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Advancements in Lentil Genomics for Enhanced Crop Breeding: A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Lentil (*Lens culinaris* Medik) is an essential pulse crop that is widely grown for its high nutritional value, notably its high protein content, making it an important dietary component for vegetarians and vegans. Despite being the world's fifth most produced pulse, with large contributions from Canada and India, lentil production confronts obstacles such as poor productivity due to limited genetic improvement against biotic and abiotic stresses under rainfed cultivation conditions. Recent advances in lentil genetics and genomics, such as the discovery of genes related to yield, disease resistance, and nutritional content, have boosted breeding efforts to generate improved lentil

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varieties. The use of contemporary genomic techniques like molecular markers, marker-assisted selection (MAS), genomic selection (GS), and next-generation sequencing (NGS) technology has sped up the discovery of quantitative trait loci (QTLs) and the production of novel cultivars with superior agronomic characteristics. Databases such as NCBI and ENA, as well as specialized resources like KnowPulse, provide critical genomic data, while the creation of lentil genome assemblies, notably the CDC Redberry variety, has improved our understanding of lentil genetics. These resources help to solve the constraints of traditional breeding, particularly for complex characteristics impacted by genotype-environment interactions, opening the way for more robust and productive lentil varieties. Although the application of advanced tools such as genetic engineering, cisgenesis, and genome editing has moved more slowly in lentils than in other crops, their potential to improve lentil output is encouraging. Recent studies on lentil genomes, together with the creation of increased genetic resources and cutting-edge techniques, offer the ability to overcome production constraints and dramatically increase lentil production and quality throughout the world.

Keywords: Genomics; genomic selection; GWAS; lentil; molecular markers.

1. INTRODUCTION

The lentil plant, scientifically known as *Lens culinaris* Medik 2n=2x=14, is a member of the Fabaceae family. It is a pulse crop that has been cultivated for thousands of years, making it one of the oldest known crops [1]. They are a staple in many cuisines around the world and are a green alternative to animal meat protein [2]. It occupies fifth position in production worldwide among the pulses, with the production of 6.65 mt out of 5.5 mha areas; around 75% of the world's production is coming from Northern America and Asia (Fig. 1). Canada and India being the largest and second largest producers with the total production of 2.3 MT and 1.26 MT, respectively [3]. Lentils are highly nutritious, rich in protein, fiber, vitamins, minerals, and complex carbohydrates [4,5,6,7]. They are an excellent source of plant-based protein, making them an important food source for vegetarians and vegans [2,8]. Additionally, lentils are low in fat and glycemic index (GI), making them a healthy choice for those looking to maintain a balanced diet [9,10]. Although there has been an increase in the production of lentils over the period, the increase in productivity has not been realized [11]. This is due to poor genetic gain in lentil improvement [12]. However, the release of short-duration lentil varieties has gained areas under lentil cultivation in Bangladesh, Morocco, and Ethiopia [13]. Among other production constraints, lentils are being grown under rainfed conditions, and several biotic and abiotic stresses are associated with lower productivity [14,15,16,17, 18,19,20].

Advances in molecular breeding techniques, such as marker-assisted selection (MAS) and marker trait association analysis, have helped improve lentil breeding efforts. There has been a renewed interest in genetics of lentils in recent years [11,13,21]. Researchers have been studying the genetic makeup of different lentil varieties to identify genes linked with important traits such as yield, disease resistance, nutrient content, and nutrient uptake [22,23,24,25,26]. These approaches enable breeders to discover and choose desired traits and genetic factors at the molecular level, hence increasing breeding efficiency. Moreover, the availability of genomics and other datasets, such as markers and genetic maps at the publicly available databases has made it easy to understand the genetic composition of lentils [27,28]. Genomic datasets generated through next generation sequencing (NGS) technologies are enabling it further. Moreover, with the development of genetic engineering, cisgenics, and genome editing tools such as CRISPR-Cas9, researchers may now directly manipulate the lentil genome [29]. These methods have the potential to speed up the production of superior lentil varieties with increased resistance to pests, diseases, and environmental challenges, as well as greater nutritional value. With a greater understanding of lentil genetics, breeders may develop novel cultivars with higher yields and resilience, assuring the crop's viability in the face of global agricultural difficulties.

This review article aimed to delve into advancements in lentil genomics that are paving the way for targeted breeding strategies that can elevate lentil productivity, nutritional quality, and environmental resilience.

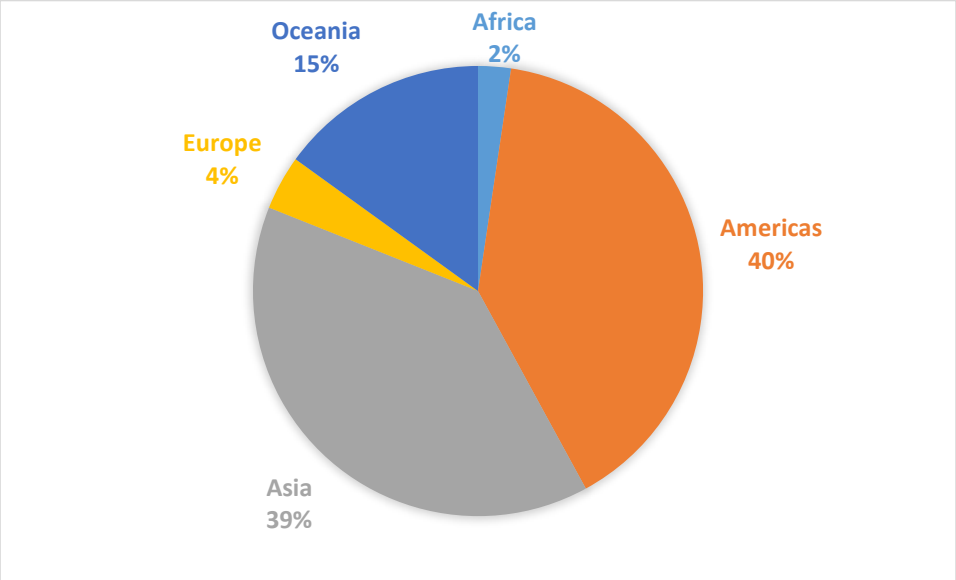

Fig. 1. Production share of lentils by different regions of the world

2. LENTIL GENOMICS: AN OVERVIEW

Improving lentil genetics is essential for enhancing crop yield [30,31,32,33,34]. Although conventional breeding strategies have been able to enhance monogenic traits by combining and selecting desirable characteristics, they are not as efficient when it comes to improving seed yield. The main reason for this is primarily the intricate nature of polygenic inheritance and the interplay between genotype and environment. Initially, the main attention has been on important characteristics such as agronomic performance, drought, heat, cold, frost tolerance, seed quality, and resistance to several fungal diseases. Nevertheless, when it comes to quantitative characteristics that are significantly affected by environmental variables and genotype-environment interactions, conventional techniques have lower accuracy and require more time [12].

Integration of genomics into the lentil improvement program is crucial for overcoming these constraints. Use of molecular markers in marker-assisted selection (MAS), marker-trait association analysis, genomic selection, and genetic engineering provide more precise and effective ways to choose superior genotypes and introduce new genetic variation and genes into the cultivated gene pool [31,35,36,37,38].

The progress in molecular markers and genome-wide association studies (GWAS) has greatly enhanced our capacity to create precise genetic maps and identify crucial quantitative trait loci (QTLs) [22,39,40]. An essential aspect of implementing these contemporary methods is accurately identifying quantitative trait loci (QTLs) and strategically integrating them into desired cultivars. NGS technology has advanced our comprehension of genetic diversity by facilitating the representative sequencing of cultivars genome over a shorter period of time through the genotyping by sequencing (GBS) approach [41,42,43]. Furthermore, the examination of the transcriptome under various situations has yielded a more profound understanding of gene expression patterns [44,45]. The progress made in this field has made it easier to identify quantitative trait loci (QTLs) and has resulted in the finding of several molecular markers, including simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) [44,45,46,47,48,49]. Apart from these, several other marker systems have also been used to assess genetic diversity and characterize *Lens* species, including inter-simple sequence repeat (ISSR), directed amplification of minisatellite DNA (DAMD), inter-primer binding site (iPBS), and sequence-related amplified polymorphism (SRAP) [50,51,52]. These markers are crucial instruments for identifying cultivars with desirable characteristics, which in turn accelerates the advancement of more robust and productive cultivars. By incorporating technologies such as MAS into breeding programs, the process of crop improvement becomes more efficient and focused, enabling the development of breeding

methods that effectively meet the requirements of modern agriculture.

Progress has also been made towards modifying the lentil genome using genetic engineering, but these developments are scarce in comparison to major crops such as rice. However, this scenario presents an opportunity to explore the use of genetic engineering as well as more efficient technologies such as cisgenesis and genome editing in directly modifying the lentils for desired traits.

3. GENOMIC RESOURCES IN LENTIL

3.1 Databases for Genes and Genomic Sequences in Lentil

There are various generic databases that cover datasets of various organisms, including lentils, and are maintained by international communities or consortia and often publicly funded. These databases, or consortia, are long-term sustainable; they work as archives for valuable data, and most of them are updated frequently, and the data stored in them can be retrieved freely. The repositories that contain datasets of lentils along with several other species include NCBI, ENA, DDBJ, Phytozome, KnowPulse, and Pulse Crop Database. The details of these databases and archives are given in Table 1.

Currently, there isn't a single database dedicated to lentils, and when we compare these databases to those for other crops such as rice and tomato, the number is significantly smaller. However, the development of new genomics datasets for lentils may lead to the inclusion of more data in new databases.

3.2 Sequencing Platform for Lentil Genomics

Rice was the first sequenced crop genome, and it paved the way for the sequencing of several other crops, including the more complex one. The rice genome was sequenced through the Sanger sequencing method following a clone-by-clone approach and was a mammoth effort of the International Rice Genome Sequencing Project (IRGSP), which started in 1997 and took about 5 years to complete, mainly due to the limited output of the Sanger sequencing technique and hardship involved [59]. The advent of next-generation sequencing (NGS) or second-generation sequencing technologies was driven by the demand for faster and more cost-effective methods to sequence vast amounts of genetic material. Over time, various NGS platforms and techniques have emerged, each employing unique chemical processes and detection strategies [60]. Despite their variations, all NGS technologies are characterized by their capacity for massively parallel processing, allowing the sequencing of thousands to millions of DNA fragments at once. This has made sequencing and re-sequencing very affordable and less time-consuming [61]. Recent advancements in sequencing technologies have resulted in the emergence of Third Generation Sequencing (TGS) technologies, which have the ability to generate substantially longer reads compared to second-generation sequencing [62]. These TGS technologies have found extensive use in genome research. Table 2 provides details on these sequencing technologies, which are also applicable to lentil genomics.

Sequence datasets generated from NGS technologies have been widely used for the creation of genetic resources such as SSRs, EST-SSRs, and SNPs [72,73]. These can be used for the development of physical maps, MAS, and GWAS. Moreover, the whole genome transcriptome analysis has resulted in the identification of genes upregulated and downregulated under various conditions or developmental stages [74]. Novel transcript isoforms, gene fusion, and splice variants can also be identified from sequenced transcriptome assembly [75,76]. Apart from these, various classes of non-coding RNAs, such as miRNAs, tRNAs, and lncRNAs, can also be detected and quantified using NGS technologies [77,78,79].

3.3 Lentil Genome Assembly

The initial version of the lentil genome assembly (the lentil genome assembly v1.0), based on the Canadian variety CDC Redberry, was released in January 2016 [80]. This assembly contains 7 pseudomolecules anchored by 6 high-density genetic linkage maps, accounting for about half of the 4.3 Gb lentil genome. The assembly was produced utilizing genomic and RNA sequencing data obtained by several institutions across the world using a variety of methods. The assembly, which includes identified potential genes, may be viewed with a genome browser (JBrowse) and accessible through the Knowpulse online portal (http://knowpulse.usask.ca) via BLAST searches.

Table 1. Databases for genes and genomic sequences in lentil

S. No.	Database	URL	Description	Species	Data Type	References
1.	NCBI	https://www.ncbi.nlm.nih.gov/	The National Center for Biotechnology Information promotes scientific and medical advancement by facilitating access to biomedical and genomic information	Lentil and other species across several kingdom	Gene, Protein, Genomic Sequences	[53]
2.	ENA	https://www.ebi.ac.uk/ena/	The European Nucleotide Archive (ENA) offers a repository of global nucleotide sequencing data, encompassing raw sequencing datasets, sequence assembly, and functional annotation	Lentil and other species across several kingdom	Gene, Protein, Genomic Sequences	[54]
3.	DDBJ	https://www.ddbj.nig.ac.jp/	The DNA Data Bank of Japan Center along with Bioinformation offers data sharing and analysis services for life science research, contributing to the advancement of scientific knowledge	Lentil and other species across several kingdom	Gene, Protein, Genomic Sequences	[55,56,57]
4.	Phytozome	https://phytozome-next.jgi.doe.gov/	Phytozome advance comparative genomics research by consolidating a vast assortment of plant genomes into one resource and performing thorough and consistent annotation and analysis, resulting in precise and informative findings	Lentil and other species	Genome Assembly and Annotation, Synteny	[27]
5	KnowPulse	https://knowpulse.usask.ca/	A website dedicated to providing comprehensive diversity data for the purpose of enhancing pulse crop development	Lentil and other legumes	Genome Assembly and Annotation	[28]
6	Pulse Crop Database	https://www.pulsedb.org/	Genome, Genetic and Breeding Recourses for Pulse crop Improvement	Lentil and other legumes	Genome Assembly and Annotation, QTL, Markers, and Traits	[58]

Table 2. Details of sequencing technologies used in genomics

Sequencing Technology Generation	Chemistry	Methodology	References
1st Generation	Chemical method (chain degradation) (Maxam and Gilbert method)	Chemical alteration of DNA followed by targeted cleavage at specific nucleotide bases	[63]
	Chain termination sequencing (Sanger method)	Targeted inclusion of labelled chain-terminating dideoxynucleotides (ddNTPs) during in vitro DNA replication	[64]
2nd Generation	Pyrosequenscing	Non-gel based DNA 'sequencing by synthesis' technique that uses light detection to detect inorganic pyrophosphate released during DNA synthesis	[65]
	Sequencing by synthesis	Incorporation of fluorescently labelled deoxyribonucleotide triphosphates (dNTPs) onto a replicating DNA strand in clusters	[66]
	Sequencing by ligation	DNA are sequenced by leveraging the mismatch sensitivity of the DNA ligase enzyme	[67,68]
3rd Generation	Single Molecule Real Time (SMRT) sequencing by the use of Zero-Mode Waveguides (ZMW)	Single Molecule Real Time (SMRT) sequencing utilizes Zero-Mode Waveguides (ZMW) technology to separate the desired fluorescent signal from the intense background fluorescence produced by unincorporated free-floating nucleotides and requires no PCR amplification	[69]
	Sequencing by Binding	By using native nucleotides and eliminating the need to remove fluorescent modifications, the sequencing strand experiences minimal alterations, leading to more accurate base calls	[70]
	Nanopore sequencing	Uses flow cells with an array of small holes, known as nanopores, embedded in an electro-resistant membrane. Each nanopore detects the electric current passing through the nanopore and identify the specific nucleotide	[71]

Utilizing the power of next-generation sequencing, the Genome Assembly v2.0 of the CDC Redberry lentil variety, which is accessible at https://knowpulse.usask.ca/bio_data/2690904, was constructed using long-read sequencing data, comprising 34x PacBio SMRT and 20x Oxford Nanopore reads [81]. The assembly's contiguity was not only validated but also enhanced with the integration of HiC data, alongside both an optical and genetic map (LR-01; ILL 1704 x CDC Robin intraspecific RIL). The completed assembly totals 3.69 Gb, organized into 7 pseudo-molecules and 2,068 unplaced unitigs.

4. USE OF LENTIL GENOMICS IN CROP IMPROVEMENT

4.1 Marker Trait Association Analysis

Conventional breeding is more effective in genetic improvement of traits with high heritability. Genetic gain of quantitative traits is low and very difficult in selection for traits governed by minor QTLs. With the advent of next-generation sequencing techniques, rapid identification of molecular markers linked with the genes, or QTLs, is possible. Higher genetic gain can be achieved for the traits governed by QTLs with small effect upon selection of traits using linked molecular markers. Genetic mapping utilizes both biparental mapping and association mapping, or genome-wide association studies (GWAS), to detect the genes or QTLs governing a trait. Biparental mapping restricts the genetic diversity between two parental lines and the limited number of recombination events. Alternatively, association mapping uses a large number of diverse parental lines; thus, it increases resolution, detects a large number of minor and major QTLs, and reduces the time spent developing mapping populations. Biparental mapping is extensively carried out in lentils for detection of QTLs of major traits including early plant vigor, heat tolerance, winter hardiness, salinity tolerance, nutritional and milling quality, disease resistance, and herbicide tolerance [35,82,83,84,85,86,87]. Grain yield remains a principal trait to breed on; thus, emphasis was given on increasing seed size of lentil. A number of genes governing seed size and weight were detected. Two QTLs for seed weight and seed size co-localized in the linkage group 4 explained phenotypic variance of 48.4% and 27.5%, respectively [88]. Breeding for these QTLs can improve simultaneously seed size and weight in lentils. Development of short-duration varieties in lentil is most suitable for the areas where the crop is grown after the harvest of rice. Delayed harvest of rice delays sowing of lentil; thus, the crop suffers heat stress and diseases appear at later stages. Growing short-duration varieties escapes the heat stress at the later growth stage and minimizes the yield loss in Indo-Gangatic plains [89]. Shivaprasad et al. (2024) detected 11 loci for extra earliness in lentil; one InDel marker (I-SP-383.9) near the *LcELF3a* gene showed 82.35% PVE (phenotypic variation explained) for earliness [46]. Biparental mapping on LG6 revealed a major flowering time locus, with one of the SSR markers, SSR212_1, closely linked to the locus, explaining 57% of the PVE [90]. Targeting these genes in breeding programs would be useful in developing early-maturing genotypes. Diseases caused significant yield loss in lentils, and the use of environmentally hazardous chemicals to control the diseases also increased the production cost. Anthracnose can cause yield losses up to 70% under favorable conditions. Major fungal diseases are ascochyta blight (*Ascochyta lentis*), stemphylium blight (*Stemphylium botryosum*), aphanomyces root rot (*Aphanomyces euteiches*), and anthracnose caused by *Colletotrichum lentis* (Damm). Breeding for disease resistance in lentils through conventional breeding approaches has achieved significant improvement. A small seeded lentil variety, “Pant Lentil 4” developed by pedigree selection, was high yielder, resistant to rust, wilt, and Ascochyta blight for North Western India [91]. A number of high-yielding varieties were developed in India, Africa, and Canada [92,93]. Genomic regions governing resistance to Ascochyta blight [94,95], stemphyllum blight [96], rust [97], anthracnose [98], and Fusarium wilt [99] were identified in Lentil. Genes conferring resistance to Ascochyta blight were detected in LG 1, 4, 5, and 9, accounting for up to 61% of PVE [94]. A major gene, LCt-2, has been designated for anthrancnose resistance [100]. Recently, Gela et al. (2021) identified major resistance loci for anthracnose on linkage groups 3 and 7, accounting for 20.1–31.2% and 8.3–18.4% of variation, respectively [101].

Association mapping, also called LD (linkage disequilibrium) mapping, was effectively used for the detection of QTLs with high resolution in lentil. LD is a non-random association of loci present at different loci on the genome. LD between a trait and marker locus indicates association of the marker with the trait phenotype. Recently, genomic tools are being utilized to unlock the genetic potential of plant

genetic resources for complex agronomic traits. Genome Wide Association Studies (GWAS) studies conducted using 96 diverse lentil genotypes detected one SSR (simple sequence repeats) PBALC 224 for seed diameter and two SSR, GLLC 614 and PBALC 29 for seed weight [102]. Lentil seed size and diameter are important parameters determining market class and price. More plump and round-shaped seeds have more efficiency of dehulling. Therefore, identification of the genomic region responsible for seed size, diameter, and plumpness in lentil is useful for improving the trait. Major genomic regions for seed diameter are reported on LG 1, 2, and 7 [103,104]. QTL clusters were detected using GWAS analysis on LG 1, 4, 5, 6, and 7 for root rot disease caused by *Aphanomyces euteiches* Drechs [24]. Utilization of global lentil germplasm to identify genotypes of important traits would assist the breeding process in realizing the fast genetic gain. One of the studies conducted using 196 ICARDA Reference Puls collection of lentils identified two flowering time loci on LG 3; eight loci for days to maturity on LG 2, 3, 5, 6, and 7; two loci for seed per pod on LG 2, and 7; and one locus on LG 1 [22]. A diverse panel of 96 lentil genotypes was used to map 24 QTLs with nine agronomic traits, including maturity, number of pods per plant, primary and secondary branches per plant, and 100 seed yield [39]. QTLs detection can be affected by the environmental variations; GWAS applied in a population evaluated under a controlled environment can be most effective in the detection of QTLs. However, conducting GWAS in a controlled environment may lead to inaccurate judgments about character expression under varying climatic conditions. Therefore, populations are tested over multiple seasons and locations. Under such circumstances, MetaGWAS has proved to be more effective than the standard to detect QTLs tested in multiple environments and having an unbalanced set of data. QTL detection through MetaGWAS analysis previously carried out in soybean [105], wheat [106], and canola [107]. Balech et al. (2024) recently used MetaGWAS analysis in lentil to detect herbicide tolerance, and four SNPs were detected to be linked with imazethapyr and metribuzin tolerance, which can be utilized in the development of herbicide tolerance lentil genotypes [35]. Biofortification is an important area of research in major food crops, including rice, wheat, maize, potatoes, and cassava. Enhancing the genetic potential of crops through which the grain mineral content increases and increasing their bioavailability is a sustainable approach to biofortification [108]. Inheritance of micronutrients is a complex trait and highly influenced by environmental fluctuation [89,108,109]. Lentil is a good source of minerals; large genetic variation for iron (Fe), zinc (Zn), and selenium (Se) was detected in the lentil germplasm [110]. Screening of lentil germplasm recorded grain iron concentrations ranging between 31.55 and 119.35 mg/kg, and that of grain Zn ranged from 7.80 to 75.45 mg/kg. Association mapping identified SSR markers PBALC 13, PBALC 206, and GLLC 563 linked with grain Fe concentration with 9% to 11% PVE and four SSR markers PBALC 353, SSR 317–1, PLC 62, and PBALC 217 linked with grain Zn concentration with 14% to 21% PVE [111]. Iron concentration in lentils is higher than in cereal crops [112]. Four QTL regions for Se concentration were identified in the LG 2 & 5, explaining phenotypic variation ranging from 6.3–16.9% [25]. For iron concentration, 21 QTLs were detected on six linkage groups (LG 1, 2, 4, 5, 6 & 7) explaining PVE of 5.9% to 14% [113]. In the recent past, twelve iron-rich biofortified lentil varieties developed at the International Center for Agricultural Research in Dry Areas (ICARDA) were released by the Harvest Plus Programme in Syria, Nepal, India, and Bangladesh [114]. Varieties IPL 220 and L4704 were released in India with 89.10 [110] and 75 mg/kg concentrations of iron, respectively. Use of molecular markers and genomic tools would be effective in developing biofortified lentil varieties.

4.2 Marker Assisted Breeding

Molecular markers are among the genomic tools used on a large scale for crop improvement programs. Molecular markers tightly linked (5 cM) to the agronomic traits, disease resistance, and quality are available in lentils suitable for marker-assisted breeding (MAB), marker-assisted backcross (MABC), marker-assisted gene pyramiding, or genomic selection. A sizable number of PCR-based makers (RAPD, SSR, SRAP, etc.) that can be detected easily with minimum cost and time are available to transfer the genes or QTLs through MAB or MABC in lentil. Tar'an et al. (2003) pyramided two genes, *ral1* and *AbR1*, to confer resistance to Ascochyta blight (*A. lentis*) and a major gene to confer resistance to anthracnose (*C. truncatum*) using RAPD markers: UBC 2271290 and RB18680 linked to *ral1* and *AbR1*, respectively, and OPO61250 linked to anthracnose resistance [115]. Successful gene introgression of anthracnose and Stemphylium blight disease

resistance was reported from wild lentil *L. ervoides* in a lentil advanced backcross population developed in the background of cultivar CDC Redberry, which can be used further as valuable genetic resources for improvement of resistance to these diseases [116]. Unlike other crops, marker-assisted breeding in lentils is limited; most of the literature is confined to the use of molecular markers for genetic diversity analysis and detection of parental lines for specific traits. Marker-assisted gene or QTL introgression or pyramiding of biotic-abiotic stress tolerance and nutritional enhancing traits is relatively higher in major field crops, particularly wheat, rice, and maize [117,118,119]. These could be attributable to the limited number of tightly linked markers (1.1 cM) identified with the target trait, the dearth of genomic data compared to the major cereals, and the less established infrastructure available for lentil breeders [120]. Ideally, the marker should be placed within the gene of interest. MAS using the linked molecular markers placed at more than 5 cM distance may lead to the detection of a false positive result. For instance, when a marker and gene are 5cM apart; then the marker prediction will wrongly predict in 5% of the progeny. Association of molecular markers with the major QTLs of agronomic traits have been detected in large number; however, lack of tightly linked markers with the QTLs limits their utilization in lentil improvement [13]. Recently, genomic information in the pulses has increased significantly, which would lead to the detection of candidate genes and their fine mapping and marker-assisted selection.

4.3 Genomic Selection

MAS is the most effective method in plant breeding for selecting desirable plant types and making changes in phenotypes through the transfer of known genetic variations. However, economically important traits like yield, disease resistance, abiotic stress tolerance, nutrient use efficiency, and quality are governed by many genes, each accounting for a minor percentage of phenotypic variations. Selection of plants for minor QTLs in breeding programs is difficult; thus, it limits the MAS for QTLs with small effects. Under such circumstances, genomic selection (GS) based on genomic estimated breeding values (GEBVs) plays a significant role, where genome-wide markers are used to estimate the genomic potential of an individual genotype. GS involves developing genomic prediction equations using phenotyping and genotyping data of the training population, which is then used for predicting the GEBV of individual populations of a testing population that have not been phenotyped [121]. GS is more effective for the traits with low heritability, given more gain per unit time than the phenotypic selection and MAS [31]. GS can increase the higher genetic gain per unit time by phenotypic selection, shortening the generation interval in lentils [122]. Genetic gain was higher from selecting plants in early generations (F_1 or F_2) than the later stages of segregating generations. During early generation selection, GS led to the loss of genetic diversity; however, the addition of additional phenotypes in F_2 families to the training populations can increase the GEBVs, genetic gain per unit time, and decrease the rate of genetic diversity loss. Genomic prediction (GP) accuracy depends on several factors, including GxE interactions, inheritance of traits, and prediction models, which are important factors to be considered. The advantage of GS is that it accounts for GxE interactions effectively, thus allowing in selecting genotypes for the untested environments. GP methods like ridge regression, Bayesian LASSO, BayesA, BayesB, kernel-based approaches, and genomic BLUP models have been used. Predication accuracy of GEBVs was recorded higher (0.34-0.83) than the BLUP estimated breeding values (EBVs) (0.22-0.54) in lentil [31]. Moderate to high prediction accuracy was observed for grain yield (0.47-0.57), Ascochyta blight (0.45-0.64), Botrytis grey mold (0.63), boron tolerance (0.47 to 0.72), and salt tolerance (0.39 to 0.52) [31]. Moderately higher prediction accuracy indicated the effectiveness of GS in improving these traits in lentil. Another study reported that BayesB has the highest prediction accuracy for traits controlled by few QTLs with relatively large effects, while incorporation of genotype-environment interactions improved prediction accuracy by up to 66% [123]. Moderate to high prediction accuracy within population (range of 0.36–0.85) and across-environment (range of 0.19–0.89), which were higher than the across-population prediction suggesting implementation of GS in lentil to predict both within population and across the environment.

5. GENETIC ENGINEERING AND GENOME EDITING

The transgenic approach has facilitated the transfer of useful genes across the gene pool in lentils through transformation. The introduction of new genes has been generally done through

particle bombardment and the *Agrobacterium tumefaciens* infection method. With the advancement of sequencing techniques, adequate information about the whole genome sequence is available in sequence databases, which can be used to develop transgenic plants to improve the lentil and to decipher the function of lentil genes either through overexpression or suppression of genes through RNAi approaches. Report is available for transformation of lentil via a number of explants, viz., shoot apices, epicotyl, root, cotyledons, and cotyledonary nodes [124]. The particle bombardment technique was used to produce first transient and stable expression in cotyledonary tissues [125]. An herbicide resistant lentil was developed using *Agrobacterium* mediated transformation containing the bar gene as a selectable marker [126]. Lentil was also transformed with the DREBA gene (derived by *rd29A* promoter) into lentil for enhancing drought and salinity tolerance [38]. A study reported the transformation of two microsperma seeded lentil varieties namely, Bari Masur-4 and Bari Masur-5 in Bangladesh using *A. tumefaciens* strain LBA4404 [127]. The development of lentil transgenic plants can also benefit from the recent advancement in next generation sequencing technologies with the identification of putative genes for traits such as drought tolerance [44,128], heat tolerance [45,129,130], cold acclimation [131], disease resistance [132,133], and agronomical traits [134] through transcriptomic analysis. Apart from these genes, functionally characterized and validated genes from several heterologous systems, such as WRKY transcription factor genes [135,136], NAC transcription factor genes [137], and DREB transcription factors genes [138], may also be used for abiotic and biotic stress tolerance.

Though, genetic engineering has been a very useful technique to introduce the useful genes across the kingdom to targeted crops, there have been several disadvantages to this approach, like the insertion of transgenes into the undesired location in the genome, leading to disruption of some functional genes; the insertion of multiple copies of the transgenes leading to gene silencing; and concern related to horizontal gene flow [139,140,141]. These concerns have prompted the utilization of alternative technologies like cisgenesis and genome editing [142]. Cisgenic plants are produced by the introduction of a natural gene from a crossable or sexually compatible plant along with their native promoter and terminator in the normalsense orientation. As cisgenic plants do not harbor any transgene, they lower some of the concern associated with transgenic plants [142]. However, on the issue of safety, regulators could treat cisgenic similer to the transgenic depending on the regulations. Though, cisgenesis have not been reported in lentil till now, cisgenesis may be exploited to improve lentil for various traits.

Genome editing is an excellent choice for precise modification of a plant genome as it allows targeting specific locations within the genome to add, remove, or alter genetic material with high accuracy, significantly advancing plant breeding compared to other methods [143,144]. Genome editing relies on the use of site-directed nucleases (SDNs) for recognizing specific DNA sequences and producing double-stranded DNA breaks (DSBs) at targeted sites. Meganucleases (MN), Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated proteins (CRISPR/Cas) are the various site-directed nucleases that are used for targeted DNA breaks [145,146,147,148,149]. However, CRISPER/Cas is the most used site-directed nucleases in comparison to meganucleases, zinc finger nucleases, and TALENs because it is simple and cheaper [150]. Though genome editing has been used towards improvement of various crops, there is no report available for lentil. However, genome editing may be an efficient choice to improve the lentils for various agronomical traits and mitigation of various biotic and abiotic stresses.

6. CONCLUSION

Recent breakthroughs in lentil genomics have greatly contributed to overcoming the constraints of traditional breeding approaches, notably in terms of poor yields, vulnerability to stress, and the problems imposed by rainfed agriculture. The use of contemporary genomic technologies, including molecular markers and next-generation sequencing, has sped the discovery of critical genes and quantitative trait loci (QTLs) linked to yield, disease resistance, and nutritional value. These developments have enabled the generation of superior lentil varieties with improved agronomic features by marker-assisted and genomic selection, resulting in increased worldwide lentil output and quality. Furthermore, we propose that sophisticated crop improvement methods, such as genetic engineering, cisgenesis, and genome editing, might be used to enhance lentil breeding in the future.

7. FUTURE PROSPECTUS

Lentil, being one of the important pulse crops in Asia and America, requires more attention in developing varieties with high yield potential, nutritional qualities, and climate resilience. Significant progress has been made in genomic research in lentil. The availability of various datasets in the large genomic databases has made faster progress in genetic improvement. Future opportunities for lentil research include increased examination of genomic resources to better understand the genetic basis of complex features like drought tolerance and nutrient efficiency. In the context of improving lentil genotypes for complex traits, this information will fasten the realized genetic gain in breeding programs. The use of gene editing technologies like CRISPR/Cas has the potential to produce lentil varieties with customized features by precisely modifying certain genes. Furthermore, incorporating phenomics, bioinformatics, and machine learning into breeding projects may result in more efficient selection procedures and the creation of climate-resilient lentil varieties. As genetic data becomes more abundant, worldwide collaboration and data sharing will be critical for driving lentil development and guaranteeing food security in the face of global problems like climate change, population expansion, and malnutrition.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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