentration of phytohormones like ABA, cytokinins, and also the expression of enzymes involved in carbon metabolism, starch accumulation, and sucrose synthesis (Ruan et al. 2010). Induced sterility is another effect seen in many plant species under heat stress. At high temperatures, a decrease in pollen germination and pollen tube growth were the most commonly reported factors. Pollen viability was considered as an indirect selection criterion for assessing heat tolerance (Wahid et al. 2007). In groundnut, the reproductive stage is heat-sensitive, while the influence of high-temperature stress on the vegetative stage is not adverse (Hall 1992).

7.3.1 Genetic and Physiological Mechanism of High-Temperature Tolerance in Groundnut

Heat tolerance is a complex trait with multi-gene inheritance which is greatly affected by environmental factors (Sadat et al. 2013) and also involves various genes, hormones, transcriptional factors, and proteins (Singh et al. 2019; Bita and

Gerats 2013). Plants have developed tolerance or avoidance mechanisms depending upon the intensity, frequency, and duration of heat stress (Wahid et al. 2007). Plants can overcome high-temperature stress by dissipating the excess heat through evaporative cooling, maintaining membrane integrity, and accelerated production of heat shock proteins (HSPs), phytohormones such as cytokinins, ABA and antioxidants, and other protective molecules (Bita and Gerats 2013).

Physiological traits such as Specific leaf area (SLA), Leaf Relative Water Content (RWC), Stress tolerance index (STI), and Stress susceptibility index (SSI) (Porch 2006) were used to determine genetic variability for heat tolerance (Weatherley 1950; Nageswara Rao et al. 2001; Porch 2006). Usually, high temperature alters the integrity and functions of the plasma membrane and in particular tertiary and quaternary structure of plasma membrane proteins and increases the fluidity of membranes which causes leakage of ions from cells and leads to cell death. Thus, cell membrane thermostability (CMT), a physiological trait, is used to determine the stability of the plasma membrane at elevated temperatures (Savchenko et al. 2002). Singh et al. (2016) reported that 18% increase in cell membrane thermos ability was observed when seedlings were exposed to high temperatures of 40/35 °C. Preconditioning of plants to high temperature or heat acclimatization can also be used to induce heat tolerance in plants by exposing them to moderate temperatures for a short period (Selvaraj et al. 2011). Membrane lipid composition, leaf hairiness and thickness, and regulation of transpiration rate are some of the factors that help to acclimatize under heat stress (Nautiyal et al. 2008).

In response to heat stress, plants produce reactive oxygen species (ROS) that act as signaling molecules under normal and stress conditions to activate, upregulate, and downregulate many genes involved in different metabolic pathways. The ROS factors like hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), superoxide radical (O_2^-), and singlet oxygen ($^1O_2$) were produced in response to heat stress in plants to cause oxidative damage (Asada 2006). Heat-tolerant plants will produce antioxidant compounds like superoxide dismutase (SOD), catalase, peroxidase, glutathione reductase, and ascorbate reductase as ROS scavenging agents (Kaushal et al. 2016). In groundnut, the oxidative stress was increased in susceptible cultivars compared to tolerant cultivars, and also higher activities of antioxidant enzymes were observed in heat-tolerant cultivars (Chakraborty et al. 2015b). Plants also accumulate low-molecular-weight organic compounds called Thermo protectants, which include sugars, polyols, proline, and glycine betaine under high temperatures (Sakamoto and Murata 2002), and these can be used as indicators to identify heat-tolerant genotypes. Various plant hormones like Auxin, Salicylic acid (SA), and Abscisic acid (ABA) have a role in plant growth and development, under heat stress. SA causes detoxification of superoxide radicals, which prevent oxidative stress and increase heat tolerance (Sakata et al. 2010). Lipid peroxidation, estimated in terms of malondialdehyde content (MDA), expressed in nmol MDA g^{-1}, was used to identify heat-tolerant genotypes (Dionisio-Sese and Tobita 1998).

7.3.2 Breeding for Heat Tolerance in Groundnut

One of the common methods of screening is measuring the pod yield and yield parameters of the genotypes in the field under normal and heat-stress environments; the heat-stress environment can be a location where the temperatures are high or by adjusting the planting date of the experiment in a way to expose the sensitive stages of flowering and pod filling to high temperatures. Temperature Induction Response (TIR), an *in vitro* technique that screens genotypes at seedling stage by exposing to various sub-lethal temperatures of 38-54 °C @ 0.5 °C/10 min for 5 hr. (induction stress), followed by lethal temperatures of 58 °C for 3 hr in WGC 450 programmable plant growth chamber followed by recovery of seedlings at 30 °C with 60% relative humidity for 48 hr. (Kokkanti et al. 2019). The recovered genotypes are classified into tolerant, moderately tolerant, and susceptible based on percentage survival of seedlings, the percentage reduction in absolute root and shoot growth. The main advantage of this technique is its ability to screen large populations in a short period. Detached Leaf Assay is another method to screen the genotypes for heat stress, wherein leaf disks of about 7.5 mm diameter are collected from a third or fourth leaf and exposed to high temperatures. Later, relative chlorophyll content was determined by using SPAD-502 chlorophyll meter, and it identifies the resistant and susceptible genotypes (Selvaraj et al. 2011). Polythene-covered tunnel structure (polytunnel) and modified Saxcil growth cabinets can also be used to screen heat-tolerant genotypes. In these structures, photo- and thermo-period of about 12 h per day is maintained and air and floral bud temperatures is recorded by using copper constantan thermocouples for every 10 seconds interval and means is recorded every 10 min using a data logger (Craufurd et al. 2003).

7.3.3 Genomic Tools for Heat Tolerance in Groundnut

The production of Heat Shock Proteins (HSP) was found in all groups of living organisms and helps in preventing denaturation of newly synthesized proteins under heat stress (Wang et al. 2004; Török et al. 2001). They act as molecular chaperons by regulating several downstream genes associated with heat tolerance (Kumar et al. 2016). Based on their molecular weight, HSPs are classified as small HSPs (15–45 kDa) and large HSPs (60–110 kDa). The expression of HSPs was confined to certain developmental stages like embryogenesis, germination, pollen development, and fruit maturation (Prasinos et al. 2005). HSPs, primarily, play an important role in the folding and conformation of cell membranes and enzymes, that is, structural and functional proteins. They also ensure the regular functioning of different cellular proteins under high temperatures. The differential expression of HSPs is a strategy to adapt to high temperatures and HSPs synthesized will confer heat tolerance (Wahid et al. 2007). Higher HSP expression was reported in heat-tolerant genotype ICGS 44 than heat-susceptible genotypes such as AK 159 and DRG 1 (Chakraborty et al. 2018). HSP 70 & HSP 90 were the predominant HSPs reported in providing stability and imparting heat tolerance in groundnut (Kokkanti et al. 2019) and Arabidopsis (Yamada et al. 2007). Genes responsible for HSP synthesis have been identified and isolated from various plant species, including tomato and maize (Liu et al. 2006; Sun et al. 2006). The heat shock transcription factors (Hsfs) regulate the expression of HSP genes by binding to the conserved motifs of heat shock elements (HSE), which are located in their promoter region (Wang et al. 2017). Hsfs have an N-terminal DNA binding domain (DBD) and oligomerization domain (OD), which is composed of two hydrophobic heptad repeats (HR-A/B). Based on the phylogenetic relationship and structural characteristics of HR-A/B domain, Hsfs were classified into A, B, and C groups. About 20–50 Hsf genes were found in plants, whereas only a few Hsf genes were found in yeast and mammals. Totally, 17 Hsfs were found in cultivated peanut species and named them as *AhHsf1*–*AhHsf16* and *AhHsfX* (Wang et al. 2017). Plants also have developed post-transcriptional mechanisms like miRNAs for developing tolerance to heat stress. Stress-regulated miRNAs can downregulate target genes in heat stress (Jenks et al. 2007).

Due to the complexity of abiotic stress tolerance and difficulty in selecting the phenotype for tolerance, MAS is an effective approach to improve stress tolerance in crops (Foolad 2005). However, this approach requires the identification of genetic markers or QTLs that were associated with genes. Meanwhile, limited research has been carried out in different plant species to identify genetic markers associated with heat tolerance. In Arabidopsis, four gene loci (QTL) determining its ability to acquire heat tolerance were determined using a panel of thermosensitive mutants (Hong and Vierling 2000). In addition, manipulating the expression of genes, proteins, enzymes, or compounds identified by the transgenic method, resulted in plant growth with improved stress tolerance in different plant species (Zhang et al. 2001; Rontein et al. 2002). Genetic screening and genome-wide expression analysis have been used to report the potential role of several genes in response to heat stress. Plants have developed post-transcriptional mechanisms like miRNAs for developing tolerance to heat stress. Stress-regulated miRNAs can down-regulate target genes in heat stress (Jenks et al. 2007). Understanding the role of miRNAs in cellular tolerance and plant phenology under stress conditions will prove useful for the future design of crop resistance mechanisms (Jenks et al. 2007).

7.4 Elevated CO_2 (eCO_2)

The carbon dioxide (CO_2) concentration in the atmosphere is increasing as a consequence of human activities and fossil fuel combustion (Black et al. 2017). Excess atmospheric CO_2 traps heat from the earth's surface and causes an increase in temperature, which in turn leads to global warming and irreversible climate change (Pacheco and Helene 1990; Solomon et al. 2009). Over the last 50 years, CO_2 levels surged from 320 to 390 parts per million volume (IPCC 2007). If current trends in global atmospheric change continue unabated, then by the end of the twenty-first century, it is estimated to even reach up to 750–1000 ppm (IPCC 2007). It is estimated that by 2050, the plants are expected to grow in an atmosphere with 50% more CO_2 than the present (Forster et al. 2007).

Plants exhibit both positive and negative responses to elevated CO_2 (eCO_2) (Misra et al. 2019). Several studies have reported that eCO_2 enhances plant growth, photosynthetic rate, and yield, and the effect on respiration is not apparent (Ainsworth and Long 2005; Vaidya et al. 2014; Jena et al. 2018; Pan et al. 2018). The response of C_3 and C_4 species varies under eCO_2 conditions due to their CO_2 fixation pathways (Hamim 2005). Studies on the effect of eCO_2 on the C_3 crop species has been widely explored in many crop species like rice, wheat, cowpea, groundnut, and soybean, wherein the CO_2 saturation point is much higher than the current atmospheric CO_2 levels (Krishnan et al. 2007; Zhu et al. 2008; Bishop et al. 2015; Dey et al. 2017; Vaidya et al. 2014). It is assumed that the excess accumulation of CO_2 in C_4 plants makes them insensitive to eCO_2 levels (Reddy et al. 2010). Although there is a dire need to boost agricultural productivity under changing climatic conditions, this increased productivity alone could lead to the depletion of nutritional quality of food crops (Broberg et al. 2017; Loladze 2014; Pilbeam 2015), thereby affecting human health. Henceforth, it is very crucial to understand the impacts of eCO_2 on crop growth and development for the future projected environment.

Studies on the responses of plants to eCO_2 have been conducted under laboratory, greenhouse, or controlled conditions. Leaf cuvettes are designed for single leaf gas exchange measurements for studying the short-time effect of eCO_2 levels on the gas exchange processes (Sinclair and Horie 1989; Sinclair and Van Houtte 1979). The controlled growth chamber studies included Sunlit Controlled Environment Chambers (SCEC) which was used for measuring photosynthesis and transpiration as a function of CO_2 concentration, light, temperature, and soil moisture conditions. Furthermore, a holistic approach was developed for CO_2 enrichment studies, namely, Soil plant atmosphere research (SPAR) by Phene et al. (1978), and the design was modified by Jones et al. (1984) to provide more accurate control of dry bulb temperature, CO_2 concentration, the humidity of the canopy air, and measurements of soil water and root conditions. Lee et al. (2000) developed a carbon dioxide temperature gradient chamber (CTGC), modified from a standard temperature gradient chamber (TGC) developed by Mihara (1971). They are made up of zinc-coated semi-circular pipes and PVC film to cover the chambers. CO_2 gas would be injected at a controlled rate automatically by an electronic mass flow controller. This gradient chamber will be useful for understanding plant response to simultaneous changes in CO_2 and temperature. Also, a portable field chamber with a non-dispersive IRGA was equipped to measure canopy photosynthetic CO_2 exchange measurements (Uprety et al. 2006). However, the controlled conditions with a single-component approach, lack of interactions with other environmental factors, wind gradients, limited replications, and cost per unit appear to be the major limitations for the application of these CO_2 enrichment techniques.

In recent times, technologies such as OTCs (Open Top Chambers) and FACE (Free Air CO_2 Enrichment) have gained a lot of popularity owing to their near-natural conditions for crop response studies. The data generated from these facilities would be more realistic for developing models to predict responses for future climatic conditions. OTCs are designed with an automatic and continuous monitoring system equipped with sensors, valves, PC linked Program Logic Control (PLC) and Supervisory Control and Data Acquisition (SCADA) (Vanaja et al. 2006). Several studies of eCO_2 experiments in natural ecosystems have been done with OTCs because they provide nearly natural environments at a reasonable cost. Despite the popularity of OTCs, their effects on microclimate are a serious limitation because of the possible interactions between eCO_2 and altered microclimate. Maini et al. (2002) described the FACE facility equipped with FACE ring (Plenum) through which CO_2-enriched air is injected into the open field, CO_2 storage and distribution system and proportional differential integral (PID) valve for regulating the quantity of CO_2 to be released based on the voltage applied. The elevated levels of CO_2 simulated in the open fields by artificially injecting CO_2 gas through the plenum is based on wind velocity and measured every second by the control system. Though FACE technology has overcome the limitations of OTC for the natural environment, but it is highly expensive.

Screen-Aided CO_2 Control (SACC), a novel technology, was developed to take advantage of both OTCs and FACE by reducing the cost involved (Leadley et al. 1997). It uses less CO_2 when compared to FACE and is superior to OTC in terms of its reduced temperature peaks, rainfall interception by chamber walls, and minimal effects on solar radiation. SACC is sorted as the best method under financial constraints and for exposing short stature vegetation to eCO_2.

In most of the crops, yield improves in response to eCO_2 but not much increase in photosynthesis is observed, as part of the fixed carbon is being utilized in producing more biomass (Reddy and Hodges 2000). In most C_3 crops, yield differs by 20–35% compared to 10–15% in the case of C_4 crop species (Ainsworth et al. 2002). Legumes are expected to respond more to CO_2 concentrations due to their ability to fix nitrogen (Vaidya et al. 2014). In groundnut, a C_3 crop, eCO_2 in the atmosphere is expected to affect in terms of yield and nutritional quality. Improved pod yield of groundnut due to eCO_2 was reported (Stanciel et al. 2000; Yadav et al. 2011; Vaidya et al. 2014; Manjula et al. 2018). Although the C_3 crops show higher yields and biomass, the magnitude of response to eCO_2 varies among the crops due to the accumulation of extra assimilates in vegetative plant parts (Ainsworth and Long 2005).

The biosynthetic pathways in plants that are affected by eCO_2 lead to changes in the seed nutrient concentrations. Earlier studies reported nutrient change as a phenomenon of the dilution effect, caused by the enhanced growth of plants, decreased stomatal conductance, reduced transpiration, and uptake of mobile elements under eCO_2 (Bunce 2001; McDonald et al. 2002; Seneweera et al. 2002). Information about the effect of elevated atmospheric CO_2 concentrations on nutritional quality in groundnut is very limited. The oil and protein content declined with eCO_2 in groundnut (Yadav et al. 2011) and soybean (Li et al. 2018). The unsaturated fatty acids like oleic acid content have risen with eCO_2 in groundnut, soybean, and oilseed rape (Yadav et al. 2011; Li et al. 2018).

7.4.1 Genetic and Physiological Responses to Elevated CO_2 in Groundnut

The effect of eCO_2 on photosynthetic and respiratory rates has direct consequences on the growth and yield of plants. Doubling of the CO_2 concentration will ameliorate photosynthesis of C_3 crop species by 30–50% (Baker and Allen 1993; Reddy and Hodges 2000). The CO_2 acclimation response is mainly due to RuBP carboxylase, the initial carboxylation enzyme in C_3 plants that can either bind to CO_2 or O_2. Increased concentration of CO_2 enables this molecule to better compete with dissolved O_2 for binding sites on the Rubisco protein, thereby contributing to greater carboxylation efficiency. Several studies reported the varied response of this enzyme from leaves of the groundnut and soybean plants to different eCO_2 levels (Vu et al. 2006). The eCO_2 enhanced the photosynthetic rate in groundnut by about 36% at 550 ppm CO_2 (Vaidya et al. 2014) and by 78% and 30% with 550 ppm and 700 ppm CO_2 in black gram (Vanaja et al. 2007). Stanciel et al. (2000) noticed an increase in carboxylation efficiency up to 800 μmol mol^{-1} CO_2 and then declining at 1200 μmol mol^{-1} CO_2 in groundnut under growth chambers. At eCO_2, higher net carbon assimilation of sucrose and starch is due to enhanced sucrose transport (Aranjuelo et al. 2009; Lee-Ho et al. 2007).

With the doubling of CO_2, the stomatal conductance is decreased by 40%, thereby lowering the transpiration of leaves due to reduced evaporation cooling. Stomatal conductance decreased in groundnut as the CO_2 concentration rose from 400 to 800 μmol mol^{-1} (Stanciel et al. 2000). Although crop transpiration might slightly decrease under eCO_2, water use will increase if temperatures rise. Under $eCO_{2,}$ the water use efficiency increased by 62%, and 72.6% due to improved photosynthetic rate coupled with low transpiration rate in groundnut (Vaidya et al. 2014; Jianlin et al. 2008).

The physiological changes caused due to eCO_2 are assumed to downregulate the antioxidant metabolism in the plants (Wustman et al. 2001). A similar observation was reported in C_3 grasses and legumes, wherein hydrogen peroxide, lipid peroxidation, lipoxygenase activity, and antioxidant enzymes decreased with eCO_2 (AbdElgawad et al. 2015). There is a significant decrease in the ROS levels, superoxide dismutase, catalase activity, and membrane disruption in mung bean cultivars under eCO_2 (Mishra and Agrawal 2014). In soybean, there is a decrease in the activities of superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione peroxidase, and glutathione reductase with an increase in CO_2 concentration (Pritchard et al. 2000).

When the plants are subjected to photosynthesis and other physiological and metabolic processes, some genes and proteins underlying such mechanisms will be differentially expressed under eCO_2 (Gamage et al. 2018). Few studies have examined the effects of eCO_2 on gene expression in crops like soybean, Arabidopsis, and rice (Ainsworth et al. 2006; Li et al. 2008; Fukayama et al. 2011). More experimental studies under eCO_2 are required in groundnut to understand the interrelation between photosynthetic capacity and the expression of genes to decipher the mechanisms under this condition.

Despite the concerns for surging atmospheric CO_2, only short-term studies have been conducted on groundnut. Hence, future research should take advantage of the available technologies to understand the underlying mechanism of the responses that enable to breed fit to the predicted climate change scenarios while also manifesting improved yield and quality attributes.

7.5 Way Forward

Developing climate-smart groundnut cultivars to adapt to the climate change effects is a feasible option, given the variability for drought and heat stress tolerance as well as other key traits required for adaptation to climate change scenarios. Photosynthetic rate, daily growth partitioned to pods, and seed-filling duration for pods are identified as the key traits for adaptation to climate change scenarios using modeling studies. Drought-tolerant varieties were developed and commercialized in groundnut, while studies on understanding responses to heat stress and elevated CO_2 are ongoing in groundnut, however, the available studies on responses to elevated CO_2 are limited. Selecting insensitive genotypes to heat stresses is a deployable option based on pod yield. Modeling studies can help in understanding the effect on yield and quality under climate change scenarios and can guide the genetic traits that can be targeted for a given agro-ecology under climate change scenarios. The climate change factors can cause various effects on groundnut and quantifying these responses, particularly under heat stress and elevated CO_2 will help in preparing efficient strategies for adaptation and breed climate-resilient groundnut varieties. Development of genomic tools to drought and heat stress tolerance increases the rate of genetic gain for pod yield and heat tolerance together with desirable quality attributes. Genomic selection tools may be appropriate given the complex nature of tolerance to drought and heat in groundnut.

References

AbdElgawad H, Farfan-Vignolo ER, deVos D, Asard H (2015) Elevated CO_2 mitigates drought and temperature-induced oxidative stress differently in grasses and legumes. Plant Sci 231:1–10

Ahmad MA, Javed R, Adeel M, Rizwan M, Yang Y (2020) PEG 6000-stimulated drought stress improves the attributes of in vitro growth, steviol glycosides production, and antioxidant activities in Stevia rebaudiana Bertoni. Plan Theory 9(11):1552

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol 165(2):351–372

Ainsworth EA, Davey PA, Bernacchi CJ, Dermody OC, Heaton EA, Moore DJ, Morgan PB, Naidu SL, Yoo Ra HS, Zhu XG, Curtis PS (2002) A meta-analysis of elevated [CO_2] effects on soybean (Glycine max) physiology, growth and yield. Glob Chang Biol 8(8):695–709

Ainsworth EA, Rogers A, Vodkin LO, Walter A, Schurr U (2006) The effects of elevated CO_2 concentration on soybean gene expression. An analysis of growing and mature leaves. Plant Physiol 142(1):135–147

Akbar A, Singh Manohar S, Tottekkaad Variath M, Kurapati S, Pasupuleti J (2017) Efficient partitioning of assimilates in stress-tolerant groundnut genotypes under high-temperature stress. Agronomy 7(2):30

Alhassan K (2018) Proximate composition and functional properties of some new groundnut accessions. (Doctoral dissertation)

Aranjuelo I, Pardo A, Biel C, Save R, Joaquim A-B, Nogues S (2009) Leaf carbon management in slow-growing plants exposed to elevated CO_2. Glob Chang Biol 15(1):97–109

Arunachalam P, Kannan P (2013) Screening for drought tolerant groundnut (*Arachis hypogaea* L.) lines suitable for rainfed Alfisol. Asian J Agric Res 7(1):35–42

Arunyanark A, Jogloy S, Wongkaew S, Akkasaeng C, Vorasoot N, Wright GC, Rachaputi RC, Patanothai A (2009) Association between aflatoxin contamination and drought tolerance traits in peanut. Field Crop Res 114(1):14–22

Arya SS, Salve AR, Chauhan S (2016) Peanuts as functional food: a review. J Food Sci Technol 53(1):31–41

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141(2):391–396

Bacharou Falke A, Hamidou F, Halilou O, Harou A (2019) Assessment of groundnut elite lines under drought conditions and selection of tolerance associated traits. Adv Agric:3034278

Baker JT, Allen LH (1993) Contrasting crop species responses to CO_2 and temperature: rice, soybean and citrus. Vegetatio 104(1):239–260

Basu MS (2004) Improving water use efficiency and drought tolerance in groundnut by trait based breeding programmes in India. JAL 2(288):98

Bhogireddy S, Xavier A, Garg V, Layland N, Arias R, Payton P, Nayak SN, Pandey MK, Puppala N, Varshney RK (2020) Genome-wide transcriptome and physiological analyses provide new insights into peanut drought response mechanisms. Sci Rep 10(1):1–6

Bishop KA, Betzelberger AM, Long SP, Ainsworth EA (2015) Is there potential to adapt soybean (*Glycine max* M err.) to future [CO_2]? An analysis of the yield response of 18 genotypes in free-air CO_2 enrichment. Plant Cell Environ 38(9):1765–1774

Bita C, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci 4:273

Black CK, Davis SC, Hudiburg TW, Bernacchi CJ, DeLucia EH (2017) Elevated CO_2 and temperature increase soil C losses from a soybean–maize ecosystem. Glob Chang Biol 23(1): 435–445

Broberg MC, Högy P, Pleijel H (2017) CO_2-induced changes in wheat grain composition: meta-analysis and response functions. Agronomy 7(2):32

Bunce JA (2001) Direct and acclimatory responses of stomatal conductance to elevated carbon dioxide in four herbaceous crop species in the field. Glob Chang Biol 7(3):323–331

Chakraborty K, Mahatma MK, Thawait LK, Bishi SK, Kalariya KA, Singh AL (2015a) Water deficit stress affects photosynthesis and the sugar profile in source and sink tissues of groundnut (*Arachis hypogaea* L.) and impacts kernel quality. J Appl Bot Food Qual 89:98–104

Chakraborty K, Singh AL, Kalariya KA, Goswami N (2015b) Physiological responses of peanut (*Arachis hypogaea* L.) cultivars to water deficit stress: status of oxidative stress and antioxidant enzyme activities. Acta Bot Croat 74(1):123–142

Chakraborty K, Bishi SK, Singh AL, Zala PV, Mahatma MK, Kalariya KA, Jat RA (2018) Rapid induction of small heat shock proteins improves physiological adaptation to high temperature stress in peanut. J Agron Crop Sci 204(3):285–297

Change IPOC (2014) IPCC. Climate change

Cole MB, Augustin MA, Robertson MJ, Manners JM (2018) The science of food security. NPJ Sci Food 2(1):1–8

Conkerton EJ, Ross LF, Daigle DJ, Kvien CS, McCombs C (1989) The effect of drought stress on peanut seed composition. II. Oil, protein and minerals. Oleagineux (France) 44(12):587–596

Craufurd PQ, Prasad PV, Kakani VG, Wheeler TR, Nigam SN (2003) Heat tolerance in groundnut. Field Crop Res 80(1):63–77

Cuc DT, Phuong PT, Loan NT, Cuong ND, Ngoan NT, Phuc NL, Anh DT, Khanh TD, Trung KH (2021) Integration of traditional and molecular breeding to improve drought tolerance of groundnut. Adv Studies Biol 13(1):29–36

Dai L, Zhang G, Yu Z, Ding H, Xu Y, Zhang Z (2019) Effect of drought stress and developmental stages on microbial community structure and diversity in peanut rhizosphere soil. Int J Mol Sci 20(9):2265

Devi MJ, Sinclair TR, Vadez V, Shekoofa A, Puppala N (2019) Strategies to enhance drought tolerance in peanut and molecular markers for crop improvement. Genomics assisted breeding of crops for abiotic stress tolerance. Vol. II, pp. 131–143.

Dey SK, Chakrabarti B, Prasanna R, Pratap D, Singh SD, Purakayastha TJ, Pathak H (2017) Elevated carbon dioxide level along with phosphorus application and cyanobacterial inoculation enhances nitrogen fixation and uptake in cowpea crop. Arch Agron Soil Sci 63(13):1927–1937

Dionisio-Sese ML, Tobita S (1998) Antioxidant responses of rice seedlings to salinity stress. Plant Sci 135(1):1–9

FAO (2002) Joint FAO/WHO Expert Committee on Food Additives, Joint FAO/WHO Expert Committee on Food Additives. Meeting, & World Health Organization. Evaluation of Certain Food Additives and Contaminants: Fifty-seventh Report of the Joint FAO/WHO Expert Committee on Food Additives, vol. 57. World Health Organization

FAO (2019) Food and Agriculture Organization of the United Nations-Statistic Division https://www.fao.org/faostat/en/#data

Farooq M, Wahid A, Kobayashi NSMA, Fujita DBSMA, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Sustain Agric:153–188

Fonceka D, Tossim HA, Rivallan R, Vignes H, Faye I, Ndoye O, Moretzsohn MC, Bertioli DJ, Glaszmann JC, Courtois B, Rami JF (2012) Fostered and left behind alleles in peanut: interspecific QTL mapping reveals footprints of domestication and useful natural variation for breeding. BMC Plant Biol 12(1):1–6

Foolad MR (2005) Breeding for abiotic stress tolerance in tomato (No. Book). Food Products Press

Forster P, Ramaswamy V, Artaxo P, Berntsen T, Betts R, Fahey DW, Haywood J, Lean J, Lowe DC, Myhre G, Nganga J (2007) Changes in atmospheric constituents and in radiative forcing. Chapter 2. In: Climate change: the physical science basis 2007

Fukayama H, Sugino M, Fukuda T, Masumoto C, Taniguchi Y, Okada M, Sameshima R, Hatanaka T, Misoo S, Hasegawa T, Miyao M (2011) Gene expression profiling of rice grown in free air CO_2 enrichment (FACE) and elevated soil temperature. Field Crop Res 121(1): 195–199

Furlan AL, Bianucci E, Giordano W, Castro S, Becker DF (2020) Proline metabolic dynamics and implications in drought tolerance of peanut plants. Plant Physiol Biochem 151:566–578

Gamage D, Thompson M, Sutherland M, Hirotsu N, Makino A, Seneweera S (2018) New insights into the cellular mechanisms of plant growth at elevated atmospheric carbon dioxide concentrations. Plant Cell Environ 41(6):1233–1246

Gautami B, Pandey MK, Vadez V, Nigam SN, Ratnakumar P, Krishnamurthy L, Radhakrishnan T, Gowda MV, Narasu ML, Hoisington DA, Knapp SJ (2012) Quantitative trait locus analysis and construction of consensus genetic map for drought tolerance traits based on three recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). Mol Breed 30(2): 757–772

Girdthai T, Jogloy S, Vorasoot N, Akkasaeng C, Wongkaew S, Patanothai A, Holbrook CC (2012) Inheritance of the physiological traits for drought resistance under terminal drought conditions and genotypic correlations with agronomic traits in peanut. Sabrao J Breed Genet 44:240–262

Guasch-Ferré M, Liu X, Malik VS, Sun Q, Willett WC, Manson JE, Rexrode KM, Li Y, Hu FB, Bhupathiraju SN (2017) Nut consumption and risk of cardiovascular disease. J Am Coll Cardiol 70(20):2519–2532

Gundaraniya SA, Ambalam PS, Tomar RS (2020) Metabolomic profiling of drought-tolerant and susceptible peanut (*Arachis hypogaea* L.) genotypes in response to drought stress. ACS Omega 5(48):31209–31219

Hall AE (1992) Breeding for heat tolerance. Plant Breed Rev 10(2):129–168

Hamidou F, Halilou O, Vadez V (2013) Assessment of groundnut under combined heat and drought stress. J Agron Crop Sci 199(1):1–11

Hamim H (2005) Photosynthesis of C_3 and C_4 species in response to increased CO_2 concentration and drought stress. Hayati J Biosci 12(4):131–131

Haro RJ, Dardanelli JL, Collino DJ, Otegui ME (2010) Water deficit and impaired pegging effects on peanut seed yield: links with water and photosynthetically active radiation use efficiencies. Crop Pasture Sci 61(5):343–352

Hashim IB, Koehler PE, Eitenmiller RR (1993) Tocopherols in runner and virginia peanut cultivars at various maturity stages. J Am Oil Chem Soc 70(6):633–635

Hong SW, Vierling E (2000) Mutants of Arabidopsis thaliana defective in the acquisition of tolerance to high temperature stress. Proc Natl Acad Sci 97(8):4392–4397

Howarth CJ (2005) of Tolerance to High Temperature. Abiotic stresses: Plant resistance through breeding and molecular approaches 1920

IPCC (2007) Climate change 2007: the physical science basis. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Jackson CL, Hu FB (2014) Long-term associations of nut consumption with body weight and obesity. Am J Clin Nutr 100(Suppl. 1):408–411

Jain AK, Basha SM, Holbrook CC (2001) Identification of drought-responsive transcripts in peanut (*Arachis hypogaea* L.). Electron J Biotechnol 4(2):3–4

Janila P, Nigam SN, Pandey MK, Nagesh P, Varshney RK (2013) Groundnut improvement: use of genetic and genomic tools. Front Plant Sci 4:23

Jena UR, Swain DK, Hazra KK, Maiti MK (2018) Effect of elevated [CO_2] on yield, intra-plant nutrient dynamics, and grain quality of rice cultivars in eastern India. J Sci Food Agric 98(15): 5841–5852

Jenks MA, Hasegawa PM, Jain SM (eds) (2007) Advances in molecular breeding toward drought and salt tolerant crops. Springer, Dordrecht, pp 1–32

Jiang C, Li X, Zou J, Ren J, Jin C, Zhang H, Yu H, Jin H (2021) Comparative transcriptome analysis of genes involved in the drought stress response of two peanut (*Arachis hypogaea* L.) varieties. BMC Plant Biol 21(1):1–4

Jianlin W, Guirui Y, Quanxiao F, Defeng J, Hua Q, Qiufeng W (2008) Responses of water use efficiency of 9 plant species to light and CO_2 and their modeling. Acta Ecol Sin 28(2):525–533

Jones P, Allen LH Jr, Jones JW, Boote KJ, Campbell WJ (1984) Soybean canopy growth, photosynthesis, and transpiration responses to whole-season carbon dioxide enrichment 1. Agron J 76(4):633–637

Junjittakarn J, Girdthai T, Jogloy S, Vorasoot N, Patanothai A (2014) Response of root characteristics and yield in peanut under terminal drought condition. Chilean J Agric Res 74(3):249–256

Kadiyala MDM, Nedumaran S, Padmanabhan J, Gumma MK, Gummadi S, Srigiri SR, Robertson R, Whitbread A (2021) Modeling the potential impacts of climate change and adaptation strategies on groundnut production in India. Sci Total Environ 776:145996

Kakeeto R, Melis R, Biruma M, Sibiya J (2020) Gene action governing the inheritance of drought tolerance and selected agronomic traits in Ugandan groundnut (*Arachis hypogaea* L.) lines under drought environment. Euphytica 216(1):1–21

Kambiranda DM, Vasanthaiah HK, Katam R, Ananga A, Basha SM, Naik K (2011) Impact of drought stress on peanut (*Arachis hypogaea* L.) productivity and food safety. Plants Environ:249–272

Kaushal N, Bhandari K, Siddique KH, Nayyar H (2016) Food crops face rising temperatures: an overview of responses, adaptive mechanisms, and approaches to improve heat tolerance. Cogent Food Agric 2(1):1134380

Kokkanti RR, Hindu V, Latha P, Vasanthi RP, Sudhakar P, Usha R (2019) Assessment of genetic variability and molecular characterization of heat stress tolerant genes in *Arachis hypogaea* L. through qRT-PCR. Biocatal Agric Biotechnol 20:101242

Krishnan P, Swain DK, Bhaskar BC, Nayak SK, Dash RN (2007) Impact of elevated CO_2 and temperature on rice yield and methods of adaptation as evaluated by crop simulation studies. Agric Ecosyst Environ 122(2):233–242

Kumar R, Singh AK, Lavania D, Siddiqui MH, Al-Whaibi MH, Grover A (2016) Expression analysis of ClpB/Hsp100 gene in faba bean (*Vicia faba* L.) plants in response to heat stress. Saudi J Biol Sci 23(2):243–247

Lal C, Ajay BC, Chikani BM, Gor HK (2019) AMMI and GGE biplot analysis to evaluate the phenotypic stability of recombinant inbred lines (RILs) of peanut under mid-season water stress conditions. Indian J Genet 79(2):420–426

Leadley PW, Niklaus P, Stocker R, Körner C (1997) Screen-aided CO_2 control (SACC): a middle ground between FACE and open-top chambers. Acta Oecol 18(3):207–219

Leal-Bertioli SC, Moretzsohn MC, Roberts PA, Ballén-Taborda C, Borba TC, Valdisser PA, Vianello RP, Araújo ACG, Guimarães PM, Bertioli DJ (2016) Genetic mapping of resistance to Meloidogyne arenaria in Arachis stenosperma: a new source of nematode resistance for peanut. G3: Genes Genomes Genetics 6(2):377–390

Lee JS, Tetsuyuki U, Takehisa O, Lee HJ (2000) High performance of temperature gradient chamber newly built for studying global warming effect on a plant population. Korean J Ecol 23(4):293–298

Lee-Ho E, Walton LJ, Reid DM, Yeung EC, Kurepin LV (2007) Effects of elevated carbon dioxide and sucrose concentrations on Arabidopsis thaliana root architecture and anatomy. Botany 85(3):324–330

Li P, Ainsworth EA, Leakey AD, Ulanov A, Lozovaya V, Ort DR, Bohnert HJ (2008) Arabidopsis transcript and metabolite profiles: ecotype-specific responses to open-air elevated [CO_2]. Plant Cell Environ 31(11):1673–1687

Li G, Wan Y, Liu F, Zhang K (2014) Morphological and physiological traits of root in different drought resistant peanut cultivars. Acta Agron Sin 40(3):531–541

Li Y, Yu Z, Jin J, Zhang Q, Wang G, Liu C, Wu J, Wang C, Liu X (2018) Impact of elevated CO_2 on seed quality of soybean at the fresh edible and mature stages. Front Plant Sci 9:1413

Liu NY, Ko SS, Yeh KC, Charng YY (2006) Isolation and characterization of tomato Hsa32 encoding a novel heat-shock protein. Plant Sci 170(5):976–985

Lobell DB, Burke MB, Tebaldi C, Mastrandrea MD, Falcon WP, Naylor RL (2008) Prioritizing climate change adaptation needs for food security in 2030. Science 319(5863):607–610

Loladze I (2014) Hidden shift of the ionome of plants exposed to elevated CO_2 depletes minerals at the base of human nutrition. elife 3:e02245

Luis JM, Ozias-Akins P, Holbrook CC, Kemerait RC Jr, Snider JL, Liakos V (2016) Phenotyping peanut genotypes for drought tolerance. Peanut Sci 43(1):36–48

Mahantesh S, Babu HN, Ghanti K, Raddy PC (2018) Identification of drought tolerant genotypes based on physiological, biomass and yield response in groundnut (*Arachis hypogaea* L.). Indian J Agric Res 52(3):221–227

Maini HK, Tiwari MK, Bahl M, John T, Chopra P, Singh D, Yadav VS, Anand JR, Poddar N, Mitra AP, Garg SC (2002) Free air carbon dioxide enrichment facility development for crop experiments. Indian J Radio Space Phys 31:404–409

Manjula O, Srimurali M, Vanaja M (2018) Impact of elevated carbon dioxide on two groundnut genotypes (*Arachis hypogaea* L.) under open top chamber facility. Int J Environ Agric Biotechnol 3(1):239041

McDonald EP, Erickson JE, Kruger EL (2002) Research note: can decreased transpiration limit plant nitrogen acquisition in elevated CO_2? Funct Plant Biol 29(9):1115–1120

Mihara Y (1971) Proposing temperature response curve technique for field crop experiment. Agric Hortic 46:721–726

Mishra AK, Agrawal SB (2014) Cultivar specific response of CO_2 fertilization on two tropical mung bean (*Vigna radiata* L.) cultivars: ROS generation, antioxidant status, physiology, growth, yield and seed quality. J Agron Crop Sci 200(4):273–289

Misra V, Shrivastava AK, Mall AK, Solomon S, Singh AK, Ansari MI (2019) Can sugarcane cope with increasing atmospheric CO_2 concentration? Aust J Crop Sci 13(5):780–784

Mohamed A, Monnier Y, Mao Z, Lobet G, Maeght JL, Ramel M, Stokes A (2017) An evaluation of inexpensive methods for root image acquisition when using rhizotrons. Plant Methods 13(1): 1–13

Nageswara Rao RC, Talwar HS, Wright GC (2001) Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using a chlorophyll meter. J Agron Crop Sci 186(3): 175–182

Nautiyal PC, Rajgopal K, Zala PV, Pujari DS, Basu M, Dhadhal BA, Nandre BM (2008) Evaluation of wild Arachis species for abiotic stress tolerance: I. Thermal stress and leaf water relations. Euphytica 159(1):43–57

Nieuwenhuis L, van den Brandt PA (2019) Nut and peanut butter consumption and the risk of lung cancer and its subtypes: a prospective cohort study. Lung Cancer 128:57–66

Nigam SN, Dwivedi SL, Gibbons RW (1991) Groundnut breeding: constraints, achievements and future possibilities. In Plant breeding abstracts, vol. 61, No. 10. CAB, pp. 1127-1136

Nigam SN, Upadhyaya HD, Chandra S, Rao RN, Wright GC, Reddy AGS (2001) Gene effects for specific leaf area and harvest index in three crosses of groundnut (*Arachis hypogaea*). Ann Appl Biol 139(3):301–306

Nigam SN, Chandra S, Sridevi KR, Bhukta M, Reddy AGS, Rachaputi NR, Wright GC, Reddy PV, Deshmukh MP, Mathur RK (2005) Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. Ann Appl Biol 146(4):433–439

Oppong-Sekyere D, Akromah R, Ozias-Akins P, Laary JK, Gimode D (2019) Heritability studies of drought tolerance in groundnuts using the North Carolina design II fashion and variance component method. J Plant Breed Crop Sci 11(9):234–253

Pacheco MRPDS, Helene MEM (1990) Atmosfera, fluxos de carbono e fertilização por CO_2. Estudos avançados 4(9):204–220

Painawadee M, Jogloy S, Kesmala T, Akkasaeng C, Patanothai A (2009) Heritability and correlation of drought resistance traits and agronomic traits in peanut (*Arachis hypogaea* L.). Asian J Plant Sci 8(5):325–334

Pan C, Ahammed GJ, Li X, Shi K (2018) Elevated CO_2 improves photosynthesis under high temperature by attenuating the functional limitations to energy fluxes, electron transport and redox homeostasis in tomato leaves. Front Plant Sci 9:1739

Pandey MK, Upadhyaya HD, Rathore A, Vadez V, Sheshshayee MS, Sriswathi M, Govil M, Kumar A, Gowda MVC, Sharma S, Hamidou F, Kumar VA, Khera P, Bhat RS, Khan AW, Singh S, Li H, Monyo E, Nadaf HL, Mukri G, Jackson SA, Guo B, Liang X, Varshney RK (2014) Genome-wide association studies for 50 agronomic traits in peanut using the 'reference set' comprising 300 genotypes from 48 countries of the semi-arid tropics of the world. PLoS One 9(8):e105228

Pandey MK, Gangurde SS, Sharma V, Pattanashetti SK, Naidu GK, Faye I, Hamidou F, Desmae H, Kane NA, Yuan M, Vadez V, Nigam SN, Varshney RK (2020) Improved genetic map identified major QTLs for drought tolerance-and iron deficiency tolerance-related traits in groundnut. Genes 12(1):37

Pasupuleti J, Nigam SN, Pandey MK, Nagesh P, Varshney RK (2013) Groundnut improvement: use of genetic and genomic tools. Front Plant Sci 4:23

Pettit RE, Taber RA, Schroeder HW, Harrison AL (1971) Influence of fungicides and irrigation practice on aflatoxin in peanuts before digging. Appl Microbiol 22(4):629–634

Phene CJ, Baker DN, Lambert JR, Parsons JE, McKinion JM (1978) SPAR—A soil-plant-atmosphere research system. Trans ASAE 21(5):924–0930

Pilbeam DJ (2015) Breeding crops for improved mineral nutrition under climate change conditions. J Exp Bot 66(12):3511–3521

Porch TG (2006) Application of stress indices for heat tolerance screening of common bean. J Agron Crop Sci 192(5):390–394

Prasad PV, Kakani VG, Upadhyaya HD (2010) Growth and production of groundnut. UNESCO Encyclopedia:1–26

Prasinos C, Krampis K, Samakovli D, Hatzopoulos P (2005) Tight regulation of expression of two Arabidopsis cytosolic Hsp90 genes during embryo development. J Exp Bot 56(412):633–644

Pritchard SG, Ju Z, van Santen E, Qiu J, Weaver DB, Prior SA, Rogers HH (2000) The influence of elevated CO_2 on the activities of antioxidative enzymes in two soybean genotypes. Funct Plant Biol 27(11):1061–1068

Rao MV, Nigam SN, Huda AKS (1992) The thermal time concept as a selection criterion for earliness in peanut. Peanut Sci 19(1):7–10

Ratnakumar P, Vadez V (2011) Groundnut (Arachis hypogaea) genotypes tolerant to intermittent drought maintain a high harvest index and have small leaf canopy under stress. Funct Plant Biol 38(12):1016–1023

Ravi K, Vadez V, Isobe S, Mir RR, Guo Y, Nigam SN, Gowda MVC, Radhakrishnan T, Bertioli DJ, Knapp SJ, Varshney RK (2011) Identification of several small main-effect QTLs and a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L.). Theor Appl Genet 122(6):1119–1132

Reddy KR, Hodges HF (2000) Climate Change and global crop productivity

Reddy AR, Rasineni GK, Raghavendra AS (2010) The impact of global elevated CO2 concentration on photosynthesis and plant productivity. Curr Sci:46–57

Rontein D, Basset G, Hanson AD (2002) Metabolic engineering of osmoprotectant accumulation in plants. Metab Eng 4(1):49–56

Ruan YL, Jin Y, Yang YJ, Li GJ, Boyer JS (2010) Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. Mol Plant 3(6):942–955

Sadat S, Saeid KA, Bihamta MR, Torabi S, Salekdeh SG, Ayeneh GA (2013) Marker assisted selection for heat tolerance in bread wheat. World Appl Sci J 21(8):1181–1189

Sakamoto A, Murata N (2002) The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. Plant Cell Environ 25(2):163–171

Sakata T, Oshino T, Miura S, Tomabechi M, Tsunaga Y, Higashitani N, Miyazawa Y, Takahashi H, Watanabe M, Higashitani A (2010) Auxins reverse plant male sterility caused by high temperatures. Proc Natl Acad Sci 107(19):8569–8574

Sanders TH, Cole RJ, Blankenship PD, Dorner JW (1993) Aflatoxin contamination of peanuts from plants drought stressed in pod or root zones. Peanut Sci 20(1):5–8

Savchenko GE, Klyuchareva EA, Abramchik LM, Serdyuchenko EV (2002) Effect of periodic heat shock on the inner membrane system of etioplasts. Russ J Plant Physiol 49(3):349–359

Selvaraj MG, Burow G, Burke JJ, Belamkar V, Puppala N, Burow MD (2011) Heat stress screening of peanut (*Arachis hypogaea* L.) seedlings for acquired thermotolerance. Plant Growth Regul 65(1):83–91

Seneweera SP, Ghannoum O, Conroy JP, Ishimaru K, Okada M, Lieffering M, Kim HY, Kobayashi K (2002) Changes in source–sink relations during development influence photosynthetic acclimation of rice to free air CO_2 enrichment (FACE). Funct Plant Biol 29(8):947–955

Shanker AK, Maheswari M, Yadav SK, Desai S, Bhanu D, Attal NB, Venkateswarlu B (2014) Drought stress responses in crops. Funct Integr Genomics 14(1):11–22

Sharma K, Singh G (1987) Effect of water stress on yield and quality of groundnut. Environ Ecol 5(4):647–650

Sinclair TR, Horie T (1989) Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. Crop Sci 29(1):90–98

Sinclair TR, Van Houtte RC (1979) Mobile laboratory for continuous, long-term gas exchange measurements of 39 leaves. Photosynthetica 13(4):446–453

Singh D, Balota M, Collakova E, Isleib TG, Welbaum GE, Tallury SP (2016) Heat stress related physiological and metabolic traits in peanut seedlings. Peanut Sci 43(1):24–35

Singh B, Salaria N, Thakur K, Kukreja S, Gautam S, Goutam U (2019) Functional genomic approaches to improve crop plant heat stress tolerance. F1000Res:8

Solomon S, Plattner GK, Knutti R, Friedlingstein P (2009) Irreversible climate change due to carbon dioxide emissions. Proc Natl Acad Sci 106(6):1704–1709

Stanciel K, Mortley DG, Hileman DR, Loretan PA, Bonsi CK, Hill WA (2000) Growth, pod, and seed yield, and gas exchange of hydroponically grown peanut in response to CO_2 enrichment. HortScience 35(1):49–52

Sun AQ, Yi SY, Yang JY, Zhao CM, Liu J (2006) Identification and characterization of a heat-inducible ftsH gene from tomato (*Lycopersicon esculentum* Mill.). Plant Sci 170(3):551–562

Sun L, Yang YJ, Xian T, Lu ZM, Fu YF (2010) Strong enhancement of chlorophyll a concentration by a weak typhoon. Mar Ecol Prog Ser 404:39–50

Suriharn B, Patanothai A, Jogloy S (2005) Gene effects for specific leaf area and harvest index in peanut (*Arachis hypogaea* L.). Asian J Plant Sci 4(6):667–672

Thakur SB, Ghimire SK, Shrestha SM, Chaudhary NK, Mishra B (2013) Variability in groundnut genotypes for tolerance to drought. Nepal J Sci Technol 14(1):41–50

Török Z, Goloubinoff P, Horváth I, Tsvetkova NM, Glatz A, Balogh G, Varvasovszki V, Los DA, Vierling E, Crowe JH, Vígh L (2001) Synechocystis HSP17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperone-mediated refolding. Proc Natl Acad Sci 98(6):3098–3103

UNICEF (2007) Progress for children: a World fit for children: statistical review

Upadhyaya HD, Dwivedi SL, Nadaf HL, Singh S (2011) Phenotypic diversity and identification of wild Arachis accessions with useful agronomic and nutritional traits. Euphytica 182(1):103–115

Uprety DC, Garg SC, Bisht BS, Maini HK, Dwivedi N, Paswan G, Raj A, Saxena DC (2006) Carbon dioxide enrichment technologies for crop response studies. J Scientific Ind Res 65:859–866

Vaidya S, Vanaja M, Sathish P, Vagheera P, Anitha Y (2014) Impact of elevated CO_2 on growth and physiological parameters of groundnut (*Arachis hypogaea* L.) genotypes. J Plant Physiol Pathol 3:1–5

Vanaja M, Maheswari M, Ratnakumar P, Ramakrishna YS (2006) Monitoring and controlling of CO_2 concentrations in open top chambers for better understanding of plants response to elevated CO_2 levels. Indian J Radio Space Phys 35:193–197

Vanaja M, Reddy PR, Jyothi Lakshmi N, Maheswari M, Vagheera P, Ratnakumar P, Jyothi M, Yadav SK, Venkateswarlu B (2007) Effect of elevated atmospheric CO_2 concentrations on growth and yield of blackgram (*Vigna mungo* L. Hepper)—a rainfed pulse crop. Plant Soil Environ 53(2):81–88

Variath MT, Janila P (2017) Economic and academic importance of peanut. In: The peanut genome. Springer, Cham, pp 7–26

Varshney RK, Hiremath PJ, Lekha P, Kashiwagi J, Balaji J, Deokar AA, Hoisington DA (2009) A comprehensive resource of drought-and salinity-responsive ESTs for gene discovery and marker development in chickpea (*Cicer arietinum* L.). BMC Genomics 10(1):1–18

Vu JC, Allen LH Jr, Widodo W (2006) Leaf photosynthesis and Rubisco activity and kinetics of soybean, peanut, and rice grown under elevated atmospheric CO_2, supraoptimal air temperature, and soil water deficit. Curr Top Plant Biol 7:27

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61(3):199–223

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9(5):244–252

Wang P, Song H, Li C, Li P, Li A, Guan H, Hou L, Wang X (2017) Genome-wide dissection of the heat shock transcription factor family genes in Arachis. Front Plant Sci 8:106

Weatherley P (1950) Studies in the water relations of the cotton plant: I. The field measurement of water deficits in leaves. New Phytol 49(1):81–97

Wilson DM, Stansell JR (1983) Effect of irrigation regimes on aflatoxin contamination of peanut pods. Peanut Sci 10(2):54–56

Wustman BA, Oksanen E, Karnosky DF, Noormets A, Isebrands JG, Pregitzer KS, Hendry GR, Sober J, Podila GK (2001) Effects of elevated CO_2 and O_3 on aspen clones varying in O_3 sensitivity: can CO_2 ameliorate the harmful effects of O_3? Environ Pollut 115(3):473–481

Yadav SK, Vanaja M, Reddy PR, Jyothilakshmi N, Maheswari M, Sharma KL, Venkateswarlu B (2011) Effect of elevated CO_2 levels on some growth parameters and seed quality of groundnut (*Arachis hypogaea* L.). Indian J Agric Biochem 24(2):158–160

Yamada K, Fukao Y, Hayashi M, Fukazawa M, Suzuki I, Nishimura M (2007) Cytosolic HSP90 regulates the heat shock response that is responsible for heat acclimation in *Arabidopsis thaliana*. J Biol Chem 282(52):37794–37804

Zhang HX, Hodson JN, Williams JP, Blumwald E (2001) Engineering salt-tolerant Brassica plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. Proc Natl Acad Sci 98(22):12832–12836

Zhu CW, Zhu JG, Liu G, Zeng Q, Xie ZB, Pang J, Feng ZZ, Tang HY, Wang L (2008) Elevated CO_2 concentration enhances the role of the ear to the flag leaf in determining grain yield of wheat. Photosynthetica 46(2):318–320

Zhu M, Monroe JG, Suhail Y, Villiers F, Mullen J, Pater D, Hauser F, Jeon BW, Bader JS, Kwak JM, Schroeder JI, McKay JK, Assmann SM (2016) Molecular and systems approaches towards drought-tolerant canola crops. New Phytol 210(4):1169–1189

Horse Gram, An Underutilized Climate-Resilient Legume: Breeding and Genomic Approach for Improving Future Genetic Gain

8

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Abstract

Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) is an underutilized multipurpose legume crop with inherent biotic and abiotic stress tolerance, high seed protein, dietary fiber, and micronutrients, and nutraceutical properties. However, this legume crop is lagging behind other legumes in genetic resource development and genetic improvement. Thus, this chapter highlights the global importance of horse gram in terms of its food value, nutraceutical features, and other benefits and its importance for improving soil quality, maintaining agroecological systems, and providing feed and forage for livestock sustenance. We discuss breeding approaches to enhance horse gram's yield and biotic and abiotic stress tolerance and provide the current status of horse gram genomic resources, including a genetic map, molecular marker repertoire, QTL mapping of various traits of agronomic importance, draft genome sequence, and transcriptome assemblies. We conclude the chapter by highlighting the importance of horse gram as a climate-resilient crop and its future scope as a dietary grain legume for securing global food security.

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Horse gram · Genomics · Molecular marker · QTL · Underutilized legume

8.1 Introduction

Horse gram *Macrotyloma uniflorum* (Lam.) Verdc. is an important minor/underutilized, hardy legume crop grown in the semi-arid tropics (Fuller and Murphy 2018). It is an annual herb, self-pollinated, cleistogamous species, with $2n = 20$ chromosomes (Neelam et al. 2014; Mahesh et al. 2021). It belongs to the family Fabaceae and subtribe Phaseolinae in the Millettioids clade (Shirasawa et al. 2021). Horse gram is India's fifth most widely grown legume crop (Fuller and Murphy 2018). It is also cultivated in Bangladesh, Pakistan, and Myanmar (Spate and Learmonth 1967). In Australia, Taiwan, and the Philippines, it is grown as a fodder crop (Fuller and Murphy 2018). Horse gram is a multipurpose legume crop used as food, feed, forage, and green manure, and it offers nutraceutical benefits (Sudha et al. 1995; Morris 2008). It is rich in macro (phosphorus) and micronutrients, including iron and molybdenum, essential vitamins (carotene, thiamine, riboflavin) (Sodani et al. 2006), amino acids (Gautam et al. 2020a), various antioxidants and dietary fiber, and low in sodium and lipid content. It is used for preparing diets for diabetic patients (Bazzano et al. 2001). Its highly digestible protein and few digestive inhibitors make it palatable for milch animals (Mahesh et al. 2021). However, the presence of antinutrient phytochemicals (phytate, tannins, and trypsin inhibitors) limits its food value (Sreerama et al. 2012). Its inherent capability to withstand various abiotic stresses, especially drought stress, renders it a climate-resilient crop. Thus, horse gram is grown mainly on marginal land with low inputs under harsh environmental conditions. It is grown in southern India, on poor soil or lateritic soil with 40–90 cm annual rainfall, without irrigation (Kingwell-Banham and Fuller 2014). Rapid advances in next-generation sequencing have enabled the development of genomic resources in various crops, including grain legumes, thus facilitating linkage mapping and trait mapping with high resolution. However, horse gram has received little attention for developing genomic resources compared to other major grain legumes. Here, we discuss how harnessing existing horse gram genetic variability could play a critical role in improving its genetic gain and making it more climate-resilient. We discuss the progress in developing horse gram genomic resources, including molecular markers (SSRs, SNPs), genetic map, trait QTL, and draft genome assembly, and highlight the functional genomics status, including expressed sequence tags (ESTs) and RNA-seq-derived abiotic-stress-specific differentially expressed genes/candidate gene(s). We conclude the chapter by discussing the role of horse gram in combating global food insecurity.

8.2 Origin and Distribution

Horse gram is native to South Asia (Hooker 1879). However, according to Verdcourt (1971), horse gram is presumed to originate from Africa, and the northwestern Himalayan region is considered the secondary center of origin. Relying on the evidence and assessing herbarium specimens, Fuller and Harvey (2006) postulated that horse gram is of south Indian origin; however, archaeobotanical evidence suggests it is of northwest Indian origin. The evidence suggests that it was cultivated and domesticated in south Deccan and northwest India (Kingwell-Banham and Fuller 2014). It is now cultivated in temperate and subtropical regions in India, the Philippines, Bhutan, China, Pakistan, Sri Lanka, and Australia (Krishna 2010).

8.3 Horse Gram, a Wonder Legume with Nutraceutical Benefits

Horse gram is rich in protein (17.9–25.3%), carbohydrates (51.9–60.9%), and lipids (0.58–2.06%) and contains iron and molybdenum (Bravo et al. 1999; Sodani et al. 2006). It is used as whole seed and sprouts for human consumption and could be used as whole seed for cattle feed (Kadam and Salunkhe 1985). Horse gram is an excellent source of carotene, thiamine, riboflavin, niacin, and vitamin C (Sodani et al. 2006) and several bioactive compounds, including phenolic acids, flavonoids, alkaloids, tannins, and antioxidants (Siddhuraju and Becker 2007; Prasad and Singh 2015; Gautam et al. 2020a). The nutraceutical compounds can assist in curing various complex diseases, including urinary diseases, kidney stones, piles, coronary heart disease, diabetes, And obesity (Bazzano et al. 2001; Yadava and Vyas 1994; Gautam et al. 2020b).

8.4 Harnessing Horse Gram Genetic Variability to broaden Its Genetic Base for Genetic Gain

Exploring and harnessing natural genetic variation in existing crop gene pools is key to improving the genetic base of crop plants, including horse gram (Chahota et al. 2005). Substantial genetic variability has been recorded in horse gram (Chahota et al. 2005; Singh et al. 2019; Neelima et al. 2021), such as for various yield and yield-related traits in 50 horse gram genotypes (Neelima et al. 2021). Likewise, an assessment of 10 quantitative traits in 55 horse gram genotypes revealed significant genetic variability. Notably, high heritability for seed yield and pods per plant revealed scope for genetic improvement in these traits (Singh et al. 2019). Amal et al.(2020) used a machine learning method for analyzing genetic variability in various morphological traits, including plant height and shoot, pod, and seed lengths in 20 horse gram genotypes, which clustered the genotypes into four groups. Sankar et al. (2015) screened 21 horse gram genotypes in a greenhouse to identify resistant genetic resources for anthracnose disease. Relying on percentage disease index scoring, the authors identified IC470275 genotype as immune against

Colletotrichum dematium, which causes anthracnose disease in horse gram (Sankar et al. 2015). Field screening of 110 horse gram genotypes identified five (AK-38, HG-GP, DPI-2278, Paiyur-1, and Paiyur-2) resistant to horse gram yellow mosaic virus (Prema and Rangaswamy 2017). Another earlier study that screened 23 genotypes identified six (AK-38, HG-14, HG-52, HG-59, HG-63, HG-75) resistant to horse gram yellow mosaic virus and two (AK-38 and HG-46) resistant to powdery mildew disease (Parimala et al. 2011).

Desingh and Kanagaraj (2019) reported PAIYUR-2 genotype as salt-tolerant based on its enhanced antioxidant enzymatic activity of superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase under high salinity stress. This genotype also had higher proline and glycine betaine contents and lower lipid peroxidation than CO-1 under salinity stress (Kanagaraj and Sathish 2017). In another study, higher accumulation of relative water content, APX, catalase, SOD, and proline and phenolic contents rendered drought tolerance in HPK4 compared to HPKC-2 (Bhardwaj and Yadav 2012). A wild relative of horse gram, *Macrotyloma sargarhwalensis* Gaur and Dangwal (IC 212722), contained 34.88% protein, double that of cultivated lines SK-2001, VLG-1, and Raipur Local (Yadav et al. 2004). Hence, the high protein content trait could be transferred to high-yielding horse gram genotypes by incorporating this wild species into pre-breeding programs. Mutation breeding approach is an important breeding approach for creating novel genetic variation, which has been used in horse gram (Chahota et al. 2013; Bolbhat and Dhumal 2009). Substantial genetic variability for phenological traits, including flowering time, maturity, photo-insensitivity, determinacy, and semi-dwarfism, was noted in the M2 generation of HPKC 2 and VLG 1 genotypes by applying gamma radiation at 150, 250, and 350 Gy (Chahota et al. 2013). Likewise, mutagenesis through gamma rays altered plant ideotypes and reproductive traits, including anther and pollen size in horse gram (Priyanka et al. 2021).

Despite significant genetic variability in horse gram, it lacks proper germplasm exploration, collection, characterization, and a structured breeding program for rapid improvement. Thus, intervention of modern breeding tools and investment in germplasm collection and characterization is urgently needed to harness its novel traits and increase genetic gain.

8.5 Status of Genomic Resources in Horse Gram

8.5.1 Molecular Markers for Assessing Diversity

Molecular markers are important genomic resources for germplasm characterization, diversity analysis, gene(s) mapping or tagging, and marker-assisted breeding in crop improvement programs. Initial diversity assessments of horse gram accessions used RAPD and ISSR markers (Sharma et al. 2015a). Subsequent efforts developed SSR markers to characterize horse gram germplasm collected from diverse regions (Sharma et al. 2015a, b). The diversity analysis resulted in 309 amplified alleles with an average of 2.64 alleles per primer with an average polymorphism

Table 8.1 Status of genomic resources in horse gram

Chromosome no. and ploidy	$2n = 20, 22, 24$
Genome size	400 Mb (Bhardwaj et al. 2013a)
Molecular markers	SSR, intron length polymorphism (Sharma et al. 2015a; Chahota et al. 2017; Kaldate et al. 2017; Kumar et al. 2020)
	RAPD, ISSR (Sharma et al. 2015b); 6195 SSR (Bhardwaj et al. 2013a); SNP (Shirasawa et al. 2021; Mahesh et al. 2021)
Mapping populations	2 (Katoch and Chahota 2021; Chahota et al. 2020; Shirasawa et al. 2021)
Genetic map	1423.4 cM (Chahota et al. 2020); 980 cM (Shirasawa et al. 2021); 1541.1 cM (Katoch and Chahota 2021)
Trait QTLs	Drought tolerance QTL (Katoch and Chahota 2021); yield QTLs (Chahota et al. 2020)
	Phenological trait QTL (Chahota et al. 2020); powdery mildew QTL (Mahesh et al. 2021)
Draft genome sequence	HPK-4, 259.2 Mb (Shirasawa et al. 2021); 279.12 Mb (Mahesh et al. 2021)
Transcriptome assembly	Bhardwaj et al. (2013a); Shirasawa et al. (2021); Mahesh et al. (2021)

information content of 0.36 (Sharma et al. 2015b). Likewise, 117 SSRs developed from NGS were used in diversity analysis of 48 diverse horse gram lines, revealing observed heterozygosity(Ho:0.64) and PIC value of 0.46 (Kaldate et al. 2017). The mining of potential SSR motifs of horse gram identified 23,305 SSR motifs (Chahota et al. 2017). Of these, 30 SSRs were used to assess diversity in 360 horse gram genotypes, which obtained 170 alleles with PIC values ranging from 0.15 to 0.76 and an average of 0.50 (Chahota et al. 2017). Recently, transcriptome sequencing of horse gram mined 7352 SSRs, of which 150 randomly selected SSRs were used for population structure and diversity analysis in 58 diverse horse gram genotypes (Kumar et al. 2020). A total of 130 alleles were produced with PIC values ranging from 0.065 to 0.78. Another diversity analysis of 89 horse gram accessions, in association with 277 SNP markers using dd-RAD-seq analysis, resulted in two major clusters (Shirasawa et al. 2021). Most of the genotypes bred at CSK-HPAU had close relationships at the genetic level. Assembly of another horse gram genome sequence allowed in silico prediction of 77,821 SSRs, including mono, di-, tri-, tetra-, penta-, and hexa-nucleotide repeats (Mahesh et al. 2021), with potential for molecular breeding in horse gram. Likewise, ddRAD sequencing of 40 horse gram germplasm resulted in 3942 high-quality SNPs (Mahesh et al. 2021). The developed molecular markers could be used for marker-assisted breeding in horse gram. The genomic resources developed in horse gram are listed in Table 8.1.

8.6 Linkage Mapping and QTL Identification

With the availability of RAPD, ISSR, SSR, and conserved orthologous sequence markers, the first genetic map of horse gram, constructed from an intraspecific mapping population derived from HPK4 × HPKM249, measured 1423.4 cM with a mean marker interval of 9.6 cM (Chahota et al. 2020). Another genetic map, spanning 1541.1 cM and harboring 295 markers, was developed from 162 recombinant inbred lines generated from a HPK4 × HPKM249 mapping population (Katoch and Chahota 2021). Subsequently, SNP markers derived from the HK4 draft genome assembly enabled the construction of a high-density linkage map developed from an F_2-derived HPK-4 × HPKM-193 mapping population comprising 214 progenies. The map contained 1263 SNPs on ten linkage groups measuring 980 cM (Shirasawa et al. 2021).

8.7 Trait Mapping

The paucity in genomic resource development in horse gram limits trait mapping. Horse gram has the inherent capability for adapting to diverse environments, including drought stress. Thus, Katoch and Chahota (2020) mapped drought-tolerant related traits in horse gram using 162 RIL populations in association with 295 molecular markers and identified seven QTL related to drought stress tolerance, including QTL for malondialdehyde content on LG2, root length on LG3 and LG9, and proline and chlorophyll content on LG4 (Katoch and Chahota 2021). Recently, a genome-wide association study analysis of 40 horse gram genotypes using 4624 variants derived from ddRAD sequencing identified 10 significant marker-trait associations for powdery mildew resistance (Mahesh et al. 2021). More mapping populations are needed to map various traits of agronomic importance in horse gram.

8.8 Draft Genome Assembly

Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] is a diploid legume ($2n = 20$, 22). Shirasawa et al. (2021) constructed the draft genome sequence of HPK-4 and assembled 10 pseudomolecules measuring 259.2 Mb. The draft genome sequence covered 89% of the total length of the assembled sequences and predicted 36,105 genes (Shirasawa et al. 2021). A comparative genomics and macrosynteny analysis suggested that the horse gram genome sequence is more similar to the *Vigna angularis* genome than the *Phaseolus vulgaris* genome (Shirasawa et al. 2021). Subsequently, Mahesh et al. (2021) assembled the genome sequence of PHG-9 measuring 279.12 Mb with N50 size of 111,472 bp using a combination of long-read (PacBio) and short-read (Illumina) sequencing technologies. The total number of predicted genes was 24,521. These genome assemblies could help elucidate the underlying trait candidate genes for future improvements in horse gram at the genetic and genomic levels.

8.9 Status and Progress of Functional Genomics Research in Horse Gram

Functional genomics provides novel insights into the gene(s) function of various plant traits, including biotic, abiotic, and developmental biology. However, little attention has been paid to developing functional genomic resources in horse gram.

A subtracted cDNA library analysis resulted in 1050 ESTs, of which, 531 were unique (Reddy et al. 2008). Validation of selected differentially expressed genes, such as encoding heat shock cognate protein, metallothionein, dehydrin, calmodulin-binding protein, DEAD Box RNA helicase, using reverse northern and northern blot analysis indicated prominent differential expression under various abiotic stresses, including drought, cold, and salinity in horse gram (Reddy et al. 2008). Similarly, an EST-based approach was used to investigate miRNAs in horse gram (Bhardwaj et al. 2010). Eight potential miRNAs were elucidated and designated hor-miR1 to hor-miR8. The target genes of these identified miRNAs were related to zinc finger, protein kinase, and calcineurin-like phosphodiesterase, contributing to plant development and biotic and abiotic stress responses (Bhardwaj et al. 2010).

Subsequent advances in transcriptome sequencing, especially RNA-seq analysis, provided the opportunity to investigate global expression profiling of candidate genes with higher precision. De novo transcriptome analysis of drought-sensitive M-191 and drought-tolerant M-249, two contrasting horse gram genotypes, using RNA-seq identified 26,045 transcripts and 21,887 unigenes (Bhardwaj et al. 2013a). A Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis indicated the possible role of various genes involved in ribosome metabolism, hormone signaling, valine degradation, gluconeogenesis, and purine nucleotide degradation under drought stress. Moreover, the authors elucidated the involvement of various classes of transcription factor gene families, including NAC, MYB-related, and WRKY, in response to drought stress (Bhardwaj et al. 2013a). Furthermore, of the identified differentially expressed genes, 10 candidate genes, including *C103051_65* (encoding heat shock protein), *C22097_65* (encoding cysteine proteinase inhibitor 4), *C103 779_65* (encoding calmodulin-binding factor), and *C83083_65* (encoding dehydrin), were validated via qRT-PCR. Earlier, the involvement of WRKY TF regulating drought and salinity stress was reported in horse gram (Kiranmai et al. 2016). Transcriptome analysis of horse gram identified 1680 genes encoding TFs belonging to ERF, NAC, WRKY, MYB, and bZIP (Mahesh et al. 2021). In addition, genome and transcriptome assembly construction identified *Mu_g15332.t1* and *Mu_g08128.t1* genes with lipoxygenase activity conferring defense against pests and pathogens, *Mu_g18571.t1* encoding the Bowman-Birk protease inhibitor, and *Mu_g12727.t1* gene has homology with *CONSTANS* (*CO*) and *HEADING DATE1* (*Hd1*) gene contributing in photoperiod sensitivity (Mahesh et al. 2021).

miRNAs are endogenous regulatory RNAs participating in the regulation of plant development and biotic and abiotic stresses (Kim 2006). Fifteen conserved miRNAs belonging to nine families were uncovered in horse gram from expressed sequence tags and a transcriptome shotgun assembly, from which 39 putative target genes

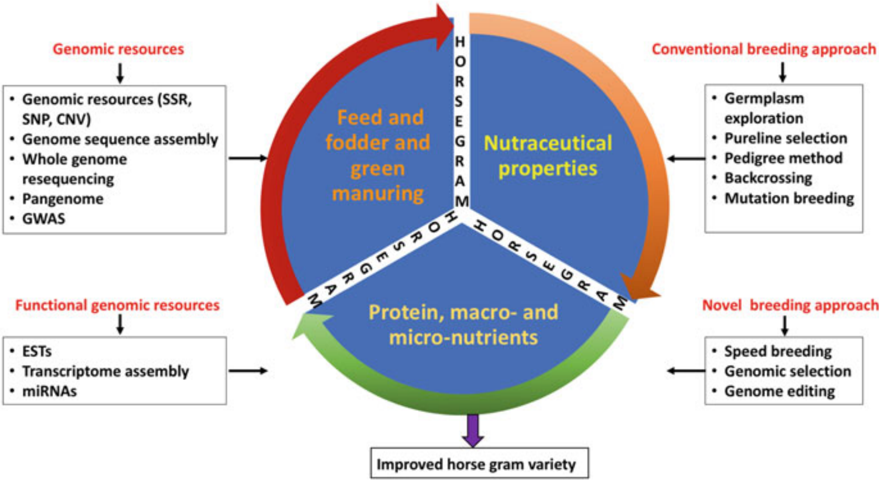

Fig. 8.1 Integration of conventional breeding, modern breeding tools, and genomic resources for improving genetic gain in horse gram, a multipurpose legume crop

were predicted using bioinformatic analysis (Yasin et al. 2020). The predicted functions of these genes were related to controlling cell wall degradation, hormone biosynthesis, glutathione, ascorbate synthesis, glycolysis, development, and biotic and abiotic stress signaling (Yasin et al. 2020). However, considering advances in functional genomics, horse gram is lagging behind the other major grain legumes. Hence, future research could provide novel insights into trait candidate genes with plausible functions in horse gram. Thus, combining a conventional breeding approach with genomics resources, functional genomic resources, and modern breeding tools could improve genetic gain in horse gram (see Fig. 8.1).

8.10 Horse Gram, a Minor Legume Yet Climate-Resilient Crop Ensuring Global Food Security

Horse gram is grown globally in temperate and subtropical countries as a cheap source of dietary protein. Being a leguminous crop, horse gram can help ameliorate soil nitrogen and thus maintain agroecological systems (Bhartiya et al. 2015). Moreover, it can survive water and salinity stress and can be grown on marginal land with low inputs due to its efficient antioxidant mechanisms, osmotic adjustment, and genetic and molecular mechanisms (Murthy et al. 2012; Ramesh et al. 2011; Bhardwaj and Yadav 2012; Bhardwaj et al. 2013a, b; Kamenya et al. 2021). Likewise, it can adapt to a wide range of temperatures where other crops fail to survive (Smartt 1985). The current urgency of global climate change and increasing human population is challenging for sustaining environments and global food security. Thus, breeding elite horse gram for frequent episodes of drought and heat stress is of great interest.

8.11 Conclusion and Future Prospects

Horse gram is an important climate-smart grain legume, adaptable to extreme weather conditions and resilient to various biotic and abiotic stresses. It also has nutraceutical benefits, is rich in essential nutrients, and can be used as feed and fodder (Prasad and Singh 2015). Despite having multiple uses, its current genetic and genomics resources limit its rapid improvement. However, with the decreasing costs of genome sequencing, some progress has been made, including decoding a draft genome sequence. Further investment in trait mapping gene(s)/QTL contributing to climate resilience is needed for future genomic-assisted breeding. Implementation of modern breeding tools, such as genome-wide association studies for unveiling marker-trait associations, whole-genome resequencing, pangenomes, genomic selection, high-throughput phenotyping, speed breeding, and CRISPR/Cas9-based genome editing techniques, could be used for the rapid genetic gain in this underutilized legume crop (Kamenya et al. 2021). Extensive germplasm exploration, germplasm conservation in gene banks, characterization, seed system strengthening, marketing, and popularization among resource-poor farmers by strengthening extension services in underdeveloped and developing countries is also needed to expedite the overall improvement of horse gram. Thus, leveraging a conventional breeding approach, modern breeding tools, and “multi-omics” based approaches, and establishing effective seed systems and extension services are urgently needed for rapid genetic gain in horse gram to meet the rising demand of protein-based foods and the sustainable growth development goals set by the United Nations.

References

Amal TC, Thottathil AT, Veerakumari KP, Rakkiyappan R, Vasanth K (2020) Morphological traits of drought tolerant horse gram germplasm: classification through machine learning. J Sci Food Agric 100:4959–4967

Bazzano LA, He J, Ogden LG, Loria C, Vupputuri S, Myers L et al (2001) Legume consumption and risk of coronary heart disease in US men and women: NHANES I epidemiologic follow-up study. Arch Intern Med 161:2573–2578

Bhardwaj J, Yadav SK (2012) Comparative study on biochemical and antioxidant enzymes in a drought tolerant and sensitive variety of horse gram (Macrotyloma uniflorum) under drought stress. Am J Plant Physiol 7:17–29

Bhardwaj J, Mohammad H, Yadav SK (2010) Computational identification of microRNAs and their targets from the expressed sequence tags of Horsegram (Macrotyloma uniflorum (Lam.) Verdc.). J Struct Funct Genom 11:233–240

Bhardwaj J, Chauhan R, Swarnkar MK, Chahota RK, Singh AK, Shankar R, Yadav SK (2013a) Comprehensive transcriptomic study on horsegram (*Macrotyloma uniflorum*): de novo assembly, functional characterization and comparative analysis in relation to drought stress. BMC Genomics 14:647–665

Bhardwaj J, Mahajan M, Yadav SK (2013b) Comparative analysis of DNA methylation polymorphism in drought sensitive (HPKC2) and tolerant (HPK4) genotypes of horse gram (Macrotyloma uniflorum). Biochem Genet 51:493–502

Bhartiya A, Aditya JP, Kant L (2015) Nutritional and remedial potential of an underutilized food legume horsegram [*Macrotyloma uni orum* (Lam.) Verdc.] :a review. J Anim Plant Sci 25:908–920

Bolbhat SN, Dhumal KN (2009) Induced macromutations in horsegram [Macrotyloma uniflorum (lam.) verdc]. Legume Res 32:278–281

Bravo L, Siddhuraju P, Saura-Calixto F (1999) Composition of underexploited Indian pulses. Comparison with common legumes. Food Chem 64:185–192

Chahota RK, Sharma TR, Dhiman KC, Kishore N (2005) Characterization and evaluation of horsegram (*Macrotyloma uniflorum* Roxb.) germplasm for Himachal Pradesh. Indian J Plant. Genet Res 18(2):221–223

Chahota RK, Sharma SK, Sharma TR, Kumar N, Kapoor C (2013) Induction and characterisation of agronomically useful mutants in horsegram (*Macrotyloma uniflorum*). Indian J Agric Sci 83(10):1105–1109

Chahota RK, Shikha D, Rana M, Sharma V, Nag A, Sharma TR, Rana JC, Hirakawa H, Isobe S (2017) Development and characterization of SSR markers to study genetic diversity and population structure of horsegram germplasm (*Macrotyloma uniflorum*). Plant Mol Biol Rep 35:550–561

Chahota RK, Sharma V, Rana M et al (2020) Construction of a framework linkage map and genetic dissection of drought- and yield-related QTLs in horsegram (*Macrotyloma uniflorum*). Euphytica 216:61

Desingh R, Kanagaraj G (2019) Differential responses to salt stress on antioxidant enzymatic activity of two horse gram[Macrotyloma uniflorum(lam.) Verdc] varieties. Int J Res Anal Rev 6:425–430

Fuller DQ, Harvey EL (2006) The archaeobotany of Indian pulses: identification, processing and evidence for cultivation. Environ Arch 11(2):219–246

Fuller DQ, Murphy C (2018) The origin and early dispersal of horsegram (*Macrotyloma uniflorum*), a major crop of ancient India. Genet Resour Crop Evol 65:285–305

Gautam M, Katoch S, Chahota RK (2020a) Comprehensive nutritional profiling and activity directed identification of lead antioxidant, antilithiatic agent from Macrotyloma uniflorum (Lam.) Verdc. Food Res Int 137(109600)

Gautam M, Datt N, Chahota RK (2020b) Assessment of calcium oxalate crystal inhibition potential, antioxidant activity and amino acid profiling in horse gram (Macrotyloma uniflorum): high altitude farmer's varieties. 3 Biotech 10(9):1–2

Hooker J (1879) Flora of British India, vol 2. L. Reeve & Co., London

Kadam SS, Salunkhe DK (1985) Nutritional composition, processing, and utilization of horse gram and moth bean. Crit Rev Food Sci Nutr 22:1–26

Kaldate R, Rana M, Sharma V, Hirakawa H, Kumar R, Singh G, Chahota RK, Isobe SN, Sharma TR (2017) Development of genome-wide SSR markers in horsegram and their use for genetic diversity and cross-transferability analysis. Mol Breed 37:103

Kamenya SN, Mikwa EO, Song B, Odeny DA (2021) Genetics and breeding for climate change in Orphan crops. Theor Appl Genet 23:1–29

Kanagaraj G, Sathish C (2017) Effect of salinity stress on carbohydrate, lipid peroxidation and proline contents of two horse gram [Macrotyloma uniflorum (Lam.) Verdc] varieties. J Sci Agric 1:37–45

Katoch M, Chahota R (2021) Mapping of Quantitative Trait Locus (QTLs) that contribute to drought tolerance in a recombinant inbred line population of horsegram (*Macrotyloma uniflorum*). bioRxiv

Kim VN (2006) MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol 6: 376–385

Kingwell-Banham E, Fuller DQ (2014) Horse gram: origins and development. In: Encyclopedia of global archaeology. Springer, New York

Kiranmai K, Gunupuru L, Nareshkumar A, Amaranatha Reddy V, Lokesh U, Pandurangaiah M, Venkatesh B, Kirankumar T, Sudhakar C (2016) Expression analysis of WRKY transcription factor genes in response to abiotic stresses in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.). Amer J Mol Biol 6:125–137

Krishna KR (2010) Agroecosystems of South India: nutrient dynamics, ecology and productivity. BrownWalker Press, Boca Raton, FL

Kumar R, Kaundal SP, Sharma V, Sharma A, Singh G, Sharma RK, Chahota RK, Sharma TR (2020) Development of transcriptome-wide SSR markers for genetic diversity and structure analysis in Macrotyloma uniflorum (Lam.) Verdc. Physiol Mol Biol Plants 26:2255–2266

Mahesh HB, Prasannakumar MK, Manasa KG, Perumal S, Khedikar Y, Kagale S, Soolanayakanahally RY, Lohithaswa HC, Rao AM, Hittalmani S (2021) Genome, transcriptome, and germplasm sequencing uncovers functional variation in the warm-season Grain Legume Horsegram Macrotyloma uni orum (Lam.) Verdc. Front Plant Sci 12:758119. https://doi.org/10.3389/fpls.2021.758119

Morris JB (2008) Macrotyloma axillare and M. uniflorum: descriptor analysis, anthocyanin indexes, and potential uses. Genet Resour Crop Evol 55:5–8

Murthy SM, Devaraj VR, Pand A, Tejavathi DH (2012) Studies on the activities of antioxidant enzymes under induced drought stress in vivo and in vitro plants of *Macrotyloma uniflorum* (Lam.) Verdc. Recent Res Sci Technol 4:34–37

Neelima G, Kumar CS, Kumar KA, Nataraj V (2021) Genetic divergence and interrelationships for yield and yield attributing traits in horsegram. Electr J Plant Breed 12:464–472

Parimala K, Meenakumari KVS, Sudhakar R, Durga KK (2011) Screening of horsegram genotypes against yellow mosaic virus and powdery mildew diseases. Indian J Plant Protect 39:160–161

Prasad SK, Singh MK (2015) Horse gram-an underutilized nutraceutical pulse crop: a review. J Food Sci Technol 52:2489–2499

Prema GU, Rangaswamy KT (2017) Field evaluation of horsegram germplasm/genotypes against Horsegram Yellow Mosaic Virus (HgYMV) Disease and biological transmission of horse gramyellow mosaic virus to different leguminous hosts through white flies. Int J Agric Sci:0975–3710

Priyanka S, Sudhagar R, Vanniarajan C, Ganesamurthy K, Souframanien J (2021) Gamma rays induced morphological, flowering and palynological modifications in horse gram (Macrotyloma uniflorum). J Environ Biol 42:1363–1369

Ramesh CK, Rehman A, Prabhakar BT, Vijay AB, Aditya SJ (2011) Antioxidant potential in sprout vs. seeds of *Vigina radiate* and *Macrotyloma uniflorum*. J Appl Pharm Sci 1:99–103

Reddy PCO, Sairanganayakulu G, Thippeswamy M, Reddy PS, Reddy MK, Sudhakar C (2008) Identification of stress-induced genes from the drought tolerant semi-arid legume crop horsegram (Macrotyloma uniflorum (Lam.) Verdc.) through analysis of subtracted expressed sequence tags. Plant Sci 175:372–384

Sankar AU, Anitha K, Sivaraj N, Kumari KM, Sunil N, Chakrabarty SK (2015) Screening of horsegram germplasm collected from Andhra Pradesh against anthracnose. Legume Res 38: 753–757

Sharma V, Rana M, Katoch M, Sharma PK, Ghani M, Rana JC, Sharma TR, Chahota RK (2015a) Development of SSR and ILP markers in horsegram (*Macrotyloma uniflorum*), their characterization, cross-transferability and relevance for mapping. Mol Breed 35:102

Sharma V, Sharma TR, Rana JC, Chahota RK (2015b) Analysis of genetic diversity and population structure in horsegram (*Macrotyloma uniflorum*) using RAPD and ISSR markers. Agric Res 4(3):221–230

Shirasawa K, Chahota R, Hirakawa H, Nagano S, Nagasaki H, Sharma TR, Isobe S (2021) A Chromosome-scale draft genome sequence of horsegram (Macrotyloma uniflorum). Gigabyte:1–23. bioRxiv

Siddhuraju P, Becker K (2007) The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Walp.) seed extracts. Food Chem 101:10–19

Singh R, Salam JL, Mandavi NC, Saxena RR, Sao A (2019) Genetic diversity estimation in horsegram [Macrotyloma uniflorum (L.) Verdcout] genotypes collected from bastar plateau. Int J Curr Microbiol App Sci 8:613–620

Smartt J (1985) Evolution of grain legumes II. Old and new world pulses of lesser economic importance. Expt Agric 21:1–18

Sodani S, Paliwal R, Jain L (2006) Phenotypic stability for seed yield in rainfed horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.]. J Arid Legumes:4:340

Spate OH, Learmonth AT (1967) India and Pakistan, a general and regional geography, 3rd edn. Methuen & Co.Ltd., London

Sreerama YN, Sashikala VB, Vishwas PM, Singh V (2012) Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: evaluation of their flour functionality. Food Chem 131:462–468

Sudha N, Mushtari-Begum J, Shambulingappa KG, Babu CK (1995) Nutrients and some anti-nutrients in horse gram (Macrotyloma uniflorum (Lam.) Verdc.). Food Nutr Bull 16:81–83

Verdcourt B(1971) Phaseoleae. In: Gillet, J.B., Polhill, R.M., Verdcourt, B. (eds) Flora of east tropical Africa. Leguminosae sub family Papilionoideae. Crown Agents. 2:581–594

Yadav S, Negi KS, Mandal S (2004) Protein and oil rich wild horsegram. Genet Resour Crop Evol 51(6):629–633

Yadava ND, Vyas NL (1994) Arid legumes. Agrobios, India

Yasin JK, Mishra BK, Pillai MA, Verma N, Wani SH, Elansary HO, El-Ansary DO, Pandey PS, Chinnusamy V (2020) Genome wide in-silico miRNA and target network prediction from stress responsive Horsegram (Macrotyloma uniflorum) accessions. Sci Rep 14:1–23

9

Understanding Abiotic Stress Responses in Lentil Under Changing Climate Regimes

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Anirban Chakraborty, and Sabhyata Bhatia

Abstract

Lentil (*Lens culinaris* Medik.) is a cool-season grain legume crop that is mainly cultivated across the semi-arid regions of Australia, South Asia, Africa, and North America. The crop is highly valued for its nutritional attributes such as dietary proteins (22–35%), carbohydrates, minerals, and fiber that play a significant role in alleviating malnutrition and micronutrient deficiencies across populations in developing countries. The last five decades have seen an upward trend in global production of lentils from 0.85 to 5.73 Mt. suggesting its increasing demand and utilization. However, various abiotic stresses such as drought, heat, cold, salinity, and nutrient deficiency impose severe threats to the global lentil yield and productivity. The current book chapter is an attempt to comprehend the morpho-physiological and biochemical changes occurring during these stresses and the developmental plasticity shown by the plant to counteract them. Furthermore, the current status of research focusing on the development of novel molecular and functional markers/tags, identification of candidate genes/QTLs responsible for abiotic stress tolerance, the intervention of high throughput genotyping and phenotyping platforms, development of populations and linkage maps, and omics studies have been discussed. Some tolerant germplasm and varieties developed through conventional and next-generation breeding approaches are also enlisted making the book chapter a concise platform for reports of abiotic stress tolerance in lentils.

Baljinder Singh and Asish Kumar Padhy contributed equally to this work.

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Keywords

Lentil · *Lens culinaris* Medik · Abiotic stress · Climate change · Omics approaches

9.1 Introduction

Lentil (*Lens culinaris* Medik.) is a self-pollinating, diploid grain legume crop with a genome size of ~4 Gbp (Arumuganathan and Earle 1991). It is a rich source of dietary proteins, fibers, minerals, and carbohydrates thereby playing important role in mitigating nutritional deficiencies in developing nations. Due to its low glycemic index, it is recommended as a remedy for diseases such as obesity, diabetes, and cardiovascular problems (Kumar et al. 2015). Like other legumes, lentil is capable of biological nitrogen fixation thereby improving soil health and providing crop rotational benefits to cereal crops. It is also able to effectively control the proliferation of insects, pests, and weeds (Kumar et al. 2021a, b). In the last five decades, global production of lentil has risen from 1.08 to 5.73 Mt. due to its nutritional and ecological benefits (FAOSTAT, 2019), (Fig. 9.1). Canada (37.7%) is the largest producer of lentil in the world followed by India (21.4%) (FAOSTAT 2019). Although the area under lentil cultivation has increased in India during the last few decades but comparatively low productivity has been achieved due to the impact of various environmental stresses. Different abiotic stresses such as heat, cold,

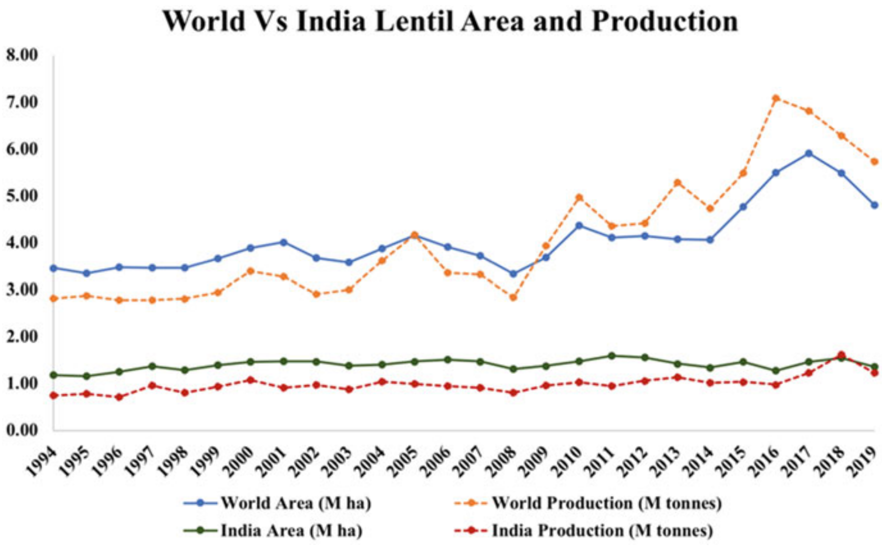

Fig. 9.1 lentil production and area in World and India

drought, mineral nutrient deficiency, and soil salinity adversely affect lentil crop production worldwide (Muehlbauer et al. 2006). Of these abiotic stresses, drought, heat (generally terminal heat stress), and cold (at the seedling stage) are considered as the most important ones over the globe while the nutrient deficiency/toxicity and salinity are of lesser importance worldwide but are of more significance and concern in localized regions (Kumar et al. 2013). In South Asia, lentil is grown generally in the post-rainy season with residual soil moisture content. Therefore, the crop confronts adverse effects of early withdrawal of rains. Lentil also faces terminal heat stress at the grain filling stage which in turn causes forced maturity and ultimately results in lower productivity. In general, spring-planted crop frequently suffers from terminal heat and drought stress whereas winter-sown crop experiences cold stress and frost injuries mainly in the early developmental stages (Kumar et al. 2013). In the Sub-Saharan region, yield loss occurs frequently due to drought and water stress at critical growth stages while in the Central and West Asia-North Africa (CWANA) region frequent drought and terminal heat stress cause severe yield loss (Kumar et al. 2013).

To overcome these abiotic stresses, research efforts have been made in lentil during the last four decades toward screening diverse lentil germplasm, utilizing promising accessions in traditional breeding programs and the release of improved varieties. Several abiotic stress-tolerant varieties have been released by different lentil breeders (Kumar et al. 2017; Sehgal et al. 2017; Kumawat et al. 2018). Although traditional breeding strategies are suitable for developing improved lentil varieties for various monogenic traits, in the case of quantitative traits they are less precise and time-consuming. However, with the development of next-generation sequencing technologies, increasing efforts are being made toward the development of molecular markers, linkage maps, transcriptome sequences, and identification of QTLs/genes controlling various agronomically important traits. These genomic resources could be utilized for lentil improvement through different biotechnological techniques such as marker-assisted breeding and genetic engineering. The book chapter summarizes various abiotic stresses, their symptoms (Fig. 9.2), abiotic stress-tolerant varieties and germplasm registered in various parts of the globe (Table 9.1), recent research progress, and future directions in lentil breeding.

9.2 Heat Stress

Global climate changes have intensified the incidences and severity of heatwaves imposing drastic effects on plants. Extreme high temperature manifests a variety of morphological, physiological, biochemical, and reproductive changes in plants by disrupting the dynamic balance of cells (Hemantaranjan et al. 2014). The heat-affected plants display greater inhibition of root and shoot growth, premature leaf abscission and senescence, declined pollen and ovule viability, reduced fertilization, and seed set. Additionally, plants subjected to heat stress exhibit severe defects in their growth and development and prolonged exposure can subsequently challenge their survival. High temperature directly impacts crucial phenomena including

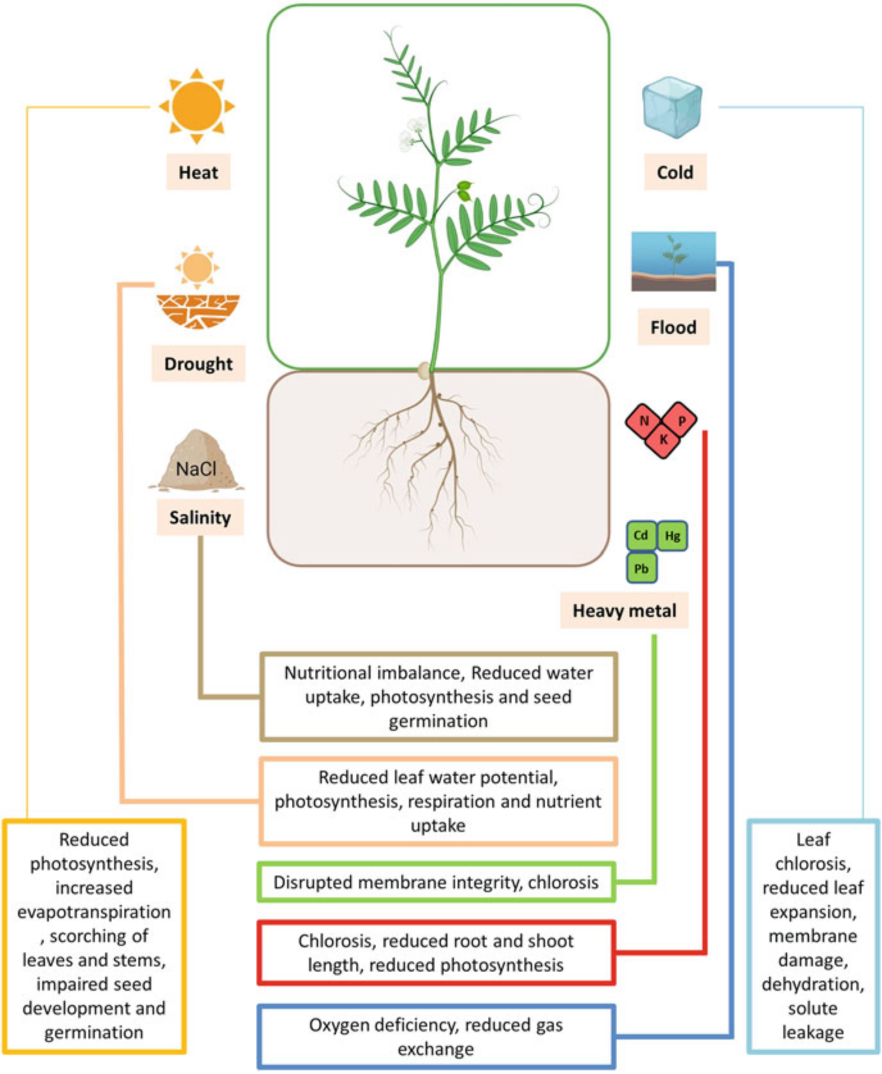

Fig. 9.2 Abiotic stresses and their effects on lentil

photosynthesis and respiration in plants by severing the functionality of important associated enzymes, namely, Ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco), affecting chlorophyll biosynthesis, disrupting chloroplast components, and suppressing carbohydrate metabolism (Redden et al. 2014). Heat stress also impacts water relations and membrane stability along with modulating the levels of hormones, primary and secondary metabolites in the affected plants (Hemantaranjan et al. 2014). In crop plants, heat stress is often associated with irreversible damages

Table 9.1 Genotypes and varieties registered and used for different abiotic stresses in lentil

Abiotic stresses	Accession/genotypes	Origin	References
Heat	IG 3745, IG 4258 and IG 5146	India	Kumar et al. (2016)
	ILL 10712 IG 2507, IG 3327, IG 3330, IG 3546, IG 4258	ICARDA India	Kumar et al. (2017)
	FLIP2009	India	Sita et al. (2018)
	IG2507, IG3263, IG3297, IG3312, IG3327, IG3546, IG3330, IG3745, IG4258, and FLIP2009	-	Sita et al. (2017)
	IG 2507 and IG 4258	-	Sehgal et al. (2017)
	LL931	India	Bhandari et al. (2016)
	PDL-2	ICARDA	Singh et al. (2019a, b)
Cold	ILL 4605—Precoz	ICARDA	Erskine (1996)
	ILL4400	Jordanian	Erskine and El Ashkar (1993)
	FLIP- 2014-135 L	ICARDA	Ghimire and Mandal (2019)
	FLIP 97-28 L, FLIP 2002-9 L, FLIP 2002-32 L, FLIP 2002-37 L, FLIP 2003-28 L, FLIP 2004-2 L, FLIP 2004-20 L and FLIP 2004-29 L	ICARDA	BICER and Sakar (2008)
	FLIP 2004-13 L	ICARDA	Shah et al. (2013)
Drought	DPL53 and JL1	India	Sehgal et al. (2017)
	HUL-57	-	Mishra et al. (2016)
	ILL 6002	ICARDA	Sarker et al. (2005)
	GP3643 and IC248956	India	Sinha et al. (2018)
	FLIP 2004-13 L	ICARDA	Shah et al. (2013)
	"ILL-10700," "ILL-10823," and "FLIP-96-51"	ICARDA	Singh et al. (2013)
	ILL4400	-	Panahyan-e-Kivi et al. (2009)
	Eston Castelluccio	Canada Italy	Muscolo et al. (2014, 2015)
	Digger, Cumra, Indianhead, ILL 5588, ILL 6002, and ILL 5582	Australia Turkey Canada Syria	Biju et al. (2018)
	ILL 6002	ICARDA	Sarker et al. (2005)
	"PDL-1" and "FLIP-96-51"	-	Singh et al. (2016a, b)
	"PDL-1" and "PDL-2"	-	Singh et al. (2016a, b)
	DPL 53, JL 1, and IPL 98/193	-	Kumar et al. (2012)
	BLX-010014-9	Bangladesh	Ahmed et al. (2016)
	ILL-6031, ILL-9893 and ILL-8095	-	Chakherchaman et al. (2009a, b)

(continued)

Table 9.1 (continued)

Abiotic stresses	Accession/genotypes	Origin	References
	ILL8 (78S26002)	Jordanian	Erskine and El Ashkar (1993)
Salinity	Pantelleria and Ustica	Italy	Muscolo et al. (2014, 2015)
	Çağıl and Altın Toprak	Turkey	Kökten et al. (2009)
	Siliana, Local oueslatia and Nefza	Tunisia	Ouji et al. (2015)
	Giza 9	Egyptian	Gaafar and Seyam (2018)
	L-4076, RLG-254, RLG-234 and RLG-258	India	Kumawat et al. (2018)
	PDL1	ICARDA	Pandey and Sengar (2020), Singh et al. (2017a, b, c, d, 2020a, b)
	PSL-9	-	Singh et al. (2017a, b, c, d, 2020a, b)
	NEL-2704	-	Mamo et al. (1996)
	Masoor 93 and Mansehra 89	-	Yasin et al. (2002)
Heavy metal			
As	L 414 and L 830	-	Talukdar (2013)
Nutrient			
(a) Micronutrients			
Fe	DL10–2, VL140, IPL219, RLG109, HPCL649, PL063, L4147, SL2–28, VL141, DPL62, LH07–26, LH84–8, VL520, IPL321, LL1190, PL099, L4704	India	Kumar et al. (2014)
	Diy-Kulp, Kahmar1, and Mar-Kız	Turkish	Karaköy et al. (2012)
	PI 299215	Chile	Khazaei et al. (2017)
	LR11–17 and LR11–133, ILL 8006/Barimasur-4 (Parent)	Canada	Aldemir et al. (2017)
	ILL 82, ILL 890, ILL 6870, ILL 6848, ILL 6281 ILL 6272, ILL 5553, ILL 5505, ILL 5416, ILL 5384, ILL 5151, ILL 4830, ILL 4606, ILL 4164, ILL 2297, ILL 1861, and ILL 224	India	Choukri et al. (2020)
Zn	L4589, DL10–2, VL140, IPL219, 4147, HPCL617, VL141 (81.547 mg/kg grain), RVL32, LH07–28, DPL62, KBL102, LL1161, LH07–26, LH84–8, VL520, IPL320, L4704	India	Kumar et al. (2014)

(continued)

Table 9.1 (continued)

Abiotic stresses	Accession/genotypes	Origin	References
	LSI88, ILL 6281, and ILL 5505	India	Choukri et al. (2020)
	Kahmar1, Adıy-kah2, and Diy-Kulp	Turkish	Karaköy et al. (2012)
	ILL 4875	Uzbekistan	Khazaei et al. (2017)
Mn	Kahmar2 and Adıy-Kah2	Turkish	Karaköy et al. (2012)
(b) Macronutrients			
P	Kahmar1, Diy-Haz, and SiirtBes	Turkish	Karaköy et al. (2012)
K	Kahmar1, MarKız, and Surfa-Siv	Turkish	Karaköy et al. (2012)
Mg	Kahmar1, Kahmar2, and Diy-Haz landraces	Turkish	Karaköy et al. (2012)
Ca	DiyDic1, Diy-Kulp, and Diy-Dic2	Turkish	Karaköy et al. (2012)
Cu	Kahmar1, Diy-Krcd, and Mar-Kız3	Turkish	Karaköy et al. (2012)

to the cellular compartments that culminate in a decline of crop yield and productivity.

Lentil is adapted to grow in low to mild temperature regimes. The optimum temperature for the lentil growth cycle varies between 18 and 30 °C with the vegetative stage requiring low temperature followed by slightly warmer conditions during maturity (Roy et al. 2012). It is usually cultivated under the crop rotation systems with rice and often, delayed sowing predisposes it to a variety of abiotic stresses, particularly heat stress (Sehgal et al. 2017). Lentil displays high sensitivity to even a slight rise in temperature ($\geq$32 °C) (Ibrahim 2011). A notable instance was recorded in 2009 in south-eastern Australia where heat stress (35 °C) for a continuous period of 6 days resulted in yield losses of 70% equating to a cost of \$1000/ha (Delahunty et al. 2015). On similar lines, lentil plants grown in a controlled experimental set up with temperature $>$ 38 °C at the reproductive phase showed a yield reduction of 87% (Bhandari et al. 2016).

The effect of high temperature on lentil production is governed by the intensity and duration of the exposure. Heat exposure during the vegetative phase of the crop reduces overall growth causing low biomass and adversely affecting the aerial plant parts which show symptoms of leaf chlorosis and mild to severe burning (Sehgal et al. 2017; Bhandari et al. 2020). The photosynthetic efficiency of lentils is challenged owing to decreased stomatal conductance and premature leaf senescence observed under extreme heat stress (Bhandari et al. 2016; Sita et al. 2017). Heat sensitivity causes membrane damage, intense oxidative injuries, and diminishes sucrose concentration in lentil leaves (Bhandari et al. 2020). Also, the nodulation efficiency in lentils is curtailed on subjection to heat stress resulting in a significantly lower number of nodules (Sita et al. 2017). Lentil is particularly susceptible to heatwaves at the reproductive and pod filling phases (Sehgal et al. 2019). High temperature accelerates reproductive growth which promotes early maturity and impairs proper seed setting leading to significant yield losses and deterioration of grain quality (Bhandari et al. 2016; Kumar et al. 2021a, b). At the onset of the reproductive phase, supra-optimal temperatures affect pollen and ovule viability, inhibit pollen tube germination and cause a reduction in the number of flowers (Bhandari et al. 2016, 2020; Sita et al. 2017). Seed filling is a critical and sensitive phase of crop production wherein various important biochemical components are channelized into developing seeds. Heat stress at this stage in lentils causes premature grain filling leading to reduced seed size and consequently, compromising the true yield potential (Tickoo et al. 2005; Barghi et al. 2012; Delahunty et al. 2015). Impairment in seed growth rate by 30–44% with a marked decrease in internal storage reserves such as starch, proteins, and fats in lentil seeds are also observed during heat stress (Sita et al. 2018). Additionally, lentil seeds exhibited a significant decrease in the concentration of micronutrients including calcium, iron, potassium, phosphorus, zinc, and amino acids such as glycine, alanine, isoleucine, leucine, and lysine when subjected to heat stress (Sita et al. 2018).

The high sensitivity of lentils to heat stress has detrimental effects and necessitates appropriate measures to combat yield losses. Several studies have been carried out in the direction to understand the genetic basis and the molecular machinery governing heat stress responses in lentil. For instance, a QTL mapping performed by Singh et al. (2017a, b, c, d) reported two QTLs, that is, *qHt_ss* and *qHt_ps* with a phenotypic variance of 12.1 and 9.23% for seedling survival and pod set during heat stress respectively. Likewise, RNA-seq analysis of two lentil genotypes IG4258 (heat tolerant) and IG3973 (heat sensitive) identified genes involved in the tryptophan pathway that could mediate tolerance to heat stress in lentil (Kumar et al. 2021a, b). The study also led to the identification of 4968 SSR markers pertaining to heat stress in the crop plant. Similarly, another transcriptome study conducted on two lentil genotypes, that is, PDL-2 (heat tolerant) and JL-3 (heat sensitive), under heat stress conditions reported a significant change in the expression of genes involved in cell wall modification and secondary metabolic pathways (Singh et al. 2019a, b). Moreover, Kumar et al. (2017) performed screening of 334 diverse lentil genotypes for different physiological and biochemical parameters and identified 11 genotypes contrasting for heat tolerance. This study reported a functional marker, ISM11257, present only in the tolerant genotypes which could be utilized in breeding programs of lentil for the selection of heat-tolerant genotypes. Furthermore, screening of diverse lentil genotypes by different groups led to the identification of some of the heat-tolerant genotypes such as IG 3745, IG 4258, IG 5146, LL931, and PDL-2 which could be a useful resource for new variation of heat tolerance in high-yielding cultivars in India (Kumar et al. 2016; Bhandari et al. 2016; Singh et al. 2019a, b).

9.3 Cold Stress

Cold stress is another major stress encountered by plants in the field and has devastating effects on plant yield and productivity globally. Cold stress in plants is of two types, that is, chilling injury (<20 °C) and freezing injury (0 °C) (Thakur et al.

2010). The symptoms of cold stress in plants are generally manifested through changes in morphological features such as stunted growth and small and deformed leaves (Öktem et al. 2008; Kahraman et al. 2004a, b). Plants exposed to cold stress also display alterations in their biochemical properties such as increased MDA (malondialdehyde), total sugar, proline, ROS (reactive oxygen species) content. Lentil is cultivated as a spring crop in higher altitudes places (above 850 m) of Central West Asia and North Africa (CWANA) to avoid harsh winter cold. An economic loss of 8–13% has been reported in various regions over the globe due to cold stress (Eujayl et al. 1999).

Screening of lentil for cold tolerance in the field usually employs calculation of the percentage of germination and damage due to cold stress (Hamdi et al. 1996). However, varying climatic conditions impedes the assessment of cold stress in field conditions and therefore requires artificial screening. The process of artificial screening involves cold induction and hardening of 10°C/0°C day/night temperature and 10 hr. photoperiod for 6–8 weeks following which, the temperature is decreased to −15°C with a cooling rate at 2–3°C/h. and exposure period of 3 hr. under controlled conditions (Ali et al. 1999).

Cold stress in lentil induces changes in morphological features such as burns with withered leaflets and branches, and under extreme conditions, it causes wilting and death of susceptible plants (Hamdi et al. 1996). However, the genetic studies for cold tolerance are very limited. Estimation of heritability of cold tolerance in lentil under control and field conditions revealed the trait to be under the control of the environment and additive genes (Ali and Johnson 2000). A significant association between QTL for leaf area and QTL for winter hardiness has been reported (Kahraman et al. 2010). Moreover, a positive correlation between anthocyanin pigmentation of leaf, stem, and cold tolerance was demonstrated by Ali and Johnson (1999) and was subsequently adopted as an indirect field screening method for winter hardiness in lentil under field conditions. Eujayl et al. (1999) reported an RAPD marker, OPS-16_{750} from a RIL population of ILL5588 (R)/ L692-16-1 (S), which could help in marker-assisted selection for tolerance against frost injury. Likewise, a consistent QTL for winter survival trait has been identified in a RIL population of WA8649090/Precoz (Kahraman et al. 2004a, b). The study also reported two ISSR markers, that is, ubc808-12 and ubc840–3, that were associated with winter survival and injury, respectively, and thus could be evaluated for their role in screening and developing cold-tolerant lentil varieties. Analysis of RNA-seq data of lentil under cold stress led to the identification of 8905 EST-SSRs. Interestingly, most of these markers were associated with the genes that are known to regulate cold tolerance in plants (Sohrabi et al. 2018). A deep super-SAGE (Serial Analysis of Gene Expression) transcriptome of lentil identified genes involved in photosynthesis, glycine-rich and proline-rich proteins, and transmembrane proteins that could confer tolerance against frost injury (Barrios et al. 2017). Recent studies have also indicated the role of ABA in cold stress wherein several genes involved in ABA biosynthesis were highly upregulated following cold stress (Sohrabi et al. 2022).

An evaluation for winter hardiness utilizing 3592 lentil accessions in Central Anatolia identified 238 lentil genotypes that were cold tolerant. Most of these germplasms are being maintained by the gene bank of ICARDA (ILL52, 465, 468, 590, 7115, 9832, 9918, etc.) Erskine et al. (1981) and have often been used to develop improved varieties in several countries including India, Turkey, Uzbek, Cifci, and Morocco. While Turkey has registered Kafkas, Morocco has developed Bichette and Zaris. Iran and Pakistan have also registered cold-tolerant lentil varieties, Gachsaran and Shiraz-96, respectively (Kumar et al. 2013). A winter hardy variety named "Morton (LC 9979010)" developed by USDA-ARS from F_6 RIL of WA8649090/ WA8649041 was registered in 2004 (Muehlbauer and McPhee 2007). Winter hardiness in lentil was first demonstrated by Hamdi et al. (1996) in fields of Syria and Turkey, where the minimum temperature dipped to −18°C during the winters. Lentil species *L. orientalis* was able to withstand the extreme cold and thus considered as the most tolerant in comparison to *L. nigricans* and *L. ervoides* that were susceptible to cold stress. Kahraman et al. (2004a, b) used lines WA8649041 (PI 547039) and WA8649090 (PI 547038) as tolerant genotypes and also categorized ILL1878 and ILL669 as tolerant lines for cold stress. These germplasm resources and molecular candidates could provide a substantial base for lentil breeding programs to develop high-yielding cold-tolerant genotypes.

9.4 Drought

Legumes in general and lentil in particular are moderately tolerant to drought but severe drought stress, especially at the reproductive phase, leading to flower abortion thereby significantly impacting yield-related traits such as number of filled pods, total number of pods, and seed weight. Drought also causes a reduction in the number of branches, plant height, shoot width, and total plant biomass. Water stress in lentil results in huge yield losses due to suppression of photosynthesis, respiration, and various metabolic processes. The magnitude of the loss of yield depends upon growth stages (vegetative or reproductive), the intensity of stress, and frequent association with other stresses especially heat (>32 °C) (Choukri et al. 2020; Zandalinas et al. 2018). It has been observed that drought stress has a negative impact on phenological traits such as flowering date, grain yield, and nutrition quality. Phenologically, drought stress causes plants to flower early leading to early podding and early maturity (Sehgal et al. 2017; Choukri et al. 2020). A decrease in the iron and zinc content levels in lentil seeds has also been observed which could be the result of decrease in nutrient absorption and uptake during drought stress (Sehgal et al. 2019). Seed nitrogen and protein content are also reported to be decreased under drought stress which could possibly be explained by the association of water stress with inhibition of protein synthesis and nitrogen fixation (Choukri et al. 2020). Moreover, the physiological and biochemical response of lentil genotype under drought stress by Sinha et al. (2018) have demonstrated the higher relative water content in drought-tolerant genotype, decreased CO_2 assimilation, production of reactive oxygen species, reduced chlorophyll content, and higher MDA content in drought susceptible plant. Additionally, accumulation of proline and anthocyanin and changes in antioxidant enzyme activities are also observed. Coupled with these, the expression of drought stress marker genes such as DREB, RD, and ERD are also upregulated. These genes further regulate the expression of downstream drought-responsive genes involved in redox homeostasis, accumulation of compatible solutes, etc. Sehgal et al. (2017) demonstrated increased cell membrane damage, reduced photosynthetic traits in heat and drought alone or in combination. Drought, as well as heat, negatively impacts the grain filling process. Stressed grains are generally small and shriveled. The grain filling process is a highly complex and coordinated process requiring coordination in embryonic stages hormonal changes and starch metabolism. Drought and temperature can cause a reduction in protein stability and activity of enzymes, involved in the synthesis of photosynthesis assimilates as well its translocation, resulting in uncoupling of reactions ROS accumulation (Sehgal et al. 2018; Awasthi et al. 2014).

Several efforts have been made for screening of drought-tolerant accessions of lentil from a diverse germplasm collection based on phenological, morphological, and yield traits, but the screening based on physiological traits utilizing high throughput phenotyping is still limited in lentil. Rajendran et al. (2020) characterized a set of 162 genetically diverse lentil accessions for heat and drought tolerance through Focused Identification of Germplasm Strategy (FIGS), linking the adaptive trait with environmental parameters. Some studies also suggest that the application of certain chemicals and seed priming before sowing may provide tolerance to heat and drought. For example, heat priming and treatment with chemicals such as γ-Aminobutyric Acid (GABA) have been reported to provide heat tolerance to lentil (Bhardwaj et al. 2021). Similarly, Farooq et al. (2020) reported that osmo-priming of seeds confers drought tolerance in lentil. Alternatively, it has been shown that silicon supplementation significantly increased ground biomass, chlorophyll concentration, relative water content, osmolyte concentration, and antioxidant enzyme activity levels in drought-stressed lentil plants (Biju et al. 2021). Therefore, it is proposed that an association analysis of markers responsible for physiological traits coupled with precise and efficient phenotyping protocols to understand the mechanism of drought tolerance in lentil would be a good strategy for drought tolerance research in lentils. However, very limited work has been done towards this. Idrissi et al. (2016) identified 14 QTLs governing different root and shoot traits related to drought tolerance in a population of 132 RILs derived from a cross between ILL6002 and ILL5888 using 252 dominant and co-dominant markers. Moreover, a transcriptome study carried out on two lentil genotypes, that is, PDL-2 (drought-tolerant) and JL-3 (drought-sensitive), under drought stress at the seedling stage reported total 18,369 transcripts with differential expression under stress conditions (Singh et al. 2017a, b, c, d). Similarly, another transcriptome study of drought-sensitive cultivar "sultan" under short and long-term drought stress was carried out by Morgil et al. (2019). This study reported 2915 and 18,327 DEGs under short and long-term stress conditions, respectively. These genes/transcripts may serve as potential candidates for future studies in lentil for drought tolerance.

9.5 Salinity Stress

Salinity stress is one of the major abiotic factors menacing food security as high salt concentrations in the soil severely affect crop performance. Salinity has a direct effect on plant germination, plant vigor, and crop yield, especially in arid and semi-arid regions of the world (Deinlein et al. 2014). Lentil which is one of the major nitrogen-fixing grain legumes cultivated in semi-arid and dry areas of the Middle East also incurs this stress. Several physiological parameters like plant germination, shoot length, seedling length, shoot fresh weight, root fresh and dry weight, seedling vigor index, and salinity tolerance index are significantly reduced during salinity stress in lentil (Keshtiban et al. 2015, Kumawat et al. 2018). Alterations in the biochemical parameters like chlorophyll content, soluble sugar, soluble protein, proline and activities of catalase (CAT), proline dehydrogenase (PDH), and ascorbate oxidase (AO) are also associated with salinity stress (El-khamissi et al. 2018). A study on five lentil cultivars under different salinity conditions revealed ascorbate-glutathione involvement in salinity tolerance mechanism in Giza 9 (salt-tolerant) (Gaafar and Seyam 2018). Screening for salinity tolerance can be performed by estimating germination percentage and salinity tolerance index after subjecting the plants to different concentrations of NaCl at the seedling stage (Kumawat et al. 2018). Turan et al. 2007 developed the method for screening of salinity tolerance at the vegetative stage by subjecting the lentil plants to salt stress up to 200 mM NaCl.

Genetic studies for salt tolerance in lentil are limited. Dissanayake et al. (2021), screened a diverse panel of 276 lentil accessions through traditional and image-based approaches under salt stress. Singh et al. (2017a, b, c, d) reported restricted uptake of Cl^- and Na^+ in wild (ILWL-137) and tolerant (PDL-1) genotype when compared to sensitive (L-4076) due to the presence of a thick layer of epidermis and endodermis. Utilizing Genome-wide association studies (GWAS), the significant marker-trait associated regions for salt tolerance traits were identified along with candidate genes like HKT potassium transporters and transcription factors regulating salinity tolerance on chromosomes 2 and 4 (Dissanayake et al. 2021). By crossing salt-sensitive (L-4147 and L-4076) and salt-tolerant (PDL-1 and PSL-9) genotypes, Singh et al. (2019a, b) generated a population for screening the genotypes for salt tolerance. They used seedling survival and FDA signal as a criterion to test the parents' F1, F2, and F3 progenies for salinity tolerance in a 120 mM NaCl salt solution. Of the 495 SSR markers that were used to screen for contrasting parents, only 11 were polymorphic of which 7 SSR markers were associated with salinity stress. QTL mapping using genotyping and phenotyping data identified QTLs explaining 65.6% phenotypic variation for salt stress tolerance. Singh et al. (2021a, b) performed comparative physiological, biochemical, and transcriptomic studies in salt-tolerant (PDL-1) and salt-sensitive (L-4076) cultivars under control (0 mM NaCl) and salinity stress (120 mM NaCl) conditions at the seedling stage that led to the identification of 17,433 DEGs between treated and control plants. DEGs associated with phytohormone-mediated signaling, nitrogen metabolism, and cellular stress signaling were enriched. This study also identified 5643 SSRs and thousands of SNPs markers for utilizing in enhancing the genetic linkage map of lentil (Singh et al. 2021a, b). Moreover, metabolomic analysis of two lentil varieties, F-56 and LC-960254 revealed a decrease in the levels of organic acids, accumulation of sugar, polyols, and several metabolites in leaves and roots of plants under salinity stress (Skliros et al. 2018). Khatib et al. (2011), performed transformation of lentil using DREB1A gene which enhanced drought and salinity tolerance of lentil.

9.6 Nutrient Stress

Plants require 14 inorganic elements known as essential nutrients to complete their life cycle. Insufficient availability of any nutrient results in its deficiency which has a characteristic effect on the plant's phenotype. Severe deficiency of nutrients may result in reduced yield and growth. The essential elements are divided into two groups, that is, macronutrients and micronutrients based on their concentration in the plant dry matter. Macronutrients are comprised of nitrogen, potassium, calcium, magnesium, phosphorous, and sulfur. On the other hand, micronutrients are required in minute quantities, generally below the 100 parts per million (ppm) levels, and contribute to less than 1% of the plant's dry weight. Essential micronutrients for plants are Iron (Fe), zinc (Zn), manganese (Mn), molybdenum (Mo), boron (B), chlorine (Cl), and copper (Cu). Some nutrients are required in trace amounts like cobalt (Co), vanadium (V), sodium (Na), and silicon (Si), and therefore there is not much concern for deficiency of these trace elements.

Nitrogen constitutes around 1–5% of total plant dry matter. It is an integral component of nucleic acids, proteins, chlorophyll, phytohormones, and several secondary metabolites. Plants such as legumes are capable of forming a symbiotic relationship with nitrogen-fixing bacteria and are capable of uptaking atmospheric nitrogen in the form of ammonium and nitrate. Nitrate is more mobile when compared to ammonium in the soil and thus is more available. Amino acids are also a source of nitrogen present in the soil for plants. The availability of different nitrogen sources varies from soil to soil; it depends on soil texture, pH, moisture, and microbial activity. As a result of this, plants have also evolved their mechanism of nitrogen uptake and metabolism. Nitrogen deficiency in plants can result in reduced growth and photosynthesis and increased accumulation of anthocyanin (Kant et al. 2011). Zakeri et al. (2013) studied the effect of different nitrogen treatments on plant yield and reported that N supply up to the mid-pod stage produces maximum yield when compared to any other treatment. In another study by Bannayan Aval et al. (2017), it was reported that 40 kg ha^{-1} of N produced maximum growth and yield in lentil cultivars. Similarly, Niri et al. (2010) reported that urea concentration of 25 kg ha^{-1} showed an increased number of filled pods per plant, seed protein, and nitrogen content. The use of nitrogen fertilizer is an efficient strategy to overcome yield losses in lentil due to nitrogen deficiency. However, the cost of fertilizer application is very high; therefore, it would be beneficial to develop nitrogen uptake efficient varieties. In a study conducted by Ashraf and Zafar (1996), nitrogen deficiency treatment of salt-tolerant (ILL 6793) and salt-sensitive (ILL 6439) accessions showed increased levels of chlorophyll b, soluble sugars, and efficient nitrogen utilization in tolerant accession when compared to the sensitive accession.

Fe-deficiency is common in high pH and calcareous soils. Fe cannot be readily mobilized from older leaves to younger leaves therefore major symptoms appear in young leaves. Symptoms of Fe deficiency include interveinal chlorosis in younger leaves, leaflets turning yellow–green, white, or light-colored necrotic patches on the leaflets of young leaves. Acute Fe deficiency can cause leaflets to wither and die. Fe deficiency symptoms are transient and disappear during reproductive growth. To cope up with iron deficiency in the soil, foliar spray of 1% $FeSO_4$ solution was found to be effective against yield loss in chickpea and lentil. Ferritin-1 and IRT-1 genes were found to be the key regulatory genes during Fe deficiency as well as toxicity (Erskine et al. 1993; sen Gupta et al. 2017). Twenty-one QTLs regulating Fe content have been identified in a lentil population segregating for seed iron concentration using genotyping by sequencing (GBS) (Aldemir et al. 2017).

Lentil is one of the cheapest sources of dietary Zn, which plays an important role in the normal growth, development, and activity of various enzymes in plants and animals. Zn-deficient soils are quite common in tropical and temperate climates. Zn deficiency progressively increases in high pH and calcareous soils but decreases with an increase in the level of soil organic matter. Zinc is crucial for the biosynthesis of various plant hormones, majorly indole acetic acid. It works as a cofactor for several crucial enzymes like alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase, and RNA polymerase. Zn deficiency negatively impacts water uptake efficiency, nodulation, and nitrogen fixation. It also leads to shortened inter-nodal length forming a rosette-like plant growth pattern. Upon Zn deficiency, initially, the younger leaves become pale green but later the upper surface of leaves and lower portions of stems show red–brown pigmentation (Islam et al. 2018). Zn toxicity also adversely affects lentil growth by reducing photosynthetic efficiency. Excess Zn in plants can disrupt normal ionic homeostasis by interfering with critical ion uptake, transport, and osmotic regulation, resulting in metabolic processes like transpiration and photosynthesis being disrupted. By producing free radicals and reactive oxygen species (ROS), zinc phytotoxicity causes oxidative stress (Abbas et al. 2010; Ibrahim 2014). Khazaei et al. (2017) conducted an association mapping analysis in lentil utilizing a panel of 138 accessions and identified two SNP markers linked with seed iron content and one marker with zinc content. Genes underlying these regions encode for iron and zinc-related functions. Similarly, another study by Singh et al. (2017a, b, c, d), performed association analysis using SSR markers in 96 diverse germplasm and identified three SSRs linked with iron content and four SSRs linked with the seed zinc content.

Phosphorus is another important macronutrient required for the proper growth of lentil. Phosphorus deficiency has profound effects on plants as it is a constituent of membrane phospholipids and nucleic acids. It also plays a significant role in energy transfer and signal transduction. Phosphorus also has an important role in nodule formation in plants. Plants under phosphorus-deficient conditions show delayed flower formation and a reduced number of flowers (Niri et al. 2010). Sarker et al. (2015) reported the development of long root hairs, reduced diameter of stem and root, and reduced number of xylem vessels in lentil plants under phosphorus-deficient conditions. External phosphorus (P_2O_5) application in soil resulted in improved yield and protein content in lentil plants in comparison to control plants (Singh et al. 2017a, b, c, d). Similarly, another study reported an increase in pods per plant, thousand-grain weight, grain yield, and seed recovery at 100 and 120 kg P ha^{-1} application (Ali et al. 2017). However, there are no reports related to the identification of genomic regions involved in phosphorus uptake. Identifying markers related to phosphorus uptake would be a beneficial resource for the development of efficient varieties.

Potassium is one of the essential and most abundant cations present in plants. It significantly contributes to plant processes that are associated with growth and development. Potassium deficiency in plants is associated with inhibition of nitrogen assimilation and reduced photosynthesis (Hafsi et al. 2014). K^+ deficiency symptoms in the plant include curling and brown scorching of leaf tips and leaf chlorosis between veins (Xiao-Lei et al. 2012). In lentil, the effect of potassium deficiency was evaluated in salt-tolerant and salt-sensitive lines, which showed increased total sugars content in salt-tolerant genotype while an antagonistic trend was observed in sensitive genotype under K^+ deficiency (Ashraf and Zafar 1997). This study suggested the role of soluble sugars in maintaining osmoregulation in plants under deficiency conditions. Jahan et al. (2009) reported an increase in lentil yield with increased potassium fertilizer application in Bangladesh. Similarly, another study reported an increase in chlorophyll content, the relative water content in plants treated with urea and potassium chloride, which mitigates terminal moisture stress in lentil plants (Yadav et al. 2021).

Mn deficiency is a condition that occurs only in certain areas. It is frequently found in patches and is related to dry, fluffy soil conditions. Mn shortage causes juvenile leaves to be pale green and mature leaves to have light brown patches on their leaflets and stipules. During Mn-deficiency, severely damaged plants lose a large number of leaflets, which leads to the mortality of young shoots, resulting in increased axillary bud growth. Mn excess has a negative impact on plant growth as well. Symptoms first begin on fresh leaves and then progress to mid and older leaves. On immature leaves, little purple dots form along the margins, and on somewhat older leaves, the spots turn scarlet (https://grdc.com.au/resources-and-publications/grownotes). QTL mapping was carried out in a biparental lentil population of 120 RILs in a work by Ates et al. (2018). Seed manganese concentration of population varied from 8.5 to 26.8 mg/kg. This analysis identified total of 6 QTLs which explained phenotypic variation of 15.3–24.1%. These QTL regions could be the basis of manganese biofortification in lentil, but work in this area needs to be strengthened.

Boron is a key component of cell walls and has been linked to enzyme activation, membrane maintenance, nucleic acid metabolism, and sugar transport (Dordas et al. 2000). Yellowing of the leaflet edges is one of the first signs of B insufficiency. Affected leaflets' tips and edges begin to die, and the terminal buds turn brown in hue. Plant roots become thick and stunted, with black tips. In lentil, boron deficiency in soil could result in yield losses. For instance, Srivastava et al. (2000) evaluated the growth of 494 exotic lentil germplasm in the Chitwan region of Nepal with boron deficiency in the soil. The study reported reduced yield, severe chlorosis, and stunting in 82% of the exotic accessions while 10 accessions gave no yield. Excess of Boron also affects Lentil growth adversely. Boron toxicity affects root growth more adversely in comparison to shoot growth. B toxicity in Lentil is characterized by the presence of chlorosis and necrosis, majorly at the margins and tips of older leaves. Total chlorophyll content decreases and the activity of antioxidant enzymes SOD, LOX, and GPX increases in Boron toxicity (Tepe and Aydemir 2011). Screening of 310 lentil genotypes under boron toxicity condition (18.20 mg/kg) displayed varying responses in terms of yield and identified two tolerant accessions, that is, ILL213A and ILL2024 with higher biomass as compared to intolerant accessions (Hobson et al. 2006). These genotypes could play a significant role in the identification of genes responsible for boron tolerance. Kaur et al. (2014), reported a single genomic region responsible for B tolerance in lentil using 546 SSRs and 768 SNPs in a biparental mapping population (Cassab × ILL2024). This region consisted of candidate genes that play role in boron tolerance and could be utilized for marker development in lentil.

Conclusively, if micronutrients are applied in conjunction with macronutrients it will positively influence the plant vigor, physiology, and morphology. Optimal concentrations of these nutrients increase the overall protein content of lentil seeds, seed number, seed weight, nodulation, and overall plant growth (Islam et al. 2018). However, there are very limited reports available related to transcriptomes and the identification of genes/QTLs playing a significant role in the uptake and translocation of these nutrients. These studies will provide important candidates for lentil improvement through MAS and transgenic approaches to develop nutrient-efficient genotypes.

9.7 Next-Generation Strategies for Lentil Improvement

9.7.1 Genomics

During the last five decades, conventional breeding approaches have been able to significantly increase the productivity of lentil from 605 kg/ha to 1038 kg/ha (FAOSTAT 2019). However, these approaches are suitable for monogenic traits but lack precision and are time-consuming when it comes to quantitative traits that are influenced by genotype and environment interaction (Kumar et al. 2015). Therefore integration of several genomic approaches (GWAS, QTL mapping, transcriptomics, BSA-seq, etc.) into lentil breeding programs would expedite the generation of markers leading to the development of lentil varieties with high yield and productivity. Genomics-assisted breeding is a robust technique to precisely identify and fix superior alleles and develop high-yielding varieties that are suitable for challenging climatic conditions.

The pace of genomic resources development in lentil has been slow in comparison to other legumes such as soybean, pigeon pea, chickpea, and common bean.

Identification of loci associated with quantitative traits necessitates the large-scale development of molecular markers. Generation of these markers in lentil initially began with hybridization-based markers such as restriction fragment length polymorphism (RFLPs) that were utilized for construction of the first linkage map of lentil using an interspecific cross between *Lens culinaris* × *L. orientalis* (Havey and Muehlbauer 1989) and gradually moved towards the use of PCR-based markers such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSRs). The first extensive linkage map in lentil was developed utilizing a RIL population generated from a cross between *L. culinaris* and *L. orientalis* (Eujayl et al. 1998). Among PCR-based markers, SSRs have been extensively used and continue to be used for genetic diversity analysis and development of linkage maps in lentil (Jin et al. 2008; Gupta et al. 2012; Kushwaha et al. 2013; Dikshit et al. 2016; Singh et al. 2019a, b; Singh et al. 2021a, b). However, the advent of NGS technologies in the past decade has facilitated the rapid development of genomic resources such as SNPs, SSRs, transcripts, genes, and QTLs in lentil that could be utilized for crop improvement in a cost-effective manner (Singh et al. 2017a, b, c, d; Temel et al. 2014; Johnson et al. 2021). SNP markers can be generated using various techniques such as deep sequencing generating short and long reads, transcriptome sequencing and genotyping by sequencing, etc. Sharpe et al. (2013) generated ~44,879 SNPs using Illumina Genome Analyzer in lentil. Availability of SNP markers led to the development of genotyping platforms such as the Illumina GoldenGate platform accommodating more than 1000 markers of lentil (Sharpe et al. 2013; Kaur et al. 2014). Besides these platforms, a huge number of SNP markers have been generated during the past few years using transcriptome sequencing and genotype by sequencing approaches (Singh et al. 2017a, b, c, d; Sudheesh et al. 2016; Khazaei et al. 2017; Ma et al. 2020; Dissanayake et al. 2021; Johnson et al. 2021). Moreover, NGS approaches have enabled the development of a draft genome assembly of lentil cultivar CDC Redberry with 23× coverage, which continues to be further enriched by additional 125× coverage (https://knowpulse.usask.ca/lentil-genome). This has essentially led to the identification of SNPs that are found abundantly in legume genomes and can generate a large number of markers in a short genomic region overcoming the problems associated with PCR-based markers. Therefore, the SNP markers can be utilized to generate high density saturated linkage maps in lentil as was demonstrated by Temel et al. (2014) where a high-density linkage map was constructed using 50,960 SNP markers. Markers generated from these high throughput genotyping techniques can be utilized to construct high-density linkage maps and identify QTLs that in turn facilitate the identification of candidate genes/markers controlling traits of interest.

9.7.2 Phenomics

Although the advent of NGS-based sequencing approaches has led to the rapid development of molecular markers, the major bottleneck that remains in developing high-yielding varieties through molecular breeding is phenotyping. Currently, phenotyping of various traits in lentil is carried out through conventional methods that suffer major drawbacks such as the development of a reliable scoring system, low efficiency, and is labor intensive due to the lack of trained staff for efficiently visualizing and scoring the phenotype. However, the problems associated with the traditional methods of phenotyping may be overcome by high throughput phenotyping strategies that aid in the fast measurement of complex traits including resistance to biotic and abiotic stresses, plant growth, yield under controlled as well as field conditions (Dissanayake et al. 2020). High-throughput phenotyping includes thermal imaging, digital Red–Green–Blue (RGB) imaging, multi/hyperspectral imaging, fluorescence scanning, and other 3D imaging techniques (ranging (LiDAR) and light detection) (Dissanayake et al. 2020). Of these imaging techniques, hyperspectral and RGB imaging are the most extensively used methods. The use of high-throughput phenotyping has increased in several crop plants including cereals and pulses, albeit the use of these techniques in lentil is very limited (Qiu et al. 2019; Hu et al. 2020; Hairmansis et al. 2014; Atieno et al. 2017; Humplík et al. 2015). In a study by Marzougui et al. (2019), screening for Aphanomyces root rot (ARR) resistance was performed under field and greenhouse conditions using RGB, hyperspectral, and multispectral imaging techniques. Similarly, Dissanayake et al. (2020) developed RGB imaging-based phenotyping method for screening salt toxicity in lentils. In comparison with conventional methods, high throughput phenotyping techniques show more precision and accuracy and hence may be employed in molecular breeding programs of lentil for efficient identification of loci(s) controlling traits of interest.

9.7.3 Transcriptomics

Transcriptome analysis serves as an excellent source for the identification of genes that are expressed during multiple stages of tissue development as well as in response to different stress conditions (Wang et al. 2020; Singh et al. 2019a, b). The NGS-based sequencing approach facilitates the identification of molecular markers associated with differentially expressed genes and thus can be beneficial for the development of markers in lentil, where the complete reference genome is not available (Kaur et al. 2011; Sharpe et al. 2013; Verma et al. 2013; Wang et al. 2020). Initially, Sanger sequencing was used for the development of expressed sequence tags (ESTs) from the mRNAs expressed in different tissues of lentil (Kumar et al. 2021a, b). For example, eight lentil cultivars with diverse seed phenotypes were used to develop an EST library by Vijayan et al. (2009). Another study generated around 5000 ESTs from *Colletotrichum truncatum* infected lentil tissues (Bhadauria et al. 2011). One of the initial NGS-based de novo transcriptome analyses of lentil by Verma et al. (2013) reported assembly of 42,196 high-quality transcripts and developed 8722 SSRs. Likewise, RNA-seq analysis of two lentil cultivars, Precoz and WA8649041 identified 50,960 SNPs that were utilized for the construction of the linkage map (Temel et al. (2015). Likewise, a total of 9949 SSRs and 8260 SNPs were identified from the transcriptome data of drought-tolerant (PDL-2) and sensitive (JL-3) lentil genotypes (Singh et al. 2017a, b, c, d). Additionally, a total of 50 of the 9949 EST-SSRs generated in the previous study were further utilized for genetic diversity analysis of 234 genotypes of lentil (Singh et al. 2020a, b). Similarly, another study by Singh et al. (2019a, b) reported 194,178 high-quality SNPs and 141,050 SSRs in the transcriptome sequencing of two lentil genotypes: PDL-2 (tolerant) and JL-3 (sensitive) under heat stress conditions. These molecular markers could provide a basis for the dissection of various agronomical traits and the development of improved lentil cultivars.

9.8 Conclusion

As the world's population continues to grow at an alarming rate, the need to solve the problem of global hunger by utilizing grain legumes such as lentil becomes crucial. The quality and yield of lentil may be improved in several ways, majorly by dissecting the abiotic stress tolerance. For this, advanced genomics-assisted breeding of lentils must be considered. The introduction of Next Generation Sequencing (NGS) technologies has enabled the availability of genomic resources to solve the lack of genomic information. Numerous DNA markers are available to investigate genetic variation, stress-tolerant lines, and genetic information related to abiotic challenges. Advanced marker applications using integrated approaches combining GWAS, QTL mapping, QTL-seq, and transcriptomics can now pinpoint specific genes or groups of genes involved in abiotic stress tolerance. Numerous genes/QTLs coding for various agriculturally significant traits have been found, which help to enhance breeding efficiency. Furthermore, transcriptome analysis of lentils under various abiotic stresses has also identified stress-responsive genes thereby allowing identification of the target gene/site to be edited using genome editing technologies. This wealth of information will aid in identifying genes that control a variety of critical features and target areas for genome editing and genetic transformation. Moreover, given the demand for ongoing improvement in lentils, sequence-based breeding approaches will allow developing stress-tolerant varieties under changing climate regimes to address the problem of hunger and malnutrition.

References

Abbas G, Hassan G, Ali MA, Aslam M, Abbas Z (2010) Response of wheat to different doses of $ZnSO_4$ under Thal desert environment. Pak J Bot 42(6):4079–4085

Ahmed B, Alam MJ, Hossain MA, Sultana M, Islam MN, Ali MR (2016) Study of selected lentil genotypes against drought. Int J Appl Res 2:95–99

Aldemir S, Ateş D, Temel HY, Yağmur B, Alsaleh A, Kahriman A, Özkan H, Vandenberg A, Tanyolac MB (2017) QTLs for iron concentration in seeds of the cultivated lentil (*Lens culinaris* Medic.) via genotyping by sequencing. Turk J Agric For 41(4):243–255

Ali A, Johnson DL (1999) Association of growth habit and anthocyanin pigment with winter hardiness in lentil. Pak J Biol Sci 2:1292–1295

Ali A, Johnson D (2000) Heritability estimates for winter hardiness in lentil under natural and controlled conditions. Plant Breed 119:283–285. https://doi.org/10.1046/j.1439-0523.2000.00491.x

Ali A, Johnson D, Stushnoff C (1999) Screening lentil (*Lens culinaris*) for cold hardiness under controlled conditions. J Agric Sci 133(3):313–319. https://doi.org/10.1017/S0021859699006917

Ali A, Ahmad B, Hussain I, Ali A, Shah FA (2017) Effect of phosphorus and zinc on yield of lentil. Pure Appl Biol (PAB) 6(4):1397–1402

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218. https://doi.org/10.1007/BF02672069

Ashraf M, Zafar ZU (1996) Effect of nitrogen deficiency on growth and some biochemical characteristics in salt-tolerant and salt-sensitive lines of Lentil (*Lens culinaris* Medic.). Arch Agron Soil Sci 40(4):231–239

Ashraf M, Zafar ZU (1997) Effect of potassium deficiency on growth and some biochemical characteristics in two lines of lentil (*Lens culinaris* Medic.). Acta Physiol Plantarum 19(1):9–15

Ates D, Aldemir S, Yagmur B, Kahraman A, Ozkan H, Vandenberg A, Tanyolac MB (2018) QTL mapping of genome regions controlling manganese uptake in lentil seed. G3: Genes, Genomes. Genetics 8(5):1409–1416

Atieno J, Li Y, Langridge P, Dowling K, Brien C, Berger B, Varshney RK, Sutton T (2017) Exploring genetic variation for salinity tolerance in chickpea using image-based phenotyping. Sci Rep 7(1):1–11

Awasthi R, Kaushal N, Vadez V, Turner NC, Berger J, Siddique KH, Nayyar H (2014) Individual and combined effects of transient drought and heat stress on carbon assimilation and seed filling in chickpea. Funct Plant Biol 41(11):1148–1167

Bannayan Aval M, Yaghoubi F, Rashidi Z, Bardehji S (2017) Effect of different nitrogen levels on phenology, growth indices and yield of two lentil cultivars under rainfed conditions in Mashhad. Iran J Field Crops Res 15(4):939–956

Barghi SS, Mostafaii H, Peighami F, Zakaria RA (2012) Path analysis of yield and its components in lentil under end season heat condition. Int J Agric: Res Rev 2(Special Issue):969–974

Barrios A, Caminero C, García P, Krezdorn N, Hoffmeier K, Winter P, de la Vega MP (2017) Deep Super-SAGE transcriptomic analysis of cold acclimation in lentil (*Lens culinaris* Medik.). BMC Plant Biol 17(1):1–15

Bhadauria V, Banniza S, Vandenberg A, Selvaraj G, Wei Y (2011) EST mining identifies proteins putatively secreted by the anthracnose pathogen Colletotrichum truncatum. BMC Genomics 12(1):1–16

Bhandari K, Siddique KH, Turner NC, Kaur J, Singh S, Agrawal SK, Nayyar H (2016) Heat stress at reproductive stage disrupts leaf carbohydrate metabolism, impairs reproductive function, and severely reduces seed yield in lentil. J Crop Improv 30(2):118–151

Bhandari K, Sita K, Sehgal A, Bhardwaj A, Gaur P, Kumar S, Singh S, Siddique KH, Prasad PV, Jha U, Nayyar H (2020) Differential heat sensitivity of two cool-season legumes, chickpea and lentil, at the reproductive stage, is associated with responses in pollen function, photosynthetic ability and oxidative damage. J Agron Crop Sci 206(6):734–758

Bhardwaj A, Devi P, Chaudhary S, Rani A, Jha UC, Kumar S, Bindumadhava H, Prasad PV, Sharma KD, Siddique KH, Nayyar H (2021) 'Omics' approaches in developing combined drought and heat tolerance in food crops. Plant Cell Rep:1–41

Bicer BT, Sakar D (2008) Studies on variability of lentil genotypes in Southeastern Anatolia of Turkey. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 36(1):20–24

Biju S, Fuentes S, Gupta D (2018) The use of infrared thermal imaging as a non-destructive screening tool for identifying drought-tolerant lentil genotypes. Plant Physiol Biochem 127:11–24

Biju S, Fuentes S, Gonzalez Viejo C, Torrico DD, Inayat S, Gupta D (2021) Silicon supplementation improves the nutritional and sensory characteristics of lentil seeds obtained from drought-stressed plants. J Sci Food Agric 101(4):1454–1466

Chakherchaman SA, Mostafaei H, Yari A, Hassanzadeh M, Jamaati-e-Somarin S, Easazadeh R (2009a) Study of relationships of leaf relative water content, cell membrane stability and duration of growth period with grain yield of lentil under rain-fed and irrigated conditions. Res J Biol Sci 4(7):842–847

Chakherchaman SA, Mostafaei H, Imanparast L, Eivazian MR (2009b) Evaluation of drought tolerance in lentil advanced genotypes in Ardabil region. Iran J Food Agric Environ 7(3/4): 283–288

Choukri H, Hejjaoui K, El-Baouchi A (2020) Heat and drought stress impact on phenology, grain yield, and nutritional quality of lentil (*Lens culinaris* Medikus). Front Nutr 7:596307

Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI (2014) Plant salt-tolerance mechanisms. Trends Plant Sci 19(6):371–379

Delahunty, A., Nuttall, J., Nicolas, M., and Brand, J. (2015). Genotypic heat tolerance in lentil. In: Proceedings of the 17th ASA Conference. pp. 20–24

Dikshit, H. K., Singh, A., Singh, D., Aski, M., Jain, N., Hegde, V. S., Basandrai, A.K., Basandrai, D., and Sharma, T. R. (2016). Tagging and mapping of SSR marker for rust resistance gene in lentil (*Lens culinaris* Medikus subsp. *culinaris*)

Dissanayake R, Kahrood HV, Dimech AM, Noy DM, Rosewarne GM, Smith KF, Cogan NO, Kaur S (2020) Development and application of image-based high-throughput phenotyping methodology for salt tolerance in lentils. Agronomy 10(12):1992

Dissanayake R, Cogan NO, Smith KF, Kaur S (2021) Application of Genomics to Understand Salt Tolerance in Lentil. Genes 12(3):332

Dordas C, Chrispeels MJ, Brown PH (2000) Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. Plant Physiol 124(3):1349–1362

El-khamissi HA, El-Hamamsy SMA, Behairy RT (2018) Mitigation of drought stress on fenugreek seedlings by application of ascorbic acid. J Biol Chem Environ Sci 13(2):343–358

Erskine W (1996) Seed-size effects on lentil (*Lens culinaris*) yield potential and adaptation to temperature and rainfall in West Asia. J Agric Sci 126(3):335–341

Erskine W, El Ashkar F (1993) Rainfall and temperature effects on lentil (*Lens culinaris*) seed yield in Mediterranean environments. J Agric Sci 121(3):347–354

Erskine W, Myveci K, Izgin N (1981) Screening a world lentil collection for cold tolerance. Lens Newslett 8:5–8

Erskine W, Saxena NP, Saxena MC (1993) Iron deficiency in lentil: yield loss and geographic distribution in a germplasm collection. Plant Soil 151(2):249–254

Eujayl I, Baum M, Powell W, Erskine W, Pehu E (1998) A genetic linkage map of lentil (Lens sp.) based on RAPD and AFLP markers using recombinant inbred lines. Theor Appl Genet 97(1): 83–89

Eujayl I, Erskine W, Baum M, Pehu E (1999) Inheritance and linkage analysis of frost injury in lentil. Crop Sci 39:639–642

Faostat, F. A. O. (2019). Statistical databases. Food and Agriculture Organization of the United Nations. Available at. http://www.fao.org/faostat/en/#data/QC, September 28, 2021

Farooq M, Romdhane L, Al Sulti MK, Rehman A, Al-Busaidi WM, Lee DJ (2020) Morphological, physiological and biochemical aspects of osmopriming-induced drought tolerance in lentil. J Agron Crop Sci 206(2):176–186

Gaafar RM, Seyam MM (2018) Ascorbate–glutathione cycle confers salt tolerance in Egyptian lentil cultivars. Physiol Mol Biol of Plants 24(6):1083–1092

Ghimire NH, Mandal HN (2019) Genetic variability, genetic advance, correlation and heritability of cold tolerance lentil (*Lens culinaris* Medic.) genotypes at high Hill of Nepal. Int J Adv Res Biol Sci 6(11):1–10

Gupta M, Verma B, Kumar N, Chahota RK, Rathour R, Sharma SK, Bhatia S, Sharma TR (2012) Construction of intersubspecific molecular genetic map of lentil based on ISSR, RAPD and SSR markers. J Genet 91(3):279–287

Hafsi C, Debez A, Abdelly C (2014) Potassium deficiency in plants: effects and signaling cascades. Acta Physiol Plantarum 36(5):1055–1070

Hairmansis A, Berger B, Tester M, Roy SJ (2014) Image-based phenotyping for non-destructive screening of different salinity tolerance traits in rice. Rice 7(1):1–10

Hamdi A, Küsmenoĝlu I, Erskine W (1996) Sources of winter hardiness in wild lentil. Genet Resour Crop Evol 43:63–67. https://doi.org/10.1007/BF00126942

Havey MJ, Muehlbauer FJ (1989) Linkages between restriction fragment length, isozyme, and morphological markers in lentil. Theor Appl Genet 77(3):395–401

Hemantaranjan A, Nishant Bhanu A, Singh MN, Yadav DK, Patel PK, Singh R, Katiyar D (2014) Heat Stress Responses and Thermotolerance. Adv Plants Agric Res 1(3):00012

Hobson K, Armstrong R, Nicolas M, Connor D, Materne M (2006) Response of lentil (*Lens culinaris*) germplasm to high concentrations of soil boron. Euphytica 151(3):371–382

Hu Y, Knapp S, Schmidhalter U (2020) Advancing high-throughput phenotyping of wheat in early selection cycles. Remote Sens 12(3):574

Humplík JF, Lazár D, Fürst T, Husičková A, Hýbl M, Spíchal L (2015) Automated integrative high-throughput phenotyping of plant shoots: a case study of the cold-tolerance of pea (*Pisum sativum* L.). Plant Methods 11(1):1–11

Ibrahim HM (2011) Heat stress in food legumes: evaluation of membrane thermostability methodology and use of infra-red thermometry. Euphytica 180(1):99–105

Ibrahim S (2014) Effect of zinc on lentil (*Lens culinaris* L.) metabolites and antioxidant enzyme activities. RADS J Biol Res Applied Sci 5(1):20–24

Idrissi O, Udupa SM, De Keyser E, McGee RJ, Coyne CJ, Saha GC, Muehlbauer FJ, Van Damme P, De Riek J (2016) Identification of quantitative trait loci controlling root and shoot traits associated with drought tolerance in a lentil (*Lens culinaris* Medik.) recombinant inbred line population. Frontiers. Plant Sci 7:1174

Islam M, Karim M, Oliver M, Hosain M, Urmi TA, Hossain M, Haque MM (2018) Impacts of trace element addition on lentil (*Lens culinaris* L.) agronomy. Agronomy 8(7):100

Jahan SA, Alim MA, Hasan MM, Kabiraj UK, Hossain MB (2009) Effect of potassium levels on the growth, yield and yield attributes of lentil. Int J Sustain Crop Prod 4(6):1–6

Jin LIU, Jian-Ping GUAN, Dong-Xu XU, Zhang XY, Jing GU, Xu-Xiao ZONG (2008) Genetic diversity and population structure in lentil (*Lens culinaris* Medik.) germplasm detected by SSR markers. Acta Agron Sin 34(11):1901–1909

Johnson N, Boatwright JL, Bridges W, Thavarajah P, Kumar S, Shipe E (2021) Genome-wide association mapping of lentil (*Lens culinaris* Medikus) prebiotic carbohydrates toward improved human health and crop stress tolerance. Sci Rep 11(1):1–12

Kahraman A, Kusmenoglu I, Aydin N, Aydogan A, Erskine W, Muehlbauer FJ (2004a) Genetics of winter hardiness in 10 lentil recombinant inbred line populations. Crop Sci 44:5–12. https://doi.org/10.2135/cropsci2004.5000

Kahraman A, Kusmenoglu I, Aydin N, Aydogan A, Erskine W, Muehlbauer FJ (2004b) QTL mapping of winter hardiness genes in lentil. Crop Sci 44:13–22. https://doi.org/10.2135/cropsci2004.1300

Kahraman A, Demirel U, Ozden M, Muehlbauer FJ (2010) Mapping of QTLs for leaf area and the association with winter hardiness in fall-sown lentil. Afr J Biotechnol 9(50):8515–8519

Kant S, Bi YM, Rothstein SJ (2011) Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. J Exp Bot 62(4):1499–1509

Karaköy T, Erdem H, Baloch FS, Toklu F, Eker S, Kilian B, Özkan H (2012) Diversity of macro- and micronutrients in the seeds of lentil landraces. Sci World J 2012

Kaur S, Cogan NO, Pembleton LW, Shinozuka M, Savin KW, Materne M, Forster JW (2011) Transcriptome sequencing of lentil based on second-generation technology permits large-scale unigene assembly and SSR marker discovery. BMC Genomics 12(1):1–11

Kaur S, Cogan NO, Stephens A, Noy D, Butsch M, Forster JW, Materne M (2014) EST-SNP discovery and dense genetic mapping in lentil (*Lens culinaris* Medik.) enable candidate gene selection for boron tolerance. Theor Appl Genet 127(3):703–713

Keshtiban RK, Carvani V, Imandar M (2015) Effects of salinity stress and drought due to different concentrations of sodium chloride and polyethylene glycol 6000 on germination and seedling growth characteristics of pinto bean (*Phaseolus vulgaris* L.). Adv Environ Biol 9(5):229–235

Khatib F, Makris A, Yamaguchi-Shinozaki K, Kumar S, Sarker A, Erskine W, Baum M (2011) Expression of the DREB1A gene in lentil (*Lens culinaris* Medik. subsp. culinaris) transformed with the Agrobacterium system. Crop Pasture Sci 62(6):488–495

Khazaei H, Podder R, Caron CT, Kundu SS, Diapari M, Vandenberg A, Bett KE (2017) Marker–trait association analysis of iron and zinc concentration in lentil (*Lens culinaris* Medik.) seeds. Plant Genome 10(2):plantgenome2017-02

Kökten K, Karaköy T, Bakoğlu A, Akçura M (2009) Determination of s nity tolerance of some lentil (*Lens culinaris* M.) varieties

Kumar J, Basu PS, Srivastava E, Chaturvedi SK, Nadarajan N, Kumar S (2012) Phenotyping of traits imparting drought tolerance in lentil. Crop Pasture Sci 63(6):547–554

Kumar S, Barpete S, Kumar J, Gupta P, Sarker A (2013) Global lentil production: constraints and strategies. SATSA Mukhapatra-Annu Tech 17:1–13

Kumar H, Dikshit HK, Singh A, Jain N, Kumari J, Singh AM, Singh D, Sarker A, Prabhu KV (2014) Characterization of grain iron and zinc in lentil (‘*Lens culinaris’* Medikus' *culinaris*') and analysis of their genetic diversity using SSR markers. Aust J Crop Sci 8(7):1005–1012

Kumar S, Rajendran K, Kumar J, Hamwieh A, Baum M (2015) Current knowledge in lentil genomics and its application for crop improvement. Front Plant Sci 6:78

Kumar J, Kant R, Kumar S, Basu PS, Sarker A, Singh NP (2016) Heat tolerance in lentil under field conditions. Legume Genomics Genet 7

Kumar J, Basu PS, Gupta S, Dubey S, Gupta DS, Singh NP (2017) Physiological and molecular characterisation for high temperature stress in *Lens culinaris*. Funct Plant Biol 45(4):474–487

Kumar J, Gupta DS, Kesari R, Verma R, Murugesan S, Basu PS, Soren KR, Gupta S, Singh NP (2021a) Comprehensive RNAseq analysis for identification of genes expressed under heat stress in lentil. Physiol Plant

Kumar J, Sen Gupta D, Baum M, Varshney RK, Kumar S (2021b) Genomics-assisted lentil breeding: current status and future strategies. Legume Science:e71

Kumawat KR, Gothwal DK, Kumawat R, Choudhary M, Kumawat S (2018) Determination of salinity tolerance in lentil (*Lens culinaris* M.) seedlings using salt tolerance index. Int J Chem Stud 6(1):1584–1589

Kushwaha UKS, Ghimire SK, Yadav NK, Ojha BR (2013) Genetic relatedness of lentil (*Lens culinaris* L.) germplasm by using SSR markers. Int J Appl Sci Biotechnol 1(3):132–136

Ma Y, Marzougui A, Coyne CJ, Sankaran S, Main D, Porter LD, Mugabe D, Smitchger JA, Zhang C, Amin M, Rasheed N, Ficklin SP, McGee RJ (2020) Dissecting the genetic architecture of Aphanomyces root rot resistance in lentil by QTL mapping and genome-wide association study. Int J Mol Sci 21(6):2129

Mamo T, Richter C, Heiligtag B (1996) Salinity effects on the growth and ion contents of some chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medic.) varieties. J Agron Crop Sci 176(4):235–247

Marzougui A, Ma Y, Zhang C, McGee RJ, Coyne CJ, Main D, Sankaran S (2019) Advanced imaging for quantitative evaluation of Aphanomyces root rot resistance in lentil. Front Plant Sci 10:383

Mishra BK, Srivastava JP, Lal JP, Sheshshayee MS (2016) Physiological and biochemical adaptations in lentil genotypes under drought stress. Russ J Plant Physiol 63(5):695–708

Morgil H, Tardu M, Cevahir G, Kavakli İH (2019) Comparative RNA-seq analysis of the drought-sensitive lentil (*Lens culinaris*) root and leaf under short-and long-term water deficits. Funct Integr Genomics 19(5):715–727

Muehlbauer F, McPhee K (2007) Registration of ‘Morton’ winter-hardy Lentil. Crop Sci 47:438–439. https://doi.org/10.2135/cropsci2005.12.0490

Muehlbauer FJ, Cho S, Sarker A, McPhee KE, Coyne CJ, Rajesh PN, Ford R (2006) Application of biotechnology in breeding lentil for resistance to biotic and abiotic stress. Euphytica 147(1): 149–165

Muscolo A, Sidari M, Anastasi U, Santonoceto C, Maggio A (2014) Effect of PEG-induced drought stress on seed germination of four lentil genotypes. J Plant Interact 9(1):354–363

Muscolo A, Junker A, Klukas C, Weigelt-Fischer K, Riewe D, Altmann T (2015) Phenotypic and metabolic responses to drought and salinity of four contrasting lentil accessions. J Exp Bot 66(18):5467–5480

Niri HH, Tobeh A, Gholipouri A, Zakaria RA, Mostafaei H, Jamaati-e-Somarin S (2010) Effect of nitrogen and phosphorous on yield and protein content of lentil in dryland condition. Am Eur J Agric Environ Sci 8(2):185–188

Öktem HA, Eyidoðan F, Demirba D, Bayraç AT, Öz MT, Özgür E, Selçuk F, Yücel M (2008) Antioxidant responses of lentil to cold and drought stress. J Plant Biochem Biotechnol 17(1): 15–21

Ouji A, El-Bok S, Mouelhi M, Younes MB, Kharrat M (2015) Effect of salinity stress on germination of five Tunisian lentil (*Lens culinaris* L.) genotypes. Eur Sci J 11(21)

Panahyan-e-Kivi M, Ebadi A, Tobeh A, Jamaati-e-Somarin S (2009) Evaluation of yield and yield components of lentil genotypes under drought stress. Res J Environ Sci 3(4):456–460

Pandey AK, Sengar RS (2020) Effect of salt stress on salt tolerant indices of morpho-physiological traits and yield attributes of lentil (*Lens culinaris* Medik.). Int J Chem Stud 8:2292–2301

Qiu Q, Sun N, Bai H, Wang N, Fan Z, Wang Y, Meng Z, Li B, Cong Y (2019) Field-based high-throughput phenotyping for maize plant using 3D LiDAR point cloud generated with a “Phenomobile”. Front Plant Sci 10:554

Rajendran K, Smouni A, Es-Safi NE, Benbrahim N, Mentag R, Nayyar H, Maalouf F, Kumar S (2020) Screening the FIGS set of lentil (*Lens Culinaris* Medikus) germplasm for tolerance to terminal heat and combined drought-heat stress. Agronomy 10(7):1036

Redden RJ, Hatfield JL, Prasad V, Ebert AW, Yadav SS, O’Leary GJ (2014) Temperature, climate change, and global food security. Temp Plant Dev 8:181–202

Roy CD, Tarafdar S, Das M, Kundagrami S (2012) Screening lentil (*Lensculinaris* Medik.) germplasms for heat tolerance. Trends Biosci 5:143–146. https://doi.org/10.3389/fpls.2017.00744

Sarker A, Erskine W, Singh M (2005) Variation in shoot and root characteristics and their association with drought tolerance in lentil landraces. Genet Resour Crop Evol 52(1):89–97

Sarker BC, Rashid P, Jarmoker JL (2015) Anatomical changes of lentil (*Lens culinaris* medik.) under phosphorus deficiency stress. Bangladesh J Bot 44(1):73–78

Sehgal A, Sita K, Kumar J, Kumar S, Singh S, Siddique KH, Nayyar H (2017) Effects of drought, heat and their interaction on the growth, yield and photosynthetic function of lentil (*Lens culinaris* Medikus) genotypes varying in heat and drought sensitivity. Front Plant Sci 8:1776

Sehgal A, Sita K, Siddique KH, Kumar R, Bhogireddy S, Varshney RK, HanumanthaRao B, Nair RM, Prasad PV, Nayyar H (2018) Drought or/and heat-stress effects on seed filling in food crops: impacts on functional biochemistry, seed yields, and nutritional quality. Front Plant Sci 9: 1705

Sehgal A, Sita K, Bhandari K, Kumar S, Kumar J, Vara Prasad PV, Siddique KH, Nayyar H (2019) Influence of drought and heat stress, applied independently or in combination during seed development, on qualitative and quantitative aspects of seeds of lentil (*Lens culinaris* Medikus) genotypes, differing in drought sensitivity. Plant Cell Environ 42(1):198–211

Sen Gupta D, McPhee K, Kumar S (2017) Development of molecular markers for iron metabolism related genes in lentil and their expression analysis under excess iron stress. Front Plant Sci 8: 579

Shah BH, Khan JAHANGIR, Khetran MA, Kurd AA, Sadiq NADEEM (2013) Evaluation and Selection of cold and drought resistant lentil genotypes for highlands of Balochistan. Sarhad J Agric 29(4):511–513

Sharpe AG, Ramsay L, Sanderson LA, Fedoruk MJ, Clarke WE, Li R, Kagale S, Vijayan P, Vandenberg A, Bett KE (2013) Ancient orphan crop joins modern era: gene-based SNP discovery and mapping in lentil. BMC Genomics 14(1):1–13

Singh D, Dikshit HK, Singh R (2013) A new phenotyping technique for screening for drought tolerance in lentil (*Lens culinaris* Medik.). Plant Breed 132(2):185–190

Singh D, Singh CK, Taunk J, Tomar RSS (2016a) Genetic analysis and molecular mapping of seedling survival drought tolerance gene in lentil (*Lens culinaris* Medikus). Mol Breed 36(5):58

Singh D, Singh CK, Tomar RSS, Taunk J, Singh R, Maurya S, Chaturvedi AK, Pal M, Singh R, Dubey SK (2016b) Molecular assortment of Lens species with different adaptations to drought conditions using SSR markers. PLoS One 11(1):e0147213

Singh A, Sharma V, Dikshit HK, Aski M, Kumar H, Thirunavukkarasu N, Patil BS, Kumar S, Sarker A (2017a) Association mapping unveils favorable alleles for grain iron and zinc concentrations in lentil (*Lens culinaris* subsp. *culinaris*). PLoS One 12(11):e0188296

Singh D, Singh CK, Kumari S, Tomar RSS, Karwa S, Singh R, Singh RB, Sarkar SK, Pal M (2017b) Discerning morpho-anatomical, physiological and molecular multiformity in cultivated and wild genotypes of lentil with reconciliation to salinity stress. PLoS One 12(5):e0177465

Singh D, Singh CK, Singh Tomar RS, Pal M (2017c) Genetics and molecular mapping of heat tolerance for seedling survival and pod set in lentil. Crop Sci 57(6):3059–3067

Singh D, Singh CK, Taunk J, Tomar RSS, Chaturvedi AK, Gaikwad K, Pal M (2017d) Transcriptome analysis of lentil (*Lens culinaris* Medikus) in response to seedling drought stress. BMC Genomics 18(1):1–20

Singh A, Dikshit HK, Mishra GP, Aski M, Kumar S (2019a) Association mapping for grain diameter and weight in lentil using SSR markers. Plant Gene 20:100204

Singh D, Singh CK, Taunk J, Jadon V, Pal M, Gaikwad K (2019b) Genome wide transcriptome analysis reveals vital role of heat responsive genes in regulatory mechanisms of lentil (*Lens culinaris* Medikus). Sci Rep 9(1):1–19

Singh D, Singh CK, Tomar RSS, Sharma S, Karwa S, Pal M, Singh V, Sanwal SK, Sharma PC (2020a) Genetics and molecular mapping for salinity stress tolerance at seedling stage in lentil (*Lens culinaris* Medik). Crop Sci 60(3):1254–1266

Singh D, Singh CK, Tribuvan KU, Tyagi P, Taunk J, Tomar RSS, Kumari S, Tripathi K, Kumar A, Gaikwad K, Yadav RK, Pal M (2020b) Development, characterization, and cross species/genera transferability of novel EST-SSR markers in lentil, with their molecular applications. Plant Mol Biol Report 38(1):114–129

Singh D, Singh CK, Taunk J, Sharma S, Gaikwad K, Singh V, Sanwal SK, Singh D, Sharma PC, Pal M (2021a) Transcriptome skimming of lentil (*Lens culinaris* Medikus) cultivars with contrast reaction to salt stress. Funct Integr Genomics 21(1):139–156

Singh J, Sirari A, Singh H, Kumar A, Jaidka M, Mandahal KS, Kumar S, Singh S (2021b) Identifying and validating SSR markers linked with rust resistance in lentil (*Lens culinaris*). Plant Breed

Sinha R, Pal AK, Singh AK (2018) Physiological, biochemical and molecular responses of lentil (*Lens culinaris* Medik.) genotypes under drought stress. Indian J Plant Physiol 23(4):772–784

Sita K, Sehgal A, Kumar J, Kumar S, Singh S, Siddique KH, Nayyar H (2017) Identification of high-temperature tolerant lentil (*Lens culinaris* Medik.) genotypes through leaf and pollen traits. Plant Sci 8:744

Sita K, Sehgal A, Bhandari K, Kumar J, Kumar S, Singh S, Siddique KH, Nayyar H (2018) Impact of heat stress during seed filling on seed quality and seed yield in lentil (*Lens culinaris* Medikus) genotypes. J Sci Food Agric 98(13):5134–5141

Skliros D, Kalloniati C, Karalias G, Skaracis GN, Rennenberg H, Flemetakis E (2018) Global metabolomics analysis reveals distinctive tolerance mechanisms in different plant organs of lentil (*Lens culinaris*) upon salinity stress. Front Plant Soil 429(1):451–468

Sohrabi SS, Ismaili A, Azarian Firouz-Abadi FN, Fallahi H (2018) Discovery of EST-SSRs markers in lentil (Lens culinaris) under cold stress. Crop Biotechnol 8(22):1–14

Sohrabi SS, Ismaili A, Nazarian-Firouzabadi F, Fallahi H, Hosseini SZ (2022) Identification of key genes and molecular mechanisms associated with temperature stress in lentil. Gene 807:145952

Srivastava SP, Bhandari TMS, Yadav CR, Joshi M, Erskine W (2000) Boron deficiency in lentil: yield loss and geographic distribution in a germplasm collection. Plant Soil 219(1):147–151

Sudheesh S, Verma P, Forster JW, Cogan NO, Kaur S (2016) Generation and characterisation of a reference transcriptome for lentil (*Lens culinaris* Medik.). Int J Mol Sci 17(11):1887

Talukdar D (2013) Bioaccumulation and transport of arsenic in different genotypes of lentil (*Lens culinaris* Medik.). Int J Pharm Bio Sci 4(1):694–701

Temel, H. Y., Gol, D., Kahriman, A., & Tanyolac, M. B. (2014). Construction of linkage map through genotyping-by-sequencing in lentil. In: Proceedings of plant and animal genome conference, vol. 22, pp. 358.

Temel HY, Göl D, Akkale HBK, Kahriman A, Tanyolac MB (2015) Single nucleotide polymorphism discovery through Illumina-based transcriptome sequencing and mapping in lentil. Turk J Agric For 39(3):470–488

Tepe M, Aydemir T (2011) Antioxidant responses of lentil and barley plants to boron toxicity under different nitrogen sources. Afr J Biotechnol 10(53):10882–10891

Thakur P, Kumar S, Malik JA, Berger JD, Nayyar H (2010) Cold stress effects on reproductive development in grain crops: an overview. Environ Exp Bot 67(3):429–443

Tickoo JL, Mishra SK, Dikshit HK (2005) Lentil (*Lens culinaris*) In India: present Status and future perspectives

Turan MA, Turkmen N, Taban N (2007) Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants. J Agron

Verma P, Shah N, Bhatia S (2013) Development of an expressed gene catalogue and molecular markers from the de novo assembly of short sequence reads of the lentil (Lens culinaris Medik.) transcriptome. Plant Biotechnol J 11(7):894–905

Vijayan, P., Vandenberg, A., and Bett, K. E. (2009). A mixed genotype lentil EST library representing the normalized transcriptome of different seed development stages

Wang D, Yang T, Liu R, Li N, Wang X, Sarker A, Zhang X, Li R, Pu Y, Li G, Huang Y, Ji Y, Li Z, Tian Q, Zong X, Ding H (2020) RNA-Seq analysis and development of SSR and KASP markers in lentil (*Lens culinaris* Medikus subsp. *culinaris*). Crop J 8(6):953–965

Xiao-lei WANG, Hai-qiu YU, Ning LIU, Bing YI, Jing WEN, Xing-tao LI, Xin-hua ZHAO, Min-jian CAO (2012) Some physiological characteristics in maize (*Zea mays* L.) inbred lines tolerant to low potassium from grain filling to maturity. Afr J Agric Res 7(11):10–5897

Yadav GS, Devi AG, Das A, Kandpal B, Babu S, Das RC, Nath M (2021) Foliar application of urea and potassium chloride minimizes terminal moisture stress in lentil (*Lens culinaris* L.) crop. Legume Res: Int J 44(6)

Yasin M, Zahid MA, Ghafoor A, Ahmad Z (2002) Genotypic behavior of lentil (*Lens culinaris* Medik) towards salinity. In: Prospects for saline agriculture. Springer, Dordrecht, pp 231–235

Zakeri H, Bueckert RA, Schoenau J (2013) Effects of nitrogen on reproductive duration and yield in lentil cultivars. J Plant Nutr 36(6):877–894

Zandalinas SI, Mittler R, Balfagón D, Arbona V, Gómez-Cadenas A (2018) Plant adaptations to the combination of drought and high temperatures. Physiol Plant 162(1):2–12

Approaches Toward Developing Heat and Drought Tolerance in Mungbean

10

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Abstract

The projected rise in the global temperature and water scarcity will increase the frequency and intensity of heat and drought stresses. Agriculture is the main sector that is severely affected by these stresses and going to threaten global food security. Mungbean is the major summer-season food legume and of paramount importance because of its protein-rich seeds and have the ability to restore soil fertility. But these stresses adversely affect the overall growth and development of this crop as revealed through altered morphology, physiology, enzymatic activities, and eventually declined its quality as well as quantity. Reproductive stage is extensively studied and more susceptible toward heat stress as various processes such as pollen germination, pollen load, pollen tube growth, stigma receptivity, ovule fertility, and seed filling are reduced to a much extent leading to poor yield. The present review summarizes the effects of heat and drought stress on the vegetative, reproductive growth, physiological functions, and cellular

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activities of mungbean. Furthermore, defense mechanisms employed by the plants that comprise elevated productions of HSPs, antioxidants, osmolytes, and secondary metabolites are also considered. Based upon such responses of the plants, various approaches, which can be employed by the breeders for developing stress-tolerant varieties comprise physiological, biochemical, molecular traits, and agronomic strategies. These approaches would prove to be valuable for getting insights into the physiological and molecular mechanisms governing heat and drought tolerance and will pave the way for engineering the plants.

Keywords

Mungbean · Heat stress · Drought stress · Climate resilience · QTL

10.1 Introduction

Mungbean or green gram (*Vigna radiata* L. wilczek) is grown on almost seven million hectares of land worldwide (Abid et al. 2018). *Vigna radiata* is the most widely distributed of the six Asiatic Vigna species (Dahiya et al. 2015). It is an important grain legume and cash crop that is widely farmed in South, East, and Southeast Asia as well as in South America and sub-Saharan Africa (Akhtar et al. 2015). Due to its photo-insensitivity and short duration, it can be used in a variety of intensive cropping techniques (Dahiya et al. 2015; Sehrawat et al. 2013). The crop is indigenous to India (Ardalani et al. 2015) and its seeds and sprouts are widely utilized as a fresh salad, vegetable, or staple meal not just in Asian countries but also in the Western world (Sehrawat et al. 2013). Around six million hectares of land are grown for the mungbean crop worldwide, accounting for around 8.5% of total pulse area (Chand et al. 2018). Mungbean is high in easily digestible proteins, carbs, fibers, minerals, vitamins, antioxidants, and other phytonutrients, making it a good candidate for malnutrition mitigation. The crop has a yield potential of roughly 2 tonnes per hectare, with average productivity of about 0.5 tonnes per hectare (Sehrawat et al. 2013). Mungbean is more heat and drought tolerant than other pulse crops, but it is still affected by severe abiotic stresses like low or high temperatures, insufficient or excessive water, high salinity, and low soil fertility (Reddy 2009).

Spring (February/March), summer (March/April), and kharif (June/July) are the three main seasons for mungbean cultivation on the Asian continent (Basu et al. 2019). When grown in rainfed areas, seedlings are exposed to water stress due to decreased water supplies throughout these months. Water scarcity causes stress in plants at any stage (Dias et al. 2010). Due to a predicted increase in the occurrence of water shortfalls, the global drought-restricted zone is expanding, posing a threat to mungbean crop production worldwide (Nair et al. 2019). For the growth of mungbean, sufficient water availability is more important than any other environmental trigger (Sehrawat et al. 2013). Water scarcity in the seedling stage makes it difficult to produce healthy seedlings and reduces overall productivity (Dahiya et al.

2015). Mungbean (*Vigna radiata* L. Wilczek) is also a unique source of protein supplement with a plethora of medicinal properties and is a climate-friendly crop (Chikukura et al. 2017). The plant is an annual legume of the Fabaceae family that plays a critical role in nutrition in all developing countries (Pratap et al. 2016). Thus, during the last few decades, there has been an increase in attempts to develop and expand the production of this pulse crop (Bazaz et al. 2016). Consuming mung beans in combination with cereals tends to improve the protein quality, as these grains are fairly high in sulfur-containing amino acids (Fang et al. 2017) and provide a cost-effective source of easily digestible protein for vegetarians. As a result, mungbean may sometimes be referred to as “poor man’s meat” (Hall 1992). Mungbean has also been claimed to be suitable for youngsters due to its decreased flatulence and hypoallergenic qualities (Dahiya et al. 2015; Ali et al. 2018).

The crop is usually grown in the summer and fall at an optimal temperature range of 27–30 °C and is primarily cultivated in dry and semiarid tropics at elevations <2000 m (Singh et al. 2017). However, the high degree of fluctuation in climatic conditions, such as rising temperatures and uncertain water scarcity situations, is restricting mungbean productivity during its cropping season (Singh et al. 2017). Heat stress is one of the primary issues influencing mungbean crop production during summers in the current global climatic condition (Martinez et al. 2012). Hence, the primary goal of mungbean breeders is to boost the genetic potential of genotypes under conditions of heat stress (Mariani and Ferrante 2017).

Numerous varieties have been produced that are resistant/tolerant to abiotic (drought, salinity, and heat) stresses (Mariani and Ferrante 2017). Drought and heat are some of the most critical threats gaining considerable attention from farmers and researchers due to their major influence on agriculture (Ashraf and Foolad 2007). The situation is worsening due to erratic changes in the nature of the environment and escalated frequency of global climate change; henceforth, it is imperious to secure food supplies for the future by increasing the survivability of important agricultural crop plants (Joshi et al. 2016). Due to their restricted genetic base, these types become prone to stressors, and their average production has remained nearly constant over the last few decades (Hanif and Wahid 2018). However, Mungbean wild relatives can be used to increase genetic diversity and to introduce beneficial characteristics into cultivated mungbean lines (Jiang et al. 2015). There is an urgent need for extensive study of genetic resources, cytological, genetic, genomic, and tissue culture research to elevate this critical crop’s vertical and horizontal base to that of other key legumes (Nair et al. 2019). In comparison to other legume crops, the publications on the development of transgenic mungbean demonstrate a lack of an efficient plant genetic transformation methodology compatible with in vitro regeneration. Heat and drought stress often occur simultaneously, and due to global climate change, this kind of phenomenon occurs more frequently and severely, which poses detrimental effects on plants (Nahar et al. 2015). Hence, it is critical to incorporate novel technologies like transgenics approaches, genome editing, conventional breeding, and embryo rescue procedures to enhance its productivity under heat and drought stress conditions (Devasirvatham et al. 2012). However, little research has been done on this issue, which should be expanded to allow for greater use of wild mungbean species.

10.2 Various Traits for Heat Stress Tolerance in Mungbean

10.2.1 Morpho-Physiological Trait Variations for Improving Heat Tolerance

Heat stress is frequently associated with drought, high solar irradiation, and high wind velocity, and the combination of these conditions can exacerbate damage even in adequately watered plants (Hall 1992). A plethora of research has established mungbean's great susceptibility to rising temperatures (Teixeira et al. 2013; Jha et al. 2017).

High-temperature stress has a detrimental effect on plant growth and development as well as on a variety of physiological activities (HanumanthaRao et al. 2016). For example, extended exposure to high temperatures may cause mungbean seeds (*Phaseolus aureus* Roxb.) to lose vigor, impairing seedling emergence and establishment (Devasirvatham et al. 2012; Kumar et al. 2011). Photosynthetic rate, which is determined by leaf area and dry matter production, is related to the number of pods and seeds. With increased leaf area, the percentage of solar radiation intercepted and the rate of dry matter synthesis enhanced (Hamid et al. 1981). Temperatures above 45 °C, which occur frequently throughout the flowering cycle, can cause flower abortion and production losses. Sharma et al. (2016) used Temperature Induction Response (TIR) and physiological screening methodologies at the seedling and whole plant levels to assess the influence of high temperature on different mungbean lines for vegetative and reproductive performance. The most promising tolerant lines were chosen for further study at the plant level. These lines were evaluated for growth and yield features at two sowings: normal sowing (NS), where day/night temperatures throughout the reproductive stage were between 40 and 28 °C, and late sowing (LS), where temperatures were higher (>40/28 °C). Leaf rolling and chlorosis were observed on the leaves of LS plants and hastened phenology resulted in a significant reduction in leaf area, biomass, flowers, and pods. Intriguingly, the length of flowering and podding was also reduced (Sharma et al. 2016).

High-temperature treatment (50 °C for 10, 20, 30 min) dramatically lowered seed germination and vigor index in mungbean (Piramila et al. 2012). Furthermore, high temperatures, particularly those more than 40/30 °C (max/min), impede growth and produce chlorosis in mungbean (Kumar et al. 2011). This is connected with a drop in leaf hydration status and increased oxidative stress, which has been observed to be alleviated by exogenous ascorbic acid treatment (Kumar et al. 2011). Sharma et al. (2016) also discovered heat-induced leaf blistering, leaf rolling, and chlorosis in mungbean plants. Other effects of heat stress on mungbean included leaf curving, leaf withering, leaf yellowing and blackening, plant height reduction, and decreased leaf, branch, and biomass numbers (Kaur et al. 2015). Heat stress also reduces the photosynthetic ability and crop productivity; however, the genetic reason for this is unknown and needs detailed insight in the future (Biswash et al. 2014).

10.2.1.1 Reproductive and Yield Traits for Heat Stress Tolerance

Although plants have the natural ability to maintain their metabolism and vegetative development under a wide range of temperature regimes, reproductive growth showed a significant sensitivity to warmer temperatures (Abou-Shleel 2014). Rawson and Craven (1979) conducted extensive research on the effects of high temperatures on blooming in several mungbean genotypes exposed to lengthy photoperiods and high mean temperatures (24–28 °C).

Extreme temperatures cause late flowering, tissue damage in male and female reproductive structures, and flower and pod abortion (Young et al. 2004; Zinn et al. 2010; Firon et al. 2012; Djanaguiraman et al. 2013). Increased temperature also affects the shape of anther and pollen, reduces pollen content, retards their dehiscence, and may result in full male sterility (Awasthi et al. 2015; Djanaguiraman et al. 2013). This modification in the anther dehiscence and pollen release process is frequently caused by high relative humidity during periods of heat stress (Bansal et al. 2014; Jiang et al. 2015). In comparison to female gametophytic tissue, elevated temperatures have a greater detrimental effect on male gametophytic development, affecting pollen germination, viability, and pollen tube elongation (Jiang et al. 2015). Across all stages of pollen development, the formation of meiocytes and microspores showed greater sensitivity to heat, as demonstrated in a variety of experiments on various crop species (Monterroso and Wien 1990; Ahmed et al. 1992; Devasirvatham et al. 2012). As a result of these irregularities in anther and pollen formation, pollination and fertilization events are disrupted, significantly reducing fruit and pod set (Bita and Gerats 2013). The assessment of pollen thermotolerance and the reaction to heat stress is a critical topic for plant geneticists, agronomists, and biologists interested in improving current germplasm (Mittler et al. 2012; Devasirvatham et al. 2012).

High temperatures (>40 °C) have a direct effect on flower maintenance and pod development in mungbean, accounting for up to 79% of flower shedding (Kumari and Verma 1983). In a similar investigation, flower preservation under heat stress was examined in 77 mutants produced from NM 92 and 51 recombinants derived from three crossings, namely, VC1482C NM92, VC1560D NM92, and NM98 VC3902A (Khattak et al. 2006). No genotype was completely resistant to flower shedding, although NM 92 was susceptible to the same feature under conditions of extreme heat (>40 °C) (Khattak et al. 2006). Additionally, shedding was detected only in opened flowers and not in pods at any developmental stage; further humidity changes had no influence on floral shedding (Khattak et al. 2006). Similarly, the detrimental effects of heat stress (45/25 °C) on two mungbean cultivars (SML 832 and SML 668) were evaluated, with a particular emphasis on the reproductive stage (Kaur et al. 2015). Their findings indicated that temperatures greater than 35/25 °C, 43/30 °C, 45/32 °C (day/night) were severely detrimental to reproductive activities and also had a significant effect on the crop's potential output (Kaur et al. 2015). In mungbean, high temperatures during the early phases of development and reproduction have a detrimental effect on seed yield, owing to pollen viability, reduced fertilization, and complete flower shedding (Kaur et al. 2015). Thus, screening and selection of mungbean genotypes capable of withstanding high temperatures throughout reproductive phases are critical for increasing the crop's growth and output (Singh and Singh 2011). Alagupalamuthirsolai et al. (2015) also investigated reproductive heat stress in 20 high-yielding mungbean cultivars using stress indices, yield-contributing attributes, and growing degree days (°C) and found that all genotypes exhibited significant variance in yield traits under heat stress.

Recently, a study was done to determine the effect of heat stress on the vegetative and reproductive functions of 41 mungbean genotypes grown under managed growth circumstances (Sharma et al. 2016). We identified a few selective heat-tolerant mungbean lines that can be used in future breeding projects (Sharma et al. 2016). Another study examined the variation in response of 28 mungbean genotypes to heat stress (45/30 °C NAc-HT, unacclimated), particularly during the reproductive stage, and to pre-acclimation of different genotypes to elevated temperatures of 35/28 °C (Ac-HT, acclimated) prior to exposing them to high temperatures. The total pollen count was dramatically reduced from 88/mm^2 in CON (28 °C/24 °C, control) to 50/mm^2 in Ac-HT and 40/mm^2 in NAc-HT plants, with evident genotypic variation, implying that acclimated plants (Ac-HT) maintained a greater pollen number and viability than non-acclimated plants (NAc-HT) (Patriyawaty et al. 2018).

Terminal heat stress is a typical problem with mungbean in India, particularly during the spring/summer season (HanumanthaRao et al. 2016). During the early growth period, high temperatures, greater than 40 °C, result in a significant reduction in yield potential due to reduced fertilization, pollen sterility, and a rapid rate of flower shedding (HanumanthaRao et al. 2016). Rainey and Griffiths (2005) demonstrated that the abscission of reproductive structures was the primary determinant of yield in a variety of annual grain legumes when subjected to heat stress. Very little research has been conducted on the effects of heat stress on mungbean, particularly on the reproductive stage (Devasirvatham et al. 2012). Therefore, to boost mungbean productivity under heat stress conditions, it is critical to characterize the genetic variation for heat tolerance in the core germplasm and to investigate the mechanisms underlying this crop's heat sensitivity (Devasirvatham et al. 2012; Kumar et al. 2011). On the basis of grain yield in normal and heat stress conditions, eight heat indices were calculated. Under both normal and heat stress conditions, the highest significant positive correlations were established between mean productivity, geometric mean productivity, stress tolerance index, and yield indices. SML 1186, NDM 12-308, IPM 02-4, and Smrat genotypes were identified as having a high stress index score and could be used in mungbean breeding efforts to introduce and produce heat-tolerant promising varieties (Ahmad et al. 2021).

Furthermore, to boost mungbean growth and productivity, genotypes that can endure high temperatures during reproductive stages must be screened and selected (Singh and Singh 2011).

10.2.2 Biochemical Traits Modulating Heat Tolerance

The mungbean (*Vigna radiata* L. Wilczek) is one of the most significant crops in the world (Kumar et al. 2013; Farooq et al. 2017). It has a production potential of around 2000 kg per hectare; however, productivity is only 842 kg per hectare (BBS 2016). The ideal temperature for mungbean yield is between 28 °C and 30 °C (Poehlman 1991). Applying heat stress during the flowering period allows partitioning to the pods and seed development. Following a period of 15 days of stress, the data were collected on seed production and quality at maturity, as well as physiological and biochemical parameters were also evaluated (Hanif and Wahid 2018). According to their report, high temperature resulted in a significant increase in H_2O_2 and MDA levels as well as a decrease in net photosynthesis, stomatal conductance, and water content (Hanif and Wahid 2018). Similarly, a significant decrease in sucrose concentration was observed in the leaves and anthers, along with a decrease in the activities of sucrose-synthesizing enzymes (sucrose synthase, sucrose phosphate synthase) and hydrolyzing enzymes (acid invertase) due to heat stress, which could be a critical factor affecting reproductive function and yield attributes (Kaur et al. 2015). In another study, an increase in MDA content was noticed in seedlings treated with deadly temperature (40 °C) at any harvest but decreased when seedlings were pretreated with 40 °C prior to lethal stress (Mansoor and Naqvi 2013). Furthermore, there was a variable response of antioxidant enzyme activity among different genotypes. POD and SOD activities increased under stress in all genotypes except NM 20-21, whereas APX activity increased in all genotypes. However, CAT activity decreased under stress for NM 19-19 and 121-123 but increased for NM 20-21 and NCM 89. Under heat stress, NM 19-19 had a low MDA concentration and elevated antioxidant enzymes, indicating that it was the most thermotolerant genotype. However, elevated MDA levels and low antioxidant enzyme activity were found in NM 20-21, suggesting that it is the least thermotolerant genotype (Mansoor and Naqvi 2013). In another study, heat stress has caused significant accumulation of H2O2 and MDA that decreased stomatal conductance and water use efficiency, chlorophyll a and b, and total chlorophylls and net photosynthesis in the sensitive varieties while increased carotenoids contents in the tolerant varieties (Hanif and Wahid 2018).

10.2.3 Multi-Omics Approaches to Understand Heat Tolerance in Mungbean

10.2.3.1 Genomics Approaches

Mungbean is a quantitative short-day legume grown across varied locations, environments, and seasons (Ohama et al. 2017). The crop has inherent intrinsic tolerance mechanisms to cope with different environmental stressors (Pratap et al. 2016). Despite its wide growth and cultivation, it is exposed to high temperatures and also faces photo-thermoperiod sensitivity. Considerable efforts have been made toward the development of input-responsive, high-yielding, disease-resistant, and short-duration varieties of mungbean in the past three to four decades (Priya et al. 2020). However, breeding techniques for heat stress resistance have largely remained untouched, consequently posing serious constraints to mungbean production (Pratap et al. 2016). In the current era, the advancements in "omics" technologies, particularly genomics, proteomics, metabolomics, and transcriptomics, have enabled unbiased and direct monitoring of the factors affecting Mungbean growth and yield in response to environmental constraints (Naveed et al. 2015). Omics tool is proving highly beneficial in deciphering the complex molecular mechanisms underlying plant growth, development, and their interactions with the environment, which ultimately determine the nutritional value, yield potential (Raina et al. 2016), and the agricultural inputs of crop. Substantial genetic and genomic resources of mungbean are available now, which can be exploited for the development of climate-resilient cultivars (Varshney et al. 2014). Different climate-smart traits can be incorporated in mungbean through current advancements in breeding techniques which will help them to adapt to varied climates and perform well across environments (Varshney et al. 2014).

At the present scenario of global warming and changing climate, breeders and plant researchers are working on the foundational work of implementation of genomic technologies (Yadav et al. 2010). The World Vegetable Centre and the Australian National Mungbean Improvement Program have dramatically increased the yields, reliability, and sustainability of mungbean crops worldwide through conventional breeding programs (Varshney et al. 2014). The genome sequencing of diverse sets of mungbean germplasm aims at quantifying the genetic diversity present among the world's mungbean gene pool collection and to decipher genes associated with agronomically beneficial traits (Kajla et al. 2015).

The World Vegetable Center (AVRDC) has created a mungbean mini-core set, which comprises a significant fraction of the variety in the WorldVeg gene bank for this species (HanumanthaRao et al. 2016). This site contains a significant genetic resource for identifying new features that can be used in breeding programs in the future. There have been a variety of breeding procedures used to increase genetic variability in green gram, but hybridization and induction of mutations are seen to be the most promising for selecting acceptable variants from a segregating population (Kalaji et al. 2016). To generate varieties with pyramided traits, combining traditional breeding methods with molecular breeding technologies is beneficial (Nair et al. 2019). With the increasing availability of genomic technologies and resources for legumes, a more complete and in-depth genome mapping of green gram is critical for genetic improvement (Pratap et al. 2015). Systematic efforts must be made to investigate the physiological and biochemical regulation of biotic and abiotic stresses, as well as the entire profile of genes, proteins, and metabolites that confer resistance/tolerance, so that they can be modified to generate improved mungbean cultivars (Nair et al. 2019). Furthermore, by linking the sequence and phenotyping data regions of the genome associated with beneficial traits, the photosynthetic pathways, and water-use efficiency can be targeted (Kaushal et al. 2016). Once identified, these pathways can be manipulated directly using genome-editing tools, hence reducing current breeding efforts by more than half (Nair et al. 2019). As abiotic stressors pose an extensive and immediate risk, these technologies and plant scientists needed to address the present issues in detail.

10.2.4 Exploring Gene Families and Transcriptional Factors as Heat Responsive Markers

To address the challenge of the hidden hunger and to ensure the nutritional security of future generations as proposed by the FAO (Food and Agriculture Organization), pulse production must be doubled by 2050; however, various environmental stressors are confronting this goal (Mannur et al. 2019; Kim et al. 2015). Among all environmental challenges, heat stress has the broadest and most far-reaching influence on legumes and is affecting agricultural crops more frequently and more severely (Hatfield and Prueger 2015). With the introduction of various physiological, molecular, and genetic bases of heat stress tolerance mechanisms, cultivated plants have gained a major attention for intense research on how they can tolerate or avoid heat stress through natural genetic variation or by using DNA technologies, mutational breeding, or genome editing to create new variations (Sgobba et al. 2015). Since past few decades, the identification of Hsf genes in many species has greatly increased our knowledge of the molecular mechanisms of plant developmental and defense processes (Guo et al. 2016; Wang et al. 2016). As mungbean is an economically important legume crop of the world, the emergence of its genetic database allows functional analysis of mungbean genes (Kang et al. 2014). Mungbean's genetic diversity has been conserved in various germplasm collection units globally. The major collection centers are developed in China, India, Philippines, Taiwan, and the United States (Liu et al. 2017) and by using genetic diversity at these centers genetic base of mungbean cultivars can be enlarged. Generally, screening of large collections for required traits is economically as well logistically challenging for mungbean breeders (Priya et al. 2020). The establishment of subsets of large germplasm collections can make screening more practical, but this procedure is laborious and costly (Schafleitner et al. 2015). In a recent study, 24 VrHsf genes were identified in mungbean and their characteristics were investigated using the mungbean genome database. Chromosome location analysis showed that out of 11 mungbean chromosomes, VrHsf genes are located on 8 and 7 duplicated gene pairs had formed between them. Furthermore, transcriptional patterns of VrHsf genes varied among different tissues, suggesting their involvement in plant growth and development. Identified multiple stress-related cis-elements in promoter regions of VrHsf and they observed that the expression of maximum VrHsf genes was influenced by different stress conditions, indicating their potential role in stress resilience pathways (Priya et al. 2020). Currently, the WRKY transcription factors are gaining importance for genomic and functional studies due to their seminal participation in plant growth, development, metabolism, and in the governance of multiple stress-defensive pathways. The recent release of draft genome sequences of Mung bean (*Vigna radiata*) has paved the way for the characterization of WRKY gene family in this crop. The genome-wide analysis of WRKY transcription factors in mungbean led to the identification of two WRKY TFs (Vradi05g21980 Vradi0158s00480) for heat, drought, and osmotic stress tolerance (Zandalinas et al. 2018).

Regardless of the immense economic importance of the mungbean crop, the genomic studies and transcriptional analysis of this crop are still inadequate and need detailed insight to develop heat resilience under stressful environmental conditions.

10.2.5 Agronomic Approaches to Understand Heat Tolerance in Mungbean

Despite all of the efforts to improve green gram cultivars, the crop's yield potential remains poor due to biotic and abiotic causes (Mariani and Ferrante 2017). Poor yield potentiality, indeterminate growth, asynchronous maturity, low harvest index, faulty plant type, low partitioning efficiency, small seed size, and vulnerability to biotic and abiotic stressors are the key restrictions (Keatinge et al. 2011). As a leguminous crop, mungbean (*Vigna radiata*) may replenish soil fertility, prevent land degradation, and boost crop productivity and livestock raising while preserving the ecosystem (Priya et al. 2020). Cultivation of such dual-purpose leguminous crops improves overall productivity, conserves natural resources, and aids in generating greater output from the agricultural system (Fedoroff et al. 2010).

Seeding time and plant population are two of the most important factors contributing to lower pulse production. Managerial methods must be adjusted in the shifting scenario of abiotic and biotic stress to ensure greater crop output (Wang et al. 2015). The agro-ecological conditions play an important influence in determining when to plant. Planting time is critical, as it has a substantial impact on growth, development, and output (Waraich et al. 2012; Osakabe et al. 2014; Rasheed et al. 2016). The best time to plant mungbean depends on the cultivar (Sakata et al. 2010). As planting timings differ greatly among cultivars, precise planting schedules must be followed to achieve maximum output. Furthermore, due to an increase in grain weight and other production features, early sowing may also improve final yield and biomass production in mungbean (Sun et al. 2014). Due to the shortened growth cycle caused by late seeding, the interception of radiations was reduced, resulting in a drop in total dry matter accumulation and, as a result, inferior yields (Rasheed et al. 2011). To have a good yield, you need to pick the right cultivar and seed it at the right time (Reardon and Qaderi 2017). Mungbean cultivars respond to sowing dates and growing seasons in different ways. As a result, various kinds of mungbean cultivars should have different optimal planting dates (Reddy 2009). Sowing at the right times after identifying high-yielding cultivars can result in higher yields (Ahmad et al. 2021). Variable planting schedules resulted in considerable changes in mungbean seed production, according to the authors (Ahmad et al. 2015). Improving agronomic management systems and cultivation techniques may further prove promising in mechanized management and increasing yield potential (Chauhan and Williams 2018).

10.2.5.1 Drought Stress and Mungbean

Water stress is mainly caused by uneven rainfall, ground water shortage, or high soil salinity, and this situation becomes severe in many parts of the world including all arid and semiarid regions (Fathi and Tari 2016). The effects of drought range from morphological to molecular levels and influence all phenological stages of plant growth (Farooq et al. 2009). Drought stress is characterized by the cessation of various physiological processes like growth, development, and cell metabolism, eventually affecting the economic yield and productivity of crops (Yuriko et al. 2014). The first notable symptom of water scarcity is impaired seedling germination and poor stand establishment as reported in pea (Okcu et al. 2005). The main consequences of drought are wilting of leaves due to loss of turgor pressure, exhilarated solute concentrations in the cytosol, consequently inhibiting growth and reproductive functions (Ranawake et al. 2011). In fact, under severe water scarcity, ion uptake and transport get impaired along with a decrease in leaf area, cell wall lignifications, and increased root shoot ratio of grain (Farooq et al. 2009). Drought stress also triggers a decrease in leaf water potential, which acts as an important hydraulic signal for stomatal closure to prevent further loss via transpiration (Chai et al. 2016). Some other consequences of limited water supply are early switching to reproductive stage, reduction in fresh and dry matter production, due to diminished photosynthetic efficiency (Sheoran and Saini 1996). Water stress during the reproduction and grain-filling stage is more detrimental and usually results in a significant loss in grain yield. Pre-anthesis drought also leads to pollen sterility and hampers flowering and seed filling in plants (Jaleel et al. 2009). It also results in decreased intake of carbon dioxide, which reduces carboxylation and directs more electrons to form ROS (reactive oxygen species) which in turn damages the photosynthetic apparatus and promotes photoinhibition (Farooq et al. 2009). The major symptom of the degradation of the photosynthetic machinery in plants is the development of chlorosis.

Plants often confront adverse drought conditions, which significantly depend on plant genetics as well as the duration and severity of drought. In response to water deficit in plants, stomatal closure occurs often triggered by phytohormones ABA to avoid further loss via transpiration, which regulates the cell metabolism by inducing expression of various stress-related genes (Fathi and Tari 2016). Acclimation of plants to drought stress is an interrelated cross-talk between molecular and physiological events, including changes in plant growth structure, accumulation of various organic and inorganic osmolytes, improved antioxidant defense activity, and reduced transpiration (Anjum et al. 2011). Due to the exaggerated vulnerability of plants to drought stress in a scenario of climate change, it is crucial to understand the morphological and physiological adaptations of plants to cope with these adverse situations (Farooq et al. 2011).

In developing nations, grain legumes constitute a major source of dietary protein as a means to provide food security and nutrition. Among grain legumes, mungbean is an important pulse crop having a short life span and is more sensitive to drought stress mainly at the reproductive stage (flower initiation and pod set), leading to reduced productivity and yield.

10.2.5.2 Morpho-Physiological Traits for Drought Tolerance in Mungbean

Plants exhibit continuous growth throughout their lives that are accomplished through processes like cell division, cell enlargement, and cell differentiation (Farooq et al. 2009). The establishment of these processes mainly depends upon the turgor pressure of cell and underwater stress, they are reduced to a larger extent affecting the overall growth of plant (Taiz and Zeiger 1991). Deficit water supply poses negative effects at any growth and development stage of the plant. Water stress during early stages reduces germination and stand establishment due to lesser water uptake and reduced water potential during the imbibition phase of germination, impaired enzyme activity, and reduced energy supply (Farooq et al. 2011). Drought critically affects the growth and development of plants with a considerable reduction in biomass accumulation and crop growth rate. Plant growth under drought stress can be restricted at various levels, most studied are seed germination, plant height, leaf area, and crop growth (Ranawake et al. 2011; Aslam et al. 2013a, b; Saima et al. 2018). Reduction in growth parameters under drought stress was reported in many crops like chickpea (Talebi et al. 2013); sunflower (Kiani et al. 2007); and barley (McMaster and Wilhelm 2003). Various effects of drought on growth traits of mungbean crops are discussed below.

10.2.6 Seed Germination

Saima et al. (2018) reported effects of drought stress that include reduction in seed germination and shoot length in 10 days old seedlings in all the seven hybrids of *Vigna radiata* along with increasing PEG-induced drought stress (5% and 10%), whereas root growth increased with increasing drought stress.

Early growth stages of 17 mungbean genotypes were evaluated for drought tolerance at the seedling stage by Aslam et al. (2013a, b). Germination percentage, root/shoot length, root/shoot ratio, shoot weight, and stem diameter were studied at different moisture levels (80%, 50%, and 30% field capacity). They reported that all the reported traits decrease with an increase in drought stress except root/shoot ratio.

10.2.6.1 Plant Height and Biomass

Other growth traits like plant height and shoot weight were measured in the three mungbean varieties (Kamway-1, VC-2010, and King) by Ahmad et al. (2015). Experiments were divided into four sets of irrigation, that is, I_1, I_2, I_3, and I_4 that irrigated at third, fifth, seventh, and ninth day, respectively, and by controlling the amount of water in each set. Plant height and shoot weight varied among the genotypes and also with the irrigation set. These traits were declined along with increasing drought stress, which clearly represents the effects of drought on the growth of mungbean genotypes. Ranawake et al. (2011) reported the response of mungbean genotypes for drought stress by imposing stress at three different stages (3WAP, 6WAP, and 8 WAP). Growth traits like average plant height, average number of leaves, average dry matter weight of root/shoot, average number of lateral roots, and average length of taproot were studied on the Harsha mungbean variety. They noted that drought stress imposed at 3 weeks after planting (Vegetative stage) and 6 WAP (reproductive stage) caused more damage to growth and crop yield than 8WAP (Podding stage). Therefore, it is recommended that mungbean be planted in such a way that drought stress ought not to coincide with 6WAP (reproductive stage).

Studies by Uddin et al. (2013) revealed similar findings that drought stress hampers the mungbean growth rate significantly. Morphological attributes including plant height, leaf area, shoot dry weight showed the lowest performance when no irrigation was applied to the crop compared to control. All above-mentioned studies imply that drought stress has retarding effect on the crop phenology involving traits like plant height, number of leaves, leaf area, and dry weight of shoots that are directly linked with low yield.

10.2.6.2 Chlorophyll Content

Drought stress environment hampers the process of photosynthesis by decreasing the concentration of various photosynthetic pigments. Degradation of pigment might be due to swelling of chloroplast membrane, distortion of lamellae, and vesiculization of lamellae (Baroowa and Gogoi 2012). Studies by Batra et al. (2014) recorded drought-induced damage by withholding water for 4 days on the chlorophyll content of three varieties of mungbean; RMG 268, K-851, and Anand. Gradual decline in total chlorophyll content up to 57% in Anand, 54% in K-851, and 39% in Anand when compared to control. These deformities further curtail the capturing of photons to the PSII by damaging D1 core protein and reducing the electron transfer. Uprety and Bhatia 1989, also reported comparable results in the three mungbean varieties; PS16, P105, Pusa Baisakhi having reduced total chlorophyll content. Higher reduction pattern recorded in Pusa Baisakhi and minimum in P105 reported during the flowering stage. Baroowa and Gogoi 2013 noted a positive correlation between soil moisture, total chlorophyll, and chlorophyll index. The rate of decline in chlorophyll content was rapid during prolonged drought stress (withholding water for 20 days).

10.2.6.3 Photosynthetic Rate (Pn)

Moderate and severe drought decreases photosynthesis rate by 25% and 50%, respectively, reported in the mungbean genotypes by Moradi et al. (2008). This reduction was accompanied by a decrease in stomatal conductance since the stomatal closure limits CO_2 availability to the mesophyll of leaves. Furthermore, they reported that drought stress imposes severe effects on the reproductive stage indicating 37% lower photosynthetic rate when compared to vegetative stage. More studies on the photosynthetic rate in mungbean genotypes demonstrate that Pn varied significantly at vegetative, pod setting, and pod filling stages. Photosynthetic rate increases with the advancement of crop stage under control but significantly decline on the application of drought treatment in all the mungbean varieties (Naresh et al. 2013). Hamid et al. assess the impacts of water stress imposed at different growth stages; pre-flowering, flowering, and pod development. Water deficits have more prominent effects in terms of photosynthesis rate, leaf area, leaf growth, dry matter accumulation during the flowering phase compared to podding stage. A reduction in yield by 6% has been reported in water-stressed plants compared to well-watered plants. Yield loss is mainly caused by inhibition of photosynthetic rate as well as lower dry matter accumulation.

10.2.6.4 Stomatal Conductance

Drought stress negatively affects photosynthesis as well as gas exchange traits that target the assimilation capacity of plants. Loss over stomatal regulation during water-deficit conditions may be responsible for a decline in assimilation capacity reported in mungbean crop (Moradi et al. 2008). The effects of severity of drought stress on different growth stages (vegetative and reproductive) of mungbean were recorded by Moradi et al. (2008). Water deficit during the vegetative and reproductive growth stage reduces stomatal conductance and transpiration rate. But the greatest effects of severe drought stress on these traits were recorded during the reproductive stage. Zarifinia et al. (2012) recorded similar observations on the mungbean genotypes that drought stress significantly reduces the stomatal conductance. Their study provided two drought-tolerant genotypes; Partow and Indian heap based on physiological traits involving stomatal conductance.

10.2.6.5 Chlorophyll Fluorescence

Chlorophyll fluorescence is the measurement of photosystem II activity and to understand the photosynthetic mechanisms. It further provides an indicator that how plants respond under environmental fluctuations. This technique gained major attention due to its use for the selection of desirable plant traits related to genetic and physiological responses for crop improvement. Responses of mungbean genotypes under drought stress were also recorded using this trait are discussed below.

Response of mungbean plants recorded at vegetative (S_2) and reproductive stage (S_3) for drought stress by Allahmoradi et al. (2011). Their results showed that drought stress reduces vegetative growth significantly as compared to the reproductive stage. A study of chlorophyll fluorescence showed a significant difference between S_2 with S_3 and S_1 (Control). Furthermore, PS II activity in S_1 and S_3 fall in a normal range but in S_2 it was out of the normal range (Allahmoradi et al. 2011). Batra et al. 2014 study the effects of drought stress on different mungbean varieties (RMG 268, K-851, and Anand) by analyzing their chlorophyll fluorescence. Their results indicated that drought stress hinders PS II activity and energy transfer by altering D1 protein of thylakoid protein. Variety RMG 268 and K-851 were more tolerant to drought stress than Anand variety judged by PS II activity.

10.2.7 Relative Water Content (RWC)

It is an important measure of the physiological water status of plants indicating leaf hydration and leaf water deficit. Maintaining water potential is important for the plant cell because it has a crucial role in maintaining all vital cellular activities. Thus an understanding of the effects of drought on leaf water relations is imperative for classifying the mechanism of drought tolerance of a plant. Therefore, it is a reliable trait for assessing the drought tolerance of plants (Chowdhury et al. 2017). Various studies conducted on mungbean are discussed below.

Effects of water deficit were examined in three mungbean varieties (PS 16, P105, and Pusa Baisakhi) by withholding irrigation at pre-flowering, flowering, post-flowering, and pod development stage (25, 35, 45, and 55 days after sowing, respectively). Relative water content was declined in all the three mungbean genotypes however the depression was less in variety P105 at pre-flowering, flowering, post-flowering stage. In contrast variety, Pusa Baisakhi exhibit maximum reduction at these stages, clearly pointing to their susceptibility toward drought stress (Uprety and Bhatia 1989).

Field studies of Bangar et al. (2019) also showed that RWC of mungbean leaves was significantly affected by drought. They screened total 25 varieties of mungbean and selected few drought-tolerant varieties (*Vigna sublobata*, MCV-1, PLM-32, LGG-407, LGG-450, TM-96-2, and Sattya) based on RWC along with other physiological traits. Drought stress significantly affects the RWC in the vegetative stage compared to the reproductive stage further decreasing pattern was more prominent in the sensitive genotypes. The lowest percentage decrease (1.5–1.82%) was recorded in tolerant genotypes (*V. sublobata* and MCV-1) and the highest percentage decrease (16.6–19.52%) noted in sensitive genotypes (PDM 139 and TARM-1).

Similarly, studies by Nazran et al. (2019) on the mungbean varieties indicate that severity of drought stress decreases leaf water content. Genotypic differences in mungbean demonstrate that BARI Mung-6 genotype maintains the maximum RWC (66.14%) while BUmug 2 genotype had the minimum RWC (55.21%).

10.2.7.1 Leaf Water Potential

Leaf water potential (LWP) and osmotic adjustment (OA) are the important traits that can be used as selection criteria for improving drought tolerance. Maintaining leaf water potential under water-deficit conditions is important for cellular activities. It is further associated with dehydration avoidance mechanisms that how plant cells manage to assess water through adjusting the osmolyte concentrations (Jongdee et al. 2002). Comparison of LWP in the mungbean varieties needs to be crucial for screening drought tolerance.

LWP of mungbean varieties was negatively affected by drought stress treatment reported in the studies of Nazran et al. (2019). Their studies showed that the highest water potential was reported in well-watered plants (−0.67 to −0.55 MPa) and it gradually decreases with the severity of drought stress (−1.64 to −1.13 MPa at 50–60% field capacity). Moreover, BARI Mung-6 variety of mungbean maintains higher water potential even at low field capacity (50–60%) and is categorized as drought stress-tolerant in terms of physiological adaptations.

In another study on mungbean, water stress significantly reduces the LWP throughout the growing period and majorly during flowering and post-flowering stages (Uprety and Bhatia 1989).

10.2.8 Biochemical Traits for Drought Tolerance in Mungbean

10.2.8.1 Oxidative Stress and Anti-oxidants

Under normal growth conditions, most of the cellular components of the plant exhibit constant homeostasis. But the exposure to drought stress imbalanced this homeostasis by reducing antioxidant production as a result of which greater production of ROS is obvious. The imbalance between ROS generation and their detoxification disrupts the redox control thus damaging the major biomolecules like carbohydrates, lipids, proteins, nucleic acids, and enzymatic activities (Hasanuzzaman et al. 2012). Water-deficit conditions make the protoplasm more viscous, denature proteins, and halting the activities of Calvin cycle enzymes. Inactivation of Calvin cycle enzymes resulted in shifting toward photorespiration, the main cycle for ROS production. Damage to PS II and lipid peroxidation are other reasons for ROS production. Oxidative stress was measured in terms of production of MDA accumulation, increased $H_2O_{2.}$ To endure oxidative stress damage, plants attain well-organized enzymatic and nonenzymatic systems. Enzymatic antioxidants in plants are superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate reductase (APX), and glutathione peroxidase (GPX). Among non-enzymatic anti-oxidants glutathione (GSH), ascorbic acid (ASA), tocopherol, alkaloids, and phenolic compounds are well recognized in plants. Improvement in antioxidant activities is effective in providing tolerance to plants against drought stress. It has been reported through various studies that higher levels of antioxidants help in reducing ROS-induced damage, thus improving drought tolerance.

Sengupta et al. (2013) studied the drought-induced ROS damage and significant production of antioxidants in the mungbean roots. Drought stress exposure (by withholding water for three (D3) and 6 days (D6) at the vegetative stage; 30 days old plant) lead to the gradual increment in $H_2O_2$and MDA content. Non-enzymatic antioxidant GSH and ASA also showed progressive increase under drought stress treatment. Their content increased slightly in the D3 but significantly higher content (twofold) recorded on D6. Hence, root responses toward drought stress are crucial to understanding that further provide some interdependence mechanisms with photosynthetic performance and plant water status. Yin et al. (2015) recorded the responses of mungbean genotypes to polyethylene glycol-induced drought stress. Three concentrations of PEG 6000 were used as 5%, 10%, and 20% to impose drought stress at the flowering period. Drought stress caused a significant increase in the levels of O_2^- and MDA with decreasing water potential. In contrast, activities of enzymatic antioxidants such as SOD and POX increased in all the drought stress treatments. Drought stress hampered photosynthetic function and enhanced the oxidative stress measured in terms of lipid peroxidation and $H_2O_{2.}$ Drought stress-induced oxidative stress reduces the pools of GSH and ASA. Maintenance of content of non-enzymatic antioxidants increases the resistance of mungbean genotypes as reported in the studies of Anjum et al. (2015).

10.2.8.2 Osmotic Adjustment

To survive under water deficit conditions, one common approach endorsed by plants is the accumulation of osmolytes or compatible solutes. Osmolytes are low molecular weight, small organic compounds synthesized by plants in the cytosol, chloroplast, and other cellular organelles having a role in the protection of cellular components against dehydration (Hasanuzzaman et al. 2019). Osmolytes mainly include proline, glycine betaine, polyamines, and sugar alcohols (Ashraf and Foolad 2007). They not only help in maintaining cell turgor but are also involved in improving ROS scavenging mechanisms, therefore buffering the cellular redox potential (Anjum et al. 2017). Therefore, it is a protective mechanism involving the accumulation of compatible osmolytes in plants to prevent cellular dehydration which maintains cell water status by cell integrity, leaf water adjustment, and osmotic adjustment (Blum 2005). Osmotic adjustment is a means by which plants adapt to water stress by the active accumulation of solutes in the cell sap and as a result of solute accumulation, the osmotic potential of the cell decreases, therefore helping in withdrawing more water from surrounding and maintaining turgor of cell (Ludlow and Muchow 1990). Due to this osmotic adjustment, all cytoplasmic activities occur normally and, in this way, help plants to perform better in terms of growth, photosynthesis, and assimilate partitioning (Subbarao et al. 2000). Among all protective osmolytes, free proline is an important beneficial solute allowing plants to increase cellular osmolarity during drought (Ashraf and Foolad 2007).

Studies by Bangar et al. (2019), reported the proline content in 25 mungbean varieties at two development stages; vegetative and reproductive stage. Proline content significantly increases in both developmental stages. The percentage increase ranged from 6.5% to 80.2% during the vegetative stage and 9.6–118.3% during the reproductive stage over the control. Further, significant variations in the proline content were recorded in all 25 varieties. Similar findings were recorded from the studies of Bhardwaj et al. (2018) that drought stress significantly increases the proline content in all the seven mungbean genotypes. However genotypes ‘IPM99–125’ accumulate highest (62%) while the lowest content was recorded in genotype “Pratap” (42%) under both the development stages. Higher proline accumulation maintains to provide maximum cell osmoprotection, thus sustaining the vital cellular activities under water deficit conditions.

10.2.8.3 Yield Traits for Drought Tolerance in Mungbean

Abovementioned effects of water scarcity are reduced plant growth and biomass accumulation altered photosynthetic efficiency, low stomatal conductance, and weaker source-sink activities. Poor performances of all these traits merged to induce severe yield losses. The magnitude of reduction in grain yield depends on the intensity and duration of stress. Drought impedes productivity at all the growth stages but its occurrence during reproduction and grain development stages are more crucial and results in significant yield fall (Farooq et al. 2017). Drought-induced yield losses were reported in cereal crops like wheat by 34% (Saeidi and Abdoli 2015); maize by 59–90% (Kamara et al. 2003); and leguminous crops like Chickpea by 45–69% (Nayyar et al. 2006), cowpea by 34–66% (Ahmed and Suliman 2010);

lentil by 24% (Allahmoradi et al. 2013) and soybean by 42% (Maleki et al. 2013). Effects of drought stress on the yield of mungbean crop were discussed in detail in this review.

Ranawake et al. (2011) evaluated the effects of drought stress imposed at different growth stages (3WAP, 6WAP, 8WAP) on yield traits like average number of pods and average grain weight. Water stress affects the grain yield at 6 WAP (flowering stage) more severely because plants were failed to initiate reproductive processes resulting in no net yield. While drought stress imposed at 8WAP (podding stage) cause comparatively less damage indicating that crop should be planted in such a way that the reproductive phase should not coincide with drought stress. Ahmad et al. (2015) also reported that drought stress affects the yield of mungbean genotypes. The reduction pattern varies with the irrigation interval (3, 5, 7, and 9) and amount of irrigation. Seed yield (kg/h) declined in all the treatments, but more reduction was observed under irrigation interval of 9 days with the lowest number and amount of irrigation. Seed yield declined from 1146 to 78 kg/ha in this treatment clearly demonstrating that water deficiency hampered the yield components. Kumar and Sharma (2009) recorded the genotypic differences in mungbean and noted that drought stress affects the seed yield by affecting dry matter partitioning. Tolerant mungbean genotypes exhibit high yield and it is correlated with higher RWC which promotes more dry matter partitioning.

10.2.9 Genomics of Drought Tolerance in Mungbean

A high-throughput platform for genotyping in association with sequencing technologies allowed the access of genetic linkage maps and quantitative trait loci (QTL) mapping of the traits related to drought tolerance (Jha et al. 2020). Isolation of drought-responsive genes and detection of QTLs studies are limited in mungbean. However the study of Liu et al. 2017 identified novel genetic elements in the mungbean providing drought tolerance. They identified 58 QTLs in the 11 linkage groups ((LG 1–11) using 313 markers. These QTLs were associated with plant height, leaf area, biomass, RWC, days to first flowering, and yield traits. Sixteen QTLs were detected for plant height associated with linkage group LG 04, LG05, and LG08. Similarly, 8 QTLs for biomass involving LG02, LG03, LG04, LG06, and LG08, 6 QTLs for RWC on LG04, LG08 and LG10, 12 QTLs for leaf area in LG02, LG03, LG04, LG08, and LG11, 8 QTLs for days to flowering in LG04 and 4 QTLs for seed yield in LG01, LG04, and LG08. Few studies reported on Genome-wide association identification of transcription factors in the mungbean genotypes to gain more insights into the molecular mechanisms governing drought tolerance. Labbo et al. (2018) identified 71 AP2/ERF (APETALA 2/Ethylene-responsive element factor binding protein) transcription factors and classified them into AP2 (16), ERF (22), RAV (2), DREB (30). Among them, DREB genes played a critical role in drought tolerance. Five DREB genes (*VrDREB 5, VrDREB12, VrDREB13, VrDREB22, VrDREB30)* exhibit higher expression under drought stress and might be considered excellent candidates for improving drought tolerance in mungbean genotypes. Genome-wide analyses of genes related to sucrose nonfermenting-1-

related protein kinase 2 family (SnRK2) were characterized in mungbean that is known to play important role in osmotic stress. 8 SnRK2 genes were reported named as *VrSnRK2.1, VrSnRK2.2a, VrSnRK 2.2b, VrSnRK 2.2c, VrSnRK 2.3, VrSnRK 2.4, VrSnRK 2.5, VrSnRK 2.6a, VrSnRK 2.6b, VrSnRK 2.6c, VrSnRK2.7* and*VrSnRK2.8.* Expression of these genes induced by drought stress indicates its importance in drought tolerance. Moreover gene *VrSnRK 2.6c* exhibit higher expressions among all the genes illustrating its critical role under drought stress tolerance (Fatima et al. 2020).

10.2.9.1 Agronomic Approaches to Combat Drought Stress

Various approaches have been developed from time to time to achieve stress tolerance in plants. Nowadays, seed priming methods are extensively used as an emerging technology to raise stress-tolerant plants. Seed priming evokes physiological, biochemical, and cellular processes in the plant that prepares them to respond to stress conditions instantly (Lal et al. 2018). It has been reported through various studies that seed priming improves the seedling emergence, seed establishment, growth traits, and yield traits under drought stress in wheat (Hussain et al. 2018); maize (Nada and Hamza 2019); chickpea (Shariatmadari et al. 2017); and sorghum (Sheykhbaglou et al. 2014). Through these studies, it is clear that seed priming improves the drought tolerance in the plant through enhanced antioxidant activities, accumulation of osmolytes, and better nutrient uptake. Case study on seed priming in mungbean to exclude drought stress effects discussed in this review.

Studies by Jisha and Puthur (2016) recorded that seed priming of β-amino butyric acid (BABA) (0.5, 1, 1.5, 2, 2.5 mM) in mungbean genotypes (Pusa Ratna, Pusa 9531, Pusa Vishal) alleviate the drought stress effects. Primed seeds exhibit higher chlorophyll fluorescence, mitochondria activity, photosynthetic activity, and seedling growth parameters (shoot length, shoot fresh and dry weight). Moreover, primed seeds have reduced MDA content, increased accumulation of proline content, total carbohydrate, total proline, nitrate reductase activity, and activity of antioxidant enzymes like SOD and guaiacol peroxidase. Similarly, seed priming with polyamines (put+spd + spm) improves the drought tolerance in mungbean genotypes by decreasing the membrane damage, increasing the proline content, soluble proteins, and soluble sugars. Hence, treated mungbean genotypes resulted in improved growth and yield under drought stress (Sadeghipour 2019).

Besides conventional plant breeding and transgenic approaches, the application of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhiza (AM) proved to be useful for improving drought tolerance in mungbean crops (Sarma and Saikia 2014; Habibzadeh et al. 2014). PRPR is a group of bacteria that are found in the rhizosphere mainly at root surfaces and in association with roots that promote the establishment of various interactions that benefit the plant growth directly or indirectly. They are widely studied because of their potential use as bio-fertilizer (Kumari and Chakraborty 2017). In a similar way mycorrhiza is a symbiotic association between fungus and plant that forms a beneficial relationship between soil and plant that increases the water absorption as well as nutrient uptake through mycorrhizal hypha (Habibzadeh et al. 2014). Studies have confirmed that inoculation of various plant species with such micro-organisms leads to the improvement of root system architecture that enhances the ability of the plant with better adaptation to stress condition (Sarma and Saikia 2014).

Elevated production of ROS scavenging enzymes, cellular osmolytes, and upregulation of genes associated with drought tolerance like Dehydration Responsive Element Binding protein (DREB2A) catalase (CAT1) and dehydrin, were reported in the mungbean plants when inoculated with arbuscular mycorrhizal fungi (*Glomus mosseae, G. intraradices*) (Habibzadeh et al. 2014). In another study, mungbean genotype; AU-M4 (overproducing mutant of PGPR) has significant drought tolerance. This mutant has enhanced ACC deaminase activity, IAA production, and inorganic phosphate solubilization compared to the wild strains. Therefore, express superior tolerance under drought stress in terms of higher plant biomass, proline accumulation, water content, and lower osmotic stress injury. Inoculation with osmoprotectant rhizobacteria isolates (A124-K and Ver5-K) produced glycine betaine that improved mungbean tolerance to drought stress (Maryani et al. 2018). Hence, to lessen the effects of drought stress, the use of PGPR and AM will be employed to ensure sufficient growth and yield of crop plants (Kumari et al. 2016).

10.3 Conclusion

To meet future food demands, plant stress tolerance must be improved. Plants express a wide range of responses to heat and drought stresses which are mostly represented by a variety of modifications in the overall growth of plant (Zhou et al. 2017). These stresses significantly affect morphological, physiological biochemical, and molecular processes resulting in major yield losses (Sehgal et al. 2018). Almost every plant process is affected by these stresses, from membrane stability, enzymatic activity, at a cellular level and decreased transpiration, stomatal conductance, and photosynthetic rate at the physiological level (Hussain et al. 2019). To minimize the damages plants have evolved various adaptive mechanisms and activated various signaling pathways for upregulation of antioxidants and accumulation of compatible solutes (Fahad et al. 2017). Aforementioned traits have successfully identified heat tolerance in mungbean crops which may provide useful information to the plant breeders. Various new technologies have been developed for assessing physiological, biochemical, and molecular traits for getting insight into the mechanisms governing heat tolerance (Chen et al. 2019). However commercial applications of these techniques are limited and require further field trials. Advances in Omics technique including genomics, transcriptomics, proteomics, and metabolomics could provide possible candidate genes, proteins, and metabolites contributing to stress tolerance (Zhou et al. 2017). Molecular breeding methods like QTLs and GWAS could also reveal the stress tolerance governing genes (Priya et al. 2019a, b). Therefore, concerted efforts are needed to enhance the efficiency of breeding programs for the rapid development of varieties with improved adaptation to heat, drought, and combined stresses and other desired traits (Fig. 10.1).

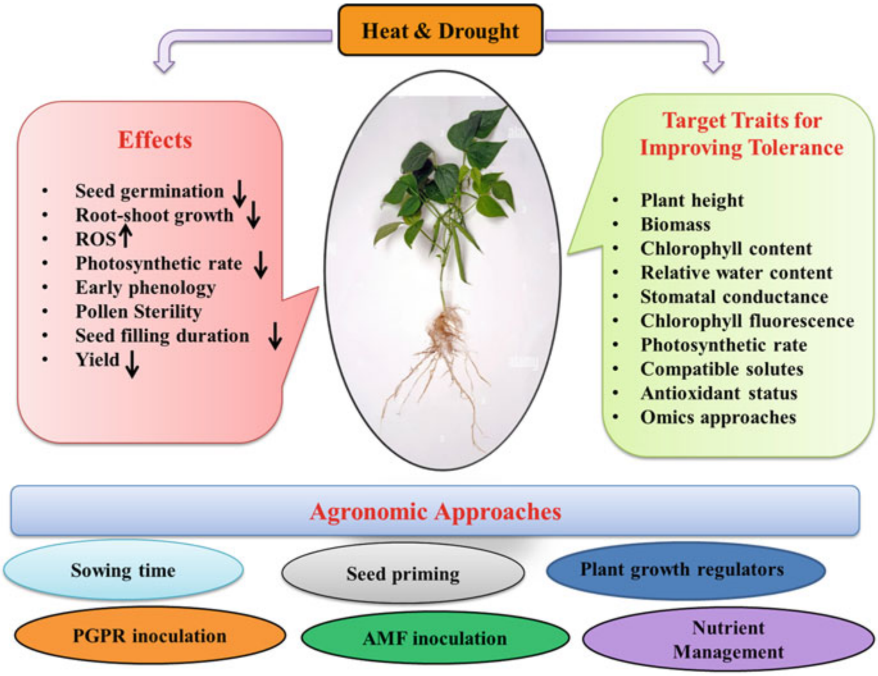

Fig. 10.1 Heat and drought stress has various negative effects on the plant performance. Overall life cycle starting from seed germination, root-shoot growth, photosynthesis, reproductive growth and seed filling stages are affected very badly leading to poor yield. But plants showed various adaptation responses under stress conditions which can be helpful to identify the underlying tolerance mechanisms and producing stress resilient varieties. Such target sites for improving plant tolerance are growth traits like plant height and biomass, morpho-physiological traits like chlorophyll content, relative water content, stomatal conductance, chlorophyll fluorescence, photosynthetic rate and biochemical traits like production of compatible solutes and antioxidants. Agronomic approaches like seed priming, sowing time, application of plant growth regulators, nutrient management, PGPR and AMF inoculation can also be employed to get better yield under stress conditions

References

Abid M, Hakeem A, Shao Y, Liu Y, Zahoor R, Fan Y, Suyu J, Ata-Ul-Karmi ST, Tian Z, Jiang D, Snider JL, Dai T (2018) Seed osmopriming invokes stress memory against post-germinative drought stress in wheat (*Triticum aestivum* L.). Environ Exp Bot 145:12–20. https://doi.org/10.1016/j.envexpbot.2017.10.002

Abou-Shleel SM (2014) Effect of air temperature on growth, yield and active ingredients of fenugreek (*Trigonella foenum-graecum*). Nat Sci 12:50–54

Ahmad A, Selim MM, Alderfasi AA, Afzal M (2015) Effect of drought stress on mung bean (*Vigna radiata* L.) under arid climatic conditions of Saudi Arabia. WIT Trans Ecol Environ 192:185–193

Ahmad J, Anwar S, Shad AA, Marwat FY, Bibi H, Ahmad F, Noor W, Sadia B (2021) Yield and nutritional status of mungbean as influenced by molybdenum and phosphorus. Pak J Agric Sci 1:34

Ahmed FE, Suliman ASH (2010) Effect of water stress applied at different stages of growth on seed yield and water-use efficiency of cowpea. Agric Biol J N Am 1:534–540

Ahmed FE, Hall AE, DeMason DA (1992) Heat injury during floral development in cowpea (*Vigna unguiculata,* Fabaceae). Am J Bot 79:784–791. https://doi.org/10.1002/j.1537-2197.1992.tb13655.x

Akhtar S, Hazra P, Naik A (2015) Harnessing heat stress in vegetable crops towards mitigating impacts of climate change. Clim Dyn Hortic Sci 1:419

Alagupalamuthirsolai M, Vijaylakshmi C, Basu PS, Singh J (2015) Physiological evaluation of mungbean (*Vigna radiata* (l.) Wilczek) cultivars for heat tolerance. Food Legum 28:30–34

Ali Q, Javed MT, Noman A, Haider MZ, Waseem M, Iqbal N, Waseem M, Shah MS, Shahzad F, Perveen R (2018) Assessment of drought tolerance in mung bean cultivars/lines as depicted by the activities of germination enzymes, seedling's antioxidative potential and nutrient acquisition. Arch Agron Soil Sci 64:84–102. https://doi.org/10.1080/03650340.2017.1335393

Allahmoradi P, Ghobadi M, Taherabadi S, Taherabadi S (2011) Physiological aspects of mungbean (*Vigna radiata* L.) in response to drought stress. In: Paper presented at the International conference on food engineering and biotechnology, Bangkok, 7–9 May 2011

Allahmoradi P, Mansourifar C, Saidi M, Honarmand SJ (2013) Water deficiency and its effects on grain yield and some physiological traits during different growth stages in lentil (*Lens culinaris* L.) cultivars. Ann Biol Res 4:139–145

Anjum SA, Xie XY, Wang LC, Saleem MF, Man C, Lei W (2011) Morphological, physiological and biochemical responses of plants to drought stress. Afr J Agric Res 6:2026–2032. https://doi.org/10.5897/AJAR10.027

Anjum NA, Umar S, Aref IM, Iqbal M (2015) Managing the pools of cellular redox buffers and the control of oxidative stress during the ontogeny of drought-exposed mungbean (*Vigna radiata* L.)—role of sulfur nutrition. Front Environ Sci 2:1–9. https://doi.org/10.3389/fenvs.2014.00066

Anjum SA, Ashraf U, Tanveer M, Khan I, Hussain S, Shahzad B, Zohaib A, Abbas F, Saleem MF, Ali I, Wang LC (2017) Drought induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids. Front Plant Sci 8:1–12. https://doi.org/10.3389/fpls.2017.00069

Ardalani S, Saeidi M, Jalali Honarmand S, Ghobadi ME, Abdoli M (2015) Effect of post anthesis drought stress on some agronomic and physiological traits related to source strength in four bread wheat genotypes. Cereal Res 5:45–65

Ashraf MFMR, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Aslam M, Ibni Zamir MS, Afzal I, Yaseen M (2013a) Morphological and physiological response of maize hybrids to potassium application under drought stress. J Agric Res 51:454–443

Aslam M, Maqbool MA, Zaman QU, Latif MZ, Ahmad RM (2013b) Responses of mungbean genotypes to drought stress at early growth stages. Int J Basic Appl Sci 13:22–27

Awasthi R, Bhandari K, Nayyar H (2015) Temperature stress and redox homeostasis in agricultural crops. Front Environ Sci 3:1–24. https://doi.org/10.3389/fenvs.2015.00011

Bangar P, Chaudhury A, Tiwari B, Kumar S, Kumari R, Bhat KV (2019) Morphophysiological and biochemical response of mungbean [*Vigna radiata* (L.) Wilczek] varieties at different developmental stages under drought stress. Turk J Biol 43:58–69. https://doi.org/10.3906/biy-1801-64

Bansal M, Kukreja K, Suneja S, Dudeja SS (2014) Symbiotic effectivity of high temperature tolerant mungbean (*Vigna radiata*) rhizobia under different temperature conditions. Int J Curr Microbiol App Sci 3:807–821

Baroowa B, Gogoi N (2012) Effect of induced drought on different growth and biochemical attributes of black gram (*Vigna mungo* L.) and green gram (*Vigna radiata* L.). J Environ Res Develop 6:584–593

Baroowa B, Gogoi N (2013) Biochemical changes in two Vigna spp. during drought and subsequent recovery. Indian J Plant Physiol 18:319–325

Basu PS, Garg AP, Gupta S, Sharma K, Tomar R, Singh NP (2019) Physiological traits for shortening crop duration and improving productivity of greengram (*Vigna radiata* L. Wilczek) under high temperature. Front Plant Sci 10:1–18

Batra NG, Sharma V, Kumari N (2014) Drought-induced changes in chlorophyll fluorescence, photosynthetic pigments, and thylakoid membrane proteins of *Vigna radiata*. J Plant Interact 9: 712–721. https://doi.org/10.1080/17429145.2014.905801

Bharadwaj N (2018) Morpho-physiological responses in different mungbean genotypes under drought stress. Res J Recent Sci 7:1–5

Biswash MR, Rahman MW, Haque MM, Sharmin M, Barua R (2014) Effect of potassium and vermicompost on the growth, yield and nutrient contents of mungbean (BARI Mung 5). Open Sci J Biosci Bioeng 1:33–39

Bita C, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci 4:1–18. https://doi.org/10.3389/fpls.2013.00273

Blum A (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Aust J Agric Res 56:1159–1168. https://doi.org/10.1071/AR05069

Chai Q, Gan Y, Zhao C, Xu HL, Waskom RM, Niu Y, Siddique KH (2016) Regulated deficit irrigation for crop production under drought stress. Agron Sustain Dev 36:1–21. https://doi.org/10.1007/s13593-015-0338-6

Chand G, Nandwal AS, Kumar N, Devi S, Khajuria S (2018) Yield and physiological responses of mungbean *Vigna radita* (L.) Wilczek genotypes to high temperature at reproductive stage. Legum Res An Int J 41:557–562. https://doi.org/10.18805/LR-3795

Chauhan Y, Williams R (2018) Physiological and agronomic strategies to increase mungbean yield in climatically variable environments of northern Australia. Agronomy 8:1–20. https://doi.org/10.3390/agronomy8060083

Chen S, Guo Y, Sirault X, Stefanova K, Saradadevi R, Turner NC, Nelson MN, Furbank RT, Siddique KHM, Cowling WA (2019) Nondestructive phenomic tools for the prediction of heat and drought tolerance at anthesis in Brassica species. Plant Phenomics 2019:1–16. https://doi.org/10.34133/2019/3264872

Chikukura L, Bandyopadhyay SK, Kumar SN, Pathak H, Chakrabarti B (2017) Effect of elevated temperature stress on growth, yield and yield attributes of mungbean (*Vigna radiata*) in semi-arid north-west India. Curr Adv Agric Sci 9:18–22. https://doi.org/10.5958/2394-4471.2017.00003.X

Chowdhury JA, Karim MA, Khaliq QA, Ahmed AU, Mondol AM (2017) Effect of drought stress on water relation traits of four soybean genotypes. SAARC J Agric 15:163–175. https://doi.org/10.3329/sja.v15i2.35146

Dahiya PK, Linnemann AR, Van Boekel M, Khetarpaul N, Grewal RB, Nout MJR (2015) Mung bean: technological and nutritional potential. Crit Rev Food Sci Nutr 55:670–688. https://doi.org/10.1080/10408398.2012.671202

Devasirvatham V, Gaur PM, Mallikarjuna N, Tokachichu RN, Trethowan RM, Tan DKY (2012) Effect of high temperature on the reproductive development of chickpea genotypes under controlled environments. Funct Plant Biol 39:1009–1018. https://doi.org/10.1071/FP12033

Dias AS, Barreiro MG, Campos PS, Ramalho JC, Lidon FC (2010) Wheat cellular membrane thermotolerance under heat stress. J Agron Crop Sci 196:100–108. https://doi.org/10.1111/j.1439-037X.2009.00398.x

Djanaguiraman M, Prasad PVV, Boyle DL, Schapaugh WT (2013) Soybean pollen anatomy, viability and pod set under high temperature stress. J Agron Crop Sci 199:171–177. https://doi.org/10.1111/jac.12005

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ, Alhraby H, Wu C, Wang D, Huang J (2017) Crop production under drought and heat stress: plant responses and management options. Front Plant Sci 8:1–16. https://doi.org/10.3389/fpls.2017.01147

Fang Y, Du Y, Wang J, Wu A, Qiao S, Xu B, Zhang SH, Siddique KHM, Chen Y (2017) Moderate drought stress affected root growth and grain yield in old, modern and newly released cultivars of winter wheat. Front Plant Sci 8:1–14. https://doi.org/10.3389/fpls.2017.00672

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29:185–212. https://doi.org/10.1051/agro:2008021

Farooq M, Bramley H, Palta JA, Siddique KHM (2011) Heat stress in wheat during reproductive and grain-filling phases. CRC Crit Rev Plant Sci 30:491–507. https://doi.org/10.1080/07352689.2011.615687

Farooq M, Gogoi N, Barthakur S, Baroowa B, Bharadwaj N, Alghamdi SS, Siddique KHM (2017) Drought stress in grain legumes during reproduction and grain filling. J Agron Crop Sci 203:81–102. https://doi.org/10.1111/jac.12169

Fathi A, Tari DB (2016) Effect of drought stress and its mechanism in plants. Int J Life Sci 10:1–6. https://doi.org/10.3126/ijls.v10i1.14509

Fatima A, Khan MJ, Awan HM, Akhtar MN, Bibi N, Sughra K, Khan MR, Ahmad R, Ibrahim M, Sadiq I (2020) Genome-wide identification and expression analysis of SnRK2 gene family in mungbean (*Vigna radiata*) in response to drought stress. Crop Pasture Sci 71:469–476. https://doi.org/10.1071/CP19392

Fedoroff NV, Battisti DS, Beachy RN, Cooper PJM, Fischhoff DA, Hodges CN, Knauf VC, Lobell D, Mazur BJ, Molden D, Reynolds MP, Ronald PC, Rosegrant MV, Sanchez PA, Vonshak A, Zhu JK (2010) Radically rethinking agriculture for the 21st century. Science 327:833–834. https://doi.org/10.1126/science.1186834

Firon N, Nepi M, Pacini E (2012) Water status and associated processes mark critical stages in pollen development and functioning. Ann Bot 109:1201–1214. https://doi.org/10.1093/aob/mcs070

Guo M, Liu J-H, Ma X, Luo D-X, Gong Z-H, Lu M-H (2016) The plant heat stress transcription factors (HSFs): structure, regulation, and function in response to abiotic stresses. Front Plant Sci 7:1–13. https://doi.org/10.3389/fpls.2016.00114

Habibzadeh Y, Evazi AR, Abedi M (2014) Alleviation drought stress of mungbean (*Vigna radiata* L.) plants by using arbuscular mycorrhizal fungi. Int J Agric Sci Nat Resour 1:1–6

Hall AE (1992) Breeding for heat tolerance. Plant Breed Rev 10:129–168

Hamid A, Kubota F, Agata W, Morokuma M (1981) Photosynthesis, transpiration, dry matter accumulation and yield. J Fac Agric Kyushu Univ 35:81–92

Hanif A, Wahid A (2018) Seed yield loss in mungbean is associated to heat stress induced oxidative damage and loss of photosynthetic capacity in proximal trifoliate leaf. Pak J Agric Sci 55:777–786. https://doi.org/10.21162/PAKJAS/18.7461

HanumanthaRao B, Nair RM, Nayyar H (2016) Salinity and high temperature tolerance in mungbean [*Vigna radiata* (L.) Wilczek] from a physiological perspective. Front Plant Sci 7:1–20. https://doi.org/10.3389/fpls.2016.00957

Hasanuzzaman M, Hossain MA, da Silva JAT, Fujita M (2012) Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: Venkateswarlu B, Shankaer AK, Shanker C, Maheswari M (eds) Crop stress and its management: perspectives and strategies. Springer, Dordrecht, pp 261–315

Hasanuzzaman M, Anee TI, Bhuiyan TF, Nahar K, Fujita M (2019) Emerging role of osmolytes in enhancing abiotic stress tolerance in rice. In: Hasanuzzaman M, Fujita M, Nahar K, Biswas JK (eds) . Advances in rice research for abiotic stress tolerance, Woodhead Publishing, pp 677–708

Hatfield JL, Prueger JH (2015) Temperature extremes: effect on plant growth and development. Weather Clim Extrem 10:4–10. https://doi.org/10.1016/j.wace.2015.08.001

Hussain M, Farooq M, Sattar A, Ijaz M, Sher A, Ul-Allah S (2018) Mitigating the adverse effects of drought stress through seed priming and seed quality on wheat (*Triticum aestivum* L.) productivity. Pak J Agric Sci 55:313–319. https://doi.org/10.21162/PAKJAS/18.5833

Hussain HA, Men S, Hussain S, Chen Y, Ali S, Zhang S, Zhang K, Li Y, Xu Q, Liao C, Wang L (2019) Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. Sci Rep 9:1–12. https://doi.org/10.1038/s41598-019-40362-7

Jaleel CA, Manivannan P, Wahid A, Farooq M, Al-Juburi HJ, Somasundaram R, Panneerselvam R (2009) Drought stress in plants: a review on morphological characteristics and pigments composition. Int J Agric Biol 11:100–105

Jha UC, Bohra A, Parida SK, Jha R (2017) Integrated "omics" approaches to sustain global productivity of major grain legumes under heat stress. Plant Breed 136:437–459. https://doi.org/10.1111/pbr.12489

Jha UC, Bohra A, Nayyar H (2020) Advances in "omics" approaches to tackle drought stress in grain legumes. Plant Breed 139:1–27. https://doi.org/10.1111/pbr.12761

Jiang Y, Lahlali R, Karunakaran C, Kumar S, Davis AR, Bueckert RA (2015) Seed set, pollen morphology and pollen surface composition response to heat stress in field pea. Plant Cell Environ 38:2387–2397. https://doi.org/10.1111/pce.12589

Jisha KC, Puthur JT (2016) Seed priming with BABA (β-amino butyric acid): a cost-effective method of abiotic stress tolerance in *Vigna radiata* (L.) Wilczek. Protoplasma 253:277–289. https://doi.org/10.1007/s00709-015-0804-7

Jongdee B, Fukai S, Cooper M (2002) Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. Field Crops Res 76:153–163. https://doi.org/10.1016/S0378-4290(02)00036-9

Joshi R, Wani SH, Singh B, Bohra A, Dar ZA, Lone AA, Pareek A, Singla-Pareek SL (2016) Transcription factors and plants response to drought stress: current understanding and future directions. Front Plant Sci 7:1–15. https://doi.org/10.3389/fpls.2016.01029

Kajla M, Yadav VK, Khokhar J, Singh S, Chhokar RS, Meena RP, Sharma RK (2015) Increase in wheat production through management of abiotic stresses: a review. J Appl Nat Sci 7:1070–1080. https://doi.org/10.31018/jans.v7i2.733

Kalaji HM, Jajoo A, Oukarroum A, Brestic M, Zivcak M, Samborska IA, Centner MD, Lukasik I, Goltsev V, Ladle RJ (2016) Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. Acta Physiol Plant 38:1–11. https://doi.org/10.1007/s11738-016-2113-y

Kamara AY, Menkir A, Badu-Apraku B, Ibikunle O (2003) The influence of drought stress on growth, yield and yield components of selected maize genotypes. J Agric Sci 141:43–50. https://doi.org/10.1017/S0021859603003423

Kang YJ, Kim SK, Kim MY, Lestari P, Kim KH, Ha B-K, Jun TH, Hwang WJ, Lee T, Lee J, Shim S, Yoon MY, Jang YE, Han KS, Taeprayoon P, Yoon N, Somta P, Tanya P, Kim KS, Gwag J-G, Moon J-K, Lee Y-H, Park B-S, Bombarely A, Doyle JJ, Jackson SA, Schafleitner R, Srinives P, Rajeev K, Varshney RK, Lee S-H (2014) Genome sequence of mungbean and insights into evolution within Vigna species. Nat Commun 5:1–9. https://doi.org/10.1038/ncomms6443

Kaur R, Bains TS, Bindumadhava H, Nayyar H (2015) Responses of mungbean (*Vigna radiata* L.) genotypes to heat stress: effects on reproductive biology, leaf function and yield traits. Sci Hortic 14:197:527

Kaushal N, Bhandari K, Siddique KHM, Nayyar H (2016) Food crops face rising temperatures: an overview of responses, adaptive mechanisms, and approaches to improve heat tolerance. Cogent Food Agric 2:1–42. https://doi.org/10.1080/23311932.2015.1134380

Keatinge JDH, Easdown WJ, Yang RY, Chadha ML, Shanmugasundaram S (2011) Overcoming chronic malnutrition in a future warming world: the key importance of mungbean and vegetable soybean. Euphytica 180:129–141. https://doi.org/10.1007/s10681-011-0401-6

Khattak GSS, Saeed I, Muhammad T (2006) Breeding for heat tolerance in mungbean (*Vigna radiata* (L.) Wilczek). Pak J Bot 38:1539–1550

Kiani SP, Grieu P, Maury P, Hewezi T, Gentzbittel L, Sarrafi A (2007) Genetic variability for physiological traits under drought conditions and differential expression of water stress-associated genes in sunflower (*Helianthus annuus* L.). Theor Appl Genet 114:193–207. https://doi.org/10.1007/s00122-006-0419-7

Kim SK, Nair RM, Lee J, Lee S-H (2015) Genomic resources in mungbean for future breeding programs. Front Plant Sci 6:1–12. https://doi.org/10.3389/fpls.2015.00626

Kumar A, Sharma KD (2009) Physiological responses and dry matter partitioning of summer mungbean (*Vigna radiata* L.) genotypes subjected to drought conditions. J Agron Crop Sci 195:270–277. https://doi.org/10.1111/j.1439-037X.2009.00373.x

Kumar S, Kaur R, Kaur N (2011) Heat-stress induced inhibition in growth and chlorosis in mungbean (*Phaseolus aureus* Roxb .) is partly mitigated by ascorbic acid application and is related to reduction in oxidative stress. Acta Physiol Plantarum 33:2091–2101. https://doi.org/10.1007/s11738-011-0748-2

Kumar S, Thakur P, Kaushal N, Malik JA, Gaur P, Nayyar H (2013) Effect of varying high temperatures during reproductive growth on reproductive function, oxidative stress and seed yield in chickpea genotypes differing in heat sensitivity. Arch Agron Soil Sci 59:823–843. https://doi.org/10.1080/03650340.2012.683424

Kumari D, Chakraborty D (2017) Drought stress mitigation in *Vigna radiata* by the application of root-nodulating bacteria. Plant Sci Today 4:209–212. https://doi.org/10.14719/pst.2017.4.4.343

Kumari P, Verma SK (1983) Genotypic differences in flower production, shedding and yield in mungbean. J Agric Sci 99:219–223

Kumari S, Vaishnav A, Jain S, Varma A, Choudhary DK (2016) Induced drought tolerance through wild and mutant bacterial strain *Pseudomonas simiae* in mung bean (*Vigna radiata* L.). World J Microbiol Biotechnol 32:1–10. https://doi.org/10.1007/s11274-015-1974-3

Labbo AM, Mehmood M, Akhtar MN, Khan MJ, Tariq A, Sadiq I (2018) Genome-wide identification of AP2/ERF transcription factors in mungbean (*Vigna radiata*) and expression profiling of the VrDREB subfamily under drought stress. Crop Pasture Sci 69:1009–1019

Lal SK, Kumar S, Sheri V, Mehta S, Varakumar P, Ram B, Borphukan B, James D, Fartyal D, Reddy MK (2018) Seed priming: an emerging technology to impart abiotic stress tolerance in crop plants. In: Rakshit A, Singh HB (eds) Advances in seed priming. Springer, Singapore, pp 41–50

Liu C, Wu J, Wang L, Fan B, Cao Z, Su Q, Zhang Z, Wang Y, Tian J, Wang S (2017) Quantitative trait locus mapping under irrigated and drought treatments based on a novel genetic linkage map in mungbean (*Vigna radiata* L.). Theor Appl Genet 130:2375–2393. https://doi.org/10.1007/s00122-017-2965-6

Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. Adv Agron 43:107–153. https://doi.org/10.1016/S0065-2113(08)60477-0

Maleki A, Naderi A, Naseri R, Fathi A, Bahamin S, Maleki R (2013) Physiological performance of soybean cultivars under drought stress. Bull Environ Pharmacol Life Sci 2:38–44

Mannur DM, Babbar A, Thudi M, Sabbavarapu MM, Roorkiwal M, Sharanabasappa BY, Bansal VP, Jayalakshmi SK, Yadav SS, Rathore A, Chamarthi SK, Mallikarjuna BP, Gaur PM, Varshney RK (2019) Super Annigeri 1 and improved JG 74: two Fusarium wilt-resistant introgression lines developed using marker-assisted backcrossing approach in chickpea (*Cicer arietinum* L.). Mol Breed 39:1–13. https://doi.org/10.1007/s11032-018-0908-9

Mansoor S, Naqvi FN (2013) Isoamylase profile of mung bean seedlings treated with high temperature and gibberellic acid. Afr J Biotechnol 12:1495–1499. https://doi.org/10.5897/AJB12.2806

Mariani L, Ferrante A (2017) Agronomic management for enhancing plant tolerance to abiotic stresses—drought, salinity, hypoxia, and lodging. Horticulturae 3:1–18. https://doi.org/10.3390/horticulturae3040052

Martínez IG, Prat C, Ovalle C, del Pozo A, Stolpe N, Zagal E (2012) Subsoiling improves conservation tillage in cereal production of severely degraded Alfisols under Mediterranean climate. Geoderma 189:10–17. https://doi.org/10.1016/j.geoderma.2012.03.025

Maryani Y, Dewi WS, Yunus A (2018) Study on osmoprotectant rhizobacteria to improve mung bean growth under drought stress. In: Paper presented at IOP Conference Series: Earth and Environmental Science, Banda Aceh, Indonesia, 26–27 September 2018

McMaster GS, Wilhelm WW (2003) Phenological responses of wheat and barley to water and temperature: improving simulation models. J Agric Sci 141:129–147. https://doi.org/10.1017/S0021859603003460

Mittler R, Finka A, Goloubinoff P (2012) How do plants feel the heat? Trends Biochem Sci 37:118–125. https://doi.org/10.1016/j.tibs.2011.11.007

Monterroso VA, Wien HC (1990) Flower and pod abscission due to heat stress in beans. J Am Soc Hortic Sci 115:631–634

Moradi A, Ahmadi AHMD, Hossain Zadeh A (2008) The effects of different timings and severity of drought stress on gas exchange parameters of mungbean. Desert 13:59–66. https://doi.org/10.22059/jdesert.2008.27176

Nada HS, Hamza JH (2019) Priming of maize seed with gibberellin (GA3) to tolerate drought stress. 2. Field emergence and its properties. Iraqi J Des Stud 9:1–12

Nahar K, Hasanuzzaman M, Alam MM, Fujita M (2015) Exogenous glutathione confers high temperature stress tolerance in mung bean (*Vigna radiata* L.) by modulating antioxidant defense and methylglyoxal detoxification system. Environ Exp Bot 112:44–54. https://doi.org/10.1016/j.envexpbot.2014.12.001

Nair RM, Pandey AK, War AR, Hanumantharao B, Shwe T, Alam AKMM, Pratap A, Malik SR, Karimi R, Mbeyagala EK, Douglas CA, Rane J (2019) Biotic and abiotic constraints in mungbean production-progress in genetic improvement. Front Plant Sci 10:1–24. https://doi.org/10.3389/fpls.2019.01340

Naresh RK, Singh SP, Dwivedi A, Kumar V (2013) Effects of water stress on physiological processes and yield attributes of different mungbean (L.) varieties. Afr J Biochem Res 7:55–62. https://doi.org/10.5897/AJBR.9000110

Naveed M, Mehboob I, Hussain MB, Zahir ZA (2015) Perspectives of rhizobial inoculation for sustainable crop production. In: Arora NK (ed) Plant microbes symbiosis: applied facets. Springer, New Delhi, pp 209–239

Nayyar H, Kaur S, Singh S, Upadhyaya HD (2006) Differential sensitivity of Desi (small-seeded) and Kabuli (large-seeded) chickpea genotypes to water stress during seed filling: effects on accumulation of seed reserves and yield. J Sci Food Agric 86:2076–2082. https://doi.org/10.1002/jsfa.2574

Nazran A, Ahmed JU, Karim AJMS, Ghosh TK (2019) Physiological responses of mungbean (*Vigna radiata*) varieties to drought stress. Bangladesh J Agr Res 44:1–11. https://doi.org/10.3329/bjar.v44i1.40899

Ohama N, Sato H, Shinozaki K, Yamaguchi-Shinozaki K (2017) Transcriptional regulatory network of plant heat stress response. Trends Plant Sci 22:53–65. https://doi.org/10.1016/j.tplants.2016.08.015

Okcu G, Kaya MD, Atak M (2005) Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). Turk J Agric For 29:237–242

Osakabe Y, Osakabe K, Shinozaki K, Tran LSP (2014) Response of plants to water stress. Front Plant Sci 5:1–8. https://doi.org/10.3389/fpls.2d4.00086

Patriyawaty NR, Rachaputi RCN, George D, Douglas C (2018) Genotypic variability for tolerance to high temperature stress at reproductive phase in Mungbean [*Vigna radiata* (L.) Wilczek]. Sci Hortic 227:132–141. https://doi.org/10.1016/j.scienta.2017.09.017

Piramila BHM, Prabha AL, Nandagopalan V, Stanley AL (2012) Effect of heat treatment on germination, seedling growth and some biochemical parameters of dry seeds of black gram. Int J Pharm Phytopharm Res 1:194–202

Pratap A, Gupta S, Malviya N, Tomar R, Maurya R, John KJ, Madhavan L, Singh NP (2015) Genome scanning of Asiatic Vigna species for discerning population genetic structure based on microsatellite variation. Mol Breed 35:1–13. https://doi.org/10.1007/s11032-015-0355-9

Pratap A, Gupta S, Tomar R, Malviya N, Maurya R, Pandey VR, Mehandi S, Singh NP (2016) Cross-genera amplification of informative microsatellite markers from common bean and scarlet runner bean for assessment of genetic diversity in mungbean (*Vigna radiata*). Plant Breed 135: 499–505. https://doi.org/10.1111/pbr.12376

Priya M, Sharma L, Kaur R, Bindumadhava H, Nair RM, Siddique KHM, Nayyar H (2019a) GABA (γ-aminobutyric acid), as a thermo-protectant, to improve the reproductive function of heat-stressed mungbean plants. Sci Rep 9:1–14. https://doi.org/10.1038/s41598-019-44163-w

Priya M, Sharma L, Singh I, Bains TS, Siddique KHM, Bindumadhava H, Nair RM, Nayyar H (2019b) Securing reproductive function in mungbean grown under high temperature environment with exogenous application of proline. Plant Physiol Biochem 140:136–150. https://doi.org/10.1016/j.plaphy.2019.05.009

Priya M, Pratap A, Sengupta D, Singh NP, Jha UC, Siddique K, Nayyar H (2020) Mungbean and high temperature stress:: responses and strategies to improve heat tolerance. In: Heat stress in food grain crops:: plant breeding and omics research. Bentham Science Publishers, pp 144–170

Raina SK, Govindasamy V, Kumar M, Singh AK, Rane J, Minhas PS (2016) Genetic variation in physiological responses of mungbeans (*Vigna radiata* (L.) Wilczek) to drought. Acta Physiol Plant 38:1–12. https://doi.org/10.1007/s11738-016-2280-x

Rainey KM, Griffiths PD (2005) Inheritance of heat tolerance during reproductive development in snap bean (*Phaseolus vulgaris* L.). J Am Soc Hortic Sci 130:700–706. https://doi.org/10.21273/JASHS.130.5.700

Ranawake AL, Dahanayaka N, Amarasingha UGS, Rodrigo WDRJ, Rodrigo UTD (2011) Effect of water stress on growth and yield of mung bean (*Vigna radiata* L). Trop Agr Res Ext 14:76–79

Rasheed R, Wahid A, Farooq M, Hussain I, Basra SMA (2011) Role of proline and glycinebetaine pretreatments in improving heat tolerance of sprouting sugarcane (Saccharum sp.) buds. Plant Growth Regul 65:35–45. https://doi.org/10.1007/s10725-011-9572-3

Rasheed A, Wen W, Gao F, Zhai S, Jin H, Liu J, Guo Q, Zhang Y, Dreisigacker S, Xia X, He Z (2016) Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. Theor Appl Genet 129:1843–1860. https://doi.org/10.1007/s00122-016-2743-x

Rawson HM, Craven CL (1979) Variation between short duration mungbean cultivars (*Vigna radiata* (L.) Wilczek) in response to temperature and photoperiod. Indian J Plant Physiol 22: 127–136

Reardon ME, Qaderi MM (2017) Individual and interactive effects of temperature, carbon dioxide and abscisic acid on mung bean (*Vigna radiata*) plants. J Plant Interact 12:295–303. https://doi.org/10.1080/17429145.2017.1353654

Reddy KS (2009) A new mutant for yellow mosaic virus resistance in Mungbean (*Vigna radiata* (L.) Wilczek) variety SML-668 by recurrent gamma-ray irradiation. In: Shu YQ (ed) Induced plant mutations genomics Era. Food Agric Organ United Nations, Rome, pp 361–362

Sadeghipour O (2019) Polyamines protect mung bean [*Vigna radiata* (L.) Wilczek] plants against drought stress. Biol Futura 70:71–78. https://doi.org/10.1556/019.70.2019.09

Saeidi M, Abdoli M (2015) Effect of drought stress during grain filling on yield and its components, gas exchange variables, and some physiological traits of wheat cultivars. J Agric Sci Technol 17:885–898

Saima S, Li G, Wu G (2018) Effects of drought stress on hybrids of *Vigna radiata* at germination stage. Acta Biol Hung 69:481–492. https://doi.org/10.1556/018.69.2018.4.9

Sakata T, Oshino T, Miura S, Tomabechi M, Tsunaga Y, Higashitani N, Miyazawa Y, Takahasti H, Watanabe M, Higashilani A (2010) Auxins reverse plant male sterility caused by high temperatures. Proc Natl Acad Sci 107:8569–8574. https://doi.org/10.1073/pnas.1000869107

Sarma RK, Saikia R (2014) Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. Plant Soil 377:111–126. https://doi.org/10.1007/s11104-013-1981-9

Schafleitner R, Nair RM, Rathore A, Wang Y, Lin C, Chu S, Lin P-Y, Chang J-C, Elbert AW (2015) The AVRDC–The World Vegetable Center mungbean (*Vigna radiata*) core and mini core collections. BMC Genomics 16:1–11. https://doi.org/10.1186/s12864-015-1556-7

Sehgal A, Sita K, Siddique KH, Kumar R, Bhogireddy S, Varshney RK, HanumanthaRao B, Nair RM, Prasad PVV, Nayyar H (2018) Drought or/and heat-stress effects on seed filling in food crops: impacts on functional biochemistry, seed yields, and nutritional quality. Front Plant Sci 9: 1–19. https://doi.org/10.3389/fpls.2018.01705

Sehrawat N, Jaiwal PK, Yadav M, Bhat KV, Sairam RK (2013) Salinity stress restraining mungbean (*Vigna radiata* (L.) Wilczek) production: gateway for genetic improvement. Int J Agric Crop Sci 6:505–509

Sengupta D, Guha A, Reddy AR (2013) Interdependence of plant water status with photosynthetic performance and root defense responses in *Vigna radiata* (L.) Wilczek under progressive drought stress and recovery. J Photochem Photobiol B Biol 127:170–181. https://doi.org/10.1016/j.jphotobiol.2013.08.004

Sgobba A, Paradiso A, Dipierro S, De Gara L, de Pinto MC (2015) Changes in antioxidants are critical in determining cell responses to short-and long-term heat stress. Physiol Plant 153:68–78. https://doi.org/10.1111/ppl.12220

Shariatmadari MH, Parsa M, Nezami A, Kafi M (2017) The effects of hormonal priming on emergence, growth and yield of chickpea under drought stress in glasshouse and field. Biosci Res 14:34–41

Sharma L, Priya M, Bindumadhava H, Nair RM, Nayyar H (2016) Influence of high temperature stress on growth, phenology and yield performance of mungbean [Vigna radiata (L.) Wilczek] under managed growth conditions. Sci Hortic 14:213–379

Sheoran IS, Saini HS (1996) Drought-induced male sterility in rice: changes in carbohydrate levels and enzyme activities associated with the inhibition of starch accumulation in pollen. Sex Plant Reprod 9:161–169

Sheykhbaglou R, Rahimzadeh S, Ansari O, Sedghi M (2014) The effect of salicylic acid and gibberellin on seed reserve utilization, germination and enzyme activity of sorghum (Sorghum bicolor L.) seeds under drought stress. J Stress Physiol Biochem 10:5–13

Singh DP, Singh BB (2011) Breeding for tolerance to abiotic stresses in mungbean. Food Legum 24:83–90

Singh B, Singh N, Thakur S, Kaur A (2017) Ultrasound assisted extraction of polyphenols and their distribution in whole mung bean, hull and cotyledon. J Food Sci Technol 54:921–932. https://doi.org/10.1007/s13197-016-2356-z

Subbarao GV, Nam NH, Chauhan YS, Johansen C (2000) Osmotic adjustment, water relations and carbohydrate remobilization in pigeonpea under water deficits. J Plant Physiol 157:651–659. https://doi.org/10.1016/S0176-1617(00)80008-5

Sun Y, Liu F, Bendevis M, Shabala S, Jacobsen SE (2014) Sensitivity of two quinoa (*Chenopodium quinoa* Willd.) varieties to progressive drought stress. J Agron Crop Sci 200:12–23. https://doi.org/10.1111/jac.12042

Taiz L, Zeiger E (1991) Plant physiology, Redwood City, Calif Benjamin/Cummings Pub

Talebi R, Ensafi MH, Baghebani N, Karami E, Mohammadi K (2013) Physiological responses of chickpea (*Cicer arietinum*) genotypes to drought stress. Environ Exp Biol 11:9–15

Teixeira EI, Fischer G, van Velthuizen H, Walter C, Ewert F (2013) Global hot-spots of heat stress on agricultural crops due to climate change. Agric For Meteorol 170:206–215. https://doi.org/10.1016/j.agrformet.2011.09.002

Uddin S, Parvin S, Awal MA (2013) Morpho-physiological aspects of mungbean (*Vigna radiata* L.) in response to water stress. Int J Agric Sci Res 3:137–148

Uprety DC, Bhatia A (1989) Effect of water stress on the photosynthesis, productivity and water status of mung bean (*Vigna radiata* L. Wilczek). J Agron Crop Sci 163:115–123. https://doi.org/10.1111/j.1439-037X.1989.tb00744.x

Varshney RK, Terauchi R, McCouch SR (2014) Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding. PLoS Biol 12:1–8. https://doi.org/10.1371/journal.pbio.1001883

Wang X, Dinler BS, Vignjevic M, Jacobsen S, Wollenweber B (2015) Physiological and proteome studies of responses to heat stress during grain filling in contrasting wheat cultivars. Plant Sci 230:33–50. https://doi.org/10.1016/j.plantsci.2014.10.009

Wang H, Wang H, Shao H, Tang X (2016) Recent advances in utilizing transcription factors to improve plant abiotic stress tolerance by transgenic technology. Front Plant Sci 7:1–13. https://doi.org/10.3389/fpls.2016.00067

Waraich EA, Ahmad R, Halim A, Aziz T (2012) Alleviation of temperature stress by nutrient management in crop plants: a review. J Soil Sci Plant Nutr 12:221–244. https://doi.org/10.4067/S0718-95162012000200003

Yadav SS, McNeil DL, Redden R, Patil SA (eds) (2010) Climate change and management of cool season grain legume crops. Springer, Dordrecht

Yin Z, Liang J, Hao X, Lu H, Hao J, Yin F (2015) Physiological response of mung bean to polyethylene glycol drought stress at flowering period. Am J Plant Sci 6:785–795. https://doi.org/10.4236/ajps.2015.65084

Young LW, Wilen RW, Bonham-Smith PC (2004) High temperature stress of *Brassica napus* during flowering reduces micro-and megagametophyte fertility, induces fruit abortion, and disrupts seed production. J Exp Bot 55:485–495

Yuriko O, Keishi O, Kazuo S, Tran L (2014) Response of plants to water stress. Front Plant Sci 5:86

Zandalinas SI, Mittler R, Balfagón D, Arbona V, Gómez-Cadenas A (2018) Plant adaptations to the combination of drought and high temperatures. Physiol Plant 162:2–12. https://doi.org/10.1111/ppl.12540

Zarifinia N, Aynehband A, Lak S, Modhej A (2012) Evaluation of physiological traits changes in drought stress, the application of potassium and their impact on the yield of mung bean cultivars and promising lines. Adv Environ Biol 6:2854–2860

Zhou R, Yu X, Ottosen CO, Rosenqvist E, Zhao L, Wang Y, Yu W, Zhao T Wu Z (2017) Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress. BMC Plant Biol 17:1–13. Doi: https://doi.org/10.1186/s12870-017-0974-x

Zinn KE, Tunc-Ozdemir M, Harper JF (2010) Temperature stress and plant sexual reproduction: uncovering the weakest links. J Exp Bot 61:1959–1968. https://doi.org/10.1093/jxb/erq053

A Review on Stress Physiology and Breeding Potential of an Underutilized, Multipurpose Legume: Rice Bean (*Vigna umbellata*)

11

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Abstract

Pulses and legumes are reliable dietary supplements for people of the developing countries. Ricebean [*Vigna umbellata (Thunb.) Ohwi and Ohashi*] is an underutilized, multipurpose legume owing to its profuse pod-bearing habit, wider adaptability, tolerance to biotic and abiotic stresses, high seed yield, and high contents of nutrients considered as a minor food and fodder crop. It is a potential leguminous crop with luxuriant growth habits and ability to produce huge quantity of nutritive green fodder as well as high seed yield under limited management inputs. Ricebean has source of high-quality proteins, calcium, phosphorus, tryptophan, and starch in its seeds. It is mostly cultivated for human consumption; however, its foliage and dry straw are nutritious livestock feed and provide higher productivity and quality fodder if sown with cereals like maize and sorghum. Ricebean thrives well in rainfed areas and also poor soils owing to its wider adaptability and resilience to various abiotic stresses. In view of the climate change scenario, ricebean has enormous potential as a climate-smart legume crop for areas having drought or salinity stress, etc. In this article, an attempt has been made to discuss aspects and scope of abiotic stress-tolerant ricebean genotypes.

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Keywords

Biotic stress · Climate-smart crop · Drought stress · Metal stress · Ricebean · Salinity stress · Stress resilience

11.1 Introduction

Ricebean [*Vigna umbellata (Thunb) Ohwi and Ohashi*] is an ancient leguminous crop of South, Southeast, and East Asia (Seehalak et al. 2006; Tomooka et al. 2002; Pattanayak et al. 2019), which is mainly grown by subsistence farmers for its ability to produce a huge quantity of nutritive green fodder under limited management inputs (Chatterjee and Dana 1977; Chatterjee and Mukherjee 1979). It is also called as "under-utilized" legume or "orphan" crops or poor man's pulse. Legumes such as mungbean, chickpea, pigeon pea, and lentils are widely cultivated in India and abroad. But the poor availability of soils and various biotic and abiotic stresses have reduced the production and productivity of the crops (Sultana et al. 2014). Farmers and consumers need a cost-effective, stress-tolerant alternative crop for their increasing demands. Crop diversification provides better opportunities for creating a uniform cropping system as well as developing resistance against biotic and abiotic stress. Underutilized legumes play an important role in such scenario providing better alternatives with high degrees of stress tolerance. One such underutilized legume is rice bean (*Vigna umbellata*) which has great potential as an alternative with high nutritional and economic value (Dhillon and Tanwar 2018). It has recently gained attention as an underutilized grain legume owing to its profuse pod-bearing habit, wider adaptability, tolerance to biotic and abiotic stresses, high seed yield, and high contents of protein, calcium, phosphorus, tryptophan as well as starch in its seeds (Singh et al. 1980; Srivastava et al. 2001). It performs well in marginal lands, rainfed areas, drought-prone areas, and exhausted soils. It is a neglected crop under diverse conditions with no additional inputs, cultivated on small areas by subsistence farmers in hill areas of Nepal, northern and northeastern India, and parts of southeast Asia.

Continuous supply of nutrients, biosynthetic capacity, and energy are required for proper cell division and normal plant growth. Restriction of any of these most important factors may cease plant growth which leads to plant death. Deviation to normal physiology, growth, developmental processes, and metabolic functions that lead to an injurious effect as well as irreversible damage to the plants can be termed stress (Pareek et al. 2009). Biotic stresses include many living organisms such as fungi, bacteria, virus, insect, and nematode, which draw nutrition parasitizing the host plants. On the other hand, non-living things on which plants grow and develop strongly depend may contribute to abiotic stresses when their supply is irrational. Modern agriculture faces many biotic and abiotic stresses that challenge crop cultivation to a great extent. Several reports highlight massive loss in crop production under environmental stresses (Godfray et al. 2010; Cramer et al. 2011).

Inadequate supply of soil water, relative humidity, excess soil salinity, acidity or basicity, heavy metals, and physiochemical properties may cause stress on plants.

Therefore, the improvement of existing commercial varieties and other plant species to ensure their survival coping with various stresses is the major thrust of plant research. Proper anticipation of physiological and molecular changes under adverse environments to bolster an effective acclamatory response is quite necessary to adopt suitable breeding strategies. Abiotic stresses and their impact on plant performances may be explored in a great variety of model and crop species from different aspects: metabolic or physiological response, signaling pathway, eco-physiology, and crop breeding studies (Pareek et al. 2009).

11.2 "Ricebean: A General Overview"

The center of origin and diversity of ricebean is considered to be Indo-China (Tamooka et al. 1991) mainly native of South and South East Asia. It is cultivated in India, Burma, Malaysia, China, Korea, Indonesia, Philippines, and to a limited extent in West Indies, Australia, United States, and East Africa. In India, it is predominantly grown in tribal regions of North-eastern hills, eastern peninsula tracts of Orissa, and chotanagpur, and in hilly regions of North Bengal and Sikkim. It is known by different local names in different parts of the country, such as Masyang, Jhilinge, Gurous, or Siltung. There are many landraces of ricebean under cultivation in different parts of the country. These landraces are mostly low yielding. Plant breeding efforts for genetic improvement and the development of new varieties of ricebean have not yet been carried out successfully (Khadka and Acharya 2009).

It is believed that ricebean evolved from its wild form, *Vigna umbellata var. gracilis*, which is an indeterminate growth habit with photoperiod sensitive, sporadic and asynchronous flowering, typically small leaved, freely branching, and strongly dehiscent pods with small and hard seeds. Ricebean is understood for its diverse distribution and range of adaptation from humid subtropical to warm and cool temperate climate. Ricebean is distributed from southern China through the north of Vietnam, Laos, and Thailand to Burma, India, and Nepal. Its successful cultivation in Queensland and East Africa has also been reported. However, there are reports that ricebean is also cultivated commonly in Honduras, Brazil, and Mexico too.

11.2.1 Diversity of Ricebean

Ricebean is an annual legume with an erect to semi-erect vine that may grow to more than 3 m height. It produces profuse branching. Leaves are tri-foliate, leaflets being comparatively broader and hairy. Flowers are conspicuously bright yellow and borne in clusters. Flowering is asynchronous, and there is a tendency to hard seeds. In many areas, landraces that retain many of these characteristics persist, in particular with regard to daylight sensitivity, growth habits, and hard seeds. Ricebean is a

Table 11.1 Different characteristics of landraces

Sl. No.	Landraces	Different characteristics
1	Early small grained	1. Greenish to yellowish in color 2. Early in maturity 3. Small-sized grain and 4. Low grain and fodder
2	White big grained	1. Big sized grain, 2. White to yellowish in color 3. Late in maturity 4. Better in taste 5. High grain and fodder yield.
3	Red grained	1. Small to medium-sized grain 2. Medium in maturity 3. Red in color 4. Least affected by rainfall 5. Coarse grain 6. Low grain yield
4	Big brown grained	1. Brown striped in color 2. Big sized grained 3. Late in maturity 4. Better taste

Source: LI-BIRD (2007)

diploid ($2n = 22$), and there is some evidence of natural out-crossing (Sastrapradja and Sutarno 1977). It has elongated, slightly curved, and beaked seeds of variable size and color with predominant hilum. In Nepal, there are very few scientific studies conducted on ricebean, and little has been done to assess its diversity and to promote it as a livelihood supporting grain legume.

Ricebean is a multipurpose crop, mainly used for human dietary uptake, with a smaller proportion used for fodder and green manuring (Joshi et al. 2006). It forms an important part of cereal-based diet as the dried grains are rich in protein, minerals, and vitamins. Besides, it carries social and cultural values in some communities in the country. The promotion of ricebean as a crop could play a vital role in improving the human diet and food security of people (Table 11.1).

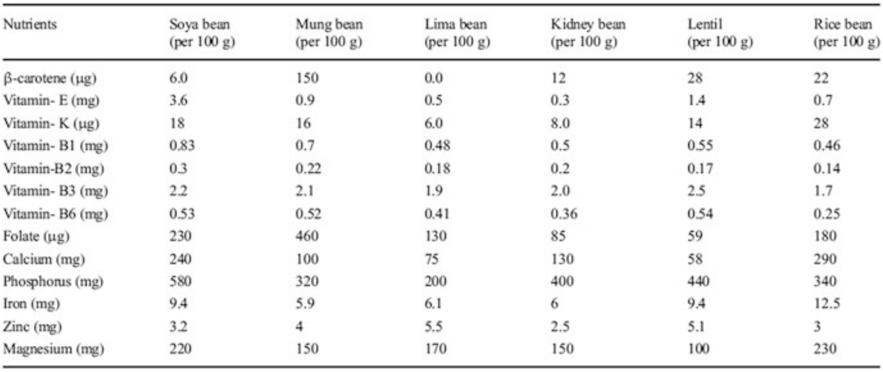
11.2.2 Nutritional Factors

In the human diet, legumes are an excellent source of macronutrients, not only carbohydrates but particularly protein as well as healthy fats and dietary fiber. They are also a good source of micronutrients and vitamins. Ricebean is highly nutritious. In terms of nutritionary worth, rice bean is proportional supplementary low-fat grain pulses. The dry seeds of ricebean are good sources of carbohydrates, proteins, minerals, and vitamins. Protein content in ricebean is rich in limiting amino acids tryptophan and methionine (De Carvalho and Vieira 1996). Other amino acids including tyrosine, valine, and lysine (Mohan and Janardhan 1994) are very high in the seeds of ricebean (Table 11.2).

Table 11.2 Biochemical constituents of ricebean

Constituent	Range (%)
Carbohydrate	58.2–72.0
Crude protein	18.3–32.2
Ash	3.5–4.9
Soluble ether extract	0.1–0.5
Crude fiber	3.6–5.5

Source: Buergelt et al. (2009)



Nutrients	Soya bean (per 100 g)	Mung bean (per 100 g)	Lima bean (per 100 g)	Kidney bean (per 100 g)	Lentil (per 100 g)	Rice bean (per 100 g)
β-carotene (μg)	6.0	150	0.0	12	28	22
Vitamin- E (mg)	3.6	0.9	0.5	0.3	1.4	0.7
Vitamin- K (μg)	18	16	6.0	8.0	14	28
Vitamin- B1 (mg)	0.83	0.7	0.48	0.5	0.55	0.46
Vitamin-B2 (mg)	0.3	0.22	0.18	0.2	0.17	0.14
Vitamin- B3 (mg)	2.2	2.1	1.9	2.0	2.5	1.7
Vitamin- B6 (mg)	0.53	0.52	0.41	0.36	0.54	0.25
Folate (μg)	230	460	130	85	59	180
Calcium (mg)	240	100	75	130	58	290
Phosphorus (mg)	580	320	200	400	440	340
Iron (mg)	9.4	5.9	6.1	6	9.4	12.5
Zinc (mg)	3.2	4	5.5	2.5	5.1	3
Magnesium (mg)	220	150	170	150	100	230

Fig. 11.1 Micro-nutrient and vitamin content of ricebean in comparison with other pulses and legumes. Source: Anonymous (2004)

Vitamins such as niacin, riboflavin, thiamine, and ascorbic acid (Joshi et al. 2006) are present in ricebean seed. A laboratory experiment by Buergelt (2009) has also proven ricebean to be a good source of various nutrients. Biochemical constituents of ricebean are carbohydrate 58.2–72%, crude protein 18.3–32.2%, ash 3.5–4.9%, soluble ether extract 0.1–0.5%, and crude fiber 3.6–5.5% (Source: Buergelt et al. 2009) (Fig. 11.1; Table 11.3).

11.2.3 Ricebean as a Fodder Crop

Ricebean is a multipurpose underutilized legume. The green forage ricebean is good source of crude protein and crude fiber, which can be fed fresh or processed into hay, and seeds are used as concentrate feed. Ricebean foliage is highly nutritious animal fodder. Ricebean straw after seed harvested, including the stems, leafy portions, empty pods, and seeds can be used as fodder (Singh et al. 2020). Ricebean is valuable as a high-class fodder that is known to increase milk production in livestock.

Table 11.3 Nutritional composition of ricebean grains

Component	Content
Crude protein (%)	14.00–26.10
Amino acids (mg/100 g)	
Arginine	4.32–7.12
Alanine	3.26–6.60
Aspartic acid	10.39–13.50
Glutamic acid	12.36–17.00
Glycine	2.96–4.26
Lysine	5.38–8.75
Methionine	0.90–2.88
Proline	2.54–8.36
Tryptophan	1.23–2.00
Tyrosine	2.12–3.31
Valine	4.40–5.89
Minerals (mg/100 g)	
Sodium	6.00–347.40
Potassium	610.40–2875.00
Calcium	111.5–598.23
Magnesium	73.0–356.12
Zinc	2.45–10.44
Iron	3.72–9.25
Manganese	2.04–5.0
Copper	0.68–4.97
Phosphorous	124.0–567.69
Fatty acid (%)	
Palmitic acid	5.60–16.88
Stearic acid	2.10–5.87
Linoleic acid	7.50–18.98
Oleic acid	15.62–68.00
Linolenic acid	39.89–44.38

Source: Chandel et al. (1978), Bepary et al. (2017)

11.2.4 Importance of Rice Bean in Crop Cultivation

Pulses and legumes are reliable dietary supplements for people of developing countries (Katoch 2013). It is a cheap source of high-quality proteins and adds variety to the palate. Legumes such as moong bean, chickpea, pigeon pea, and lentils are widely cultivated in India and abroad. But the poor availability of soils and various biotic and abiotic stresses have reduced the production and productivity of the crops (Sultana et al. 2014). Farmers and consumers need a cost-effective, stress-tolerant alternative crop for their increasing demands. Crop diversification provides better opportunities for creating a uniform cropping system as well as developing resistance against biotic and abiotic stress. The use of pesticides to mitigate the biotic stresses does not fit well due to higher economic inputs Underutilized legumes play an important role in such scenario providing better alternatives with high degrees of stress tolerance. One such underutilized legume is ricebean (*Vigna umbellata*) which has great potential as an alternative with high nutritional and economic value (Dhillon and Tanwar 2018). Here, the major potential of rice bean as a stress-tolerant crop and physiological aspects under different stress are discussed below.

11.2.5 Ricebean on Abiotic Stress

11.2.5.1 Response to Drought Stress

Drought can be defined as a period without significant rainfall and it is one of such major abiotic stresses that contributes to a huge reduction in crop yield throughout the world (Vinocur and Atman 2005). Plant shows a broad range of physiological, morphological, and biochemical changes such as reduced photosynthetic accumulation and altered gene expression under the drought stress which ultimately cause reduced growth as well as poor grain yield (Turner 1986; Bray 1993; Baroowa et al. 2016; Maheswari et al. 2016). An adverse effect of drought stress is related to the disturbances of essential physiological properties such as leaf water potential (ψ), RWC (relative water content), OP (osmotic potential), SC (stomatal conductance), and TR (transpiration rate) (Anjum et al. 2011; Subramanian and Maheswari 1990a, b; Shanker et al. 2014). Such water imbalance inside the plant system may be responsible for loss in turgor pressure and impairment of cell enlargement.

Osmotic adjustment is the most common form of plant defense under water stress. Accumulation of osmolytes having low molecular mass such as proline, betaine, and polyols is one of the major responses under drought stress (Hanson 1992). Osmolytes play a key role in protecting the macromolecular structures and their functions by adjusting the osmotic balances (Timasheff 1992). Several reports are available for the accumulation of cyclic polyols such as D-pinitol (1d-3-Omethyl-chiro-inositol) and D-ononitol (D-4-O-methyl-myo-inositol) by different plant species including legumes under drought stress (Richter and Popp 1992; Keller and Ludlow 1993; Wanek and Richter 1997). These two substances are methylated derivatives of myo-inositol which is biosynthetically produced from Glucose-6-P by myo-inositol-1-phosphate synthase (m1PS) and myo-inositol monophosphatase (cf. Wanek and Richter 1997; Noiraud et al. 2001). Methylation of myo-inositol is mediated by a methyl transferase enzyme, myo-inositol-6-O-methyl transferase (m60MT) which is encoded by IMT1 gene (Vernon and Bohnert 1992; Rammesmayer et al. 1995; Wanek and Richter 1997).

Wanek and Richter (1997) purified and characterized m60MT from *V. umbellata* and further they reported (1997) a significant increase in ononitol content in the leaves upon imposition of drought stress by withholding water for nine days. However, neither significant accumulation of ononitol nor any activity of m60MT was found in rice bean roots, whereas a remarkable increase in activity was observed in stems. Higher activity of m60MT in stems and greater accumulation of ononitol in leaves suggested possible transport of this substance from stem to leaves (Wanek and Richter 1997). Ricebean, although performing well under humid conditions, was also tolerant to drought (Chatterjee and Mukherjee 1979; Mukherjee et al. 1980; NAS 1979) and high temperatures. Jana et al. (2016) previously reported that Bidhan Ricebean-1 shows tolerance to drought stress.

11.2.5.2 Response to Salinity Stress

Salinity is one of the most influential stressors for increasing production in cropping areas throughout the world. It is an environmental factor that also limits crop productivity or damages biomass. Salinity stress in soil occurs when an excess concentration of soluble salts (Na^+, K^+, Ca^{2+}, Mg^{2+}) in the root zone of plants makes constrains to uptake nutrients and water from the soil and causing plant injury (Szabolcs 1989). The injurious effects of salinity on plant growth are specific ion toxicity, nutrition deficiency and attributed to a decrease in osmotic potential of the growing medium (Greenway and Munns 1980). Salinity stress affects plant growth because the high concentration of salts in the soil solution interferes with the balanced absorption of essential nutritional ions by plants (Tester and Davenport 2003). Salinity can affect plants mainly in three ways. Initially salt makes it very difficult for plants to withdraw water from soil due to very low osmotic potential. In effect, the plants suffer from a sort of osmotic stress which restricts plant growth causing yield reduction. Secondly, the Na^+ and Cl^- ions taken up by plants from saline water are toxic to plants. Due to the absorption of these ions along with water in high concentration, plants suffer from cytotoxicity, resulting in the reduction of growth, leaf burn, and plant death. Thirdly, the presence of high concentration of Na + and Cl- ion reduces the availability of other ions like K^+, Ca^{2+}, and Mg^{2+}, thus causing other nutritional disorders (Atta et al. 2020, 2021). There are few researchers who have done their work on salinity stress on ricebean.

There are six subgenera under genus *Vigna* (Leguminosae) distributed in Australia, Africa, America, and Asia (Verdcourt 1970; Maréchal et al. 1978). In Asia, most of the *Vigna* species belong to the subgenus Ceratotropis, which is also known as Asian *Vigna* (Tomooka et al. 2002), and ricebean [*Vigna umbellata (Thunb.) Ohwi et Ohashi*] is one of them. Avoidance (excluder type) and tolerance (includer type) are the two mechanisms (Levitt 1980; Munns and Tester 2008) of plant salt tolerance. The "includer type" plants take up Na^+ ions with a relatively lower toxicity, while "excluder type" plants exclude toxic ions from internal plant tissue (Johnson et al. 1991). *Vigna* genus only reported "excluder type" salt tolerance mechanisms so far, where *Vigna unguiculata (L.) Walp.* (cowpea) and mung bean prevent Na^+ migration from the root to the aerial part or restrict Na^+ uptake from the root (Jacoby 1964; Lessani and Marschner 1978; Fernandes de Melo et al. 1994; West and Francois 1982; Bernardo et al. 2006). Yoshida et al. (2016) previously reported, while working on different concentrations of NaCl stress, that *Vigna umbellata* showed slight salinity tolerance at the early stages of seedlings and later on as the duration of stress increases, the tolerance of the salinity became more prominent. The germplasm KRB-272 and KRB-274 was reported by Jana et al. (2017) as resistant to salinity stress when tested with various growth parameters for salinity tolerant index. Atta et al. (2019) while working on Bidhan Ricebean-1 in germinating seeds shows some tolerance to salinity stress when compared with unstressed control.

11.2.5.3 Response to Cold Stress

Cold temperature stress is also a limiting factor, which comes under abiotic stress. Chilling injury and freezing injury are the classified forms of low-temperature injury based upon the severity of cold. Basically, when the temperature remains above freezing point (>0 °C) chilling injury occurs and freezing injury occurs at a temperature below freezing point (0 °C). Both the low-temperature injury caused detrimental effects on plants, which includes cell membrane disarranging, retarded pollen germination and impeded in pollen formation (Steponkus et al. 1993; McKersie and Bowley 1997), photosynthesis disturbances (Bell 1993), disruptions in electron transport chain (Hallgren and Oquest 1990), and also CO_2 fixation involved enzymes (Sassenrath et al. 1990). ROS (reactive oxygen species) activities increased as usual due to chilling temperature, as a result chilling injury occurs (Omran 1980; Hodgson and Raison 1991; Prasad et al. 1994).

There are a series of biochemical and physiological changes that involve cold tolerance mechanism, that cause alteration in lipid composition in cell membrane (Graham and Patterson 1982; Murata and Yamaya 1984), increase in ABA (Rikin and Richmond 1976; Ciardi et al. 1997; Morgan and Drew 1997), and also the changes in osmolytes and increase in antioxidants (Fridovich 2006). Low-temperature stress is more frequent in the temperate region making a significant threat to vegetative growth by necrosis of leaf tip, chlorosis, and curling of the whole leaf. Likewise, reproductive stage constitutes the most vulnerable phase where damaging events may take place, such as, the juvenile buds drop, reduced pollen viability, aborted pods and stigma receptivity, reduced pollen tube growth, and finally, deteriorated seed quality and seed yield (Kumar et al. 2007, 2010). There are few research works done till now on cold stress in ricebean. Jana et al. (2016) reported that Bidhan Ricebean-1 shows tolerance to cold stress.

11.2.5.4 Ricebean on Metal Stress

Heavy metals at toxic level on plants trigger a wide range of physiological and metabolic alterations. The most widespread visual evidence of heavy metal toxicity could be a reduction in plant growth (Sharma and Dubey 2007) including leaf chlorosis, necrosis, turgor loss, a decrease in the rate of seed germination, and a crippled photosynthetic apparatus, often correlated with the acceleration of senescence processes and plant death (Dalcorso et al. 2010; Carrier et al. 2003). All these effects are related to ultra-structural, biochemical, and molecular changes in plant tissues and cells brought about by the presence of heavy metals (Gamalero et al. 2009).

11.2.5.5 Response to Aluminum Stress

Al toxicity is a growing concern in modern agriculture, especially for soils having acidic pH (Kochian 1995). Plant productivity can seriously be affected due to the onset of Al stress, even a micromolar concentration is sufficient to limit water and nutrient uptake of many species by rapidly inhibiting root elongation (Yang et al. 2007). However, plants evolved with their own defense mechanism to cope with such abiotic stresses. Major crops like wheat, maize, and soybean are found to display a wide range of variation for Al tolerance that may be utilized to improve other commercially grown susceptible plant species (Ryan et al. 2001). Exclusion of excess Al externally or through internal tolerance mechanism is the most common form of defense (Ma et al. 2001). Tolerant genotypes are reported to release organic anions from their roots to chelate Al ions in the rhizosphere by forming nontoxic complexes (Ryan et al. 2001). Depending on the time of secretion of organic ions from roots as a response to Al stress plants may be broadly categorized into two patterns: Pattern I (secretes organic ions as soon as Al stress emerges) and pattern II (take few hours to respond) (Ma et al. 2000). Wheat, buckwheat, and tobacco follow pattern I to activate organic anion efflux through malate, oxalate, and citrate secretion, respectively, whereas, along with *Cassia tora*, triticale and rye (Ryan et al. 2001), rice bean (*Vigna umbellata*) which is a potent leguminous crop that grows well in acidic soils also can withstand Al toxicity following pattern II citrate secretion from root apex in order to detoxify Al externally (Yang et al. 2006; Fan et al. 2014). The delay in showing such physiological response suggests the presence of some intermediate steps between reception of stimulus and anion efflux (Ma et al. 2000).

Although several Al responsive genes from different species (*Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, *Glycine max*) have been reported so far (Goodwin and Sutter 2009; Yamaji et al. 2009; Tsutsui et al. 2012; Mattiello et al. 2010; You et al. 2011), true association with Al tolerance were recognized for very few genes (Delhaize et al. 2012). Such contradiction was raised probably because of improper characterization of plant symptoms under severe Al toxicity which not only inhibits root growth but also imposes some secondary effects that are difficult to distinguish from the primary targets (Kochian 1995).

Cloning of two Aluminum stress-responsive citrate transporter genes *VuMATE1* and *VuMATE*2 coding for multidrug and toxic compound extrusion (MATE) protein (Yang et al. 2006; Liu et al. 2018) using RACE (Rapid Amplification of cDNA ends) technology opened a new horizon in comprehending molecular mechanism of rice bean Al toxicity tolerance. *VuMATE1* gene transcription at root apex was only observed under Al stress (Liu et al. 2013), although not consequently, rather after 6 h of exposure to Al stress. However, further investigation with a potential role of *VuMATE2* gene in rice bean suggested a biphasic citrate secretion from root apex under Al stress where an earlier expression of *VuMATE2* is reported (Liu et al. 2018). Tissue-specific expression of *VuMATE2* gene was observed under Al stress. 5 μM of external Al concentration was found sufficient to upregulate *VuMATE2* gene throughout the root (both at basal and tip region), whereas no such increase in gene expression was detected in leaf tissues. In addition to the different expression patterns of *VuMATE1* and *VuMATE*2, Liu et al. (2018) also speculated a contrasting signal transduction pathway between these two genes. Where de novo synthesis of a transcriptional activator is supposed to initiate *VuMATE1* transcription (Liu et al. 2013, 2016), Al^{-}-induced degradation of repressor supports *VuMATE*2 expression.

Al toxicity also decreases the Mg concentration in root cells. However, Mg plays a crucial role in maintaining ATPase protein (H^+-ATPase) activity on plasma membrane (Brooker and Slayman 1983). Micromolar concentrations of Mg have been reported to increase the Al^- stress-responsive citrate efflux in rice bean roots due to the upregulation of Mg-dependent plasma membrane H^+-ATPase activity (Yang et al. 2007). Further extension of research in this area may help us to find further explanations of Mg-associated Al stress tolerance in rice bean and other crops as well.

11.2.6 Ricebean on Biotic Stress

Biotic stress is stress that occurs as a result of damage done to an organism by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants. Plants react to biotic stress by a defense system. Innate response and systemic response are the classified forms of defense mechanism. After infection, generation of ROS occurs and oxidative bursts limit pathogen spread (Atkinson and Urwin 2012). As pathogen attack occurs, plants increase cell lignification and this mechanism blocks invasion of parasites and reduces host susceptibility. The defenses include structural and morphological barriers, proteins, chemical compounds, and enzymes. These converse resistance or tolerance to biotic stresses by protecting products and by giving them strength and rigidity. Crop diversification provides better opportunities for creating a uniform cropping system as well as developing resistance against biotic and abiotic stress. Underutilized legumes play an important role in such scenario providing better alternatives with high degrees of stress tolerance.

11.2.6.1 Storage Bruchid Pest Resistance

The bruchid weevils are a serious pest of many leguminous crops. The major species of brucid pests that pose a threat to the cultivation of commercial leguminous crops are *Callosobruchus maculatus*, also known as cowpea weevil; *Callosobruchus chinensis*, azuki bean weevil, and *Callosobruchus analis*, graham bean weevil (Kashiwaba et al. 2003). Most of the ricebean varieties have shown resistance to this weevil attack, especially during the vegetative growth stage (Tomooka et al. 2000). There are two major factors that confer resistance to a crop from insects, namely, antixenosis factor and antibiosis factor (Painter 1951). Antixenosis factor depends upon the seed size, seed coat thickness, seed hardiness, etc., whereas biochemical factors such as enzymes and toxins relate to antibiosis factor (Edwards and Singh 2006). In an experiment conducted by Seram et al. (2016), eight ricebean landraces were taken and inoculated with bruchid pest *C. maculatus*. None of the ricebean landraces were rejected by bruchid species for egg laying and seed coat penetration, although the degree of egg laying varied due to differences in the level of compounds present in the seed coat. Despite such heavy inoculation, very few to no larval emergence were observed. The hatching percentage varied from 39.2% to 49.2% with a mean value of 45.25 ± 3.23%. This proved that antixenosis factor was not responsible for bruchid resistance but antibiosis factors present in the cotyledon. Another experiment showed the same results where the bruchid species penetrated the seed and laid eggs but the larvae died in the cotyledons in the first and second larval stages (Kashiwaba et al. 2003). Srinivasan and Durairaj (2007) conducted experiments to calculate the amount of antibiosis enzymes present in ricebean species. The inhibitory activity was expressed as Trypsin/chymotrypsin/cysteine protease inhibitors (TIU/CIU/CPIU) per gram of sample, where one unit of activity was equivalent to 50% inhibition. The data recorded were as follows: TIU ranged between 1576 and 3120 as compared to 680 in check variety (CO 6). CIU ranged 380–583 as compared to 314 in check variety. CPIU ranged 2061–3069 as compared to 686 in check. The role of protease inhibitors has been well documented by Shukle and Murdock (1983) who reasoned that protease inhibitors were capable of inactivating digestive enzymes of the insects and also reduced the quality of proteins that can be digested. This results in nutritional deficiency in insects which ultimately results in stunted growth and death of the insects. Thus it can be concluded that the resistance attributed to ricebean species is due to protease inhibitors, namely, trypsin, chymotrypsin, and cysteine protease inhibitors.

11.2.6.2 Yellow Mosaic Virus Resistance

The Yellow Mosaic Virus is a serious disease of most leguminous plants, especially mungbean (Nene 1973). The disease is caused by a begomovirus with bipartite genome. It is a white-fly transmitted single-stranded DNA virus (Khattak et al. 2000). The symptoms of the disease emerge as yellow specs on the leaves near the veins, which later turn chlorotic as the disease progresses (Qazi et al. 2007). YMV infection at the reproductive stage produces either empty pods or yellow colored pods with unviable or infected seeds (Sehrawat and Yadav 2014). Various studies have shown that ricebean is significantly resistant to YMV attack (Kashiwaba et al. 2003; Sudha et al. 2015). Many approaches to incorporate resistance gene from *Vigna umbellata* to other susceptible *Vigna* species have been quite successful. Studies have shown significant cross-compatibility of ricebean with *Vigna mungo* and *V. sublobata* (Sehrawat and Yadav 2014). Genetic similarity, same chromosome number ($n = 11$), and other morphological similarities between ricebean and mungbean (*V. radiata*) allow breeders to make successful interspecific crosses (Bharathi et al. 2006; Pandiyan et al. 2010; Chaisan et al. 2013; Bhanu et al. 2017; Mathivathana et al. 2019). Modern approaches such as Marker Assisted Breeding (MAB) give pace to the conventional breeding taking advantage of molecular markers (Ashraf and Foolad 2013). Micro-satellite markers or commonly known as SSRs (Simple Sequence Repeats) are widely used in the hybridity confirmation, phylogeny, gene maps, and associated marker-traits establishment in a segregating population. This further helps in selecting specific genes for resistance to incorporate in desired species (Michelmore et al. 1991). In a research conducted by Mathivathana et al. (2019), a population of 108 inter-specific recombinant inbred line (RIL) population from a cross between VRM (Gg) 1(Mungbean) and TNAU RED (Ricebean), designated VRMTNAU, was developed and used to construct the genetic linkage map and detect the QTLs.

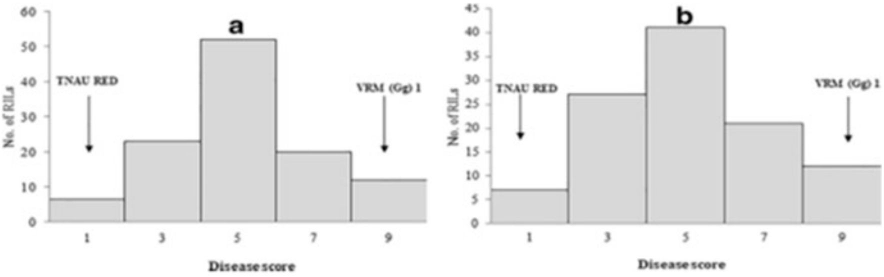


Fig. 11.2 Frequency distribution of MYMV resistance in F_9 RIL population derived from the cross between VRM (Gg) 1 and TNAU RED. (**a**) 2015 and (**b**) 2016 (Mathivathana et al. 2019)

Normal distribution of pattern (Fig. 11.2) obtained by phenotypic disease scoring (1–9 scale) of the RIL population confirmed the quantitative control of the resistance gene. The RIL population was also found to have high heritability of MYMV incidence with significant consistency for two consecutive years (0.75 and 0.78, respectively). A total of four QTLs were detected, namely, qMYMV4_1, qMYMV5_1, qMYMV6_1, and qMYMV10_1 distributed on chromosomes 4, 5, 6, and 10. The QTL on chromosome 4 (qMYMV4_1) was identified as the major QTL, present in all segregating populations consistently, showing resistance. Using mungbean genome as the reference, 83 annotated genes coding for known, unknown, or hypothetical proteins were found to be associated with the qMYMV4_1 region. It was observed that none of the resistant lines encoded the nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins. The R gene proteins that were encoded were serine/threonine protein kinase super family; MYB transcription factor; WRKY family transcription factor; and zinc finger RING/FYVE/PHD-type protein. A few small protein-encoding sites were also observed for small GTP-binding protein, receptor-like kinase (RLKs) protein, and jasmonic acid carboxyl methyltransferase (JMT) proteins. In conclusion, it was observed that about four QTLs were responsible for disease resistance against the virus. Of these qMYMV4_1 was a stable and major QTL for resistance to YMV. Therefore, further exploration may open the prospect for gene cloning followed by introgression to other cross-compatible species as well to facilitate crop improvement.

11.3 Conclusion

Clearly, ricebean is a potentially valuable multipurpose (grain, fodder, and green manure) crop for farmers in the marginal hill areas of Nepal and northern India, as well as in third countries with similar environments. Considering ricebean as a climate as well as pest resilient crop it can be concluded that this crop has tremendous potential in agronomic cultivation as well as in scientific research. Biotic as well as abiotic stress resistance can be incorporated into the background of other commercially important legumes to improve their defense against different stresses. Future research may be focused on further elaboration of molecular mechanism underlying drought tolerance of ricebean. Germplasm collection and screening for other stress resistance will pave the way for further research of this crop. Additionally, rice bean cultivation as an economically important crop can also be promoted by growing interest among the farmers for this pulse crop.

References

Anjum SA, Xie XY, Wang LC, Saleem MF, Man C, Lei W (2011) Morphological, physiological and biochemical responses of plants to drought stress. Afr J Agric Res 6(9):2026–2032. https://doi.org/10.5897/AJAR10.027

Anonymous (2004) Food composition database. Laboratory of Food Function, Department of Food and Nutrition, School of Life Studies, Sugiyama Jogakuen University, Japan. http://database.food.sugiyamau.ac.jp/index asia.php

Ashraf M, Foolad MR (2013) Crop breeding for salt tolerance in the era of molecular markers and marker-assisted selection. Plant Breed 132:10–20

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. J Exp Bot 63:3523–3543

Atta K, Karmakar S, Dutta D, Pal AK, Jana K (2019) Comparative physiology of salinity, drought and heavy metal stress during seed germination in ricebean [*Vigna umbellata (Thunb.) Ohwi and Ohashi*]. J Crop Weed 15:145–149. https://doi.org/10.22271/09746315.2019.v15.i3.1251

Atta K, Chettri P, Pal AK (2020) Physiological and biochemical changes under salinity and drought stress in Ricebean [*Vigna umbellata (Thunb.) Ohwi and Ohashi*] seedlings. Int J Environ Clim Change 10:58–64. https://doi.org/10.9734/ijecc/2020/v10i830218

Atta K, Pal AK, Jana K (2021) Effects of salinity, drought and heavy metal stress during seed germination stage in ricebean [*Vigna umbellata (Thunb.) Ohwi and Ohashi*]. Plant Physiol Rep 26:109–115. https://doi.org/10.1007/s40502-020-00542-4

Baroowa B, Gogoi N, Farooq M (2016) Changes in physiological, biochemical and antioxidant enzyme activities of green gram (*Vigna radiata L.*) genotypes under drought. Acta Physiol Plant 38:1–10. https://doi.org/10.1007/s11738-016-2230-7

Bell MJN (1993) Low night temperature response in peanut (*Arachis hypogaea L.*). PhD Thesis. University of Guelph, pp. 170

Bepary RH, Wadikar DD, Neog SB, Patki PE (2017) Studies on physico-chemical and cooking characteristics of rice bean varieties grown in NE region of India. J Food Sci Technol 54:973–986

Bernardo MA, Enrique TD, Jose LGH, Raúl LA, Narciso YAS, Sergio ZS, Edgar ORP, Cengiz K (2006) Effect of NaCl salinity in the genotypic variation of cowpea (*Vigna unguiculata*) during early vegetative growth. Sci Hort 108:423–432

Bhanu AN, Kumar P, Singh MN, Srivastava K, Hemantaranjan A (2017) Assessment of genetic purity of inter-specific F_1 hybrids involving *Vigna radiata* and *Vigna umbellata*. J Exp Biol Agric Sci 5:636–643

Bharathi A, Vijay SKS, Veerabadhiran P, Subba Lakshmi B (2006) Cross ability barriers in mungbean (*Vigna radiata L. Wilczek*): with its wild relatives. Indian J Crop Sci 1:120–124

Bray EA (1993) Molecular responses to water deficit. Plant Physiol 103:1035. https://doi.org/10.1104/pp.103.4.1035

Brooker RJ, Slayman CW (1983) Effects of Mg^{2+} ions on the plasma membrane [H^+]-ATPase of *Neurospora crassa*. II. Kinetic studies. J Biol Chem 258:8833–8838. https://doi.org/10.1016/S0021-9258(18)32131-8

Buergelt D, Von Oppen M, Yadavendra JP (2009) Quality parameters in relation to consumer's preferences in ricebean. In: Presentation at the International Conference on Grain Legumes: Quality Improvement, Value Addition and Trade, February 14–16, 2009, Kanpur, India

Carrier P, Baryla A, Havaux M (2003) Cadmium distribution and micro-localization in oilseed rape (Brassica napus) after long-term growth on cadmium-contaminated soil. Planta 216:939–950. https://doi.org/10.1007/s00425-002-0947-6

Chaisan T, Somta P, Srinives P, Chanprame S, Kaveeta R, Dumrongkittikule S (2013) Development of tetraploid plants from an interspecific hybrid between mungbean (*Vigna radiata*) and ricebean (*Vigna umbellata*). J Crop Sci Biotechnol 16:45–51

Chandel KPS, Joshi BS, Arora RK, Pant KC (1978) Rice bean a new pulse with high potential. Indian Farm

Chatterjee BN, Dana S (1977) Rice bean [(*Vigna umbellata* (Thumb.) Owhi and Ohashi)]. Trop Gr Leg Bull N°10, 1977; Int. Grain Leg. Info Center. http://books.google.fr/books?id=_NJ6TJ308GMC&pg=PA22

Chatterjee BN, Mukherjee AK (1979) A new rice bean for fodder and grain pulse. Indian Farm 29(5):29–31

Ciardi JA, Deikman J, Orzolek MD (1997) Increased ethylene synthesis enhances chilling tolerance in tomato. Physiol Plant 101:333–340

Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol 11:163. https://doi.org/10.1186/1471-2229-11-163

Dalcorso G, Farinati S, Furini A (2010) Regulatory networks of cadmium stress in plants. Plant Signal Behav 5:1–5. https://doi.org/10.4161/psb.5.6.11425

De Carvalho NM, Vieira RD (1996) Rice bean (Vigna umbellata (Thunb.) Ohwi et Ohashi). In: Food and feed from legumes and oilseeds, pp 222–228. https://doi.org/10.1007/978-1-4613-0433-3_25

Delhaize E, Ma JF, Ryan PR (2012) Transcriptional regulation of aluminium tolerance genes. Trends Plant Sci 17:341–348. https://doi.org/10.1016/j.tplants.2012.02.008

Dhillon PK, Tanwar B (2018) Rice bean: a healthy and cost-effective alternative for crop and food diversity. Food Secur 10:525–535. https://doi.org/10.1007/s12571-018-0803-6

Edwards O, Singh KB (2006) Resistance to insect pests: what do legumes have to offer? Euphytica 147:273–285

Fan W, Lou HQ, Gong YL, Liu MY, Wang ZQ, Yang JL, Zheng SJ (2014) Identification of early Al-responsive genes in rice bean (*Vigna umbellata*) roots provides new clues to molecular mechanisms of Al toxicity and tolerance. Plant Cell Environ 37(7):1586–1597. https://doi.org/10.1111/pce.12258

Fernandes de Melo D, Jolivet Y, Rocha AF, Gomes FE, Silva LM, Dizengremel P (1994) Effect of salt stress on mitochondrial energy metabolism of *Vigna unguiculata* cultivars differing in NaCl tolerance. Plant Physiol Biochem 32:405–412

Fridovich I (2006) Superoxide dismutases. Adv Enzymol Relat Areas Mol Biol 58:61–97. https://doi.org/10.1002/9780470123041.ch2

Gamalero E, Lingua G, Berta G, Glick BR (2009) Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can J Microbiol 55:501–514. https://doi.org/10.1139/w09-010

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF et al (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818. https://doi.org/10.1126/science.1185383

Goodwin SB, Sutter TR (2009) Microarray analysis of Arabidopsis genome response to aluminium stress. Biol Plant 53:85–99. https://doi.org/10.1007/s10535-009-0012-4

Graham D, Patterson BD (1982) Responses of plants to low, non-freezing temperatures: proteins, metabolism, and acclimation. Annu Rev Plant Physiol 33:347–372. https://doi.org/10.1146/annurev.pp.33.060182.002023

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31:149–190. https://doi.org/10.1146/annurev.pp.31.060180.001053

Hallgren JE, Oquest G (1990) Adaptation to low temperatures. In: Alscher RG, Cumming JR (eds) Stress responses in plants: adaptation and acclimation mechanisms. Wiley Liss Inc., New York, pp 265–293

Hanson AD (1992) Compatible solute synthesis and compartmentation in higher plants. In: Water and life. Springer, Berlin, Heidelberg, pp 52–60

Hodgson RA, Raison JK (1991) Superoxide production by thylakoid during chilling and its implication in the susceptibility of plants to chilling induced photo inhibition. Planta 183: 222–228

Jacoby B (1964) Function of bean roots and stems in sodium retention. Plant Physiol 39:445–449. https://doi.org/10.1104/pp.39.3.445

Jana K, Kundu CK, Sarkar S, Banerjee J, De DK (2016) Ricebean: nutritionally rich grain legume and grown under diverse cropping system in drought prone areas of Indian sub-continent to maintain food security. Souvenir, National Group Meet, Rabi, 2016–17, AICRP on Forage Crops and Utilization held at KAU, Vellayani, September 5–6, 2016, pp. 50–59

Jana K, De DK, Sarkar S, Banerjee J, Kundu CK (2017) Ricebean germplasm variability: Development of molecular markers and variety for salinity and other abiotic stress conditions. Souvenir, National Group Meet, Kharif, 2017, AICRP on Forage Crops and Utilization held at CSKHPKV, Palampur, April 18–19, 2017, pp. 25–32

Johnson SL, McPhee L, Birch LL (1991) Conditioned preferences: young children prefer flavors associated with high dietary fat. Physiol Behav 50:1245–1251. https://doi.org/10.1016/0031-9384(91)90590-k

Joshi KD, Bhandari B, Gautam R, Bajracharya J, Hollington PA (2006) Ricebean: a multipurpose underutilised legume

Kashiwaba K, Tomooka N, Kaga A, Han OK, Vaughan DA (2003) Characterization of resistance to three bruchid species (*Callosobruchus spp., Coleoptera, Bruchidae*) in cultivated ricebean (*Vigna umbellata*). J Econ Entomol 96:207–213. https://doi.org/10.1093/jee/96.1.207

Katoch R (2013) Nutritional potential of rice bean (*Vigna umbellata*): an underutilized legume. J Food Sci 78:C8–C16. https://doi.org/10.1111/j.1750-3841.2012.02989.x

Keller F, Ludlow MM (1993) Carbohydrate metabolism in drought-stressed leaves of pigeonpea (*Cajanus cajan*). J Exp Bot 44:1351–1359. https://doi.org/10.1093/jxb/44.8.1351

Khadka K, Acharya BD (2009) Cultivation practices of rice bean. Research and Development (LI-BIRD), local initiatives for biodiversity, Pokhara, Nepal

Khattak GSS, Haq MA, Ashraf M, Elahi T (2000) Genetics of mungbean yellow mosaic virus (MYMV) in mungbean (*Vigna radiata (L.) Wilczek*). J Genet Breed 54:237–243

Kochian LV (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. Ann Rev Plant Physiol Plant Mol Biol 46:237–260. https://doi.org/10.1146/annurev.pp.46.060195.001321

Kumar S, Kaur G, Nayyar H (2007) Exogeneous application of abscisic acid improves cold tolerance in chickpea (Cicer arietinum L.). J Agron Crop Sci 194:449–456

Kumar S, Nayyar H, Bhanwara RK, Upadhyaya HD (2010) Chilling stress effects on reproductive biology of chickpea. J SAT Agric Res 8:1–14

Lessani H, Marschner H (1978) Relation between salt tolerance and long distance transport of sodium and chloride in various crop species. Aust J Plant Physiol 5:27–37. https://doi.org/10.1071/PP9780027

Levitt J (1980) Responses of plants to environmental stress. Volume II: water, radiation, salt, and other stresses. Academic Press, New York

LI-BIRD. 2007. Food security through ricebean research in India and Nepal (FOSRIN). Periodic activity report. Local Initiatives for Biodiversity, Research and Development (LI-BIRD), Pokhara, Nepal

Liu MY, Chen WW, Xu JM, Fan W, Yang JL, Zheng SJ (2013) The role of VuMATE1 expression in aluminium-inducible citrate secretion in rice bean (*Vigna umbellata*) roots. J Exp Bot 64: 1795–1804. https://doi.org/10.1093/jxb/ert039

Liu MY, Xu JM, Lou HQ, Fan W, Yang JL, Zheng SJ (2016) Characterization of VuMATE1 expression in response to iron nutrition and aluminium stress reveals adaptation of rice bean (*Vigna umbellata*) to acid soils through cis regulation. Front Plant Sci 7:511. https://doi.org/10.3389/fpls.2016.00511

Liu MY, Lou HQ, Chen WW, Piñeros MA, Xu JM, Fan W et al (2018) Two citrate transporters co-ordinately regulate citrate secretion from rice bean root tip under aluminium stress. Plant Cell Environ 41:809–822. https://doi.org/10.1111/pce.13150

Ma JF, Taketa S, Yang ZM (2000) Aluminium tolerance genes on the short arm of chromosome 3R are linked to organic acid release in triticale. Plant Physiol 122:687–694. https://doi.org/10.1104/pp.122.3.687

Ma JF, Ryan PR, Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273–278. https://doi.org/10.1016/s1360-1385(01)01961-6

Maheswari M, Tekula VL, Yellisetty V, Sarkar B, Yadav SK, Singh J et al (2016) Functional mechanisms of drought tolerance in maize through phenotyping and genotyping under well-watered and water stressed conditions. Eur J Agron 79:43–57

Maréchal R, Mascherpa JM, Stainer F (1978) Etude taxonomique d'un groupe complexe d'espe'ces des genres Phaseolus et Vigna (Papilionaceae) sur la base de donne'es morphologiques et polliniques, traite'es par l'analyse informatique. Boissiera 28:1–273

Mathivathana MK, Murukarthick J, Karthikeyan A, Jang W, Dhasarathan M, Jagadeeshselvam N et al (2019) Detection of QTLs associated with mungbean yellow mosaic virus (MYMV) resistance using the interspecific cross of *Vigna radiata*× *Vigna umbellata*. J Appl Genet 60: 255–268. https://doi.org/10.1007/s13353-019-00506-x

Mattiello L, Kirst M, da Silva FR, Jorge RA, Menossi M (2010) Transcriptional profile of maize roots under acid soil growth. BMC Plant Biol 10:196. https://doi.org/10.1186/1471-2229-10-196

McKersie BD, Bowley SR (1997) Active oxygen and freezing tolerance in transgenic plants. In: Li PH, Chen THH (eds) Plant cold hardiness, molecular biology, biochemistry and physiology. Plenum, New York, pp 203–214

Michelmore RW, Paranand I, Kessele RV (1991) Identification of markers linked to disease resistance genes by bulk segregant analysis: a rapid method to detect markers in specific genome using segregant population. Proc Nat Acad Sci 88:9828–9832

Mohan VR, Janardhan K (1994) Chemical composition and nutritional evaluation of raw seeds of six rice bean varieties. J Indian Bot Soc 73:259–263

Morgan PW, Drew MC (1997) Ethylene and plant response to stress. Physiol Plant 100:620–630

Mukherjee AK, Roquib MA, Chatterjee BN (1980) Ricebean for the scarcity period. Indian Farm 30:26–28

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Ann Rev Plant Biol 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Murata N, Yamaya J (1984) Temperature-dependent phase behaviour of phosphatidylglycerols from chilling-sensitive and chilling-resistant plants. Plant Physiol 74:1016–10124

National Academy of Sciences (NAS) (1979) Ricebean. In: Tropical legumes, resources for the future, pp 80–85

Nene YL (1973) Viral diseases of some warm weather pulse crops in India. Plant Dis Rep 57:463–467

Noiraud N, Maurousset L, Lemoine R (2001) Transport of polyols in higher plants. Plant Physiol Biochem 39:717–728. https://doi.org/10.1016/S0981-9428(01)01292-X

Omran RG (1980) Peroxidase levels and the activities of catalase, per-oxidase and indole acetic acid oxidase during and after chilling cucumber seedling. Plant Physiol 65:407–408

Painter RH (1951) Insect resistance in crop plants. Mac Millan Co., New York

Pandiyan M, Senthil N, Ramamoorthi N, Muthiah AR, Tomooka N, Duncan V, Jayaraj T (2010) Interspecific hybridization of *Vigna radiata* × 13 wild *Vigna* species for developing MYMV donar. Electron J Plant Breed 1:600–610

Pareek A, Sopory SK, Bohnert HJ (2009) Abiotic stress adaptation in plants. Springer, Dordrecht, Switzerland

Pattanayak A, Roy S, Sood S, Iangrai B, Banerjee A, Gupta S, Joshi DC (2019) Rice bean: a lesser known pulse with well-recognized potential. Planta 250:873–890. https://doi.org/10.1007/s00425-019-03196-1

Prasad TK, Anderson MD, Martin BA, Steward CR (1994) Evidence for chilling induced oxidative stress maize seedling and a regulatory role for hydrogen peroxide. Plant Cell 6:65–75

Qazi J, Ilyas M, Mansoor S, Briddon B (2007) Legume yellow mosaic viruses: genetically isolated begomo viruses. Mol Plant Pathol 8:343–348

Rammesmayer G, Pichorner H, Adams P, Jensen RG, Bohnert HJ (1995) Characterization of IMT1, myo-inositol O-methyltransferase, from *Mesembryanthemum crystallinum*. Arch Biochem Biophy 322:183–188. https://doi.org/10.1006/abbi.1995.1450

Richter A, Popp M (1992) The physiological importance of accumulation of cyclitols in *Viscum album L.* New Phytol 121:431–438. https://doi.org/10.1111/j.1469-8137.1992.tb02943.x

Rikin A, Richmond AE (1976) Amelioration of chilling injuries in cucumber seedlings by abscisic acid. Physiol Plant 38:95–97

Ryan PR, Delhaize E, Jones DL (2001) Function and mechanism of organic anion exudation from plant roots. Ann Rev Plant Biol 52:527–560. https://doi.org/10.1146/annurev.arplant.52.1.527

Sassenrath GE, Ort GE, Portis AR (1990) Impaired reductive activation of stomatal bio-phosphatases in tomato leaves following low temperature exposure at high light. Arch Biochem Biophy 282:302–308

Sastrapradja S, Sutarno H (1977) *Vigna umbellata* in Indonesia. Annales Bogorienses 6:155–167

Seehalak W, Tomooka N, Waranyuwat A, Thipyapong P, Laosuwan P, Kaga A, Vaughan DA (2006) Genetic diversity of the Vigna germplasm from Thailand and neighboring regions revealed by AFLP analysis. Genet Resour Crop Evol 53:1043–1059. https://doi.org/10.1007/s10722-004-7939-2

Sehrawat N, Yadav M (2014) Screening and cross-compatibility of various *Vigna* species for yellow mosaic virus resistance. J Innov Biol 1(1)

Seram D, Senthil N, Pandiyan M, Kennedy JS (2016) Resistance determination of a South Indian bruchid strain against rice bean landraces of Manipur (India). J Stored Products Res 69:199–206

Shanker AK, Maheswari M, Yadav SK, Desai S, Bhanu D, Attal NB, Venkateswarlu B (2014) Drought stress responses in crops. Funct Integr Genomics 14:11–22

Sharma P, Dubey RS (2007) Lead toxicity in plants. Braz J Plant Physiol 17:35–52

Shukle RH, Murdock LL (1983) Lipoxygenase Trypsin inhibitor, and lectin from soybeans: effects on larval growth of *Manduca sexta* (Lepidoptera: Sphingidae). Environ Entomol 12:787–791

Sing SP, Misra BK, Chandel KPS, Pant KC (1980) Major food constituent of ricebean (*Vigna umbellata*). J Food Sci Technol 17:238–240

Singh M, Rundan V, Onte S (2020) Ricebean: high valued fodder crop. Indian Farm 70(6):27–31

Srinivasan T, Durairaj C (2007) Biochemical basis of resistance to in rice bean *Vigna umbellata Thunb. (Ohwi and Ohashi*) against *Callasobruchus maculatus F*. J Entomol 4:371–378. https://doi.org/10.3923/je.2007.371.378

Srivastava RP, Srivastava GK, Gupta RK (2001) Nutritional quality of ricebean (*Vigna umbellata)*. Indian J Agric Biochem 14:55–56

Steponkus PL, Uemura M, Webb MS (1993) A contrast of the cryostability of the plasma membrane of winter rye and spring oat-two species that widely differ in their freezing tolerance and plasma membrane lipid composition. In: Steponkus PL (ed) Advances in low temperature biology. JAI Press, London, pp 211–312

Subramanian VB, Maheswari M (1990a) Physiological responses of groundnut to water stress. Indian J Plant Physiol 33:130–135

Subramanian VB, Maheswari M (1990b) Stomatal conductance, photosynthesis and transpiration in green gram during, and after relief of, water stress. Indian J Expt Biol 28:542–544

Sudha M, Karthikeyan A, Shobhana VG, Nagarajan P, Raveendran M, Senthil N (2015) Search for *Vigna* species conferring resistance to Mungbean yellow mosaic virus in mungbean. Plant Genet Res 13:162–167

Sultana R, Choudhary AK, Pal AK, Saxena KB, Prasad BD, Singh R (2014) Abiotic stresses in major pulses: current status and strategies. In: Approaches to plant stress and their management. Springer, India, pp 173–190

Szabolcs I (1989) Salt-affected soils. CRC Press, Boca Raton, FL

Tester M, Davenport R (2003) Na^+ tolerant and Na^+ transport in higher plants. Ann Bot 91:503–527. https://doi.org/10.1093/aob/mcg058

Timasheff SN (1992) A physicochemical basis for the selection of osmolytes by nature. In: Water and life. Springer, Berlin, Heidelberg, pp 70–84

Tomooka N, Lairungreang C, Nakeeraks P, Egawa Y, Thavarasook C (1991) Mungbean and the genetic resources. TARC, Japan (cited in Lawn, 1995)

Tomooka N, Kashiwaba K, Vaughan DA, Ishimoto M, Egawa Y (2000) The effectiveness of evaluating wild species: searching for sources of resistance to bruchid beetles in the genus *Vigna* subgenus Ceratotropis. Euphytica 115:27–41

Tomooka N, Vaughan DA, Moss H, Maxted N (2002) The Asian *Vigna*: genus *Vigna* subgenus Ceratotropis genetic resources. Kluwer Academic Publishers, Dordrecht

Tsutsui T, Yamaji N, Huang CF, Motoyama R, Nagamura Y, Ma JF (2012) Comparative genome-wide transcriptional analysis of Al-responsive genes reveals novel Al tolerance mechanisms in rice. PLoS One 7:e48197. https://doi.org/10.1371/journal.pone.0048197

Turner NC (1986) Adaptation to water deficits: a changing perspective. Austr J Plant Physiol 13: 175–190

Verdcourt B (1970) Studies in the Leguminosae Papilionoideae for the flora of tropical East Africa: IV. Kew Bull 24:507–569. https://doi.org/10.2307/4102859

Vernon DM, Bohnert HJ (1992) A novel methyl transferase induced by osmotic stress in the facultative halophyte *Mesembryanthemum crystallinum*. EMBO J 11:2077–2085. https://doi.org/10.1002/j.1460-2075.1992.tb05266.x

Vinocur B, Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr Opin Biotechnol 16:123–132. https://doi.org/10.1016/j.copbio.2005.02.001

Wanek W, Richter A (1997) Biosynthesis and accumulation of D-ononitol in *Vigna umbellata* in response to drought stress. Physiol Plant 101:416–424. https://doi.org/10.1111/j.1399-3054.1997.tb01016.x

West DW, Francois LE (1982) Effects of salinity on germination, growth and yield of cowpea. Irrig Sci 3:169–175. https://doi.org/10.1007/BF00446005

Yamaji N, Huang CF, Nagao S, Yano M, Sato Y, Nagamura Y, Ma JF (2009) A zinc finger transcription factor ART1 regulates multiple genes implicated in aluminum tolerance in rice. Plant Cell 21:3339–3349

Yang JL, Zhang L, Li YY, You JF, Wu P, Zheng SJ (2006) Citrate transporters play a critical role in aluminium-stimulated citrate efflux in rice bean (*Vigna umbellata*) roots. Ann Bot 97:579–584. https://doi.org/10.1093/aob/mcl005

Yang JL, You JF, Li YY, Wu P, Zheng SJ (2007) Magnesium enhances aluminum-induced citrate secretion in rice bean roots (*Vigna umbellata*) by restoring plasma membrane H^+-ATPase activity. Plant Cell Physiol 48:66–73. https://doi.org/10.1093/pcp/pcl038

Yoshida Y, Marubodee R, Ogiso-Tanaka E, Iseki K, Isemura T, Takahashi Y et al (2016) Salt tolerance in wild relatives of adzuki bean, *Vigna angularis (Willd.) Ohwi et Ohashi*. Genet Resour Crop Evol 63:627–637. https://doi.org/10.1007/s10722-015-0272-0

You J, Zhang H, Liu N, Gao L, Kong L, Yang Z (2011) Transcriptomic responses to aluminium stress in soybean roots. Genome 54:923–933. https://doi.org/10.1139/g11-060

Physiological Traits Based Breeding to Achieve Higher Yield in Soybean Crop

12

Maharaj Singh, Shivani Nagar, Akanksha Singh, and G. K. Satpute

Abstract

Currently, the feeding of 1.39 billion people of India can only be possible by doubling the current agricultural crop production, and this target has been heavily challenged by climate change and the environmental impact on agricultural systems. Soybean (*Glycine max* L. Merril) is the world's most important and leading seed legume, contributing to 25% of the global edible oil, about two-thirds of the world's protein concentrate for livestock feeding. At present, India ranks fifth in the area and production of soybean in the world. The rate of yield increase is not high enough to satisfy global demand for food (Godfray et al. Science (Washington, DC) 327:812–818, 2010). One strategy to increase the rate of genetic progress is the application of trait-based hybridization in breeding programs (Reynolds and Langridge, Curr Opin Plant Biol 31:162–171, 2016). The genetic progress would be attained by improving those physiological traits that theoretically have the highest positive impact on yield. There have been several studies revealing genetic differences in various physiological parameters ranging from pollen germination, canopy temperature, chlorophyll fluorescence, chlorophyll content, and leaf antioxidants. A strong correlation between traits and seed yield showed the possibility that improvements have occurred physiologically while selections were made for seed yield. Osmotic adjustment, accumulation and remobilization of stem reserves, superior photosynthesis, heat- and desiccation-tolerant enzymes, and so on are important physiological traits (PTs)

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in a breeding program either by direct selection or in the course of a substitute such as molecular markers. The key steps in physiological breeding are (a) crop design, (b) genetic resource exploration, (c) phenotyping mainly in field environments, (d) genetic analysis to enable marker-assisted breeding, (e) hybridization and progeny selection, (f) evaluation of genetic gains via multi-location testing systems, and (g) informatics services underpinning all activities. Thus, the present review urges the need for incorporating physiological breeding strategies into food legumes improvement pipelines; addresses the gap between breeders and physiologists; focuses on physiological traits that are to be targeted; yield gains in food legumes; the impact of abiotic stress and the importance of roots and root phenotyping in food legumes breeding programs.

Keywords

Soybean · Physiological traits · Abiotic stress · Genetic resource

12.1 Introduction

Currently, the feeding of 1.39 billion people in India can only be possible by doubling the current agricultural crop production. This increase in crop production target has been heavily challenged by climate change and the environmental impact on agricultural systems. Breeders and the agricultural community around the world will have to expand the capacity of current breeding programs to increase yield potentials. In a changing world, attempting to increase yield stability, rather than just practicing a traditional empirical approach, plant breeders will also have to focus on the physiological aspects of crops to achieve their yield goals.

Physiology-based phenotyping for traits of specific interests is significant in crop improvement programs of the twenty-first century. An increase in yield depends on a myriad number of physiological traits including but not limited to light, water, and nutrient use efficiency of crops. Root architecture, its sink strength and translocation, water and nutrient acquisition and a canopy’s efficiency in capturing light, and photosynthesis efficiency are some of the factors that largely contribute to crop yield (Parry and Hawkesford 2012). The importance of translating the beneficial traits that are screened during drought in controlled environmental conditions to field environments to be useful to breeders has been emphasized by physiologists (Passioura 2012). These traits can then be used either directly in the selection or used as surrogates to identify superior crop varieties. “Phenomics” or next-generation phenotyping techniques will play a pivotal role in unblocking the bottlenecks in phenotyping physiological traits. To narrow the genotype to phenotype information gap, there are newer technologies such as red, green, and blue (RGB), laser, stereo, multispectral, thermal, hyperspectral, fluorescence imaging, magnetic resonance imaging (MRI), and computed tomography (CT)-based screening that can be employed in field environments (Araus and Cairns 2014; Großkinsky et al. 2015). These technologies, coupled with physiological breeding and when used to screen new crop ideotypes generated from diversified genetic resources, will significantly improve crop genetic gains (Reynolds and Langridge 2016).

Soybean (*Glycine max* L. Merril) is the world's most important seed legume, which contributes to 25% of the global edible oil, about two-thirds of the world's protein concentrate for livestock feeding. At present, India ranks fifth in the area and production in the world after United States, Brazil, Argentina, and China. The soybean yield in United States, Brazil, Argentina, and China increased across years, associated with genetic yield improvements (de Felipe et al. 2016; Specht et al. 2014; Wang et al. 2016). However, the rate of yield increase is not high enough to satisfy the global demand for food (Godfray et al. 2010). One strategy to increase the rate of genetic progress is the application of trait-based hybridization in breeding programs (Reynolds and Langridge 2016). The highest genetic progress would be attained by improving those physiological traits that theoretically have the highest positive impact on yield. Retrospective genetic gain studies are key in determining which traits to manipulate. Although soybean yield genetic progress has been reported, the description of relevant traits is more limited.

There have been several studies revealing genetic differences in various physiological parameters ranging from pollen germination (Kakani et al. 2002; Koti et al. 2004; Kakani et al. 2005; Salem et al. 2007), canopy temperature (Blum and Mayer 1982; Harris et al. 1984; McKinney et al. 1989b; Kashiwagi et al. 2008; Mutava et al. 2011; Lopes et al. 2012; Xiao et al. 2012), chlorophyll fluorescence (Sthapit et al. 1995; Moffatt et al. 1990; Mutava et al. 2011), chlorophyll content (Saitoh et al. 2004; Xiao et al. 2012), and leaf antioxidants (Emmons and Peterson 2001; Dasgupta and De 2004). These results show support for further analysis of physiological traits in other species and the relation that these traits might have to the improvement in seed yield. A strong correlation between traits and seed yield shows the possibility that improvements have occurred physiologically while selections were made for seed yield. There have been many studies that link changes in physiological traits to the improvement found from increased seed yield. Morrison et al. (1999a, b) found a positive relationship with photosynthesis, stomatal conductance, and chlorophyll concentration with an increase in seed yield of soybean. Jin et al. (2010) found a positive correlation between soybean seed yield and year of release with an average increase of 0.58% annually and a 0.59% increase per year in photosynthesis of cultivars. Reduced plant height and lodging have also been noted in soybeans with improvement in seed yield (Jin et al. 2010).

The present review urges the need for incorporating physiological breeding strategies into food legumes improvement pipelines; addresses the gap between breeders and physiologists; focuses on physiological traits that are to be targeted; yields gains in food legumes; the impact of abiotic stress and the importance of roots and root phenotyping in food legumes breeding programs. It also discusses the introduction of physiological traits into molecular breeding programs and the potential of genome engineering of those traits for next-generation food legumes' breeding.

12.2 Yield Framework

There are different theoretical frameworks to functionally dissect seed yield into underlying physiological mechanisms. The simplest approach is to describe seed yield using the Donald and Hamblin (1976) framework:

$$\text{Seed yield}\left(\text{kg}\,\text{ha}^{-1}\right)=\text{Total biomass}\ \times\ \text{HI}$$

where total biomass (kg ha^{-1}) is aboveground plant dry mass at physiological maturity, and HI (%) is harvest index defined as the proportion of seed dry mass to total plant dry mass.

Early genetic gain studies in wheat (*Triticum aestivum* L.) showed increases in yield associated with higher HI but more modern studies are showing predominant importance of total biomass production (Hall and Richards 2013). For soybean in the northern United States, Suhre et al. (2014) reported a 1.2% yr.$^{-1}$ yield genetic progress and HI increase of only 0.1% yr.$^{-1}$. However, evidence from the same region showed that both total aboveground biomass and HI increased in modern cultivars (De Bruin and Pedersen 2008; Koester et al. 2014).

Total aboveground biomass accumulated at physiological maturity can be further described in terms of radiation capture and utilization during the growing cycle. Monteith (1977) provided the theoretical framework to describe biomass accumulation:

$$\text{Total biomass}\left(\text{kg}\,\text{ha}^{-1}\right)=\text{PARinc}\ \times\ \text{ei}\ \times\ \text{RUE}$$

where PARinc (MJ m^{-2}) is the incident photosynthetic active radiation at the top of the canopy accumulated during the growing season, ei (%) is the radiation interception efficiency, and RUE (kg MJ^{-1}) is the radiation use efficiency. The PARinc depends on days to maturity, which is controlled by E genes associated with maturity groups (MGs) (Bernard 1971).

Optimum days to maturity should be long enough to maximize radiation capture but short enough to minimize losses associated with excessive vegetative growth and consequent HI reduction (Egli 2011). Beyond shifts in days to maturity, genetic progress information for ei and RUE is limited. Koester et al. (2014) reported a genetic gain in ei and RUE when evaluating 28 cultivars in a Midwestern location. The increase in ei was mostly associated with a higher proportion of late-maturing cultivars in the most recently released genotypes (Koester et al. 2014). Changes in respiration or photosynthesis could underpin the increase in RUE as suggested by Canadian and Chinese germplasm studies showing that leaf-level photosynthesis has improved with release year (Jin et al. 2010; Morrison et al. 1999a, b). However, Koester et al. (2016) showed that maximum photosynthetic capacity, mesophyll conductance, and night-time respiration have not changed with a cultivar release date. Regardless of actual increases in field-level RUE in the United States, as reported by Koester et al. (2014), no information is available for other breeding programs or regions.

An alternative to the RUE framework presented above is to explain total aboveground biomass as a function of total N uptake and nitrogen use efficiency (NUE) (Novoa and Loomis 1981):

$$\text{Total biomass}\left(\text{kg}\,\text{ha}^{-1}\right)=\text{Nup}\quad\times\quad\text{NUE}$$

where Nup is (g N m^{-2}) is total aboveground N uptake accumulated from emergence to physiological maturity and NUE is the aboveground biomass produced per unit of captured nitrogen (kg kg^{-1}). The Nup is strongly correlated with yield, resulting from a combination of mineral N uptake and biological N_2 fixation (Rotundo et al. 2014; Salvagiotti et al. 2008; Santachiara et al. 2017a). Kumudini et al. (2002) reported that newer soybean cultivars, compared to older ones, accumulate more N during the seed-filling period. The high correlation between soybean yield and Nup can be explained by the dependence of C assimilation on leaf N (Rotundo et al. 2014; Santachiara et al. 2017b; Sinclair and Horie 1989).

12.3 Nature of Physiological Traits

Plant physiology is the study of the functions and processes occurring in plants, which include the various aspects of plant life such as yield, photosynthetic capacity, cold tolerance, water relations, mineral nutrition, growth, developmental stages, and response to environmental stimuli as well as their interactions inter se. Understanding the genetic control of physiological traits and the linkage of these physiological characteristics to molecular markers on chromosomes, and ultimately the gene(s) underlying the trait is the future of plant breeding. Mapping and cloning of genes involved in various physiological traits is a rather complicated process though due to the complexity of plant functions. In contrast, the accelerated pace of gene sequence discovery has surpassed our capability to understand the biological function of many genes. Sequencing technology has provided a wealth of genomic (total DNA) and expressed sequence (DNA that is transcribed and translated into protein) information, which has been accompanied by projects to locate, annotate, and assign biological functions. Despite these efforts, only a fraction of these annotated genes is associated with a phenotype that provides a predictive framework for understanding and manipulation. Complex traits such as pest and disease resistance mechanisms and quality traits such as flavor, texture, and appearance are poorly understood at the molecular/biochemical level. A renewed focus on linking genes to phenotypes is required to ensure our understanding of commercially acceptable traits.

12.4 Breeder-Physiologist Gap

Plant breeding in the past has been dependent on generating variations by multiple crosses and screening progenies to identify cultivars with higher yield potentials. The role of a physiologist in a breeding program has always been on the pre-breeding aspects, suggesting specific physiological parameters that could be used as a screening tool. It has been more than 20 years since the gap between breeders and physiologists was identified toward the physiological understanding in breeding programs (Jackson et al. 1996). This gap is still to be bridged, at least with respect to food legume breeding programs. From a breeder's perspective, a physiological parameter needs to be heritable, stable across multiple environments, and at the same time correlate with key economic traits that are of their specific interests for them to be incorporated into their programs. However, most of the physiological traits are highly variable and require high throughput screening methodologies to screen them. The reductionist approach followed by physiologists and geneticists to dissect specific traits identifies key genes associated with the traits, which can then be used by plant breeders (Edmeades et al. 2004). In the case of drought breeding, physiologists will have to design experiments to select drought adaptive cultivars based on the mechanism in question. For example, is the experiment measuring soil water deficit or stress avoidance? (Gilbert, and Medina 2016). One of the main challenges of integrating plant physiological studies with breeding programs lies in the laborious and time-sensitive measurement of certain physiological traits. For this reason, a limited number of genotypes are often used in physiological studies. An example of laborious and time-sensitive measurements of physiological traits is measuring photosynthetic parameters using portable photosynthesis measuring units such as Li-Cor. The main challenge is that the efficiency of photosynthesis changes over time, so the measurement should be completed within a limited amount of time, which is not practical for a breeding study that usually involves hundreds of individuals. High-throughput phenotyping offers potential in this regard. New cameras, sensors, and automatic mechanical devices such as drones (unmanned airborne vehicles, UAVs) have enabled fast, accurate, and non-destructive phenotyping at the whole-plant and canopy level. For example, high-throughput chlorophyll fluorescence imaging has been used as a proxy for the underlying process of photosynthesis (Fahlgren et al. 2015). Identification of appropriate traits and their proper phenotyping are critical in physiological breeding strategies.

12.5 Traits to Be Targeted in Physiological Breeding

Crop physiology, combined with crop modeling and phenotyping relevant traits, can lead to a better understanding of a particular plant mechanism. To double global wheat yields, screening for the stability of traits across wider environments, canopy architecture and its function, photosynthesis efficiency, crop phenology, and source to sink relationship in partitioning the resources available is of paramount importance (Malcolm et al. 2013). In the context of drought phenotyping, in addition to the

Table 12.1 Traits and parameters that are to be targeted in physiological breeding programs. Both non-invasive and invasive types of measurements are listed*

Traits/parameters	Measurement
Aboveground biomass, root architecture, seedling vigor, canopy structure and growth dynamics, normalized difference vegetative index (NDVI)	Aboveground biomass and canopy structure: Manual measurement Root architecture: root scanner Normalized difference vegetative index (NDVI): NDVI meter
Photosynthetic parameters	Infra-red gas analyzer
Surface temperature	IR Thermometer
Water content, leaf and canopy water status	Manually by measuring the fresh, turgid, and dry weight of plant sample and then calculated
Water stress or water potential	Pressure chamber, water potential measurement system
Chlorophyll content	SPAD meter
Anti-oxidant enzymes	Chemical estimation in lab
LAI or leaf area	Leaf area meter
Chlorophyll fluorescence	Fluorescence meter

*0.5%

above-mentioned traits, root architecture, early vigor, flowering time, carbon isotope discrimination (CID), stomatal conductance, abscisic acid (ABA), osmolytes, chlorophyll concentration, and remobilization of water-soluble carbohydrates are to be screened to draw meaningful correlations (Tuberosa 2012). Among these, root architecture plays an important role and is discussed exclusively elsewhere in this review.

Efforts are underway to improve photosynthesis in crop plants aiming at increasing crop yields to meet the world's goods and bioenergy demand. Improvement in light and carbon capture and conversion in addition to designing smarter crop canopies have been identified as potential opportunities to redesign crop photosynthesis (Ort et al. 2015).

An efficiently planned and careful phenotyping, backed up by relevant experimental designs, will narrow the gap between genotype and phenotype (Tuberosa 2012). The influence of engineering and other technologies has introduced many next-generation phenotyping platforms and facilitates noninvasive measurements of growth and developmental traits (Fiorani and Schurr 2013). A list of traits that can be included in a physiological breeding program is shown in Table 12.1.

12.6 Root Traits

Substantial phenotypic variation in root architecture systems between genotypes in soybean germplasm has been reported. Morphology parameters can be used to classify roots into different types and correlate root type to environmental advantages, such as nutrient acquisition and drought or flood tolerance. Because of this difficulty and root trait(s) genetic complexity, these generally are not used as a breeding criterion. Root traits collected include the number of root branches (V1, R8), volume of root tissue, length of root branches, surface area of the root system, secondary root branch angle from the primary root, and 3D structure and volume displaced by the root architecture (R8 extracted roots only).

Carter (1989) suggested that root systems that enhance soil water extraction would be the most promising target for improving soybean drought tolerance. However, the root, which is referred to as the "hidden half" of a plant (Waisel et al. 2002), is challenging to study, major reasons being the phenotypic plasticity of roots in response to physical, chemical, and biological factors in the soil, lack of high-throughput and cost-effective screening methods, and difficulty to harvest roots from the soil without significant root loss (Poorter and Nagel 2000; Fitter 2002; Manschadi et al. 2008). The role of a root system in improving water and nutrient use efficiencies is well recognized in legume crops, including soybean (Carter 1989; Gaur et al. 2008; Ao et al. 2010; Liang et al. 2010). Genetic variability of root traits and its relationship with water and nutrient acquisition have been documented in legumes such as common bean (*Phaseolus vulgaris* L.) (Sponchiado et al. 1989, chickpea (*Cicer arietinum* L.) (Gaur et al. 2008) and lentil (*Lens culinaris* L.) (Gahoonia et al. 2006). Very limited research has taken place to evaluate genetic variability for root traits in this crop. As a result, limited progress has been made in improving the root system morphology and architecture of this crop that will increase resource acquisition. Exploring genetic variability of root traits will identify contrasting genotypes for root traits that can be included in crop improvement programs and help develop varieties with drought tolerance and/or resource capture. Shoot dry weight and chlorophyll index were positively related to total root length, surface area, volume, and fine root length (Correlation coefficient, $r \geq 0.60$ and P-value <0.0001 for shoot dry weight and $r \geq 0.37$ and P value <0.01 for chlorophyll index]. Plant height was negatively correlated with total root surface area, total root volume, and average root diameter ($|r| \geq 0.29$, P-value <0.05). Seed size was not correlated with any root traits. The genetic variability identified in this research for root traits and penetration is critical for soybean improvement programs in choosing genotypes with improved root characteristics to increase yield in stressful or optimum environments.

12.7 Flower and Pod Set

Among the two main yield components (i.e., the number of seeds and seed size) of soybean (*Glycine max* (L.) Merr.), the number of seeds, which primarily depends on the number of pods, accounts for most of the variation in yield (Calviño et al. 2003). The number of pods is determined by the number of flowers and pod-set ratio; therefore, environmental conditions during the flowering and pod set period—from the beginning of flowering (R1) to the beginning of seed filling (R5)—are closely associated with the numbers of pods and seeds (Jiang and Egli 1993; Puteh et al. 2013). Late developing flowers have higher levels of abortion compared to the early developing flowers (Gai et al. 1984; Heitholt et al. 1986; Huff and Dybing 1980). This may result because most of the available assimilate may be used by rapidly growing pods from the early-opened flowers (Egli and Bruening 2002a, b). Meanwhile, synchronous flowering is reported to increase the pod set (Egli and Bruening 2002a, b), indicating that the temporal pattern of flowering may play an important role in determining the pod number of soybeans (Egli and Bruening 2002a, b; Egli 2005). However, the high temperature, i.e., above 26.9 °C, decreased the overall pod-set ratio (Table 12.2) by reducing the pod-set ratio of the early-opened flowers pod-set ratio of soybean decreased by increasing the day/night temperature from 30/20 °C to 30/26 °C, 30/29 °C, and 39/20 °C for 10 days at the flowering stage (Djanaguiraman et al. 2013). High temperatures extend flowering duration (Kumagai and Sameshima 2014), the extension of which may arise due to the formation of flowers on sub-racemes in the late flowering period (Egli 2005), the pod-set ratio, and pod number of the late-opened-flowers were not significantly increased by high temperatures and were consistently low regardless of the temperature. These may have arisen from the nutrient deficiency during late growth. The top-dressing of nitrogen around flowering is reported to improve the pod-set ratio of soybean (Brevedan et al. 1978; Oko et al. 2003). Thus, the temperature response may differ under the growing conditions free of nutrient deficiency. However, in South Korea, top-dressing is often not applied in soybean fields due to economic (e.g., additional fertilizers and labors) and environmental issues although it is recommended for high yield.

Soybeans tend to abort a large percentage of flowers and pods throughout the growing season (Van Schaik and Probst 1958; Jiang and Egli 1993). These reports have led to suggestions that the seed yield for soybeans can be enhanced by reducing factors that limit pod and seed sets (Hansen and Shibles 1978). Improving pollen germination may have a positive impact on pod set and seed yield. Significant genotypic differences were found with the nodulating genotype having 20% higher pollen germination than the nonnodulating genotype. No other agronomic or physiological measurements were presented.

12.8 Canopy Temperature

Early work with infrared thermometers has proven successful in monitoring evapotranspiration rates in crops (Stone and Horton 1974) and in the observation of daily crop temperatures (Blad and Rosenberg 1976) Leaf and canopy temperatures have been fast and easy to measure with infrared thermometers and have been related to plant water stress. Blad and Rosenberg (1976) compared crop surface temperatures measured with an infrared thermometer to plant temperatures measured with leaf thermocouples throughout the course of a day. Results found that measurements performed by infrared thermometers produced accurate readings when the canopy was completely full. When canopy coverage was not full, heat from the exposed soil affected the readings of the plant surfaces. Harris et al. (1984) used a hand-held infrared thermometer to evaluate 20 soybean genotypes by measuring leaf canopy

Table 12.2 Adaptive traits related to abiotic stresses in legume crops

Stress	Trait	Crop	Reference
Drought	High water use efficiency	Alfalfa, faba bean	Ray et al. (1998), Nerkar et al. (1981)
	Vigorous root growth	Alfalfa, chickpea	Kang et al. (2011), Gaur et al. (2015)
	Osmolyte accumulation/ osmotic adjustment/turgor maintenance	Alfalfa, faba bean	Kang et al. (2011), Khan et al. (2007)
	Accumulation of antioxidants	Alfalfa	Kang et al. (2011)
	Increased leaf cuticular wax	Alfalfa	Zhang et al. (2005)
	Early flowering/maturity	Chickpea, common bean, cowpea, faba bean, lentil	Bueckert and Clarke (2013), Rehman et al. (2011)
	Low leaf conductance/ stomatal regulation/ transpiration	Faba bean	Kang et al. (2011), Khan et al. (2007), Nerkar et al. (1981)
	Delayed leaf senescence	Alfala	Kang et al. (2011)
	Changed leaf orientation	Soybean	(68)
	Reduced canopy temperature	Chickpea, Faba bean, soybean	Rehman et al. (2011), Khazaei et al. (2013), Bai and Purcell (2018)
	Relative water content	Faba bean	Khazaei et al. (2013)
Salinity	Accumulation of osmolytes/ osmotic adjustment/turgor maintenance	Faba bean	Shahid et al. (2012)
Flooding	High stomatal conductance	Lentil	Stoddard et al. (2006)
	Large air-spaces and aerenchyma in roots	Lentil, field pea, soybean	Stoddard et al. (2006), Shimamura et al. (2016)
Heat	Increased pollen germination under stress	Field Pea	Jiang et al. (2018)
	Reduced canopy temperature	Chickpea	Rehman et al. (2011), Bueckert and Clarke (2013)
	Accumulation of leaf cuticular wax	Fieldpea	Sánchez et al. (2001)
	Early flowering/maturity	Lentil, field pea, chickpea	Bueckert and Clarke (2013)
	Indeterminate growth habit	Field pea, chickpea	Rehman et al. (2011)
Cold	Presence of dehydrin protein	Cowpea	Ismail et al. (1997)
	Increased osmoprotectants	Faba bean	Link et al. (2010)
	Increased fatty acid desaturation of membrane lipids	Faba bean	Link et al. (2010)
	Maintenance of photosynthesis	Fieldpea	Grimaudv et al. (2013)

temperatures. Canopy temperature differentials were found by subtracting the air temperature from the canopy temperature and were used in the analysis. Significant differences in the average canopy temperature differentials were found across years. A negative correlation between canopy temperature differentials and seed yield was found to be significant for both irrigated and dryland plots With the proper setup and growing season climate, infrared thermometers may be successfully implemented into breeding programs for selection purposes. McKinney et al. (1989a) evaluated the use of canopy temperature for selections in a soybean breeding program, within six soybean populations by using a hand-held infrared thermometer. McKinney et al. (1989b), evaluations were made on thirty elite soybean lines and cultivars replicated three times where the five warmest and five coolest lines were selected and used in the following two years. Measurements were made with a hand-held infrared thermometer for canopy temperature and an aspirated psychrometer for vapor pressure deficit (VPD). Significant differences were found for mean canopy temperature differentials among the genotypes and a significant negative correlation with seed yield of -0.72 across all environments.

In a more in-depth slow-wilting soybean study, Ries et al. (2012) measured canopy temperature, among many other traits on three slow-wilting, one intermediate wilting, and five fast-wilting genotypes in an effort to relate delayed wilting to soil moisture conservation. An infrared thermometer (model OS562, Omega Engineering Inc., Stamford, CT) was used to measure canopy temperature by averaging ten random measurements of each plot between R2 and R5 growth stages (Fehr et al. 1971). Canopy temperature depression was calculated using the built-in thermocouple to measure ambient air temperature and then subtracting that from the canopy temperature (Idso 1982).

12.9 Chlorophyll Fluorescence

As the light energy is absorbed by a chlorophyll molecule in a leaf, the energy is either utilized by photochemical or by non-photochemical processes. The photochemical process is known as photosynthesis, while the non-photochemical processes include energy dissipating as infrared radiation (heat) and as red/far-red radiation, which is chlorophyll fluorescence. Of all the light absorbed, only about 1% or 2% is re-emitted as chlorophyll fluorescence. This light is best measured with a chlorophyll fluorometer, which will provide information about photosystem II (PSII) and the extent to which PSII is using the energy absorbed by chlorophyll, and the extent to which it is being damaged by excess light (Maxwell and Johnson 2000). With the use of handheld chlorophyll fluorometers the ability to quickly measure plants in the field has increased. The fluorometer measurements are typically taken on a dark-adapted leaf that receives controlled amounts of light energy from the instrument. At exposure to light, leaf fluorescence rises to the minimal level of fluorescence (Fo), which is the fluorescence level obtained when the PSII reaction centers are in the “open” state (capable of photochemistry).

Physiological measurements were made with a pulse-modulated fluorometer after 30 min of dark adaption of the third trifoliate leaf from the main stem apex between 11:00 and 13:00 h at R2 growth stage. This measurement allowed for the determination of the ratio of variable fluorescence to maximum fluorescence (Fv/Fm) and the ratio of minimum fluorescence to maximum fluorescence (Fo/Fm). Results were significant at the $P < 0.001$ value and showed a 33% increase in thylakoid membrane damage (Fo/Fm) in the HT plants over the OT plants. This study characterized the response of one genotype and suggested further evaluations of additional genotypes to determine the potential genetic variability of HT stress tolerance.

12.10 Chlorophyll Content

Chlorophyll concentrations have been measured nondestructively by absorbance methods. A commonly used absorbance chlorophyll meter is the Minolta SPAD-502 meter (Konica Minolta Optics, Inc., Tokyo, Japan). Monje and Bugbee (1992) conducted an experiment in which the dual-wave SPAD-502 chlorophyll meter was tested against a custom-built single-wave meter and then destructive colorimetric measurement was performed to find in vitro chlorophyll concentration to compare the absorbance measurements. There were no significant differences found between the single-wavelength and SPAD-502 meters, although results showed that the SPAD-502 meter produced a correlation of 93% with the destructive measurement slightly higher than the single wavelength meter of 90%.

HT stress decreased the chlorophyll content compared with the OT at a significance value of $P < 0.001$In. A self-calibrating Minolta SPAD-502 chlorophyll meter (Konica Minolta Optics, Inc., Tokyo, Japan) was used on the topmost fully expanded leaf three times throughout the season from booting stage through maturity. No significant differences between genotypes or relationships with seed yield were found in this study.

12.10.1 Leaf Antioxidant

In plants, water shortage leads to the excessive production of reactive oxygen species (ROS) such as $^1O_2$, O_2^- and H_2O_2, which are very reactive and lead to rapidly injury to living tissues and macromolecules (e.g. DNA, lipids, proteins, and carbohydrates), eventually resulting in induced programmed cell death (PCD) processes 9. The risk of irrecoverable injuries within green tissues because of ROS production may increase under severe stress 7,10. Reactive oxygen species (ROS) are highly active and are produced by the normal operation of photosynthesis and electron transport as well as abiotic stresses, but are balanced by scavenging antioxidants and enzymes (Taiz and Zeiger 2010). Besides these enzymes, certain carotenoids and glutathione can also play part in the antioxidant system as non-enzymatic components. The enzymes such SOD, POD, and CAT either directly scavenge the ROS or protect plants indirectly by managing non-enzymatic defense

(Anjum et al. 2011). In response to ROS, increased content of malondialdehyde has been reported which is a pure indicator of drought-induced oxidative damage (Møller et al. 2007). Therefore, maintenance of the higher levels of the anti-oxidants can be a good strategy by the plants to counter the negative effects of ROS (Sharma and Dubey 2005). Phytohormones are also natural defense molecules in plants that maintain higher levels of anti-oxidants under stress. They help plants to acclimatize to varying environments by mediating growth, development, source/sink transitions, and nutrient allocation (Fahad et al. 2015).

Sugars, (such as glucose, fructose, and sucrose), sugar alcohols (such as mannitol), and amino acids (such as proline) accumulate under drought stress conditions in different plant species and function not only as osmolytes but also as antioxidants, helping in ROS detoxification, membrane protection, and enzyme/protein stabilization, ultimately improving plant resistance against abiotic stresses. High-temperature stress, which causes increased ROS, has been shown to increase membrane damage, measured by electrolyte leakage, in grain sorghum (Djanaguiraman et al. 2010) and soybeans (Djanaguiraman et al. 2010). Measurements of antioxidant enzyme activity have shown decreases under high temperatures (Djanaguiraman et al. 2010; Djanaguiraman and Prasad 2010).

Oxidative damage is usually a subsequent stage of most of the abiotic stresses in plants. Exposure of plants to drought stresses initially causes oxidative damage by the formation of ROS. These ROS pose a serious threat to the cell's functioning by damaging lipids and proteins. The ROS are mainly produced in the chloroplast (Reddy et al. 2004), however, the reaction of oxygen with the components of the electron transport chain in mitochondria also results in the generation of ROS (Moller 2001). The mechanisms involved in the generation of ROS can be either enzymatic or non-enzymatic (Apel and Hirt 2004). The production of ROS has also been reported under high-temperature stress (Liu and Huang 2000; Wahid et al. 2007).

12.11 Criteria for Using Physiological Traits in Breeding Programs

Osmotic adjustment, accumulation and remobilization of stem reserves, superior photosynthesis, heat- and desiccation-tolerant enzymes, and so on are important physiological traits (PTs) in a breeding program either by direct selection or in the course of a substitute such as molecular markers. Several pieces of information on important PTs may be collected on potential parental lines that engross screening of whole crossing block, or a set of commonly used parents, thus bringing into being an index of useful PTs which have to imperatively establish their heritability and genetic correlation with yield in target environments that can be used advantageously in designing crosses, which bring together desirable traits through increasing transgressive segregation events.

It is vital that the application of the trait as a selection criterion be definite when significant genetic diversity for a physiological trait in a germplasm collection for the given species is established. Subsequently, breeding strategies are effective only when these traits are rightly defined in terms of the stage of crop development to using specific attributes of the target environment and their potential contribution to yield.

Key steps in a physiological breeding pipeline: (a) crop design, (b) genetic resource exploration, (c) phenotyping mainly in field environments, (d) genetic analysis to enable marker-assisted breeding, (e) hybridization and progeny selection, (f) evaluation of genetic gains via multi-location testing systems, like the International Wheat Improvement Network, and (g) informatics services underpinning all activities. The latter underlines the inter-dependence of each step where, for example, results from multi-location trials combined with genetic analysis permit dissection of genotype × environment interaction, better-targeted phenotyping of genetic resources, and refined crossing strategies.

12.12 Molecular Breeding of Physiological Traits

Facilitating marker-assisted selection (MAS), the genomics resources have also contributed to the development and improvement of newer techniques such as genome-wide association studies (GWAS), genotype by sequencing (GBS), genomic selection (GS), and genome editing in plants and have advanced plant breeding to the next generation. Though marker-based strategies have tremendous potential in expediting breeding, the effect of the environment on genotypes and their interactions (G × E) can make them unsuccessful as crops or undergo significant changes in gene expression patterns during various morpho-physiological and stress-related changes (Xu and Crouch 2008). Insufficient diversity in the primary gene pool of the accessions screened can also lead to failed MAS inbreeding (Jitendra et al. 2011). These phenomena will be more evident when it comes to physiological traits, as they are influenced by changes in the environment and gene expression, which makes the incorporation of these traits into molecular breeding programs a little complex. MAS is particularly successful only when the heritability of the trait of interest is high, which is not the case generally in physiological traits as they are influenced by multiple factors. QTL mapping of physiological traits may result in landing coincidentally on morphological and phenological trait QTLs as these traits are heavily controlled by physiological mechanisms. A list of physiological traits and/or parameters that can be mapped using QTL mapping techniques in crops is listed in Reference (Graham et al. 2009). The photosynthetic assimilation rate, chlorophyll content, stomatal resistance, and transpiration rate to map photosynthesis, root mass, root depth, root axis length, and lateral root branching to correlate to nutrient absorption and assimilation, root nodule number, shoot mass, symbiotic nitrogen fixation (Sym) genes and various other regulatory genes for nitrogen fixation, reduction in chlorophyll content, number of late-coloring leaves per panicle determining senescence, photoperiod and flowering-related locus for mapping flowering, Na + and K+ concentration for salt tolerance, carbon isotope ratio, abscisic acid concentration, CID, water-soluble carbohydrate concentration, osmotic potential for drought tolerance, photosynthetic parameters and leaf pigment composition for cold tolerance can all used to identify QTLs associated with their respective traits.

12.13 Gene Editing Key Physiological Traits

The new gene-editing technology CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR associated protein 9) provides great promise for crop improvement employing precise editing of nucleotides although the acceptability of the technology is still under consideration (Gao 2018). This technology combats the limitations of conventional breeding by accelerating germplasm development by creating heritable and desired mutations in the genome without undesirable background mutations as observed in traditional mutagenesis in crop plants. Furthermore, the generation of transgene-free plants through segregation of traits in the F1 or F2 generations with intact heritable mutations can generate plants identical to those obtained by conventional breeding with a reduced cost and time (Gao 2018). The CRISPR/Cas9 nuclease directed by the guide RNA creates double-stranded breaks in the DNA, which are subsequently repaired by DNA repair mechanisms: Error-prone non-homologous end-joining (NHEJ) or homology-directed repair (HDR). The NHEJ repair mechanism is error-prone and can lead to insertion or deletion mutations upstream of the PAM site thereby altering the coding sequence. In comparison, the HDR mechanism generates specific point mutations or can be used to insert nucleotides as provided in the donor-specified DNA template (Sandar and Joung 2014).

The CRISPR/Cas9 technology has been continuously optimized for improved genomic editing since the first report and the abilities of this technology have been constantly expanded, which include guide RNA engineering for use with multiple guide RNAs (Cermak et al. 2017), targeted base editing (Kang et al. 2018), and more recently epigenome editing (Klann et al. 2017). The most widely used and characterized nuclease is Cas9 (CRISPR associated nuclease 9); however, utilization of cpf1 has also been recently reported (Gao 2018). Although the utilization of CRISPR/Cas9 in legumes is still in its infancy, important reports from other crops showing the efficient utilization of CRISPR/Cas9 have been explained in this chapter. The examples provided below provide evidence for modifying physiological traits using CRISPR/Cas9 in other crops, which can be used to edit traits in legume crops. CRISPR/Cas9 can be efficiently used to target or modify qualitative traits where a desired loss-of-function outcome has been validated and previously studied. Mutations in four yield-related genes grain number 1a (Gn1A), dense and erect panicle (DEP1), grain size (GS3), and ideal plan. Architecture (IPA1) was induced in the rice cultivar Zhonghua 11 (Li et al. 2016). Mutant lines edited with CRISPR/Cas9 (T2) for Gn1A resulted in an increased number of flowers per panicle (184–199) in comparison to wild-type plants (104 flowers per panicle). These mutant plants also showed an increased plant height and panicle length in comparison to wild-type plants. Mutant plants for DEP1 had shorter panicles with significantly more flowers per panicle than wildtype plants.

References

Anjum SA, Farooq M, Wang LC, Xue LL, Wang SG, Wang L (2011) Gas exchange and chlorophyll synthesis of maize cultivars are enhanced by exogenously-applied glycinebetaine under drought conditions. Plant Soil Environ 577:326–331

Ao J, Fu J, Tian J, Yan X, Liao H (2010) Genetic variability for root morph-architecture traits and root growth dynamics as related to phosphorus efficiency in soybean. Funct Plant Biol 37:304–312

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399

Araus JL, Cairns JE (2014) Field high-throughput phenotyping: the new crop breeding frontier. Trends Plant Sci 19:52–61

Bai H, Purcell LC (2018) Aerial canopy temperature differences between fast- and slow-wilting soya bean genotypes. J Agron Crop Sci 204:243–251

Bernard RL (1971) Two major genes for time of flowering and maturity in soybean. Crop Sci 11:242–244

Blad BL, Rosenberg NJ (1976) Measurement of crop temperature by leaf thermocouple, infrared thermometry, and remotely sensed thermal imagery. Agron J 68:635–641

Blum AJ, Mayer GG (1982) Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. Field Crops Res 5:137–146

Brevedan RE, Egli DB, Leggett JE (1978) Influence of N nutrition on flower and pod abortion and yield of soybeans. Agron J 70:81–84

Bueckert RA, Clarke JM (2013) Review: annual crop adaptation to abiotic stress on the Canadian prairies: six case studies. Can J Plant Sci 93:375–385

Calviño PA, Sadras VO, Andrade FH (2003) Development, growth and yield of late-sown soybean in the southern Pampas. Eur J Agron 19:265–275

Carter TE Jr (1989) Breeding for drought tolerance in soybean: where do we stand? In: Pascale AJ (ed) Proc. World Soybean Conf., IV, Buenos Aires, Argentina. 5–9 March 1989, pp 1001–1008

Cermak T, Curtin SJ, Gil-Humanes J, Cegan R, Kono TJY, Konecna E, Belanto JJ, Starker CG, Mathre JW, Greenstein RL (2017) A multipurpose toolkit to enable advanced genome engineering in plants. Plant Cell 29:1196–1217

Dasgupta N, De B (2004) Antioxidant activity of piper betle L. leaf extract in vitro. Food Chem 88: 219–224

De Bruin JL, Pedersen P (2008) Effect of row spacing and seeding rate on soybean yield. Agron J 100:704–710

de Felipe M, Gerde JA, Rotundo JL (2016) Soybean genetic gain in maturity groups III to V in Argentina from 1980 to 2015. Crop Sci 56:3066–3077

Djanaguiraman M, Prasad PVV (2010) Ethylene production under high temperature stress causes premature leaf senescence in soybean. Funct Plant Biol 37:1071–1084

Djanaguiraman M, Prasad PVV, Seppanen M (2010) Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol Biochem 48:999–1007

Djanaguiraman M, Prasad PVV, Schapaugh WT (2013) High day- or nighttime temperature alters leaf assimilation, reproductive success, and phosphatidic acid of pollen grain in soybean [*Glycine max* (L.) Merr.]. Crop Sci 53:1594–1604

Donald CM, Hamblin J (1976) The biological yield and harvest index of cereals as agronomic and plant breeding criteria. Adv Agron 28:361–405. https://doi.org/10.1016/S0065-2113(08)60559-3

Edmeades GO, McMaster GS, White JW, Campos H (2004) Genomics and the physiologist: ridging the gap between genes and crop response. Field Crops Res 90:5–18

Egli DB (2005) Flowering, pod set and reproductive success in soya bean. J Agron Crop Sci 191: 283–291

Egli DB (2011) Time and the productivity of agronomic and crop stems. Agron J 203:743–750

Egli DB, Bruening WP (2002a) Flowering and fruit set dynamics at phloem-isolated nodes in soybean. Field Crop Res 79:9–19

Egli DB, Bruening WP (2002b) Synchronous flowering and fruit set at phloem-isolated nodes in Soybean. Crop Sci 42:1535–1540

Emmons C, Peterson D (2001) Antioxidant activity and phenolic content of oat as affected by cultivar and location. Crop Sci 41:1676–1681

Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, Khan FA, Khan F, Chen Y, Wu C, Tabassum MA, Chun MX, Afzal M, Jan A, Jan MT, Huang J (2015) Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. Environ Sci Pollut Res Int 22:4907–4921

Fahlgren N, Gehan MA, Baxter I (2015) Lights, camera, action: high-throughput plant phenotyping is ready for a close-up. Curr Opin Plant Biol 24:93–99

Fehr WR, Caviness CE, Burmood DT, Pennington JS (1971) Stage of development descriptions for soybeans, Glycine max (L.) Merrill. Crop Sci 11(1971):929–931

Fiorani F, Schurr U (2013) Future scenarios for plant phenotyping. Annu Rev Plant Biol 64:267–291

Fitter AH (2002) Characteristics and functions of root systems. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots: the hidden half. Marcel Dekker, New York, pp 249–259

Gahoonia TS, Ali O, Sarker A, Nielsen NE, Rahman MM (2006) Genetic variation in root traits and nutrient acquisition of lentil genotypes. J Plant Nutr 29:643–655

Gai J, Palmer RG, Fehr WR (1984) Bloom and Pod set in determinate and indeterminate soybeans grown in China. Agron J 76:979–984

Gao C (2018) The future of CRISPR technologies in agriculture. Nat Rev Mol Cell Biol 19:275–276

Gaur PM, Krishnamurthy L, Kashiwagi J (2008) Improving drought-avoidance root traits in chickpea (*Cicer arietinum* L.)—Current status of research at ICRISAT. Plant Prod Sci 11:3–11

Gaur PM, Krishnamurthy L, Kashiwagi J (2015) Improving drought-avoidance root traits in chickpea (*Cicer arietinum* L)—current status of research at ICIRISAT. Plant Prod Sci 11:3–11

Gilbert ME, Medina V (2016) Drought adaptation mechanisms should guide experimental design. Trends Plant Sci 21:639–647

Godfray HCJ, Beddington JR, Crute IR, Hadded L, Lawrence D, Muir JF et al (2010) Food security: the challenge of feeding 9 billion people. Science (Washington, DC) 327:812–818

Graham J, Ratnaparkhe MB, Powell W (2009) Molecular mapping and breeding of physiological traits. In: Kole C, Abbott AG (eds) Principal and practices of plant genomics. Molecular breeding. CRC Press, Boca Raton, FL, pp 217–241

Grimaudv F, Renaut J, Dumont E, Sergeant K, Lucau-Danila A, Blervacq AS, Sellier H, Bahrman N, Lejeune-Henaut I, Delbreil B (2013) Exploring chloroplastic changes related to chilling and freezing tolerance during cold acclimation of pea (*Pisum sativum* L.). J Proteome 80:145–159

Großkinsky DK, Svensgaard J, Christensen S, Roitsch T (2015) Plant phenomics and the need for physiological phenotyping across scales to narrow the genotype-to-phenotype. knowledge gap. J Exp Bot 66:5429–5440

Hall AJ, Richards RA (2013) Prognosis for genetic improvement of yield potential and water-limited yield of major grain crops. Field Crop Res 143:18–33

Hansen WR, Shibles R (1978) Seasonal log of the flowering and podding activity of field-grown soybeans. Agron J 70:47–50

Harris DS, Schapaugh WT, Kanemasu ET (1984) Genetic diversity in soybeans for leaf canopy temperature and the association of leaf canopy temperature and yield. Crop Sci 24:839–884

Heitholt JJ, Egli DB, Leggett JE (1986) Characteristics of reproductive abortion in soybean. Crop Sci 26:589–595

Huff A, Dybing CD (1980) Factors affecting shedding of flowers in soybean (Glycine max (L.) Merrill). J Exp Bot 31:751–762

Idso SB (1982) Non water stressed baseline: a key to measuring and interpreting plant water stress. Agric Meteorol 27:59–70

Ismail AM, Hall AE, Close TJ (1997) Chilling tolerance during emergence of cowpea associated with a dehydrin and slow electrolyte leakage. Crop Sci 37:1270–1277

Jackson P, Robertson M, Cooper M, Hammer G (1996) The role of physiological understanding in plant breeding; from a breeding perspective. Field Crops Res 49:11–37

Jiang H, Egli DB (1993) Shade induced changes in flower and Pod number and flower and fruit abscission in soybean. Agron J 85:221–225

Jiang Y, Bueckert R, Warkentin T, Davis AR (2018) High temperature effects on in vitro pollen germination and seed set in field pea. Can J Plant Sci 98:71–80

Jin J, Liu X, Wang G, Mi L, Shen Z (2010) Agronomic and physiological contributions to the yield improvement of soybean cultivars released from 1950 to 2006 in northeast China. Field Crops Res 115:116–123

Jitendra K, Arbind CA, Ramesh KS, Aditya P (2011) Towards marker-assisted selection in pulses: a review. Plant Breed 130:297–313

Kakani VG, Prasad PVVP, Craufurd Q, Wheeler TR (2002) Response of in vitro pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. Plant Cell Environ 25:1651–1661

Kakani VG, Reddy KR, Koti S, Wallace TP, Prasad PVV, Reddy VR (2005) Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Ann Bot 96:59–67

Kang Y, Han Y, Torres-Jerez I, Wang M, Tang Y, Monteros M, Udvardi M (2011) System responses to long-term drought and re-watering of two contrasting alfalfa varieties Plant. J For Cell Mol Biol 68:871–889

Kang BC, Yun JY, Kim ST, Shin Y, Ryu J, Choi M, Woo JW, Kim JS (2018) Precision genome engineering through adenine base editing in plants. Nat Plants 4:427–431

Kashiwagi J, Krishnamurthy L, Upadhyaya HD, Gaur PM (2008) Rapid screening technique for canopy temperature status and its relevance to drought tolerance improvement in chickpea. J SAT Agric Res 6:105–104

Khan HUR, Link W, Hocking TJ, Stoddard FL (2007) Evaluation of physiological traits for improving drought tolerance in faba bean (*Vicia faba* L.). Plant Soil 292:205–217

Khazaei H, Street K, Bari A, Mackay M, Stoddard FL (2013) The FIGS (focused identification of germplasm strategy) approach identifies traits related to drought adaptation in vicia faba genetic resources. PLoS One 8:e63107

Klann TS, Black JB, Chellappan M, Safi A, Song L, Hilton IB, Crawford GE, Reddy TE, Gersbach CA (2017) Crispr-cas9 epigenome editing enables high-throughput screening for functional regulatory elements in the human genome. Nat Biotechnol 35:561–568

Koester RP, Skoneczka JA, Cary TR, Diers BW, Ainsworth EA (2014) Historical gains in soybean (glycine max merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. J Exp Bot 65:3311–3321

Koester RP, Nohl BM, Diers BW, Ainsworth EA (2016) Has photosynthetic capacity increased with 80 years of soybean breeding? An examination of historical soybean cultivars. Plant Cell Environ 39:1058–1067

Koti S, Reddy KR, Kakani VG, Zhao D, Reddy VR (2004) Soybean (*Glycine max* L.) pollen germination characteristics, flower and pollen morphology in response to enhanced ultraviolet-B radiation. Ann Bot 94:855–864

Kumagai E, Sameshima R (2014) Genotypic differences in soybean yield responses to increasing temperature in a cool climate are related to maturity group. Agric For Meteorol 198:265–272

Kumudini S, Hume G, Chu G (2002) Genetic improvement in short season soybeans: II. Nitrogen accumulation, remobilization, partitioning. Crop Sci 42:141–145

Li M, Li X, Zhou Z, Wu P, Fang M, Pan X, Lin Q, Luo W, Wu G, Li H (2016) Reassessment of the four yield-related genes Gn1a, dep1, GS3, and IPA1 in rice using a CRISPR/Cas9 system Front. Plant Sci 7:377

Liang Q, Cheng X, Mei M, Yan X, Liao H (2010) QTL analysis of root traits as related to phosphorus efficiency in soybean. Ann Bot 106:223–234. pmid:20472699

Link W, Balko C, Stoddard FL (2010) Inter hardiness in Faba bean: physiology and breeding. Field Crops Res 115:287–296

Liu X, Huang B (2000) Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. Crop Sci 40:503–510

Lopes MS, Reynolds MP, Manes Y, Singh RP, Crossa J (2012) Genetic yield gains and changes in associated traits of CIMMYT spring bread wheat in a "historic" set representing 30 years of breeding. Crop Sci 52:1123–1131

Malcolm JH, Jose-Luis A, Robert P, Daniel C, Daniel M, Tianmin S, Jianping Z, Martin AJP (2013) Prospects of doubling global wheat yields. Food Energy Secur 2:34–48

Manschadi AM, Hammer GL, Christopher JT, deVoil P (2008) Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). Plant Soil 303:115–129

Maxwell K, Johnson GN (2000) Chlorophyll fluorenes – a practical guide. J Exp Bot 51:659–668

McKinney NV, Schapaugh WT, Kanemasu ET (1989a) Canopy temperature, seed yield, and vapor-pressure deficit relationships in soybean. Crop Sci 29:1038–1041

McKinney NV, Schapaugh WT, Kanemasu ET (1989b) Selection for canopy temperature differential in six populations of soybean. Crop Sci 29:255–259

Moffatt JM, Sears RG, Cox TS, Paulsen GM (1990) Wheat high-temperature tolerance during reproductive growth II. genetic-analysis of chlorophyll fluorescence. Crop Sci 30:886–889

Moller IM (2001) Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu Rev Plant Physiol 52:561–591. https://doi.org/10.1146/annurev.arplant.52.1.561

Møller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481

Monje O, Bugbee B (1992) Inherent limitations of nondestructive chlorophyll meters: a comparison of two types of meters. Hortic Sci 27(1):69–71

Monteith JL (1977) Climate and the efficiency of crop production in Britain. Philos Trans Royal Soc Lond B Biol Sci 281:277–297

Morrison MJ, Voldeng HD, Cober ER (1999a) Physiological changes from 58 years of genetics improvement of short-season soybean cultivars in Canada. Agron J 91:685–689

Morrison M, Voldeng H, Cober E (1999b) Physiological changes from 58 years of genetic improvement of short-season soybean cultivars in Canada. Agron J 91:685–689

Mutava RN, Tuinstra MR, Kofoid MD, Yu J (2011) Characterization of sorghum genotypes for traits related to drought tolerance. Field Crops Res 123:10–18

Nerkar YS, Wilson D, Lawes DA (1981) Genetic variation in stomatal characteristics and behaviour, water use and growth of five *Vicia faba* L. Genotypes under contrasting soil moisture regimes. Euphytica 30:335–345

Novoa R, Loomis RS (1981) Nitrogen and plant production. Plant Soil 58:177–204

Oko BFD, Eneji AE, Binang W, Irshad M, Yamamoto S, Honna T, Endo T (2003) Effect of foliar application of urea on reproductive abscission and grain yield of soybean. J Plant Nutr 26:1223–1234

Ort DR, Merchant SS, Alric J, Barkan A, Blankenship RE, Bock R (2015) Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proc Natl Acad Sci U S A 112:8529–8536

Parry MAJ, Hawkesford MJ (2012) An integrated approach to crop genetic improvement. J Integr Plant Biol 54(4):250–259

Passioura JB (2012) Phenotyping for drought tolerance in grain crops: when is it useful to breeders? Funct Plant Biol 39:851–859

Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. Aust J Plant Physiol 27: 595–607

Puteh AB, Thuzar M, Mondal MMA, Abdullah NAPB, Halim MRA (2013) Soybean [*Glycine max* (L.) Merrill] seed yield response to high temperature stress during reproductive growth stages. Aust J Crop Sci 7:1472–1479

Ray I, Townsend MS, Henning JA (1998) Variation for yield, water-use efficiency and canopy morphology among nine alfalfa germplasms. Crop Sci 38:1386–1390

Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161:1189–1202. https://doi.org/10.1016/j.jplph.2004.01.013

Rehman AU, Malhotra RS, Bett K, Tar'an B, Bueckert R, Warkentin TD (2011) Mapping qtl associated with traits affecting grain yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. Crop Sci 51:450–463

Reynolds M, Langridge P (2016) Physiological breeding. Curr Opin Plant Biol 31:162–171

Ries L, Purcell L, Carter TE, Edward JT (2012) Physiological traits contributing to differential canopy wilting in soybean under drought. Crop Sci 52(1):272–281

Rotundo JL, Borras L, De Bruin J, Pedersen P (2014) Soybean nitrogen uptake and utilization in Argentina and United States cultivars. Crop Sci 54:1153–1165

Saitoh K, Nishimura K, Kuroda T (2004) Comparison of leaf photosynthesis between wild and cultivated types of soybean. Plant Prod Sci 7:277–279

Salem MA, Kakani VG, Koti S, Reddy KR (2007) Pollen-based screening of soybean genotypes for high temperatures. Crop Sci 47:219–231

Salvagiotti F, Cassman KG, Specht JE, Walters DT, Weiss A, Dobermann A (2008) Nitrogen uptake, faxation and response to fertilizer nitrogen in soybeans: a Review. Field Crop Res 108: 1–13

Sánchez FJ, Manzanares MA, De Andrés EF, Tenorio JL, Ayerbe L (2001) Residual transpiration rate, epicuticular wax load and leaf colour of pea plants in drought conditions. Influence on harvest index and canopy temperature. Eur J Agron 15:57–70

Sander JD, Joung JK (2014) Crispr-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32:347–355

Santachiara G, Borras L, Rotundo JL (2017a) Physiological processes leading to similar yield in contrasting soybean maturity groups. Agron J 109:158–167

Santachiara G, Borras L, Salvagiotti F, Gerde JA, Rotundo JL (2017b) Relative importance of biological nitrogen fixation and mineral uptake in high yielding soybean cultivars. Plant Soil 418:191–203

Shahid MA, Balal RM, Pervez MA, Abbas T, Ashfaq M, Ghazanfar U, Afzal M, Rashid A, Garcia-Sanchez F, Mattson NS (2012) Differential response of pea (*Pisum sativum* L.) genotypes to salt stress in relation to the growth, physiological attributes antioxidant activity and organic solutes. Aust J Crop Sci 6:828–838

Sharma P, Dubey RS (2005) Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regul 46:209–221. https://doi.org/10.1007/s10725-005-0002-2

Shimamura S, Nishimura T, Koshiba T, Yamamoto R, Hiraga S, Nakamura T, Komatsu S (2016) Effects of anti-auxins on secondary aerenchyma formation in flooded soybean hypocotyls. Plant Prod Sci 19:154–160

Sinclair TR, Horie T (1989) Leaf nitrogen, photosynthesis and crop radiation use efficiency: a review. Crop Sci 29:90–98

Specht JE, Diers BW, Nelson RL, Francisco J, De Toledo F, Torrison JA, Grassini P (2014) Soybean. In: Smith S, Diers B, Specht J, Carver B (eds) Yield grains in major U.S Field Crop. ASA, CSSA, and SSSA, Madison, WI, pp 311–356

Sponchiado BN, White JW, Castillo JA, Jones PG (1989) Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Exp Agric 25:249–257

Sthapit BR, Witcombe JR, Wilson JM (1995) Methods of selection for chilling tolerance in nepalese rice by chlorophyll fluorescence analysis. Crop Sci 35:90–94

Stoddard FL, Balko C, Erskine W, Khan HR, Link W, Sarker A (2006) Screening techniques and sources of resistance to abiotic stresses in cool-season food legumes. Euphytica 147:167–186

Stones IR, Horton ML (1974) Estimating evapo-transpiration using canopy temperature: field evaluation. Agron J 66:450–455

Suhre JJ, Weidenbenner NH, Rowntree SC, Wilson EW, Naeve SL (2014) Soybean yield partitioning changes revealed by genetic gain and seeding rate interactions. Agron J 106:1631–1642. https://doi.org/10.2134/agronj14.0003

Taiz L, Zeiger E (2010) Plant physiology. Sinauer Associates Inc., Sunderlands, MA

Tuberosa R (2012) Phenotyping for drought tolerance of crops in the genomics era. Front Physiol 3:347

Van Schaik PH, Probst AH (1958) Effects of some environmental factors on flower production and reproductive efficiency in soybeans. Agron J 50:192–197

Waisel Y, Eshel A, Kafkafi U (2002) Plant roots: the hidden half, 3rd edn. Marcel Dekker, New York, p 1120

Wang C, Wu T, Sun S, Xu R, Ren J, Wu C, Han T (2016) Seventy five years of improvement of yield and agronomic traits of soybean cultivars released in the Yellow-Huai-Hai river valley. Crop Sci 56:2354–2364

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011

Xiao YG, Qian ZG, Wu K, Liu JJ, Xia XC (2012) Genetic gains in grain yield and physiological traits of winter wheat in Shandong province, China, from 1969 to 2006. Crop Sci 52:44–56

Xu Y, Crouch JH (2008) Marker-assisted selection in plant breeding: from publications to practice all rights reserved. Crop Sci 48:391–407

Zhang JY, Broeckling CD, Blancaflor EB, Sledge MK, Sumner LW, Wang ZY (2005) Overexpression of wxp1, a putative medicago truncatula ap2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (medicago sativa). Plant J 42:689–707

Sunn Hemp: A Climate-Smart Crop

13

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Abstract

Sunnhemp (*Crotalaria juncea* L.), primarily a crop of tropical and subtropical parts of the world, is a multipurpose leguminous crop valued for diversified uses. Fibre extracted from its bark is the main economic product. Its fibres are used in the preparation of ropes, cordage, fishing nets, twines, etc. Chemical attributes of its fibres, namely high cellulose and negligible lignin content, make it an excellent source of raw material for paper and pulp industries and specialty paper industries in its fibres. It is used for culinary and forage purposes to a limited extent. It is immensely popular as a green manure and cover crop in different parts of the world. It improves soil physical, chemical and biological properties in a natural way. It adds a large amount of organic matter into the soil. The nitrogen fixed by the symbiotic process is able to meet a large fraction of nitrogen requirements even to the succeeding crop. It is a potential tool for Integrated Pest Management (IPM) on account of its inherent antihelminthic properties. It has the potential to be used in bio-fuel and in bio-composites/geotextiles. It is an excellent carbon sequestration agent. It has the potential to reduce the usage of synthetic chemicals in terms of fertilizers, insecticides, etc. from the present agricultural system. In addition to providing employment to rural households and sustainability to the

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agricultural system, it minimizes the ill-effects of modern commercial agriculture, thereby offering several ecological advantages and holding special relevance in mitigating the effects of climate change.

Keywords

Sunnhemp · Climate change · Mitigation · Sustainable agriculture

13.1 Introduction

Sunnhemp (*Crotalaria juncea* L.), utilized in many ways in Indian households and industries, is a native of India and offers several economical and ecological advantages to the farmers and environment, respectively. It is a diploid, annual species with a disomic chromosome number of 16. It is a member of Leguminosae family and crotalarieae tribe. It is the only cultivated species in the tribe crotalarieae. India is the major producer of sunnhemp. It is widely distributed in tropical and subtropical regions of India, Brazil, Bangladesh, Nepal, Sri Lanka, Southern Africa, etc. (Chaudhary et al. 2016). In India, it is cultivated in almost all the states, particularly for green manure purposes. Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Andhra Pradesh, Rajasthan, Maharashtra, Karnataka are the major sunn hemp producing states.

Sunnhemp is integral to Indian civilization since prehistoric times (Montgomery 1954). The archaeological evidence indicates its mention during the period of Indus civilization (Good 2007) and in the literature of the sixteenth century by Bhavamisra under the name 'shanapushpika' (Chunekar and Pandey 1998) and in 'Ain-I-Akbari'. It finds its mention in different names as shahnapushpika, sana, etc. as early as 400 BC. The mention of fibre plant with yellow fibres in Ain-I-Akbari is considered to be referred to sunnhemp only (Sarkar et al. 2015). 'Law of Menu' describes it as 'Pabitra Rasa'. Ancient literature mentions that the sacred thread (janeu) worn by Kshatriyas should be made of sunn hemp.

13.2 Economic Importance

Sunnhemp is a multipurpose crop with diversified uses ranging from household usage to specialized industries. Being a member of Leguminosae family, its root characteristically harbours nitrogen-fixing rhizobium bacteria enriching the soil with organic nitrogen. Its rapid growth, extensive foliage, high biomass and ideal C:N ratio in its plant parts deems it perfect for cover crop and green manure crop. It has unparalleled weed-suppressing ability owing primarily to its growth characteristics and allelopathic effect to some extent. Fibres extracted from its stem are used in making ropes, cordage, fishing nets, twines, etc. for household uses. Its fibres are resistant to the action of water and are supposed to be stronger when wet. Its flowers are relished as vegetables and pakoras (fritters) in the states of Jharkhand and Uttar

Pradesh. Succulent parts of the plants are often used as forage albeit to a limited extent due to the presence of pyrrolizidine alkaloids harmful to animals (Mannetje 2004) predominantly found in flowers, pods and seeds. Garzon et al. (2011) reported that sunn hemp harvested during the vegetative growth stage (no seeds present) might be a viable feedstock for cattle production. Different varieties/accessions are reported to have variable alkaloid content (Ji et al. 2005). Hence, the selection of varieties and stage of crop must be taken care when the crop is used for forage purposes. Paper and pulp industries use it for handmade paper, cigarette paper, tissue paper, currency paper, etc. High protein content (30–35%) in its seeds makes it suitable as a source of adhesive for plywood industries.

13.3 Botanical Description

Sunnhemp is a member of genus crotalaria genus comprising about 600 species, of which *C. juncea* (sunn hemp) is the only cultivated species. It is an annual, short day, erect herbaceous plant and its plant height ranges from 2.5 to 3.0 m. It has a tap root system with root nodules – the characteristic feature of the family. The stem is erect, ribbed, hairy and succulent. The leaves are simple, stipulated, entire, oblong, alternate, pubescent and spirally arranged. The inflorescence is a raceme with an indeterminate flowering habit. Its flowers are bisexual, zygomorphic, pentamerous with a typical standard floral arrangement. Its flowers have 10 free stamens (in two whorls of five each) and gynoecium is represented by a single pistil. Its pods are cylindrical in shape containing 6–12 seeds. Seeds are small, kidney-shaped, flattened, yellow and dark brown to black in colour. The flowers, pods and seeds of sunnhemp are depicted in Fig 13.1. Insect-mediated cross-pollination is almost a rule in sunn hemp which can be ascribed due to self-incompatibility (Bhandari et al. 2016; Jitendramohan 1971). Pollination is brought about by insect visitors having relatively large bodyweight like *Xylocopa fenistroides*, *X. latipies* and *Megachile lanata* (Jitendramohan 1971; Abrol 1993).

13.4 Current Status of Crop

Sunnhemp cultivation remained an important part of every household till the popularization of synthetic fibres in India. The advent of Green revolution in 1960s in India ushered in commercial agriculture dominated by important cereal and cash crops (like paddy, wheat, cotton, sugarcane). Availability of irrigation led to multiple cropping of these crops and the area under sunnhemp shrank considerably from nearly 200,000 hectares in 1960s to merely 10,000 hectares in 2017–2018. In contrast, a sharp improvement in yield level is noted since 2009–2010 and maximum yield levels were recorded during 2017–2018 (Fig. 13.2). Some of the underlying reasons for reduction in area under its cultivation are (i) competition from major crops, (ii) availability of synthetic fibres, (iii) unavailability of water bodies for retting, (iv) tedious retting process, (v) unavailability of quality seeds, (vi) grazing

Fig. 13.1 Flower, pods and seeds of sunn hemp

problem by stray animals particularly Nilgai in northern India, (vi) unorganized market, etc. With increased awareness on sustainable farming, its demand has surged for green manuring purposes.

13.5 Breeding and Varietal Improvement

Varietal improvement in sunnhemp has been an arduous task on account of its complex floral and breeding behaviour. Though all the structural (bisexual, complete flowers) and temporal floral features (synchrony in anther maturity and stigma receptivity, pollen dispersal before anthesis of flowers) are indicative of the possibility of self-pollination, selfing results in failure of pod formation and insect-mediated out-crossing is almost a rule in this crop. This self-incompatibility may be ascribed due to the presence of membrane on stigmatic surface, a characteristic feature of the tribe crotalarieae. The insects visiting the flowers bring about pollination by rupturing this membrane. However, only the insects with larger bodyweight (Megachile spp., Xylocopa spp.) are effective pollinators as their weight causes the stigma to protrude out upon landing of insects on flowers, leading to contact of stigma with body parts of insects (Bhandari et al. 2016; Rakesh et al. 2019). In some

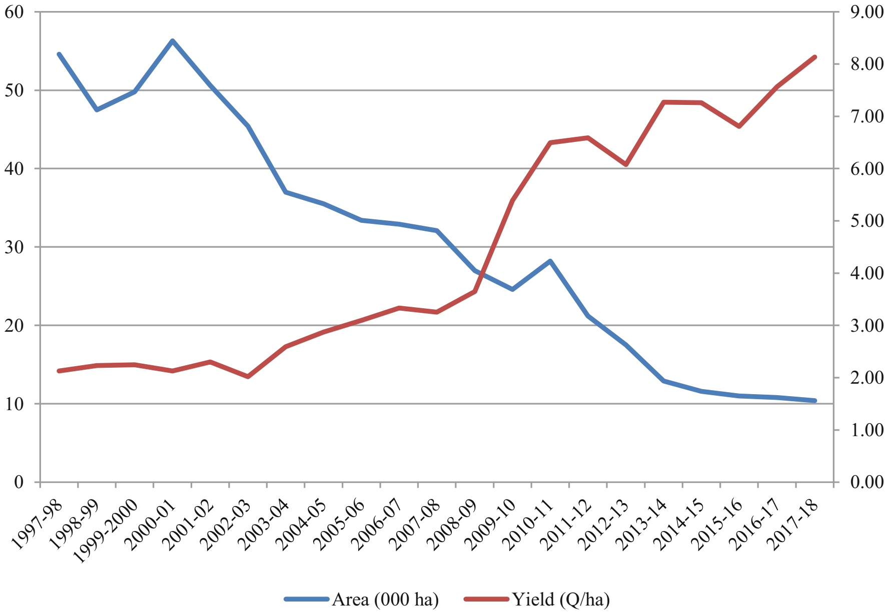

Fig. 13.2 Trend on production and productivity of sunn hemp fibre crop (Source: DAC and FW 2019a, b)

cases, however, delayed autonomous self-pollination has been reported in absence of pollinator insect visits (Kumar et al. 2019).

The complexity of self-incompatibility coupled with extensive out-crossing limits the choice of breeding methodologies in sunnhemp, and the mass selection or random mating is exclusively used in variety development in this crop. Other techniques like mutation, polyploidy and inter-specific hybridization have remained largely unsuccessful. Maintenance of genetic purity in sunnhemp requires large physical isolation between different varieties. Temporal isolation is not effective because of photo-sensitive nature of its varieties which flower simultaneously under short days.

As the crop has diversified uses, breeding objectives varies with its end uses. For fibre crops, the major breeding objectives are high fibre yield, quality fibres, early maturity and tolerance to biotic stresses. Rapid growth rate and high biomass are the target traits for green manure and cover crops. Enhanced root nodulation and increased nitrogen fixation are the major target traits for developing varieties suitable for green manuring ability which can be achieved through screening and selection of genotypes possessing higher nodulating capacity and symbiotic tolerance towards nitrate – the limiting factor in root nodulation. Mutation can be utilized for enhanced root nodulation. Wild species of crotalaria can be used as donors for introgressing better root nodulating capacity and higher biomass.

Initial varietal development works resulted in some improved strains like K-12 (also termed as K-12 black), M-35, M-19, T-6, Chhindwara, etc. Variety

Table 13.1 List of improved varieties of sunn hemp in India

S. No.	Variety	Year of release	Features
1.	K 12 Black	1926	100–120 days for fibre purpose, wilt and shoot borer resistant
2.	K 12 Yellow	1971	Selection from K-12, wilt resistant, wide adaptability, Good fibre quality (16.04 g/tex)
3.	SH-4 (Sailesh)	2005	Suitable for Uttar Pradesh, Madhya Pradesh, West Bengal, Good fibre quality (15.49 g/tex)
4.	SUIN 053 (Swastik)	2009	High yielding variety, has wider adaptability
5.	SUIN 037 (Ankur)	2013	90–100 days for fibre purpose, 140–145 days for seed purpose, Resistant to biotic and abiotic stresses
6.	JRJ 610 (Prankur)	2015	Suitable for irrigated and rainfed conditions, Better fibre quality (13.19 g/tex)
7.	SUIN 03 (Kavita)	2019	Suitable for both irrigated and upland conditions, better fibre quality (16.40 g/tex), tolerant to biotic stresses particularly wilt

development programmes received major impetus in the last two decades. The major work in this direction was carried out at Sunnhemp Research Station (ICAR-CRIJAF), Pratapgarh, Uttar Pradesh. Some of the varieties suitable for fibre purpose are listed below (Table 13.1).

In the United States, the variety ‘Tropic Sun’ was released by the University of Hawaii and USDA in 1982. Auburn University released ‘AU Golden’ and ‘AU Durbin’ varieties. The varieties ‘Crescent Sunn’ and ‘Ubon’ were developed in Ubon Ratchathani, Thailand. The varieties CO-1, PAU 1691 and ND-1 were released for green manuring purposes.

13.6 Cultivation

The crop is primarily suitable for tropical and sub-tropical areas and can be grown in the summer and rainy seasons. It cannot withstand low temperatures and frost conditions (Korrapati and Manasa 2017). However, the possibility of sunnhemp cultivation in Virginia and mid-Atlantic regions of the United States was demonstrated by Bhardwaj et al. (2005). It is a highly photosensitive crop and grows vertically with no branches under long-day conditions. Under short-day conditions, its vertical growth is arrested and the reproductive phase is initiated. When grown for bast fibres (extracted from its stem), tall, erect ideotype is favoured. Branching is not desirable as it causes defects in fibre characteristics. This type of growth is favoured by long days and high temperatures. Hence, fibre crop is grown in the summer season (April–May), though it can be grown in kharif season also. The crop sown in the summer season is not suitable for seed purposes due to different photo-period requirements. In contrast, pre-rabi season (Mid-Aug) is preferred for seed production. The prevalent photo-period and other environmental factors favour profuse flowering and pod setting. Accordingly, agronomic practices for fibre crops and seed crops are entirely different in this crop.

13.6.1 Fibre Crop Cultivation

Summer season is most suited for fibre crops. Fibre crop requires 30–35 °C temperature and nearly 400 mm rainfall. Well-drained alluvial light soils (loam or sandy loam) are most suitable. It requires little moisture. Excess moisture or water logging is harmful to the crop. Neutral to slightly alkaline soil is considered good for better root nodulation. Lower or acidic pH hampers nodule formation. It requires little nitrogen in the early seedling stage. In addition to major nutrients (nitrogen, phosphorous and potassium), calcium, magnesium, zinc, manganese, molybdenum, cobalt, iron, and boron favour its growth. The land should be ploughed and levelled well. Organics like farmyard manure or poultry manure at the rate of 5 tonnes per hectare must be applied for enhanced yield. The optimum sowing time for fibre purposes is mid-April to first week of May under north Indian conditions (Tripathi et al. 2013a). Row-to-row spacing of 15 cm and plant-to-plant distance of 10 cm is ideal for high fibre yield (Tripathi et al. 2013a). At this rate, the seed requirement is 30–40 kg per hectare. Application of rhizobium, phosphorous solubilizing bacteria can reduce the use of inorganic fertilizers (Tripathi et al. 2013c). Seeds should be sown at depth of 3–5 cm. Fertilizers should be applied at the rate of 20:40:40 kg NPK per hectare. Its roots develop nodules that harbour nitrogen-fixing bacteria and hence nitrogen is applied as the basal application only. It is fast-growing crop and develops extensive foliage within a very short span of time and suppresses weed efficiently except nut sedges. Hence, weeding is generally not required. Two-three light irrigations are required.

The fibre crop gets ready for harvest within 90–120 days. Tripathi et al. (2013a) found similar fibre yield when the crop is harvested at 90, 100, 110 and 120 days of sowing. Fibre quality and yield are reduced if the plant is allowed to age beyond 120 days. The crop is harvested at the ground level and the top portion of the plants is cut. Then the plants are bundled and left for one night. The next day, the bundles are put in ponds for retting. Retting in flowing water is beneficial as it results in quality fibre.

The bundle of plants is tied and loaded with some heavy matter (like bricks, stones, etc.) so that the bundle remains submerged in water to a depth of 10–15 cm. The fibre can be extracted after 72 h which depends upon the prevailing temperature and microbial load of the water. The sunn hemp plant contains nearly 3% fibre on a total green weight basis and nearly 13% on a dry weight basis (Chaudhury et al. 2000).

The fibre bundles in sunnhemp originate from primary vascular bundles and are termed as primary fibre. Extraction of fibres is done stalk-by-stalk from the lower end upwards. The fibre is washed in water and dried under the sun. Different steps in retting and fibre extraction are depicted in Fig. 13.3. On an average, 10 q fibre per hectare can be obtained. Quality of fibres depends on stage/age of the crop at harvest

Fig. 13.3 (**a**) Fibre crop. (**b**) Bundling and steeping. (**c**) Fibre extraction. (**d**) Fibre

and retting conditions, Its fibres have a fineness of 5.5–17.0 tex and tenacity of 30–40 g/tex. Its fibres have high cellulose (78.3%), low lignin (4.0%), low nitrogenous matter (1.4%) and negligible ash content (0.3%).

13.6.2 Seed Crop Cultivation

The timely availability of quality seeds in adequate quantity is a must for realizing the genetic potential of a variety of particular crop. Availability of quality seed of sunnhemp in India is one of the main constraints underlying reduced acreage under the crop (Chittapur and Kulkarni 2003). In India, its seed production is localized and not organized. Farmers generally meet their seed requirements from their seeds produced by them in small patches of otherwise unutilized lands, bunds, etc. Seed production of sunnhemp is confined to some hilly areas of Uttar Pradesh (Mirzapur, Sonbhadra) and Madhya Pradesh states.

In India, maintenance and multiplication of seeds of improved varieties of sunnhemp have been entrusted to Central Seed Research Station for Jute & Allied Fibres (Burdwan, West Bengal), under the aegis of Central Research Institute for Jute & Allied Fibres (ICAR-CRIJAF), Kolkata, West Bengal. The agro-techniques for seed production in India have been standardized at Sunnhemp Research Station (ICAR-CRIJAF), Pratapgarh, Uttar Pradesh.

Maximization of seed yield requires less vertical growth with increased lateral branching. This leads to more number of flowers and pods per plant. Seed crop requires well-prepared, levelled land. Heavy textured, acidic soils and low-lying lands should be avoided. Soils rich in calcium and phosphorous are considered good. The seed crop requires hot and humid conditions initially for vegetative growth and later on low temperature and humidity during the reproductive phase. Climatic factors of temperate regions hinder seed production (Mosjidis and Wehtje 2011). The problem of unavailability of seed in the United States was overcome by the development of two varieties 'AU Golden' and 'AU Durbin' which were able to flower and set seed in subtropical/temperate areas of continental USA (Mosjidis et al. 2013).

The agronomic practices may differ slightly in different locations. The optimum sowing time of sunnhemp seed crop is within the first fortnight of August. In southern India, where sunshine is more and temperatures are high, sowing can be delayed up to September. Delay in sowing causes a considerable reduction in yield. Row-to-row spacing of 45 cm and plant-to-plant distance of 20 cm is preferred to induce an increased number of branches, number of pods per plant, pod weight, number of seeds per pod and consequently higher seed yield per plant (Tripathi et al. 2013b). At this spacing, 25 kg of seed per hectare is required. Fertilizers should be applied at the rate of 20:40:40 kg NPK per hectare. An inter-row spacing of 30 cm and inter-plant spacing of 15 cm and fertilizer dose of 20:60:60 kg NPK per hectare were found optimum in Aduthurai, Tamil Nadu (Kavin et al. 2018). Two-three light irrigations as per the requirement may be provided. The process of topping is often adopted in sunnhemp where the apical portion of the crop is removed at 40–45 days of crop stage to suppress apical dominance and induce lateral branches. In a late sown crop, it is generally not required. Irrigation at the flowering/pod formation stage is a must to ensure higher yields and quality seeds. In the initial stage, one weeding may be required which can be performed manually or through a nail weeder.

Flowering starts at 60–655 days of crop age. The blooming crop of sunnhemp is depicted in Fig. 13.4. Foliar spray of micronutrients at the flowering stage enhances seed yield (Mandal et al. 2017). It is recommended to avoid insecticide spray during the flowering stage as insects are the main agent for pollination in this crop. The seed crop becomes ready for harvest in nearly 150 days after sowing. After harvesting,

Fig. 13.4 Seed crop of sunn hemp

threshing, winnowing should be done before storage. The moisture content in the seed should be nearly 10% before storage. Seed yield of 12–15 quintals per hectare can be obtained following all the recommended packages of practices.

Maintenance of genetic purity demands special attention in seed production, which becomes a herculean task in this crop on account of its obligatory out-crossing behaviour. Seed production should be preferred in areas where the crop is not grown commercially. Seed production of plots of different varieties should be sufficiently isolated to avoid insect-mediated genetic contamination. Roguing of off-types is a must for genetic purity maintenance.

13.6.3 Green Manure Crop

In modern agriculture, sunnhemp is highly valued for the purpose of green manure. Ram and Singh (2011) mentioned it as one of the most outstanding green manure crop suitable to almost all parts of India. Sunnhemp as a green manure crop can be sown before 1–1.5 months prior to main crop. Most of the varieties released for fibre purposes can be utilized for this purpose. Some varieties bred specifically for green manure purposes are CO-1 developed at Tamil Nadu Agricultural University (Coimbatore, India), PAU 1691 developed at Punjab Agricultural University (Ludhiana, India), ND-1 developed at Narendra Dev University of Agriculture & Technology (Faizabad, India). Dense planting (40–50 kg seed per hectare) is better for this purpose. Nearly 60 kg of seed rate has been found suitable in sweet potato (Ossom et al. 2011). Cho et al. (2013) reported that seed rate of 50 kg per hectare was enough for this purpose. They demonstrated that among its plant parts, the flower had maximum nitrogen content followed by leaves, roots and stems. One-month-old crop can be incorporated into the soil. Sunn hemp biomass requires nearly 5–7 days for degradation depending upon prevailing temperature and soil moisture.

13.6.4 Protection Measures

Several insect pests and diseases attack the crop and reduce the yield. Some of the important insect pests and their control are listed in the Table 13.2 below.

13.7 Importance of Sunnhemp in Context of Climate Change

Recent decades have evidenced many turbulent changes in climatic conditions worldwide. Temperatures have risen abnormally high during summer; rainy seasons have experienced uneven rains and winters are abnormally hot. These changes have been attributed to global climate change which, in turn, is dependent on the

Table 13.2 Important insect pests and diseases of sunn hemp and their management

S. No.	Name of the insect pest/diseases	Symptom/damage	Control
1.	Top shoot borer (*Cydia tricentra* Meyr)	Larvae bore into the top portion of young plants, Apical buds distorted into galls, Stunted plants,	Deep summer ploughing, foliar spray of neem-based formulation @ 2 ml/L. Two sprays of profenophos 2 ml/L at 15 days interval.
2.	Hairy caterpillar/Bella moth (*Utethesia pulchella* Linn.)	Feed on the folige and skeletonize them completely, Bore into pods and damage the seeds	2 sprays of profenophos @ 2 ml/L or cypermethrin 10EC @ 1 ml/L
3.	Vascular wilt (*Fusarium udum* f. sp. crotalariae)	Whithering plants, drooping leaves, wilting of plants, drying of stem	Avoid waterlogging, Application of neem cake in soil @ 5 t/ha, Seed treatment with carbendazim @ 2 g/kg of seed, Adoption of crop rotation, Timely sowing (April–May)
4.	Anthracnose (*Colletotrichum curvatum*)	Discoloured spots on leaves including cotyledonary leaves, Falling off of leaves	Spray of bavisitn @ 2.5 g/Litre of water
5.	Leaf mosaic	Patches of yellow and green colour on leaves, crinkled leaves, reduced plant growth	Seed treatment with imidachloprid 1 g/kg of seed, Spray of imidachloprid @ 0.4 ml/L to control vectors, Timely sowing in dry season (April–May), uprooting and burning of severely infected plants

greenhouse gas (GHG) concentrations in the atmosphere. Some of the major indicators of climate change are: (i) increased global temperature, (ii) rapid melting of glaciers, (iii) increased water levels of the ocean and (iv) increased GHG emissions. Agriculture has been both the culprit and victim of climate change. On one hand, it leads to pollution by the way of agrochemicals, it is also affected greatly by the events of climate change. Agriculture production and yield levels are declining due to various factors.

With the advent of the green revolution, there has been a strategic shift in the use of agriculture inputs. The driving factor of the green revolution has been the input-responsive genotypes. Consequently, agricultural inputs become mandatory in realizing the yield gain. Several types of synthetic agricultural chemicals started to be used in agriculture. These include synthetic fertilizers, pesticides (including fungicide, insecticide and nematicides), etc. These became essential in due course of time as the natural sources were unable to meet the huge demand and had gradual effects. Thus these chemicals proved to be a boon to agriculture.

Later, the ill-effects of these synthetic chemicals gradually started appearing in the form of degraded soil, polluted water bodies, etc. These synthetic chemicals contribute to environmental pollution either directly or indirectly. Direct pollution is in the form of energy consumption and pollutants released from manufacturing units. Many types of suspended particulate matters and gases emitted raise serious concerns (Thakkar 2013). Indirect pollution is caused by agrochemicals consequent upon their indiscriminate usage. Much of the applied chemicals undergo several types of losses and reach water bodies thereby causing harm to various life forms by eutrophication or other ways.

Sunnhemp can be employed to mitigate change by various ways. In India, it is cultivated by rural people for their household purpose, like green manuring, for fibres and for forage to a limited extent. It can be employed as a tool in sustainable agriculture, organic farming, integrated nutrient and pest management (Sarkar et al. 2015). In the following paragraph, a detailed information regarding sunnhemp is given in the context of mitigating climate change.

13.7.1 As a Substitute for Synthetic Fertilizers

Total synthetic fertilizer use in the world is increasing at the rate of 1.9% and that of N fertilizers use around 1.6% annually (International Fertilizer Industry Association 2015). India ranks second in consumption of nitrogenous fertilizer and accounts for 15.3% of the world's share (International Fertilizer Industry Association 2015). India needs to incur an expenditure of nearly 8000 crore Rs. per year for import of urea (DAC and FW 2019a, 2019b). Synthetic fertilizers have helped in making our nation self-reliant in food production. However, its consequences like unbalanced soil structure, change in soil pH towards acidic range, air and water pollution from effluents from the manufacturing units, water pollution from run-off of chemicals, loss of nitrate fertilizer by various ways, retardation of soil-microbial activity, etc.

have been realized recently. The loss of nitrate fertilizer may go upto the extent of 80% in case of submergence (Sahu and Samant 2006).

Urea is the major fertilizer used indiscriminately in India. The urea manufacturing units contribute to environmental degradation by the emission of ammonia and particulate matter. Uncontrolled discharge of particulate matter may be of the order of 23–109 kg per tonne of urea manufactured. Ammonia is the major raw material used for the manufacture of urea. Nearly 80% of the total energy required in the manufacture of urea goes towards the synthesis of ammonia. Several greenhouse gases like CO_2, NO_x, SO_2 and CO are emitted to the extent of 500 kg per tonne of ammonia (World Bank Group 1998). These gases are hazardous and add to Green House Gas effect.

In the above context, sunnhemp can be employed as an alternate and organic source of nitrogen as it is the best suitable crop for green manuring (Sarkar and Ghoroi 2007). It is reported to add nearly 80–120 kg of nitrogen per hectare. It also adds a large amount of biomass (15–20 t/ha) to the soil and enhances soil-microbial activity. The large amount of nitrogen fixed can meet recommended fertilizer demands for different crops, particularly in the sub-tropical and temperate regions (Ashworth et al. 2016). Upto half of the nitrogen requirement of vegetable crops can be met by sunn hemp (Schomberg et al. 2007). Ambrosano et al. (2013) reported a complete nitrogen substitution in sugarcane by sunn hemp biomass. Thus it can reduce a sufficiently large amount of synthetic nitrogen fertilizers from our agricultural system. Further, it is capable of supplying nitrogen (in organic form) to immediate and succeeding crops which are released gradually.

13.7.2 As a Soil Amendment Agent

Intensive cropping, monoculture practices and indiscriminate use of synthetic fertilizers have degraded soil physical, chemical and biological properties, which need to be restored to attain higher yield sustainably. In this direction, sunn hemp can be efficiently employed as it has many beneficial effects on soil. Its biomass has C:N ratio in ideal range (Wang et al. 2011) and promotes microbial activity. Ambrosano et al. (2013) reported a positive effect of sunn hemp crop on soil physical and chemical properties. Soil becomes pulverous and porosity increases upon incorporation of sunn hemp biomass into the soil. Its biomass improves dry aggregate stability (Muzyamba et al. 2021), Ca and Mg contents, pH, base saturation, cation exchange capacity, and decreases the potential acidity of the soil. It adds other minerals and also helps in the uptake of other minerals like phosphorous and potassium (da Silva et al. 2012). Sunn hemp incorporation into soil results in the enhancement of free-living and beneficial bacterivorous nematodes (Marahatta et al. 2012). These free-living nematodes assist in nutrient recycling (Wang et al. 2004).

Table 13.3 List of nematode species controlled by sunn hemp

S. No.	Crop	Nematode species	References
1.	Banana	Radopholus similis	Chitamba et al. (2014)
		Meloidogyne incognita	Henmi and Sharadchandra (2015)
2.	Pineapple	Rotylenchulus spp.	Wang and Sipes (2009)
		Meloidogyne incognita	
3.	Okra	Meloidogyne incognita	Wang et al. (2006)
4.	Tomato	Meloidogyne incognita	Kankam et al. (2015)
5.	Sugarcane	Meloidogyne javanica	Berry and Wiseman (2003)
6.	Squash	Quinisuculcius acutus	McSorley et al. (2009)

13.7.3 As a Substitute of Helminthicides

Many studies have reported that sunn hemp possesses anti-helminthic properties, thereby controlling and reducing nematode population and density in many crops. This anti-helminthic property is due to a compound called ‘monocrotaline’ released upon incorporation of biomass (especially foliage) in soils. This compound is reported to kill several species of nematode notably Meloidogyne sp. and Radopholus similis (Garzon et al. 2021; Wang et al. 2002). Sunnhemp being suitable for rotation with many crops can be used in reclaiming nematode-infested soils. Some examples of nematode suppressed by sunnhemp are listed in Table 13.3.

Hooks et al. (2006) mentioned the mechanism of nematode suppression by sunn hemp on account of (i) being a poor or non-host of a majority of plant-parasitic nematodes, (ii) producing chemicals toxic to nematodes, (iii) producing chemicals that are inhibitory to nematode reproduction and development, (iv) stimulating activities of other soil microbes that compete with or attack the nematodes, and (v) serving as a trap crop, attracting nematodes present in the soil.

13.7.4 As a Cover Crop

On account of its rapid growth rate, it develops extensive foliage coverage within 45–60 days of sowing. Its high growth rate and extensive foliage coverage have tremendous potential in suppressing weed and use as a cover crop (Schomberg et al. 2007). It provides an alternative to herbicide application for the control of weeds. Mosjidis and Wehtje (2011) reported weed biomass suppression to the extent of 50% at a plant density of 20–50 plants/m^2. Further, it possesses allelopathic effects on other crops/weeds by secreting a non-protein amino acid (δ-hydroxynorleucine), which is phytotoxic, inhibits hypocotyl and radicle growth during germination (Wilson and Bell 1979), thereby reducing weed development. It is reported to reduce weed on subsequent crops also. It reduces seed germination and seedling length of different crops when sown immediately after incorporation of its foliage in soil (Skinner 2012). Hence, a proper waiting period is required before sowing the next crop. Javid et al. (2015) demonstrated that all biomass from this species could potentially be used for weed suppression purposes, but leaf tissue could be the most useful for this purpose. It has the advantage of being a poor host of different insects like fall armyworm and moth pests (Meagher et al. 2004). Effective control of different weeds has been reported using this crop (Collins et al. 2007).

13.7.5 As a Source of Raw Material for the Paper Industry

Traditionally wood fibres are used for the paper and pulp industry. However, forest resources are depleting at an alarmingly high pace. Paper industries are highly taxing on environmental quality as they may lead to deforestation, the consequences of which are known to all. Forests are required for pollution-free environment as they sequester a large amount of carbon. Further, pulping of wood fibres is costlier on account of their high lignin content. Availability of wood fibres is a major constraint for paper industries as most of the forests are government-owned. A large number of raw material requirements are met by a few agroforestry units or forest fellings. Hence, non-wood fibres are a better alternative for pulp industries. India is the second-largest country in the use of non-wood fibres for pulp. Sunnhemp is a potential source of fibre for pulp on account of high cellulose (78%) and low lignin content (4%) and can provide the raw material for a major portion of the year. Its fibres are also used in making hand-made papers, currency papers, cigarette papers and tissue paper. The papermaking potential of sunnhemp has been well documented (Cunningham et al. 1978; Saikia et al. 1997; Lai et al. 1967; Mishra 1963; Nelson et al. 1961; Nieschlag et al. 1960) from whole plants and from decorticated fibres (Medina et al. 1961). Saikia et al. (1997) reported higher pulp yield from sunn hemp and desirable characteristics like tensile index and folding endurance in paper made from its pulp. In some parts of India like Rajasthan, ancient documents are being rewritten on paper made from sunnhemp. Chandra (1998) studied the pulping capacity of different non-wood fibres and found an equivalent bleached pulp yield of 0.7–2.5 tonnes per hectare from sunnhemp.

13.7.6 As a Source of Biofuel

Biofules are considered important alternatives/supplements of petroleum-based fuels which are the major pollutants. In recent years, much emphasis has been given on biofuel with an aim to reduce pollution. In this context, many crops have been evaluated as a source of bio-ethanol and bio-diesel and are being used commercially. Sunnhemp is a potential biofuel crop on account of high biomass (15–20 t/ha), providing a large amount of cellulose and hemicellulose. This biomass is capable of producing nearly 80–90 GJ of equivalent energy which is equivalent to nearly 620 gallons of gasoline (Perry 2012). Other candidate crops have nearly 30–150 GJ of energy. Further, the heating value of sunnhemp is higher than that of switchgrass, bermuda grass, alfalfa, etc. and has the ability to release greater amounts of volatile matter at a faster rate (Cantrell et al. 2010). Sadhukhan and Sarkar (2016) demonstrated the feasibility of making bio-diesel from sunnhemp oil.

13.7.7 In Biocomposite and Geo-Engineering

Sunnhemp fibres have some of the ideal mechanical properties and hence, can be blended with industrial synthetic fibres (Singh et al. 2015). It can be blended with cotton to weave fabrics for various applications like curtains, sofa covers, canvas, floor matting, etc. It can also be used for preparing different household articles, toys, sports items, false ceilings, etc. Its fibres have the potential as reinforcing material in waste polythene bags to produce inexpensive thermoplastic composite materials with high toughness for various applications like door panels, furniture, packaging, etc. and thereby can reduce environmental pollution due to waste polyethylene bags (Chaudhary et al. 2010). Its fibres are durable and possess fire- and flame-retardant properties and hence sunn hemp fibre-based or blended fabrics can be used in insulation, packaging and mulching (Sengupta and Debnath 2018). It can be used in construction like building and roads, etc. It can also be used in slope management, erosion control, soil conservation and stabilization of earthen embankments.

13.7.8 As a Carbon Sequestration Agent

Terrestrial vegetation is one of the methods for carbon sequestration. On account of its high photosynthetic capacity coupled with rapid growth rate, it can fix a considerable amount of carbon and reduce CO_2 concentration in the atmosphere. Sunnhemp is reported to sequester a large amount of carbon (481 g/m^2). Nearly 58% of the carbon fixed in soil is retained after 127 days of decomposition (Wang et al. 2010). In this way, the crop fixes carbon and converts it into soil organic carbon with higher stability.

13.8 Future Prospects

In recent times, sunnhemp crop has attained much importance among agricultural crops due to its potential diverse uses. Its fibres are of high quality and better than other fibre crops. It is highly valued as a green manure crop with the potential of reducing the input cost and increasing profit. It has the potential to reduce synthetic chemicals from the agricultural system and thereby reduce environmental degradation. It is the inseparable component of sustainable agriculture. Its utilization in non-conventional areas (like biofuels, pulp industry, etc.) will further make it more popular. With increasing demand of this crop in modern agriculture, seed production needs to be strengthened and organized. In addition to yield and quality, the breeding programme needs to be focused on specialty traits like enhanced nitrogen fixation, increased biomass, pulp yield, increased biofuel yield, etc.

References

Abrol DP (1993) Insect pollination and crop production in Jammu and Kashmir. Curr Sci 65(3): 265–269

Ambrosano EJ, Cantarella H, Ambrosano GMB, Dias FLF, Rossi F, Trivelin PCO, Muraoka T (2013) The role of green manure nitrogen use by corn and sugarcane crops in Brazil. Agril Sci 4(12A):89–108

Ashworth AJ, Keyser PD, Allen FL, Tyler DD, Taylor AM, West CP (2016) Displacing inorganic nitrogen in lignocellulosic feedstock production systems. Agron J 108:109–116

Bank Group. (1998) Pollution prevention and abatement handbook. http://www.ifc.org/wps/wcm/connect. Assessed on 17/04/2016

Berry SD, Wiseman EJ (2003) The effect of green manure crops on plant parasitic nematodes in the South African sugar industry. Proc S Afr Sug Technol Ass 77:114–117

Bhandari HR, Tripathi MK, Chaudhary B, Sarkar SK (2016) Sunnhemp breeding: challenges and prospects. Ind J Agril Sci 86(11):1391–1398

Bhardwaj HL, Webber CL, Sakomoto GS (2005) Cultivation of kenaf and sunn hemp in the mid-Atlantic United States. Ind Crop Prod 22:151–155

Cantrell KB, Bauer PJ, Ro KS (2010) Utilization of summer legumes as bioenergy feedstocks. Biomass Bioenergy 34(12):1961–1967

Chandra M (1998) Use of nonwood plant fibers for pulp and paper Industry in Asia: potential in China. Thesis Submitted to the Faculty of Virginia Polytechnic Institute and State University

Chaudhary SN, Borkar SP, Mantha SS (2010) Sunnhemp fiber-reinforced waste polyethylene bag composites. J Reinforced Plastics Composites 29(15):2241–2252

Chaudhary B, Tripathi MK, Bhandari HR, Pandey SK, Meena D, Prajapati SP (2016) Problems and prospects of sunnhemp cultivation in rural areas. In: Pant H, Singh MK (eds) Natural resource management for sustainable agriculture and rural development, pp 108–119

Chaudhury J, Singh DP, Hazra SK (2000) Sunnhemp (*Crotalaria juncea* L.). Central Research Institute for Jute and Allied Fibres, India

Chitamba J, Manjeru P, Chinheya CC, Handiseni M (2014) Evaluation of legume intercrops on the population dynamics and damage level of burrowing nematode (*Radopholus similis*) in banana (Musa spp.). Arch Phytopath Plant Protect 47:761–773

Chittapur BM, Kulkarni SS (2003) Effect of sowing dates on the performance of sunnhemp. J Maharashtra Agril Uni 28(3):331

Cho HS, Seong KY, Park TS, Seo MC, Kim MH, Kang HW, Lee HJ (2013) Effect of seeding rate of Crotalaria (*Crotalaria juncea* L.) on green manure yield and nitrogen production in upland Soil. Korean J Soil Sci Fert 46(6):445–451

Chunekar KC, Pandey GS (1998) Bhavaprakash nighantu (Indian Materia Medica) of Sri Bhavamisra (1500–1600 AD). Chaukhamba Bharati Academy, Varanasi, Uttar Pradesh

Collins AS, Chase CA, Stall WM, Hutchinson CM (2007) Competitiveness of three leguminous cover crops with yellow nutsedge (*Cyperus esculentus*) and smooth pigweed (*Amaranthus hybridus*). Weed Sci 55:613–618

Cunningham RL, Clark TF, Bagby MO (1978) Crotalaria juncea- annual source of papermaking fiber. TAPPI 61(2):37–39

da Silva EC, Muraoka T, Franzini VI, Villanueva FCA, Buzetti S, Moreti D (2012) Phosphorus utilization by corn as affected by green manure, nitrogen and phosphorus fertilizers. Pesq Agropec Bras Brasilia 47(8):1150–1157

Department of Agriculture, Cooperation & Farmers Welfare (2019a) Agricultural statistics at a Glance 2018

Department of Agriculture, Cooperation & Farmers Welfare (2019b) Pocket book of agricultural statistics

Garzon J, Vendramini JMB, Silveira ML, Moriel P, da Silva HMS, Dubeux JCB Jr, Kaneko M, Carnelos C, Mamede PA (2021) Harvest management and genotype effects on sunn hemp forage characteristics. Agron J. https://doi.org/10.1002/agj2.20465

Good I (2007) Invisible exports in Aratta: Enmerkar and the three tasks. In: Gillis C, Nosch ML (eds) Ancient textiles: production, craft and society. Oxbox Books Oxford, pp 168–173

Henmi VH, Sharadchandra P, Marahatta (2015) Impacts of sunnhemp and pigeon pea on plant-parasitic nematodes, Radopholus similis and Meloidogyne spp. and beneficial bacterivorous nematodes. Int J Phytopathol 4(1):29–33

Hooks CR, Wang KH, Fallon D (2006) An Ally in the war against nematode pests: using sunn hemp as a cover crop to suppress root-knot nematodes. CTAHR Coop Ext Serv Plant Disease 32:1–3

International Fertilizer Industry Association (2015)

Javid MM, Bhan M, Johnson JV, Rathinasabapathi B, Chase CA (2015) Biological and chemical characterizations of allelopathic potential of diverse accessions of the cover crop sunn hemp. J Am Soc Hort Sci 140(6):532–541

Ji X, Khan I, Mosjidis JA, Wang H, Livant P (2005) Variability for the presence of pyrrolizidine alkaloids in *Crotolaria juncea* L. Pharmazie 60(8):620–622

Jitendramohan KV (1971) Outcrossing in sunnhemp. Ind J Genet Pl Breed 31(3):395–396

Kankam F, Suen FA, Joseph AJ (2015) Nematicidal effect of sunn hemp Crotalaria juncea leaf residues on Meloidogyne incognita attacking tomato (*Solanum lycopersicum*) roots. Crop Prot 4(2):241–246

Kavin S, Subrahmaniyan K, Mannan SK (2018) Optimization of plant geometry and NPK levels for seed and fibre yield maximization in sunnhemp [*Crotalaria juncea* (L.)] genotypes. Madras Agric J 105(4–6):156–160

Korrapati N, Manasa P (2017) A brief review on sunn hemp as the source of enhanced bioethanol production. Academia J Biotech 5(10):164–168

Kumar V, Belavadi VV, Revanasidda TKB, Srinivasa YB (2019) Stamen elongation in sunn hemp appears to allow delayed selfpollination in the absence of pollinators – a case of bet-hedging? South Afr J Bot 127:110–116

Lai ML, Yang CC, Ching PS (1967) Introduction and selection of the dual-purpose varieties of sunn hemp and their fiber extraction and paper manufacturing experiments. Taiwan Agr Quart 3:34–46

Mandal S, Dutta A, Bhattacharya PK (2017) Growth, yield and seed quality of sunnhemp (*Crotalaria juncea* L.) as influenced by foliar nutrition and pinching. Int J Bio-resource Stress Manage 8(5):617–621

Mannetje L (2004). http://www.fao.org/ag/AGP/AGPC/doc/GBASE/data/pf000475.htm

Marahatta SP, Wang KH, Sipes BS, Hooks CRR (2012) Effects of the integration of sunn hemp and soil solarization on plant-parasitic and free-living Nematodes. J Nematol 44(1):72–79

McSorley R, Seal DR, Klassen W, Wang KH, Hooks CRR (2009) Non-target effects of sunn hemp and marigold cover crops on the soil invertebrate community. Nematropica 39(2):235–245

Meagher RL, Nagoshi RN, Stuhl C, Mitchell ER (2004) Larval development of fall armyworm (Lepidoptera: Noctuidae) on different cover crop plants. Florida Entomol 87:454–460

Medina JC, Ciaramello D, Pettinelli A (1961) Resultados experimentals com a cultura de *Crotalaria juncea* L. Como planta produtora de celulose para papel. Bragantia 20:659–668

Mishra ND (1963) Chemical pulp from sunn hemp stalks. Indian Pulp Paper 18(3):211–214

Montgomery B (1954) Sunn fibre. In: Mauersberger HR (ed) Textile fibres, 6th edn. Wiley, New York

Mosjidis JA, Wehtje G (2011) Weed control in sunn hemp and its ability to suppress weed growth. Crop Prot 30(1):70–73

Mosjidis JA, Balkcom KS, Burke JM, Casey P, Hess JB, Wehtje G (2013) Production of the sunnhemp cultivars 'AU Golden' and 'AU Durbin' developed by Auburn University. Technical Report, Alabama Agricultural Experiment Station. https://doi.org/10.13140/2.1.5187.0402

Muzyamba K, Phiri E, Kalala D, Chishala BH (2021) Organic amendments effects on soil dry aggregate stability in Chipata, Zambia. African J Agril Res 17(2):346–354

Nelson GH, Nieschlag HJ, Daxenbichler ME, Wolf IA, Perdue RE (1961) A search for new fiber crops. III Laboratory scale pulping studies. TAPPI 44:319–325

Nieschlag HJ, Nelson GH, Wolf IA, Perdue RE (1960) A search for new fiber crops. TAPPI 43: 193–201

Ossom EM, Mavuso NS, Seyoum T, Nkambule TP (2011) Influence of sunnhemp (*Crotalaria juncea* L.) on agronomic and sensory characteristics of sweet potato [Ipomoea batatas (L.) Lam] in Swaziland. American-Eurasian J Agric Env Sci 11(4):581–592

Perry A (2012) There's a new biofuel crop in town. Agricultural Research 17. Academic OneFile. Assessed on 23rd July 2016

Rakesh SR, Meenakshi GN, Srinivasan MR, Kumar M (2019) Studies on diversity, abundance and pollination efficiency of insect pollinators and pollination mechanism involved in sunnhemp (*Crotalaria juncea* L.). Electronic J Plant Breed 10(2):838–851

Ram H, Singh G (2011) Growth and seed yield of sunnhemp genotypes as influenced by different sowing methods and seed rates. World J Agril Sci 7(1):109–112

Sadhukhan S, Sarkar U (2016) Production of Biodiesel from Crotalaria juncea (sunn-hemp) oil using catalytic trans-esterification: process optimisation using a factorial and box-Behnken design. Waste Biomass Valorization 7(2):343–355

Sahu SK, Samant PK (2006) Nitrogen loss from rice soils in Orissa. Orissa Rev:34–36

Saikia CN, Goswami T, Ali F (1997) Evaluation of pulp and paper making characteristics of certain fast growing plants. Wood Sci Tech 31:467–475

Sarkar SK, Ghoroi AK (2007) Sunnhemp as green manure. Int J Agric Sci 3(1):244–248

Sarkar SK, Hazra SK, Sen HS, Karmakar PG, Tripathi MK (2015) Sunnhemp in India. ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, West Bengal. 136 pp

Schomberg HH, Martini NL, Diaz-Perez JC, Phatak SC, Balkcom KS, Bhardwaj HL (2007) Potential for using sunn hemp as a source of biomass and nitrogen for coastal plain regions of the southeastern USA. Agron J 99:1448–1457

Sengupta S, Debnath S (2018) Development of sunnhemp (*Crotalaria juncea*) fibre based unconventional fabric. Ind Crops Prod 116:109–115

Singh SK, Sinha PK, Prakash EV (2015) Fabrication & characterization of bio composite materials based on sunnhemp fibre. Int J Modern Engin Res 5(4):22–29

Skinner EM (2012) Allelopathic effects of sunnhemp *(Crotalaria juncea* L.) on germination of vegetables and weeds. Hort Sci 47(1):138–142

Thakkar A (2013) Analysis of air pollution parameters of fertilizer industries specially reference to G.S.F.C. Vadodara, Gujarat. Int J Theor Appl Sci 5(2):109–113

Tripathi MK, Chaudhary B, Sarkar SK, Singh SR, Bhandari HR, Mahapatra BS (2013a) Performance of Sunnhemp (*Crotalaria juncea* L.) as a summer season (Pre monsoon) crop for fibre. J Agric Sci 5(3):236–242

Tripathi MK, Majumdar B, Bhandari HR, Chaudhary B, Saha AR, Mahapatra BS (2013b) Integrated nutrient management in Sunnhemp (*Crotalaria juncea*)-rice cropping sequence in Eastern Uttar Pradesh, India. Ind J Agril Res 47(3):253–257

Tripathi MK, Chaudhary B, Singh SR, Bhandari HR (2013c) Growth and yield of sunnhemp (*Crotalaria juncea* L.) as influenced by spacing and topping practices. Afr J Agril Res 8(28): 3744–3749

Wang KH, Sipes BS (2009) Solarization and cover cropping as alternatives to soil fumigants for nematode management in Hawai'i's pineapple fields. Soil Crop Manage Coll Trop Agric Hum Resources 29:1–4

Wang KH, Sipes BS, Schmitt DP (2002) Crotalaria as a cover crop for nematode management: a review. Nematropica 32:35–57

Wang KH, Mc Sorley R, Marshall AJ, Gallaher RN (2004) Nematode community changes following decomposition of Crotalaria juncea amendment in litterbags. Appl Soil Ecol 27:31–45

Wang KH, McSorley R, Marshall A, Gallaher RN (2006) Influence of organic Crotalaria juncea hay and ammonium nitrate fertilizers on soil nematode communities. Appl Soil Ecol 31:186–198

Wang Q, Li Y, Alva A (2010) Growing cover crops to improve biomass accumulation and carbon sequestration: a phytotron study. J Env Prot 1:73–84

Wang KH, Sipes BS, Hooks CRR, Leary J (2011) Improving the status of sunn hemp as a cover crop for soil Health and Pest Management. Hanai‘Ai/The Food Provider1-3

Wilson MF, Bell EA (1979) Amino acids and related compounds as inhibitors of lettuce growth. Phytochemistry 18:1883–1884

Status of Faba Bean (*Vicia faba* L.) in the Mediterranean and East African Countries

14

Lynn Abou-Khater, Fouad Maalouf, and Diego Rubiales

Abstract

Faba bean (*Vicia faba* L.) was first domesticated in the Fertile Crescent of the Near East. It ranks fourth among the cool season food legumes and is now grown in more than 66 countries. Faba bean is a versatile crop; besides being an important source of protein for food and feed, it offers many services for the ecosystem. The Mediterranean and East African countries account for nearly 32% of the global faba bean production. However, the interest in growing faba bean in these countries does not seem to meet their demand even though it is a crop of great economic and social importance there and it is well adapted to Mediterranean-like environments and to the Highlands of subtropical environments. Numerous factors affect the production of faba bean in the Mediterranean and East African countries including the limited financial support that is posing a challenge to the crop improvement for the major production and adoption constraints such as the biotic and abiotic stresses and the antinutritional components. While major achievements have been made toward the development of faba bean varieties having desired traits, there is still a lot of work to be done. The high level of genetic diversity in faba bean accessions and the available molecular markers will considerably help tighten the gap between the production and the demand in the Mediterranean and East African region.

Keywords

Faba bean · Mediterrenean region · East Africa · Biotic stresses · Abiotic stresses

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14.1 Introduction

Faba bean (*Vicia faba* L.) also known as broad bean, field bean, fava bean, or horse bean ranks fourth among the cool-season food legumes (FAOSTAT 2021). It is well adapted to various climatic zones including Mediterranean-like environment and highlands of subtropical environments. The ideal soil type is a deep, well-drained loam. Faba bean grows best under temperatures between 15 °C and 20 °C and can tolerate cool temperatures as low as −6 °C. It suits well the medium to high rainfall environments as it requires an average of well distributed 400 mm during its growth. Otherwise, it needs supplemental irrigation. Faba bean is well adapted to a wide range of climatic conditions, being widely cultivated across the globe (Mínguez and Rubiales 2020). Under Mediterranean climates, with mild winters and dry and warm summers, faba beans are sown in autumn and harvested in late spring to early summer. In cooler region, faba beans are sown in spring to avoid frost damage, although cultivars with winter hardiness can be sown in autumn. Under subtropical environments, it can be grown during the two rainy seasons from February to June/July (Meher) and from June to October/November (Belg). Nowadays, faba bean is cultivated in more than 66 countries with China being the largest producer followed by Ethiopia, United Kingdom, Australia, Germany, Lithuania, Sudan, France, Italy, Latvia, and Egypt (FAOSTAT 2021). Globally, faba bean acreage has shrunk in the period between 1961 and 1990, since then it has stabilized but the production has increased as illustrated in Fig. 14.1 (FAOSTAT 2021), which can be adscribed to breeding and agronomic achievements.

Since faba bean is primarily used dry, the FAOSTAT data related to faba bean include only the dry seeds and data about large-seeded faba bean types that are consumed as green grains and green pods in several Mediterranean countries and in other countries like Ethiopia are not available which may result in underestimating

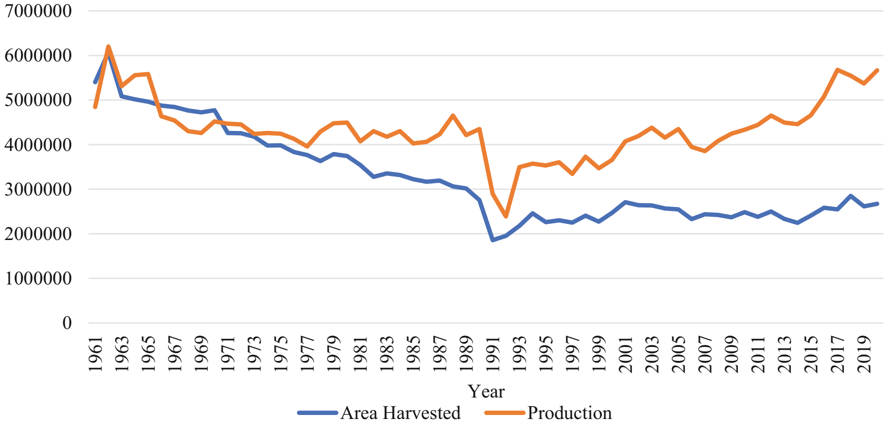

Fig. 14.1 Evolution of the global area harvested (ha) and production (tons) of faba bean between 1961 and 2020

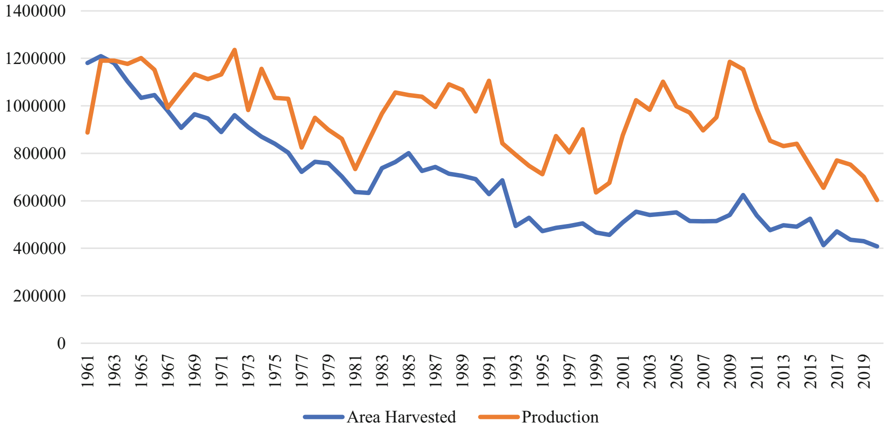

Fig. 14.2 Evolution of the area harvested (ha) and production (tons) of faba bean between 1961 and 2020 in the Mediterranean countries

the global acreage and production of the crop. Despite being well adapted to the Mediterranean climates and highly valued in many Mediterranean countries, faba bean cultivated area has decreased between 1961 and 2020 with a marked 2xfold decrease in the period 1961–1990 and a more moderate one since then, but still slightly decreasing. This contradicts with the stabilization in acreage experienced at global level since 1990. The declining trend of faba bean cultivated area is due to several reasons that made it unattractive for cultivation including the numerous biotic and abiotic constraints, technological requirements, lack of extension and prices instability and decrease (Abobatta et al. 2021). Moreover, the fluctuation in production is more noticeable in the region than at global level as seen in Fig. 14.2 (FAOSTAT 2021), probably being more affected by biotic and abiotic stresses there. However, the crop improvement efforts that led to reduced susceptibility to the biotic/abiotic stresses are behind the increase in the crop yield and the relatively small decline in production in comparison with the decline in the cultivated area. The following four Mediterranean countries Morocco, Italy, Tunisia, and France are among the top ten countries in the world that have the largest faba bean acreage but only France and Italy make it to the top ten producers. Since 1985, the faba bean production in the Mediterranean region does not seem to meet the demand of the region. As a result, the majority of the Mediterranean countries are net importer with an import quantity equal to 0.37 million tons in 2020 far exceeding the export quantity that was equal to 0.11million tons in the same year (Table 14.1) (FAOSTAT 2021). Among the Mediterranean countries, statistics show that Egypt led the import and the export numbers in 2020 followed by France (Table 14.1) (FAOSTAT 2021).

Contrary to the decreasing trend in the Mediterranean region, faba bean cultivation in East Africa is markedly increasing, with production improving markedly since 2000 (Fig. 14.3). Ethiopia and Sudan are the main producers with Ethiopia being the production leader since 1961 and continues to be so till today (FAOSTAT

Table 14.1 Area harvested, production, yield, import, and export of faba bean during 2020 in the major producing countries of the Mediterranean and East African regions (FAOSTAT 2021)

Region	Country	Area Harvested (ha)	Production (tons)	Yield (tons/ ha)	Import (tons)	Export (tons)
Mediterranean region	Algeria	39,849	49,857	1.25	4576	4
	Egypt	24,420	88,109	3.61	250,092	61,388
	France	76,540	148,410	1.94	25,368	41,270
	Greece	2480	4360	1.76	1381	126
	Italy	67,520	134,930	2.00	33,217	1558
	Morocco	107,408	48,973	0.46	28,779	410
	Spain	21,150	29,740	1.41	3897	4339
	Syrian Arab Republic	14,917	28,842	1.93	356	4478
	Tunisia	48,200	58,038	1.20	1	949
	Turkey	3488	9135	2.62	9333	2875
East Africa	Ethiopia	504,570	1,070,637	2.12	19	12,639
	Sudan	91,595[a]	173,115	1.89[a]	3376	84

[a] Calculated yield average between 2012 and 2016 based on FAOSTAT data

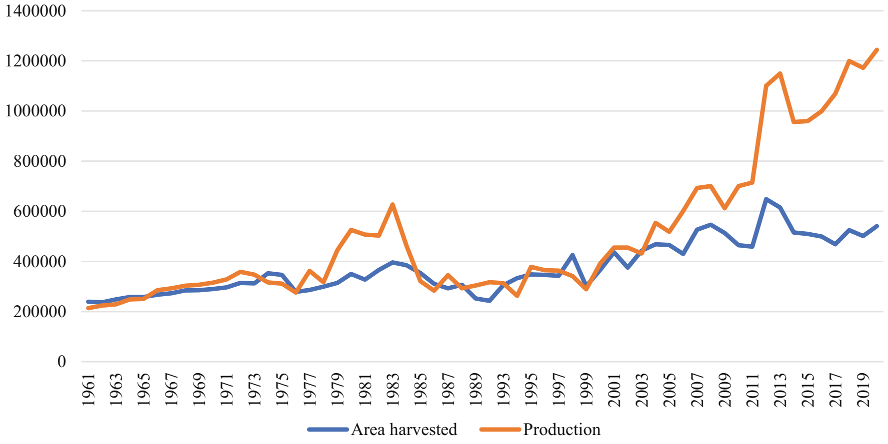

Fig. 14.3 Evolution of the area harvested (ha) and production (tons) of faba bean between 1961 and 2020 in East Africa

2021). In Ethiopia, faba bean takes the largest share of area under pulses production where around one million tons were produced in 2020 from nearly 0.50 million hectares (Table 14.1). Despite ranking second among the faba bean producing countries and encountering a remarkable crop yield increase of almost double since 1993, the crop yield in the country was equal to 2.12 tons/ha in 2020 which is almost the same as the global average but still far below one of other producing countries like Egypt. Many factors might be behind the low faba bean yield in

Ethiopia including water scarcity, *Orobanche crenata*, disease pressure, and acidic soils.

14.2 Origin and Genetic Diversity

Faba bean belongs to the Fabaceae family and to the vetches *Vicia* genus even though some scientists argue that it should be classified in a separate monotypic genus, *Faba* (Miller 1754). In light of many archeological findings, faba bean is considered one of the earliest domesticated crops. Its domestication dates back to the early Neolithic era around 8–10,000 BC (Tanno and Willcox 2006; Caracuta et al. 2016). Faba bean was first domesticated in the Fertile Crescent of the Near East and afterwards its cultivation has spread across the globe (Cubero 1974). Based on the seed size, Muratova (1931) suggested the presence of four subspecies namely: *paucijuga*, *minor*, *equina,* and *major* but since there is high cross compatibility between their different seeds, Cubero (1974) considered them later as four botanical groups of the same species. Although the wild progenitor is yet to be identified, there is high diversity within *V. faba*, as reflected in terms of morphology and agroecosystem adaptability what might be the result of the specific adaptation that faba bean gained during its dispersal (Alghamdi et al. 2012). While over 38,000 faba bean accessions are conserved globally (Duc et al. 2015; GENESYS 2022), ICARDA conserves the largest collection which is around 32% of the total global collection.

Faba bean is diploid ($2n = 12$) with six large chromosomes pairs and a genome of 13,000 Mbp, one of the largest among crop legumes (Soltis et al. 2003). The large genome of faba bean is 2.9, 3.0, 15.9, and 25 times large compared to the ones of pea, lentil, chickpea, and the model *Medicago truncatula* respectively, but is currently being assembled (Faba Bean Genome Consortium 2022). Despite the difficulties imposed by its genome size, transcriptome data have provided efficient resources (e.g., Ocaña et al. 2015) and a number of genetic maps have been generated so far (reviewed by Khazaei et al. 2021) using several types molecular markers that also allowed to discriminate different faba bean landraces based on their geographical origin's, ecological behaviors, and response to diverse biotic/abiotic stresses (Avila et al. 2004; Díaz-Ruiz et al. 2009a, b; Zong et al. 2009; Kwon et al. 2010; Wang et al. 2012; Kaur et al. 2014; Göl et al. 2017; Ocaña-Moral et al. 2017; Gutiérrez and Torres 2021; Mulugeta et al. 2021; Maalouf et al. inprint). To date, the most saturated consensus genetic map was the one reported by Carrillo-Perdomo et al. (2020). In addition to linkage mapping using biparental populations, genome-wide association studies (GWAS) are being performed to maximize the allele frequency and genetic diversity lacking in biparental populations (Faridi et al. 2021; Abou-Khater et al. 2022a).

14.3 Faba Bean Uses

14.3.1 Human and Animal Nutrition

Faba bean is nutrient dense. It has a valuable content of protein, energy, soluble and insoluble dietary fibers, vitamins, and minerals like Iron Fe, Copper Cu, Zinc Zn, and Manganese Mn (Duc 1997; Crépon et al. 2010; Singh et al. 2014). Faba bean is also a valuable source of amino acids; its nutritional profile includes bioactive compounds, such as antioxidants, phenols, and γ-aminobutyric acid (GABA) (Khazaei et al. 2019) and many essential amino acids such as arginine, lysine, and leucine (Koivunen et al. 2016), which are essential for both human and monogastric diets (Khazaei et al. 2019). Among the many studies that assessed the nutritional profile of faba bean, a study conducted by Khan et al. (2015), evaluated the nutritional profile of various faba bean genotypes and found a variation in the nutrient compositions among the genotypes (Table 14.2).

14.3.1.1 Human Nutrition and Medicine

Faba bean green pods and seeds are consumed by humans as vegetable, whereas dry seeds are consumed in different forms depending on the seed quality, color, and size. Faba bean is a staple food in the Mediterranean and East African cuisine where the most popular dishes are Medamis, Falafel, Habitas con jamón, Bissara, Shahan Ful, and Nabet soup.

Faba bean is consumed mainly as a source of protein in human diets with a protein content of almost twice the one in cereals (Rahate et al. 2021) and higher than the one of other legumes. The protein content of faba bean seeds ranges between 31.5% and 37.7% depending on the variety (Khan et al. 2015) with an average of 33.6% (Table 14.2), which makes it a good candidate for partially replacing animal proteins and lowering the climate footprint of food production. The genetic variation and high estimated heritability for mineral concentrations and protein content of the faba bean seeds (Khazaei and Vandenberg 2020) make the genetic biofortification through plant breeding possible for improving the nutritional values of faba bean in a sustainable, cost-effective, and long-term approach.

Being an inexpensive and affordable source of lean protein, many diets around the world rely on faba bean as a source of protein especially in the lower economy countries like Ethiopia, Sudan, and Egypt where about 75% of daily per capita protein intake in Egypt is of plant-based mostly faba beans as Egyptians consume about 14 g per capita per day of faba beans, which accounts for about 3 g of protein. However, in the diet of higher economy countries, faba bean is underutilized as human food as the meat is the major source of protein (Tijhuis et al. 2012) but recently the popularity of plant-based protein consumption is rising with the rising desire of people to eat a healthier diet especially that pulses are included in all dietary guidelines throughout the world. Furthermore, the carbon footprint of faba bean protein is 80–90% lower compared to dairy proteins per kilogram protein, which means that consuming faba bean-based protein reduces the negative effect of food consumption on the environment (Heusala et al. 2020).

Table 14.2 Mean values of the nutritive components of different faba bean genotypes (Khan et al. 2015)

Type	Nutrient	Nutrient value	Type	Nutrient	Nutrient value	Type	Nutrient	Nutrient value
Principal (g/100 g)	Carbohydrates	45.7 g	**Minerals (mg/100 g)**	Potassium	752.9	**Amino acids (g/kg)**	Arginine	10.118
	Protein	33.56 g		Calcium	37.11		Leucine	6.942
	Moisture	7.38		Copper	2.49		Phenylalanine	5.625
	Ash	3.84		Iron	15.18		Valine	4.705
	Total fat	1.86 g		Magnesium	7.73		Lysine	4.1725
				Manganese	2.48		Threonine	3.845
				Phosphorous	68.88		Histidine	3.465
				Zinc	6.4		Methionine	1.15

In addition to being a popular source of protein, faba bean protects against many diseases. The high potassium and low sodium contents of faba bean seeds (Table 14.2) are optimum for people adopting low-sodium diet like the ones suffering from hypertension. Moreover, faba bean is a good source of *levadopa* (*L-dopa*), a precursor of dopamine and can be potentially used as medicine for the treatment of Parkinson's disease that affects persons unable to synthesize dopamine that regulates motor cells (Lawes 1980; Vered et al. 1997; Etemadi et al. 2019) affected. L-dopa is also a natriuretic agent, which might help in controlling hypertension (Jambunathan et al. 1994). Faba bean can protect from *Plasmodium falciparum* malaria. Several studies suggested that the G6PD deficiency resulting from "favism" protects against malaria (Hussein and Saleh 1985; Ruwende and Hill 1998; Arese 2006; Louicharoen et al. 2009; Khamassi et al. 2013; Mbanefo et al. 2017), which increases its consumption in many countries like Ethiopia during the rainy season (Arese 2006). Favism is a hemolytic anemia, caused by the pyrimidine glucosides vicine and convicine in genetically predisposed humans deficient in a red blood cell enzyme.

14.3.1.2 Animal Nutrition

Being a great protein and starch (energy) source, faba beans hold great importance for animal nutrition but the inclusion of faba beans in animal feeds has been limited historically due to the presence of antinutritional compounds in their seeds such as tannins, vicines, and convicine, which cause health problems for the nonruminant animals (Vidal-Valverde et al. 1998; Crépon et al. 2010; Hendawey and Younes 2013; Multari et al. 2015). Protein content and digestibility are the driving quality criteria for animal feeding. Tannins are known to reduce the digestibility and biological value of protein in the diet of monogastric animals, which leads to a decrease in their daily growth and negatively affects the absorption of several essential micronutrients (Bond 1976; Griffiths 1979; Multari et al. 2015). High levels of vicine and convicine can increase the risk of erythrocyte hemolysis for laying hens and cause a significant reduction in the efficiency of production systems for broiler chickens, laying hens, and pigs (Muduuli et al. 1982; Grosjean et al. 2000; Vilariño et al. 2009; Crépon et al. 2010; Lessire et al. 2017) in addition to having detrimental effects on the performance levels and egg characteristics but they have little effect on pigs (Crépon et al. 2010; Lessire et al. 2017). Tannins are associated with the seed coat, sprouting the seeds or dehulling generally reduces them. Moreover, many foods processing methods, including domestic actions such as soaking and cooking at high temperatures, markedly reduce and often eliminate them totally thus limiting any detrimental consequences (Luo et al. 2009). Low tannin content can be reduced also by breeding as it is coded by two recessive genes *zt-1* and *zt-2* (Cabrera and Martin 1986), and as it is a trait easy to select because it is linked with the white color of flowers. Cultivars with low tannin content are available in the Mediterranean and East African areas like the two low tannin cultivars namely Nubaria-1 and Wadi-1 that are available in Egypt and Sudan, respectively. On the other hand, vicine/convicine are thermostable and therefore difficult to breakdown by processing. However, their content can also be reduced by breeding, as a recessive gene (*vc*-) is responsible for a lowered (10- to 20-fold) concentration. Selection can also be made by a rapid colorimetric test (Duc et al. 2004) and with SNP markers tightly linked to the trait (Khazaei et al. 2017). Low vicine/convicine cultivar is available in France under the market name FEVITA.

The development of faba bean varieties with reduced amount of anti-nutrients, the use of enzymes, and the advances in animal feed processing technologies like pelletizing have proven that faba bean has the potential to partially replace soybean in the diet of monogastric and ruminant animals (Crépon et al. 2010; Ivarsson and Wall 2017; Cherif et al. 2018; Alagawany et al. 2019) and therefore diminishing the dependence of the Mediterranean countries on soybean imports that was equal to 15.5 million tons in 2020 according to FAOSTAT (2021). Livestock producers of these countries have many reasons to grow faba bean including its protein content that is slightly lower than the one of soybean, the well adaptation of faba bean to different climatic zones, and the ability to home grow it in the Mediterranean countries in contrast with soybean that requires warm weather. Adding to that is the possibility of processing faba bean on-farm since it contains little oil.

Furthermore, faba bean plant makes an excellent forage and can be grazed or used to make good quality hay and silage (Jensen et al. 2010; McVicar et al. 2013). Faba bean straw contains 5–11% protein and is considered cash crop in Egypt and Sudan (Muehlbauer and Abebe 1997).

14.3.2 Agroecosystem

Diverse agronomic, environmental, and ecological services are expected from integrating faba bean in the cropping system. Being an entomophilous species and a partly allogamous crop, faba bean plays an important role in conserving wild pollinators that are threatened worldwide. Faba bean plants form with their pollinators a symbiotic relationship where the pollinators benefit by feeding on the nectar and pollen provided by faba bean flowers and the faba bean plants benefit by having their pollen distributed to the flowers of other faba bean plants and therefore be able to reproduce. The role of faba bean in conserving wild pollinators can be enhanced by adopting the approach called Farming with Alternative Pollinators (FAP) as it induces benefits for pollinator diversity and increases the crop yield by enhancing pollination services while producing marketable products (Christmann and Aw-Hassan 2012; Christmann et al. 2021; Sentil et al. 2021).

Moreover, like other legumes, faba bean can fix atmospheric nitrogen (N_2) in its root nodules through *Rhizobium leguminosarum* symbiosis. This will lower the need for synthetic nitrogen fertilizers and therefore lower the energy used to manufacture, transport, and supply the fertilizers that are derived mainly from fossil fuels. The reduction in the energy used can substantially reduce the greenhouse gas emissions of the entire cropping system. Noting that the amount of nitrogen fixed by faba bean which is around 154 kg/ha depending on the cultivar and environmental conditions, is greater than the one fixed by many other legumes like lentils and chickpeas (Jensen et al. 2010).

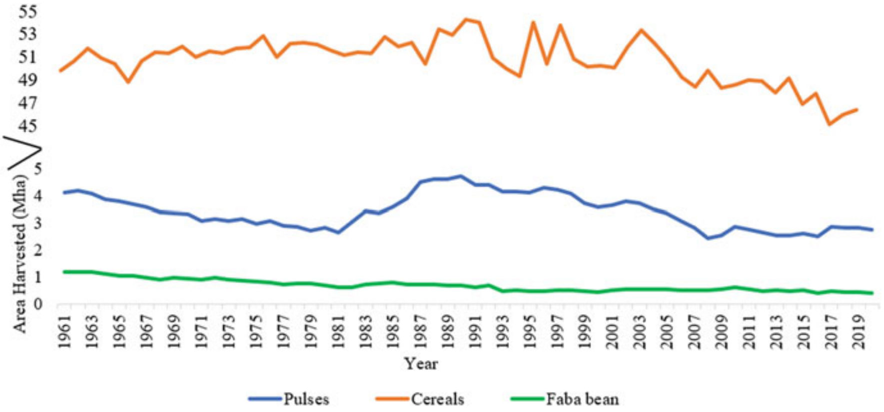

Fig. 14.4 Total area harvested (Mha) of cereals, pulses, and faba bean in the Mediterranean region over the years

In addition to improving the soil fertility through supplying it with Nitrogen that can be taken up by subsequent crops, faba bean improves the physical, chemical, and biological properties of the soil (Aschi et al. 2017). Moreover, the introduction of faba bean in the cropping system through rotation or intercropping leads to a more diversified and sustainable cropping system, an increase in crop yield and stability, and present a break crop for a better pests and weeds control (Jensen et al. 2010; Fernández-Aparicio et al. 2011a, b; Abera et al. 2015; Landry et al. 2015a, b; Shtaya et al. 2021). Furthermore, intercropping faba bean with other crops can help reduce the production gap and reduce the import in many countries like Egypt, Sudan, France, and Spain.

Despite the many benefits that legumes in general and faba bean in a particular offer to the ecosystem, statistics show that Mediterranean and East African countries are still adopting the cereal monocropping system (Figs. 14.4 and 14.5). In 2020, the total areas under cereals were equal to 46.5 and 20.4 million ha, while the total areas under pulses were almost similar and equal to 2.8 and 2.7 in the Mediterranean and East African regions, respectively (Figs. 14.4 and 14.5) (FAOSTAT 2021). The season to season yields fluctuations that farmers are encountering due to climate change and the absence of modern faba bean varieties that exhibit traits like mechanical harvesting, tolerance to biotic and abiotic stresses, and other seed quality traits explain the lack of inclusion of faba bean in the cropping system. Hence, greater scientific and national efforts to develop faba bean varieties with farmer-desired traits along with extension to raise awareness of the economic and ecological benefits of the faba bean–cereal cropping system are needed.

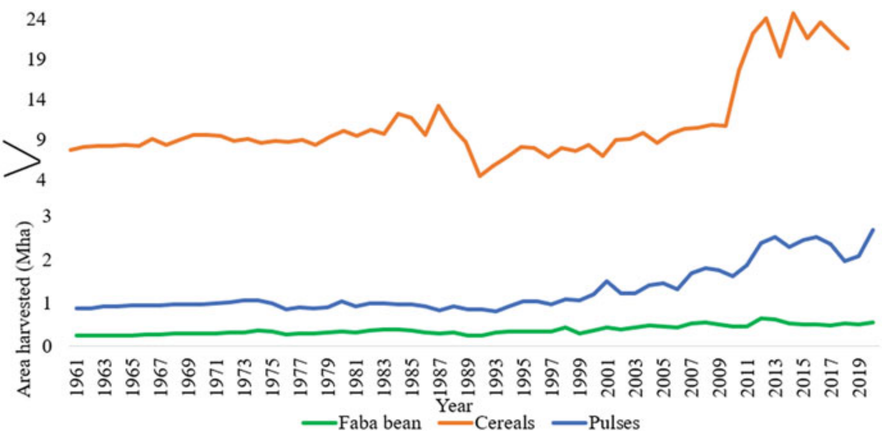

Fig. 14.5 Total area harvested (Mha) of cereals, pulses, and faba bean in the East African region over the years

14.4 Faba Bean Constraints

Faba bean production in the Mediterranean and East African regions is threatened by a number of biotic and abiotic constraints (Fig. 14.6) causing a gap between its yield potential and its actual yield.

14.4.1 Abiotic Stresses

The most common abiotic stresses affecting faba bean production in the Mediterranean and East African regions are frost, heat, drought, waterlogging, soil acidity, and soil salinity.

14.4.1.1 Frost

Faba bean is a cool season grain legume than can tolerate frost better than many other pulses. However, frost can still affect faba bean early in the crop season and can cause yield reduction when planted as a winter crop like in the Mediterranean countries where temperatures can drop to as low as −10 °C. The severity of frost damage depends not only on the tolerance level of each genotype but also on the timing of the frost event, the length, and the frequency of exposure. Under favorable environmental conditions, faba bean can recover from frost damage that occurred in the vegetative phase by generating new tillers. Winter hardiness exists in faba bean cultivars and there is potential for expanding the range of winter faba bean through breeding for improved hardiness (Herzog and Saxena 1988; Flores et al. 2012). Winter hardiness has allowed faba beans to survive European (−15 °C) and Mediterranean winters (−10 °C) (Herzog 1989). Hardening is a crucial adaptation that

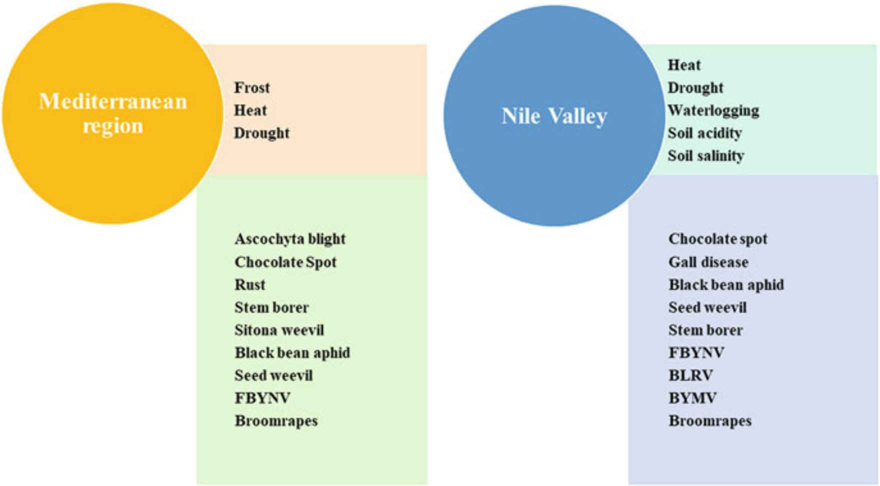

Fig. 14.6 Major abiotic and biotic constraints affecting faba bean production in the Mediterranean and East African region

allows faba bean cultivars to match their development to the cool rainy seasons in Mediterranean environments when water is available and will allow faba beans to be cultivated in a wider area than at present (Stoddard et al. 2006; Link et al. 2010; Ali et al. 2016).

14.4.1.2 Heat

In addition to frost, faba bean is susceptible to heat stress in the East African and in the Mediterranean area where the crop can be exposed to a temperature of 32 °C and above during the productive stage. Heat waves during the vegetative stage lead to the development of small, short, stemmed plants with few branches as is the case in other legume crops (Abdelmula and Abuanja 2007). Moreover, heat waves during the flowering and pod development stages increase the number of flowers but decrease the number of pods and seeds (Sekara et al. 2001) as they damage the pollen and reduce its germination (Bishop et al. 2016). The impact of heat on faba bean depends on the genotypes, plant tissues, and on the frequency, duration of exposure, and severity of heat (Abdelmula and Abuanja 2007). With the projected climate change and the high and frequent temperature anomalies, the development of heat-tolerant faba bean lines is very important to ensure yield stability especially in the Mediterranean region that is identified as one of the most vulnerable regions to heat stress that results from climate change (García-Ruiz et al. 2011; Cook and Lewandowsky 2016; Mansour et al. 2018). Many studies reported the identification of heat-tolerant faba bean accessions namely C.52/1/1/1, IG11743, IG11843, VF351, VF420, VF522, VF626, and INRA1631 (Abdelmula and Abuanja 2007; Maalouf et al. 2018). An increase in the faba bean cultivated area and production was observed in Sudan where heat stress is the major constraint, after the release of two improved faba bean varieties Hudeiba-93 and Basabeer (Maalouf et al. 2018). In addition, Maalouf et al. (inprint) were the first to identify SNPs associated with heat tolerance in faba bean.

14.4.1.3 Drought

Faba bean is also sensitive to drought that reduces the growth parameters and the chlorophyll content which ultimately impairs its growth, performance, and yield potential (Siddiqui et al. 2015; Mansour et al. 2021). Since climate change is anticipated to further decrease rainfall and drought stress is projected to become more severe and frequent, an enhancement of the faba bean tolerance to drought should be achieved particularly in the regions where faba bean is grown under rainfed conditions as it is the case in many Mediterranean and East African countries like Morocco and Ethiopia. The identification of early flowering and early maturing genotypes that can escape terminal drought is much needed to ensure yield stability under Mediterranean-type climate with the frequent occurrence of terminal drought. The genotypic variation in the response of faba bean to drought (Abdelmula et al. 1999; Amede et al. 2003; Abdellatif et al. 2012; Maalouf et al. 2015; Siddiqui et al. 2015) is the key toward the identification of sources for drought tolerance in faba bean. During the evaluation of faba bean lines under drought and normal conditions, Maalouf et al. (2015) selected FLIP06-010FB as the highest yielded and most stable accession across environments.

14.4.1.4 Waterlogging

Although unfavorable temperature and drought are the major biotic constraints for faba bean production, waterlogging is considered a notable limiting factor in many high rainfall areas like vertisol area in Ethiopia (Keneni et al. 2001) and irrigated Nile River basin in Sudan and Egypt. Despite being able to tolerate waterlogging better than other cool season food legumes (Solaiman et al. 2007), waterlogging during flowering and pod setting can cause noticeable drop in flowers and pods, which leads to severe growth reduction and yield losses (Pampana et al. 2016; Tadele 2020). Moreover, a prolonged waterlogging affects plant growth and escalates foliar diseases at any stage of the plant life cycle. While a well-aerated soil and a drainage using broad bed and furrow (BBF) cultivation enable management of excess water (Keneni et al. 2001), breeding for productive genotypes under waterlogging conditions could be the best solution to solve this problem. To address the problem of waterlogging, the national faba bean research in Ethiopia developed and released six faba bean varieties (Selale, Wayu, Wolki, Hachalu, Didi'a, Ashebeka) that still give high yield under waterlogging environments (Tadele 2020).

14.4.1.5 Soil Acidity

Besides the mentioned biotic constraints, soil acidity also is a potential constraint for faba bean production in East Africa and particularly in Ethiopia where faba bean has been replaced by cereal rye cropping and livestock grazing in the agricultural zones where soil acidity is severe (Genanew et al. 2012). Besides the simple matter of low pH, soil acidity decreases the availability of essential nutrients and increases that of toxic elements such as Aluminum (Al^{3+}) (Agriculture Victoria n.d.), which leads to an aggravation in disease susceptibility, reduction in the nitrogen-fixation efficiency of the rhizobia and reduction in plant vigor and yield (Chen et al. 1993; Zahran 1999; Tadele 2020). Acid soil can be managed by the application of lime, but this management option is not always applicable especially in regions where farmers are poor like in Ethiopia so the breeding for soil acidity stress tolerance is still the most effective and sustainable approach to grow faba bean on acid soils (Tadele 2020). Hence, the existence of diverse faba bean landraces increases the chance of developing acid-tolerant genotypes. Belachew and Stoddard (2017) identified three faba bean accessions (Dosha, NC 58, Kassa) originated from Ethiopia and one (GLA 1103) originated from Austria that was tolerant to acid soils.

14.4.1.6 Soil Salinity

Soil salinity is the most serious problem facing semi-arid agriculture systems as it restricts the ability of the plant to access water and nutrients. Around 35% of agricultural lands in Egypt are salt-affected especially the northern part of Nile Delta and it is increasing because of the climate change effect (Omar et al. 2021) and the erroneous cultivation practices (Rengasamy 2010). Soil salinity is the result of different natural and human activities like saltwater intrusion, land clearing, excessive irrigation, irrigation with low-quality water, and an inadequate field drainage (Kotb et al. 2000). Faba bean is very sensitive to saline soil that causes yield losses ranging between 20% and 50% (Farooq et al. 2017) by threatening the seed germination, plant growth, rhizobium symbiosis, development of root nodule, and nitrogen-fixation capacity (Delgado et al. 1994; Tavakkoli et al. 2010; Abeer et al. 2014; Hussein et al. 2017). Many strategies were recommended to mitigate the effect of salt stress on faba bean including leaching, treating salty drainage water, applying bio stimulants like biochar, jasmonic acid (El Nahhas et al. 2021); melatonin, exopolysaccharide-producing bacteria (El-Ghany et al. 2020), and salicylic acid (Ahmad et al. 2018) and biofertilizer like the soil yeast *Rhodotorula glutinis* (Gaballah and Gomaa 2004) and *Rhizobium leguminosarum* bv. *viciae* (El-Akhdar 2020) as well as treating the seeds with gamma γ radiation (15, 25, and 35Ray) prior to sowing (El-Awadi et al. 2017). However, most of these suggestions can be applied at postemergence stages and the seed germination of faba bean is known to be sensitive to salt stress. Therefore, planting improved faba bean cultivars that tolerate salt stress during germination stage is an optimal strategy. Genotypic variation for salinity tolerance has been reported in faba bean (Tavakkoli et al. 2012; El-Bastawisy et al. 2018) and many cultivars like G429, G843, and M1 were recommended for use in the development of salt-tolerant genotypes (Abdelhamid et al. 2010) their salinity tolerance. Yang et al. (2020) explained the mechanism of genes that regulates seed germination under salinity and Asif and Paull (2021) identified quantitative trait loci associated with salt tolerance in faba bean. The findings will greatly benefit the breeding of salt-tolerant faba bean cultivars.

14.4.2 Biotic Stresses

The most important biotic constraints that which affect faba bean production in the Mediterranean and East African regions include foliar diseases, pests, viruses, and parasitic weeds.

14.4.2.1 Foliar Diseases

Faba bean is susceptible to many foliar diseases caused by pathogenic fungi including mainly ascochyta blight (*Ascochyta fabae*), chocolate spot (*Botrytis fabae*), rust (*Uromyces viciae-fabae*), and gall disease (*Physioderma viciae*). Ascochyta blight is widespread in regions where faba beans are grown during the winter like in the Mediterranean region (Stoddard et al. 2010). It spreads most rapidly in the early spring at the pre flowering stage and can cause up to 90% yield losses when the environmental conditions are suitable for disease development (Hanounik 1980). Chocolate spot is most prevalent in regions characterized with high rainfall such as Ethiopia, Egypt, France, and the Maghreb countries. Under favorable conditions, chocolate spot can reduce the faba bean yield by up to 90% (Bouhassan et al. 2004; Tivoli et al. 2006; Beyene et al. 2018) and can affect the quality of the seeds and hence reduce their market value. Rust can attack faba bean in almost every growing area. It is considered as a major disease of faba bean in the Mediterranean region. It usually occurs late in the growing season at the podding stage and causes moderate to high yield losses up to 70% in early infection (Marcellos et al. 1995; Jellis et al. 1998; Emeran et al. 2011). Gall disease has been threatening faba bean production in Ethiopia during the past 10 years. It causes severe yield losses ranging between 30% and 100% (Dereje et al. 2012; Abebe et al. 2014). Not until recently that the scientists discovered that *Physoderma*, not *Olpidium is* the real agent behind this devastating disease (You et al. 2021).

The severity of foliar diseases depends on the resistance level of the genotypes and on the environmental factors that determine the epidemic development of disease. Since the optimal environmental conditions for each disease are well documented, epidemics can be forecasted which facilitate the timely application of disease management techniques. A successful foliar disease management relies on the application of an integrated approach that combines a variety of practices including cultural practices (early planting, crop rotation, intercropping), fungicides application (foliar application or seed treatment), the use of clean seeds, and the use of resistant cultivars (Stoddard et al. 2010; Ahmed et al. 2016; Kassa et al. 2020; Mengesha et al. 2022). Lines with resistance to the key diseases were developed, recommended for use, or released in many Mediterranean and East African countries (Sillero et al. 2010; Rubiales et al. 2012; Alehegn et al. 2018; Beyene et al. 2018; Rubiales and Khazaei 2022) but they usually provide partial resistance only as the presence of pathogen variability in these diseases complicate the development of complete resistance. For example, L-831818, V-26, and V-958 were identified by Rubiales et al. (2012) for their consistent resistance to Ascochyta blight across environments. Moreover, Gora, Moti, Gebelcho, Obsie, Gora, Didea, and Walki were released in Ethiopia as resistant cultivars to Chocolate spot and Walki was recommended by Alehegn et al. (2018) as resistant to faba bean gall. The current climate change emphasizes the need to develop faba bean lines with combined resistance to the previously mentioned diseases. In this regard, lines with multiple disease resistance were identified by Villegas-Fernández et al. (2011) and Maalouf et al. (2016a, b) for further use in breeding programs. Moreover, in the aim to facilitate the development of disease-resistant cultivars and to allow a faster and improved selection that is not influenced by environmental conditions efforts were made to identify molecular markers associated with resistance to the major diseases affecting faba bean. Several molecular works identified genes, QTLs and/or markers associated with resistance to Ascochyta blight (Avila et al. 2004; Díaz-Ruiz et al. 2009a, b; Atienza et al. 2016; Ocaña-Moral et al. 2017; Faridi et al. 2021; Gutiérrez and Torres 2021), rust (Avila et al. 2003; Ijaz et al. 2021) and chocolate spot (Gela et al. 2021) but to the best of our knowledge no QTLs or markers have been published so far for faba bean gal despite its importance in the region.

14.4.2.2 Insects

Of all insects attacking faba bean crop, faba bean stem borer (*Lixus algirus*), sitona weevil (*Sitona lineatus*), black bean aphid (*Aphis fabae*), and seed weevil (*Bruchus rufimanus*) are the most serious and more prevalent in the Mediterranean and East African region (Weigand and Bishara 1991; Stoddard et al. 2010; Béji et al. 2013; Carrillo-Perdomo et al. 2019). Out of the listed insects, the first three cause significant damage to the crop during the different growth stage by using them as a host plant, directly feeding on the phloem and by being vectors of many viruses. However, seed weevil damages the embryo and spreads bacterial and fungal infections, which leads to a decrease in seed viability (Sallam 2013). Currently, the management of insects in faba bean fields depends to a large extend on the use of insecticides but this control method has many limitations so an integrated pest management that combines agronomical, cultural, biological, chemical, and genetical options is recommended as it is more efficient and sustainable. Recently, IG 72498 and IG 11561 were identified by Ait taadaouit et al. (2021) as new sources for resistance to the stem borer that is considered a dangerous biotic stress in some Mediterranean areas like Morocco and sources of resistance to the seed weevil were identified by Carrillo-Perdomo et al. (2019) and will be used to develop resistant cultivars.

14.4.2.3 Viruses

In addition to the many biotic stresses threatening faba bean production, many viruses can infect Faba bean through seeds, mechanical transmission, or insects such as aphids. Viral diseases are sometimes perceived as more dangerous than fungal diseases due to the lack of effective control management and to the absence of treatment once the infection occurs. The most common and of major economic importance in the Mediterranean and East African regions are Faba Bean Necrotic Yellow Virus (FBYNV), Bean Leaf Roll Virus (BLRV), Bean Yellow Mosaic Virus (BYMV) (Makkouk et al. 2002; Makkouk et al. 2003; Kumari and Makkouk 2007; Kraberger et al. 2018; Younes et al. 2021). The first two exhibit yellowing, stunting, and necrosis symptoms, while the last two exhibit mosaic/mottling (Kumari and Makkouk 2007). In addition to the viruses mentioned above, other viruses were reported in specific Mediterranean or East African regions, such as Tomato spotted wilt virus (TSWV) in Spain (Babin et al. 2000), Chickpea chlorotic stunt virus (CCSV) in Ethiopia (Abraham et al. 2006) and broad bean phyllody has been reported in Sudan (Jones et al. 1984). Crop losses due to viral infection vary from almost none to a complete crop failure depending on the susceptibility of the crop to the infection, the incidence of the infection, the growth stage of the crop at which the infection occur, and on the field location as the virus incidence increases when the crop is grown under warm environment as it favors the development and movement of aphids. Yield losses due to FBNYV reached 90% in Egypt during 1992 as reported by Kumari and Makkouk (2007). Some viruses can also affect the quality of the seeds making them less attractive to the consumers (Kaiser and Schalk 1973). However, nowadays losses have been reduced; thanks to the progress in developing resistant varieties and improving the detection methods and the management practices. An efficient virus disease management starts with an early detection and correct identification of the virus and involves an integration of hygienic practices such as the use of virus-free seeds and roguing, cultural practices such as amendment of planting date, seed rate, row spacing, and planting nonviruses' host crop as a border, chemical sprays such as insecticides to manage the vectors and herbicides to manage weeds acting as viruses' host and genetic resistance such as selection and breeding for virus resistance (Kumari and Makkouk 2007). Faba bean genotypes resistant to BLRV (Robertson and Saxena 1993; Makkouk et al. 2002) and BYMV were reported (Robertson and Saxena 1993; Makkouk and Kumari 1995; Robertson et al. 2000) and 27 resistant genotypes to FBYNV have been recently identified at ICARDA (Kumari et al. 2018).

14.4.2.4 Weeds

Last but not least, weeds are considered one of the major constraints to growing faba bean successfully in the Mediterranean and East African region. Faba bean is very sensitive to weed competition particularly during the initial growth stages, since its slow growth favors the emergence and establishment of weeds before the crop gets established, cover the interrow spaces, and get a good shading and consequently a better ability to suppress weeds (Frenda et al. 2013; GRDC 2017). Faba bean weeds competition depends greatly on many factors such as site location, climatic conditions, management strategies, weed flora, weed density, and weed emergence time (Rajcan and Swanton 2001). In addition to competing faba bean for water nutrients and lights, weeds emerging in faba bean fields damage the crop by acting as a harbor for many diseases and for insect vectors of these diseases (Parihar et al. 2017), releasing chemical compounds that suppress the growth of faba bean (Klingman et al. 1982) and by causing problems at sowing and harvesting (GRDC 2017). Annual weeds including *Convolvulus arvensis*, *Lolium multiflorum*, *Sinapsis arvensis,* and *Chenopodium album* tend to be the most common annual weeds in faba bean fields in the Mediterranean and East African region (Cubero 1974; Garcia De Arevalo et al. 1992) but the major weeds of concern are the parasitic weeds.

Parasitic Weeds

Broomrapes are the most harmful root parasitic weeds for faba bean production along the Mediterranean area and East Africa (Abebe et al. 2013; Parker 2009; Rubiales 2020; Gebru and Mesganaw 2021). They are achlorophyllous and depend totally on faba bean plants for organic carbon, water, and nitrogen. Faba bean is susceptible to several species of broomrapes; crenate broomrape (*Orobanche crenata*) is widespread throughout the Mediterranean and East African regions whereas fetid broomrape (*O. foetida*) is of local importance and a major threat for faba bean production in Tunisia (Kharrat et al. 1992; Pérez-de-Luque et al. 2010). Faba bean yield penalty due to orobanche infestation can reach up to 100% depending on the infection severity, cultivar resistance, and environmental factors such as temperature and seedbank density (Fernández-Aparicio et al. 2016). Broomrapes damage the host before emerging above the soil which complicates their diagnosis and control (Rubiales et al. 2009; Fernández-Aparicio et al. 2016).

Besides broomrapes, the stem parasitic weed dodder (*Cuscuta* sp.) is becoming a serious problem for faba bean production in the Mediterranean basin (Rubiales and Fernández-Aparicio 2012; Adhikari et al. 2021). Like broomrape, dodder is characterized by the reduced amounts or complete absence of chlorophyll and is considered an obligate holoparasite that depends totally on the host to complete its life cycle (García et al. 2014). When parasitizing faba bean, dodder inhibits the pod setting and negatively affects the pod development (Wolswinkel 1974).

Since one technique rarely achieves complete and effective weed control, management of weeds requires an integrated approach that combines cultural, biological, and chemical practices along with genetic resistance (Joel et al. 2007; Rubiales et al. 2009; Pérez-de-Luque et al. 2010; Rubiales and Fernández-Aparicio 2012). Among the cultural practices that have been recommended to control parasitic weeds in faba bean we cite hand weeding, late planting (Pérez-de-Luque et al. 2004; Grenz et al. 2005; Rubiales et al. 2012; Mishra et al. 2016), mulching (Boutagayout et al. 2020), intercropping (Fernández-Aparicio et al. 2008 2010; Abbes et al. 2019), solarization (Mauromicale et al. 2001), minimum tillage and no-tillage (Ghersa and Martınez-Ghersa 2000; López-Bellido et al. 2009), crop rotation (Karkanis et al. 2016), and deep ploughing (Van Delft et al. 2000). As for the biological control, many scientists highlighted the importance of allelopathic effect of some plant species as a bioherbicide for controlling annual and parasitic weeds infecting faba bean (Evidente et al. 2007; Alsaadawi et al. 2017; Cimmino et al. 2018; Messiha et al. 2018; El-Dabaa et al. 2019; Ahmed et al. 2020). Other bioherbicidal agents including insects (Klein and Kroschel 2002; Rubiales et al. 2012) and mycoherbicides (Müller-Stöver and Kroschel 2005; Mabrouk et al. 2007; Ghannam et al. 2007; Dor et al. 2009). Weeds are also controlled by the application of a range of chemical methods like spraying herbicides and applying chemicals to induce suicidal germination of the parasites (Yoneyama et al. 2010; Evidente et al. 2011; Cimmino et al. 2014).

In addition to the weed control methods listed above, the development of weed resistance in faba bean is one of the most effective strategies for managing weeds in faba bean fields (Rubiales 2018). In this regard, sources for *Orobanche crenata* and

Orobanche foetida were identified and released in many Mediterranean and East African countries like Spain (Rubiales et al. 2016), Morocco (Maalouf et al. 2011), Tunisia (Kharrat et al. 2010), Egypt (Ashrie et al. 2010), and Ethiopia (MoANR 2016). Although none of the identified lines have complete resistance to *Orobanche* some of them like Baraca, Quijote, Navio, 674/154/85 L3-4, or 402/29/84 showed combined resistance against both orobanche species (Kharrat and Halila 1994; Abbes et al. 2007; Fernández-Aparicio et al. 2012). The development of marker-assisted selection (MAS) techniques for broomrape resistance is a promising approach since broomrape screenings are difficult, expensive, and sometimes unreliable due to the complexity of the disease. QTLs associated with resistance to *O. crenata* and *O. foetida* in faba bean were identified and validated (Díaz-Ruiz et al. 2009a, b; Gutiérrez et al. 2013; Ocaña-Moral et al. 2017) and recently Gutiérrez and Torres (2021) proposed a more saturated map with reduced QTL intervals and identified candidate genes conferring resistance against *O. crenata*. Whereas significant progress toward the broomrape resistance was achieved, as of today no sources of resistance to dodder have been reported in faba bean.

Among the weed management techniques described above, herbicide treatment still appears as the most efficient especially in conventional agriculture for it is the least time-consuming and the least costly (Garcia De Arevalo et al. 1992; Gressel 2000). Metribuzin and imazethapyr are commonly available chemical herbicides that can control most weeds including broomrapes and dodders. However, like other legumes, faba bean cultivars are sensitive to these herbicides, with severe phytotoxicity (Fig. 14.7) and negative effect on the crop cycle and crop production as reported earlier by many scientists (Taran et al. 2010; Taran et al. 2013; Sajja et al. 2015; Jefferies et al. 2016; Sharma et al. 2016; Gupta et al. 2017; Sharma et al. 2018). Herbicide tolerance in faba bean has been explored by Maalouf et al. (2016a, b) and later by Abou-Khater et al. (2021) resulting in the identification of a number of tolerant lines. The stability of this tolerance has been validated in multiple environments (Abou-Khater et al. 2022b) and associated SNPs markers identified by GWAS analysis (Abou-Khater et al. 2022a).

14.5 Conclusions

Faba bean is of great value for both human and animal nutrition and for the ecosystem. Although great achievements were made in improving faba bean traits, the increasing interest in sustainable agriculture and healthy diets in the Mediterranean and East African area promotes the cultivation of faba bean as a multipurpose crop. In addition, the projected climate change and its impact on crop production support the need to conduct additional research that addresses all the nutritional, biotic, and abiotic constraints described above and to develop nutritious, climate-smart, and pest-resistant varieties. The high level of genetic diversity in faba bean accessions and the available molecular markers will greatly facilitate the development of varieties having a combination of traits desired by both farmers and consumers.

Fig. 14.7 Metribuzin tolerant plot (left) and susceptible plot (right)

References

Abbes Z, Kharrat M, Delavault P, Simier P, Chaïbi W (2007) Field evaluation of the resistance of some faba bean (*Vicia faba* L.) genotypes to the parasitic weed *Orobanche foetida* Poiret. Crop Prot 26(12):1777–1784

Abbes Z, Trabelsi I, Kharrat M, Amri M (2019) Intercropping with fenugreek (*Trigonella foenum-graecum*) enhanced seed yield and reduced *Orobanche foetida* infestation in faba bean (*Vicia faba*). Biol Agric Hortic 35(4):238–247

Abdelhamid MT, Shokr MM, Bekheta MA (2010) Growth, root characteristics, and leaf nutrients accumulation of four faba bean (*Vicia faba* L.) cultivars differing in their broomrape tolerance and the soil properties in relation to salinity. Commun Soil Sci Plant Anal 41(22):2713–2728

Abdellatif KF, El Sayed A, Zakaria AM (2012) Drought stress tolerance of faba bean as studied by morphological traits and seed storage protein pattern. J Plant Studies 1(2):47

Abdelmula AA, Abuanja IK (2007) Genotypic responses, yield stability, and association between characters among some of Sudanese Faba bean (*Vicia faba* L.) genotypes under heat stress. In: Conference on International Agricultural Research for Development, University of Kassel, Witzenhausen and University of Goettingen, Chöttingen

Abdelmula AA, Link W, Kittlitz EV, Stelling D (1999) Heterosis and inheritance of drought tolerance in faba bean, *Vicia faba* L. Plant Breed 118(6):485–490

Abebe T, Beyene H, Nega Y (2013) Distribution and economic importance of broomrape (*Orobanche crenata*) in food legumes production of south Tigray, Ethiopia. Crop Prod 2(3): 101–106

Abebe T, Birhane T, Nega Y, Workineh A (2014) The prevalence and importance of faba bean diseases with special consideration to the newly emerging faba bean gall in Tigray, Ethiopia. Discourse J Agric Food Sci 2(2):33–38

Abeer H, Abd Allah EF, Alqarawi AA, El-Didamony G, Alwhibi M, Egamberdieva D, Ahmad P (2014) Alleviation of adverse impact of salinity on faba bean (*Vicia faba* L.) by arbuscular mycorrhizal fungi. Pak J Bot 46(6):2003–2013

Abera T, Semu E, Debele T, Wegary D, Kim H (2015) Effects of faba bean break crop and N rates on subsequent grain yield and nitrogen use efficiency of highland maize varieties in Toke Kutaye, western Ethiopia. Am J Res Communn 3(10):32–72

Abobatta WF, El-Hashash EF, Hegab RH (2021) Challenges and opportunities for the global cultivation and adaption of legumes. J Appl Biotechnol Bioeng 8(5):160–172

Abou-Khater L, Maalouf F, Patil SB, Balech R, Nacouzi D, Rubiales D, Kumar S (2021) Identification of tolerance to metribuzin and imazethapyr herbicides in faba bean. Crop Sci 61(4): 2593–2611

Abou-Khater L, Maalouf F, Jighly A, Alsamman AM, Rubiales D, Rispail N, Hu J, Ma Y, Balech R, Hamwieh A, Baum M, Kumar S (2022a) Genomic regions associated with herbicide tolerance in a worldwide faba bean (*Vicia faba* L.) collection. Sci Rep 12:158

Abou-Khater L, Maalouf F, Jighly A, Rubiales D, Kumar S (2022b) Adaptability and stability of faba bean (*Vicia faba* L.) accessions under diverse environments and herbicide treatments. Plan Theory 11:251

Abraham AD, Menzel W, Lesemann DE, Varrelmann M, Vetten HJ (2006) Chickpea chlorotic stunt virus: a new polerovirus infecting cool-season food legumes in Ethiopia. Phytopathology 96(5):437–446

Adhikari KN, Khazaei H, Ghaouti L, Maalouf F, Vandenberg A, Link W, O'Sullivan DM (2021) Conventional and molecular breeding tools for accelerating genetic gain in Faba Bean (*Vicia Faba* L.). Front Plant Sci 12:744259

Agriculture Victoria. (n.d.). https://agriculture.vic.gov.au/farm-management/soil/soil-acidity. Accessed on 25 January, 2022

Ahmed S, Abang MM, Maalouf F (2016) Integrated management of Ascochyta blight (*Didymella fabae*) on faba bean under Mediterranean conditions. Crop Prot 81:65–69

Ahmad P, Alyemeni MN, Ahanger MA, Egamberdieva D, Wijaya L, Alam P (2018) Salicylic acid (SA) induced alterations in growth, biochemical attributes and antioxidant enzyme activity in faba bean (Vicia faba L.) seedlings under NaCl toxicity. Russ J Plant Physiol 65(1):104–114

Ahmed SAA, Messiha NK, El-Dabaa MAT, El-Masry RR (2020) The residual allelopathic effects of two Brassicaceae seed powder on the following summer plants and associated weeds. Middle East J 9(2):479–490

Ait Taadaouit N, El Fakhouri K, Sabraoui A, Maalouf F, Rohi L, El Bouhssini M (2021) First sources of resistance in faba bean (*Vicia faba* L.) to the stem borer weevil, *Lixus algirus* L. (Coleoptera: Curculionidae). Phytoparasitica 49(3):349–356

Alagawany M, Abd El-Hack ME, Ashour EA, Salah AS, Hussein ESO, Al Alowaimer A et al (2019) Raw faba bean (*Vicia faba*) as an alternative protein source in laying hen diets. J Appl Poult Res 28(4):808–817

Alehegn M, Tiru M, Taddess M (2018) Screening of faba bean (*Vicia faba*) varieties against faba bean gall diseases (*Olpidium viciae*) in east Gojjam zone, Ethiopia. J Biol Agric Healthc 8(1): 50–55

Alghamdi SS, Migdadi HM, Ammar MH, Paull JG, Siddique KHM (2012) Faba bean genomics: current status and future prospects. Euphytica 186(3):609–624

Ali MB, Welna GC, Sallam A, Martsch R, Balko C, Gebser B et al (2016) Association analyses to genetically improve drought and freezing tolerance of faba bean (*Vicia faba* L.). Crop Sci 56(3): 1036–1048

Alsaadawi IS, Tawfiq AA, Malih HM (2017) Effect of allelopathic Sorghum Mulch on growth and yield of Faba Bean (*Vicia faba*) and companion weeds. J Plant Protect 12:123–127

Amede T, Schubert S, Stahr K (2003) Mechanisms of drought resistance in grain legumes I: osmotic adjustment. SINET: Ethiopian J Sci 26(1):37–46

Arese P (2006) How genetics and biology helped humanity to survive falciparum malaria. Parassitologia 48:553–559

Aschi A, Aubert M, Riah-Anglet W, Nélieu S, Dubois C, Akpa-Vinceslas M, Trinsoutrot-Gattin I (2017) Introduction of Faba bean in crop rotation: Impacts on soil chemical and biological characteristics. Appl Soil Ecol 120:219–228

Ashrie AMA, Mohamed EA, Helal AA, Abdel-Tawab YM, EL-Harty EH (2010) Performance of six faba bean genotypes under free and Orobanche soils. Egypt J Plant Breed 14(2):189–205

Asif MA, Paull JG (2021) An approach to detecting quantitative trait loci and candidate genes associated with salinity tolerance in faba bean (*Vicia faba*). Plant Breed 140(4):643–653

Atienza SG, Palomino C, Gutiérrez N, Alfaro CM, Rubiales D, Torres AM, Ávila CM (2016) QTLs for ascochyta blight resistance in faba bean (*Vicia faba* L.): validation in field and controlled conditions. Crop Pasture Sci 67(2):216–224

Avila CM, Sillero JC, Rubiales D, Moreno MT, Torres AM (2003) Identification of RAPD markers linked to the *Uvf-1* gene conferring hypersensitive resistance against rust (*Uromyces viciae-fabae*) in *Vicia faba* L. Theor Appl Genet 107(2):353–358

Avila CM, Satovic Z, Sillero JC, Rubiales D, Moreno MT, Torres AM (2004) Isolate and organ-specific QTLs for ascochyta blight resistance in faba bean (*Vicia faba* L). Theor Appl Genet 108(6):1071–1078

Babin M, Ortiz V, Castro S, Romero J (2000) First detection of Faba bean necrotic yellow virus in Spain. Plant Dis 84(6):707–707

Béji B, Bouktila D, Mezghani-Khemakhem M, Bouhachem-Boukhris S, Makni M, Makni H (2013) Genetic structure of *Aphis fabae* Scopoli (Hemiptera, Aphididae) in Tunisia, inferred from RAPD markers. Romanian Agric Res 30:307–315

Belachew KY, Stoddard FL (2017) Screening of faba bean (*Vicia faba* L.) accessions to acidity and aluminium stresses. PeerJ 5:e2963

Beyene AT, Derera J, Sibiya J (2018) Genetic variability of faba bean genotypes for chocolate spot (*Botrytis fabae*) resistance and yield. Euphytica 214(8):1–17

Bishop J, Potts SG, Jones HE (2016) Susceptibility of faba bean (*Vicia faba* L.) to heat stress during floral development and anthesis. J Agron Crop Sci 202(6):508–517

Bond DA (1976) EEC seminar on protein quality from leguminous crops. Dijon, France

Bouhassan A, Sadiki M, Tivoli B (2004) Evaluation of a collection of faba bean (*Vicia faba* L.) genotypes originating from the Maghreb for resistance to chocolate spot (*Botrytis fabae*) by assessment in the field and laboratory. Euphytica 135(1):55–62

Boutagayout A, Nassiri L, Bouiamrine EH, Belmalha S (2020) Mulching effect on weed control and faba bean (*Vicia faba* L. Minor) yield in Meknes region, Morocco. In: E3S web of conferences, vol 183. EDP Sciences, p 04002

Cabrera A, Martin A (1986) Variation in tannin content in *Vicia faba* L. J Agric Sci 106(2):377–382

Caracuta V, Weinstein-Evron M, Kaufman D, Yeshurun R, Silvent J, Boaretto E (2016) 14,000-year-old seeds indicate the Levantine origin of the lost progenitor of faba bean. Sci Rep 6(1):1–6

Carrillo-Perdomo E, Raffiot B, Ollivier D, Deulvot C, Magnin-Robert JB, Tayeh N, Marget P (2019) Identification of novel sources of resistance to seed weevils (*Bruchus* spp.) in a faba bean germplasm collection. Front Plant Sci 9:1914

Carrillo-Perdomo E, Vidal A, Kreplak J, Duborjal H, Leveugle M, Duarte J et al (2020) Development of new genetic resources for faba bean (*Vicia faba* L.) breeding through the discovery of gene-based SNP markers and the construction of a high-density consensus map. Sci Rep 10(1): 1–14

Chen H, Richardson AE, Rolfe BG (1993) Studies of the physiological and genetic basis of acid tolerance in *Rhizobium leguminosarum* biovar *trifolii*. Appl Environ Microbiol 59(6): 1798–1804

Cherif C, Hassanat F, Claveau S, Girard J, Gervais R, Benchaar C (2018) Faba bean (*Vicia faba*) inclusion in dairy cow diets: effect on nutrient digestion, rumen fermentation, nitrogen utilization, methane production, and milk performance. J Dairy Sci 101(10):8916–8928

Christmann S, Aw-Hassan AA (2012) Farming with alternative pollinators (FAP)—an overlooked win-win-strategy for climate change adaptation. Agric Ecosyst Environ 161:161–164

Christmann S, Bencharki Y, Anougmar S, Rasmont P, Smaili MC, Tsivelikas A, Aw-Hassan A (2021) Farming with alternative pollinators benefits pollinators, natural enemies, and yields, and offers transformative change to agriculture. Sci Rep 11(1):1–10

Cimmino A, Fernández-Aparicio M, Andolfi A, Basso S, Rubiales D, Evidente A (2014) Effect of fungal and plant metabolites on broomrape (*Orobanche* and *Phelipanche* spp.) seed germination and radicle growth. J Agric Food Chem 62:10485–10492

Cimmino A, Masi M, Rubiales D, Evidente A, Fernández-Aparicio M (2018) Allelopathy for parasitic plant management. Nat Prod Commun 13(3):289–294

Cook J, Lewandowsky S (2016) Rational irrationality: modeling climate change belief polarization using Bayesian networks. Top Cogn Sci 8(1):160–179

Crépon K, Marget P, Peyronnet C, Carrouée B, Arese P, Duc G (2010) Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. Field Crop Res 115(3):329–339

Cubero JI (1974) On the evolution of *Vicia faba* L. Theor Appl Genet 45(2):47–51

De Arevalo RCG, Lusarreta CA, Neyra CB, Sanchez MA, Algarra PJ (1992) Chemical control of annual weeds in field beans (*Vicia faba*) in Central Spain. Weed Sci 40(1):96–100

Delgado MJ, Ligero F, Lluch CL (1994) Effects of salt stress on growth and nitrogen fixation by pea, faba-bean, common bean and soybean plants. Soil Biol Biochem 26(3):371–376

Dereje G, Wendafrash M, Gemechu K (2012) Faba Bean Galls: a new disease of faba bean in Ethiopia. https://docs.google.com/file/d/0B6qFhb82WH8MWXJxdFJZZXlRcEE/edit. Accessed on 20 December 2021

Díaz-Ruiz R, Satovic Z, Ávila CM, Alfaro CM, Gutierrez MV, Torres AM, Román B (2009a) Confirmation of QTLs controlling *Ascochyta fabae* resistance in different generations of faba bean (*Vicia faba* L.). Crop and Pasture. Science 60(4):353–361

Díaz-Ruiz R, Torres A, Gutierrez MV, Rubiales D, Cubero JI, Kharrat M et al (2009b) Mapping of quantitative trait loci controlling *Orobanche foetida* Poir. resistance in faba bean (*Vicia faba* L.). Afr J Biotechnol 8(12)

Dor E, Hershenhorn J, Andolfi A, Cimmino A, Evidente A (2009) *Fusarium verticillioides* as a new pathogen of the parasitic weed *Orobanche* spp. Phytoparasitica 37(4):361–370

Duc G (1997) Faba bean (*Vicia faba* L.). Field Crop Res 53(1–3):99–109

Duc G, Marget P, Page D, Domoney C (2004) Facile breeding markers to lower contents of vicine and convicine in faba bean seeds and trypsin inhibitors in pea seeds. In: Proceedings of the fourth international workshop on antinutritional factors in legume seeds and oilseeds. Toledo, Spain, 8–10 March 2004

Duc G, Aleksić JM, Marget P, Mikic A, Paull J, Redden RJ et al (2015) Faba bean. Grain Legumes:141–178

El Nahhas N, AlKahtani MD, Abdelaal KA, Al Husnain L, AlGwaiz HI, Hafez YM et al (2021) Biochar and jasmonic acid application attenuates antioxidative systems and improves growth, physiology, nutrient uptake and productivity of faba bean (*Vicia faba* L.) irrigated with saline water. Plant Physiol Biochem 166:807–817

El-Akhdar I (2020) Improvement of faba bean production in salt-affected soils by *Rhizobium leguminosarum* bv. *viciae* inoculation and phosphorus application. In Situ 12:11

El-Awadi ME, Sadak M, Dawood MG, Khater MA, Elashtokhy MMA (2017) Amelioration the adverse effects of salinity stress by using γ-radiation in faba bean plants. Bull NRC 41(1): 293–310

El-Bastawisy ZM, El-Katony TM, Abd El-Fatah SN (2018) Genotypic variability in salt tolerance of *Vicia faba* during germination and early seedling growth. J King Saud Univ-Sci 30(2): 270–277

El-Dabaa MAT, Ahmed SAE, Messiha NK, El-Masry RR (2019) The allelopathic efficiency of *Eruca sativa* seed powder in controlling *Orobanche crenata* infected *Vicia faba* cultivars. Bullet Natl Res Centre 43(1):1–8

El-Ghany A, Mona F, Attia M (2020) Effect of exopolysaccharide-producing bacteria and melatonin on faba bean production in saline and non-saline soil. Agronomy 10(3):316

Emeran AA, Sillero JC, Fernández-Aparicio M, Rubiales D (2011) Chemical control of faba bean rust (*Uromyces viciae-fabae*). Crop Prot 30:907–912

Etemadi F, Hashemi M, Barker AV, Zandvakili OR, Liu X (2019) Agronomy, nutritional value, and medicinal application of faba bean (*Vicia faba* L.). Hortic Plant J 5(4):170–182

Evidente A, Fernández-Aparicio M, Andolfi A, Rubiales D, Motta A (2007) Trigoxazonane, a monosubstituted trioxazonane by *Trigonella foenum-graecum* root exudate, inhibiting agent of *Orobanche crenata* seed germination. Phytochemistry 68:2487–2492

Evidente A, Andolfi A, Cimmino A (2011) Relationships between the stereochemistry and biological activity of fungal phytotoxins. Chirality 23(9):674–693

Faba Bean Genome Consortium (2022). https://projects.au.dk/fabagenome. Accessed 15 Jan 2022

FAOSTAT (2021) Food and Agriculture Organization of the United Nations. www.faostat.fao.org. Accessed 20 December 2021

Faridi R, Koopman B, Schierholt A, Ali MB, Apel S, Link W (2021) Genetic study of the resistance of faba bean (*Vicia faba*) against the fungus *Ascochyta fabae* through a genome-wide association analysis. Plant Breed 140:442–452

Farooq M, Gogoi N, Hussain M, Barthakur S, Paul S, Bharadwaj N et al (2017) Effects, tolerance mechanisms and management of salt stress in grain legumes. Plant Physiol Biochem 118:199–217

Fernández-Aparicio M, Emeran AA, Rubiales D (2008) Control of *Orobanche crenata* in legumes intercropped with fenugreek (*Trigonella foenum-graecum*). Crop Prot 27:653–659

Fernández-Aparicio M, Emeran AA, Rubiales D (2010) Inter-cropping with berseem clover (*Trifolium alexandrinum*) reduces infection by *Orobanche crenata* in legumes. Crop Prot 29: 867–871

Fernández-Aparicio M, Shtaya M, Emeran AA, Allagui MB, Kharrat M, Rubiales D (2011a) Effects of crop mixtures on chocolate spot development on faba bean grown in Mediterranean climates. Crop Prot 30:1015–1023

Fernández-Aparicio M, Westwood JH, Rubiales D (2011b) Agronomic, breeding, and biotechnological approaches to parasitic plant management through manipulation of germination stimulant levels in agricultural soils. Botany 89:813–826

Fernández-Aparicio M, Moral A, Kharrat M, Rubiales D (2012) Resistance against broomrapes (*Orobanche* and *Phelipanche* spp.) in faba bean (*Vicia faba*) based in low induction of broomrape seed germination. Euphytica 186:897–905

Fernández-Aparicio M, Flores F, Rubiales D (2016) The effect of *Orobanche crenata* infection severity in faba bean, field pea, and grass pea productivity. Front Plant Sci 7:1409

Flores F, Nadal S, Solis I, Winkler J, Sass O, Stoddard FL, Link W, Raffiot B, Muel F, Rubiales D (2012) Faba bean adaptation to autumn sowing under European climates. Agron Sustain Dev 32:727–734

Frenda AS, Ruisi P, Saia S, Frangipane B, Di Miceli G, Amato G, Giambalvo D (2013) The critical period of weed control in faba bean and chickpea in Mediterranean areas. Weed Sci 61(3): 452–459

Gaballah MS, Gomaa AM (2004) Performance of Faba Bean varieties grown under salinity. J Appl Sci 4(1):93–99

García MA, Costea M, Kuzmina M, Stefanović S (2014) Phylogeny, character evolution, and biogeography of Cuscuta (dodders; Convolvulaceae) inferred from coding plastid and nuclear sequences. Am J Bot 101(4):670–690

García-Ruiz JM, López-Moreno JI, Vicente-Serrano SM, Lasanta-Martínez T, Beguería S (2011) Mediterranean water resources in a global change scenario. Earth Sci Rev 105(3–4):121–139

Gebru A, Mesganaw M (2021) Improving faba bean production and productivity through the integrated management of *Orobanche crenata* at Kutaber, Amhara Region, Ethiopia. Int J Agric Sci Food Technol 7(1):114–117

Gela TS, Bruce M, Chang W, Stoddard FL, Schulman AH, Vandenberg A, Khazaei H (2021) Genomic regions associated with chocolate spot (Botrytis fabae Sard.) resistance in faba bean (Vicia faba L.). bioRxiv. https://doi.org/10.1101/2021.11.22.469473

Genanew T, Argaw M, Adgo E (2012) Farmers soil management practices and their perceptions to soil acidity at Ankesha District of Awi Zone, Northwestern Ethiopia. Libyan Agric Res Center J Int 3(2):64–72

GENESYS. (2022). https://www.genesys-pgr.org/. Accessed 17 February, 2022

Ghannam I, Al-Masri M, Barakat R (2007) Biological Control of Egyptian Broomrape (*Orobanche aegyptiaca*) Using *Fusarium* spp. Phytopathol Mediterr 46:177–184

Ghersa CM, Martınez-Ghersa MA (2000) Ecological correlates of weed seed size and persistence in the soil under different tilling systems: implications for weed management. Field Crop Res 67(2):141–148

Göl Ş, Doğanlar S, Frary A (2017) Relationship between geographical origin, seed size and genetic diversity in faba bean (*Vicia faba* L.) as revealed by SSR markers. Mol Gen Genomics 292(5): 991–999

Grains Research and Development Corporation (GRDC). (2017) GRDC Grownotes—Faba Bean Section 6: weed control. https://grdc.com.au/__data/assets/pdf_file/0030/369525/GrowNote-Faba-Bean-North-6-Weed-control.pdf

Grenz JH, Manschadi AM, Uygur FN, Sauerborn J (2005) Effects of environment and sowing date on the competition between faba bean (*Vicia faba*) and the parasitic weed *Orobanche crenata*. Field Crop Res 93(2-3):300–313

Gressel J (2000) Molecular biology of weed control. Transgenic Res 9(4):355–382

Griffiths DW (1979) The inhibition of digestive enzyme by extracts of field bean (*Vicia faba*). J Sci Food Agric 30:458–462

Grosjean F, Bourdillon A, Rudeaux F, Bastianelli D, Peyronnet C, Duc G, Lacassagne L (2000) Valeur alimentaire pour la volaille de féveroles isogéniques (*Vicia faba* L) avec ou sans tannins et avec ou sans vicine. Sci Tech Avicoles 32:17–23

Gupta M, Bindra S, Sood A, Singh I, Singh G, Gaur PM, Chatuverdi SK, Dixit GP, Singh S (2017) Identifying new sources of tolerance to post emergence herbicides in chickpea (*Cicer arietinum* L.). J Food Legumes 31:5–9

Gutiérrez N, Torres AM (2021) QTL dissection and mining of candidate genes for *Ascochyta fabae* and *Orobanche crenata* resistance in faba bean (*Vicia faba* L.). BMC Plant Biol 21(1):1–12

Gutiérrez N, Palomino C, Satovic Z, Ruiz-Rodríguez MD, Vitale S, Gutiérrez MV et al (2013) QTLs for *Orobanche* spp. resistance in faba bean: identification and validation across different environments. Mol Breed 32(4):909–922

Hanounik S (1980) Effect of chemical treatments and host genotypes on disease severity/yield relationships of Ascochyta blight in faba beans (*Vicia faba*). FABIS Newslett (ICARDA) 2:50

Hendawey MH, Younes AMA (2013) Biochemical evaluation of some faba bean cultivars under rainfed conditions at El-Sheikh Zuwayid. Ann Agric Sci 58(2):183–193

Herzog H (1989) Freezing resistance and performance of faba bean populations during winter seasons in Northern Germany. J Agron Crop Sci 162:225–235

Herzog H, Saxena MC (1988) Winter hardiness in Mediterranean populations of faba bean. Fabis Newslett 21:19–25

Heusala H, Sinkko T, Sözer N, Hytönen E, Mogensen L, Knudsen MT (2020) Carbon footprint and land use of oat and faba bean protein concentrates using a life cycle assessment approach. J Clean Prod 242:118376

Hussein LA, Saleh M (1985) Antinutritional factors in faba beans [*Vicia faba*]. In: International Workshop on Faba Beans, Kabuli, Chickpeas, and Lentils in the 1980's, Aleppo (Syria), 16–20 May 1983. International Center for Agricultural Research in the Dry Areas

Hussein M, Embiale A, Husen A, Aref IM, Iqbal M (2017) Salinity-induced modulation of plant growth and photosynthetic parameters in faba bean (*Vicia faba*) cultivars. Pak J Bot 49(3): 867–877

Ijaz U, Sudheesh S, Kaur S, Sadeque A, Bariana H, Bansal U, Adhikari K (2021) Mapping of two new rust resistance genes Uvf-2 and Uvf-3 in faba bean. Agronomy 11(7):1370

Ivarsson E, Wall H (2017) Effects of toasting, inclusion levels and different enzyme supplementations of faba beans on growth performance of broiler chickens. J Appl Poult Res 26(4):467–475

Jambunathan R, Blain HL, Dhindsa KS, Hussein LA, Kogure K, Li-Juan L, Youssef MM (1994) Diversifying use of cool season food legumes through processing. In: Expanding the production and use of cool season food legumes. Springer, Dordrecht, pp 98–112

Jefferies ML, Willenborg CJ, Tar'an B (2016) Response of conventional and imidazolinone-resistant chickpea (*Cicer arietinum* L.) cultivars to imazamox and/or imazethapyr applied post-emergence. Can J Plant Sci 96(1):48–58

Jellis GJ, Bond DA, Boulton RE (1998) Diseases of faba bean. In: Allen DJ, Lenné JM (eds) The pathology of food and pasture legumes. CAB International and ICRISAT, Wallingford, UK, pp 371–422

Jensen ES, Peoples MB, Hauggaard-Nielsen H (2010) Faba bean in cropping systems. Field Crop Res 115(3):203–216

Joel DM, Hershenhorn J, Eizenberg H, Aly R, Ejeta G, Rich PJ et al (2007) Biology and management of weedy root parasites. Hortic Rev 33:267–350. John Wiley & Sons, Inc

Jones P, Cockbain AJ, Freigoun SO (1984) Association of a mycoplasma-like organism with broad bean phyllody in the Sudan. Plant Pathol 33(4):599–602

Kaiser WJ, Schalk JM (1973) Pea leaf roll virus: transmission by aphids to pulse crops in Irán. J Econ Entomol 66:1131–1132

Karkanis A, Travlos IS, Bilalis DJ, Tabaxi EI (2016) Integrated weed management in winter cereals in Southern Europe. In: Weed and pest control: molecular biology, practices and environmental impact. Nova Science Publishers, Inc., USA, pp 1–15

Kassa Y, Ayele T, Worku Y, Teferra B (2020) Participatory evaluation of faba bean gall disease (*Olpidium viciae*) management options in the highland disease hotspot areas of South-Eastern Amhara Region, Ethiopia: an integrated approach. Cogent Food Agric 6(1):1801216

Kaur S, Kimber RB, Cogan NO, Materne M, Forster JW, Paull JG (2014) SNP discovery and high-density genetic mapping in faba bean (*Vicia faba* L.) permits identification of QTLs for ascochyta blight resistance. Plant Sci 217:47–55

Keneni G, Asmamaw B, Jarso M (2001) Efficiency of drained selection environments for improving grain yield in faba bean under undrained target environments on vertisol. Euphytica 122(2): 279–285

Khamassi K, Jeddi FB, Hobbs D, Irigoyen J, Stoddard F, O'Sullivan DM, Jones H (2013) A baseline study of vicine–convicine levels in faba bean (*Vicia faba* L.) germplasm. Plant Genetic Resources 11(3):250–257

Khan MA, Ammar MH, Migdadi HM, El-Harty EH, Osman MA, Farooq M, Alghamdi SS (2015) Comparative nutritional profiles of various faba bean and chickpea genotypes. Int J Agric Biol 17(3):449–457

Kharrat M, Halila MH (1994) Orobanche species on faba bean (*Vicia faba* L.) in Tunisia: problems and management. In: Biology and management of Orobanche. Proceedings of the third international workshop on Orobanche and related Striga research, Amsterdam, Netherlands, 8-–12 November 1993. Royal Tropical Institute, pp 639–643

Kharrat M, Halila MH, Linke KH, Haddar T (1992) First report of *Orobanche foetida* Poiret on faba bean in Tunisia. Fabis Newslett 30:46–47

Kharrat M, Abbes Z, Amri M (2010) A new faba bean small seeded variety Najeh tolerant to orobanche registered in the Tunisian catalogue. Tunis J Plant Prot 5(1)

Khazaei H, Vandenberg A (2020) Seed mineral composition and protein content of faba beans (*Vicia faba* L.) with contrasting tannin contents. Agronomy 10(4):511

Khazaei H, Purves RW, Song M, Stonehouse R, Bett KE, Stoddard FL, Vandenberg A (2017) Development and validation of a robust, breeder-friendly molecular marker for the vc-locus in faba bean. Mol Breed 37(11):1–6

Khazaei H, Purves RW, Hughes J, Link W, O'Sullivan DM, Schulman AH et al (2019) Eliminating vicine and convicine, the main anti-nutritional factors restricting faba bean usage. Trends Food Sci Technol 91:549–556

Khazaei H, O'Sullivan DM, Stoddard FL, Adhikari KN, Paull JG, Schulman AH et al (2021) Recent advances in faba bean genetic and genomic tools for crop improvement. Legume Sci 3 (3):e75

Klein O, Kroschel J (2002) Biological control of *Orobanche* spp. with *Phytomyza orobanchia*, a review. BioControl 47(3):245–277

Klingman GC, Ashton FM, Noordhoff LJ (1982) Weed Science: principles and practices (No. 632.954 K5 1982). Wiley, New York

Koivunen E, Partanen K, Perttilä S, Palander S, Tuunainen P, Valaja J (2016) Digestibility and energy value of pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.) and blue lupin (narrow-leaf) (*Lupinus angustifolius*) seeds in broilers. Anim Feed Sci Technol 218:120–127

Kotb TH, Watanabe T, Ogino Y, Tanji KK (2000) Soil salinization in the Nile Delta and related policy issues in Egypt. Agric Water Manag 43(2):239–261

Kraberger S, Kumari SG, Najar A, Stainton D, Martin DP, Varsani A (2018) Molecular characterization of faba bean necrotic yellows viruses in Tunisia. Arch Virol 163(3):687–694

Kumari SG, Makkouk KM (2007) Virus diseases of faba bean (*Vicia faba* L.) in Asia and Africa. Plant Viruses 1(1):93–105

Kumari, S.G., Van Leur, J., Maalouf, F. (2018). Screening faba bean (*Vicia faba* L.) germplasm for resistance to persistently aphid transmitted viruses. In: Presented at Seventh International Food Legumes Research Conference, Marrakesh, Morocco, 6–8 May.2018. Oral presentation in Workshop 3. pp. 100

Kwon SJ, Hu J, Coyne CJ (2010) Genetic diversity and relationship among faba bean (*Vicia faba* L.) germplasm entries as revealed by TRAP markers. Plant Genetic Resources 8(3):204–213

Landry EJ, Coyne CJ, Hu J (2015a) Agronomic performance of Spring-Sown Faba Bean in Southeastern Washington. Agron J 107(2):574–578

Landry EJ, Lafferty JE, Coyne CJ, Pan WL, Hu J (2015b) Registration of four winter-hardy faba bean germplasm lines for use in winter pulse and cover crop development. J Plant Registr 9(3): 367–370

Lawes DA (1980) Recent developments in understanding, improvement and use of *Vicia faba*. In: Summerfield RJ, Bunting AH (eds) Advances in legumes science. Royal Botanic Gardens, Kew, Richmond, UK, pp 625–636

Lessire M, Gallo V, Prato M, Akide-Ndunge O, Mandili G, Marget P et al (2017) Effects of faba beans with different concentrations of vicine and convicine on egg production, egg quality and red blood cells in laying hens. Animal 11(8):1270–1278

Link W, Balko C, Stoddard FL (2010) Winter hardiness in faba bean: physiology and breeding. Field Crop Res 115(3):287–296

López-Bellido RJ, Benítez-Vega J, López-Bellido L (2009) No-tillage improves broomrape control with glyphosate in Faba-Bean. Agron J 101(6):1394–1399

Louicharoen C, Patin E, Paul R, Nuchprayoon I, Witoonpanich B, Peerapittayamongkol C et al (2009) Positively selected G6PD-Mahidol mutation reduces *Plasmodium vivax* density in Southeast Asians. Science 326(5959):1546–1549

Luo Y, Gu Z, Han Y, Chen Z (2009) The impact of processing on phytic acid, in vitro soluble iron and Phy/Fe molar ratio of faba bean (*Vicia faba* L.). J Sci Food Agric 89(5):861–866

Maalouf F, Khalil S, Ahmed S, Akintunde AN, Kharrat M, El Shama'a K et al (2011) Yield stability of faba bean lines under diverse broomrape prone production environments. Field Crop Res 124(3):288–294

Maalouf F, Nachit M, Ghanem ME, Singh M (2015) Evaluation of faba bean breeding lines for spectral indices, yield traits and yield stability under diverse environments. Crop Pasture Sci 66(10):1012–1023

Maalouf F, Ahmed S, Shaaban K, Bassam B, Nawar F, Singh M, Amri A (2016a) New faba bean germplasm with multiple resistances to Ascochyta blight, chocolate spot and rust diseases. Euphytica 211(2):157–167

Maalouf F, Patil SB, Rajendra K, Hamwieh A, Goyal A, Kumar S (2016b) Breeding for post-emergence herbicide tolerance in cool-season food legumes. In: Presented at International Conference on Pulses for Health, Nutrition, and Sustainable Agriculture in Drylands, Marrakesh, Morocco, 18–20 Apr. 2016. ICARDA

Maalouf F, Ahmed S, Somanagouda P (2018) Developing improved varieties of faba bean. In: Sivasankar S, Bergvinson D, Gaur PM, Kumar S, Beebe S, Tamo M (eds) Achieving sustainable cultivation of grain legumes, Improving cultivation of particular grain legumes, vol 2. Burleigh Dodds Science Publishing, Cambridge, UK, pp 253–267

Maalouf F, Abou-Khater L, Babiker Z, Hu J, Ma Yu, Jighly A, Alsamman MA, Rispail N, Balech R, Baum M, Kumar S (inprint). Genetic dissection of heat stress tolerance in faba bean (*Vicia faba* L.) using GWAS [Manuscript submitted for publication]

Mabrouk Y, Zourgui L, Sifi B, Belhadj O (2007) The potential of Rhizobium strains for biological control of *Orobanche crenata*. Biologia 62(2):139–143

Makkouk KM, Kumari SG (1995) Screening and selection of faba bean (*Vicia faba* L.) germplasm for resistance to bean yellow mosaic potyvirus. J Plant Dis Protect:461–466

Makkouk KM, Kumari SG, van Leur JA (2002) Screening and selection of faba bean (*Vicia faba* L.) germplasm resistant to Bean leafroll virus. Aust J Agric Res 53(9):1077–1082

Makkouk KM, Hamed AA, Hussein M, Kumari SG (2003) First report of Faba bean necrotic yellows virus (FBNYV) infecting chickpea (*Cicer arietinum*) and faba bean (*Vicia faba*) crops in Sudan. Plant Pathol 52(3):412–412

Mansour E, Moustafa ES, Qabil N, Abdelsalam A, Wafa HA, El Kenawy A et al (2018) Assessing different barley growth habits under Egyptian conditions for enhancing resilience to climate change. Field Crop Res 224:67–75

Mansour E, Desoky ESM, Ali MM, Abdul-Hamid MI, Ullah H, Attia A, Datta A (2021) Identifying drought-tolerant genotypes of faba bean and their agro-physiological responses to different water regimes in an arid Mediterranean environment. Agric Water Manag 247:106754

Marcellos H, Moore KJ, Nikandrow A (1995) Influence of foliar-applied fungicides on seed yield of faba bean (*Vicia faba* L.) in northern New South Wales. Aust J Exp Agric 35(1):97–102

Mauromicale G, Restuccia G, Marchese M (2001) Soil solarization, a non-chemical technique for controlling *Orobanche crenata* and improving yield of faba bean. Agronomie 21(8):757–765

Mbanefo EC, Ahmed AM, Titouna A, Elmaraezy A, Trang NTH, Phuoc Long N et al (2017) Association of glucose-6-phosphate dehydrogenase deficiency and malaria: a systematic review and meta-analysis. Sci Rep 7(1):1–10

McVicar R, Panchuk D, Brenzil C, Hartley S, Pearse P, Vandenberg A (2013). Faba bean. Gov. Saskatchewan. Agriculture, Crops

Mengesha GG, Terefe H, Cheleko DC (2022) Progression of chocolate spot (*Botrytis fabae*) and grain yield of faba bean as influenced by integration of fungicide rate and host resistance in Southern Ethiopia. J Crop Sci Biotechnol 25(1):73–90

Messiha NK, El-Dabaa MAT, El-Masry RR, Ahmed SAA (2018) The allelopathic influence of *Sinapis alba* seed powder (white mustard) on the growth and yield of *Vicia faba* (faba bean) infected with *Orobanche crenata* (broomrape). Middle East J Appl Sci 8(2):418–425

Miller P (1754) The gardeners dictionary, 4th edn. Rivington, London

Mínguez I, Rubiales D (2020) Faba bean (Chapter 15). In: Sadras VO, Calderini DF (eds) Crop physiology: case histories for major crops. Academic Press, pp 453–482. ISBN 978-0-12-819194-1. https://doi.org/10.1016/B978-0-12-819194-1.00015-3

Mishra JS, Rao AN, Singh VP, Kumar R (2016) Weed management in major field crops. Adv Weed Manage Indian Soc Agron:1–23

MoANR (Ministry of Agriculture and Natural Resources). (2016) Pest management support services strategy for Ethiopia, pp. 1–88

Muduuli DS, Marquardt RR, Guenter W (1982) Effect of dietary vicine and vitamin E supplementation on the productive performance of growing and laying chickens. Br J Nutr 47(1):53–60

Muehlbauer FJ, Abebe T (1997). *Vicia faba* L. New crop factsheet. Center for New Crops and Plant Products. Purdue University, USA

Müller-Stöver D, Kroschel J (2005) The potential of *Ulocladium botrytis* for biological control of *Orobanche* spp. Biol Control 33(3):301–306

Multari S, Stewart D, Russell WR (2015) Potential of fava bean as future protein supply to partially replace meat intake in the human diet. Compr Rev Food Sci Food Saf 14(5):511–522

Mulugeta B, Tesfaye K, Keneni G, Ahmed S (2021) Genetic diversity in spring faba bean (*Vicia faba* L.) genotypes as revealed by high-throughput KASP SNP markers. Genet Resour Crop Evol 68(5):1971–1986

Muratova VS (1931) Boby (*Vicia faba* L.). Izd Instituta rastenievodstva

Ocaña S, Seoane P, Bautista R, Palomino C, Claros GM, Torres AM, Madrid E (2015) Large-scale transcriptome analysis in faba bean (Vicia faba L.) under Ascochyta fabae infection. PLoS One 10(8):e0135143

Ocaña-Moral S, Gutiérrez N, Torres AM, Madrid E (2017) Saturation mapping of regions determining resistance to Ascochyta blight and broomrape in faba bean using transcriptome-based SNP genotyping. Theor Appl Genet 130(11):2271–2282

Omar MEDM, Moussa AMA, Hinkelmann R (2021) Impacts of climate change on water quantity, water salinity, food security, and socioeconomy in Egypt. Water Sci Eng 14(1):17–27

Pampana S, Masoni A, Arduini I (2016) Response of cool-season grain legumes to waterlogging at flowering. Can J Plant Sci 96(4):597–603

Parihar AK, Dixit GP, Kumar N, Nath CP, Singh AK, Chaturvedi SK, Singh NP (2017) P-637: a post emergence herbicide (Metribuzin) tolerant genotype of fieldpea (*Pisum sativum* L.). J Food Legumes 31(1):66–68

Parker C (2009) Observations on the current status of Orobanche and Striga problems worldwide. Pest Manage Sci: Formerly Pest Sci 65(5):453–459

Pérez-de-Luque A, Sillero JC, Moral A, Cubero JI, Rubiales D (2004) Effect of sowing date and host resistance on the establishment and development of *Orobanche crenata* in faba bean and common vetch. Weed Res 44(4):282–288

Pérez-de-Luque A, Eizenberg H, Grenz JH, Sillero JC, Ávila C, Sauerborn J, Rubiales D (2010) Broomrape management in faba bean. Field Crop Res 115(3):319–328

Rahate KA, Madhumita M, Prabhakar PK (2021) Nutritional composition, anti-nutritional factors, pretreatments-cum-processing impact and food formulation potential of faba bean (*Vicia faba* L.): a comprehensive review. LWT 138:110796

Rajcan I, Swanton CJ (2001) Understanding maize–weed competition: resource competition, light quality and the whole plant. Field Crop Res 71(2):139–150

Rengasamy P (2010) Soil processes affecting crop production in salt-affected soils. Funct Plant Biol 37(7):613–620

Robertson LD, Saxena MC (1993) Problems and prospects of stress resistance breeding in faba bean. In: Singh KB, Saxena MC (eds) Breeding for stress tolerance in cool seasons food legumes. John Wiley & Sons, Chichester, UK, pp 37–50

Robertson LD, Sadiki M, Matic R, Li-juan L (2000) *Vicia* spp: conserved resources, priorities for collection and prospects. In: Linking Research and marketing opportunities for pulses in the 21st century. Springer, Dordrecht, pp 623–633

Rubiales D (2018) Can we breed for durable resistance to broomrapes? Phytopathol Mediterr 57(1): 170–185

Rubiales D (2020) Broomrape threat to agriculture. Outlooks Pest Manage 31(3):141–144

Rubiales D, Fernández-Aparicio M (2012) Innovations in parasitic weeds management in legume crops. A review. Agronr Sustain Dev 32(2):433–449

Rubiales D, Khazaei H (2022) Advances in disease and pest resistance in faba bean. Theor Appl Genet. https://doi.org/10.1007/s00122-020-03751-5. (in press)

Rubiales D, Verkleij J, Vurro M, Murdoch AJ, Joel DM (2009) Parasitic plant management in sustainable agriculture. Weed Res 49(Suppl. 1):1–5

Rubiales D, Ávila CM, Sillero JC, Hybl M, Narits L, Sass O, Flores F (2012) Identification and multi-environment validation of resistance to *Ascochyta fabae* in faba bean (*Vicia faba*). Field Crop Res 126:165–170

Rubiales D, Rojas-Molina MM, Sillero JC (2016) Characterization of resistance mechanisms in faba bean (*Vicia faba*) against broomrape species (*Orobanche* and *Phelipanche* spp.). Frontiers. Plant Sci 7:1747

Ruwende CYRIL, Hill A (1998) Glucose-6-phosphate dehydrogenase deficiency and malaria. J Mol Med 76(8):581–588

Sajja S, Samineni S, Gadekar M, Jayalakshmi V, Vijayakumar A, Yasin M, Varshney RK (2015) Effect of post-emergence herbicide imazethapyr on phenological and agronomic traits in chickpea breeding lines. In: Proceedings of the International Plant Breeding Congress (IPBC) and Eucarpia – Oil and Protein Crops Section Conference, Antalya, Turkey, 1–5 Nov. 2015. CGIAR

Sallam MN (2013) INSECT DAMAGE: damage on post-harvest. Food and Agriculture Organization of the United Nations. https://www.fao.org/3/av013e/av013e.pdf

Sekara A, Poniedzialek M, Ciura J, Jedrszczyk E (2001) The effect of meteorological factors upon flowering and pod setting of faba bean [*Vicia faba* L.] at different sowing times. Vegetable Crops Res Bullet 54(1):65–68

Sentil A, Lhomme P, Michez D, Reverté S, Rasmont P, Christmann S (2021) "Farming with Alternative Pollinators" approach increases pollinator abundance and diversity in faba bean fields. J Insect Conserv:1–14

Sharma SR, Singh S, Aggarwal N, Kaur J, Gill RK, Patil SB, Kumar S (2016) Effect of post-emergence herbicide metribuzin application on morpho-physiological traits, yield and yield components in lentil (*Lens culinaris* Medik.). In: Presented at International Conference on Pulses for Health, Nutrition, and Sustainable Agriculture in Drylands, Marrakesh, Morocco, 18–20 Apr. 2016. ICARDA

Sharma SR, Singh S, Aggarwal N, Kaur J, Gill RK, Kushwah A, Patil SB, Kumar S (2018) Genetic variation for tolerance to post-emergence herbicide, imazethapyr in lentil (*Lens culinaris* Medik.). Arch Agron Soil Sci 64:1818–1830. https://doi.org/10.1080/03650340.2018.1463519

Shtaya MJY, Emeran AA, Fernández-Aparicio M, Abu Qaoud H, Abdallah J, Rubiales D (2021) Effects of crop mixtures on rust development on faba bean grown in Mediterranean climates. Crop Prot 146:105686

Siddiqui MH, Al-Khaishany MY, Al-Qutami MA, Al-Whaibi MH, Grover A, Ali HM et al (2015) Response of different genotypes of faba bean plant to drought stress. Int J Mol Sci 16(5): 10214–10227

Sillero JC, Villegas-Fernández AM, Thomas J, Rojas-Molina MM, Emeran AA, Fernández-Aparicio M, Rubiales D (2010) Faba bean breeding for disease resistance. Field Crop Res 115(3):297–307

Singh AK, Bhardwaj R, Singh IS (2014) Assessment of nutritional quality of developed faba bean (*Vicia faba* L.) lines. J AgriSearch 1(2)

Solaiman Z, Colmer TD, Loss SP, Thomson BD, Siddique KHM (2007) Growth responses of cool-season grain legumes to transient waterlogging. Aust J Agric Res 58(5):406–412

Soltis DE, Soltis PS, Bennett MD, Leitch IJ (2003) Evolution of genome size in the angiosperms. Am J Bot 90(11):1596–1603

Stoddard FL, Balko C, Erskine W, Khan HR, Link W, Sarker A (2006) Screening techniques and sources of resistance to abiotic stresses in cool-season food legumes. Euphytica 147:167–186

Stoddard FL, Nicholas AH, Rubiales D, Thomas J, Villegas-Fernández AM (2010) Integrated pest management in faba bean. Field Crop Res 115(3):308–318

Tadele M (2020) Impact assessment of abiotic production constrains of Faba Bean (*Vicia faba* L.) and breeding mechanisms for acid soil, drought and waterlogging affected environments. J Biol Agric Healthc 10(11):49–56

Tanno KI, Willcox G (2006) The origins of cultivation of Cicer arietinum L. and Vicia faba L.: early finds from Tell el-Kerkh, north-west Syria, late 10th millennium BP. Veg Hist Archaeobotany 15(3):197–204

Taran B, Warkentin TD, Vandenberg A, Holm FA (2010) Variation in chickpea germplasm for tolerance to imazethapyr and imazamox herbicides. Can J Plant Sci 90(1):139–142

Taran B, Holm F, Banniza S (2013) Response of chickpea cultivars to pre-and post-emergence herbicide applications. Can J Plant Sci 93(2):279–286

Tavakkoli E, Rengasamy P, McDonald GK (2010) High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. J Exp Bot 61(15):4449–4459

Tavakkoli E, Paull J, Rengasamy P, McDonald GK (2012) Comparing genotypic variation in faba bean (*Vicia faba* L.) in response to salinity in hydroponic and field experiments. Field Crop Res 127:99–108

Tijhuis, M. J., Ezendam, J., Westenbrink, S., van Rossum, C., & Temme, L. (2012). Replacement of meat and dairy by more sustainable protein sources in the Netherlands: quality of the diet

Tivoli B, Baranger A, Avila CM, Banniza S, Barbetti M, Chen W et al (2006) Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. Euphytica 147(1):223–253

Van Delft GJ, Graves JD, Fitter AH, Van Ast A (2000) Striga seed avoidance by deep planting and no-tillage in sorghum and maize. Int J Pest Manage 46(4):251–256

Vered Y, Grosskopf I, Palevitch D, Harsat A, Charach G, Weintraub MS, Graff E (1997) The influence of Vicia faba (broad bean) seedlings on urinary sodium excretion. Planta Med 63(03): 237–240

Vidal-Valverde C, Frias J, Sotomayor C, Diaz-Pollan C, Fernandez M, Urbano G (1998) Nutrients and antinutritional factors in faba beans as affected by processing. Zeitschrift für Lebensmitteluntersuchung und-Forschung A 207(2):140–145

Vilariño M, Métayer JP, Crépon K, Duc G (2009) Effects of varying vicine, convicine and tannin contents of faba bean seeds (*Vicia faba* L.) on nutritional values for broiler chicken. Anim Feed Sci Technol 150(1-2):114–121

Villegas-Fernández AM, Sillero JC, Emeran AA, Flores F, Rubiales D (2011) Multiple-disease resistance in Vicia faba: multi-environment field testing for identification of combined resistance to rust and chocolate spot. Field Crop Res 124(1):59–65

Wang HF, Zong XX, Guan JP, Yang T, Sun XL, Ma Y, Redden R (2012) Genetic diversity and relationship of global faba bean (*Vicia faba* L.) germplasm revealed by ISSR markers. Theor Appl Genet 124(5):789–797

Weigand S, Bishara SI (1991) Status of insect pests of faba bean in the Mediterranean region and methods of control. In: Options Mediterraneennes Serie A: seminaires Mediterraneens (CIHEAM)

Wolswinkel P (1974) Complete inhibition of setting and growth of fruits of *Vicia faba* L. resulting from the draining of the phloem system by Cuscuta species. Acta Bot Neerlandica 23(1):48–60

Yang F, Chen H, Liu C, Li L, Liu L, Han X et al (2020) Transcriptome profile analysis of two Vicia faba cultivars with contrasting salinity tolerance during seed germination. Sci Rep 10(1):1–10

Yoneyama K, Awad AA, Xie X, Yoneyama K, Takeuchi Y (2010) Strigolactones as germination stimulants for root parasitic plants. Plant Cell Physiol 51(7):1095–1103

You MP, Eshete BB, Kemal SA, van Leur J, Barbetti MJ (2021) Physoderma, not Olpidium, is the true cause of faba bean gall disease of Vicia faba in Ethiopia. Plant Pathol 70(5):1180–1194

Younes HA, Abd El-Aziz MH, Zeid AH (2021) Detection of Bean yellow mosaic potyvirus in infected faba bean plants (*Vicia faba* L.) from Northern Egypt. Arch Phytopathol Plant Protect 54(19–20):1633–1648

Zahran HH (1999) Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63(4):968–989

Zong X, Liu X, Guan J, Wang S, Liu Q, Paull JG, Redden R (2009) Molecular variation among Chinese and global winter faba bean germplasm. Theor Appl Genet 118(5):971–978