Lentil Breeding: Present State and Future Prospects

Abstract

Lentil (*Lens culinaris* Medik.) is one of the vital grain legume crops that originated from *L. culinaris* Medik.ssp. *orientalis* in the Near East. It is an essential food source that is capable of contributing energy, proteins and iron to human diet. Major concern of lentil producing countries is to have a stable high seed yield in addition to resistance against abiotic and biotic stresses. The activities of domestication and selection in the course of crop evolution have led to reduction in genetic variation in the current cultivars and species, which is a major drawback for lentil breeding. The improvement of many monogenic traits has been carried out by conventional breeding techniques like selection and recombination. However, these improvements still lack in addressing economic traits like yield as they are governed by polygenes and G x E interaction. Various species in the genus *Lens* can be aid to enhancement of genetic variation for target lentil traits to develop new varieties. Induced mutagenesis is another vital breeding method which can ease the availability of lentil genomic resources. Commendable success stories in applications of molecular markers and biotechnological techniques will further speed-up the development of improved varieties. This review article an outline on present status on genetic improvement in lentil and a compendium of important aspects of lentil diversity, genetics and breeding with the way forward.

The human population growth has shown an unprecedented rate which has resulted to reduction in the per capita food availability. This calls for urgent demands to attain enhanced productivity, especially in the developing countries like India, Pakistan and Bangladesh. The global population is expected to reach over 9 billion by 2050 which will require 70% more demand to feed the people (FAO). The present state of decline in agricultural land as a result of decrease in water resources and addition to urbanization and industrialization demands acceleration in the targets of food production with limited agricultural inputs. The need of the situation is to generate high yielding improved crop varieties that are genetically diverse, input use-efficient, climate-change resilient, that is bio-fortified and versatile to various farming practices and agro-ecosystems. Implication of plant breeding strategies for the development of diverse suite of such crop varieties for crucial food crops will be solution to fight the global food crisis.

The limited genetic variability of major food crops such as cereals and pulses, especially grain legumes, is a bottleneck to crop improvement. It is necessary to induce of innovative breeding tools for generating new genetic variability in the desirable traits of these crops. Lentil (*Lens culinaris* Medikus) is a highly nutritious and climate resilient pulse crop that fits well into the class of target food crops or *smart crops* grown throughout the subtropics. Although being one of the oldest domesticated grain legumes, genetic improvement in lentil still requires appropriate scientific investment.

Introduction

Origin and Taxonomy

Lentil is an annual, self-pollinated, diploid (2n = 2x = 14) species of the family Fabaceae (Leguminosae). It is a cold-season legume with a haploid genome size of 4063 Mbp (Arumuganathan and Earle 1991). Lentil seems to be domesticated from its wild progenitor *Lens culinaris spp. orientalis* in Syria and Turkey approximately 8500 BC (Cubero 1981).

Many taxonomist worked in order to classify lentils, but the most accepted latest classification recommends following seven taxa in four *Lens* species (Ferguson and Erskine 2001; Ferguson et al. 2000). In 1787, Medikus, a German botanist is given the credit to the scientific name of lentil as *Lens culinaris* (Fratini et al. 2014).

1. *Lens culinaris* Medik.
    ssp. *culinaris*
    ssp. *orientalis* (Boiss.) Ponert
    ssp. *tomentosus* (Ladiz.) M.E. Ferguson& al.
    ssp. *odemensis* (Ladiz.) M.E. Ferguson& al.
2. *Lens ervoides* (Brign.) Grande
3. *Lens nigricans* (M. Bieb.) Godron
4. *Lens lamottei* Czefr.

Distribution and Diversity

It has a global production of 6.33 million metric tons from an area of 6.10 million ha with Canada, India, Turkey, and USA being the top producers (FAO 2018). In India, lentil is grown on 2.22 million ha land giving a production of 1.62 million tones with Madhya Pradesh, Uttar Pradesh, Bihar and West Bengal as the major growing states. The average productivity in India comes about to be 731 kg/ha which is still lesser compared to global average of 1038 kg/ha (FAO 2018).

The lentils were classified into two groups based on seed size by Barulina (1930) viz. microsperma / small seeded type (<6 mm diameter) and macrosperma / large seeded types (>6 mm diameter). Lentil grains are important source of nutritious dietary protein (22–35%), carbohydrates, minerals and vitamins in human nutrition. They are mainly consumed as dhal, fried snacks, in the form of soup in or as sprouts. Lentil grains and straw are also utilized as animal feed. It is considered a high value crop option for farmers as it provides the benefits of crop rotation favorable to weed and pest management.

Bottlenecks in lentil productivity

The major obstacles in good productivity of lentil are narrowed genetic base of released cultivars, shorter growing period, biotic stresses like wilt, rust and abiotic stresses like drought and heat (Amin et al. 2015; Asnake and Bejiga 2003; Muehlbauer et al. 2006; Sarker and Erskine 2006). A number of breeding programs have been taken up in lentil for increased productivity. Unfortunately, little progress was made due to environmental effects, G x E interactions and repeated use of similar lines in breeding programmes (Kumar and Ali, 2006). The domestication in lentil has led upto 40% loss in its genetic diversity (Alo et al. 2011). Genetic improvement and genetic variation can thus be a key to increase crop. The pedigree analysis of Indian lentil

varieties proved their narrow genetic base, which can be broadened by use of Mediterranean land races and crossable wild subspecies having desirable traits (Kumar et al. 2014).

Another barrier for lentil growing farmers is heat stress. It is a period of prolonged hot temperature causing irreversible damage to growth and development of crop plants (Delahunty et al. 2018). In key growing areas, the crop is exposed to >35°C temperature at flowering and pod filling stages during maturity (Delahunty et al. 2015). This problem becomes even more serious in rice-lentil crop rotations, since the late harvesting of rice delays lentil sowing. As a result, the reproductive phase of lentil is placed under high temperature stress (Subbarao et al. 2001). High temperature results into drastic losses of yields attributable to poor grain filling in lentil. In addition to the above, high temperature deteriorates physiological processes leading to forced grain maturity (Redden et al. 2014). A yield reduction of 87% was observed in lentil plants grown under field conditions in the pots when exposed to a temperature of >38/23°C (average day/night temperature) during the reproductive phase (Bhandari et al., 2016). This could be worse as higher temperatures by reason of global warming is ever challenging for lentil production and productivity (Kaur et al. 2015).

Germplasms Conservation

Accessions of genus *Lens* comprising of wild relatives, landraces and advanced breeding materials. Globally, *Lens* has leading 41 collections together holding 43,214 accessions. Official world germplasm collections of lentils for breeding purposes are maintained by ICARDA, the Indian Agricultural Research Institute, the Vavilov Research Institute of Plant Industry and USDA collection at the Regional Plant Introduction Station, Pullman, Washington. The global status of *Lens* ex situ genetic resources is recorded b The Global Crop Diversity Trust (https://www.croptrust.org) upcoming with new conservation strategies. ICARDA leads in conserving the global *Lens* germplasm accessions with 24% of total accessions including 583 wild accessions. The south Asian sub-region conserved 3022 lentil accessions with the largest collection at NBPGR, India and the working collection at IIPR, India. The Global Seed Vault in Svalbard, Norway, famous as the Doomsday Vault, is also preserving a wide variety of plant seeds including lentil seeds collected from operational gene banks worldwide since 2008. A number of other gene banks with large lentil collections are working to conserve diverse accessions of lentil genetic resources to be assessed for future breeding and research.

Breeding Objectives

The key breeding goals in lentil breeding are higher and stable seed yield, disease resistance and better seed quality (Muehlbauer et al. 1995). Genetically diverse parents are used in lentil breeding to generate new gene combinations with traits of interest like increased yield and stress resistance (Sarker et al. 2009). Pureline selection in heterogeneous landraces was the most common cultivar development method during initial phases of lentil improvement (Muehlbauer 1992). The application of pureline selection developed genetically uniform and locally adaptable lentil cultivars from local landraces or introduced germplasm. Species from these selection efforts had decreased pod dehiscence and dormant seeds, with a considerable

increase in erect plant habits and seed size (Zohary 1996). Hybridization and selection then took over as methods preferred for quick development of new cultivars/genotypes in lentil over the past few decades. Lentil being predominantly self-pollinating species has very low out crossing rate. Therefore, the breeding methods in lentil alike other self-pollinated crops viz. pure line selection or hybridization followed by the bulk method, the pedigree method, the single seed descent, or a modification of these procedures along with mutation breeding (Muehlbauer et al. 2009; Toker et al. 2007). Genetic improvement of any trait through plant breeding is only successful if crop gene pool is available with sufficient variability for a trait.

Plant Introduction

Plant introduction is a method of obtaining high yield and wide adaptability of lentil cultivars from within or outside the country. The soil and climatic conditions of the new location where cultivar is introduced, alters success of plant introduction. Homozygous pure lines are preferred as they are adaptable compared to a heterozygous segregating population. This is because the latter requires recognizing productive line with target traits. Plant introduction is a quick and economic way of developing cultivars. For developing countries, it is even more feasible due to availability of lesser area under crop cultivation, economic curtailments or the lack of skilled personnel. Lentil is reported to be introduced into South Asia around 2000 BC from West Asian regions via Afghanistan (Cubero 1981; Materne and Siddique 2009). Selection-introduction-acclimatization of lentil is being done at different institutes globally, ICARDA being the leading one. In order to widen the narrow genetic base of small-seeded Pilosae type, bold-seeded Precoz type, of Argentine origin, was introduced from ICARDA (Erskine et al. 1998). Lentil cultivars Vipasha and VL 507 in India; Simal, Sikhar, Khajura Masuro 1 and Khajura Masuro 2 in Nepal; Mansehra 89 and Shiraz 96 in Pakistan were also introduced from ICARDA (Rahman et al. 2009).

Hybridization

Hybridization is a method of integrating desirable traits from two or more parents into progeny cultivar. The key objective of hybridization is to elevate the degree of genetic variation.

- Selection of Parents

The selection of appropriate parents plays a crucial role in the success of hybridization. Depending on the objective, the choice of parents is made. One or two parents are selected if higher yield and wider adaptability is the improvement criteria. If broadening of genetic base is desired, diverse parents are selected. Biometrical approaches can be deployed to analyze diversity and combining ability of genotypes to be used in hybridization. DNA-based markers facilitate precise estimation of genetic diversity through germplasm characterization. Lentil diversity assessments studies in recent times using SNP markers, suggested the selection of contrasting parental genotypes for lentil breeding programs (Lombardi et al. 2014).

- Crossbreeding Techniques

Ladizinsky (1992) defined crossability as the potential of creating fertile F_1 hybrid from intercrossing between individuals belonging to different taxa. Crossbreeding in lentil is a cumbersome task with a success range of mere 20–50% owing to tiny and fragile flowers being

prone to injury during emasculation and pollination. Favorable results in artificial hybridization also depends upon the genotypes involved, and climatic conditions prevailing like temperature and humidity. For successful hybridization,

- Selection of appropriate size flower buds should be done
- Lateral buds should be preferred over the terminal ones (Sindhu et al. 1981);
- Complete care should be ensured during emasculation and pollination to keep away any mechanical injury to the floral parts
- Timings of pollination and fertilization are key determining factors for the success rate. Low temperatures, afternoon emasculation and pollination during the following morning are recommended. In case of high temperatures, morning emasculation followed by prompt pollination at same time is also successful (Bejiga and Tessema 1981; Pundir and Reddy 1984).

Inter-specific hybridization within the genus *Lens* shows crossability barriers both between and within the species (Ferguson et al. 2000; Ladizinsky 1997). The high crossability potential for accessions of *L. culinaris* ssp. *orientalis* and *L. culinaris* ssp. *culinaris* suggested ssp. *orientalis* as the wild progenitor of lentil (Ladizinsky et al. 1984). Davey et al. (2005) suggested that Inter-specific hybridization hurdles can be overcome via techniques like somatic hybridization and protoplast fusion. However, there are limited successful reports on application of these in lentil.

Mutation Breeding

Prolonged use of traditional breeding methods has narrowed the genetic variability. Other than recombination, mutation includes all types of heritable genetic changes in an organism. Mutations can aid plant breeding programs by creating novel genetic variability. Induced mutation breeding techniques are swift and cost-effective method for augmenting the genetic base and recreating genetic variability in lentil. Many successful studies have been carried out in this field (Toker et al. 2007; Nakagawa et al. 2011; Shu et al. 2012; Mba 2013; Tomlekova et al. 2014; Riaz and Gul 2015; Oladosu et al. 2016; Jankowicz-Cieslak et al. 2017). The success of mutation breeding depends upon the productiveness of induced mutations, a desirable frequency and spectrum of mutation and then the accuracy of breeders in their screening them effectively (Khan and Siddiqui 1992a,b; Manju and Gopimony 2009). The advantage of this method is that shorter time is required to breed improved varieties and the process does alter only a small portion and not the whole genome. The technique thus became a highly convenient tool for plant breeders to generate the much-needed genetic variation in different crop species, thereby providing them access to unexplored allelic combinations within the crop genome. In lentil mutation breeding, careful selection of mutants with altered morphological architecture is focused to find elite breeding lines for yield and yield-attributing traits. Induced mutagenesis is believed to be the best approach done in lentils for broadening the genetic base and to produce useful new mutants overcoming the genetic bottlenecks. Amongst the different plant breeding methods for self-pollinated crops, mutation breeding along with advanced modern genomic techniques is highly recommended to broaden the genetic base of lentil (Erskine et al.1998; Toker et al. 2007). Some studies have confirmed the effectiveness of chemical mutagenesis in

lentil (Toker et al. 2007; Gaikwad and Kothekar 2004; Sarker and Sharma 1989; Solanki and Sharma 1994, 1999). A combination of physical and chemical mutagen also has shown useful results for improvement of lentil and other pulses.

- *Past Achievements*

In the current era of climate change, sustainable agricultural intensification is a necessity to meet food demand and supply. Lentil is a great dietary staple owing to its high protein content and nutrient density. Generating polygenic variability for quantitative traits in lentils, like yield, is highly recommended. Mutation breeding has contributed to the development of 18 mutant varieties worldwide. Two cultivars, Ranjan and Rajendra Masoor 1, released from India, have different improved traits like high yield and spreading type, tolerance to low temperatures, early maturity and suitable for late sowing.

Applications of biotechnological advances

Success has been achieved by lentil breeders in the improvement of a number of monogenic traits using the conventional plant breeding techniques via selection and recombination. However, major economic traits like seed yield are quantitative with a complex polygenic mode of inheritance. Thus, execution of conventional plant breeding techniques for the improvement of these traits faces difficulties in precision, management and time durations. With the advances in biotechnological techniques like recombinant DNA technologies, molecular-marker based technologies and bioinformatics, lentil breeders can now use novel genetic variability into the cultivated gene pool. They can identify the background genetic network and introgress desirable traits into cultivars using genomics-assisted breeding (GAB) and genetic engineering techniques more precisely in lesser time.

In Vitro Culture

The application of *in vitro* culture techniques can be an efficient tool for management of genetic variability and acceleration of conventional breeding process in pulse crops (Gatti et al. 2016). One of the essential necessities for success in genetic transformation using *in vitro* culture is a reliable regeneration protocol. In comparison to the success attained in other grain legumes, this technique is difficult in lentil due to its recalcitrant nature (Sarker et al. 2003; Gatti et al. 2016). In the past few decades, techniques have improved progressively. The first partial success of using meri-stem tips as explants and obtaining lentil regenerants was achieved by Bajaj and Dhanju (1979). A lentil regeneration protocol from the hypocotyl and epicotyl-derived callus cells was put forth by Williams and McHughen (1986). Polanco et al. (1988) reported multiple shoot formation from shoot tips. As the techniques in such kind of studies advanced, better work as seen. A quick, effective and reproducible protocol for *in vitro* shoot regeneration by employing various explants and different concentrations of BAP (6-Benzylaminopurine, benzyl adenine) was reported by Omran et al. (2008). The results revealed successful *in vitro* shoot regeneration with a slight modification in the Murashige and Skoog (MS) medium. To a great extent, higher levels of BAP facilitated shoot regeneration in lentil genotypes. Also, decapitated embryos were the ideal explants for the highest shoot regeneration. MS medium supplemented with 25 mg/l indole butyric acid (IBA) showed 30% rooting efficiency (Sarker et al. 2003). The

use of cytokinin in the initial explants for multiple shoots regeneration has been linked to problems of root induction at a later stage (Mohamed et al. 1992). *In vitro* induction of flowering followed by pod formation and seed set directly from *in vitro* regenerated shoots was successfully demonstrated in lentil, which is remarkable for improving and shortening the breeding process (Sarker et al. 2012; Das et al. 2012). The *in vitro* embryo rescue technique has been applied for introgression of resistance to diseases like anthracnose, ascochyta blight and stemphylium blight (Fiala et al. 2009; Tullu et al. 2013; Saha et al. 2015). The *Agrobacterium tumefaciens* biolistic transformations including electroporation and particle bombardment are reported in lentil (Chowrira et al. 1996; Lurquin et al. 1998; Gulati et al. 2002; Mahmoudian et al. 2002). Transgenic lentils were produced with genes like *nptII*, *gus*A and *DREB1A* successfully (Akcay et al. 2009; Khatib et al. 2011). Transgenic lentil shoots were developed in two microsperma varieties using *A. tumefaciens* (Subroto et al. 2012). An efficient and reproducible protocol for *in vitro* shoot regeneration from cotyledonary node explants was reported by Bermejo et al. (2012) from which subsequent transgenic lentils were developed later (Bermejo et al. 2016).

Marker-Assisted Selection (MAS)

Marker is a tag that can identify a particular location within the plant genome. MAS gives an exceptional and efficient path to breeders by which they can select plants with elite gene combinations. A number of molecular markers for genetic analysis viz. RFLP, RAPD, AFLP, SSR, ISSR, have been used by lentil geneticists and breeders (Sharma et al. 1996; Havey and Muehlbauer 1989; Ferguson et al. 1998; Sonnante and Pignone 2001; Toklu et al. 2009; El-Nahas et al. 2011; Alghamdi et al. 2014; Dikshit et al. 2015; Idrissi et al. 2015; Tsanakas et al. 2018). However, due to inadequate availability and slow development of lentil genomic resources, the application of MAS in lentil-breeding programs is hindered and remains limited compared to other major legumes (Kumar et al. 2014).

Linkage analysis experiments were initiated in lentils by Zamir and Ladizinsky (1984) while the first DNA marker-based genetic map was developed by efforts of Havey and Muehlbauer (1989). The advent of PCR-based markers expanded genetic map studies in lentil. Eujayl et al. (1998) first published an extensive linkage map in lentil utilizing RAPD, AFLP, RFLP and morphological markers with an inter-specific cross between *Lens* ssp. *culinaris* and ssp. *orientalis*. The first intra-specific lentil map comprised of 114 RAPD, inter simple sequence repeats (ISSR) and resistance gene analog (RGA) markers (Rubeena and Taylor 2003). The first genomic library was constructed using lentil cultivar ILL5588 (Hamwieh et al. 2005a, b). SSR markers are preferred over other DNA markers for studying genetic diversity, population structure, phylogenetic relationships, construction of frame-work linkage maps, QTL interval mapping, map-based gene cloning, MAS, etc, owing to their reproducibility, co-dominant multi-allelic nature, high degree polymorphism, locus specific inheritance, relative abundance, and better genome coverage (Powell et al. 1996; Hendre et al. 2007). Several polymorphic and functional SSR markers were also evolved for genetic diversity studies and genomic library construction in lentil (Hamwieh et al. 2009; Verma et al. 2014; Andeden et al. 2015).

A molecular linkage map with 11 linkage groups of lentil was reported by Tanyolac et al. (2010) making use of AFLP, ISSR, RAPD markers covering 1396.3 cM with an average map distance of 8.4 cM between markers. Several genetic linkage maps have been constructed from various lentil mapping populations, but the marker density is low and span in cM is higher, making them impractical. Ates et al. (2018a) used Diversity Arrays Technology (DArT) makers to construct a high-density consensus linkage map comprising of seven linkage groups, representing seven chromosomes on lentil genome covering a total of 977.47 cM with an average inter-maker distance of 0.10 cM. Altogether, genetic maps reported in lentil have helped in the understanding of lentil genome (Eujayl et al. 1998; Kahraman et al. 2004; Tanyolac et al. 2010; Saha et al. 2010a,b; Ates et al. 2018a,b).

Qualitative markers were identified for morphological traits like cotyledon (Yc), anthocyanin in the stem (Gs), pod indehiscence (Pi), seed coat pattern (Scp), flower color (W), radiation frost tolerance locus (Rf), early flowering (Sn) and ground color of the seed (Gc), as they exhibited monogenic dominant inheritance (Tullu et al. 2003; Duran et al. 2004; Hamwieh et al. 2005a,b). Quantitatively inherited traits were mapped by Duran et al. (2004) which could identify QTLs for height of the first ramification, flowering time, plant height, pod dehiscence, shoot number and seed diameter. A study using RIL population of 106 lines derived from WA8649090 × Precoz identified QTLs that established winter survival and winter injury (Kahraman et al. 2004). A QTL that conveyed resistance to *Stemphylium* blight and rust diseases was identified using RIL populations (Saha et al. 2010a,b). Such advance breeding populations give higher efficacy in mapping studies and are still fewer such materials developed in lentil till date.

With advancements in biotechnological tools and molecular markers, there are novel concepts coming up like consensus linkage maps using multiple mapping populations. Although, the possibility and success of these still need to be worked in lentil. In present times, DNA chip-based markers using single nucleotide polymorphisms (SNPs) are gaining popularity over PCR-based markers in next generation sequencing (NGS) approaches with several success reports of identifying candidate genes for desirable traits (Sharpe et al. 2013; Temel et al. 2014; Bett et al. 2016). This headway will assist breeders to implement MAS in lentil breeding.

Next Generation Technologies

In the past, breeders' efforts in bringing success to breeding programs were not maximized due to narrow genetic base and unavailability of genomic resources in lentil. The progress in next generation sequencing (NGS) technology has provided new opportunities for lentil genome sequencing projects and speedy sequence-based marker development. Owing to the next generation DNA sequencing, some genome aasemblies, genomic map, QTLs have also been reported in different studies (Gupta et al. 2012; Bett et al. 2016). Transcriptome profiling of lentil has revealed key defense response genes against *Ascochyta* blight via RNA-seq analysis and quantitative real time-PCR (RT-qPCR approaches (Khorramdelazad et al. 2018; Sari et al. 2018). Identification of QTLs for Fe and Mn concentrations in the lentil genome through NGS will enhance future biofortification attempts in the crop (Aldemir et al. 2017; Ates et al. 2018b) The

high frequency of genomic variations induced by physical and chemical mutagens is accurately detectable by screening SNPs at the molecular level. High-throughput technique viz. target-induced local lesions in genomes (TILLING), can identify mutations in single nucleotide, contributing significantly to an understand gene(s) function.

Conclusion and Future Prospects

Lentil is predominantly auto-gamous legume crop with >1–6% out-crossing. Therefore, breeding methods applicable to both self- and cross-pollinated crops could be applied to its enhancement. The major breeding objectives in lentil is ultimately yield and yield stability in addition to resistance for biotic and abiotic stresses, higher grain protein quality, earliness and wider adaptability. Seed yield being a complex polygenic trait with indirect mode of inheritance, direct selection for yield is likely ineffective. To attain this, breeders need to identify new traits with major influence on yield. Studies cracking the genetics of complex traits by gene tagging of target traits are in demand. The advent in cutting edge advanced techniques have added pace to plant breeding through utilizing molecular markers, DNA sequencing, genomics and statistical and bioinformatics based tools. Crop improvement work plan and strategies have become automated, cost effective and reliable. DNA sequencing and various analysis methods are now permitting larger sample size in shorter with higher precision. The last decade witnessed development of linkage maps and identification of QTLs for the traits of interest in lentil. In recent times, clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) systems have gained much interest to plant geneticists and crop scientists (Rojo et al. 2018). The incorporation of genetic variability is of major concern in order to success a plant breeding program to develop a crop genotype with target traits. The count of use of markers for MAS and marker-assisted backcrossing (MABC) in lentil breeding is still on finger tips. These advances will simultaneously encourage lentil breeders in developing countries to incorporate MAS and MABC in lentil breeding programs. The ultimate aim is to select lines with genes for traits that lead to better yield and introgression of more desirable traits like resistance to stresses, multipodding or a determinate growth habit, nutrition and adaptability in lentil.

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