

Jitendra Kumar, Rohit Kant, Syed Mohd Quatadah,
Shiv Kumar, and Ashutosh Sarker

Abstract

Micronutrient deficiency affects more than two billion population worldwide especially in sub-Saharan Africa and South Asia. The deficiency in human body is commonly described as “hidden hunger” leading to a range of health hazards including low birth weight, anemia, learning disabilities, increased morbidity and mortality rates, and high health-care costs. Biofortification of food crop varieties with essential micronutrients is one of the potential means to address micronutrient deficiencies through crop breeding techniques. Grain legumes constitute the prime source of vegetarian diet in the developing world. Among grain legumes, lentil is a rich source of protein and other minerals including iron, zinc, selenium, folates, carotenoids, and vitamins. A wide range of genetic variability has been reported among the lentil germplasm with regard to nutrient-related traits. Therefore, lentil has been identified an ideal crop for micronutrient biofortification and a possible whole food solution to the global micronutrient malnutrition. Here, we discuss the current status of breeding interventions in nutritional enhancement of lentil varieties.

Keywords

Biofortification • Lentil • Nutrient • Genetic variability • Variety

J. Kumar (✉) • R. Kant • S.M. Quatadah
Division of Crop Improvement, ICAR-Indian Institute of
Pulses Research, Kanpur, Uttar Pradesh 208 024, India
e-mail: jitendra73@gmail.com; rohitkant.gpb@gmail.com;
squatadah@gmail.com

S. Kumar
International Center for Agricultural Research in the Dry
Areas, Beirut 1108-2010, Lebanon
e-mail: sk.agrawal@cgiar.org

A. Sarker
International Center for Agricultural Research in the Dry
Areas, CGIAR Block, NASC Complex, New Delhi
110 012, India
e-mail: A.Sarker@cgiar.org

12.1 Introduction

More than 22 mineral nutrients are required by the human body for its normal function (White and Broadley 2005). These minerals include calcium (Ca), phosphorus (P), sodium (Na), chlorine (Cl), potassium (K), magnesium (Mg), iron (Fe), copper (Cu), cobalt (Co), iodine (I), zinc (Zn), manganese (Mn), molybdenum (Mo), fluoride (F), chromium (Cr), selenium (Se), sulfur (S), boron (B), silicon (Si), arsenic (As), and nickel (Ni) (Murray et al. 2000; Eruvbetine 2003). Based on the quantity required by our body, these minerals can be classified as macro- and micronutrients or ultra micro elements. Micronutrient deficiency affects more than two billion population worldwide (Welch and Graham 1999), which is rampant in sub-Saharan Africa and South Asia. Micronutrient malnutrition is commonly known as “hidden hunger” and causes many health hazards including low birth weight, anemia, learning disabilities, increased morbidity and mortality rates, low work productivity, and high health-care costs especially in developing nations (Welch and Graham 1999; Batra and Seth 2002). Although deficiency of any of these essential elements can be seen in world population, Fe, Zn, Se, and I deficiencies are predominant in rural areas of Southeast Asia. Recent estimates indicate that about 60 % of world population are Fe deficient and 33 % are Zn deficient (Hotz et al. 2004) and 15 % are Se deficient (FAOSTAT 2011). Zn deficiency is generally prevalent in areas where soil is generally poor in available form. These areas include India, Pakistan, China, Iran, and Turkey (Hotz et al. 2004; Khan et al. 2008). Preschool children and pregnant women are mostly affected by Fe and Zn deficiencies due to excessive reliance on cereal-based diets, which are low in these essential micronutrients. In India, every year, 330,000 children die due to vitamin A deficiency and 22,000 people, mainly pregnant women, from severe anemia. Besides, 6.6 million children are born mentally impaired every year due to I deficiency, intellectual capacity is reduced in 15 %

of people due to I deficiency, and 200,000 babies are born every year with neural tube defects due to folic acid deficiency (Kotecha 2008). Therefore, it is essentially required to overcome these deficiencies by supplying the enriched diet with these minerals and vitamins. Development of staple food cultivars with enriched micronutrients is one of the viable options for combating global micronutrient malnutrition (Thavarajah et al. 2011). Biofortification of food crop varieties with essential micronutrients is one of the means to combat micronutrient deficiencies through classical plant genetic improvement.

12.2 Crop Biofortification Using Breeding Techniques

In genetic biofortification, plant breeding techniques are used to produce staple food crops with higher micronutrient levels, reducing levels of antinutrients and increasing the levels of substances that promote nutrient absorption in addition to higher yield (Bouis 2003). Plant breeders screen existing accessions in global germplasm banks to determine whether sufficient genetic variation exists to breed for a particular trait. They then selectively breed nutritious cultivars of major staples rich in Zn and Fe concentrations and with substances that promote the bioavailability of Zn and Fe. Biofortification of plant foods through genetic improvement has been introduced as a long-term and sustainable solution for increasing the bioavailability of minerals especially for lentils. This strategy has been widely accepted as a cost-effective way to minimize the extent of mineral deficiencies.

12.3 Lentil: A Nutritious Pulse Crop and Solution of Malnutrition

Lentil (*Lens culinaris* subsp. *culinaris* Medikus) is a cool season food legume. It is mostly cultivated in warm temperate, subtropical, and tropical regions of 52 countries on 3.6 million

Table 12.1 Nutritional value of lentil seeds

Protein	20–25 %
Carbohydrate	50–60 %
Fat	0.7–0.8 %
Ca	60–70 mg/100 g
Fe	7–9 mg/100 g
Zn	4–5 mg/100 g
Se	42–67 µg/100 g
Folate	261–290 µg/100 g

ha area with annual production of 3.6 million tons (FAOSTAT 2011). India is the major lentil producer in the world with 0.9 million tons of production harvested from 1.6 million ha area. Research done in Canada and the USA has revealed suitability of lentil crop for micronutrient biofortification as it holds the potential to provide solution to menacing problem of global micronutrient malnutrition (Thavarajah et al. 2011). It is a rich source of protein and other minerals including iron, zinc, selenium, folates, carotenoids, and vitamins (Thavarajah et al. 2011; Johnson et al. 2013; Gupta et al. 2013). Previous studies provided the proportions of micronutrients in lentil crop which is presented in Table 12.1. It has been shown that these values of micronutrients in 100 g lentil seeds could provide a minimum of 41–113 % of the recommended daily allowance (RDA) of Fe, 40–68 % of the RDA of Zn, and 77–122 % of the RDA of Se. Besides, lentils have been shown to be rich in beta-carotene and found 2–12 µg/g in lentils grown in the USA. Phytic acid (PA) is an antinutrient and high PA levels reduce mineral bioavailability. Lentils are naturally low in PA (2.5–4.4 mg/g), and these levels are lower than the level of PA found in other crops such as rice (1.22–2.23 mg/g), soybean (1.77–4.86 mg/g), wheat (1.24–2.51 mg/g), maize (3.3–3.7 mg/g), and common bean (0.52–1.38 mg/g). Low PA is a favorable factor to increase lentil mineral bioavailability. The genetic variability has also been observed among lentil genotypes, and certain lentil genotypes (e.g., CDC Milestone and CDC Redberry) are observed rich in highly bioavailable Fe and Se (Thavarajah et al. 2011). Lentils

cook faster (less than 10 min) and are therefore a better solution to reduce energy and cooking time demands. These observations clearly show that lentils could be an ideal crop for micronutrient biofortification and a possible whole food solution to the global micronutrient malnutrition (Thavarajah et al. 2011).

12.4 Geographical Variation for Micronutrients in Lentil

Mineral levels of lentils are known to vary with growing location and soil conditions. It has been shown among international lentil samples that concentration of micronutrients varies from one geographical region to other. For example, high concentrations of Fe has been observed in seed sample of Syria (63 mg/kg), Turkey (60 mg/kg), the USA (56 mg/kg), and Nepal (50 mg/kg), while it was observed low in seed sample of Australia (46 mg/kg) and Morocco (42 mg/kg). Similarly, higher Zn was found in lentils grown in Syria (36 mg/kg), Turkey (32 mg/kg), and the USA (28 mg/kg) and lowest in Australia (18 mg/kg) and Morocco (17 mg/kg). Examination of Se concentrations in lentil genotypes grown in eight major world regions indicated considerable variation. For example, Nepal and Australia showed greater Se concentrations of 180 and 148 µg/kg, respectively. Syria, Morocco, and Turkey had the lowest values of 22, 28, and 47 µg/kg, respectively. Turkey landraces showed higher amount of Ca in their seeds (0.48–1.28 g/kg), and analysis of Indian samples showed high to low Fe (>60 mg/kg) and Zn (20–40 mg/kg) in their seeds. Concerning genotypes, CDC Greenland had higher selenium content with low iron and zinc content compared to Merritt. However, Red Chief was low in selenium and high in iron, zinc, calcium, and potassium compared to the other cultivars. Spanish brown cultivar Pardina was rich in iron and zinc but low in selenium as it was mostly grown in low-selenium soils. Generally, green lentil cultivars were low in iron and zinc compared to selenium.

12.5 Presence of Substantial Genotype \times Environment ($G \times E$) Interactions

Soils of growing location, weather, and other environmental conditions affect mineral levels in lentil. A study conducted in the USA for green and red lentil genotypes for over 2 years for a number of micronutrients revealed significant differences in concentration of micronutrients over the years. The evaluation of seven lentil genotypes over four locations along with a farmer's filed survey conducted in Bangladesh revealed significant genotype and location differences for seed selenium concentration but genotype \times location interaction was nonsignificant. Similar results were also observed when 12 genotypes of lentil were evaluated over seven locations in Australia (Rahman et al. 2014). In other crops such as wheat, breeding for high Zn concentration is complicated by environmental conditions, particularly soil composition (Trethowan 2007). Therefore, despite advances in breeding for uptake efficiency or mobilization to the grain, grain Zn concentration is limited by Zn availability in the soil (Ortiz-Monasterio et al. 2007, 2011). Significant genotype \times location interactions were observed for Zn and Fe in wild and improved wheat cultivars (Oury et al. 2006; Ortiz-Monasterio et al. 2007; Trethowan 2007; Gomez-Becerra et al. 2010). However, a recent study tested biofortified wheat lines at multiple locations in South Asia and revealed high heritability and high genetic correlation between locations for grain Zn suggesting that $G \times E$ interactions may not be a serious issue in breeding high Zn wheat genotypes (Velu et al. 2012). However, mineral characteristics of the crop plants are largely influenced by the genetic and environmental factors such as soil fertility, soil type, seed characteristics, seed composition, and climatic factors; hence further studies are required to conduct experiments under different environmental conditions to validate the results.

12.6 Breeding for Improved Nutrient Content

Naturally, lentil is a storehouse of most of the essential nutrients for human health. However, there is still scope to enhance the nutritive value of its edible part because a range of variability has been observed among the lentil germplasm including cultivars, breeding lines, landraces, and wild species. This gives an opportunity to make selection of nutritionally appropriate breeding materials, which can be used directly for cultivation at farmer's fields or can be used as donors in breeding programs. Development of improved variety with enhanced nutritional value is a onetime investment and cost-effective strategy to overcome the malnutrition. Its cost is estimated on average of about \$400,000 per year per crop over a 10-year period, globally. Once biofortified varieties have been developed, in country trials and local adaptation research costs are incurred, after which routine maintenance breeding to ensure the trait remains stable is put in place. Development of lentil cultivars enriched with minerals and vitamins makes this as a biofortified crop. These cultivars can be developed by using the different approaches of breeding involving both conventional and modern techniques for increasing concentration and bioavailability of these essential elements in lentil (Welch 2002). Therefore, focus has been made on lentil biofortification, and different breeding strategies are being utilized for developing biofortified varieties as given below.

12.6.1 Screening of Existing Variability of Cultivated Species for Identification of Nutritionally Enriched Genotypes

Identification of natural variants having favorable alleles for enhanced micronutrient content in the germplasm is one way to develop the

improved cultivars for cultivation. Earlier significant variability has been observed for micronutrients in the germplasm from Turkey, Syria, Canada, and Pakistan (Thavarajah et al. 2011). These studies have identified lentil germplasm with favorable nutritional traits including folates, macro-, and micronutrients. Many landraces of lentil are grown in particular regions due to their some specific characters including taste preferences. These landraces are a potential genetic resource for biofortification of lentils with increased micronutrients. Initially, under the HarvestPlus Challenge Program, more than 1600 germplasm, breeding lines, released cultivars, and wild relatives of lentil were analyzed for iron and zinc contents. A wide variation of 43–132 ppm of iron and 22–95 ppm of zinc was found in these materials. Earlier studies mainly exploited the variability for micronutrients in the breeding materials generated from ICARDA, and no source of variability was identified in Indian germplasm (Thavarajah et al. 2011; Gupta et al. 2013). However recently we studied the variability among breeding lines, varieties and parental lines, and exotic lines. We observed a range of variability for ten macro- and micronutrients. In Indian germplasm, the amount of Ca and Mg was recorded much lower than Turkish germplasm, while the availability of P (3907.0–7261.58 mg) and K (4771.43–9633.90 mg) was observed higher compared to Turkish landraces (2860–5330 mg for P and 6380–9500 mg for K). Higher amount of Fe, Zn, and Se was observed in Indian varieties and breeding lines compared to exotic lines. These findings encouraged breeders to improve iron and zinc contents in lentil through recombination breeding having significant amount of all macronutrients. Also, ICARDA research with partners has identified several released varieties of lentil in Bangladesh, Ethiopia, Nepal, Morocco, Turkey, Syria, Lesotho, and Portugal that had high iron and zinc contents. These varieties are being disseminated to farmers on a fast-track mode through national program. In Bangladesh, the government has taken a massive dissemination program to promote promising

lentil varieties such as Barimasur 4 (86 ppm Fe and 63 ppm Zn) and Barimasur 5 (86 ppm Fe, 59 ppm Zn) and Barimasur 6 (86 ppm Fe and 63 ppm Zn) in traditional and nontraditional areas. Similarly, in Nepal lentil varieties such as Sishir (98 ppm Fe, 64 ppm Zn), Khajurah-2 (94 ppm Fe, 59 ppm Zn), Khajurah-1 (zn 58 ppm), and shital (59 ppm Zn) are spreading fast in the Terai region. In India, variety Pusa Vaibhav contains 102 ppm of Fe, and it is being grown by farmers in the northwest plain zone (ICARDA 2012).

12.6.2 Lentil Biofortification Through Pre-breeding

Wild species are a rich reservoir of useful alien genes, which are no longer available within the cultivated gene pool (Hawkes 1977; Doyle 1988; Tanksley and McCouch 1997). Continuous efforts have been underway to collect and conserve wild relatives of various food legume crops in national and international gene banks (Plucknett et al. 1987; FAO 1996). Over the years, ICARDA has collected and conserved 587 accessions representing six wild *Lens* species from 26 countries. In the past years, efforts have been made to search for genes imparting resistance to various stresses and other traits within the cultivated species and their wild relative. However, success of introgression of alien genes from wild relatives has been limited to a few diseases and insect pests, which are controlled by major gene(s), and could be available in crossable primary gene pool (Knott and Dvorak 1976; Stalker 1980; Ladizinsky et al. 1988; Prescott-Allen and Prescott-Allen 1986, 1988; Hajjar and Hodgkin 2007). To diversify and broaden the genetic base of cultivated germplasm, introgression of alien genes from wild species needs to be persuaded vigorously not only to minimize the risk of stress epidemics but also to make discernible yield advances in lentil. The advances in tissue culture techniques can now help to make feasible alien gene introgression easy. Moreover, evolutionary forces can make changes in accessions of wild species toward the possible cross-

compatibility with cultivated species. Therefore earlier cross-incompatible wild species may now cross with cultivated species. Therefore, pre-breeding efforts are urgently required involving particularly those wild species, which carry useful alien genes for improving yield, quality, and stress resistance.

12.6.2.1 Crossability Group and Gene Pool

Knowledge of crossability between cultivated and wild species is essential for successful pre-breeding activities. Previously on the basis of crossability between the lentil species, crop species of lentil have been grouped into primary (*Lens culinaris* ssp. *culinaris*, *L. culinaris* ssp. *orientalis*, *L. odemensis*), secondary (*L. ervoides*, *L. nigricans*), and tertiary gene pools (*L. lamottei*, *L. tomentosus*) (Ladizinsky et al. 1984; Ladizinsky 1999; Muehlbauer and McPhee 2005). Crossing between species of primary gene pool and *L. nigricans* and *L. ervoides* produces nonviable seed in hybrids due to irregular meiosis (Ladizinsky et al. 1984, 1985). However, use of embryo rescue can produce viable seed in hybrids derived from crossing between *L. culinaris* and *L. ervoides* (Ladizinsky et al. 1985). *L. tomentosus* was put in tertiary gene pool, as a single species group, and this species does not produce viable seed in hybrids derived by crossing with other group of species. Although useful genes identified in the primary gene pool have readily been used for making genetic improvement by generating new variability, frequency of occurrence of the useful genes has been observed much more in the species of the secondary and tertiary gene pools (Collard et al. 2001; Mallikarjuna et al. 2006; Tullu et al. 2006). To use these gene pools in the improvement of lentil, it requires much effort on deployment of novel techniques. During the past years thus Fratini and Ruiz (2006) suggested that hybrid between *L. culinaris* ssp. *culinaris* and *L. nigricans*, *L. ervoides*, and *L. odemensis* can be viable through embryo rescue with a rate of 3–9 %. Based on these observations, *L. odemensis* has been considered secondary gene pool and *L. nigricans* and *L. ervoides* were

classified in the tertiary gene pool (Ladizinsky 1993). However, Cubero et al. 2009 suggested placing *L. odemensis* in secondary gene pool, and *L. nigricans* and *L. ervoides* can be part of secondary gene pool by means of embryo rescue, while there is need to study hybridization in order to establish place of *L. tomentosus* and *L. lamottei* in secondary or tertiary gene pool.

Releasing of new hidden variability present in the background of wild species for nutritional traits depends upon the cross-compatibility of these species with cultivated species. Therefore, efforts have been made over the past years in lentil to study the cross-compatibility of wild species with cultivated species. The wild species *L. culinaris* ssp. *orientalis* and *L. odemensis* have been shown crossable with cultivated lentil (Ladizinsky et al. 1984; Abbo and Ladizinsky 1991, 1994; Fratini et al. 2004; Fratini and Ruiz 2006; Muehlbauer et al. 2006), although the fertility of the hybrids depends on the chromosome arrangement of the wild parent (Ladizinsky 1979; Ladizinsky et al. 1984). Most accessions of *L. culinaris* ssp. *orientalis* cross readily with *L. culinaris*, and both are genetically isolated from the other species. *Lens nigricans* and *L. ervoides* are not readily crossable with the cultivated lentil using conventional crossing methods due to hybrid embryo breakdown (Abbo and Ladizinsky 1991, 1994; Gupta and Sharma 2005). Crosses are possible between *L. culinaris* and the remaining species, but they are characterized by a high frequency of hybrid embryo abortion, albino seedlings, and chromosomal rearrangements that result in hybrid sterility, if these seedlings reach maturity (Abbo and Ladizinsky 1991, 1994; Ladizinsky 1993; Gupta and Sharma 2005).

12.6.2.2 Introgression of Alien Genes for Nutritional Traits from Wild Relatives

The wild relatives are important source for generation of new variability through recombination breeding approaches. The evidences from other crops such as wheat indicated that wild relatives have substantial variation for nutritional traits. For example, >3000 germplasm accessions,

including hexaploid, tetraploid, and diploid sources from the International Maize and Wheat Improvement Center (CIMMYT) gene bank, have been screened for Zn and Fe variation (Monasterio and Graham 2000). Highest Zn and Fe concentrations are observed among accessions belonging to progenitors of wheat and landraces (Cakmak et al. 2000; Ortiz-Monasterio et al. 2007) and little variation in improved adapted wheat varieties. Therefore more focus has been given on a more in-depth evaluation of wheat landraces (Monasterio and Graham 2000) and secondary gene pool in wheat for identification of the most promising sources of high Fe and Zn grain concentration (Cakmak et al. 2000). In lentil, however, limited efforts were invested to identify the wild relatives enriched with minerals and vitamins. The study conducted at IIPR, Kanpur, with ten accessions of lentil wild species revealed protein content of 29.7 %, phenol content 8.9 mg/100 g, and higher total antioxidant activity (16.17 μ mole TE/g), which was higher than that found among the cultivated accessions. Therefore, concerted efforts are required to screen more wild resources of lentil for minerals and other nutritional traits. These identified resources can be utilized in wide hybridization for transferring useful genes from alien species into cultivated species. It is well recognized that gene transfer through wide crosses is a long and tedious process due to lack of homology between chromosomes of participating species in the cross and pre- and post-zygotic crossability barriers between wild and cultivated species. Utilizing wild gene pool in breeding program is also constrained by collection gaps in wild species with poor or no information about genome relationship, poor/limited screening of wild species, linkage drag, and genetic complexity of the traits. Therefore, improvement through distant hybridization often takes longer time in order to recover genotypes with acceptable agronomic background, and thus, it requires a long-term approach to be in place.

12.6.2.3 Limitations in Pre-breeding

In lentil, strong crossability barriers limit utilization of wild gene pool for lentil improvement.

Among these, problem of chromosome pairing was observed between the participating genomes in the cross between *L. culinaris* and *L. tomentosus* (Ladizinsky 1979). Only retardation of the growth of hybrid embryo was noticed, while no sign was detected concerning endosperm disintegration in some *L. culinaris* \times *L. culinaris* ssp. *orientalis* crosses (Ladizinsky 1993). Later on, Abbo and Ladizinsky (1991) observed either absence or abnormal endosperm in *L. culinaris* \times *L. culinaris* ssp. *orientalis* crosses. Hybrids showed varying degrees of fertility usually due to chromosome translocations and subsequent problems with chromosome pairing at meiosis in *Lens culinaris* \times *L. nigricans* (Goshen et al. 1982; Ladizinsky et al. 1984). Fertility is often very low with less viable pollen produced in anthers and the extent varied depending on the accession in *L. culinaris* \times *L. culinaris* ssp. *orientalis* crosses from 2 % to 69 % (Ladizinsky et al. 1984). These problems can occur in the F₁ and also persist in later generations causing partial or complete sterility. Additionally, albino seedlings can also occur in the F₁ generation and thus prevent hybridization success (Ladizinsky and Abbo 1993). Another common problem is that hybrid embryos cease to grow about 7–14 days after pollination due to endosperm degeneration and thus need rescuing in order to obtain viable hybrids (Ahmad et al. 1995; Ladizinsky et al. 1985). Hence, *L. culinaris* \times *L. ervoides* or *L. culinaris* \times *L. nigricans* crosses need embryo rescue techniques in order to develop mature hybrid plants (Abbo and Ladizinsky 1991; Cohen et al. 1984).

12.7 Markers Assisted Breeding for Biofortification in Lentil

Marker-assisted selection (MAS) is an effective alternative to genetic engineering strategies for biofortification of crops. Successful examples include increased levels of provitamin A (beta-carotene) in maize; a team of plant geneticists

and crop scientists recently identified genetic markers that are associated with higher levels of provitamin A in maize. PCR-based DNA markers distinguishing alleles of three key genes of maize endosperm carotenoid biosynthesis (PSY1, lcyE, and crtRB1) have been developed, which can help to assess provitamin A concentration in maize kernels easily. Thus maize varieties with increased provitamin A contents to combat vitamin A deficiencies can easily be bred using MAS.

Significant progress can be made through conventional breeding; 85 % of HarvestPlus resources are currently devoted to conventional breeding. However, transgenic approaches are in some cases necessary and, in some cases, potentially advantageous compared with conventional breeding. The best-known example is Golden Rice; b-carotene has not been identified in the endosperm of any rice variety, and an advanced transgenic line containing 37 mg/g carotenoid, of which 31 mg/g is b-carotene, is now available (Paine et al. 2005). Ongoing transgenic research is exploring the use of an endosperm-specific promoter to deposit iron within the endosperm of rice so that it is not milled away (Goto et al. 1999). This approach is necessary because most of the iron in rice grains is deposited in the aleurone layer, which is removed when rice is milled to produce polished rice, a practice widely used in many countries (Gregorio et al. 2000). The QTL has also been identified in rice for biofortification of Zn. The functional markers associated with Zn content in RIL populations showed significant variation among RIL population with a phenotypic variation of 4.5 %, 19.0 %, 5.1 %, and 10.2 %, respectively. However no significant efforts were made in lentil. Initially we screened the parental lines of a mapping population that identified significant differences for Fe and Zn in the mapping populations.

12.8 Future Directions

As discussed above, more than three billion people worldwide suffer from mineral deficiencies.

Lentil is an indispensable supplementary food in many countries, particularly in South Asia, West Asia, North and East, and North Africa. In South Asia, particularly in Pakistan, Nepal, Bhutan, India, and Bangladesh, red lentil is an integral part of daily diet, most importantly among rural population. Owing to its abundant nutritional and functional components, red lentil is very popular in Turkey and other Mediterranean countries. Thus, even a small increase in the nutritive value of lentil seed can have remarkable impacts on human nutrition. In summary, considerable variation in the macro- and micronutrient contents of lentil landraces and cultivars was observed. Identification of genetic variation is essential for achieving improvements in the mineral content of crops. Our results provide a useful foundation for the development of new cultivars of lentil with high mineral content. In particular, some of the landraces that were studied could be used to develop lentil varieties with more nutrient contents to address mineral deficiencies in developing countries. The observed genetic variation can also be used to identify quantitative trait loci associated with mineral uptake and transport. The data provided in this study gives us an important basis for improvement of micronutrient contents in lentil.

12.9 Conclusion

Lentil being a nutritious grain legume crop provides an appropriate candidate crop for nutritional enrichment. To serve the purpose, a range of scientific interventions were proposed of which crop breeding techniques remain cost effective and accessible, providing solutions to the growing problem of food insecurity worldwide. In the context, reasonable amount of genetic variation for nutrient traits has been reported so far in lentil, which constitutes the robust base for the downstream breeding methods. Among various conventional methods, utilization of wild relatives offers the most potential researchable area, and significant progress has been made toward this end. More importantly, unlike transgenic technologies, enriching

nutrients in edible parts of crops using breeding protocols does not face any legislative or socio-political challenge. Once investment has been done in developing a nutrient-dense cultivar, the nutritional benefits can be reaped in a sustainable and economically viable manner.

References

- Abbo S, Ladizinsky G (1991) Anatomical aspects of hybrid embryo abortion in the genus *Lens* L. *Bot Gaz* 152:316–320
- Abbo S, Ladizinsky G (1994) Genetical aspects of hybrid embryo abortion in the genus *Lens* L. *Heredity* 72:193–200
- Ahmad M, Fautrier AG, McNeil DL, Burritt DJ, Hill GD (1995) Attempts to overcome post-fertilization barrier in interspecific crosses of the genus *Lens*. *Plant Breed* 114:558–560
- Batra J, Seth PK (2002) Effect of iron deficiency on developing rat brain. *Indian J Clin Biochem* 17:108–114
- Bouis HE (2003) Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? *Proc Nutr Soc* 62:403e411
- Cakmak I, Ozkan H, Braun H-J, Welch RM, Romheld V (2000) Zinc and iron concentrations in seeds of wild primitive and modern wheats. *Food Nutr Bull* 21:e401–e403
- Cohen D, Ladizinsky G, Ziv M, Muehlbauer FJ (1984) Rescue of interspecific *Lens* hybrids by means of embryo culture. *Plant Cell Tissue Org* 3:343–347
- Collard BCY, Ades PK, Pang ECK, Brouwer JB, Taylor PWJ (2001) Prospecting for sources of resistance to ascochyta blight in wild *Cicer* species. *Australas Plant Path* 30:271–276
- Cubero JI, Perez de la Vega M, Fratini R (2009) Origin phylogeny domestication and spread. In: Erskine W, Muehlbauer FJ, Sarker BS (eds) *The lentil: botany production and uses*. CABI Oxfordshire, UK, pp 13–33
- Doyle JJ (1988) 5S ribosomal gene variation in the soybean and its progenitor. *Theor Appl Genet* 75:621–624
- Eruvbetine D (2003) Canine nutrition and health. A paper presented at the seminar organized by Kensington Pharmaceuticals Nig Ltd Lagos. Accessed 21 Aug 2003
- FAO (1996) State of the world's plant genetic resources for food and agriculture. Food and Agriculture Organization, Rome, p 131
- FAOSTAT (2011) <http://faostat.fao.org/>. Accessed 14 Mar 2015
- Fratini R, Ruiz ML (2006) Interspecific hybridization in the genus *Lens* applying in vitro embryo rescue. *Euphytica* 150:271–280
- Fratini R, Ruiz ML, Perez de la Vega M (2004) Intra-specific and inter-sub-specific crossing in lentil (*Lens culinaris* Medik). *Can J Plant Pathol* 84:981–986
- Gomez-Becerra HF, Yazici A, Ozturk L, Budak H, Peleg Z, Morgounov A, Fahima T, Saranga Y, Cakmak I (2010) Genetic variation and environmental stability of grain mineral nutrient concentrations in *Triticum dicoccoides* under five environments. *Euphytica* 171:39–52
- Goshen D, Ladizinsky G, Muehlbauer FJ (1982) Restoration of meiotic regularity and fertility among derivatives of *Lens culinaris* × *L nigricans* hybrids. *Euphytica* 31:795–799
- Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F (1999) Iron fortification of rice seed by the soybean ferritin gene. *Nat Biotechnol* 17:282–286
- Gregorio GB, Senadhira D, Htut H, Graham RD (2000) Breeding for trace mineral density in rice. *Food Nutr Bull* 21:382–386
- Gupta D, Sharma SK (2005) Embryo-ovule rescue technique for overcoming post-fertilization barriers in interspecific crosses of *Lens*. *J Lentil Res* 2:27–30
- Gupta DS, Thavarajah D, Knutson P, Thavarajah P, McGee RJ, Coyne CJ, Kumar S (2013) Lentils (*Lens culinaris* L) a rich source of folates. *J Agric Food Chem* 61:7794–7799
- Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement a survey of developments over the last 20 years. *Euphytica* 156:1–13
- Hawkes JG (1977) The importance of wild germplasm in plant breeding. *Euphytica* 26:615–621
- Hotz C, Brown KH, Rivera JA et al (2004) Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr Bull* 25:S94–S204
- ICARDA (2012) Biofortified lentils to enhance nutritional security in South Asia. ICARDA-South Asia and China regional program, New Delhi, p 2
- Johnson C, Thavarajah D, Thavarajah P (2013) The influence of phenolic and phytic acid food matrix factors on iron bioavailability potential in 10 commercial lentil genotypes (*Lens culinaris* L). *J Food Compos Anal* 31:82–86
- Khan MA, Fuller MP, Baloch FS (2008) Effect of soil applied zinc sulphate on wheat (*Triticum aestivum* L) grown on a calcareous soil in Pakistan. *Cereal Res Commun* 36:571–582
- Knott DR, Dvorak J (1976) Alien germplasm as a source of resistance to diseases. *Annu Rev Phytopathol* 14:211–235
- Kotecha PV (2008) Micronutrient malnutrition in India: let us say “no” to it now. *Indian J Community Med* 33:9–10
- Ladizinsky G (1979) The origin of lentil and its wild gene pool. *Euphytica* 28:179–187

- Ladizinsky G (1993) Wild lentils. *Crit Rev Plant Sci* 12:169–184
- Ladizinsky G (1999) Identification of the lentil's wild genetic stock. *Genet Resour Crop Evol* 46:115–118
- Ladizinsky G, Abbo S (1993) Cryptic speciation in *Lens culinaris*. *Genet Resour Crop Evol* 40:1–5
- Ladizinsky G, Braun D, Goshen D, Muehlbauer FJ (1984) The biological species of the genus *Lens* L. *Bot Gaz* 145:253–261
- Ladizinsky G, Cohen D, Muehlbauer FJ (1985) Hybridization in the genus *Lens* by means of embryo culture. *Theor Appl Genet* 70:97–101
- Ladizinsky G, Pickersgill B, Yamamoto K (1988) Exploitation of wild relatives of the food legumes. In: Summerfield RJ (ed) *World crops cool season food legumes*. Kluwer, Dordrecht, pp 967–987
- Mallikarjuna N, Jadhav D, Reddy P (2006) Introgression of *Cajanus platycarpus* genome into cultivated pigeon pea *C. cajan*. *Euphytica* 149:161–167
- Monasterio I, Graham RD (2000) Breeding for trace minerals in wheat. *Food Nutr Bull* 21:393e396
- Muehlbauer FJ, McPhee KE (2005) Lentil (*Lens culinaris* Medik). In: Singh RJ, Jauhar PP (eds) *Genetic resources chromosome engineering and crop improvement grain legumes*. Taylor & Francis, Boca Raton, pp 219–230
- Muehlbauer FJ, Cho S, Sarker A, McPhee KE, Coyne CJ, Rajesh PN, Ford R (2006) Application of biotechnology in breeding lentil for resistance to biotic and abiotic stress. *Euphytica* 147:149–165
- Murray RK, Granner DK, Mayes PA, Rodwell VW (2000) *Harper's biochemistry*, 25th edn. McGraw-Hill Health Profession Division, USA pp. 780–786
- Ortiz-Monasterio I, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena RJ (2007) Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *J Cereal Sci* 46:293e307
- Ortiz-Monasterio I, Trethowan R, Holm PB, Cakmak I, Borg S, Tauris BEB, Brinch-Pedersen H (2011) Breeding transformation and physiological strategies for the development of wheat with high zinc and iron grain concentration. In: Bonjean AP, Angus WJ, Van Ginkel M (eds) *The world wheat book: a history of wheat breeding*, vol 2., pp 951–977
- Oury FX, Leenhardt F, Révész C, Chanliaud E, Duperrier B, Balfouriera F, Charmet G (2006) Genetic variability and stability of grain magnesium zinc and iron concentration in bread wheat. *Eur J Agron* 25:177–185
- Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G et al (2005) A new version of golden rice with increased pro-vitamin A content. *Nat Biotechnol* 23:482–487
- Plucknett DL, Smith NJH, Williams JT, Anishetty NM (1987) *Gene banks and the world's food*. Princeton University Press, New Jersey
- Prescott-Allen C, Prescott-Allen R (1986) *The first resource: wild species in the north American economy*. Yale University, New Haven
- Prescott-Allen C, Prescott-Allen R (1988) *Genes from the wild: using wild genetic resources for food and raw materials*. International Institute for Environment and Development, London
- Rahman MM, Erskine W, Siddique KHM, Thavarajah P, Thavarajah D, Zaman MS, Materne MA, McMurray LM (2014) Selenium biofortification of lentil in Australia and Bangladesh. In: 6th international food legume research conference, TCU Place, Sakatoon, 7–11 July 2014
- Stalker HT (1980) Utilization of wild species for crop improvement. *Adv Agron* 33:111–147
- Tanksley SD, McCouch SR (1997) Seed banks and molecular maps unlocking genetic potential from the wild. *Science* 277:1063–1066
- Thavarajah D, Thavarajah P, Wejesuriya A, Rutzke M, Glahn RP, Combs GF Jr, Vandenberg A (2011) The potential of lentil (*Lens culinaris* L) as a whole food for increased selenium iron and zinc intake: preliminary results from a 3 year study. *Euphytica* 180:123–128
- Trethowan RM (2007) Breeding wheat for high iron and zinc at CIMMYT: state of the art challenges and future prospects. In: *Proceedings of the 7th international wheat conference*, Mar del Plata
- Tullu A, Buchwaldt L, Lulsdorf M, Banniza S, Barlow B, Slinkard AE, Sarker A, Tar'an TD, Warkentin TD, Vandenberg A (2006) Sources of resistance to anthracnose (*Colletotrichum truncatum*) in wild *Lens* species. *Genet Resour Crop Evol* 53:111–119
- Velu G, Singh RP, Huerta-Espino J, Peña-Bautista RJ, Arun B, Mahendru-Singh A et al (2012) Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations. *Field Crop Res* 137:261–267
- Welch MR, Graham DR (1999) A new paradigm for world agriculture: meeting human needs productive sustainable nutritious. *Field Crop Res* 60:1–10
- Welch RM (2002) Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. *J Nutr* 132:495S–499S
- White PJ, Broadley MR (2005) Biofortifying crops with essential mineral elements. *Trends Plant Sci* 10:588–593