Genetics and Genomics of Drought and Heat Tolerance in Cowpea, Mung Bean and Black Gram

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Abstract

Drought and heat stresses are the most important abiotic stresses that are the major stumbling blocks in the scientific endeavour to develop climate-smart legumes. These unpredictable abiotic stresses influence almost all facets of the plant right from germination to maturity and at extremes could be lethal. In India, with a predominant vegetarian population, pulses or grain legumes are the prime and affordable sources of dietary proteins. The protein-rich pulses play a significant role in alleviating protein malnutrition. There is an urgent need to sustain and accelerate the productivity of these pulses that are highly vulnerable to abiotic stresses, owing to their cultivation mostly under rainfed conditions with limited natural resources. The complex genetic architecture of the tolerance traits in conjunction with the ensuing climate change and narrow genetic base of the legumes poses a challenge to the breeding community in the redressal mechanism for ensuring food and nutritional security. Cowpea, mung bean and black gram are significant short-duration hardy pulses in India that are laden with the potential to perform better under challenging environmental vagaries in comparison to others. The limited success of conventional breeding in addressing the drought and heat stress tolerance could be negated by revisiting and adopting suitable holistic strategies to understand and breed for these complex traits. The prospect of developing resilience against drought and heat stress in these crops in the backdrop of the genetic and genomic resources currently available is discussed. With the advent of whole-genome sequencing, advanced phenotyping platforms and reducing cost of next-generation sequencing, it could be expected

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that success strides witnessed in cereal crops like rice and wheat could be replicated in these orphans yet paramount crops as well.

Keywords

Cowpea · Mung bean · Black gram · Drought tolerance · Heat tolerance · Genetics · Genomics · Water stress · High-temperature stress

8.1 Introduction

Plants are unveiled to a variety of ecological stresses, which influence almost all facets of plants right from the time of germination to physiological maturity. Depending on their biological nature, stresses could be classified as biotic (pathogen, pests, weeds, etc.) or abiotic (excess or deficit moisture, high or low temperature, salinity, deficiency or toxicity of minerals, soil pH, etc.). These stresses interfere with the complete expression of the plant's genetic potential (Atkinson and Urwin 2012). The plant's sensitivity and response to these environmental stresses are highly complex and vary with the phenological stage of the plant, their genetic potential, and the extent and severity of the stresses (Zhu 2002). The erraticism of these stresses is further aggravated by the present-day climate change phenomenon. The farm breeding systems are necessitated to be more prolific, ensuring viable agricultural outputs counteracting climatic vagaries and safeguarding the food and nutritional security of the burgeoning population across the globe that is likely to cross over nine billion by the middle of this century (Santos et al. 2020).

Food legumes or pulses are indispensable in safeguarding food security as they serve as an inexpensive source of dietary proteins, essential vitamins and minerals (Bohra and Singh 2015). They form a unique and essential component of the diet by complementing staple cereals with proteins (two to three times that of wheat and rice). With their innate ability to grow on a range of edaphic and environmental conditions, pulses contribute vastly to the sustainability of the farming systems. Besides being an intrinsic unit of assorted cropping systems, they enrich soil productiveness through biological N fixation and help liberate soil-adsorbed phosphorus (Souframanien et al. 2020). In the global context, India preponderates with its highest production (25%), consumption (27%) and import (14%) of pulses. Despite the concerted research efforts of the plant breeders and the policies of the Government of India, 2.5 million tonnes of pulses were imported during 2018–2019 (DAC & FW 2020), ensuing a misfortune of 1.3 billion USD forex to the exchequer. Grain legumes are highly vulnerable to abiotic stresses, owing to their cultivation mostly under rainfed conditions with limited natural resources (Ahmad et al. 2005). Among the abiotic stresses, dearth of water and high temperature assume prime importance in the sustained endeavour to develop climate-smart legume crops. There has been meagre prosperity in the efforts to mitigate the repercussions of abiotic stresses in food legumes (Deshmukh et al. 2014). This slow progress could be attributed to the intricate genetic constitution of abiotic stress tolerance governed by several minor genes/quantitative loci coupled with environmental interference (Fleury et al. 2010). Further, breeding for abiotic stress tolerance is extremely complex as the crop plants are confronted simultaneously by several abiotic stresses.

Drought, a challenging constraint to legume productivity, is characterized by a lack of precipitation for a protracted period, resulting in water scarcity, which destructively affects the physiology and biochemistry of metabolic processes that eventually hamper crop yield. Extreme drought conditions could also ultimately lead to total crop failure. Another abiotic stress, as challenging as drought, is the high temperature. As per a prediction, 0.2 °C rise in the atmospheric temperature is expected each decade that would cause a 1.8–4.0 °C upsurge in the current temperature levels by the beginning of the next century (IPCC 2007). Heat stress triggered by high perpetual temperatures might hamper crop phenology, can diminish the yields and could prove mortal beyond certain levels (Janni et al. 2020). Lack of adequate water and high temperature are the principal unpredictable abiotic stresses which adversely impact global food production. According to an estimate, there could be a 50% yield decrement owing to these stresses, primarily in the arid and semi-arid zones (Nam et al. 2001). For sustained legume production, it is imperative to induce genetic tolerance against water stress and elevated temperature in legumes. Drought and/or heat tolerance are complex traits that endure the plants to survive, develop and substantially yield under water deficit (Singh and Matsui 2002) and/or high temperatures. Plants deploy one or more of the following three mechanisms to cope with drought and high-temperature stresses: escape (eluding the effects of stress by altering the crop ontogeny), avoidance (any plant machinery shunning the effects of stress) and tolerance (endurance under stress conditions) (Mitra 2001; Osmond et al. 1987).

Among the short-duration pulses in India, cowpea (*Vigna unguiculata* (L.) Walp.), mung bean (*Vigna radiata* (L.) Wilczek) and black gram (*Vigna mungo* (L.) Hepper) are pivotal legume crops, producing relatively higher yields than other cultivated legume species under stress conditions. Within these three crops, cowpea is the most drought and heat stress tolerant followed by mung bean and black gram (Singh et al. 2018). Nevertheless, the productivities of these crops are also highly disrupted under stress conditions as evident from the ramifications of drought stress on yield reduction in cowpea (Ahmed et al. 2010), mung bean and black gram (Kulkarni et al. 2016) that varied up to 66%, 71% and 74%, respectively. Therefore, it becomes imperative to address these issues and induce drought and temperature stress resilience into these crops before it escalates into an imperious situation warranted by climate change.

The limited success of the conventional breeding approaches in tackling the multigenic drought and heat stress tolerance traits advocates a cautious reconsideration in the game plan to comprehend and develop genotypes for these complex traits. A multidisciplinary, integrated approach involving sundry methodologies encompassing breeders, physiologists, and bioinformaticians would be beneficial considering the intricate interactions between various stresses and plant phenological traits, and amalgamating the genetic and genomic tools to effectively induce water and heat stress tolerance in food legumes. In this chapter, we have comprehensively reviewed the genetics and genomics of drought and heat stress tolerance in cowpea, mung bean and black gram. The prevalence of whole-genome sequence reckoners in these pulse crops (Jegadeesan et al. 2021; Kang et al. 2014; Lonardi et al. 2019) has extended great avenues to the pulse breeders for extensive exploration of stress tolerance traits.

8.2 Independent and Collective Effects of Drought and Heat Stress

Drought adversely affects crop production worldwide. Water stress prevails when low humidity levels in the soil and atmosphere coupled with high environmental temperature result in a disparity between evapotranspiration loss and water absorption from soil (Lipiec et al. 2013). The intricacy of drought stress alters the physiology, morphology, biochemistry and molecular biology of the plant system (Salehi-Lisar and Bakhshayeshan-Agdam 2016). Drought causes numerous catastrophic aftermaths by perturbing different plant metabolisms such as carbon fixation, cellular turgor potential, reactive oxygen species, leaf gas exchange, leaf morphogenesis, enzyme activity and electro-neutrality of ions and also negatively influences the characteristics and quantum of plant growth and yield. Water stress has also been reported to negatively impact seed traits such as germinability, viability, vigour, seed count, produce and other qualities. In grain legumes, drought is also well known to intervene in the biological nitrogen fixation process through its influence on the host-rhizobium symbiotic relationship and root nodulation as well. The response reaction of plants to water stress depends not only on the extent and duration of water dearth, but also on the nature of the species involved, its age and its ontogenic stage of drought exposure (Rao et al. 2006). The yield penalty varied from 34% to 68% in cowpea (Farooq et al. 2017) and 71–74% in mung bean and black gram (Kulkarni et al. 2016), as conditioned by the developmental timing of the water stress. Among the different stages of plant growth, the phase involving reproduction happens to be highly vulnerable to water stress. Jha et al. (2020a) may be referred for backreferences on the various effects of drought on plants. Mung bean is highly susceptible to water deficiency during the pod-filling phase. Yield reduction consequent to water stress was in the range of 10–33% in the course of the vegetative phase, while it varied by 5–27% amid flowering and 53–75% during the initial pod-filling stage in comparison to the unstressed plants. Mung bean compensated for a reduced number of pods during drought stress by redirecting carbon assimilates to the remnant pods, increasing individual grain size (Wenham et al. 2020).

Heat stress causes permanent harm to plant growth and development when the soil and air temperature rises for a minimum amount of time beyond a threshold level (Lamaoui et al. 2018). The threshold temperatures for heat stress in cowpea, mung bean and black gram are given in Table 8.1. Heat stress adversely affects a majority of the key plant growth parameters, including electron transport activity, photosystems I and II, respiration, chloroplast thylakoid membranes and biological

Table 8.1 Critical temperature range of cowpea, mung bean and black gram for high-temperature stress (updated from Sita et al. 2017)

Legume crop	Critical temperature (°C)	Reference
Cowpea	18–28	Laing et al. (1984), Craufurd et al. (1997)
Mung bean	28–35	Kumar et al. (2011)
Black gram	25–35 27–35	Shirsath and Bhosale Agro India Ltd. (2017) Divyaprasanth et al. (2020)

nitrogen fixation. The adversity of high-temperature stress in plants is more pronounced during the phase of reproduction in comparison to the pre-reproductive phase (Hall 1992). Male reproductive structures as against the female reproductive structures are more vulnerable to high-temperature stress. Also, pre-fertilization stages are highly susceptible to elevated temperatures than post-fertilization stages. Jha et al. (2017) may be referred for backreferences on the above ramifications of heat stress on plants. The whole reproductive course from gametogenesis to syngamy, embryogenesis and seed development are highly sensitive to heat stress. Disruption of microspore formation due to damaged tapetal layer and disparity of nutrients in the developing pollen leads to sterile reproductive organs. Impairment of fertilization due to reduced viable pollens, less receptive stigmas and poor pollen tube growth consequently lead to lower seed set, enhanced ovule abortion and shrunken seeds. Heat stress ultimately results in declined photosynthetic rates and diminished supply of photosynthates to developing seeds, causing severe yield losses (Sita et al. 2017 and references therein). In cowpea, it has been reported that for every incremental rise in night temperature beyond 16 °C, the number of pods and grain production were offset by 4–14% (Hall 2004). The grain production in cowpea was significantly affected when heat waves coincided with anthesis and pod-setting phases (Ntare 1991). Commonly, reduced pod bearing, abysmal harvest index, high flower abscission, pollen sterility, anther indehiscence, browning of seed coat and reduced biological nitrogen fixation beyond 40 °C have been described in cowpea subjected to heat stress (Jha et al. 2017 and references therein).

Also, under natural conditions, the legumes are mostly exposed to heat and water stresses concurrently during seed filling. Though these two stresses have overlapping effects, they act differently on various physiological processes. For example, in the investigation by Sehgal et al. (2017, 2019), the following was observed. RuBisCo activity and stomatal conductance were elevated under high temperatures but were depressed under water-stress conditions. Hydrolysis of sucrose, though increased independently under high-temperature and drought stresses, was found to be subdued under the combination of these stresses. Heat stress had contradicting effects on the starch levels in the leaves and seeds, wherein there was an increase in the former and a reduction in the latter. However, there was a drastic decline of the starch in seeds under water stress alone or combinedly with high temperature. The reduction in seed weights was more pronounced under water-stress conditions than under high temperatures. Therefore, it would be pertinent to decipher candidate genes discretely for high-temperature and water stresses in legumes.

8.3 Genetic Variability for Heat and Drought Tolerance

Tapping the genetic variability available in any crop germplasm could be ideally suitable for enhancing tolerance with yield stability to different abiotic stresses, inclusive of drought and heat. In this context, the landraces could come handy as they are a potential reservoir of rare alleles (Lopes et al. 2015). Though substantial progress has been accomplished in developing heat stress-tolerant cereal crops through the exploitation of natural genetic variation, it has remained comparatively untapped in grain legumes (Thudi et al. 2014). Therefore, there is a dire necessity to incorporate the stress tolerance-imparting genes/QTL(s) in these stress-sensitive legume cultivars with high yield potential.

The genus *Vigna* with significant diversity for drought tolerance might be exploited not only for genetic improvement of pulse crops against water stress but also to comprehend their mechanism of action (Iseki et al. 2018). Mai-Kodomi et al. (1999b) discerned type I and type II water stress tolerance in cowpea, wherein the former delayed senescence in both the true and primary leaves while in the latter the true leaves were relatively more tolerant to wilting in comparison to the cotyledonary leaves. IITA reported significant diversity among the 1200 germplasm lines concerning yield penalty in response to imposed water stress. They identified a total of 190 lines that showed enhanced levels of drought tolerance, which was further narrowed down to the best 10 for use in breeding (Boukar et al. 2019). Similarly, various researchers identified different cowpea genotypes for drought tolerance: ‘C47’ (Iran), ‘C56’ and ‘C11’ (Portugal) (Santos et al. 2020); ‘PI293469’, ‘PI349674’ and ‘PI293568’ (Ravelombola et al. 2018); ‘17-61’, ‘17-86’, ‘Early Scarlet’ and ‘AR Black eye #1’ (Cui et al. 2020); and ‘C11’, ‘C18’, ‘C44’, ‘C46’, ‘C47’, ‘C50’ and ‘C54’ (Carvalho et al. 2019b). The stress tolerance index was found to be the most superior benchmark for assessing genotypic variability for response to drought tolerance through biplot analysis (Batieno et al. 2016). In mung bean, the cultivars ‘NM-2006’ and ‘NM-8005’ were identified to be drought tolerant as assessed by seed germination parameters in conjunction with antioxidative potential and nutrient uptake of seedlings under water stress (Ali et al. 2018), and the variety ‘Pratap’ was found promising against drought by employing biochemical traits (Baroowa and Gogoi 2014). Based on high seed yield and physiological water-stress tolerance traits, the mung bean genotypes, *Vigna sublobata*, ‘MCV-1’, ‘PLM-32’, ‘LGG-407’, ‘LGG-450’, ‘TM-96-2’ and ‘Sattya’ genotypes (Bangar et al. 2019), and the Egyptian genotypes ‘L4’, ‘L18’, ‘L19’ and ‘L21’ (El-Nabarawy et al. 2016) were identified to possess drought tolerance. In black gram, the genotypes ‘RU8-705’, ‘PALAVAYAL-LOCAL’, ‘T 9’, ‘PHM 8’, ‘ADT 3’, ‘CBG-09-06’, ‘VANNIYUR-LOCAL’, ‘CBG-09-13’ (Prakash et al. 2018), ‘Uttara’, ‘NP 16’, ‘PU 99’, ‘UH85-4’ and ‘No. 13/11’ (Kumar et al. 2019) were identified as drought tolerant under rainfed conditions. The black gram genotype ‘CBG-09-06’ also performed well under moisture-stress conditions based on the dynamics of root and gas-exchange parameters and seems promising against drought stress. Morphophysiological and biochemical parameters imparted drought tolerance in the black gram genotypes ‘PGRU95016’, ‘COBG05’, ‘IPU99209’, ‘IPU941’, ‘IPU243’ (Gurumurthy et al. 2019) and ‘T 9’ (Baroowa and Gogoi 2014).

Concerning heat stress, significant genetic variation was unravelled in cowpea at pod-filling and anthesis stages under varied photoperiodic conditions. The genotype ‘Prima’ with a higher pod set was delineated as heat stress tolerant under hot and short days. The superior yields exhibited by two photoperiod-sensitive genotypes (‘B 89-200’ and ‘TN 88-63’) in response to hot short-day conditions rendered them valuable for developing elite cowpea genotypes with high yield potential (Ehlers and Hall 1998). Similarly, the possession of high-temperature tolerance at the reproductive phase enabled the genotype ‘California Blackeye No. 27’ (‘CB27’) to produce superior yields. Yield reduction was less pronounced in ‘TVu 4552’ and ‘Prima’ when exposed to high nocturnal temperatures at the flowering stage than in ‘CB5’. A total of 268 cowpea accessions from the USA, India and Nigeria were categorized into eight discrete groups primarily based on their differential responses to high temperatures during flowering and pod-setting phases. Such classification may aid breeders in choosing appropriate genotypes for introgressive breeding aimed at incorporating heat tolerance in cowpea. Sunayana and Yadav (2016) identified mung bean genotypes ‘MH 805’, ‘MH 736’, ‘MH 421’, ‘IPM 02-3’, ‘MH 721’, ‘MH 810’, ‘IPM 409-4’, ‘Ganga 8’, ‘IPM 03-3’ and ‘IPM 06-5’ to possess drought- and heat-stress tolerance that could be used in breeding programmes. The black gram varieties ‘J.L’, ‘PDU-1’ (Dash and Shree 2013), ‘VBG-07-001’ and ‘VBG-06-010’ (Partheeban et al. 2017) were found to perform best in high-temperature regimes. Gupta et al. (2021) studied a panel of 97 diverse black gram genotypes for yield under stress and non-stress conditions in the field and identified 8 highly tolerant lines (‘UPU 85-86’, ‘IPU 94-2’, ‘IPU 98/36’, ‘NO-5731’, ‘PGRU 95014’, ‘PGRU 95016’, ‘PLU 1’, ‘BGP 247’). Some of the important genotypes identified for drought and heat tolerance in cowpea, mung bean and black gram are enlisted in Table 8.2.

8.4 Genetics of Heat and Drought Tolerance

A priori knowledge on the genetics governing the inheritance of desirable traits is obligatory for any breeding programme. Drought tolerance in cowpea has largely been deciphered to be a complex, multigenic, quantitative trait and is one of the most difficult traits to study and characterize (Carvalho et al. 2017; Ravelombola et al. 2021). It is highly influenced by G × E interactions. The classical genetic studies like diallel or generation mean that analyses were largely based on yield or yield-attributing traits under irrigated or water-stressed conditions that have provided us with a fair idea of the genetic control of water-stress tolerance in cowpea. The wooden box technique (Mai-Kodomi et al. 1999b) for screening drought tolerance has been widely used for studying genetics in cowpea. Mai-Kodomi et al. (1999a) in their study found that the ‘type I’ and ‘type 2’ drought-tolerant reactions were governed by monogenic dominant genes, *Rds1* and *Rds2*, and that *Rds1* was dominant over *Rds2*. Olubunmi (2015) reported that grain yield and auxiliary

Table 8.2 Important drought- and heat-tolerant genotypes identified in cowpea, mung bean and black gram (modified from Jha et al. 2017, 2020a)

Crop	Tolerant resources	Basis of drought tolerance	Institute/source	Reference
Cowpea	Ein El Gazal	Early flowering	Institut Senegalais de Recherches Agricoles and University of California	Hall and Patel (1985)
	Mouride	Early flowering		Cisse et al. (1995)
	Melakh	Early flowering		Cisse et al. (1997)
	California Blackeye 5	High seed yield, high biological yield, early maturity	Delmarva Region of the USA	Dadson et al. (2005)
	Texas Cream 8, Elite Mississippi Silver			
	Gorom Local, Mouride TN88-63	Higher net photosynthesis		Hamidou et al. (2007)
	BRS-Paraguacu	Chlorophyll content, LAI	Embrapa Meio-Norte's germplasm bank	Bastos et al. (2011)
	Pingo-de-ouro-1-2 Pingo-de-ouro-2	Low reduction in pods/plant and grain yield		
Mung bean	NM-2006, 8005	Higher activity of SOD and POD	The University of Agriculture, Faisalabad	Ali et al. (2018)
Black gram	CBG-09-13	Better root dynamics and gas exchange	Annamalai University, India	Prakash et al. (2018)
	VBN-4, K1	Increased synthesis of ABA, proline and lipid peroxidase	Tamil Nadu Agricultural University	Sai and Chidambaranathan (2019)
	UPU 85-86	Fast quenching of Fm, high antioxidant activity, high membrane stability, high ETR, leaf NBI	ICAR—Indian Institute of Pulses Research	Gupta et al. (2021)

Crop	Genotype name	Basis of heat stress tolerance	Yield (Y)/survival (S) trait under HS	Reference
Cowpea	TN88-63	Pod set	Y	Ntare (1991)
Cowpea	CB27	Reproductive stage	Y	Ismail and Hall (1998), Ehlers et al. (2000)
Cowpea	B89-200 and TN88-63	High yield under heat stress	Y	Ehlers and Hall (1998)

(continued)

Table 8.2 (continued)

Crop	Genotype name	Basis of heat stress tolerance	Yield (Y)/ survival (S) trait under HS	Reference
Cowpea	Tvu 4552 and Prima	Seed yield	Y	Nielsen and Hall (1985)
Cowpea	–	Delayed leaf senescence	Y	Ismail et al. (2000)
Cowpea	Tvu 4552 and Prima Lower	Flower abscission	Y	Nielsen and Hall (1985)
Cowpea	IT93K-452-1, IT98K-1111-1, IT93K-693-2, IT97K-472-12, IT97K-472-25, IT97K-819-43, IT97K-499-38	Yield-related traits	Y	Timko and Singh (2008)
Mung bean	Binamoog-1	Antioxidant defence and methylglyoxal (MG) detoxification	S	Nahar et al. (2015)
Black gram	VBG-07-001, VBG-06-010	Cellular response post-TIR	Y	Partheeban et al. (2017)

water stress-adaptive characters were under the governance of additive and non-additive gene effects in cowpea. Though both the types of gene actions were observed, dominance and/or dominance × dominance effects played a predominant role in genetically controlling the traits related to water-stress tolerance (Olajide and Ilori 2018). The majority of the studies indicate the quantitative nature of drought tolerance traits in cowpea (Boukar et al. 2016; Muchero et al. 2009, 2013) and mung bean (Liu et al. 2017). However, the development of water stress-tolerant legume varieties is hindered by the passive selection response to various drought tolerance traits.

The poor understanding of the genetic mechanisms underlying high-temperature tolerance in grain legumes could be attributed to limited genetic inheritance studies and also to the complex nature of the trait. Genetic analyses to discern the genetics governing the high-temperature tolerance in grain legumes have been carried out on the basis of both classical and quantitative genetics. Initially, genetic inheritance of important agronomic traits contributing directly or indirectly to yield performance under heat stress and administered largely by a single dominant (Marfo and Hall 1992) or recessive gene (Hall 1993) has been worked out in cowpea. Browning of the seed coat (Patel and Hall 1988) and the abscission rate of reproductive organs (Rainey and Griffiths 2005) resulting from heat stress were reported to be under monogenic control in cowpea. Later, Marfo and Hall (1992) proclaimed two dominant genes in cowpea to primarily control most of the heritable tolerance to high temperature at the pod-filling stage. However, their findings also hinted towards QTLs controlling high-temperature tolerance, which was also reiterated by Lucas et al. (2013).

8.5 Breeding Strategies for Improving Drought and Heat Tolerance

Breeding for water-stress tolerance would be a tenable approach to subside the hazards of crop loss by enhancing the crop's ability to extricate water from the deeper layers of soil by altering the root morphology, by reducing the crop water requirements (improved water-use efficiency) or by improving the crop's endurance to withstand longer water-stress periods, consequently leading to improved yields under dryland conditions (Sofi et al. 2019). Breeding programmes involving biparental crosses have limited scope for drought tolerance improvement in legumes due to the narrow genetic base, and therefore, intercrosses involving multiparental advanced generations (MAGIC) should be exploited for introgression of drought tolerance and other desirable agronomic traits (Ravelombola et al. 2021). Classical breeding approaches involving intergeneric and interspecific crosses and induced mutations for isolating novel drought- and heat-tolerant traits could be capitalized on for stress tolerance improvement (Briglia et al. 2019). Iseki et al. (2018) in their studies highlighted that tolerance of domesticated species in genus *Vigna* could be enhanced through pre-breeding efforts. Physiological trait breeding has also been observed to bestow crops with better performance potential under water stress (Jha et al. 2020a). Heat-stress tolerance traits could be improved through different breeding strategies by exploiting the existing genetic variability in crop germplasm. The development of high temperature-tolerant cultivars could be accelerated by taking advantage of novel breeding techniques, such as developing multiparent advanced generation intercross populations, marker-assisted selections, accelerated breeding techniques and CRISPR/Cas9-based genome editing systems (Jha et al. 2020b). Inclusion of candidate genes transcribing heat-shock proteins in cultivar development programmes could be a judicious strategy in the redressal mechanism of high-temperature stress (Feder and Hofmann 1999). In cowpea, profuse flowering and copious pod bearing under high nocturnal temperatures and long photoperiods are used as selection criteria in breeding for high-temperature tolerance (Marfo and Hall 1992). Such breeding efforts culminated in the development of high temperature-tolerant high-yielding cowpea variety 'California Blackeye 27' ('CB27') (Janni et al. 2020). The mung bean selections with quick germination and rapid growth were observed to combat terminal heat stress probably due to improved population stand, earliness and increased yield (Hanumantharao et al. 2016). Selections with reduced leaf electrolyte leakage under high temperature were also associated with high pod set (Hall 2004). The yield-contributing characters such as number of pods, number of seeds and seed size in terms of test weight are suggested as three key traits, which could be beneficial in screening and breeding genotypes for high-temperature tolerance along with better seed yield in black gram (Anitha et al. 2016).

8.6 Screening of Target Traits for Drought- and Heat-Stress Tolerance

The intricacies involved in drought and high-temperature tolerance forbid the efficient screening of these traits in plants. It would be appropriate to have a gamut of selection indices so that several traits contributing to stress tolerance could be effectively screened and introgressed into the elite genetic background with good agronomical value. An array of parameters imparting water-stress tolerance in cowpea have been employed for screening cowpea genotypes (Carvalho et al. 2019a; Iseki et al. 2018; Matsui and Singh 2003; Muchero et al. 2008). Shoot biomass (Iseki et al. 2018), deep root systems (Matsui and Singh 2003) and increased root mass have been used widely (Santos et al. 2020). Slabbert et al. (2004) established other protocols for screening cowpea for water-stress tolerance like proline and ABA accruals, tetrazolium assays, membrane stability based on electrolyte leakage, relative water content (RWC), water potential and area of leaves, chlorophyll and carotenoid contents, chlorophyll fluorescence, enzymatic assays for studying antioxidative responses (SOD, glutathione reductase, ascorbate peroxidase) and wooden boxes for evaluating drought tolerance at early vegetative stage. The screening for the content of osmoprotectants (e.g. proline, trehalose, fructans, mannitol, glycine betaine) has also been used for screening water-stress tolerance (Carvalho et al. 2017). The agronomical traits such as early maturity and 'stay green' have also been widely used (Fatokun et al. 2012). The drought avoidance mechanisms such as stomatal closure, paraheliotropic movement of leaves and enhanced water conductivity of roots (Agbicodo et al. 2009) could also be exploited. Cowpea has been found to modify its metabolic activities under water-stress conditions for accommodating the demanding tolerance functions through the interactive shikimate and arginine/proline pathways, leading to manipulations in the levels of metabolites like proline, galactinol and quercetin 3-*O*-6′′-malonylglucoside (Goufo et al. 2017) with drought response manifestations. Ravelombola et al. (2018) and Verbree et al. (2015) have claimed water-stress tolerance to be correlated with stem diameter in cowpea seedlings. The depression of canopy temperature from the atmospheric temperature (CTD) expedites the screening of crop response to heat and water stresses (Sofi et al. 2019). Positive CTD resulting from cooler canopies has been correlated with high yield in various crops (Fischer et al. 1998; Singh and Kanemasu 1983). In mung bean, polyethylene glycol (PEG) has also been used to simulate water-stress conditions for laboratory screening (Islam et al. 2019). In black gram, several traits, viz. photosynthetic efficiency, conductance of stomata, rate of transpirational water loss, contents of photosynthetic pigments, prolines and activities of peroxidases, have been reported to be useful for screening water-stress tolerance (Gurumurthy et al. 2019).

Thiaw and Hall (2004) were of the view that a blend of traits rather than targeting a single trait proved beneficial in developing high temperature-tolerant cowpea genotypes. They observed that the traits such as abundant flowering and copious podding in combination led to the identification of summer-suitable cowpea genotypes with better performance potential under long photoperiods and hot conditions. Similarly, the selection of genotypes with high membrane stability and low electrolyte leakage proved beneficial for developing heat stress-tolerant winter-suitable cowpeas (Jha et al. 2017). The studies associated with stomatal performance and metabolite contents such as that of prolines and anthocyanins could efficiently discriminate the genotypes for high-temperature tolerance (Carvalho et al. 2019a, b). A number of traits affecting the physiology of plants such as rate of photosynthesis, germinability of pollen grains, cell membrane integrity, water potential in leaves and relative water content (RWC) have been explored extensively in discriminating high temperature-tolerant lines from the sensitive ones (Janni et al. 2020; Kumar et al. 2020a; Siddiqui et al. 2015). In mung bean, the genotypes with the genetic potential of retaining maximum flowers and sustaining higher productive pods during extremes of temperature (>40 °C) were found to be relatively more heat stress tolerant, and thus, these traits are valuable selection indices for screening (Khattak et al. 2006; Singh and Singh 2011). Alternatively, the preponderance of fertile pollen and sucrose synthase (*SuSy*) enzymatic activity have also been used for screening mung bean heat-tolerant lines at high temperature (40 °C). The identified heat-tolerant line also showed substantial variations for photoperiod-temperature photosynthetic response, Fv/Fm chlorophyll fluorescence parameter reflecting the maximum quantum efficiency of photosystem II, and photosynthetic electron transport rate (ETR) (Basu et al. 2019). As far as possible, an array of traits need to be screened for ascertaining the drought and heat tolerance of potential putative genotypes. Partheeban et al. (2017) standardized temperature induction response technique (TIR) in black gram (induction at 36–46 °C for 3 h and lethal temperature at 52 °C for 3 h) and studied the cellular response as a rapid and reliable technique for thermotolerance. Gupta et al. (2021) compared a set of highly heat-sensitive black gram genotypes with that of highly tolerant genotypes with respect to physiological and biochemical traits and found significant genotypic variability for leaf nitrogen balance index (NBI), chlorophyll (SPAD), epidermal flavonols and anthocyanin contents under 42/25 °C max/min temperature. The heat-tolerant lines also exhibited high membrane stability index, high electron transport rate, fast quenching of Fm following fluorescence kinetics and high antioxidation activity resulting in scavenging of ROS. The susceptible lines also displayed reduced quantum yield of PSII leading to reduced photosynthetic efficiency.

8.7 Genomics for Improving Drought and Heat Tolerance

Genomics pertains to the study of the complete genome of an organism; it involves DNA sequencing methods in conjunction with bioinformatics for sequencing, assembling and analysing their structural, functional and evolutionary aspects and opens avenues for mapping and editing of genomes. These genomic tools enable the generation of extensive and exhaustive data sets related to the differential gene expression patterns and proteomic and metabolomic differences resulting from exposure to water and high-temperature stresses. The various genomic tools available for genetic improvement of drought- and heat-stress tolerance in pulses are discussed below.

8.7.1 Quantitative Trait Locus (QTL) Mapping

Drought tolerance, being an intricate quantitative trait, is under the confluence of many genes and gene families and presents a highly unfeasible circumstance for simultaneous selection. Such quantitative traits could be discerned through QTL analysis. QTL analysis has been in vogue since 1997 to associate regions of chromosomes responsible for water-stress mitigation (Teulat et al. 1997). These QTLs are useful in mapping genes and finding applications in marker-assisted breeding programmes. Muchero et al. (2009) divulged the mapping of ten QTLs associated with water-stress tolerance in the cowpea seedlings of a RIL population under greenhouse conditions. These ten QTLs were confirmed through field experiments and accounted for 4.7–24.2% of phenotypic variance. Four QTLs pertaining to per-peduncle pod number under high-temperature conditions were also recognized and exploited in marker-assisted breeding (Lucas et al. 2013; Pottorff et al. 2014). Cloning QTL (first attempted by Salvi and Tuberosa 2005), though being technology, resource and time intensive, offers great scope in developing elite productive cultivars through marker-assisted selections. The hotspots within QTLs may harbour candidate genes and demonstrate differential gene expression. Similarly, the transcriptome atlases developed in various legume crops like common bean (PvGEA), cowpea (VuGEA), groundnut (AhGEA) and soybean could assist in deeper understanding of unannotated gene functions related to water stress (see Jha et al. 2020a for cross reference). Five QTLs concerning stay-green trait under water-stress conditions have been reported in cowpea (Muchero et al. 2013). Six stable QTLs associated with different water-stress tolerance traits were discerned and localized onto the SSR-based novel genetic linkage map developed in mung bean (Liu et al. 2017). Unfortunately, limited QTLs associated with high-temperature tolerance have been established in pulses. Five QTLs (*Cht-1*, *Cht-2*, *Cht-3*, *Cht-4* and *Cht-5*) conferring 11.5–18.1% of the total phenotypic variation for high-temperature tolerance have been identified in cowpea (Lucas et al. 2013). Significantly, these QTLs contained the candidate heat tolerance-imparting genes, viz. DNA J heat-shock proteins, heat-shock proteins (HSP) and heat-shock transcription factors (HSTF). Pottorff et al. (2014) discovered one major QTL *Hbs-1* explaining 77.3% of the total phenotypic variance and two minor QTLs *Hbs-2* and *Hbs-3* contributing 12.3% and 6.8% of the total variance, respectively, that were associated with high temperature-induced browning of seed testa in cowpea. Interestingly, the QTL *Hbs-1* was found to have syntenic correspondence with ethylene-forming enzyme (EFE) coding regions of other legumes, and SNP markers associated with *Hbs-1* gene were identified for use in MAS (Pottorff et al. 2014). In forthcoming years, such QTLs shall be promising in the efforts towards the development of drought- and high temperature-tolerant genotypes.

8.7.2 Association Studies

Genome-wide association studies (GWAS) are powerful tools for precisely detecting chromosomal regions tightly linked with the trait of interest by correlating the phenotypic and genotypic data generated on a relatively large set of natural variations existing in the germplasm (Brachi et al. 2011). The preponderance of molecular markers in pulses including cowpea (Muñoz-Amatriaín et al. 2017; Xu et al. 2017) has enabled in-depth study of water-stress tolerance. GWAS involving 383 genotypes and a biparental population-based QTL analysis independently confirmed the involvement of three prime QTLs (*Dro-1*, *Dro-3* and *Dro-7* for delayed senescence, biomass and grain yield) attributing stay-green trait for water-stress tolerance in cowpea (Muchero et al. 2013). Ravelombola et al. (2021) utilized GWAS on a MAGIC population comprising 305 F_8 RILs for evaluating drought tolerance index based on plant phenology, maturity, days to flowering, seed test weight and seed yield and identified many SNPs linked with water stress-associated plant growth habit (14), maturity (18), days to flower (5), seed test weight (5) and seed yield (35). The outcome of this study would aid in the comprehension of genes governing water stress tolerance and would be of help in genetic improvement of cowpea through marker-aided and genomic selections. GWAS was employed in mung bean, and eight *SnRK2* genes (sucrose non-fermenting-1-related protein kinase 2 family) were predicted. It was found that most of these *VrSnRK2* genes were upregulated post-induction of drought, suggestive of their role in water-stress mitigation response. The gene *SnRK2.6c* exhibited the highest differential gene expression (12-fold) under water-stress conditions, indicative of its crucial role in moderating the effects of drought stress (Fatima et al. 2020). Noble et al. (2017) have developed the mung bean nested association mapping (NAM) population that could prove to be a valuable resource for studying complex traits such as water and high-temperature stresses. As part of NAM, 560 mung bean, black gram and wild accessions (*Vigna sublobata* var. *sublobata*) have been genotyped. In addition, the mung bean diversity panel developed by Queensland University would be a treasured resource for accelerating genetic improvement of mung bean by providing deeper insights into the genetic architecture of traits of agronomic importance (Noble et al. 2018).

8.7.3 Comparative Genomics

The growing abundance of whole-genome reference sequences in various food crops including pulses has empowered legume researchers to gain deeper knowledge on the various traits of interest (Bohra and Singh 2015; Varshney et al. 2019). Concurrently, the reference genome sequences could act as a launch pad for performing comparative genomic studies for elucidating chromosomal regions imparting tolerance against water and high-temperature stresses in legumes. For example, the analyses of whole genomes have led to the recognition of 111 and 109 drought-responsive genes in pigeon pea (Varshney et al. 2012) and soybean (Schmutz et al.

2010), respectively. To this end, more recent whole-genome reference sequencing efforts in grain legumes have expounded unique genomic identities regulating important functional traits including water-stress tolerance in food legumes (referenced in Jha et al. 2020a, b). With the next-generation sequencing facilities and increasing availability of hybrid assemblers, relatively better quality genome assemblies are being made in those crops wherein quality reference genomes are unavailable. In legume crops like black gram, with the available information on genetic variation in conjunction with these assemblies, rapid strides in the development of promising varieties through marker-aided selections could be achieved. The comparative genomics in these orphan legume crops from the structural, functional and evolutionary perspectives should be possible in near future with the increasing availability of these valuable genomic resources (Pootakham et al. 2021).

8.7.4 Candidate Genes

Candidate genes with a potential role in alleviating water stress have been identified in several legumes including *CPRD8, CPRD12*, *CPRD14* and *CPRD22* genes in cowpea. *SKP1* (S-phase kinase-associated protein 1) gene is involved in proteolysis and has a determinative role in stress tolerance. The *Vigna radiata*-specific *SKP1* (*VrSKP1*) ORF comprising 550 bp and corresponding to 114 amino acids was isolated and cloned for the first time from water stress-tolerant mung bean variety 'Pratap' and was found to be significantly upregulated, thereby validating their role as a candidate gene for drought tolerance (Bharadwaj et al. 2019). In water stress-tolerant cowpea, a unique drought-responsive element-binding protein 2-type transcription factor *VuDREB2A* mediates DRE-dependent expression of stress-responsive genes and confers enhanced drought resistance (Sadhukhan et al. 2014). Carvalho et al. (2017) have reviewed in detail the various candidate genes that have been identified in cowpea by various researchers. Glutathione reductase (GR) is involved in water-stress response in cowpea as was evident from the dual-targeted (*dtGR*) and cytosolic (*cGR*) glutathione reductase leaf-expressed genes. Among the plant explicit families of transcription factors (TF), APETALA2/ethylene-responsive element factor-binding proteins (*AP2*/*ERF*) play a determinative role in water-stress tolerance. In silico analysis performed by Labbo et al. (2018) revealed 71 AP2/ERF TFs in the *Vigna radiata* genome. Constituents of *DREB* subfamily are known to perform crucial functions in water-stress tolerance. The differential expression of *VrDREB* genes under water-stress conditions was studied, and five candidate genes (*VrDREB5*, *VrDREB12*, *VrDREB13*, *VrDREB22*, *VrDREB30*) were identified that were upregulated under water stress. The drought stress-induced genes like *VrP5CS*, *VrRAB18*, *VrDHN3*, *VrDREB* and *VrNCED* were highly expressed under combined phosphorus- and water-stress conditions, while *VrDHN3* and *VrNCED* were specific to drought (Meena et al. 2021). The list of various candidate genes identified in cowpea and mung bean is given in Table 8.3. Given the tremendous developments in the 'omics' field, it is expected that the identification of prime candidate genes having manifestations in the intricate abiotic stresses should be

Table 8.3 Candidate genes associated with drought and high-temperature tolerance identified in cowpea and mung bean (modified and updated from Carvalho et al. 2017)

Crop	Gene designation	Gene function	Author
Cowpea	*CPRD8*, *CPRD14*, *CPRD22*, *CPRD12*	Response to dehydration stress	Iuchi et al. (1996a)
	CPRD46	Neoxanthin cleavage enzyme involved in ABA biosynthesis	Iuchi et al. (1996b)
	VuNCED1	9-*Cis*-epoxycarotenoid dioxygenase involved in a key step of ABA biosynthesis	Ajayi et al. (2021), Iuchi et al. (2000)
	VuABA1	Zeaxanthin epoxidase involved in early step of ABA biosynthesis	Iuchi et al. (2000)
	VuPLD1	Putative phospholipase D, a major lipid-degrading enzyme in plant	El Maarouf et al. (1999)
	VuPAP-α, *VuPAP-β*	Putative phosphatidate phosphatase, important for the enzymatic cascade leading to membrane lipid degradation under environmental stresses or senescence	Marcel et al. (2000)
	VuPAT1	Galactolipid acyl hydrolase involved in membrane degradation induced by drought stress	Matos et al. (2001)
	VuC1	Protein inhibitor of cysteine proteinase belonging to the papain family	Diop et al. (2004)
	dtGR	Dual-targeted glutathione reductase, a key enzyme involved in detoxification of AOS	Contour-Ansel et al. (2006)
	cGR	Cytosolic glutathione reductase, a key enzyme involved in detoxification of AOS	Contour-Ansel et al. (2006)
	VucAPX	Cytosolic ascorbate peroxidase, a key enzyme involved in detoxification of AOS	D'Arcy-Lameta et al. (2006)
	VupAPX	Peroxisomal ascorbate peroxidase, a key enzyme involved in detoxification of AOS	D'Arcy-Lameta et al. (2006)
	VusAPX	Stromatic ascorbate peroxidase, a key enzyme involved in detoxification of AOS	D'Arcy-Lameta et al. (2006)
	VutAPX	Thylakoidal ascorbate peroxidase, a key enzyme involved in detoxification of AOS	D'Arcy-Lameta et al. (2006)
	GST	Glutathione-*S*-transferase, a well-recognized stress-related gene	Gazendam and Oelofse (2007)
	PR-1	Pathogenesis-related protein 1, a well-recognized stress-related gene	Gazendam and Oelofse (2007)
	VuNSR4	Digalactosyldiacylglycerol synthase 1	da Silva et al. (2012)
	VuNSR10	Kinase protein calcium dependent	da Silva et al. (2012)
	VuNSR44	CPRD12 protein	da Silva et al. (2012)
	VuNSR47	CPRD8 protein 'old yellow' enzyme	da Silva et al. (2012)

(continued)

Table 8.3 (continued)

Crop	Gene designation	Gene function	Author
	VuNSR49	CPRD65 protein	da Silva et al. (2012)
	VuDREB2A	DRE-dependent expression of stress-responsive genes	Sadhukhan et al. (2014)
	VuHsp17.7	sHSP family class I protein	Carvalho et al. (2019b)
	Hbs-1	Ethylene-forming enzymes EFE	Pottorff et al. (2014)
	Hbs-3	ACC synthase 1	Pottorff et al. (2014)
Mung bean	*VrbZIP*	Drought-responsive gene	Wang et al. (2018)
	codA	Improve abiotic stress tolerance	Baloda et al. (2017)
	VrWRKY	Enhance abiotic stress tolerance	Srivastava et al. (2018)
	VrSKP1	Ubiquitin-proteasome system component	Bharadwaj et al. (2019)

feasible in field crops. The whole-genome sequence of black gram could be used to identify candidate genes for water- and high temperature-stress tolerance using genome-wide association studies (Pootakham et al. 2021; Souframanien et al. 2020).

8.7.5 Genes for Heat-Shock Proteins

Under conditions of high temperature, plants produce unique types of molecular chaperon proteins commonly referred to as heat-shock proteins (HSPs). These 10–200 kDa HSPs prevent the functional proteins from getting aggregated and denatured, ensuring the effectiveness of various biological membranes and metabolic processes including photosynthesis, assimilate apportioning, water and nutrient balance in plants having tolerance to elevated temperatures. Therefore, the inclusion of genes underlying these HSPs in procreating cultivars with tolerance against high temperatures could be an important accommodative tactic for heat-stress redressal in plants (reviewed in Kumar et al. 2020a). Heat-shock transcription factors (Hsfs) are essential signal-transducing elements that mediate gene expression in retortion to various abiotic stresses. With the growing emergence of genomic resources now permitting functional analysis of genes, Li et al. (2019) dissected the mung bean *Hsfs* through genome-wide association and differential expression analyses. They studied the evolutionary and conserved domains of 24 *VrHsf* genes and categorized them into three sets (A, B and C). The promoters of these highly conserved *VrHsf* motifs are known to house *cis*-elements against multiple stresses. The *VrHsf* genes expressed differentially under varying stresses, suggestive of their plausible roles in stress alleviation. Thus, *Hsfs* motifs are considered to be vital in plants as regulatory elements transducing signals and facilitating the expression of different genes involved in tolerance against numerous abiotic stresses such as low temperature, water, salt and high temperature (reviewed in Li et al. 2019).

8.7.6 Genomic-Assisted Breeding

The increasing adoption of marker technologies in breeding programmes such as marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS) has resulted in the rapid advancement of water-stress and high-temperature tolerance-contributing traits in various crop species. The MAS/MABC is effective in transferring small number of major QTLs in grain legumes that have profound phenotypic influence (Varshney et al. 2019). However, MABC could be quite challenging in the improvement of intricate traits like yield under drought stress that are under the control of numerous minor QTLs having hitherto little phenotypic influence. To address this situation, researchers are progressively inclined towards genomic selections (GS), which could be performed due to the ease of access to millions of single nucleotide polymorphisms (SNPs) across the genome as a result of de-escalating cost of sequencing. Promising genotypes could be identified from the breeding population as GS permits swift, precise and effectual selection. The application of GS models in food legumes has divulged enhanced prediction efficiency for intricate traits (reviewed in Jha et al. 2020a, b). MABC has been used to introgress water stress-tolerant *Striga* and *Meloidogyne* nematode resistance QTLs into an extensively cultivated cowpea landrace widely preferred by the peasants in Burkina Faso. A set of 184 genome-wide SNPs deduced by expressed sequence tags extending over an average span of 2 cM intervals and abutting known annotated loci on either side were used for construing the genotypes of backcross progenies by utilizing the cowpea KASP genotyping platform. This study proclaimed the utility of highly efficient SNPs in performing foreground and background selections under a MABC system for bettering a widely cultivated cowpea variety by introgressing water-stress tolerance and biotic stress resistance genes (Batieno et al. 2016). In black gram, Gupta et al. (2021) used a set of 21 genetic markers for establishing genetic differences between the heat-tolerant and -sensitive lines. VigSatDB, the world's first exhaustive SSR database of genus *Vigna*, comprising more than 875 thousand (772,354 simple and 103,865 compounds) presumed microsatellite markers identified from six genome assemblies belonging to three *Vigna* species, viz. *Vigna radiata* (mung bean), *Vigna angularis* (adzuki bean) and *Vigna unguiculata* (cowpea), could be a treasured tool in legumes for marker discovery and genomic-assisted breeding (Jasrotia et al. 2019).

8.7.7 Transcriptome Analysis

Transcriptome profiling is highly useful in enhancing our understanding of the regulatory mechanisms imparting tolerance to various stresses. Though advanced techniques like RNA-seq have permitted profound expression studies, thereby unravelling several high temperature tolerance-imparting candidate genes in different crops (see Jha et al. 2017), only limited work has been conducted via transcriptome analysis in grain legumes for abiotic stress tolerance. Numerous genes, their pathways and metabolic processes involved in a plant's reaction to many abiotic or biotic stresses have been deciphered through transcriptomics, thus providing avenues for genetic enhancement of stress tolerance. Candidate genes formerly reported to impart water-stress tolerance in other related crops have been used to decipher many drought tolerance-bestowing genes in cowpea, which were later authenticated by differential gene expression studies in response to water stress (Carvalho et al. 2017). In mung bean, Kumar et al. (2020b) carried out transcriptome profiling of contrasting genotypes for water-stress tolerance and identified differentially expressed genes that were mainly mapped to phytohormone signal transduction, carbon metabolism and flavonoid biosynthesis. Tian et al. (2016) reported differential expression of several TFs (MYB, AP2 and NAC), HSPs, late embryogenesis abundant proteins and genes coding methyltransferases and histones in mung bean in response to desiccation. Transcriptome sequencing in black gram has revealed a rich reserve of molecular markers like SSRs and SNPs, which could be exploited for identifying candidate genes for drought and high-temperature tolerance as well as for marker-assisted selection for these traits (Raizada and Souframanien 2019; Souframanien and Reddy 2015).

8.7.8 MicroRNAs (miRNA)

MicroRNAs are small non-coding RNA molecules of 20–24 nucleotides that are post-transcription repressors of genes primarily through recognition, base complementation and cleavage or deadenylation of target RNAs and the genes thereof. Several miRNAs have been reported to have implications in different processes governing plant development and also have definitive roles in a plant's response to various biotic and abiotic stresses. In cowpea, 44 of the 157 miRNAs detected were related to water stress that targeted genes encoding zinc finger family proteins, serine/threonine protein kinases and Kelch repeat-containing F-box proteins (Barrera-Figueroa et al. 2011). Cowpea miRNAs isolated from leaves and roots of plants post-subjection to water stress were corroborated with qPCR studies, and it was observed that the miRNAs exhibited differing tissue-specific responses to water stress treatment (Carvalho et al. 2017). Participation of various miRNAs in water-stress tolerance has been demonstrated in other legumes like chickpea and soybean. Besides miRNAs, there is a rising indication of involvement of long non-coding RNAs (lncRNAs) in retortion to water-stress conditions that have been demonstrated through differential gene expression studies (Jha et al. 2020a).

Recently, miRNAs have also been detected in response to high-temperature stress from several susceptible and tolerant cultivars in different crops, albeit not in legumes. The homeobox leucine-zipper protein and SOD have been reported to be regulated through miRNAs (for details, see Janni et al. 2020). It could be just a matter of time before the role of miRNAs in heat tolerance is elucidated in legumes.

8.8 Metabolite Changes

A comprehensive view of the response of plant metabolism and their regulatory mechanism to various abiotic stresses like water stress has been demonstrated by the rapid progress being made in plant metabolomics. Numerous reviews on techniques determining metabolite variations in retortion to different stresses are available (reviewed in Jha et al. 2020a, b). Significant variations have been reported in different metabolites involved in various pathways in response to water stress. The prime metabolites showing dynamic build-up under water stress include various sugars, proline and γ-aminobutyric acid (GABA) that aid in the maintenance of osmotic potential under water-stress conditions. Plant metabolites including proline, galactinol and quercetin have been found to play prominent roles in acclimatization in response to water stress in cowpea, and their correlation with yield indicated beneficial effects (Goufo et al. 2017). Obata and Fernie (2012) emphasized that integrated comprehensive studies involving metabolite profiling, transcriptomics, genomics and proteomics will help understand the regulation of various metabolic events cascading from the impact of plant's exposure to stresses. Polyamines were also found to protect mung bean plants against drought stress (Sadeghipour 2019). Proline accumulation was found to increase in cowpea, mung bean, black gram and other legumes under water stress (Carvalho et al. 2019a, b; Jha et al. 2020a, b; Pandiyan et al. 2017). Declining levels of GABA (γ-aminobutyric acid) in the cells of heat-stressed mung bean plants have been found to increase the heat sensitivity and external application of GABA served as a thermo-protectant (Priya et al. 2019).

8.9 Genome Editing

Genome editing is a fast-evolving branch of genomics that has solid applications in the evolution of abiotic stress-tolerant cultivars. The genome editing tools such as the CRISPR/Cas9 system, which selectively edits the target genes, have immense potential in breeding better yielding crops under high temperature- and water-stress conditions. Gene editing has proven applications in the manoeuvring of root and nodule traits in cowpea, photoperiod flowering pathway, *GmDrb2a* and *GmDrb2b* genes in *Glycine max*, *SPL9* gene in *Medicago sativa* and Hua enhancer1 (*MtHen1*) gene in *Medicago truncatula* (see Jha et al. 2020a). Therefore, this potential technology could be exploited for custom editing of food legume genomes so as to develop yield-sustaining stress-tolerant genotypes with enhanced ameliorative capabilities under water and high-temperature stresses (Li et al. 2017). However, the rapid generation of novel edited genotypes through CRISPR should also be accompanied with suitable field breeding experiments under stress conditions (Janni et al. 2020).

8.10 Transgenics

Transgenic approaches, albeit controversial, may contribute immensely to the attempts towards developing pulse cultivars with tolerance against abiotic stresses. Transgenics validating cloned genes associated with abiotic stress tolerance could greatly aid functional genomics studies. The legume crops being highly recalcitrant to regeneration, in vitro culture techniques and transformation technologies gained momentum at a slower pace. However, in black gram, the *ALDRXV4* gene was overexpressed through a transgenic approach and engineered multiple stress tolerance. The transgenic lines accumulated more reactive oxygen species and showed increased protection against drought and salinity through sustained photosynthetic efficiency, maintaining increased relative water content and reduced photooxidative damage (Singh et al. 2016). The HSPs and HSFs have been targeted through transgenics for increasing the endurance of crops like wheat, maize, tomato and rice to high temperatures. In cotton, the heat-shock protein, *AtHSP101*, was upregulated in pollen through transgenic events resulting in enhanced pollen germination and improved pollen tube growth under heat stress, consequently leading to significant enhancement in the endurance of high-temperature stress and reduced yield losses (Burke and Chen 2015). Janni et al. (2020) have surveyed lines developed for tolerance towards high-temperature stress through transgenics in several crops. Once the regeneration protocols are standardized in pulses, transgenics could be developed to tackle abiotic stress tolerance.

8.11 Mutation Breeding

Mutational breeding is one of the important tools available to breeders for generating genetic variation in plants and exploiting the created variations for the betterment of agriculture. Mutation breeding has been in vogue since the 1950s and has been variously employed for improving the abiotic stress tolerance of crop plants and also to a limited extent in legumes. The long-root mutant identified in mung bean was found to draw more water compared to its parent and could be utilized under the receding water level, a condition normally experienced under field conditions (Dhole and Reddy 2010). With the advent of TILLING (targeting-induced local lesions in the genome), an innovative approach was introduced to the benefit of breeders, which empowers them to locate mutant lesions within a target locus of known sequence irrespective and independent of their contribution to phenotype (Uauy 2017). The success of TILLING primarily depends on the availability of mutant populations typically generated through chemical mutagenesis such as EMS that generally produce random point mutations across the genome, unlike the physical mutagens that largely produce deletion mutations. In addition, there is also a prerequisite of sequence knowledge about the locus of interest and a well-defined screening protocol for phenotyping the mutants carrying mutations in the region of interest. Unlike genome editing, the induced mutations in TILLING are not targeted and are random and hence do not provide the flexibility envisaged in genome editing. However, the crops developed through TILLING-based chemical or physical mutagenesis do not come under the purview of transgenic regulations. Recently, a set of four novel small *Hsp26* (*sHsp26*) alleles were identified in durum wheat with the potential of augmenting high-temperature tolerance in durum wheat through in silico and in vivo TILLING approaches. Similarly, in tomato also, a mutated HSP identified through TILLING was demonstrated to ameliorate high-temperature tolerance. HSP genes contributing towards heat-stress tolerance were explored for mutations using a TILLING population in *Oryza*, and a number of promising lines exhibiting higher tolerance to heat stress were identified for further breeding (see Janni et al. 2020). A similar application of TILLING to identify HSP genes could be replicated in legumes.

8.12 Next-Generation Platforms

The daunting task of estimating drought tolerance quantitatively is complicated by the intricacy of genetic governance and the prominence of environmental interference. Nevertheless, the advent of new technologies provides us with the sophistication of phenotyping in a non-invasive manner. An array of methodologies in this regard include image analysis software-based platforms, leaf or canopy reflectance spectrometry, analytical instrumentations based on thermal IR-near IR-visible-UV spectrum signatures and satellite-based GIS systems among others. These instruments provide us with the luxury of accurately measuring physiological responses under abiotic stresses over time and space without losing the seed materials. A highly productive phenotyping assay ‘legume shovelomics’ for examining root traits under water-stress conditions has been established for food legumes including bean and cowpea by integrating visual, manual and image analysis platforms. In *Cicer*, a PVC pipe-based phenotyping protocol has been developed for genotypic identification of water stress tolerance-imparting traits such as increased root biomass and long root lengths. Likewise, therapeutic radiological techniques like magnetic resonance imaging (MRI) and positron-emission tomography (PET) are also rendering enormously in studies related to the dynamics of photoassimilates under water-stress conditions. Also, nuclear magnetic resonance (NMR) technique is proving to be an immense help in gauging water movement and sucrose apportionment via ^{13}C-labelled sucrose (see Jha et al. 2020a for details).

8.13 Conclusion

Drought and heat tolerance are highly complex traits governed by multiple genes and pose a formidable challenge to the plant breeders to accomplish their task of accurate screening of the constituent traits and induction of resistance against these stresses in plants. Adding to the woes is the climate change and the narrow genetic base of the legumes like cowpea, mung bean and black gram, which further make the improvement of these traits a daunting task through conventional breeding. However, with the advent of modern breeding techniques coupled with high-throughput phenotyping facilities, next-generation sequencing and cues from the accomplishments in cereals like rice and wheat, it is envisaged that the goal of achieving drought- and heat-stress tolerance in legumes is possible in near future. Moreover, the identification and sourcing of untapped adaptive traits from native cultivars and landraces through pre-breeding efforts should be fast-tracked as they are known to house genes against drought and heat stress. The pace of transgenic approaches in legumes should also be accelerated so that the translation genetics could be exploited and transgenics are readily available when the regulations are relaxed. Avenues of improving the genetic gain under drought and heat stress in legumes through physiological trait-based breeding should be explored. The powerful tool of mutation breeding in generating variability for abiotic stress tolerance traits could be deployed in complementation with other breeding techniques. Furthermore, evolving ‘omics’ sciences, inclusive of genomics, transcriptomics, proteomics and metabolomics, have potential implications in improving our present knowledge on abiotic stress tolerance mechanisms and in furthering the understanding of candidate genes and complex genetic and signalling pathways associated with water and high-temperature stresses in pulse legumes. For enhancing the sustainability of legume production in the context of climate change wherein the crops are exposed to various unpredicted stresses including drought and heat, it becomes imperative to leverage and consolidate the information generated through numerous genetical, physiological, biochemical and ‘omics’ studies related to stress tolerance.

Conclusively, a cohesive approach integrating genomics with high-throughput phenotyping and genotyping is desired to comprehend the vital mechanisms associated with water and high-temperature stresses, which could eventually augur the development of climate-smart legume cultivars for ensuring food and nutritional security.

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