Genomics-Assisted Breeding Approaches in Lentil (*Lens culinaris* Medik)

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Abstract

Lentil is an important *Rabi* season food legume crop, commonly known as "poor man's meat", globally cultivated as rainfed or in residual moisture condition. Conventional breeding techniques are suffering from narrow genetic base, lack of genomic information and accurate selection procedure which restricts the lentil breeders in achieving the major breeding objectives. The accessibility of genomic tools and technologies, i.e. gene-specific markers (simple sequence repeat (SSR) or single nucleotide polymorphism (SNP)), expressed sequenced tags (EST), genome sequences, transcriptome sequences, Quantitative trait locus (QTL) maps and next generation sequencing (NGS), have unlocked a novel means to assist the lentil breeding programs by deploying diverse genomic resources which eventually changed the legume status from orphan to rich in genomic information. The transcriptome analysis identifies the ESTs derived functional markers and intron-targeted primers (ITP) in lentil. There is urgent need of high-throughput phenotyping (HTP) technologies for rapid, highly efficient, high quality and accurate prediction and measurement of complex quantitative traits in modern plant breeding. Functional genomics approaches help in tagging the unknown or targeted genes controlling the traits of interest, whereas comparative genomics approaches improved the cross-species transferability of genetic information among legume species. Marker-assisted selection (MAS) and DNA-based

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selection of desirable plant are remarkable ways to introduce QTLs/genes associated with yield enhancement, biotic or abiotic traits.

Keywords

Lentil · NGS · HTP · MAS functional · Comparative genomics

10.1 Introduction

Lentil commonly called as "poor man's meat" is an important *Rabi* season food legume crop, globally cultivated as rainfed or in residual moisture after monsoon season (Erskine et al. 2011). It is the earliest domesticated pulse crop around 8000 years ago at the same time as wheat, barley and pea (Dhuppar et al. 2012; Zohary and Hopf 1973); indigenous to south western Asia and Mediterranean region (Cokkizgin and Shtaya 2013) and known as by several names around the world namely *masser* in India, *messer* in Ethiopia, das in Arabic, *mercimek* in Turkey and *heramame* in Japan (Muehlbauer and McPhee 2005). It is cultivated in major parts of Africa, America, Asia and Australia continents, and India is the world's largest lentil producer, the other major producers of world includes Canada, Turkey, Australia and the United States (FAOSTAT 2019). In India, two types of lentil cultivars/ varieties is growing commonly known as *masur* or *malk masur* (bold seeded cultivars) in Bundelkhand region of Uttar Pradesh (UP), Madhya Pradesh (MP) and in Maharashtra (MH); and *masuri* (small seeded cultivars) are popular in Bihar, Eastern Uttar Pradesh, West Bengal and Assam.

Lentil is a self-pollinated crop belongs to pea family (Leguminosae or Fabaceae) with diploid chromosome number of $2n = 2x = 14$ and haploid genome size of about 4 Gbp (Ogutcen et al. 2018). Table 10.1 lists the taxonomic details of lentils-based USDA, Plants Database 2019.

The genus *Lens* comprised of five annual species out of them four were wild species *viz*., *L. ervoides*, *L. orientalis*, *L. montbretti* (Ladizinsky 1979; Zohary 1972) and *L. nigricans*, and fifth species was only cultivated *L. culinaris* Medik (Ferguson 2000). However, latest classification of lentil included four species which consisted of seven taxa (Ferguson and Erskine 2001; Sarker and Erskine 2006). *Lens culinaris*

Table 10.1 Taxonomic classification of lentil (USDA 2019)

Kingdom	Plantae
Sub-kingdom	*Tracheobionta*
Super-division	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Order	*Fabales*
Family	*Fabaceae/Leguminosae*
Genus	*Lens*
Species	*Culinaris* Medik.

Medik (ssp. *Culinaris*, ssp. *orientalis* (Boiss.) Ponert, ssp. *odemensis* (Ladiz.) M.E. Ferguson& al and, ssp. *tomentosus* (Ladiz.) M.E. Ferguson& al.); *Lens ervoides* (Brign.) Grande; *Lens lamottei* Czefr; *Lens nigricans* (M. Bieb.) Godron.

Lentil seeds play important role in overcoming the problems of micronutrient deficiencies and malnutrition especially in poor people due to cheapest and excellent source of dietary protein, fibres, energy, lysine amino acid, vitamin, carbohydrates and minerals *viz*., iron (Fe: 73–90 mg/kg), zinc (Zn: 44–54 mg/kg), selenium (Se: 425–673 μg/kg) potassium (K), calcium (Ca) and potassium (P), and also contain oligosaccharides, protease inhibitors and tannins (Kumar et al. 2016; Ray et al. 2014; Thavarajah et al. 2011; USDA-Agricultural Research Service 2015). Their seeds are richest source of high-quality crude protein, ranged from 23.8 to 29.0% with an average of about 26% (Khazaei et al. 2019). However, their seeds are deficient in methionine and cysteine amino acids, but could form a complete protein diet when consumed with cereals (Cash et al. 2001). In addition to these, lentil exhibited low glycemic index which is recommended as a remedy for a number of chronic diseases suffering from diabetes, obesity and cardiovascular problems (Srivastava and Vasishtha 2012).

Lentil is mostly grown as a main crop; although, intercropping can also be done with other crops such as wheat, rice, barley, sugarcane, mustard, linseed and castor bean (Andrews and Mckenzie 2007). It helps in improving soil health by symbiotic nitrogen fixation and net carbon sequestration in cereal-based cropping systems by lowering carbon footprint, i.e. −552 kg CO_2 eq/ha especially in lentil–wheat cropping system as well as provide rotational benefits in controlling weeds, diseases and insect pests effectively (Gan et al. 2014; Kumar et al. 2013). It is a climate resilient cool loving leguminous crop, grown in low annual rainfall (minimum 10 inches) and high temperature (warm temperate, subtropical and high altitude tropical climate) regions of the world (Cash et al. 2001; Muehlbauer et al. 1995). However, these factors could reduce the seed yield if they coincide during flowering and seed setting stages (Muehlbauer et al. 2006). It can be grown in wide ranges of soil with good drainage (Elzebroek and Wind 2008) and broad soil pH condition ranges from 4.4 to 8.2, capable to tolerate moderate alkaline or saline soil conditions (Muehlbauer et al. 2006) but ideally adapted towards neutral range of soil pH from 5.5 to 7 (Elzebroek and Wind 2008). Lentil has an inherent capability to survive along with high yield potential under moisture stress and problematic soil conditions, reveals wide adaptability of this crop (Mishra et al. 2007).

During last few decades, conventional breeding methods along with good agronomic practices in lentil cultivation have significantly improved the crop productivity from 605 to 1038 kg/ha (FAOSTAT 2018). Lentil crop productivity has increased by use of short duration improved cultivars in Bangladesh with the use of BARIMasur series of varieties such as BARIMasur-3, -4, -5, -6, -7 and -8 (Yigezu et al. 2019); in Morocco, yield advancement of 35 kg/ha/year (i.e. around 16–67% over local check from 1989 to 2018) (Idrissi et al. 2019, and in Ethiopia, yield enhancement of 18–28 kg/ha/year from 1980 to 2010) (Bogale et al. 2017). However, currently in developing countries like India, the average crop productivity of lentil is still below the average productivity of 2 ton/ha standard in some countries

around the world (FAOSTAT 2019) due to several constraints in lentil yield. The currently identified major constraints in lentil crop are rainfed cultivation under changing climatic and complex edaphic conditions, low annual genetic gain ($<0.7\%$), high drop rate of flowers and shedding rate of pods, compounded with several biotic (Anthracnose, *Ascochyta* blight, *Fusarium* wilt, rust, *Stemphylium* blight, collar and root rots) and abiotic (terminal drought, intermittent heat, cold and frost) stresses during crop cultivation period (Kumar 2016; Sharpe et al. 2013). Moreover, selection for quantitatively inherited traits resulted into poor or no genetic gain in lentil crop due to environmental influences and higher G $\times$ E interaction on the phenotypic expression of breeder traits of interest (Kumar and Ali 2006), that make the greatest task for breeder to develop climate-resilient superior performing lentil varieties. The conventional breeding approaches are less efficient and more time consuming in improving of polygenitically inherited several complex yield traits in lentil (Kumar 2016).

The lentil genomic resources are now available to integrate the genomics-assisted breeding approaches in lentil breeding programme which is widely used for genetic relationship/diversity studies (Dissanayake et al. 2020a; Khazaei et al. 2016; Kumar 2016; Lombardi et al. 2014; Wong et al. 2015). Just now, genomics-assisted breeding approaches have become a crucial to break the yield plateau and accelerate the genetic gain in lentil breeding by identification, selection and fixation of superior alleles in breeding populations in more precisely, effectively and rapidly manner than conventional breeding methods (Kumar 2016). In recent times, development of genomic resources with the use of gene-based markers genotyping such as single nucleotide polymorphism (SNP) and/or simple sequence repeats (SSR), and next generation sequencing (NGS) techniques have made possible to access the genomic sequences, transcriptomic sequences and genes/QTLs linked to important yield contributing traits. Thus, there is an urgent need to integrate genomics-assisted breeding approaches for more challenging breeding to develop climate resilient high yielding and resistant cultivars in lentil breeding programme.

In lentil, the earliest genetic map was constructed in early 1980s on the basis of morphological and biochemical (isozyme) markers (Tadmor et al. 1987; Zamir and Ladizinsky 1984). The first comprehensive linkage map was developed using morphological and 177 RAPD, AFLP & RFLP markers with recombinant inbred lines (RILs) population from inter-specific cross (*L. culinaris* $\times$ *L. orientalis*) in lentil (Eujayl et al. 1998). Subsequently, the first genomic library developed in a cultivated accession ILL 5588 by using RFLP markers with restriction endonuclease enzyme *Sau3AI* (*Staphylococcus aureus 3A*) and probes *viz.* $(GA)_{10}$, $(GAA)_8$, $(GC)_{10}$, $(GT)_{10}$, $(TA)_{10}$ and $(TAA)_5$ (Hamwieh et al. 2005). For the development of lentil genome maps used diverse PCR-based markers, and among them, SSR markers have contributed more significantly which is now being replaced quickly by DNA chip-based markers, especially with SNPs. The SNPs are plentiful and universally available across the genome of legume crops (Chagné et al. 2007). Transcriptome assemblies are used to identify the expressed sequenced tags (ESTs) derived from SSR and SNP markers, and intron-targeted primers (ITPs). The first EST-based library was constructed from a varietal blend of eight cultivars

with changing seed morphology (Vijayan et al. 2009) and the second cDNA library from *Colletotrichum truncatum* inoculated leaflets of a Canadian cultivar "Eston" (Kumar et al. 2014). Thus, the era of genomic tools and technologies has opened new opportunities in lentil breeding programs by deploying the diverse genomic resources of lentil. In this review, the recent developments and future potentials of genomics-assisted lentil breeding have been discussed in detail.

10.2 Origin and Types of Races

Lentil crop was domesticated at Near East in the Fertile Crescent, thereafter; expand to Europe, Northern Africa, Middle East and then the Gangetic plains of India (Ford et al. 2007). The Middle East is considered as primary centre of genetic diversity (Cubero 1981; Zohary 1972). It is originated in Near East from wild progenitor *L. orientalis* (Cubero 1981; Ladizinsky 1979; Zohary 1972). Vavilov centre of origin is the Mediterranean region and southwest Asia (Vavilov 1949; Zohary 1995).

The conventionally cultivated species are grouped into two classes based on seed size by Barulina (Cubero 1981) but this view has been renewed as race by Cubero (1981) and their contrasting traits are defined by Cubero et al. (2009):

1. Micro-sperma race: small podded (6–15 × 3.5–7 mm) with small seeds (3–6 mm) and 1000 seeds weight ≤25 g, small flowers (5–7 mm long) white to violet colour, plant height (15–35 cm).
2. Macro-sperma race: large podded (15–20 × 7.5–10.5 mm) with large seeds (6–9 mm), 1000 seed weight 25–50 g, large flowers (7–8 mm long) white colour, plant height (25–75 cm).

Europe, North Africa and America are mostly cultivated macro-sperma race (Chilean types), whereas, micro-sperma race (Persian types) is in cultivation of Asia, Egypt and Ethiopia and both of them are grown in the regions of south-eastern Europe and western Asia (Ford et al. 2007).

10.3 Floral Biology (Singh 1991)

Lentil is strictly self-pollinated due to chasmogamy flowering nature (Fig. 10.1) and outcrossing ranged from <1 to 6.6% by thrips and other insects (Baum et al. 1997; Wilson and Law 1972).

Inflorescence: Raceme
Flower: Typical papilionaceous
Calyx: 5 sepals
Corolla: 5 petals (one standard, two wings and two keels)
Androecium: Diadelphous (9 + 1) or polyadelphous
Gynoecium: Monocarpellary, sub-sessile ovary, swollen and hairy stigma

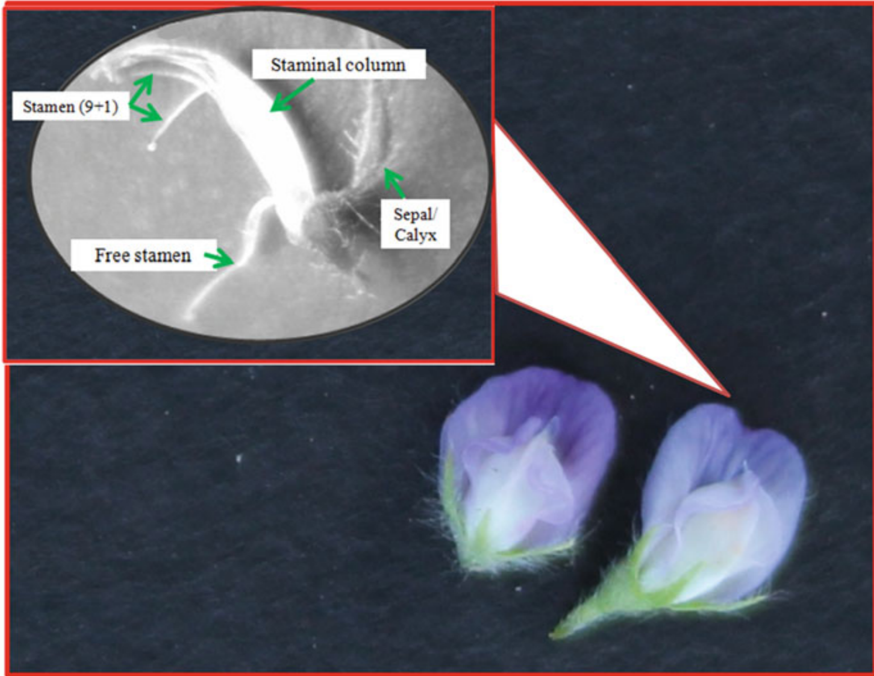

Fig. 10.1 Typical papilionaceous lentil flower and flower organs

10.4 Cytogenetics

Lentil cultivar and its wild relatives have a diploid number of chromosome ($2n = 2x = 14$) with length ranged from 3.0 to 9.2 μ (Mishra et al. 2007). Their chromosome structures have shown to vary from species to species and within species also. Sindhu et al. (1983) represented the chromosomal karyotype of lentil and observed acrocentric (three pairs), metacentric (one pair) and sub-metacentric (three pairs) chromosomes. The lentil species, *L. orientalis*, karyotype was quite similar to *L. culinaris* (Ladizinsky et al. 1984) but varied from *L. nigricans* due to the presence of three inter-changes (Gupta and Bahl 1983). Harlan and de Wet (1971) proposed gene pool concepts on the basis of crossing abilities/hybridization among species. Ladizinsky (1999) allocated *Lens* species into three categories: 1° (primary), 2° (secondary) and 3° (tertiary) gene pools based on the end products of hybridization presented in Table 10.2.

Table 10.2 Ladizinsky (1999) classified lentil gene pool based on hybridization among *Lens* species

Gene pools	Lentil species	Hybridization	F_1 fertility
Gene pool 1	Cultivated races, landraces and wild and weedy relatives: *L. culinaris* ssp. *culinaris*, *orientalis*, *odemensis*	Easy	Fertile
Gene pool 2	Wild and weedy species: *L. ervoides*, *L. Nigricans*	Moderate	Fertile/ sterile
Gene pool 3	*L. lamottei*, *L. culinaris* spp. *Tomentosus*, *L. culinaris* spp. *Orientalis*	Difficult	Sterile

10.5 Breeding Objectives

The milestone goal in breeding of lentil crop is to develop superior varieties with higher yield, better quality, and diseases and insects resistance (Muehlbauer et al. 1995). However, its breeding objectives are varying place to place with the farmer problems and consumer preferences in the specific region of the world. Various stresses of biotic and abiotic causes greatly limit the genetic improvement in seed yield and its component traits (Tomlekova 1998; Yankova and Sovkova-Bobcheva 2009). In addition to theses, agronomic complications such as lodging, pod dehiscence and inadequate crop management are important constraints in a wild progenitor of lentil, *L. culinaris* ssp. *orientalis*, which is cultivated as landrace by farmers. The major breeding objectives in lentil crop are to breed varieties with following:

1. High and stable yielding
2. Early and synchronous maturing
3. Resistance to shattering
4. Resistant to biotic stresses (insect-pest, diseases and weeds)
5. Tolerant to abiotic stresses (terminal drought, heat and frost)
6. Better quality seeds rich in protein content
7. Bold seeded seeds with less cooking time

Biotic Stresses Symbiotic nitrogen fixation enriches the legumes with nitrogen and phosphorus, therefore, these attribute attracts more pests and diseases (Sinclair and Vadez 2012). Lentil suffering from different types of biotic stresses and the important insect pests and diseases are listed in Table 10.3.

Abiotic Stresses The yield of lentil crop is globally hampered by abrupt rise in temperature with exhausting available soil moisture at terminal stages of crop, especially at grain filling stage causes forced maturity and reduction in crop yield. In India, throughout the different production zones of lentil, commonly experiences on an average of 42% yield gap (Ali and Gupta 2012; Reddy and Reddy 2010), due to intermittent or terminal drought, cold and heat stress at seedling and flowering stages of crop (Farooq et al. 2009; Silim et al. 1993; Wery et al. 1994). The drought

Table 10.3 Important insect pests and diseases of lentils

	Insect pests	Diseases
1.	*Aphis cracivora* Koch. (aphids)	*Ascochyta lentis* (*Ascochyta* blight)
2.	*Etiella zinckenella* treit (pod borer)	*Botrytis fabae* and *B. cinerea* (Botrytis grey mould)
3.	*Sitona* spp. (leaf weevil)	*Uromyces viciae-fabae* (Pers.) Schroet (rust)
4.	*Agrotis ipsilon* (cut worm)	*Fusarium oxysporum f. sp. lentis* (wilt)
5.	*Frankiniella* spp. (thrips)	Beet western yellows virus/BWYV bean leaf roll virus/BLRV or subterranean clover red leaf virus/SCRLV
6.	*Cydia nigricana* (pea moth)	(Lentil yellows disease)

and cold are key abiotic constraints of lentil causing major yield loss in crop, although other factors, such as salinity stress, nutrient deficiency and/or toxicity, could also reduce the lentil production and productivity (Muehlbauer et al. 2006; Tivoli et al. 2006).

1. Drought
2. Low temperature (cold and frost)
3. High temperature (heat stress)
4. Salinity
5. Nutrient deficiency and toxicity

10.6 Lentil Genetic Diversity

Lentil breeding started with germplasm collection and evaluation, among them genetically diverse parents for target traits is crossed to breed novel combinations of gene, genetic improvement and widening the genetic base of lentil gene to overcome the limitation of lentil breeding. In the world, different gene banks hold around 58,407 lentil accessions (Khazaei et al. 2016), among them, International Centre for Agricultural Research in the Dry Areas (ICARDA), Lebanon, preserve the largest lentil accessions in the world (About 13,907 cultivated and 603 wild species accessions) and has a prominent intent in the collection and characterization of landraces (Singh and Chung 2016) followed by Australian Temperate Field Crops Collection around 5254 accessions (Malhotra et al. 2019). In India, National Bureau of Plant Genetic Resources, New Delhi (NBPGR) has almost 2655 accessions comprised of around 2083 indigenous collection with 572 exotic accessions (Singh and Chung 2016). The ICARDA landraces were characterized and documented into four major regional groups, namely the Indian subcontinent group, the Ethiopian group, the northern group (Greece, Iran, Turkey, USSR, Chile), the Levantine group (Egypt, Jordan, Lebanon and Syria) around the world

(Erskine et al. 1989). The global germplasm collection further structured into three main groups based on eco-geographical origin by Khazaei et al. (2016): subtropical savannah, Mediterranean and northern temperate. Indian and Ethiopian landraces both come under *pilosae* group which has *pilosae* trait, i.e. the presence of pubescence on the vegetative parts and short/rudimentary tendril at the tip of leaf (Vandenberg and Slinkard 1990). Indian lentils are conventionally micro-sperma type (small seeds), more polymorphous, more pigmented and typically designated with early in flowering and maturity, less in biological yield and height (Erskine et al. 1998), whereas Ethiopian materials with the endemic elongated pod apex. Pakistan germplasm was included into *Pilosae* group for qualitative traits but intermediate between Afghanistan and India for quantitative traits. Afghanistan, Iran and Turkey germplasm were very late types in flowering and maturity among them Afghan germplasm is latest one.

The crop wild relatives (CWR) are ultimate sources of novel traits/alleles used for broadening of genetic base of lentil crop because they are found in adverse agro-climatic conditions and adapted to diversified habitats. They are characterized and documented for diverse agro-morphological, phenological, physiological, biochemical traits as well as different biotic and abiotic stresses (Singh et al. 2020; Malhotra et al. 2019). The core and FIGS (Focused Identification of Germplasm Strategy) sets were developed to display representative genetic diversity for desirable traits for better use and accessibility of available germplasm within genebanks. The FIGS strategy is successfully demonstrated the characterization of several agro-morphological and biotic resistant (insect-pest and diseases) and abiotic tolerance (heat and drought) in lentil at ICARDA using robust geo-graphical datasets. Recently, 162 lentil accessions were screened and identified heat and drought tolerant germplasm using a FIGS set (El haddad et al. 2020), and a core set of 96 accessions developed from 405 accessions of wild *Lens* species which were used to identify the key traits of nutrition, diseases, insect pest or abiotic stresses (Kumar et al. 2018d; Singh et al. 2020). The bi-parental and association mappings were used to detect the QTLs/genes within genetically diverse cultivated and wild germplasm of lentil available for several agro-morphological traits, nutritional quality (Se, Fe and Zn) as well as biotic and abiotic stresses (Kumar et al. 2018d; Khazaei et al. 2018; Polanco et al. 2019). Image-based phenotyping used for rapid screening and precise characterization of useful accessions/traits in lentil germplasm that could accelerate lentil breeding programs. For example, *Aphanomyces* root rots resistant lentil genotypes were identified by screening of lentil germplasm using this technique (Marzougui et al. 2019). The multi-parent breeding populations such as nested association mapping (NAM) and multi-parent advanced generation inter-cross (MAGIC) including both cultivated and wild species increased allelic diversity through novel recombinants in the populations from diverse parents (Scott et al. 2020) that provide a unique platform for selection of desirable recombinants of genes/mapping of QTLs of useful traits in advanced generations (Varshney et al. 2019; von Wettberg et al. 2018). In lentil, efforts for developing a MAGIC population using genetically diverse eight-parents are in progress at ICARDA.

Table 10.4 Genetic diversity in lentil germplasm for yield and its component traits

Sr. No.	Yield and its component traits	Sakthivel et al. (2019)	Kamaluddin et al. (2020)	Satpathy and Debnath (2020)	Iqbal et al. (2021)
1.	Days to 50% flowering	61–80	71–98	53–72	[a]
2.	Days to maturity	105–127	108–124	83–107	[a]
3.	Plant height (cm)	21–28	18–58	20–31	27.38–71.12
4.	Primary branches/plant	2–5	1.32–5.47	1.76–2.43	[a]
5.	Secondary branches/plant	[a]	2.88–29.23	1.42–9.80	[a]
6.	Pods/plant	9–37	18–113	6–44	2–3
7.	Seeds/pod	[a]	1–2	1.22–1.49	1–3
8.	100 seed weight (g)	1.97–3.38	1–3	1.29–1.67	1.05–3.65
9.	Seed yield/plant (g)	0.26–0.77	1.29–4.43	0.82–1.20	[a]
10.	Biological yield/ plant (g)	0.66–1.83	2.09–18.54	0.96–1.72	[a]

[a]Data were not calculated for particular trait

The fundamental of any breeding programme is the presence of ample amount of accessible genetic diversity within their crop gene pool. Narrow genetic diversity in lentil crop is bottleneck for the genetic dissection of a particular trait by conventional breeding. Conventional breeding methods in lentil has been successful in improving the crop yield and dealing with the major yield constraints by developing of cultivars resistant to major diseases and insect-pests or tolerant to important abiotic stresses (Materne and McNeil 2007; Muehlbauer et al. 2006; Sarker and Erskine 2006); however, classical lentil breeding programme has certain limitations, i.e. narrow genetic diversity, insufficient genetic information and absence of precise selection techniques which limit the breeders to reach their targets and pursue the achievable supplementary breeding objectives. Therefore, availability of sufficient amount of genetic variability for particular trait is essential for overall genetic improvement. The lentil genetic resources have sufficient variability for numerous key traits, yet not available for some other economically important traits, which limits the plant breeder efforts to address several obstacle. Lentil cultivated in South Asia, exclusively of *pilosae* types, has narrow genetic base, low photoperiod and high temperature sensitivity than the native landraces from West Asia (Erskine et al. 1998). The "daylength bottleneck" restricted the spread of lentil gene pool into the areas of Indo-Gangetic plain compounded with low yield and susceptible to an array of diseases. Currently, the scarcity in genetic variability limits the scope of selection, heterosis, new gene recombination and transgressive segregation. The genetic diversity for important seed yield and its attributing traits were shown in Table 10.4.

List of lentil breeding research institutes and their headquarters:

1. ICARDA: International Centre for Agricultural Research in the Dry Areas, Lebanon, Syria
2. IIPR: Indian Institute of Pulses Research, Kanpur, India
3. NBPGR: National Bureau of Plant Genetic Resources, New Delhi, India
4. Akdeniz, The University, Turkey
5. Bangladesh Agricultural Research Institute, Bangladesh
6. Nepal Agriculture Research Council, Nepal
7. University of Saskatchewan, Canada
8. Washington State University, USA
9. Instituto de Agricultura Sostenible (CSIC), Spain
10. University of Córdoba (UCO), Spain
11. The University of Western Australia, Australia
12. University of Birmingham, UK

10.7 Conventional Lentil Breeding Approaches

The prime objective of any breeding programme is to develop high yielding cultivars of crop plants by exploiting genetic diversity either by selection or hybridization among selected lines. Lentil crop fits remarkably well into genre of a "smart crop", therefore, appropriate breeding strategy is required for genetic improvement in the varieties for higher and stable yields. The major breeding methods in lentil are similar to the breeding methods of autogamous crops includes pure line selection or plant hybridization followed by bulk, pedigree, backcross or single seed descent methods (SSD), or a modification of these procedures, accompanied with mutation breeding for specific purposes (Muehlbauer et al. 2009; Singh and Pedapati 2015; Toker et al. 2007). Pure line selection in landraces is the largely used breeding scheme during initial period of cultivar development in lentil (Muehlbauer 1992). In India, varietal improvement programme in lentil was initiated in 1924 with single plant selection in locally collected mixed seed lots. Subsequently, the lentil breeding programme boosted under All India Coordinated Pulse Improvement Project (AICRP) in 1970s and several important cultivars have been released for various states. The selections led to achieving varieties with erect growth habit, increased seed size and diversity in colour accompanying of decreased dormant seeds and pod dehiscence (Zohary 1996). In the last few centuries, plant selection and hybridization have become the chief assets in crop improvement programme of lentil especially in developed countries; however, heterosis breeding is preferred over selection for rapid development of new varieties with increased global demand.

10.7.1 Plant Introduction

It is the simplest and quickest approach for the genetic enhancement of lentil cultivars by obtaining high yielding widely adaptable lentil cultivars from different geographical regions of within or outside of India. For example, lentil materials

"Precoz (ILL 4605)" introduced from West Asia into the Indo-Gangetic plains which is early in flowering and macro-sperma seeds (4.5 g/100 seeds). The successful plant introduction relies on genetic constitution and adaptability of introduced variety in new agro-climatic condition. Appropriate plant materials in plant introduction are homozygous pure lines due to better adaptability in new environment than heterozygous segregating lines, which necessitate further selection of introduced line with specific desirable traits. Some important lentil cultivars were introduced from ICARDA to different parts of world such as in India (VL-507 & Vipasha), in Nepal (Sikhar, Simal, Khajura Masuro-1 & -2), in Pakistan (Shiraz 96 & Mansehra 89) etc. (Rahman et al. 2009).

10.7.2 Plant Hybridization

It is the breeding procedure of combining desirable traits/alleles from genetically dissimilar parents into a cultivar. The diverse genetic makeup and geographical evolution of micro-sperma and macro-sperma races of lentils are judiciously used in hybridization programmes followed by selection of desirable recombinants in segregating generations by practicing pedigree method for combining desirable traits into developing high yielding, stress (biotic or abiotic stress or both) resistance and wider adaptability varieties for broadening and enhancing the existing genetic base of lentil crop (Chahota et al. 2007; Kumar et al. 2009; Chuni et al. 2000). The selection of parents depend on the objectives, level of genetic diversity and combining ability, however, knowledge of parental genetic relationships during selection of parents is extremely important to achieve genetic gain in lentil crop improvement programs. The efforts have also been made to extend the photoperiod length (18 h) of flowering in lentil at ICARDA to improve the synchronization by extensive crossing of *Pilosae* types with other origin lentils.

10.7.3 Mutation Breeding

The bottleneck in genetic base, variability in traits and low-yielding potential of current cultivars are the major restrictions handicapped the lentil breeding programs for long period of time (Amin et al. 2015; Asnake and Bejiga 2003). Induced mutation would be a quick, cost-effective and complementary breeding strategy for widening the base of lentil. A variety of mutagen (Gamma "γ" rays/n-nitroso-n-methyl-urea "NMU") could be applied to break the major bottlenecks in lentil morphological, phenological or yield-attributing traits and key stresses resistance to broaden the genetic diversity (Erskine et al. 1998; Toker et al. 2007). In recent times, a novel lentil mutant "multipodding (mp)" has been screened and identified which has a huge impact on inflorescence for seed yield and their stability (Amin-Laskar et al. 2018). A number of reports supported the usefulness of combination of physical and chemical mutagenic treatments instead of individual treatment for the progress in lentil breeding (Khan and Tyagi 2010, 2005). Although, the achievement

of mutation breeding for genetic enhancement in lentil crop mainly depend upon the efficiency and nature of the mutagenic agents and the precision in screening and identification of mutants (Khan and Tyagi 2005; Manju and Gopimony 2009). Mutations led to development of varieties with greater seed yield potential and quality, consequently advancement in the key agro-morphological traits with greater consumer acceptance (Ahloowalia et al. 2004). In India, "S-256 (Ranjan)" is high yielding and spreading type lentil variety developed in 1981 by X-rays irradiation and "Rajendra Masoor 1", low temperature tolerance and early maturing lentil variety recommended for late sowing developed in 1996 by Gamma rays (100 Gy).

10.7.4 Polyploidy Breeding

The genetic base of lentil cultivars is mainly confined to crossing within the GP-1/ primary gene pools, therefore, exploitation of GP-2/secondary and GP-3/tertiary gene pools are necessary for widening the genetic base of lentil. Ploidy refers to an organism with more than one set of homologous chromosomes in a cell, it is divided into two groups, autopolyploidy: polyploidy developed by doubling of haploid genome from single parent, whereas, allopolyploidy: by doubling of haploid genome from multiple parents (Aversano et al. 2012). Blakeslee and Avery (1937) used colchicine alkaloid for doubling of haploid genome in plants by disrupting the spindle fibre formation during cell cycle (Ye et al. 2010), thereafter, for producing of auto- and allopolyploids (Chen and Ni 2006). Polyploids are generally coupled with several commercial profits such as giant and seedless fruits, more vegetative growth and better resistance to diseases, and in fact the major field and horticultural crops are polyploids in nature including major cereals (Wheat, maize, oats), oilseeds (Groundnut, mustard, rape), forage (alfalfa), fibre (cotton), tobacco, sugarcane, potato, sweet potato, strawberry, banana, pear etc. (Harper et al. 2016; Rehman 2017; Emery et al. 2018). Shahwar et al. (2019) artificially induced phenotypic diversity in lentil with the use of heavy metals (lead and cadmium nitrate) in traits like plant height, plant growth, leaf and flower characteristics and pod size in lentil crop population.

10.7.5 Quality Breeding

In lentil breeding, simultaneous increase in both quantitative traits, i.e. seed yield and protein contents, is a major challenge while maintaining the progress of diseases, insect pest resistance or abiotic stress tolerance because both the traits are negatively correlated (Erskine et al. 1989; Hamdi et al. 1991; Lizarazo et al. 2015). This suggested the independent selection in any one of the traits lead to reduction in one trait by gaining in other during lentil breeding. The seeds of lentil are outstanding source of dietary protein containing approximately 26% high-quality crude protein (Khazaei et al. 2019) with estimated genetic variation of 24.30–30.20% by using a NIR Systems 6500 analyser calibrated in respect of Dumas Combustion method (Wang and Daun 2006); 23.80–29.30% by Dumus Combustion method

(Tahir et al. 2011); 21.80–27.10% by Kjeldahl method (Zaccardelli et al. 2012); 24.60–30.00% by a variety of methods (Heuzé et al. 2014) and 10.50–27.10% using Kjeldahl method (Kumar et al. 2016). These variations in protein content may be variation in locations/agro-climatic conditions, protein estimation methods, genetic cause or G × E interaction for protein content and amino acid composition. Furthermore, seed protein concentration also affected by nitrogen fixing ability of *Rhizobium* species in legumes. The improvement in nutritional quality of lentil could be achieved by upgrading the quality of protein and its composition as well as processing factors as quick expanding human consumption crop globally.

10.8 Drawbacks of Conventional Lentil Breeding Approaches

Since over the years, conventional lentil breeding approaches of selection-recombination-selection have led to fivefold raise in global crop production from 0.85 to 5.73 Mt and productivity from around 60.5 to 119.5 kg/ha (FAOSTAT 2019) during the former five decades through improvement in a number of simple monogenic/oligogenic traits (Nutritional quality, color, shape and taste). Traditional breeding techniques in lentil have been successful in dealing with the important yield constraints by developing superior cultivars, resistant/tolerant to major diseases, insect-pests or abiotic stresses *viz*., drought, heat, salinity etc. (Materne and McNeil 2007; Muehlbauer et al. 2006; Sarker and Erskine 2006). However, the majority of economically important traits like seed yield and its contributing traits are complex, govern by quantitative/polygenic genes, highly influenced by environment and exhibit wide range of genotypic × environmental interaction (GEI) led to little genetic gain in conventional lentil breeding programme (Kumar and Ali 2006).

Conventional breeding techniques are suffering from narrow genetic base, lack of genomic data and precise selection procedure which confine the lentil seed yield and restricts the lentil breeders to achieve the major breeding objectives (Kumar and Ali 2006). The execution of conventional breeding techniques is time consuming, less precise and difficult in the improvement of agronomically important quantitatively inherited complex traits (Kumar 2016). Thus, there is a necessary to incorporate genomics-assisted lentil breeding approaches such as molecular marker and genomic resources techniques in order to find, select and fix the greater recombination of genes more accurately and efficiently (Kumar 2016). It is quick and more powerful technique in developing high yielding varieties which also helps in genetic diversity assessment, gene-targeted marker-assisted selection (MAS) and gene-specific genetic engineering (GE) in crop breeding program of lentil to enhance the genetic gain and genetic diversity for developing high yielding cultivars.

10.9 Genomics-Assisted Lentil Breeding Approaches

The genomics-assisted lentil breeding is a potent tool, enabled incorporation of genomic tools and high-throughput phenotyping (HTP) with conventional breeding techniques. The presence of existing accessible genetic diversity is the fundamental for mining of beneficial alleles in gene banks which is essential for development of better-quality advanced cultivars with greater performance within defined environments. The accessibility of genomic tools and technologies such as gene-specific markers (SSR or SNP), EST, genome sequences, transcriptome sequences, QTLs maps and NGS have unlocked a novel means to assist the lentil breeding programs by deploying diverse genomic resources (Kumar et al. 2018a, b, c; Singh et al. 2019). The ongoing lentil breeding programs have limited use of genomic resources that cause boundation of the potential implementation of MAS. The slow progress in the development of lentil genomic resources (Kumar et al. 2014), vast genome dimension, low density linkage map, narrow genetic base, shortage and detection problems of candidate genes are the central limiting factors in genomics-assisted lentil improvement. The advent of NGS technologies accelerated the genomic resources development with time and cost effective manner, and changed the legume status from orphan to rich in genomic information (Singh et al. 2017, 2019; Varshney et al. 2005), which led to the development of an initial draft of 23× coverage later refined with further 125× coverage and have been widely used for a variety of purposes mostly for genetic relationship/diversity studies (Dissanayake et al. 2020a); not only in lentil crop but also in diverse legume crops like chickpea, pigeon pea, groundnut common bean, cowpea etc. (Afzal et al. 2020; Bauchet et al. 2019; Varshney et al. 2019).

10.9.1 Drafting the Genome Sequence and Developing of Gene-Specific Markers

The lentil genome sequence has been drafted in V1.2 for the CDC cultivar Redberry using bulk sequencing technique and chromosomal “pseudo-molecules” assembly representing about assembling of seven pseudomolecules and 120,000 scaffolds to a sum total of about 2.6 Gbp. The genes were tagged and annotated for genomics studies in lentil, i.e. open and accessible online on the KnowPulse (http://knowpulse.usask.ca) web portal (Bett et al. 2016). Moreover, the lentil cultivars/accessions have been analysed by re-sequencing to aware of the potential genetic and genomic information and data existing within the lentil germplasm. For example, Australian cultivar “PBA Blitz”. genome had drafted, of which included seven pseudo-molecules, a sum of 337.7 Gbp (c. 85× coverage) of top-level genome sequences and its assemblage of about 352,065 scaffolds and 444,011 singletons resulting a sum of about 2.3 Gbp. This drafted genome of lentil cultivar has a resemblance of 99% with respect to genome sequence of CDC cultivar (Kaur et al. 2016). The development of gene-specific markers linked with target traits are being developed in lentil to illustrate the genetic diversity within the available germplasm and their

association with diverse agro-morphological traits, such as disease resistance, seed quality and micronutrient concentration (Khazaei et al. 2018). The first locus-specific RFLP markers based on restriction endonuclease enzymes and hybridization with labelled probes was used to construct lentil linkage maps (Eujayl et al. 1998). For example, the first genomic library was created using restriction digestion of genomic DNA of a cultivated accession "ILL 5588" with *Sau3AI* (*Staphylococcus aureus* 3A) and hybridized with radio-labelled probes $(GT)_{10}$, $(GA)_{10}$, $(GC)_{10}$, $(GAA)_8$, $(TA)_{10}$ and $(TAA)_5$ then sequenced with M13 primers on LI-COR DNA sequencer (Hamwieh et al. 2005). However, requirement of high technical skills and difficulties in handling of radioactivity make it impracticable. Later, with the advent of PCR-based markers such as Random Amplified Polymorphic DNA (RAPD), Sequence Characterized Amplified Region (SCAR) and SSR have accelerated the use of corresponding markers in genome characterization of lentil crop (Ates et al. 2018; Singh et al. 2019; Mbasani-Mansi et al. 2019; Polanco et al. 2019). However, these marker techniques are usually labour intensive, time consuming and difficult in large scale application in plant breeding programme. At present, the development of gene-specific SSR markers has become quicker and efficient in cost through transcriptomic analysis or NGS techniques (Kaur et al. 2011; Singh et al. 2019). In current scenario, SNP markers are more popular than PCR-based markers due to even distribution across the genome, high in abundance and high detection ability with high throughput (HTP) techniques (Mammadov et al. 2012).

10.9.2 DNA Sequencing Techniques

The three generations of DNA sequencing techniques are shown in Table 10.5. First-generation/Sanger sequencing technique has been exploited to create ESTs, gene- or

Table 10.5 The generation, basis and techniques of DNA sequencing in lentil

Generations	Basis of sequencing	DNA sequencing techniques
First generation	Sequencing based on cloned DNA fragments into host cells	Sanger sequencing
Second generation	Linker- and/or adapters-based template libraries	Roche 454 pyrosequencing Illumina/Solexa HiSeq and MiSeq sequencing SOLiD (sequencing by oligo-nucleotide ligation and detection) DNA nanoball and ion torrent sequencing
Third generation	Chromosome conformation capture, optical mapping and DNA dilution-based technologies	SMRT (single molecule real time) sequencing Helicos sequencing Nanopore sequencing (MinION and PromethION) NGS by electron microscopy

genome-specific SSR and SNP markers, and re-sequence unigene-derived amplicons (Kumar 2016). For instance, the kompetitive allele-specific PCR (KASP) procedure is utilized to discover the SNPs from existing EST database in lentil (Sharpe et al. 2013; Fedoruk et al. 2013). The various second-generation techniques in lentil has been exploited to draft the sequence of genome and transcriptomes (Kaur et al. 2011; Khorramdelazad et al. 2018; Polanco et al. 2019; Sharpe et al. 2013; Singh et al. 2019). The third-generation DNA sequencing technique can be used to quickly fill the large gaps by generating longer DNA sequence in cost effective manner that is formed in drafting the lentil genome using second-generation techniques and also help in increasing the accuracy of SNPs detection (Stapley et al. 2010). The major limiting factors restricting the progress and purposes of genomics-assisted lentil breeding are narrow genetic base, too big genome size, low density linkage map and lack of candidate genes (Kumar 2016). The NGS and genotyping by sequencing (GBS) tools have unfolded new avenues for quick progress in developing sequence-based markers and sequencing platforms for lentil genome throughout the world (Elshire et al. 2011).

In recent times, NGS techniques have developed quickly which is valuable for identification and validation of unigenes, transcript, functional markers, and enriching genetic diversity, phylo-genetic studies and mapping of QTLs (Li et al. 2010). It has made feasible of SNPs mining in quick and economic manner for gene/QTL mapping and advancement in array-based high-throughput (HTP) genotyping techniques in lentil crop (Kaur et al. 2011; Sharpe et al. 2013). Several studies of NGS technologies were carried out, for example, NGS sequencing data produced on lentil cultivar CDC Redberry (Bett et al. 2014), about 44,879 SNP markers discovered using Illumina Genome Analyzer (Sharpe et al. 2013) and high-density linkage map constructed using 50,960 SNPs in lentil (Temel et al. 2015) which encouraged the progress of Illumina Golden Gate (GG) platforms for genotyping in lentil (Kaur et al. 2014; Sharpe et al. 2013), advancement in linkage maps and detection of genetic diversity and association of markers with traits of economic importance (Ates et al. 2018; Khazaei et al. 2018; Lombardi et al. 2014; Pavan et al. 2019). However, a large number of SNPs from coding regions are discovered in current years through NGS transcripts analysis in the genome of lentil (Kaur et al. 2014; Sharpe et al. 2013; Singh et al. 2019).

10.9.3 Transcriptome Analysis

The analysis of transcriptome assemblies offer an excellent way to identify the ESTs-derived functional markers like SSR, SNP markers and ITPs in lentil (Kumar 2016). In recent past, the classical Sanger sequencing technique has been employed to sequence 150–400 bp cDNA clones at particular crop stage and to generate ESTs from mRNA sequences. The progression in high throughput functional genomics techniques such as NGS and SAGE (Serial analysis of gene expression) has accelerated the designing of ESTs, functional markers based on EST and identification of candidate genes through homologous sequences across

Table 10.6 NGS platforms in lentil used for formation of EST-based functional markers

NGS platforms	SSRs	SNPs	Transcripts	Reads/ESTs	References
454 pyrosequencing	–	44,879	–	1,030,000	Sharpe et al. (2013)
Roche 454 GS-FLX Titanium	2415	–	25,592	1,380,000	Kaur et al. (2011)
Illumina HiSeq2500 platform	9949	8260	–	58,621,121	Singh et al. (2017)
	–	6306	48,453	–	Polanco et al. (2019)
	–	6693	–	46,700,000	Pavan et al. (2019)
			20,009	119,855,798	Verma et al. (2013)
Illumina HiSeq 2000 platform	141,050	194,178	–	26,165,023	Singh et al. (2019)
	–	50,960	–	111,105,153	Temel et al. (2015)
Ion proton sequencer	–	–	317,412	–	Khorramdelazad et al. (2018)

crop species. The first EST library based on seed phenotypes made from a mixture of eight cultivars (Vijayan et al. 2009), subsequently, second cDNA library was set up from the leaflets of "Eston" a Canadian cultivar inoculated with fungal pathogen *Colletotrichum truncatum* (Bhadauria et al. 2017; Kumar et al. 2014). At present, available ESTs for lentil in public domain are approximately 33,371 as accounted by NCBI, June 2020. EST-based databases are valuable genomic assets for the designing of SSR, CAPS, SNP and RFLP markers through different bioinformatics tools such as MISA for SSR detection and Snipper for SNP discovery (Kota et al. 2003; Thiel et al. 2003; Varshney et al. 2005). Kaur et al. (2011) generated 1.38×10^6 unigene/EST assembly, 3470 SNP and EST-SSR markers, contigs (15,354) and single tons (68,715) de novo assembly through transcriptome sequencing by using second-generation Roche 454 GS-FLX Titanium technique from tissue-specific cDNA samples of six lentil genotypes (Indianhead, Northfield, Digger, ILL 2024, ILL 7537 and ILL 6788). The NGS platform used for formation of EST-based functional markers and genomic resources in lentil in quick and cost-effective manner (Table 10.6).

Currently, Illumina GA/GAIIx sequencing technique is a future prospective alternative for development of transcriptome cDNA library. Using this technique Sharpe et al. (2013) developed 1536 SNP Illumina GG array to build a SNP-based linkage map and their population employed for mapping in *L. culinaris*. Similarly, Verma et al. (2013) de novo assembled the transcriptome assemblies of lentil using Illumina GAII and designed SSR markers for diversity analysis. Temel et al. (2015) generated a SNP-based genetic linkage map and their RILs mapping population using Illumina CASAVA pipelines in two lentil cultivars, Precoz and WA 8649041. Singh et al. (2017) assembled 77,346 contigs generated from overall 58,621,121 reads over four treatments of drought tolerant and susceptible lentil genotypes through de novo transcriptome analysis that led to the designing of SSR (9949)

and SNP (8260) markers. Subsequently, Singh et al. (2020) generated a sub set of about 50 EST-SSR markers which exploited for the diversity assessment among 234 lentil genotypes and cross species transferability of markers across the legumes. In another study, Singh et al. (2019) generated 96,824 contigs developed from on average 26,165,023 reads across 12 heat tolerant and sensitive lentil genotypes by de novo transcriptome analysis. More recently, Wang and Daun (2006) designed PCR-based KASP markers by converting 78 SNPs out of 276 EST-based SSR markers evaluated from 26,449 EST-SSR and 130,073 SNP markers across 94 accessions of lentil which were developed through RNAseq analysis.

10.9.4 Mapping Techniques

The stepwise flowchart of genomics-assisted lentil breeding has been depicted in Fig. 10.2.

10.9.4.1 Mapping Population (MP)

The ICARDA, Lebnana (Syria) and national lentil programmes in several countries including India were attempted to develop mapping population for genetic dissection of key QTLs/genes associated with target traits of economic significance in order to locate their relative position and genetic distance by using molecular markers (Kumar et al. 2018b, 2011). Intra-specific mapping populations is characterized

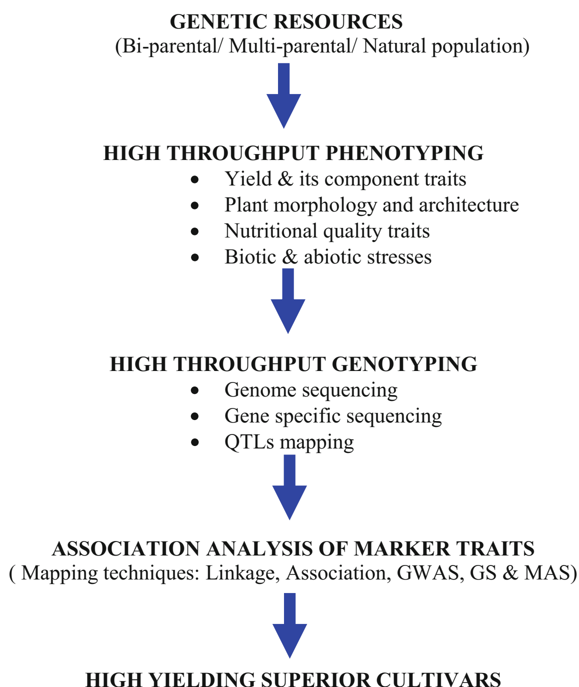

Fig. 10.2 Stepwise flowchart of genome-assisted breeding in lentil

Table 10.7 Mapping populations (MPs) derived from various crosses for different traits in lentil

S. No.	Target traits	Parental cross	MP size	References
1.	Drought	ILL 7946 × ILL 7979 ILL6002 × ILL5888	174 133	Kumar et al. (2016) Idrissi et al. (2015)
2.	Cold	ILL 4605 × ILL 10657	153	Kumar et al. (2016)
3.	Earliness	ILL 7115 × ILL 8009	150	Kumar et al. (2016)
4.	Rust	ILL 5888 × ILL 6002	152	Kumar et al. (2016)
5.	*Fusarium* wilt	ILL 213 × ILL 5883 ILL 5588 × L 692-16-1(s)	150 86	Kumar et al. (2016) Hamwieh et al. (2005)
6.	*Aschochyta* blight	ILL5588 × ILL7537 ILL7537 × ILL6002 *L. culinaris* (Alpo) × *L. odemensis* (ILWL 235)	150 153 78	Rubeena and Ades (2006) Polanco et al. (2019)
7.	Zn content	ILL 5722 × ILL 9888 ILL 9888 × ILL 5480	177 149	Kumar et al. (2016)
8.	Fe content	ILL 9932 × ILL 9951	193	Kumar et al. (2016)
9.	Milling quality	CDC Robin × 964a-46	127	Subedi et al. (2018)

with low genetic diversity lead to less profusely incorporated markers for gene-based maps (Bohra et al. 2012). Therefore, inter-specific RILs population were generated in Lentil by crossing Alpo cultivar (*L. culinaris*) and ILWL 235 accession (*L. odemensis*) for resistance of *Ascochyta* blight (Polanco et al. 2019). The back-cross inbred line (BIL) population was also generated from a cross IPL 220 cultivar (*L. culinaris*) and ILWL 118 accession (*L. orientalis*) to identify the genes for yield traits (Kumar et al. 2019). The RILs (Recombinant Inbred Lines) mapping populations in lentil for key agronomic important and, biotic and abiotic traits *viz*., early growth vigour, earliness, root traits, drought, zinc (Zn) and iron (Fe) were developed at ICARDA, Lebnan (Kumar 2016). In lentil, resistance for *Aphanomyces* root rot (ARR), generated the 189 RILs population by crossing between partial resistant and susceptible lines (Ma et al. 2020). The RILs population for quality traits have been developed from three bi-parental crosses (PI320937 × Eston; ILL8006 × CDC Milestone; and CDC Redberry × ILL7502) to recognize the QTLs for iron (Fe), manganese (Mn), and selenium (Se) uptake (Aldemir et al. 2017; Ates et al. 2016, 2018). The various mapping populations (MPs) derived from various crosses for different traits in lentil have shown in Table 10.7.

10.9.4.2 High Throughput Phenotyping (HTP)

Since plant genomics already revolutionized with the advent of NGS techniques, whereas, plant phenomics are still facing a greatest challenge in practical plant breeding due to technology availability and high initial cost. Conventional phenotyping approaches have done by manual assessments and measurements requires breeder skill and expertise, a lot of energy, labour intensity, time and accompanied with human error. A large-scale phenotyping of complex quantitative

Table 10.8 High-throughput phenotyping platform (HTPP) for various economically important traits

Sr. No.	HTPP technology	Economically important traits	Condition	Reference
1.	Imaging	Growth parameter and chlorophyll fluorescence	Controlled condition	Jansen et al. (2009)
2.	Imaging	Leaf area	Field condition	Montes et al. (2011)
3.	Automated RGB imaging	Cold tolerance, shoot biomass and PS-II efficiency	Controlled condition	Humplík et al. (2015)
4.	RGB and hyper-spectral imaging	*Aphanomyces* root rot (ARR)	Controlled condition	Marzougui et al. (2019)
5.	RGB imaging	Salt tolerance	Controlled condition	Dissanayake et al. (2020b)
6.	Unmanned aerial system and satellite imagery	Vegetation indices	Field condition	Sankaran et al. (2019)
7.	Camera	Growth parameter of leaf	Controlled condition	Massonnet et al. (2010)
8.	Camera	Presence of rice bugs	Field condition	Fukatsu et al. (2012)
9.	Spectro-radiometer	Drought stress	Controlled condition	Lu et al. (2011)
10.	Visual	Root architectural traits	Field condition	Trachsel et al. (2011)
11.	Hydraulic push press	Root distribution pattern and depth	Field condition	Wasson et al. (2012)
12.	Sensor	Height of canopy	Field condition	Andrade-Sanchez et al. (2014)

traits or unbiased scoring of diverse biotic and abiotic stresses is quite difficult in modern plant breeding (Furbank and Tester 2011). Therefore, there is urgent need of HTP technologies for rapid, highly efficient, high quality and accurate measurement and prediction of complex quantitative traits in modern plant breeding (Marko et al. 2018; Zhang et al. 2017). In recent years, rapid advancement in image-based HTP approaches has been furnished excellent opportunities to distinguish the plants for various stresses (Hu et al. 2020; Sun et al. 2018; Wang et al. 2019). For example, advancement in a range of digital imaging sensors have been progressed such as red–green–blue (RGB), fluorescence, hyperspectral and 3D imaging techniques (Light detection and ranging-LiDAR) and unmanned aerial vehicle remote sensing (Pratap et al. 2019).

Quick progress towards high-throughput phenotyping platforms (HTPPs) have been made due to technological evolution in computing and robotics which leads to establishment of plant phenomics. It has potential to non-destructive phenotyping and, high-quality data recording and analysis predominantly for detailed and reliable characterization of plants (Table 10.8). Moreover, an international association

established the major plant phenotyping center “International Plant Phenomics Network (IPPN)” for HTP of major plants via robotic and non-invasive imaging technique in short-duration crops/model plants throughout their life cycle (Muraya et al. 2017). The integration of LiDAR, high-resolution camera and hyperspectral imager are able to quick, precise and efficient phenotyping of numerous traits *viz*., plant height, leaf length, width and angle under controlled condition in the greenhouse (Guo et al. 2017). Zhang et al. (2017) quantified the dynamic range of growth characters from seedling to tasseling stage in maize RIL population using a HTPP under greenhouse. Undoubtedly, HTP together with genomic selection will increase our understanding of crop physiology for complex traits (e.g., disease resistance, stress tolerance and seed yield) (Reynolds and Langridge 2016). Recently, Marzougui et al. (2019) quantitative evaluated ARR resistance in lentil using image-based phenotyping approaches in controlled condition (351 lines and 191 RILs using digital RGB and hyperspectral imaging) and field (173 RILs using multispectral imaging) conditions. Similarly, Dissanayake et al. (2020b) applied image-based HTP methodology for screening of 276 accessions for salt tolerance in lentils using RGB images on a LemnaTec Scanalyzer 3D phenomics platform.

10.9.4.3 Linkage Mapping

The different marker types and linkage maps have been developed in lentil (Table 10.9). In ancient times, linkage maps have been generated in lentil by using both intra- and inter-specific bi-parental mapping populations (Kumar et al. 2019). For example, a molecular linkage map in lentil was constructed from a inter-specific cross between Precoz × WA 8949041 using 94 RILs population with 166 RAPD and ISSR markers, which comprised of 11 linkage groups covering 1396.30 cM and distance between framework markers ranged from 50.90 to 436.50 cM with an average of 8.40 cM (Tanyolac et al. 2009). Till now, several researches related to genetic linkage map had published, however, the first linkage map generated by exploiting the DNA-based markers during 1989 (Havey and Muehlbauer 1989). Thereafter, development of genetic linkage maps has been progressed with several molecular (RAPD, RFLP, AFLP, ITAP and SSR) and morphological markers (Andeden et al. 2013; Gupta et al. 2012b; de la Vega et al. 2011; Verma et al. 2015) and in fact first such map constructed with the help of these markers and RILs population of a cross between *L. culinaris* and *L. orientalis* (Eujayl et al. 1998). A high-density linkage map constructed by utilizing bi-parental populations with 5385 DArT markers, covered approximately 973.1 cM genetic map distance with 0.18 cM average distance between markers (Ates et al. 2018).

Currently, evolution of NGS techniques has replaced the chip-based method used with SNP markers and made greater availability in number of SNP markers which lead to the generation of high-density linkage maps. Further, low expenses of sequencing guided to the construction of a SNP-based vast number of high-density linkage maps in lentil reflected in Table 10.10 (Bhadauria et al. 2017; Ma et al. 2020; Polanco et al. 2019; Sudheesh et al. 2016). The SSR and SNP markers developed from parts of DNA expressing by NGS-based transcriptome analysis which acts as functional DNA markers and led to construction of high density linkage map from an

Table 10.9 Linkage map in lentil development based on molecular markers and mapping population

Parental crosses	MP size	Molecular markers	Marker loci (number)	Map distance (cM)	References
L. culinaris sp. *culinaris* × *L. c. sp. orientalis*	113 F_2	RAPD, ISSR, AFLP, CAPS, SSR, SRAPS	200	2234	Fratini et al. (2004) de la Puente García et al. (2013)
ILL 5588 × ILL 7537	150 F_2	RAPD, ISSR RGA	114	784	Ford and Taylor (2003)
L 830 × ILWL 77	114 F_2	SSR, ISSR, RAPD	199	3843	Gupta et al. (2012b)
ILL 5588 × L 692-16-1(s)	86 RIL	SSR, AFLP	283	751	Hamwieh et al. (2005)
Eston × PI 320937	94 RIL	AFLP, RAPD, SSR	207	1868	Tullu et al. (2006, 2008)
Precoz × WA 8649041	94 RIL	AFLP, ISSR, RAPD	166	1396	Tanyolac et al. (2009)
ILL 6002 × ILL 5888	206 RIL	SSR, RAPD, SRAP	139	1565	Saha et al. (2013)
Cassab × ILL 2024	126 RIL	SSR, SNP	318	1178	Kaur et al. (2014)
L. culinaris cv. Precoz × *L. culinaris* cv. L830	126 RIL	SSR	216	1183.70	Verma et al. (2015)
Indianhead × Northfield Indianhead × Digger Northfield × Digger	RIL 117 112 114	SNP, SSR	689	2429.61	Sudheesh et al. (2016)
ILL 8006 × CDC Milestone (LR11) CDC Redberry × ILL 7502 (LR8) PI 320937 × Eston (LR39)	RIL 96 120 118	DArT, SNP	50	977.47	Ates et al. (2018)

interspecific (*L. culinaris* × *L. odemensis*) mapping population consisted of 6153 markers grouped into 4682 unique bins, positioned on 10 LGs and covered about 5782 cM length (Polanco et al. 2019). A high-density linkage map formed via transcriptome sequencing with 61 SSR and 264 SNP markers from a lentil EST database communicated to seven linkage groups (LGs) and three satellites covered around 1178 cM with an average of one locus per 3.70 cM (Kaur et al. 2014). A high-density consensus map generated in lentil from three RILs population (Table 10.7) covered 977.47 cM genetic distance and consisted of 9793 SNP markers which were grouped in seven LGs with an average distance between markers of 0.10 cM (Ates et al. 2018). Recently, a linkage map constructed with

Table 10.10 Linkage maps in lentil constructed specifically with SNP molecular markers

Parental crosses	MP size	Total number of SNP loci	Map distance (cM)	Average distance between markers	References
DC Robin × 964a-46	144	542 (included six SSRs)	834.7	1.54	Sharpe et al. (2013)
Precoz × WA 8649041	103	388	432.8	1.11	Temel et al. (2015)
Indianhead × digger Indianhead × northfield Northfield × digger	117 112 114	689	2429.6	3.5	Sudheesh et al. (2016)
L 01-827A × IG-72815 (*L. ervoides*)	94	543	740.9	1.36	Bhadauria et al. (2017)
PI 320937 × eston	96	1784 (Included four SSRs)	1,784	2.3	Ates et al. (2016)
ILL8006 × CDC milestone	118	4177	497.1	0.12	Aldemir et al. (2017)
ILL8006 × CDC milestone CDC Redberry × ILL 7502 PI320937 × Eston	118 120 96	9793	977.47	0.10	Ates et al. (2018)
CDC Redberry × ILL 7502	120	5385	973.1	0.18	Ates et al. (2018)
Alpo (*L. culinaris*) × ILWL 235 (*L. odemensis*)	78	6306 (4682 bins)	5782.19	0.91	Polanco et al. (2019)

265 markers taken in the lentil (433 SSRs) and other legumes genome (250 RAPDs, 145 SSRs and 25 ISSR) distributed on seven LGs with map distance of 809.4 cM and an average of 3.05 cM (Mane et al. 2020).

10.9.4.4 QTLs Mapping

QTLs are genomic regions which is responsible for the variation in the quantitative trait of interest. In a study from Ma et al. (2020) identified and highlighted 19 QTL by QTL mapping and 38 QTL by association mapping linked with ARR resistance in lentil, of which seven QTL clusters and 15 putative genes within these clusters identified on six chromosomes, and also validated these results through expression analysis. Kumar et al. (2018c) identified 24 marker trait associations (MTAs) in nine agronomic important traits of lentil using association mapping at $P < 0.01$ through MTA analysis. The phenotypic variability (%) ranged from 7.30 to 25.80% explaining the marker associated with each specific agronomic trait (days to maturity, secondary branches/plant, pods/plant, reproductive duration, yield/plant and

Table 10.11 QTLs identified for economically important traits using NGS techniques in lentil

Traits	QTLs	Linkage group	1.8 LOD score	Phenotypic variation (%)
Stem pigmentation	SP	LG 1	79.00–145.70	33.96
Time to flowering	TF	LG 6	199.00–222.00	55.73
Flower colour	FC	LG 6	31.30–34.60	84.02
Seed coat spotting	Scp	LG 6	30.07–33.96	85.07
Seed size	Q1, Q2, Q3	LG 1, LG5a, LG5b	81.00–141.00	35.48
Ascochyta blight	AS-Q1, AS-Q2, AS-Q3	LG 6	180.00–222.00	28.46

100 seed weight), among them QTLs for primary branches/per plant explained the leading percentage of total phenotypic variation (23.1–25.8%). Subedi et al. (2018) mapped several QTLs for milling quality traits using 534 SNP markers, seven SSR markers and four morphological markers. In 6 of 7 linkage groups (LGs), the most stable QTLs were grouped on LGs 1, 2, 3 and 7 for dehulling efficiency (DE) and milling recovery (MR), whereas, on LGs 4, 5, 6 and 7 for football recovery (FR). Gupta et al. (2012a) documented three QTLs for *Ascochyta* blight (*Ascochyta lentis*) resistance at seedling and pod/maturity stages. Lately, Khorramdelazad et al. (2018) identified major defence response genes during host-pathogen interactions of *Ascochyta* blight by transcriptome profiling of lentil lines ILL 7537 (resistant) and ILL 6002 (susceptible) via a targeted RNA-Seq technique, while, Sari et al. (2018) concluded the usefulness of designing molecular markers in the gene pyramiding of *Ascochyta* blight resistance through quantitative real time-PCR (RT-qPCR) and RNA-seq analysis. Ates et al. (2018) identified the six QTLs for Mn (Manganese) concentration via composite interval mapping (CIM) which explained total phenotypic variation ranged from 15.30 to 24.10% with LOD scores from 3.00 to 4.42. Polanco et al. (2019) detected the QTLs for economically important traits through RNAseq methodology (NGS technique) in lentil RILs population derived from the inter-specific cross between *L. culinaris* cultivar Alpo and *L. odemensis* accession ILWL 235. There is a list of QTLs identified for yield and other economically important traits of interest in lentil by using NGS techniques (Table 10.11).

10.9.5 Functional Genomics

Functional genomics are comprehensive technique used to identify the functions and their interactions of candidate gene(s)/QTLs responsible for principal trait of interest. The gene cloning approach, resistant gene analogue (RGA) and cross species sequence homology (Synteny and co-linearity) helps in tagging the unknown or

targeted genes controlling the trait of agronomic importance or biotic resistance or abiotic tolerance. The lentil genomic resources with cDNA from ESTs and cloned genes could speed up the progress of functional markers to be used in MAS. The functional genomics techniques like SAGE enhanced the cloning of genes in several crops including legumes. In lentil, RGAs have been recognized (Yaish et al. 2004). For example, Sari et al. (2018) identified two RGAs *viz*., *RGA1* and *RGA71* with putative roles as receptors/transcription factors by NCBI BlastX tool, orthologues of *ABI1*-B and *DDB1-CUL4* involved in the signal transduction pathways and an orthologue of *PRH*, as a transcription factor involved in the transcription activation of *PR* proteins. They identified candidate defence response genes through RNA-seq analysis were "nucleotide-binding site- leucine rich repeat (*NBS-LRR*) and *RLK* gene families" tentatively involved in the *Ascochyta* blight pathogen recognition and resistance, and differentially expressed among the *Ascochyta* blight resistance genotypes "CDC Robin", "964a-46" and susceptible check "Eston". The transcription factor (TF) genes were identified in *Arabidopsis* which is also found in legumes based on cross reference sequence homology (Li et al. 2018; Udvardi et al. 2007). The cross-species distribution of functionally related or differentially expressing TF genes between legume and non-legume or in contrasting genotypes can also be identified or confirmed by functional genomics approaches. Ford et al. (2007) assessed differential gene transcript profiles in lentil resistance (ILL7537) and susceptible (ILL6002) genotypes after inoculation with *Ascochyta lentis*. Functional genomics analysis can be used to identify several defence responsive candidate genes. For example, Cao et al. (2019) identified the function of candidate genes coding for calcium-transporting *ATPase* and glutamate receptor 3.2 for *Stemphylium* blight disease resistance in lentil and validated by BSA (Bulk segregant analysis). Currently, Dissanayake et al. (2020a) identified five candidate genes from Region-1 encoding for metal and ABC transporter, and a mitogen-activated protein kinase 3 (*MAPKK* 3) using cross reference SNP markers.

10.9.6 Comparative Genomics

The synteny among the genomes of lentil and other crops of legume have shown different levels of conservation during course of evolution which has been potentially utilized by comparative genomics approach through designing of PCR-based DNA markers such as SSR and ITAP (Intron-Targeted Amplified Polymorphism) in lentil (Choi et al. 2004; Zhu et al. 2005; Choudhary et al. 2009; Phan et al. 2007). The comparative genomics approaches have improved the cross-species transferability of genetic information among legume crop species (chickpea, pigeon pea, soybean *Trifolium*, common bean and *Medicago*) that has led to the development of SSRs and establishment of phylogenetic relationship among lentil species (Alo et al. 2011; Datta et al. 2011; Gupta et al. 2012a; Phan et al. 2007; Reddy et al. 2010). The synteny and co-linearity among the genome of legume crop species have potential to offer SSR marker transferability to lentil because the latter has limited availability of SSR markers, for example, chickpea-specific STMS (Sequence Tagged Marker

Satellite) primers has 5% transferability to lentil (Pandian et al. 2000); the 62% successful amplification of *Trifolium*; 36% of *Medicago* and 25% of *Pisum* markers in lentil (Reddy et al. 2010); 19 STMS markers transferability from chickpea, pigeon pea, common bean and soybean to lentil (Datta et al. 2011); and also has potential to EST-based SSR marker transferability from model genome *M. truncatula* to lentil crop (Gupta et al. 2012a).

Comparative genomics can also be exploited to detect the cross-species candidate genes responsible for traits of agronomic importance with the help of functional markers such as EST-SSR or EST-SNP or gene-based markers. Weller et al. (2012) identified orthologous gene loci "*ELF3*" responsible for photoperiod response in lentil from pea which is one of the two genes "*HR*" (High response to photoperiod) and "*ELF3*" controlling the difference in photoperiod response between wild and domesticated pea. Kaur et al. (2014) tagged genes for micro-nutrient tolerance of boron in lentil through comparative genomics from the comparative genome of *Arabidopsis thaliana* and *M. trucatuala* by using flanking markers SNP 20002998 and SNP 20000246. In another study by Kaur et al. (2011) developed EST-based SSRs associated with flowering time in lentil led to discovery of genes responsible for flowering time in other legumes through comparative mapping technique (Kumar et al. 2018b). Recently, a study conducted by Singh et al. (2020) screened a subset of 50 out of 9949 EST-based SSR markers developed through transcriptome analysis which is detected different candidate genes associated with different metabolic activities in lentil and 12 legume genera. Thus, these functional markers could be used for identification of candidate genes underlying traits of agronomic interest through comparative mapping in lentil.

10.9.7 Marker-Assisted Selection (MAS)

Conventional breeding methods is largely reliable on selection of desirable plants in F_2 and onwards segregating generation contain appropriate combinations of genes or as a parent or variety based on complex quantitative traits which is highly influenced by the genotype, environment and interaction between them. MAS and DNA-based selection of desirable plant are remarkable ways to introduce QTLs/genes associated with yield enhancement, biotic resistant or abiotic tolerant traits. Currently, the implementation of MAS in lentil-breeding is limited due to insufficient availability, accessibility and slow progress in the enhancement of lentil genomic resources (Kumar et al. 2014). MAS breeding strategy has already been successfully applied and documented in broad range of cereals (rice, wheat, maize, sorghum and barley), pulses (chickpea, pigeon pea and beans), oilseeds (groundnut, mustard and sunflower) and vegetable crops (cassava, cowpea and potato), and other crops as well (Cobb et al. 2019). Till now, the two schemes of MAS, i.e. MABB (marker-assisted backcrossing) and MAP (marker-assisted gene pyramiding) employed extensively for a numerous of complex quantitative traits including abiotic stress (Steele et al. 2006) and grain number (Ashikari et al. 2005). The morphological markers exhibiting monogenic dominant inheritance were classified as qualitative markers

such as cotyledon (Yc), seed coat pattern (Scp), anthocyanin pigmentation in stem (Gs), early flowering (Sn), flower color (W), pod indehiscence (Pi), radiation frost tolerance (Rf) and ground color of seed (Gc) in lentil (Duran et al. 2004; Hamwieh et al. 2005; Tullu et al. 2003). Till now, several QTLS have been mapped and documented for various yield traits including disease resistance and abiotic stresses that will facilitate the introgression of theses genomic regions in lentil via MAS. Furthermore, quantitative traits are highly affected by both genetic and environmental factors, thus, RILs or NILs (near-isogenic lines) mapping population are more precise to accurately dissect the specific QTLs.

10.10 Conclusion

The milestone goal in breeding of lentil crop is to develop superior varieties with higher yield, better quality and diseases and insects resistance. In recent past, hybridization and selection have become the chief assets in lentil improvement, however, conventional breeding techniques are suffering from several bottlenecks which restricts the lentil breeders in achieving the major breeding objectives. In recent times, development of genomic resources with the use of gene-based markers genotyping such as SNP and/or SSR, and NGS techniques have made possible to access the genomic sequences, transcriptomic sequences and genes/QTLs linked to important yield contributing traits. Therefore, there is a necessary to incorporate genomics-assisted lentil breeding approaches such as molecular marker and genomic resources techniques in order to identify, select and fix superior recombinants more accurately and efficiently. The genomics-assisted lentil breeding is a powerful tool, enabled integration of genomic tools and HTP with conventional breeding techniques. There is need to establish HTP platform to accelerate the accurate phenotyping the complex quantitative traits. The newly developed genetic and genomic tools and techniques will augment the conventional plant breeding evaluation and selection procedures. The genomics-assisted lentil breeding will broaden the genomic resources, generate a large quantity of next-generation sequencing information, transcriptomes and cross references genomic information among legumes for mining of candidate gene information for traits of agronomic interest, biotic and abiotic stresses. The HTP, high-throughput genotyping and next-generation sequencing platforms pledge to further revolutionize our perceptive of genetic diversity and in designing breeding strategies to utilize precise genomic information in further improvement of lentil crop yield in future breeding programme. MAS and genomics-assisted breeding approaches have great potential to improve lentil breeding at genome level and take crop breeding into new heights of achievements in future. Lentil crop fits remarkably well into genre of a "smart crop", and now considered as target crop for research and development among pulses.

References

Afzal M, Alghamdi SS, Migdadi HH et al (2020) Legume genomics and transcriptomics: from classic breeding to modern technologies. Saudi J Biol Sci 27(1):543–555

Ahloowalia BS, Maluszynski M, Nichterlein K (2004) Global impact of mutation-derived varieties. Euphytica 135(2):187–204

Aldemir S, Ateş D, Temel HY et al (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 M, Gupta S (2012) Carrying capacity of Indian agriculture: pulse Crops. Curr Sci 102:874–881

Alo F, Furman BJ, Akhunov E et al (2011) Leveraging genomic resources of model species for the assessment of diversity and phylogeny in wild and domesticated lentil. J Hered 102(3):315–329

Amin R, Laskar RA, Khan S (2015) Assessment of genetic response and character association for yield and yield components in lentil (lens culinaris L.) population developed through chemical mutagenesis. Cogent Food Agric 1(1):1000715

Amin-Laskar R, Wani MR, Raina A et al (2018) Morphological characterization of gamma rays induced multipodding mutant (mp) in lentil cultivar Pant L 406. Int J Radiat Biol 94(11): 1049–1053

Andeden EE, Derya M, Baloch FS (2013) Development of SSR markers in lentil. In: Proceedings of plant and animal genome conference Vol 21. Conference XXIP0351, San Diego, CA, p P0351, 12 Jan 2013

Andrade-Sanchez P, Gore MA, Heun JT et al (2014) Development and evaluation of a field-based high-throughput phenotyping platform. Funct Plant Biol 41(1):68–79

Andrews M, Mckenzie BA (2007) Adaptation and ecology. In: Yadav SS, McNeil DL, Stevenson PC (eds) Lentil-an ancient crop for modern times. Springer, Dordrecht, pp 23–32

Ashikari M, Sakakibara H, Lin S et al (2005) Cytokinin oxidase regulates rice grain production. Science 309(5735):741–745

Asnake F, Bejiga G (2003) Breeding lentil for wider adaptation in forage and food legumes of Ethiopia: progress and prospects. In: Kemal A, Gemechu K, Seid A et al (eds) Proceedings of the workshop on food and forage legumes. EIAR and ICARDA, Aleppo/Addis Ababa, pp 80–86

Ates D, Sever T, Aldemir S, Yagmur et al (2016) Identification QTLs controlling genes for Se uptake in lentil seeds. PLoS One 11(3):e0149210

Ates D, Aldemir S, Yagmur B et al (2018) QTL mapping of genome regions controlling manganese uptake in lentil seed. G3 8(5):1409–1416

Aversano R, Ercolano MR, Caruso I (2012) Molecular tools for exploring polyploid genomes in plants. Int J Mol Sci 13(8):10316–10335

Bauchet GJ, Bett KE, Cameron CT (2019) The future of legume genetic data resources: challenges, opportunities, and priorities. Legume Sci 1(1):e16

Baum M, Erskine W, Ramsay G (1997) Biotechnology and genetic resource of grain legumes: lentil and faba beans. In: Watanabe KN, Pehu E (eds) Plant biotechnology and plant genetic resource for sustainability and productivity. RG Landes Company/Academic Press Inc, Austin, pp 117–132

Bett K, Ramsay L, Sharpe A et al (2014) Lentil genome sequencing: establishing a comprehensive platform for molecular breeding. In: Proceedings of international food legumes research conference (IFLRC-VI) and ICCLG-VII, vol 19. Crop Development Center), Saskatoon

Bett K, Ramsay L, Chan C et al (2016) The lentil genome – from the sequencer to the field. In: International conference on pulses, Marrakesh, Morocco, 18–20 April, 2016 Abstract

Bhadauria V, Vijayan P, Wei Y, Banniza S (2017) Transcriptome analysis reveals a complex interplay between resistance and effector genes during the compatible lentil-*Colletotrichum lentis* interaction. Sci Rep 7(1):1–3

Blakeslee AF, Avery AG (1937) Methods of inducing doubling of chromosomes in plants: by treatment with colchicine. J Hered 28(12):393–411

Bogale DA, Mekbib F, Fikre A (2017) Genetic improvement of lentil [*Lens culinaris* Medikus] between 1980 and 2010 in Ethiopia. Malays J Med Biol Res 4(2):129–138
Bohra A, Saxena RK, Gnanesh BN et al (2012) An intra-specific consensus genetic map of pigeonpea [*Cajanus cajan* (L.) Millspaugh] derived from six mapping populations. Theor App Genet 125(6):1325–1338
Cao Z, Li L, Kapoor K et al (2019) Using a transcriptome sequencing approach to explore candidate resistance genes against stemphylium blight in the wild lentil species *Lens ervoides*. BMC Plant Boil 19(1):1–6
Cash D, Lockerman R, Bowman H et al (2001) Growing lentils in Montana. In: Montana state university extension guide MT199615. Montana State University, Bozeman, MT
Chagné D, Carlisle CM, Blond C et al (2007) Mapping a candidate gene (MdMYB10) for red flesh and foliage colour in apple. BMC Genomics 8(1):1–1
Chahota RK, Kishore N, Dhiman KC et al (2007) Predicting transgressive segregants in early generation using single seed descent method-derived micro-macrosperma genepool of lentil (*Lens culinaris* Medikus). Euphytica 156(3):305–310
Chen ZJ, Ni Z (2006) Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. Bioessays 28(3):240–252
Choi HK, Mun JH, Kim DJ et al (2004) Estimating genome conservation between crop and model legume species. Proc Natl Acad Sci U S A 101(43):15289–15294
Choudhary S, Sethy NK, Shokeen B et al (2009) Development of chickpea EST-SSR markers and analysis of allelic variation across related species. Theor Appl Genet 118(3):591–608
Chuni L, Sharma SK, Chahota RK (2000) Combining ability and heterosis for growth parameters in microsperma× macrosperma lentil. Crop Res 19(1):115–121
Cobb JN, Biswas PS, Platten JD (2019) Back to the future: revisiting MAS as a tool for modern plant breeding. Theor Appl Genet 132(3):647–667
Cokkizgin A, Shtaya MJ (2013) Lentil: origin, cultivation techniques, utilization and advances in transformation. Agric Sci 1(1):55–62
Cubero JI (1981) Origin, taxonomy and domestication. In: Webb C, Hawtin GC (eds) Lentils. Commonwealth Agricultural Bureau, Slough, pp 15–38
Cubero JI, Perez De La Vega M, Frantini R et al (2009) Origin, phylogeny, domestication and spread. In: Erskine W, Muehlbauer FJ, Sarker A, Sharma B (eds) Lentil – botany, production and uses. CABI, Wallingford, pp 13–33
Datta S, Tiwari S, Kaashyap M et al (2011) Genetic similarity analysis in lentil using cross-genera legume sequence tagged microsatellite site markers. Crop Sci 51(6):2412–2422
de la Puente García R, García PG, de la Puente CG et al (2013) An improved intersubspecific genetic map in lens including functional markers. Span J Agric Res 1:132–136
de la Vega MP, Torres AM, Cubero JI et al (eds) (2011) Genetics, genomics and breeding of cool season grain legumes. CRC Press, Boca Raton
Dhuppar P, Biyan S, Chintapalli B et al (2012) Lentil crop production in the context of climate change: an appraisal. Indian Res J Ext Educ 2:33–35
Dissanayake R, Braich S, Cogan NO et al (2020a) Characterization of genetic and allelic diversity amongst cultivated and wild lentil accessions for germplasm enhancement. Front Genet 11:546
Dissanayake R, Kahrood HV, Dimech AM et al (2020b) Development and application of image-based high-throughput phenotyping methodology for salt tolerance in lentils. Agronomy 10(12): 1992
Duran Y, Fratini R, Garcia P et al (2004) An intersubspecific genetic map of lens. Theor Appl Genet 108(7):1265–1273
El Haddad N, Rajendran K, Smouni A et al (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
Elshire RJ, Glaubitz JC, Sun Q et al (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6(5):e19379
Elzebroek T, Wind K (2008) Oil crops. Guide to cultivated plants. CABI, Wallingford, pp 182–225

Emery M, Willis MM, Hao Y et al (2018) Preferential retention of genes from one parental genome after polyploidy illustrates the nature and scope of the genomic conflicts induced by hybridization. PLoS Genet 14(3):e1007267

Erskine W, Adham Y, Holly L (1989) Geographic distribution of variation in quantitative traits in a world lentil collection. Euphytica 43(1):97–103

Erskine W, Chandra S, Chaudhry M et al (1998) A bottleneck in lentil: widening its genetic base in South Asia. Euphytica 101(2):207–211

Erskine W, Sarker A, Ashraf M (2011) Reconstructing an ancient bottleneck of the movement of the lentil (*Lens culinaris ssp. culinaris*) into South Asia. Genet Resour Crop Evol 58(3):373–381

Eujayl I, Baum M, Powell W et al (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

FAOSTAT (2018) Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#data/QC

FAOSTAT (2019) Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#data/QC

Farooq M, Wahid A, Kobayashi NS et al (2009) Plant drought stress: effects, mechanisms and management. Sustain Agric:153–188

Fedoruk MJ, Vandenberg A, Bett KE (2013) Quantitative trait loci analysis of seed quality characteristics in lentil using single nucleotide polymorphism markers. Plant Genome 6(3):5

Ferguson M (2000) *Lens spp*: conserved resources, priorities for collection and future prospects. In: Linking research and marketing opportunities for pulses in the 21st century. Springer, Dordrecht, pp 613–620

Ferguson M, Erskine W (2001) Lentils (*Lens* L.). In: Plant genetic resources of legumes in the Mediterranean. Springer, Dordrecht, pp 125–133

Ford R, Taylor PW (2003) Construction of an intraspecific linkage map of lentil (*Lens culinaris ssp. culinaris*). Theor Appl Genet 107(5):910–916

Ford R, Redden RJ, Materne M et al (2007) Lentil. In: Pulses, sugar and tuber crops. Springer, Berlin, pp 91–108

Fratini R, Ruiz ML, de la Vega MP (2004) Intra-specific and inter-sub-specific crossing in lentil (*Lens culinaris* Medik.). Can J Plant Sci 84(4):981–986

Fukatsu T, Watanabe T, Hu H et al (2012) Field monitoring support system for the occurrence of *Leptocorisa chinensis* Dallas (Hemiptera: Alydidae) using synthetic attractants, field servers, and image analysis. Comput Electron Agric 80:8–16

Furbank RT, Tester M (2011) Phenomics–technologies to relieve the phenotyping bottleneck. Trends Plant Sci 16(12):635–644

Gan Y, Liang C, Chai Q et al (2014) Improving farming practices reduces the carbon footprint of spring wheat production. Nat Commun 5(1):1–3

Guo D, Juan J, Chang L et al (2017) Discrimination of plant root zone water status in greenhouse production based on phenotyping and machine learning techniques. Sci Rep 7(1):1–2

Gupta PK, Bahl JR (1983) Cytogenetics and origin of some pulse crops. In: Swaminathan MS, Gupta PK, Sinha U (eds) Cytogenetics of crop plants. Macmillan India, New Delhi

Gupta D, Taylor PW, Inder P et al (2012a) Integration of EST-SSR markers of *Medicago truncatula* into intraspecific linkage map of lentil and identification of QTL conferring resistance to *Ascochyta* blight at seedling and pod stages. Mol Breed 30(1):429–439

Gupta M, Verma B, Kumar N et al (2012b) Construction of inter-sub-specific molecular genetic map of lentil based on ISSR, RAPD and SSR markers. J Genet 91(3):279–287

Hamdi A, Erskine W, Gate P (1991) Relationships among economic characters in lentil. Euphytica 57:109–116

Hamwieh A, Udupa SM, Choumane W (2005) A genetic linkage map of *Lens sp*. based on microsatellite and AFLP markers and the localization of *Fusarium* vascular wilt resistance. Theor Appl Genet 110(4):669–677

Harlan JR, de Wet JM (1971) Toward a rational classification of cultivated plants. Taxon 20(4): 509–517

Harper AL, Trick M, He Z et al (2016) Genome distribution of differential homoeologue contributions to leaf gene expression in bread wheat. Plant Biotechnol J 14(5):1207–1214

Havey MJ, Muehlbauer FJ (1989) Linkages between restriction fragment length, isozyme, and morphological markers in lentil. Theor Appl Genet 77(3):395–401

Heuzé V, Tran G, Noziere P et al (2014) Oats. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO

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 et al (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

Idrissi O, Houasli C, Udupa SM et al (2015) Genetic variability for root and shoot traits in a lentil (*Lens culinaris* Medik.) recombinant inbred line population and their association with drought tolerance. Euphytica 204(3):693–709

Idrissi O, Sahri A, Houasli C, Nsarellah N (2019) Breeding progress, adaptation, and stability for grain yield in Moroccan lentil improved varieties. Crop Sci 59(3):925–936

Iqbal MS, Hussain SA, Akbar M (2021) Estimating genetic variability among diverse lentil collections through novel multivariate techniques. https://doi.org/10.21203/rs.3.rs-135543/v1

Jansen M, Gilmer F, Biskup B et al (2009) Simultaneous phenotyping of leaf growth and chlorophyll fluorescence via GROWSCREEN FLUORO allows detection of stress tolerance in Arabidopsis thaliana and other rosette plants. Funct Plant Biol 36:902–914

Kamaluddin RK, Sharma V, Saini HK et al (2020) Variability studies for yield and its component traits in Lentil (*Lens culinaris* Medikus spp. *Culinaris*) under Bundelkhand region. J Pharmacogn Phytochem 9(6):1436–1441

Kaur S, Cogan NO, Pembleton LW et al (2011) Transcriptome sequencing of lentil based on second-generation technology permits large-scale unigene assembly and SSR marker discovery. BMC Genomics 12(1):1

Kaur S, Cogan NO, Stephens A et al (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

Kaur S, Webster T, Sudheesh S et al (2016) Lentil genome sequencing effort: a comprehensive platform for genomics assisted breeding

Khan MH, Tyagi SD (2005) Induced variation in quantitative traits due to physical (gamma rays), chemical (EMS) and combined mutagen treatments in soybean [*Glycine max* L.) Merrill]

Khan MH, Tyagi SD (2010) Studies on effectiveness and efficiency of gamma rays, EMS and their combination in soybean [*Glycine max* (L.) Merrill.]. J Plant Breed Crop Sci 2(3):55–58

Khazaei H, Caron CT, Fedoruk M et al (2016) Genetic diversity of cultivated lentil (Lens culinaris Medik.) and its relation to the World's agro-ecological zones. Front. Plant Sci 7:1093

Khazaei H, Fedoruk M, Caron CT et al (2018) Single nucleotide polymorphism markers associated with seed quality characteristics of cultivated lentil. Plant Genome 11(1):170051

Khazaei H, Subedi M, Nickerson M et al (2019) Seed protein of lentils: current status, progress, and food applications. Foods 8(9):391

Khorramdelazad M, Bar I, Whatmore P et al (2018) Transcriptome profiling of lentil (*Lens culinaris*) through the first 24 hours of *Ascochyta lentis* infection reveals key defence response genes. BMC Genomics 19(1):1–21

Kota R, Rudd S, Facius A et al (2003) Snipping polymorphisms from large EST collections in barley (*Hordeum vulgare* L.). Mol Genet Genomics 270(1):24–33

Kumar S (2016) Current knowledge in lentil genomics and its application for crop improvement. Crop Breed 25:331–356

Kumar S, Ali M (2006) GE interaction and its breeding implications in pulses. Botany 56:31–36

Kumar N, Chahota RK, Sood BC (2009) Component analysis for seed yield and yield traits in microsperma× macrosperma derivatives of lentil (*Lens culinaris* Medik.). Agric Sci Dig 29(3): 163–168

Kumar J, Choudhary AK, Solanki RK et al (2011) Towards marker-assisted selection in pulses: a review. Plant Breed 130(3):297–313

Kumar S, Barpete S, Kumar J et al (2013) Global lentil production: constraints and strategies. SATSA Mukhapatra 17:1–3

Kumar S, Hamwieh A, Manickavelu A et al (2014) Advances in lentil genomics. In: Legumes in the omic era. Springer, New York, pp 111–130

Kumar J, Singh J, Kanaujia R (2016) Protein content in wild and cultivated taxa of lentil (*Lens culinaris ssp. culinaris* Medikus). Indian J Genet Plant Breed 76:631–634

Kumar J, Basu PS, Gupta S et al (2018a) Physiological and molecular characterisation for high temperature stress in *Lens culinaris*. Funct Plant Biol 45(4):474–487

Kumar J, Gupta S, Biradar RS et al (2018b) Association of functional markers with flowering time in lentil. J Appl Genet 59(1):9–21

Kumar J, Gupta S, Gupta DS (2018c) Identification of QTLs for agronomic traits using association mapping in lentil. Euphytica 214(4):1–5

Kumar S, Choudhary AK, Rana KS et al (2018d) Bio-fortification potential of global wild annual lentil core collection. PLoS One 13(1):e0191122

Kumar J, Choudhary AK, Gupta DS et al (2019) Towards exploitation of adaptive traits for climate-resilient smart pulses. Int J Mol Sci 20(12):2971

Ladizinsky G (1979) The origin of lentil and its wild genepool. Euphytica 28(1):179–187

Ladizinsky G (1999) Identification of the lentil's wild genetic stock. Genet Resour Crop Evol 46(2): 115–118

Ladizinsky G, Braun D, Goshen D et al (1984) The biological species of the genus *Lens* L. Bot Gaz 145(2):253–261

Li F, Liu W, Tang J et al (2010) Rice dense and erect panicle 2 is essential for determining panicle outgrowth and elongation. Cell Res 20(7):838–849

Li Y, He L, Li J et al (2018) Genome-wide identification, characterization, and expression profiling of the legume BZR transcription factor gene family. Front Plant Sci 9:1332

Lizarazo CI, Lampi AM, Liu J et al (2015) Nutritive quality and protein production from grain legumes in a boreal climate. J Sci Food Agric 95(10):2053–2064

Lombardi M, Materne M, Cogan NO et al (2014) Assessment of genetic variation within a global collection of lentil (*Lens culinaris* Medik.) cultivars and landraces using SNP markers. BMC Genet 15(1):1

Lu Y, Hao Z, Xie C et al (2011) Large-scale screening for maize drought resistance using multiple selection criteria evaluated under water-stressed and well-watered environments. Field Crop Res 124(1):37–45

Ma Y, Marzougui A, Coyne CJ et al (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

Malhotra N, Panatu S, Singh B et al (2019) Genetic resources: collection, conservation, characterization and maintenance. In: Lentils. Academic, Cambridge, pp 21–41

Mammadov J, Aggarwal R, Buyyarapu R et al (2012) SNP markers and their impact on plant breeding. Int J Plant Genomics 2012:728398

Mane R, Katoch M, Singh M et al (2020) Identification of genomic regions associated with early plant vigour in lentil (*Lens culinaris*). J Genet 99(1):1–8

Manju P, Gopimony R (2009) Anjitha-A New Okra Variety through Induced Muta-tion in Inter-specific Hybrids of *Abelmoschus spp*. Induced plant mutations in the genomics era. Food Agric Org United Nations 1:87–90

Marko D, Briglia N, Summerer S et al (2018) High-throughput phenotyping in plant stress response: methods and potential applications to polyamine field. In: Polyamines. Humana Press, New York, NY, pp 373–388

Marzougui A, Ma Y, Zhang C et al (2019) Advanced imaging for quantitative evaluation of *Aphanomyces* root rot resistance in lentil. Front Plant Sci 10:383

Massonnet C, Vile D, Fabre J et al (2010) Probing the reproducibility of leaf growth and molecular phenotypes: a comparison of three Arabidopsis accessions cultivated in ten laboratories. Plant Physiol 152(4):2142–2157

Materne M, McNeil DL (2007) Breeding methods and achievements. In: Lentil. Springer, Dordrecht, pp 241–253

Mbasani-Mansi J, Ennami M, Briache FZ et al (2019) Characterization of genetic diversity and population structure of Moroccan lentil cultivars and landraces using molecular markers. Physiol Mol Biol Plants 25(4):965–974

Mishra SK, Sharma B, Sharma SK (2007) Genetics and cytogenetics of lentil. In: Lentil. Springer, Dordrecht, pp 187–208

Montes JM, Technow F, Dhillon BS et al (2011) High-throughput non-destructive biomass determination during early plant development in maize under field conditions. Field Crop Res 121(2):268–273

Muehlbauer FJ (1992) Use of introduced germplasm in cool-season food legume cultivar development. In: Shands HL, Weisner LE (eds) Use of plant introductions in cultivar development. Part 2. Crop Science Society of America (CSSA) special publication, vol 20. CSSA, Madison, pp 49–73

Muehlbauer FJ, McPhee KE (2005) Lentil (*Lens culinaris* Medik.). In: Singh RJ, Jauhar PP (eds) Genetic resources, chromosome engineering and crop improvement. CRC Press, Boca Raton, pp 219–230

Muehlbauer FJ, Kaiser WJ, Clement SL, Summerfield RJ (1995) Production and breeding of lentil. Adv Agron 54:283–332

Muehlbauer FJ, Cho S, Sarker A et al (2006) Application of biotechnology in breeding lentil for resistance to biotic and abiotic stress. Euphytica 147(1):149–165

Muehlbauer FJ, Mihov MA, Vandenberg A et al (2009) Improvement in developed countries. In: Erskine W, Muehlbauer FJ, Sarker A, Sharma B (eds) Lentil-botany, production and uses. CABI, Wallingford, pp 137–154

Muraya MM, Chu J, Zhao Y et al (2017) Genetic variation of growth dynamics in maize (Zea mays L.) revealed through automated non-invasive phenotyping. Plant J 89:366–380

Ogutcen E, Ramsay L, von Wettberg EB et al (2018) Capturing variation in *Lens* (Fabaceae): Development and utility of an exome capture array for lentil. Appl Plant Sci 6(7):e01165

Pandian A, Ford R, Taylor PW (2000) Transferability of sequence tagged microsatellite site (STMS) primers across four major pulses. Plant Mol Biol Report 18(4):395

Pavan S, Bardaro N, Fanelli V et al (2019) Genotyping by sequencing of cultivated lentil (*Lens culinaris* Medik.) highlights population structure in the Mediterranean gene pool associated with geographic patterns and phenotypic variables. Front Genet 10:872

Phan HT, Ellwood SR, Hane JK et al (2007) Extensive macrosynteny between *Medicago truncatula* and *Lens culinaris* ssp. *culinaris*. Theor Appl Genet 114(3):549–558

Polanco C, Sáenz de Miera LE, González AI et al (2019) Construction of a high-density interspecific (*Lens culinaris* x *L. odemensis*) genetic map based on functional markers for mapping morphological and agronomical traits, and QTLs affecting resistance to *Ascochyta* in lentil. PLoS One 14(3):e0214409

Pratap A, Gupta S, Nair RM et al (2019) Using plant phenomics to exploit the gains of genomics. Agronomy 9(3):126

Rahman MM, Sarker A, Kumar S et al (2009) Breeding for short season environments. The lentil: botany, production and uses. CAB International, Wallingford, pp 121–136

Ray H, Bett K, Tar'an B et al (2014) Mineral micronutrient content of cultivars of field pea, chickpea, common bean, and lentil grown in Saskatchewan, Canada. Crop Sci 54(4):1698–1708

Reddy A, Reddy GP (2010) Supply side constrains in production of pulses in India: case study of lentils. Agric Econ Res Rev 23:129–136

Reddy MR, Rathour R, Kumar N et al (2010) Cross-genera legume SSR markers for analysis of genetic diversity in *Lens* species. Plant Breed 129(5):514–518

Rehman RU (2017) Future prospects in polyploidy research. In: Polyploidy: recent trends and future perspectives. Springer, New Delhi, pp 101–104

Reynolds M, Langridge P (2016) Physiological breeding. Curr Opin Plant Biol 31:162–171

Rubeena TPW, Ades PK (2006) QTL mapping of resistance in lentil (*Lens culinaris ssp. culinaris*) to *Ascochyta* blight (*Ascochyta lentis*). Plant Breed 125(5):506–512

Saha GC, Sarker A, Chen W et al (2013) Inheritance and linkage map positions of genes conferring agromorphological traits in *Lens culinaris* Medik. Int J Agron 2013:618926

Sakthivel G, Jeberson S, Singh NB et al (2019) Genetic variability, correlation and path analysis in lentil germplasm (*Lens culinaris* medik). Pharma Innov J 8(6):417–420

Sankaran S, Quirós JJ, Miklas PN (2019) Unmanned aerial system and satellite-based high resolution imagery for high-throughput phenotyping in dry bean. Comput Electron Agric 165: 104965

Sari E, Bhadauria V, Ramsay L (2018) Defense responses of lentil (*Lens culinaris*) genotypes carrying non-allelic *Ascochyta* blight resistance genes to *Ascochyta lentis* infection. PLoS One 13(9):e0204124

Sarker A, Erskine W (2006) Recent progress in the ancient lentil. J Agric Sci 144(1):19–29

Satpathy S, Debnath S (2020) Genetic analysis of yield and its attributing traits in lentil. J Pharmacogn Phytochem Res 9(2):713–718

Scott MF, Ladejobi O, Amer S et al (2020) Multi-parent populations in crops: a toolbox integrating genomics and genetic mapping with breeding. Heredity 125(6):396–416

Shahwar D, Ansari MY, Choudhary S (2019) Induction of phenotypic diversity in mutagenized population of lentil (*Lens culinaris* Medik) by using heavy metal. Heliyon 5(5):e01722

Sharpe AG, Ramsay L, Sanderson LA (2013) Ancient orphan crop joins modern era: gene-based SNP discovery and mapping in lentil. BMC Genomics 14(1):1–3

Silim SN, Saxena MC, Erskine W (1993) Adaptation of lentil to the Mediterranean environment. I. Factors affecting yield under drought conditions. Exp Agric 29(1):9–19

Sinclair TR, Vadez V (2012) The future of grain legumes in cropping systems. Crop Pasture Sci 63(6):501–512

Sindhu JS, Slinkard AE, Scoles GJ (1983) Studies on variation in *Lens* I. karyotype. LENS 10(1):14

Singh DP (1991) Lentils in India–genetics and breeding of pulse crops. Kalyani Publishers, Ludhiana, pp 217–238

Singh RJ, Chung GH (2016) Landmark research for pulses improvement. Indian J Genet 76(4): 399–409

Singh AK, Pedapati A (2015) Assessment of exchange of crop in view of change climate and International Treaties. J Environ Biol 36(1):309

Singh D, Singh CK, Taunk J (2017) 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 et al (2019) Association mapping for grain diameter and weight in lentil using SSR markers. Plant Gene 20:100204

Singh D, Singh CK, Tribuvan KU et al (2020) Development, characterization, and cross species/ genera transferability of novel EST-SSR markers in lentil, with their molecular applications. Plant Mol Biol Report 8(1):114–129

Srivastava RP, Vasishtha HI (2012) Saponins and lectins of Indian chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*). Indian J Agric Biochem 25(1):44–47

Stapley J, Reger J, Feulner PG et al (2010) Adaptation genomics: the next generation. Trends Ecol Evol 25(12):705–712

Steele KA, Price AH, Shashidhar HE et al (2006) Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. Theor Appl Genet 112(2):208–221

Subedi M, Bett KE, Khazaei H et al (2018) Genetic mapping of milling quality traits in lentil (*Lens culinaris* Medik.). Plant Genome 11(2):170092

Sudheesh S, Verma P, Forster JW et al (2016) Generation and characterisation of a reference transcriptome for lentil (*Lens culinaris* Medik.). Int J Mol Sci 17(11):1887

Sun S, Li C, Paterson AH et al (2018) In-field high throughput phenotyping and cotton plant growth analysis using LiDAR. Front Plant Sci 9:16

Tadmor Y, Zamir D, Ladizinsky G (1987) Genetic mapping of an ancient translocation in the genus lens. Theor Appl Genet 73(6):883–892

Tahir M, Lindeboom N, Båga M et al (2011) Composition and correlation between major seed constituents in selected lentil (*Lens culinaris* Medik) genotypes. Can J Plant Sci 91(5):825–835

Tanyolac B, Ozatay S, Kahraman A et al (2009) Linkage mapping of Lentil (*Lens culinaris* L.) genome using recombinant inbred lines revealed by AFLP, ISSR, RAPD and some morphologic markers. J Appl Biol Sci 3(2):179–185

Temel H, Göl D, Akkale H et al (2015) Single nucleotide polymorphism discovery through Illumina-based transcriptome sequencing and mapping in lentil. Turk J Agric For 39(3): 470–488

Thavarajah P, Wejesuriya A, Rutzke M et al (2011) The potential of lentil (*Lens culinaris* L.) as a whole food for increased selenium, iron, and zinc intake: preliminary results from a 3 year study. Euphytica 180(1):123–128

Thiel T, Michalek W, Varshney R et al (2003) Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor Appl Genet 106(3):411–422

Tivoli B, Baranger A, Avila CM 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

Toker C, Yadav SS, Solanki IS (2007) Mutation breeding. In: Lentil. Springer, Dordrecht, pp 209–224

Tomlekova NB (1998) The mechanism of resistance to (*A. obtectus*) in beans (*P. vulguris* L.). Agric Sci 5:19–21

Trachsel S, Kaeppler SM, Brown KM et al (2011) Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. Plant Soil 341(1):75–87

Tullu A, Buchwaldt L, Warkentin T et al (2003) Genetics of resistance to anthracnose and identification of AFLP and RAPD markers linked to the resistance gene in PI 320937 germplasm of lentil (*Lens culinaris* Medikus). Theor Appl Genet 106(3):428–434

Tullu A, Tar'an B, Breitkreutz C et al (2006) A quantitative-trait locus for resistance to *Ascochyta* blight [*Ascochyta lentis*] maps close to a gene for resistance to anthracnose [*Colletotrichum truncatum*] in lentil. Can J Plant Pathol 28(4):588–595

Tullu A, Tar'an B, Warkentin T et al (2008) Construction of an intraspecific linkage map and QTL analysis for earliness and plant height in lentil. Crop Sci 48(6):2254–2264

Udvardi MK, Kakar K, Wandrey M et al (2007) Legume transcription factors: global regulators of plant development and response to the environment. Plant Physiol 144(2):538–549

USDA (2019) Plants database. https://plants.usda.gov

USDA-Agricultural Research Service (2015) National nutrient database standard release. USDA-ARS, Washington, DC. Available at: http://ndb.nal.usda.gov/ndb/foods/show/4782?fgcd=&manu=&lfacet=&format=&count=&max=35&offset=&sort=&qlookup=lentil

Vandenberg A, Slinkard AE (1990) Genetics of seed coat color and pattern in lentil. J Hered 81(6): 484–488

Varshney RK, Graner A, Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23(1):48–55

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(3):797–816

Vavilov NI (1949) The origin, variation, immunity and breeding of cultivated plants. Chron Bot 13: 1–54

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* M edik.) transcriptome. Plant Biotechnol J 11(7):894–905

Verma P, Goyal R, Chahota RK et al (2015) Construction of a genetic linkage map and identification of QTLs for seed weight and seed size traits in lentil (*Lens culinaris* Medik.). PLoS One 10(10):e0139666

Vijayan P, Vandenberg A, Bett KE (2009) A mixed genotype lentil EST library representing the normalized transcriptome of different seed development stages

von Wettberg EJ, Chang PL, Başdemir F et al (2018) Ecology and genomics of an important crop wild relative as a prelude to agricultural innovation. Nat Commun 9(1):1–3

Wang N, Daun JK (2006) Effects of variety and crude protein content on nutrients and anti-nutrients in lentils (*Lens culinaris*). Food Chem 95(3):493–502

Wang J, Badenhorst P, Phelan A et al (2019) Using sensors and unmanned aircraft systems for high-throughput phenotyping of biomass in perennial ryegrass breeding trials. Front Plant Sci 10: 1381

Wasson AP, Richards RA, Chatrath R et al (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J Exp Bot 63(9):3485–3498

Weller JL, Liew LC, Hecht VF et al (2012) A conserved molecular basis for photoperiod adaptation in two temperate legumes. Proc Natl Acad Sci U S A 109(51):21158–21163

Wery J, Silim SN, Knights EJ et al (1994) Screening techniques and sources of tolerance to extremes of moisture and air temperature in cool season food legumes. In: Expanding the production and use of cool season food legumes. Springer, Dordrecht, pp 439–456

Wilson VE, Law AG (1972) Natural crossing in *Lens esculenta* Moench. Am Soc Hort Sci J 97: 142–143

Wong MM, Gujaria-Verma N, Ramsay L et al (2015) Classification and characterization of species within the genus Lens using genotyping-by-sequencing (GBS). PLoS One 10(3):e0122025

Yaish MW, Saenz de Miera LE, Perez De La Vega M (2004) Isolation of a family of resistance gene analogue sequences of the nucleotide binding site (NBS) type from lens species. Genome 47(4): 650–659

Yankova V, Sovkova-Bobcheva S (2009) Studying of bean varieties (*Phaseolus vulgaris* L.) reaction to bean weevil infestation (*Acanthoscelides obtectus* Say). Ann RepBean Improv Coop 52:144–145

Ye YM, Tong J, Shi XP et al (2010) Morphological and cytological studies of diploid and colchicine-induced tetraploid lines of crape myrtle (*Lagerstroemia indica* L.). Sci Hortic 124(1):95–101

Yigezu YA, Alwang J, Rahman MW et al (2019) Is DNA fingerprinting the gold standard for estimation of adoption and impacts of improved lentil varieties? Food Policy 83:48–59

Zaccardelli M, Lupo F, Piergiovanni AR et al (2012) Characterization of Italian lentil (*Lens culinaris* Medik.) germplasm by agronomic traits, biochemical and molecular markers. Genet Resour Crop Evol 59(5):727–738

Zamir D, Ladizinsky G (1984) Genetics of allozyme variants and linkage groups in lentil. Euphytica 33(2):329–336

Zhang X, Huang C, Wu D et al (2017) High-throughput phenotyping and QTL mapping reveals the genetic architecture of maize plant growth. Plant Physiol 173(3):1554–1564

Zhu H, Choi HK, Cook DR et al (2005) Bridging model and crop legumes through comparative genomics. Plant Physiol 137(4):1189–1196

Zohary D (1972) The wild progenitor and the place of origin of the cultivated lentil: *Lens culinaris*. Econ Bot 26(4):326–332

Zohary D (1995) Lentil: *lens culinaris* (Leguminoseae–Papilionoideae). In: Evolution of crop plants. Longman Scientific & Technical, Harlow, pp 271–274

Zohary D (1996) The mode of domestication of the founder crops of the Southwest Asian agriculture and pastoralism in Eurasia. University College London Press, London, pp 142–158

Zohary D, Hopf M (1973) Domestication of pulses in the old world: legumes were companions of wheat and barley when agriculture began in the Near East. Science 182(4115):887–894