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REVIEW

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Genomics-assisted lentil breeding: Current status and future strategies

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Abstract

Genomics-assisted breeding has become a powerful tool to develop high-yielding climate-resilient varieties for adaptation to adverse environmental conditions such as heat and drought stresses. Previously, efforts have been made to develop genomic resources in lentil, leading to the development of many trait-specific mapping populations, cores and mini-cores, and single nucleotide polymorphism and simple sequence repeat markers. Molecular markers have been used in genetic diversity analyses and to clarify genetic relationships in lentil. However, availability of cost-effective next-generation sequencing and genotyping-by-sequencing technologies has provided unprecedented opportunities for advancing genetics research and breeding applications. For instance, it has become possible to assemble the large and complex genome, develop high-density genetic maps for high-resolution QTL mapping, and deploy genome-wide association study in lentil. Furthermore, a range of cost-effective marker genotyping platforms have been developed. These developments offer ample opportunities to modernize current breeding programs in lentil for accelerating genetic gains. This review discusses the current status and future possibilities of genomics-assisted breeding to develop and deploy lentil cultivars suitable for changing climatic conditions.

KEYWORDS

genetic gain, genetic/genomic resources, genotyping platform, lentil, linkage maps, product profile, QTLs/genes

1 | INTRODUCTION

Lentil (*Lens culinaris* Medik.) is a self-pollinated legume crop belonging to family Fabaceae with a diploid chromosome number of $2n = 2X = 14$ and genome size of ~4 Gbp (Ogutcen, Ramsay, von Wettberg, & Bett, 2018). It is cultivated worldwide on water-limited areas as a cool weather-loving crop. More than 52 countries grow this crop, which has 6.10 Mha area and produces 6.33 Mt of grains with an average productivity of 1038 kg/ha in the world (FAOSTAT, 2018). It is a rich source of dietary proteins, fiber, prebiotic carbohydrates, minerals, and vitamins (Kumar et al., 2016). Iron, zinc, and folate are some important ingredients that are naturally present in enough quantity in lentil grains. Therefore, it plays an important role in overcoming the problem of malnutrition and micronutrient deficiencies among the inhabitants of developing countries especially those who cannot afford costly animal protein-based diets (Kumar et al., 2016). In addition to this, legume consumption has been suggested as a remedy for several chronic diseases including diabetes, obesity, and

cardiovascular problems (Srivastava & Vasishtha, 2012). Therefore, health conscious people have shown a preference for plant protein-based diets. As a result, the lentil crop has experienced higher *per capita* consumption, with a global area expansion from 1.66 to 6.10 Mha (3.7 times) and production from 1.0 to 6.33 Mt in the past five decades (FAOSTAT, 2018). Moreover, inclusion of lentil in cereal-based cropping systems helps improve soil health by enhancing net carbon sequestration leading to a lower carbon footprint as reported in a recent study that the lentil–wheat cropping system produced the lowest carbon footprint at −552 kg CO_2 eq/ha compared to other cropping systems (Gan et al., 2014). Lentil like other legumes has the ability to symbiotically fix nitrogen, thereby improving soil health. When integrated in cereal-based systems, it acts as a break for the proliferation of weeds, diseases, and insect pests in cereal crops and helps to improve the livelihood of smallholder farmers in dry land areas of developing countries (Kumar et al., 2013).

Conventional plant breeding efforts along with evolution of management practices have significantly increased lentil productivity from 605 to 1038 kg/ha in the last five decades (FAOSTAT, 2018). Past breeding efforts have had a positive impact on the production and productivity of lentil globally. Productivity gains have increased when considered with the markedly reduced crop duration of current varieties. This has enhanced their per day productivity relative to other rainfed crops, and they can be introduced into new niches and incorporated into existing cropping systems. For example, the adoption of short-duration lentil varieties, such as BARIMasur-3, BARIMasur-4, BARIMasur-5, BARIMasur-6, BARIMasur-7, and BARIMasur-8, has improved productivity in Bangladesh. A recent fingerprinting study showed that 99% of the lentil area in Bangladesh is now under improved varieties (Yigezu et al., 2019), delivering an additional 55,000 t of lentil, valued at US$ 38 million annually. A genetic trend study on lentil in Morocco reported yield gains of 35 kg/ha/year from 1989 to 2018 (Idrissi et al., 2019), with the yield advantage of improved varieties over the local check increasing from 16% to 67%. In Ethiopia, 11 lentil varieties released between 1980 and 2010 had an estimated yield gain of 18–28 kg/ha/year in a two-location study (Bogale et al., 2015). However, the current annual genetic gains of <0.7% reported will not meet the growing demands of lentil. This is due to the fact that the current average productivity of lentil in developing countries like India is still low compared to the average productivity of 2 t per ha realized in some countries (FAOSTAT, 2018). Among many production constraints, cultivation of lentil as a rainfed crop under difficult edaphic conditions compounded with biotic (ascochyta blight, fusarium wilt, anthracnose, stemphylium blight, rust, white mold, collar and root rots, *Orobanche* weeds) and abiotic (terminal drought, cold, frost, and intermittent heat) stresses during growth and development has been identified as serious yield constraint (Kumar et al., 2015; Sharpe et al., 2013). Moreover, environmental conditions and their interaction with genotypes strongly impact the expression of quantitatively inherited complex traits leading to poor genetic gain in lentil through conventional breeding approaches (Kumar & Ali, 2006). Now, it has become more challenging to breed climate-resilient cultivars under changing climatic conditions. Thus, the conventional selection–recombination–selection approach of breeding is more time consuming and less precise for manipulating agronomically useful quantitatively inherited complex traits in lentil (Kumar et al., 2015). Recently, genomics-assisted breeding has become a potential way to increase the genetic gain in lentil by identifying, fixing, and selecting superior alleles with more precision and rapidity in breeding populations (Kumar et al., 2015). Therefore, there is a need to integrate genomics in lentil breeding for developing climate-resilient and highly productive cultivars.

In the recent past, increasing effort has been given to developing genomic resources in lentil including the development of single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers, genome sequences, transcriptome sequences, and genes/QTLs controlling important traits (Bett et al., 2014; Kumar, Basu, et al., 2018; Kumar, Gupta, Biradar, et al., 2018; Kumar, Gupta, Gupta, et al., 2018; Kumar et al., 2015; D. Singh et al., 2017; D. Singh et al., 2019). Next-generation sequencing (NGS) technologies have helped to accelerate the development of genomic resources rapidly and cost effectively (D. Singh et al., 2017; D. Singh et al., 2019). NGS has led to the development an initial draft of 23× coverage, which further enriched with additional 125× coverage (University of Saskatchewan 2016, https://knowpulse.usask.ca/lentil-genome). NGS technologies have been instrumental in changing the status of legumes from orphan to rich with respect to genomic resources (Varshney et al., 2013; Varshney et al., 2015) and have been used widely for different purposes in legumes such as chickpea, pigeonpea, common bean, cowpea, and groundnut (Afzal et al., 2020; Bauchet et al., 2019; Varshney et al., 2019). Although genomic resources are available in lentil for integration in lentil breeding and used widely for genetic diversity/relationship studies (Dissanayake et al., 2020; Khazaei et al., 2016; Kumar et al., 2015; Lombardi et al., 2014; Wong et al., 2015), limited efforts have been made in current breeding pipelines as compared to other food legumes like chickpea and pigeonpea (Roorkiwal et al., 2020). Thus, genomic tools and technologies have opened new avenues for deploying genomic resources in lentil breeding programs. This review has been made to assess the current development and future opportunities for genomics-assisted breeding in lentil.

2 | GENETIC RESOURCES FOR GENOMICS-ASSISTED BREEDING

Availability of diverse genetic resources is the foremost requirement for developing improved cultivars with superior performance in target environments. Mining of useful alleles from the existing genetic diversity stored in genebanks is vital for making genetic improvement and for diversifying the cultivated gene pools. Different genebanks around the globe hold 58,405 accessions of lentils (Khazaei et al., 2016). ICARDA holds 13,907 accessions of cultivated species and 603 accessions of wild *Lens* species (R. J. Singh & Chung, 2016). ICAR-National Bureau of Plant Genetic Resources (NBPGR) of India has 2655 accessions including 2083 indigenous and 572 exotic accessions (R. J. Singh & Chung, 2016). The second largest holding of lentil

germplasm is with the Australian Temperate Field Crops Collection having 5254 accessions, followed by 3000 accessions by the Seed and Plant Improvement Institute of Iran and 2875 accessions by the USDA (Malhotra et al., 2019). These collections also include a sizable number of crop wild relatives (CWR), which are excellent sources of novel traits/alleles due to their historical record of adaptation to a diverse range of habitats.

Parts of these germplasm collections have been characterized for morphological, phenological, agronomic, and nutritional traits in addition to biotic and abiotic stresses (D. Singh et al., 2016; Gorim & Vandenberg, 2017; M. Singh et al., 2014; M. Singh et al., 2020; Malhotra et al., 2019). To facilitate accessibility and better use of the germplasm available within genebanks, efforts have been made to develop core and FIGS (Focused Identification of Germplasm Strategy) sets in order to evaluate the representative diversity easily for useful traits and make it accessible for users. A core set with 287 accessions developed by the USDA based on country origin was evaluated for agro-morphological traits by Tullu et al. (2001). The FIGS strategy is being pursued at ICARDA using robust geographical datasets, which has proven successful for various adaptive traits such as tolerance to heat, drought, and resistance to diseases in lentil. Recently, screening of a FIGS set of 162 lentil accessions resulted in identification of heat -and drought-tolerant germplasm (El haddad et al., 2020).

CWRs offer scope for widening the genetic base of lentils as they allow recovery of alleles left behind in the process of domestication. A set of 405 accessions belonging to the wild *Lens* species (*L. culinaris* ssp. *culinaris*, *L. culinaris* ssp. *orientalis*, *L. culinaris* ssp. *odemensis*, *L. culinaris* ssp. *tomentosus*, *L. nigricans*, *L. ervoides*, and *L. lamottei*) was evaluated for agronomic traits and disease reactions, resulting in identification of sources for useful traits (M. Singh et al., 2014). This collection was further used to develop a core set of 96 accessions comprising all above mentioned *Lens* species, which were validated for nutritional and key biotic/abiotic traits (Kumar, Choudhary, et al., 2018; M. Singh et al., 2020). The genetic diversity available within wild and cultivated lentil germplasm has been assessed for nutrition uptake and concentration (Se, Fe, and Zn), biotic and abiotic stresses, and agronomic traits (Bhadauria, Vijayan, et al., 2017; Gorim & Vandenberg, 2017; Khazaei et al., 2017; Kumar, Basu, et al., 2018, Kumar, Gupta, Biradar, et al., 2018; Kumar, Gupta, Gupta, et al., 2018; Kumar, Gupta, Dubey, et al., 2018; Thavarajah et al., 2017) and used for identification of QTLs/genes controlling important traits using biparental and association mappings (Bhadauria, Ramsay, et al., 2017; Khazaei et al., 2017; Kumar, Choudhary, et al., 2018; Kumar, Gupta, Biradar, et al., 2018; Polanco et al., 2019). Rapid and precise screening of germplasm can accelerate its use in lentil breeding programs. For this, there is a need to develop the imaging-based advanced phenotyping techniques for identification of useful accessions for target traits (resistance/tolerance to drought, heat, wilt, rust, etc.). Recently, advanced imaging-based phenotyping was used to identify resistant genotypes through quantitative evaluation of *Aphanomyces* root rots (Marzougui et al., 2019).

One way of harnessing the genetic potential of different accessions carrying different traits of agronomic interest is the development of breeding populations from multiple parents including wild relatives. In these populations, allelic diversity increased due to combining different alleles of genes from diverse parents leading to the availability of novel recombinants in the populations (Huang et al., 2012; Scott et al., 2020). Such populations form a broad genetic base due to increased genetic diversity that provides an opportunity for selection of useful recombinants in advanced generations and mapping of useful QTLs for multiple traits (see Varshney et al., 2019; von Wettberg et al., 2018). Multi-parent nested association mapping (NAM) and multi-parent advanced generation intercross (MAGIC) populations have been developed in chickpea by making inter-crosses between multiple (4, 8, or 16) parental lines of diverse origin (Roorkiwal et al., 2020). In lentil, efforts are underway at ICARDA for the development of a MAGIC population with eight parents of diverse origin. These multi-parent populations could be outstanding genetic resources for gene identification, isolation, and transfer of key candidate genes and development of widely adapted climate-resilient lentil cultivars.

3 | ACCELERATION IN THE DEVELOPMENT OF GENOMIC RESOURCES FOR ELEVATING LENTIL FROM ORPHAN STATUS

3.1 | Molecular markers (from hybridization based to sequence based)

Molecular markers are required to establish locus association with traits of breeders' interest. These molecular markers have been classified on the basis of timeline (past–present–future), generation of datapoints per single run (high and low throughput), and technique used (hybridization–PCR–sequencing) by several workers (Kumar, Choudhary, et al., 2019; Kumar et al., 2015; Mir et al., 2012; Mir, Bhat, et al., 2013; Mir, Hiremath, et al., 2013; Mir & Varshney, 2012). Molecular markers are discussed here based on techniques used in lentil. The first hybridization-based RFLP markers were used to develop linkage maps in lentil (Eujayl et al., 1998; Havey & Muehlbauer, 1989). Subsequently, the use of RFLP markers remained limited in lentil because it required high technical skills for their development. The advent of markers based on PCR (random amplified polymorphic DNA [RAPD], sequence characterized amplified region [SCAR], and SSR) has accelerated their use widely in lentil breeding programs (Kumar et al., 2015), and they are still in use (A. Singh et al., 2016; Ates, Aldemir, Alsaleh, et al., 2018; D. Singh et al., 2016; D. Singh et al., 2019; Gupta et al., 2016; Kumar, Choudhary, et al., 2018; Kumar, Gupta, Biradar, et al., 2018; Mbasani-Mansi et al., 2019; Polanco et al., 2019; Tsanakas et al., 2018). In lentil, a set of 122 genomic SSR markers were developed for utilization in breeding programs (Verma et al., 2014). In recent years, development of PCR-based genic-SSR markers has become faster and more cost effective through transcriptome analysis following NGS (Kaur et al., 2011; D. Singh et al., 2017; D. Singh et al., 2019).

In recent years, SNP markers have become more popular than PCR-amplified markers. SNPs are found in large number across legume genomes (Chagné et al., 2007), and different strategies have been used to identify and validate them (Mammadov et al., 2012). Initially, Sanger sequencing technology was used to re-sequence unigene-derived amplicons, or expressed sequence tag (EST) data were used for discovering gene-based SNPs that subsequently were validated by converting them to PCR-based markers (Batley et al., 2003; Wright et al., 2005). For example, in lentil, kompetitive allele specific PCR (KASP) methodology has been used to detect SNPs identified using available EST data (Fedoruk et al., 2013; Sharpe et al., 2013). NGS technologies have made feasible rapid and cost-effective mining of SNPs for mapping genes and QTL in lentil (Kaur et al., 2011; Sharpe et al., 2013). In a recent study, data generated using Illumina Genome Analyzer yielded ~44,879 SNP markers in lentil (Sharpe et al., 2013). Another study discovered a set of 50,960 SNPs, which were used to construct a high-density linkage map in lentil (Temel et al., 2014). Thus, availability of high-density SNP markers promoted the development of Illumina GoldenGate (GG) platforms having >1000 SNPs for genotyping in lentil (Kaur et al., 2014; Sharpe et al., 2013). However, during the past few years, transcriptome analysis using NGS platforms has provided a greater number of SNPs from coding regions of the lentil genome (Kaur et al., 2014; Sharpe et al., 2013; D. Singh et al., 2017; D. Singh et al., 2019). These SNPs have been used as makers to develop linkage maps and to identify genetic diversity as well as their association with traits of breeder interest (Ates, Aldemir, Alsaleh, et al., 2018; Ates, Aldemir, Yagmur, et al., 2018; Khazaei et al., 2017; Khazaei et al., 2018; Lombardi et al., 2014; Pavan et al., 2019; Sudheesh, Rodda, et al., 2016). In a recent study, 6693 SNPs were detected through genotyping by sequencing (GBS) in lentil that differentiated the Mediterranean gene pool according to geographical patterns and phenotypic traits. This study also identified the routes of introduction of lentil cultivation in Mediterranean countries after domestication and showed that selection activities are responsible for further shaping the population structure (Pavan et al., 2019).

3.2 | EST and contigs/unigenes/transcripts

Conventional (i.e., Sanger Sequencing) and advanced NGS technologies have provided availability of nucleotide sequences of coding region expressed in different tissues and plant growth stages. These sequences have provided opportunities to develop the functional or genic markers including SSR, SNP, and intron-targeted polymorphism (ITP) markers (Kumar et al., 2015). Initially, conventional sequencing was used to sequence 150–400 bp cDNA clones corresponding to mRNAs expressed in a particular tissue and/or stage of crop plants leading to the development of ESTs. Development of ESTs has been further accelerated by using Serial Analysis of Gene Expression (SAGE). For example, ESTs were identified for an amino oxidase gene in lentil (Rossi et al., 1992). The EST library was generated from eight cultivars having diverse seed phenotypes (Vijayan et al., 2009). One of the studies generated 5000 ESTs in lentil from leaflet tissues infected with *Colletotrichum truncatum* (Bhadauria et al., 2011). Currently, 33,371 ESTs are publicly available for lentil (http://www.ncbi.nlm.nih.gov/nucest/?term=lentilNCBI, June 2020). EST databases are useful resources for the development of SSR and SNP markers using different bioinformatics tools like MISA and Snipper besides their use in development of CAP and RFLP markers (Kota et al., 2003; Thiel et al., 2003; Varshney et al., 2005).

NGS-based RNA or transcriptome sequencing has further supported the generation of ESTs and unigenes/transcripts in lentil, which were used to identify SSR and SNP markers. For example, 1.38×10^6 ESTs were generated from tissue-specific cDNA of six genotypes in lentil and identified 15,354 contigs and 68,715 singletons through *de novo* assembly (Kaur et al., 2011). This resource was used for identification of 25,592 unigenes and 2,393 EST-SSR markers. Validation of a subset of 192 EST-SSR markers from this set resulted in 47.5% polymorphism across a panel of 12 cultivated genotypes in lentil (Kaur et al., 2011). Further, deep and diverse transcriptome resources for lentil have been developed from wild and cultivated accessions using 454 pyrosequencing leading to the development of 1.03×10^6 ESTs (Sharpe et al., 2013). This provided a base assembly consisting of 50,146 contigs, which further produced a reference assembly of 27,921 contigs after filtering based on duplication, overlap, and size. These transcriptome resources led to identification of 44,879 SNPs. Among these, a subset comprising 1536 SNPs was used to develop a high-throughput GG array platform for genotyping SNPs in lentil, and the array was used to construct an SNP-based genetic map of *L. culinaris* mapping population (Sharpe et al., 2013). In another study, use of 119,855,798 short reads generated by Illumina paired-end sequencing resulted in development of 20,009 non-redundant transcripts through *de novo* transcriptome assembly. These transcripts helped to generate 5673 SSR markers that were used in diversity analyses (Verma et al., 2013). The Illumina platform was used for transcriptome sequencing of two lentil cultivars, Precoz and WA8649041, and generated 111,105,153 ESTs that assembled into 97,528 high-quality contigs (Temel et al., 2015). These contigs were used to develop 50,960 SNP markers that were used to generate a linkage map. Transcriptome analysis of drought-tolerant and susceptible genotypes generated on average 58,621,121 reads across four treatments and *de novo* assembling of these reads led to development of 77,346 contigs in lentil (D. Singh et al., 2017). SSR (9949) and SNP (8260) markers were also developed from these contigs, and a set of 50 EST-SSR markers were used subsequently to determine genetic diversity among 234 genotypes and transferability across the lentil species and 12 genera of legumes (D. Singh et al., 2020). GBS analysis of 188 lentil genotypes generated about 467 million read pairs that were used to identify 410,637 SNPs using the Universal Network Enabled Analysis Kit bioinformatics pipeline for genetic diversity analysis (Pavan et al., 2019). *De novo* transcriptome analysis of heat-tolerant and heat-sensitive genotypes generated on average 26,165,023 reads and 96,824 contigs across 12 samples in lentil (D. Singh et al., 2019). From these genomic resources, 194,178 SNPs, 141,050 SSRs, and 7388 insertion/deletions were detected

(D. Singh et al., 2019). More recently, 26,449 EST-SSR and 130,073 SNP markers were developed in lentil through RNAseq analysis. Among these, 276 EST SSR markers were evaluated across 94 accessions of lentil, whereas 78 SNPs were converted to PCR-based KASP markers (Wang et al., 2020). These genomic resources are summarized in Table 1.

3.3 | Progress toward the development of trait-specific recombinant inbred line mapping populations

Quantitative traits of agronomic importance are under the control of many minor genes and highly influenced by the environments. Therefore, genetic dissection of these traits requires growth over multiple years and locations in order to identify genes/QTLs interacting with environments (Kumar et al., 2011; Kumar, Gupta, Biradar, et al., 2018). Therefore, attention has been given to develop the mapping populations, and considerable progress has been made worldwide to develop trait-specific RILs for key traits (i.e., earliness, early growth vigor, root traits, drought, Fe and Zn) in lentil, which have been reviewed earlier by Kumar et al. (2015). Further development of RILs has been focused on other traits including quality and disease resistance. The recombinant inbred line (RIL) populations have been developed from three biparental crosses ([CDC Redberry × ILL7502], [ILL8006 × CDC Milestone], and [PI320937 × Eston]) for quality traits and used to construct linkage maps and identify the QTLs for Fe, Mn, and Se uptake (Aldemir et al., 2017; Ates et al., 2016; Ates, Aldemir, Alsaleh, et al., 2018; Ates, Aldemir, Yagmur, et al., 2018). A mapping population composed of 189 RILs has been developed from a cross between partial resistant and susceptible breeding lines for genetic dissection of *Aphanomyces* root rot resistance in lentil (Ma et al., 2020). Intraspecific mapping populations in general have low genetic diversity, and only a limited number of markers can be incorporated to genetic maps (Bohra et al., 2012). Therefore, one interspecific RIL population has also been developed from a cross between *L. culinaris* cultivar Alpo and *L. odemensis* accession ILWL235. This population has been used to identify the association between functional markers and morphological and agronomical traits and resistance to *Ascochyta* (Polanco et al., 2019). Currently, this population is in the BC_2F_5 generation. Wild species may carry favorable alleles of genes for yield and traits related to biotic and abiotic resistance that may be hidden due to linkage with unfavorable genes (Tanksley & Nelson, 1996). Therefore, efforts have been made to develop backcross inbred line (BIL) populations to identify favorable genes for yield traits. This mapping population was developed from a cross between a cultivar IPL 220 and an accession ILWL 118 belonging to wild species (*L. orientalis*) (Kumar, Gupta, et al., 2019).

3.4 | Development of sequenced-based high-density linkage maps

Previously, several genetic linkage maps have been developed using biparental inter- and intraspecific mapping populations in lentil, and a comprehensive list of these linkage maps has been discussed in our earlier reviews (Kumar, Kumar, et al., 2019; Kumar et al., 2015). The first genetic linkage map using molecular markers was constructed in 1989 (Havey & Muehlbauer, 1989). Subsequently, several genetic linkage maps using RAPD, AFLP, RFLP, SSR, ITAP gene-based, and morphological markers have been developed (Andeden et al., 2013; de la Vega et al., 2011; Duran et al., 2004; Eujayl et al., 1998; Gupta, Taylor, et al., 2012; Gupta, Verma, et al., 2012; Hamwieh et al., 2005; Phan et al., 2007; Rubeena et al., 2003, 2006; Verma et al., 2015). However, NGS has made feasible the availability of SNP markers in large numbers in lentil, which were used in the development of high-density linkage maps (Table 2). First, these markers were integrated

TABLE 1 A list of select genomic resources (ESTs, contigs, unigenes, and transcripts) in lentil

Sequencing platform/ chemistry	Plant parts or stage used	Reads/ESTs	Contigs/nonredundant transcripts/unigenes	Reference
GS-FLX Titanium shotgun chemistry	Leaf, stem, flowers, immature pods, mature pods, immature seeds, root germinants, and shoot germinants	1,380,000	25,592	Kaur et al., 2011
454 Titanium chemistry	2-week-old leaf, stem before flowering, 1-week-old etiolated seedling, mixed flower stages, and developing seed at mixed stages	1,030,000	27,921	Sharpe et al., 2013
Illumina paired-end sequencing	Seedlings, leaf and root, tissue samples	119,855,798	20,009	Verma et al., 2013
Illumina paired-end sequencing	Roots, shoots, leaves, branches, and flowers	111,105,153	97,528	Temel et al., 2015
Illumina paired-end sequencing	Seedlings	58,621,121	77,346	D. Singh et al., 2017
Illumina paired-end sequencing	Young leaf	46,700,000	-	Pavan et al., 2019
Illumina paired-end sequencing	Seedlings	26,165,023	96,824	A. Singh et al., 2019

TABLE 2 Available SNP-based genetic linkage maps in lentil

Cross name	Population size	Total map distance covered (cM)	No. of SNP loci	Average distance between markers (cM)	References
DC Robin × 964a-46	144	834.7	542 (including six SSR markers)	1.54	Sharpe et al., 2013
Precoz × WA 8649041	103	432.8	388	1.11	Temel et al., 2014
Indianhead × Northfield; Indianhead × Digger; Northfield × Digger	117, 112, 114	2429.6	689	3.5 cM	Sudheesh, Rodda, et al., 2016
L01-827A (*L. ervoides*) × IG 72815 (*L. ervoides*)	94	740.9	543	1.36	Bhadauria, Vijayan, et al., 2017
PI 320937 × Eston	96	1,784	1784 (including four SSR markers)	2.3	Ates et al., 2016
ILL8006 × CDC Milestone	118	497.1	4177	0.12	Aldemir et al., 2017
CDC Redberry × ILL7502; ILL8006 × CDC Milestone; PI320937 × Eston	120, 118, 96	977.47	9793	0.10	Ates, Aldemir, Alsaleh, et al., 2018
CDC Redberry × ILL7502	120	973.1	5385	0.18	Ates, Aldemir, Alsaleh, et al., 2018
L. culinaris cv. Alpo × *L. odemensis* accession ILWL235	78	5,782.19	6306 (4682 bins)	0.91	Polanco et al., 2019

with other markers for developing high-density linkage maps in lentil (Sharpe et al., 2013). This map is composed of 534 SNP markers, seven SSR markers, and four morphological markers and used subsequently to map QTLs for milling quality traits (Subedi et al., 2018). A high-density genetic linkage map was constructed with 264 SNP and 61 SSR markers. The SNP markers were identified from a lentil EST database, which was generated through transcriptome sequencing (Kaur et al., 2014). This high-density map covered 1178 cM with an average density of one locus per 3.7 cM and corresponds to seven linkage groups (LG) and three satellites (Kaur et al., 2014). More recently, 433 SSRs from the lentil genome, 145 SSRs from other legumes, 25 ISSR, and 250 RAPD markers have been used for the development of a linkage map consisting of 265 markers. These markers were assigned on seven LGs with a total genetic distance of 809.4 cM with an average marker density of 3.05 cM (Mane et al., 2020). Further reduction in the cost of sequencing has led to the development of a greater number of high-density linkage maps based only on SNP markers in lentil (Aldemir et al., 2017; Bhadauria, Ramsay, et al., 2017; Gujarai-Verma et al., 2014; Ma et al., 2020; Polanco et al., 2019; Sudheesh, Rodda, et al., 2016; Temel et al., 2014). A high-density consensus map of lentil comprising 9793 SNP markers covering a total of 977.47-cM distance was developed from three mapping populations. This map has seven LGs and on average 0.10 cM distance between two markers (Ates, Aldemir, Yagmur, et al., 2018). One of the biparental populations of this consensus map was also used to construct a high-density linkage map with 5385 DArT markers. This map covered 973.1-cM distance and had 0.18-cM distance between two markers (Ates, Aldemir, Alsaleh, et al., 2018). NGS-based transcriptome analysis identified SSR and SNP markers from coding regions of the genome, and hence, some of these markers may act as functional markers. This led to the development of a high-density linkage map consisting of markers developed from gene-based SNPs in lentil using an interspecific (*L. culinaris* × *L. odemensis*) mapping population. This map comprises 6153 markers grouped in 4682 unique bins and placed on 10 LG with a coverage of 5782 cM length (Polanco et al., 2019).

3.5 | Development of reference genome sequence

A draft genome sequence V1.2 for the CDC cultivar Redberry including bulk sequencing, assembly of chromosomal "pseudomolecules", and gene prediction and annotation is available on the KnowPulse web portal (http://knowpulse.usask.ca) for facilitating in-depth genetic and genomics studies in lentil (Bett et al., 2016). This draft version represents seven pseudomolecules and 120,000 scaffolds, assembled into a total of 2.6 Gbp. Further, lentil accessions from around the globe have been re-sequenced to understand the potential genetic information available within the genetic resources of lentil. Another effort has also been made to develop a draft genome, that of the Australian cultivar PBA Blitz. This draft genome has a total of 337.7 Gbp (c. 85× coverage) of high-quality sequences, and its assembly is composed of 352,065 scaffolds and 444,011 singletons with N50 value of 94.4 kb, resulting a total of 2.3 Gbp. This draft genome also represented by seven pseudomolecules having a similarity of 99% with earlier reference genome sequence of lentil (Kaur et al., 2016). These draft genome sequences can help to reshape modern breeding in lentil

through identification of genes and genomic regions that control agronomic traits.

3.6 | Functional genomics toward identification of candidate genes

3.6.1 | Comparative genomics

Comparative genomics has taken advantage of synteny between lentil and other legume crops (Choudhary et al., 2009; Phan et al., 2007; Simon & Muehibauer, 1997; Weeden et al., 1992). Cross-species transferability of SSR and ITAP markers from *Trifolium*, *Medicago*, common bean, chickpea, pigeonpea, and soybean has led to the development of SSR markers for lentil (Alo et al., 2011; Datta et al., 2011; Gupta et al., 2012; Pandian et al., 2000; Phan et al., 2007; Reddy et al., 2010). Comparative genomics can also help identify candidate genes for traits of agronomic importance. For this, functional markers, that is, EST-SSR or EST-SNP, or gene-based markers have been used to study their association with agronomic traits through biparental or association mapping. The genomic sequences, from which a linked marker was developed, are used for comparative mapping in order to identify the underlying candidate genes. Two major loci (*HR* and *ELF3*) controlling differences in photoperiod response between wild and domesticated peas have been identified through comparative mapping in a biparental population using gene-based markers. Subsequently, orthologous gene loci of *ELF3* were identified through comparative mapping in lentil (Weller et al., 2012). Recently, Kaur et al. (2014) made a comparison of the flanking markers SNP_20002998 and SNP_20000246 in lentil for boron tolerance with *Arabidopsis thaliana* and *Medicago truncatula* genome sequences, leading to candidate genes for boron tolerance. In another study, EST-SSRs (developed earlier by Kaur et al., 2011) were shown to associate with flowering time in lentil. Comparative mapping of EST sequences of these associated markers led to identification of genes controlling flowering time in other legumes (Kumar, Choudhary, et al., 2018). Therefore, functional markers can be useful to identify candidate genes underlying a trait of agronomic interest through molecular mapping followed by comparative mapping in lentil. Recently, a subset of 50 EST-SSR markers from 9949 EST-SSRs developed through transcriptome analysis and validated across lentil species and 12 legume genera were associated with different candidate genes involved in different metabolic activities (D. Singh et al., 2020). These markers are useful genomic resources for identification of their roles in controlling agronomic traits through molecular mapping.

3.6.2 | Functional characterization of gene sequence through annotation

The draft genome sequence of lentil and gene sequences with known function of other legumes are now available in the public domain. These are useful resources for identification of candidate genes for traits of interest. Knowledge of gene sequences of other species has facilitated their identification in lentil using sequence homology. For example, resistance gene analogues (RGAs) have been identified in lentil (Yaish et al., 2004), whereas genes coding transcription factors (TF) identified in *Arabidopsis* have been identified in legumes on the basis of sequence homology (Li et al., 2018; Udvardi et al., 2007). The comparative mapping of *MLO* genes of other legume species with a lentil draft genome sequence has identified two likely candidates, *LcMLO1* and *LcMLO3*. These genes showed differences within and between the species only for SNPs and small indels in introns but produce identical amino acid sequences. However, two amino acid substitutions have been observed between *L. culinaris* and *L. orientalis* for the *MLO3* candidate gene, whereas amino acid substitutions and indels were observed in the carboxyl intracellular domain of this gene among three species (*L. odemensis*, *L. ervoides*, and *L. lamottei*). These genes belonging to the *MLO* gene family have been shown to involve in controlling powdery mildew response in other species (Polanco et al., 2018) and hence can be useful for breeding powdery mildew resistance in lentil. Differential expression of genes between contrasting genotypes identified through transcriptome/RNAseq/SAGE analysis has pinpointed genes expressed under the target trait. The annotation of these genes led to identification of candidate genes responsible for targeted traits in lentil, including abiotic and biotic stresses. Differential gene transcript profiles of resistant (ILL7537) and susceptible (ILL6002) lentil genotypes at different stages of inoculation with *Ascochyta lentis* infection identified a number of differentially regulated genes in both genotypes at different stages (see Mustafa et al., 2009). Among these genes, were several candidates with defense-responsive functions such as β-1,3-glucanase, a pathogenesis-related protein from the Bet V I family, a pea disease resistance response protein 230 (DRR230-a), a disease resistance response protein (DRRG49-C), a PR4-type gene, and a gene encoding an antimicrobial SNAKIN2 protein (Mustafa et al., 2009). A microarray chip developed from ESTs of different pulses including chickpea (565 ESTs), *Lathyrus* (156 ESTs), lentil (41 ESTs), and RGAs (Coram & Pang, 2006) was used to identify gene networks underlying the expression of ascochyta blight resistance in lentil (Mustafa et al., 2006). Transcriptome analysis using NGS has identified up- and down-regulated genes expressed at seedling stage under drought and heat conditions (D. Singh et al., 2017; D. Singh et al., 2019). In another study, the 75 up-regulated and 46 down-regulated genes were identified at reproductive stage under heat stress in fields (J. Kumar, personal communication). For drought tolerance, candidate genes identified through transcriptome analysis showed association with functional groups including molecular, cellular and biological processes (D. Singh et al., 2017). Comparing the database of lentil genes and proteins available in the public domain with unigenes identified through transcriptome analysis has resulted in identification of several candidate genes for involvement in the boron toxicity tolerance and flowering time (Sudheesh, Verma, et al., 2016). NGS-based transcriptome analyses have also been used to uncover the generic basis of disease resistance and to identify candidate genes involved in defense–response in lentil (Khorramdelazad et al., 2018; Vaghefi et al., 2013). For example, differential gene expression between resistant and susceptible

genotypes has identified several key genes that played an important role in providing resistance against ascochyta blight. These genes were involved in different defense response functions (see Table S1). In this study, it was observed that the resistant genotype had the ability to detect and respond to disease infection much earlier and faster than the susceptible genotype and structural defense-related genes are overexpressed in lentil (Khorramdelazad et al., 2018). In lentil, NGS has been used to identify the genes encoding disease resistance proteins in host and virulence proteins (i.e., effectors) in the pathogen. A complex interaction between resistance and effector genes has been observed for developing anthracnose disease in lentil (Bhadauria, Ramsay, et al., 2017). In this study, 26 resistance genes including *suppressor of npr1-1*, *constitutive 1* (*NBS-LRR*) and *dirigent* (resistance response protein) have been identified in the host after the infection with an isolate of the virulent race 0 of *Colletotrichum lentis* (Bhadauria, Ramsay, et al., 2017). Transcriptome analysis identified several defense-responsive nonallelic candidate genes that imparted resistance to ascochyta blight after infecting plants with *A. lentis* pathogen at different stages (Sari et al., 2018). Another study identified candidate genes encoding calcium-transporting ATPase and glutamate receptor 3.2 for resistance and another candidate gene with unknown function for the susceptibility of stemphylium blight disease in lentil through transcriptome analysis (Cao et al., 2019). These candidate genes have been validated through bulk segregant analysis in a mapping population used previously for identification of QTL (Cao et al., 2019). These candidate genes can be used for different purposes including identification of genes for pathway-specific expression analysis, genetic modification approaches, development of resources for genotypic analysis, and assistance in the annotation of a future lentil genome sequence and can be useful for developing diagnostic functional markers for breeding. In a recent study, 422,101 high-confidence SNP markers were identified against the reference lentil genome (cv. CDC Redberry) using transcriptome analysis of 467 wild and cultivated accessions of lentil at the seedling stage. Genetic diversity analysis showed major differences among studied genotypes on six genomic regions with the largest being on Chromosome 1 and identified potential candidate genes associated with these genomic regions by making comparison with the reference lentil genome. As a result, five candidate genes have been identified from Region 1, and further gene annotation showed their functionality as metal transporter, ABC transporter, and a mitogen-activated protein kinase 3 (MAPKK 3) (Dissanayake et al., 2020).

4 | EFFORTS TOWARD GENOMICS-ASSISTED BREEDING

Genomics-assisted breeding can be used for selection of parents for crossing, confirming purity of F_1s, mapping traits for introgression, and molecular profiling of breeding populations for selecting improved types. Molecular markers have been used widely in lentil to determine the extent of genetic diversity in cultivated and wild gene pools (Abo-Elwafa et al., 1995; Ahmad & McNeil, 1996; Alo et al., 2011; Ford et al., 1997; Hamwieh et al., 2009; Havey & Muehlbauer, 1989; Kumar et al., 2014; Sharma et al., 1995; Sharma et al., 1996; Yadav et al., 2016), establishing progenitors (Alo et al., 2011), taxonomic classification of gene pools (Verma et al., 2014), establishing the hybridity of F_1 in plant breeding programs (Solanki et al., 2010), and knowing the diversification of the Indian gene pool using exotic germplasm (Kumar et al., 2014) and trends of genetic diversity changes in Indian gene pool over decades (Kumar, Gupta, Dubey, et al., 2018). Further, cost-effective NGS has accelerated the development of molecular markers and their use for assessing the genetic diversity and phylogenic relationship among different species. Species belonging to the genus *Lens* have been categorized into four gene pools on the basis of 5389 and 422,101 SNPs in two studies. These studies put *L. tomentosus* and *L. orientalis* with *L. culinaris* in a primary gene pool due to close molecular similarity as well as their interspecific crossing. *L. lamottei* and *L. odemensis* have been placed in the secondary gene pool as their closeness with species of primary gene pool and reported *L. odemensis* as a sister clade to *L. lamottei*, whereas *L. ervoides* has been put in the tertiary gene pool due to its cross incompatibility with the species of primary and secondary gene pools. As crosses between *L. culinaris* and *L. nigricans* are unsuccessful beyond the F_1 generation, the latter was placed in the quaternary gene pool (Dissanayake et al., 2020; Wong et al., 2015). Further sequence-based SNP markers have been used to characterize cultivars and landraces according to their geographical origin (Khazaei et al., 2016; Lombardi et al., 2014). One of the studies showed no grouping of landraces according to their geographical origin, whereas a high level of genetic diversity has been observed in the landraces belonging to the Mediterranean region, especially from Greece and Turkey (Lombardi et al., 2014). Also, 352 accessions of 54 diverse countries including accessions from Mediterranean Basin (also including the Nile valley from Egypt to Ethiopia), subtropical Asia, and northern temperate regions could be grouped broadly according to their geographical origin (Khazaei et al., 2016). Another study used 6693 SNPs following GBS analysis to characterize 349 accessions of the Mediterranean gene pool, leading to their association with geographic patterns and phenotypic traits (Pavan et al., 2019). They also identified routes of lentil cultivation in Mediterranean countries and a role of selection of improved types in shaping the structure of lentil populations (Pavan et al., 2019). Thus, sequence-based SNP markers have been efficient in identification of significant level of genetic diversity among genotypes of the cultivated gene pool. Therefore, these markers can be used to mine diverse genotypes for hybridization in breeding programs for genetic improvement (Khazaei et al., 2016).

Trait mapping is one of the most important functions of genomics in breeding. For this, molecular markers are used to map genes/QTL controlling the target traits. During the past years, association of molecular markers with different agronomically important traits has been established in lentil by several workers and comprehensively reviewed earlier (Kumar et al., 2015). Although major QTLs explaining a large proportion of phenotypic variation for quantitatively inherited traits have been identified in these marker–trait association (MTA) studies, no report is available of their use in lentil breeding program. Thus, these MTA studies have so far been academic than practical.

TABLE 3 Molecular markers linked to QTL/genes for key traits in lentil

Traits	Mapping population	QTL name and number of QTLs	Flanking marker(s)	Converge distance cM	Phenotypic variation explained by the QTL (%)	References
Cotyledon color class	CDC Robin × 964a-46	*Yc*	LcC13114p356	1.0	23	Fedoruk et al., 2013
Seed diameter	CDC Robin × 964a-46	Three QTLs	LcC13114p356, LcC00853p101, and LcC00890p1387	<1.0	Up to 60	Fedoruk et al., 2013
Seed plumpness	CDC Robin × 964a-46	Three QTLs	LcC13114p356, LcC00890p1387, LcC00853p101	<1.0	Up to 50	Fedoruk et al., 2013
Days to 50% flowering	CDC Robin × 964a-46	Three QTLs	LcC06044p758, LcC09496p566, LcC23363p108	<1.0	Up to 34	Fedoruk et al., 2013
	ILL6002 × ILL5888	$QLG4_{83}$	RAPD_UBC 34 and RAPD_UBC 1	1.00	20.2	Saha etal., 2013
100 seed weight	ILL6002 × ILL5888	$QLG4_{82}$	RAPD_UBC 34 and RAPD_UBC 1	1.00	17.5	Saha et al., 2013
Seed diameter	ILL6002 × ILL5888	$QLG4_{82}$	RAPD_UBC 34 and RAPD_UBC 1	1.00	32.6	Saha et al., 2013
Stemphylium blight resistance	ILL6002 × ILL5888	$QLG4_{80-81}$	ME4XR16c	0.20	46	Saha et al., 2010
Se uptake	"PI 320937" × "Eston"	*SeQTL2.1*	Cluster of six SNPs	7.3	6.6–11.5	Ates et al., 2016
		SeQTL5.2 5	Cluster of eight SNPs	18.1	6.3–10.3	Ates et al., 2016
		SeQTL5.3	Cluster of 10 SNPs	17.0	6.4–10.0	Ates et al., 2016
		SeQTL5.1	Cluster of 13 SNPs	7.3	6.5–16.9	Ates et al., 2016
Ascochyta blight resistance	Indianhead × Northfield	*AB_IH1*	PBA_LC_0629 and SNP_20005010	1.00	47	Sudheesh, Rodda, et al., 2016
		AB_IH2.1	SNP_20002370 and SNP_20002371	1.30	15	Sudheesh, Rodda, et al., 2016
	Indianhead × Digger	*AB_IH1*	SNP_20005010, PBA_LC_0629, and SNP_20004695	3.5	30	Sudheesh, Rodda, et al., 2016
		AB_IH2.2	SNP_20000505 and SNP_20000553	1.8	22	Sudheesh, Rodda, et al., 2016
Anthracnose	L01-827A × IG 72815	*qSB-2.2*	Contig313227p47568	0.00	18.29	Bhadauria, Vijayan, et al., 2017
	L01-827A × IG 72815	*qANTH1-3.2*	Contig142466p23623	0.00	24.75	Bhadauria, Vijayan, et al., 2017
		qANTH0-5.2	Contig23853p125770	0,00	18.82	Bhadauria, Vijayan, et al., 2017
Manganese uptake	CDC Redberry" (P1) × "ILL7502"	*MnQTL3.1*	Cluster of 24 SNPs	0.60	18.0	Ates, Aldemir, Alsaleh, et al., 2018
		MnQTL3.2	Cluster of 10 SNPs	1.10	22.4	Ates, Aldemir, Alsaleh, et al., 2018
		MnQTL3.3	Cluster of 103 SNPs	9.50	21.6	Ates, Aldemir, Alsaleh, et al., 2018

(Continues)

TABLE 3 (Continued)

Traits	Mapping population	QTL name and number of QTLs	Flanking marker(s)	Converge distance cM	Phenotypic variation explained by the QTL (%)	References
Dehulling efficiency	CDC Robin × 964a-46	1 QTL	LcC4611p576	0.00	15.5	Subedi et al., 2018
Football recovery	CDC Robin × 964a-46	5 QTLs	LcC22183p646, LcC10086p103, LcC20366p221, LcC03203p214, LcC10025p199	0.00	20–35	Subedi et al., 2018
Seed coat spotting	*Lens culinaris* cv. ALPO × *L.* odemensis ILWL235	*Scp*	S044140 (within gene Lc25388 encoding an MYB transcription factor MIXTA-like protein) and S061401 (within gene Lc26377 encoding phosphate transporter PHO1-like protein)	0.00	85.07	Polanco et al., 2019
Flower color		*FC*	S061401 (within gene Lc26377 encoding phosphate transporter PHO1-like protein)	0.00	84.20	Polanco et al., 2019
Stem pigmentation		*SP*	S006811l (within gene Lc01979 gene [a sulfite exporter TauE/SafE family protein])	0.00	33.96	Polanco et al., 2019
Time to flowering		*TF*	Cluster of eight markers	3.9	55.73	Polanco et al., 2019
Seed size		*SSQ1*	loc120 (flanked by genes Lc03317 and Lc01979)	5.9	28.26	Polanco et al., 2019
		SSQ2	loc279 (flanked by genes Lc21173 and Lc23619)	7.23	18.63	Polanco et al., 2019
		SSQ3	S078816 (within gene Lc23140)	0.00	23.13	Polanco et al., 2019
Ascochyta severity		*AS-Q1*	S046029 bin (two markers from genes Lc27513 [ATP-dependent helicase BRM] and Lc25909 [plant/F1M20-13 protein])	0.00	27.14	Polanco et al., 2019
		AS-Q2	loc219 (between gene Lc27329 and a bin having three genes Lc25078, Lc26336, and Lc27664)	5.12	25.53	Polanco et al., 2019
		AS-Q3	S005268 bin having three genes Lc25349 (homeobox-leucine zipper ROC6), Lc25206 (methyltransferase PMT16), Lc26436 (uncharacterized protein)	0.00	23.13	Polanco et al., 2019

One important reason for this is the loose association of a marker with a trait. Practically, a molecular marker should have tight linkage having a distance of <1.0 cM from the genes/QTL controlling a trait of interest and explain high phenotypic variation. Recently, significant progress has been made in the development of gene-based SSR and SNP markers due to the availability of draft genome sequence of lentil and cost-effective sequencing of functional regions of lentil genome. This has led to the development of high-density linkage maps and tight association of molecular markers with genes/QTLs pertaining to different desirable traits including resistance/tolerance to biotic and abiotic stresses and agronomic, seed quality, and nutrition uptake traits (Table 3). These MTA studies provide an opportunity to use them in lentil breeding programs. For example, SNP markers associated with cotyledon color, seed diameter, seed plumpness, and days to 50% flowering explained 23%–60% of the total phenotypic variation and have tight linkage with 1.0 or <1.0-cM distance (Fedoruk et al., 2013). Likewise, SNP makers having a tight linkage (1–3.5 cM) with QTLs for ascochyta blight resistance explaining 15%–47% of total phenotypic variation are useful for marker-assisted breeding (Sudheesh, Rodda, et al., 2016). Several sequence-based trait mappings have also led to identification of SNP or clusters of SNPs within or surrounding the targeted genes/QTLs. Three QTLs, namely, *qSB-2.2*, *qANTH1-3.2*, and *qANTH0-5.2*, explaining 18.29, 24.75, and 18.82, respectively, have been identified to be associated with SNP-containing contigs (Bhadauria, Vijayan, et al., 2017). In another study, molecular mapping with SNP markers identified major QTLs for seed coat spotting (Scp), flower color (FC), and stem pigmentation (SP) explaining 85.07%, 84.20%, and 33.96% of total phenotypic variation, respectively. These SNP markers were found within genes encoding an MYB TF and MIXTA-like protein phosphate transporter PHO1-like protein (Polanco et al., 2019). Another major QTL for flowering time explaining 55.73% of phenotypic variance has been associated with a cluster of eight SNP markers with coverage of 3.9-cM distance (Polanco et al., 2019). Thus, significant development has been made through sequence-based trait mapping for marker-assisted breeding in lentil. However, breeders are required to screen large breeding populations for identification of useful recombinants. In fact, in crops like chickpea, genomics-assisted breeding has already been successfully used to develop cultivars with higher drought tolerance (Varshney et al., 2013) and disease resistance (Mannur et al., 2019). Therefore, it is anticipated that integration of markers will be common practice in lentil breeding soon. However, markers associated with a trait of interest must be breeder friendly so that they can be used in marker-assisted selection, and hence, more focus should be on converting the SNP markers into PCR-based KASP markers in lentil (Wang et al., 2020).

5 | FUTURE WAYS FOR GENOMICS-ASSISTED BREEDING

Current progress in lentil genomics has advanced our efforts for making genomics-assisted breeding a reality in coming years (Kumar & Gupta, 2020). Figure 1 presents future ways of genomics-assisted breeding in lentil. This includes precise and efficient discovery and deployment of traits using tools and techniques of genomics and phenomics in current lentil breeding programs. This will enable maximum genetic gains by developing climate-resilient high-yielding cultivars with precision.

5.1 | Trait mapping using sequencing

Rapid advancement in genome sequencing technologies has greatly reduced the cost of sequencing multiple accessions. This provides ample opportunities for sequence-based genotyping of whole mapping populations or pool sequencing from extreme bulks for the trait of interest (see Varshney et al., 2019). In lentil, transcriptome sequencing has been done for the whole mapping population, leading to the development of high-density linkage maps (Table S1; see Section 3.4) and identification of SNP markers/candidate genes associated with traits of interest (Polanco et al., 2019). The GBS analysis is one of the most effective approaches to genotype many individuals even in those species that have no reference genome. It has been used to identify whole genome SNPs and used to genotype large collection of lentil germplasm (Pavan et al., 2019) and genetic mapping in pea (Boutet et al., 2016). In chickpea, whole genome re-sequencing and QTLseq approaches have been used successfully to narrow down genomic regions harboring *QTL hotspot*, leading to identification of candidate genes related to drought tolerance (Das et al., 2015; Deokar et al., 2019). Therefore, sequence-based trait mapping is opening a future way for identification of candidate genes and facilitation toward the development of gene-based markers for marker-assisted breeding in lentil.

5.2 | Development of functional markers and genotyping platforms

Re-sequencing of many candidate genes has identified SNPs associated with candidate genes related to target traits (Kaur et al., 2014). Transcriptome analysis identified candidate genes expressed under biotic and abiotic stresses in addition to EST-SSR and EST-SNP markers (see Table 1). In lentil, comparative genomic mapping of flanking sequences of SNPs with the genome sequences of other species (*M. truncatula*, soybean, and *A. thaliana*) has led to identification of candidate genes that are functionally associated with boron toxicity tolerance (Sudheesh, Verma, et al., 2016). Significant progress has been made in development of such functional genomic resources also in lentil. However, their use will only be possible by breeders if they are easily able to genotype large breeding populations for identification of useful recombinants. For the future, we need more focus on the development of breeder-friendly PCR-based functional EST-SSR markers as done by D. Singh et al. (2020) or on converting SNPs into PCR-based KASP markers for marker-assisted breeding in lentil (Wang et al., 2020). DNA chips and exome arrays based on 16 wild

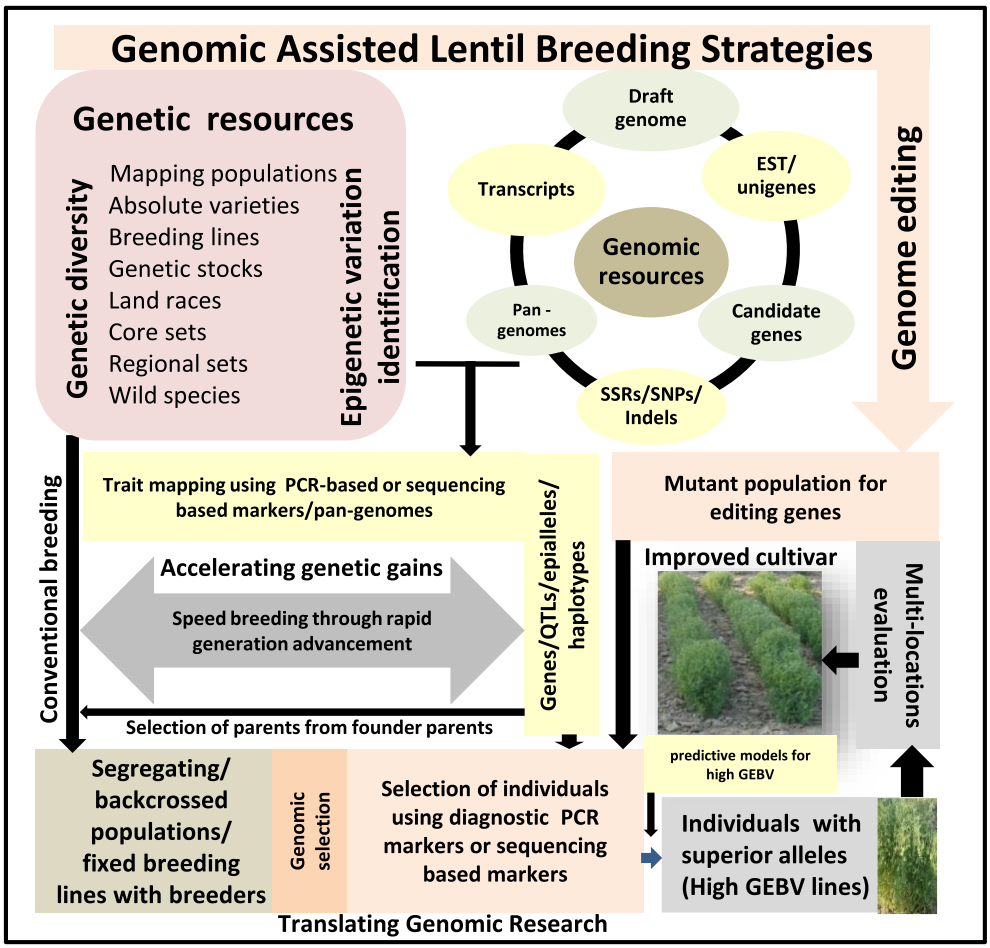

FIGURE 1 Genomics-assisted lentil breeding strategies for developing improved cultivars in the context of climate change. Genotyping of targeted genetic resources like biparental or multi-parental mapping populations or other natural or mutant populations with PCR-based or sequencing-based makers together with trait phenotyping may be used for trait mapping, leading to identification of candidate genes/QTLs/haplotypes. Epigenetic variation can also be searched among available genetic resources for a trait, and epialleles can be mapped using genomic resources. Transcriptome sequencing of genetic resources can lead to identification of SSR or SNPs within candidate genes involved in control of the target trait that can be used in breeders' populations either genotyping through sequencing or PCR. Availability of the draft genome in lentil can help with whole-genome re-sequencing (WGRS) or high- to low-density genotyping following different genotyping platforms such as genotyping-by-sequencing (GBS) and chip-based genotyping and in development of a pan-genome for capturing the entire set of genes from *Lens* species. A pan-genome analysis identifies variable genome regions that are functionally associated with adaptive traits such as resistance to biotic and abiotic stresses. It bridges the genome-to-phenome gap and identifies superior alleles for genetic improvement through genome-wide association analysis. Further integration of rapid generation advancement can help to accelerate genetic gains through development of mapping populations or breeding populations. Identified marker–trait associations as well other genomic resources can be used for translating genomic research through selection of parents for breeding, marker-assisted backcross breeding, selection of recombinants in early segregating generations, and genomic selection in fixed breeding lines. Predictive models can also be used for selecting breeding lines with high GEBV. Further, genome editing can be performed to generate mutants for targeted genes. These genes can be identified on the basis of their involvement in the control of desirable agronomic traits identified in previous studies. Consequently, breeding lines with superior alleles can be evaluated over multi-locations for yield, resistance to biotic and abiotic stresses, and nutritional traits leading to development of improved cultivars fitted to different climatic conditions and enhanced genetic gains in farmers' fields through translational research

lentils and 22 cultivar accessions developed from genic regions by previous workers (Kaur et al., 2014; Ogutcen et al., 2018; Sharpe et al., 2013) can help to accelerate genomics-assisted breeding in lentil. Moreover, candidate genes identified through transcriptome sequencing can be used to develop a DNA chip platform having genes for biotic and abiotic stresses.

5.3 | Accelerating genetic gain

Now, it is feasible to scan the entire genome or part of it through sequencing or re-sequencing of many individuals of a breeding population. Thus, high-throughput genome-wide SNP genotyping platforms such as GBS can help to identify the genotypes having high breeding

value from a breeding population on the basis of their genotypic constitution (Poland et al., 2012) and have been used for genomic selection (GS) in crop plants (Bassi et al., 2016). GBS scans a large proportion of the genome and captures specific genomic regions related to a population/family (Bhat et al., 2016). In addition to this, flexibility, low cost, and higher prediction accuracy for genomics estimated breeding value (GEBV) make GBS attractive for GS (Poland et al., 2012). Sequencing-based genotyping showed higher prediction accuracy (0.1–0.2) of GEBV compared to other genotyping methods due to high SNP density (Poland et al., 2012). GS based on the profiling of an entire genome focuses not only on major genes/QTL but also selects minor genes/QTLs for targeted traits (Crossa et al., 2017). Thus, GS is useful for those traits that have low heritability and high G × E interactions. For example, prediction accuracy of GEBV for grain yield was estimated to be 0.64 in soybean, indicating good potential of using GS for this low heritable trait in this crop (Jarquín et al., 2014). In lentil, different GS models and prediction scenarios have been evaluated for GS by including G × E interactions and multiple traits (Haile et al., 2020). In this study, GS was found useful to accelerate genetic gain within population and across environments (Haile et al., 2020). Therefore, GBS, which has already been used for genotyping the large number of genotypes, can be useful to speed up genetic gain in lentil.

Genetic gains through GS can further accelerate breeding. Conventional breeding may take seven to eight generations to develop an improved variety. Shortening breeding time of a lentil variety can help to harvest the genetic gains more rapidly through GS following the genetic gain equation ($\Delta G = (\sigma_a)(i)(r)/L$), where σ_a = square root of the additive genetic variance, i = selection intensity, r = response to selection, and L = length of breeding cycle interval or generation. Now, it is possible to accomplish six to seven generations per year in lentil by adjusting physiological parameters like temperature, humidity, photoperiod, and harvesting/germination of immature seeds (Ghosh et al., 2018). However, in recent studies, immature seeds have been germinated under *in vitro* culture using plant growth regulators (Bermejo et al., 2016). The extended photoperiod has reduced generation, leading to three generations per year under controlled conditions (Idrissi, 2020). This can help to develop the breeding population for GS within just a few years, leading to productivity gains.

5.4 | Development of pan-/super pan-genomes

The draft genome sequence of lentil provides an opportunity to develop a pan-/super pan-genome through whole genome sequencing of several accessions belonging to cultivated and wild species. A pan-genome has the sum of the all genes (i.e., core and dispensable genes) available in individuals of a species (Tettelin et al., 2005). A pan-genome analysis helps to differentiate conserved (core) and dispensable genes. Conserved genes are usually housekeeping genes that are responsible for essential cellular functions (Tao et al., 2019), whereas dispensable genes are not present across the individuals but available either in a specific individual or few individuals. Functionally dispensable genes control various adaptive traits such as tolerance to biotic and abiotic stresses, receptor and antioxidant activity, gene regulation, and signal transduction (Golicz et al., 2016; Gordon et al., 2017; Hurgobin et al., 2018; Li et al., 2014; McHale et al., 2012; Schatz et al., 2014). Therefore, these genes are fast evolving and contribute more to the diversity of a species and may be more useful for breeding (Tao et al., 2019). In lentil, substitutions of synonymous and non-synonymous SNPs in the coding regions cause genetic variation (Sharpe et al., 2013). The pan-genome analysis enables the capturing of genetic variation caused by structural changes available in the gene content of individuals belonging to the same species (Tettelin et al., 2005). Structural variations (SVs) include presence/absence variations, copy number variations (CNVs), and other form of variations such as inversion, transversions, and inter-/intrachromosomal translocations (Cook et al., 2012; Feuk et al., 2006; Qi et al., 2014; Wang et al., 2015). Using the information of a draft genome sequence, re-sequencing of diverse accessions can help to identify the prevalence of SVs in lentil, as was observed in soybean (Lam et al., 2010; Zhou et al., 2015) and pigeonpea (Varshney et al., 2017). This will help to identify the dispensable genes of agronomic importance for lentil. Therefore, efforts are required toward the development of pan-/super pan-genome in lentil.

5.5 | Exploitation of genic regions through genomics-assisted breeding

It is a challenging task to capture the genetic variation across whole genome through whole genome sequencing of many genotypes due to the presence of gene duplications, chromosomal arrangements, and repetitive elements in the complex lentil genome (Ogutcen et al., 2018). As coding regions of the genome are more useful for breeding than noncoding regions (Bamshad et al., 2011), it would be better to capture the genetic variation available in the coding regions of the genome that bear genes of agronomic importance. According to one study, 3.2% of the genome (i.e., 130 Mbp) carries genic regions, which are important for lentil research (Ogutcen et al., 2018). Therefore, more focus is required in the future to capture genetic variations of these regions. For this, exome capture arrays comprising 85 Mbp have been developed in lentil (Ogutcen et al., 2018). This exome capture array can help to sequence only the protein coding regions of the genome rather than the whole genome, making it a cost-effective sequencing method (Hodges et al., 2007). This exome array has been used to characterize 38 diverse accessions belonging to wild and cultivated species in lentil (Ogutcen et al., 2018). In the future, genomics-assisted breeding can focus on genic regions carrying useful genes for traits of breeders' interest.

5.6 | Moving toward the use of new genomic tools

Genetic transformation has been successfully reported in lentil using different methods of gene transfer (Kumar et al., 2015). The *DREB1A*

gene has been transferred successfully through *Agrobacterium*-mediated genetic transformation into lentil and developed transgenic plants with enhanced drought and salinity tolerance (Khatib et al., 2011). These developments provide a platform for gene editing in lentil. A genome editing approach has been used to understand the basic mechanisms underpinning legume–rhizobia interactions (Wang et al., 2017). In the recent past, a number of candidate genes expressing under abiotic and biotic stress conditions as well as other agronomic traits have been identified in lentil (Table S1). The function of these genes can be validated through genome editing as were the candidate genes controlling quantitative variations for nodulation (Curtin et al., 2017). Genome editing targeting candidate genes generates a mutant population that can provide useful genetic resources for breeding improved cultivars through screening under different environmental conditions.

Lentil, like other pulses, is cultivated in a wide range of environmental conditions and faces many stresses throughout its life cycle, and hence, expression of quantitative traits is highly influenced by environments (Kumar, Choudhary, et al. 2019). These changes in the expression of traits could be caused by environmentally induced epigenetic variations. Epigenetic variations occur due to methylation of gene sequences rather than alteration of DNA sequence and can be both heritable and nonheritable (Haig, 2004). These epigenetic variations help plants to adapt in wider range of environmental conditions. Therefore, breeding for epigenetic variations can be more useful for developing climate-resilient lentil cultivars. Epigenetic variations have been explored and exploited for increasing yield and stability in soybean (Raju et al., 2018). Therefore, there is a need to identify epialleles (alleles that control epigenetic variations) interaction with particular environmental conditions for epigenetic breeding in lentil.

6 | CONCLUDING REMARKS

Significant progress has been made for genomics-assisted breeding in lentil including development of genomic resources, high-density linkage maps, identification of candidate genes for functional genomics, development of draft genomes, and identification of SNP and SSR markers linked tightly with traits of breeders' interest. In spite of this, these genomic resources have as yet been utilized for marker-assisted breeding in lentil compared to other popular grain legumes like chickpea or common bean. NGS has opened new opportunities for sequence-based trait mapping, cost-effective genotyping of a large number of individuals, and identification of candidate genes (Kumar & Gupta, 2020). Therefore, a sequence-based holistic breeding approach can be integrated for modernization of lentil breeding in order to develop improved varieties with accelerated genetic gains through GS.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

This work did not involve any human or animal ethics issues to be considered.

AUTHOR CONTRIBUTION

JK and SK conceived the idea and drafted the sections of the manuscript. JK and DSG prepared tables and figures. SK, MB, and RKV made a critical revision of the content of the manuscript. All authors contributed to the final reading and approved the submitted version.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

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