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Full Length Review Article

F_1 seed mutagenesis: A novel technique of creating genetic variability in lentil

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Abstract

Plant genetic resources are the guarantee of the world food security. Genetic resources possess genetic variability contained in traditional varieties, cultivars, wild crop relatives and other wild species. Hybridization is the conventional method used to create genetic variation. Induced mutations are also a good source of creating genetic variability. In F_1 seed mutagenesis, F_1M_0 seeds are mutated with different doses of mutagens (EMS or Gamma rays) and are planted in the field to get F_2M_1 generation and then F_2M_1 seeds are planted in the field to get F_2M_3 generation. Segregating generation (F_2M_3) creates a large amount of genetic variation desired by the plant breeders to develop high-yielding and disease resistant varieties. We have recommended that combination of both these approaches i.e. hybridization and induced mutations (F_1 seed mutagenesis) will create a large amount of genetic variation in lentil. Breeding scheme for F_1 seed mutagenesis was also given. Breeders should use this novel strategy for creating genetic variation in lentil as well as in other crops.

Keywords: Hybridization, induced mutations, *Lens culinaris*.

Introduction

Plant genetic resources are the guarantee of world food security. Genetic resources possess genetic material variability contained in traditional varieties, cultivars, wild crop relatives and other wild species (Farshadfar and Farshadfar, 2008; Aslam *et al*., 2013). The lens is a small Mediterranean genus that comprises the cultivated lentil (*Lens culinaris* Medikus sub sp. *culinaris*) and six related taxa (Bermejo *et al*., 2010). Lentil plays a vital role in nutritional and food security of million low-income Asian families because it has high protein content in seed (Erskine *et al*., 2011). A big amount of foreign exchange is being spent every year on lentil import to meet local demand. It is planted on 17.1 thousand hectares, with a production of 8.1 thousand tons in Pakistan. Lentil production is continuously decreasing from previous ten years, and it decreased by 5.8% during 2014-2015 (Anonymous, 2014-15). Also, low genetic variation and useless exotic germplasm (due to lower adaptability and attack of viruses) are the key reasons for the low yield potential of lentil (Ali *et al*., 2010). It is, therefore, very crucial to increase lentil production either through increasing its area or by developing high yielding varieties. The amount of genetic variation is crucial than total (phenotypic) variation (Total variation contains genotypic as well as environmental variation. Therefore, genetic variation is more important than total (phenotypic) variation for the breeders) to enhance yield potential of a crop (Ali *et al*., 2010). Therefore, it is the need of the hour to evaluate various methods of creation of genetic variation and to select the most appropriate one which may assist the breeders to create genetic variation in various morphological traits of lentil. In this review article, we focused on genetic variability and hybridization, induced mutations and F_1 seed mutagenesis as sources of creating genetic variability in lentil. To our knowledge this review topic is unique, and little information is available till now.

Genetic Variability

The presence of genetic variability for valuable traits is mandatory for effective use of plant breeding in the improvement of crop plants (Maqbool *et al.*, 2015; Aslam *et al.*, 2016; Maqbool *et al.*, 2016; Aslam *et al.*, 2017; Asadullah *et al.*, 2017). The narrow genetic base of the present cultivars and damages due to biotic and abiotic stresses are the main threats to the lentil

improvement. Introgression of genes into macrosperma (large-seeded) and microsperma (small seeded) groups of lentil from wild relatives like *Lens Culinaris* sub-spp. Orientalis and use of biotechnological techniques, wherever essential, have been suggested by several researchers to expand the genetic base of this crop (Singh *et al*., 2011). Polygenic variations among the individuals may be phenotypic, genotypic and environmental and their coefficients provide an idea to assess the magnitude of variability (Nausherwan *et al*., 2008).

Hybridization as a source of creating genetic variation

Normally, hybridization is used to create genetic variation in lentil, but it requires tiresome exertions. Lentil has small flowers due to which its emasculation and pollination is tough and needs more time that limit the use of hybridization for the creation of genetic variability in it (Akhtar *et al*., 2015). Hybridization between two genetically different individuals (parents) produces desirable recombinants. Genetic relationship between wild relatives and their cultivated species provide an additional valuable source of variations for desirable characters (Pandiyan *et al*., 2012). Some model examples about the use of hybridization in lentil are given subsequently. Lentil is grown about 55 countries in the world. Top ten (10) lentil producing countries are Canada, India, Turkey, Australia, Nepal, Bangladesh, United States of America, Ethiopia, China mainland and China (http://www.mapsofworld.com/world-top-ten/lentil-producing-countries.html). In India, 13 varieties of lentil were developed by hybridization mostly having related parents (Singh *et al*., 2011). The latest lentil varieties developed through hybridization with trait improved, year of release in Pakistan and India are presented in Table 2. Chauhan and Singh (1998) used six (6) parental lines and 15 F_{1s} as an experimental material to study genetic variation, inheritance and genetic advance for yield and 15 other traits in lentil. Maximum genetic variability was noted for pods/plant, days to maturity and harvest index. Anbessa *et al*. (2006) studied three F_2 sib populations: 272-2×CDC-Anna, 298T-9×CDC-Anna, 298T-9×CDC-Fronteir. F_3 and F_4 populations were evaluated and found the significant genetic variance for days to flower and days to maturity in chickpea.

Studies on genetic variability in F_3 population for various morphological traits through the determination of genetic parameters like coefficients of variability, heritability, and genetic advance have been reported in the literature (Kausar, 2005; Abbas *et al*., 2016). Heritability magnitude was 98% for days taken to maturity and number of pods per plant, 97% for seed yield per plant and 93% for plant height and 100-seed weight (Akhtar *et al*., 2015). Kishore and Gupta (2002) evaluated progenies (F_3 and F_4 generation) of crosses between micro and macrosperma lentil in augmented design and found highest genetic variability for biological yield per plant, hundred seed weight, seed yield per plant, days to flowering, days to maturity, and harvest index. Higher values of genotypic coefficient of variation (it is the ratio of square root of genotypic variance to mean multiplied by 100. It shows how much variation is due to genetic effects) and the genetic advance was also observed for seed yield per plant and biological yield per plant.

The work was done by El-Titi (1988) on F_2, F_3, F_4 generations to estimate genetic variation in five crosses of lentil revealed a large amount of genetic variation between crosses and among generations, and transgressive segregants were observed in 100-seed weight and seed yield per plant. Broad sense heritability was calculated for hundred seed weight, a number of pods per plant and plant height and ranged from 0.03-0.93, 0.04-0.89 and 0.02-0.92 respectively. Seed yield per plant and number of pods per plant showed genetic advance ranging from 3.8-29.0% and 7.1-54.5%, respectively. Moreover, the desired variability does not exist most of the time. Therefore, radiation-induced mutations can be used to produce genetic variability from which desirable mutants may easily be chosen.

Induced Mutations as a source of creating genetic variation

Induced mutations are useful to create genetic variability for crop improvement (Gandhi *et al*., 2012; Satpute and Fultambkar, 2012). Mutation breeding is another suitable methodology for crop improvement (Solanki and Sharma, 2002) which plays a significant role in plant breeding (Ali and Shaikh, 2007). The mutation causing agents can induce variation in the genetic makeup of an individual, disrupt linkages and create novel traits responsible for crop improvement (Shah *et al*., 2008). It creates genetic variability for traits inherited either quantitatively or qualitatively (Khan *et al*., 2004). Extensive use of mutation breeding in cereal crops has been mostly reported. But its use in the improvement of pulses was comparatively less (Shah *et al*., 2008; Abbas *et al*., 2016). However, during last 70 years, more than 2252 mutant varieties of cereals, oilseeds, pulses, vegetables, fruits, fibers, and ornamentals have been officially released in most of the countries (Maluszynski *et al*., 2000). The lentil varieties developed through mutation breeding with trait improved, year of release and country of origin are presented in Table 1. Variation for important morphological traits, using mutation breeding approaches has been reported in the literature (Singh *et al*., 2011). Two diverse genotypes of

lentil, Masoor 85 and ICARDA-8 were exposed to a range of radiations (100 to 600 Gy) by Ali and Shaikh (2007), and a mutant strain (AEL 49/20) in an M_2 generation was found. Same genotypes were also treated with 100 to 600 Gy gamma rays to create genetic variability for drought resistance in an M_2 generation (Ali *et al.*, 2010). A drought resistant mutant strain (AEL 23/40) developed after that revealed the best performance. Another evidence of studies on trait relationships, heritability and the genetic advance of carefully chosen induced mutants of lentil in an M_4 generation has been reported in the literature (Singh *et al*., 2007). It has been recorded that an induced mutant line MP-4 of lentil had 1.5 times more seed yield than control and revealed a vast range of phenotypic and genotypic coefficient of variation and genetic advance (Singh *et al.*, 2007).

An experiment was performed by Shweta (2008) using induced mutations to determine the extent and nature of genetic variability in the M_4 generation of soybean. One hundred and twenty-three mutants of JS-335 and 120 mutants of KHSb-2 soybean genotypes were used. Genetic parameters like genotypic and phenotypic coefficients of variability and genetic advance were computed for various morphological characters such as the number of branches per plant, plant height, pod weight per plant, the number of pods per plant and seed yield. Higher values of genotypic and phenotypic coefficients, heritability and genetic advance were observed for the number of branches per plant, plant height, pod weight per plant, the number of pods per plant and seed yield. While, low values of genotypic and phenotypic coefficients, heritability and genetic advance were recorded for days to flower and days to mature. Satpute and Fultambkar (2012) calculated the effectiveness and efficiency of mutagens (EMS and gamma rays) based on biological damage in two varieties of soybean (MAUS-71 and JS-335) and observed higher frequency and range of mutations induced by both of the mutagens. Their effectiveness increased at lower doses while decreased at higher concentrations. Kavithamani *et al*. (2008) also induced mutations in two soybean genotypes i.e. Himso 1563 and TS 82 by treating the seeds with different doses of gamma rays and concentrations of EMS and concluded that EMS was found effective and gamma rays found efficient. The comparison of effectiveness and efficiency of mutagens like gamma rays, EMS, and their combined treatment has been mostly reported in the literature (Khadr and Shukry, 1972). It has been found that combined treatment, in general, proved to be more effective followed by individual treatments of EMS and Gamma rays in an M_2 generation in chickpea and it causes less biological damage thus inducing maximum amount of mutations (Wani, 2009). A similar comparison of mutagenic treatments was also performed in cowpea in the M_2 generation, and greater frequency of mutations in combined treatment was recorded (Girija and Dhanaval, 2009). Begum and Dasgupta (2010) compared chemical and physical mutagens in sesame (*Sesamum indicum* L.) in the M_3 generation and found that EMS is more effective than Gamma rays. It may be concluded from these studies that the order of mutagenic efficiency could be Gamma rays + EMS > EMS > Gamma rays.

F_1 seed mutagenesis as a source of creating genetic variation

In literature, the reports of comparison of different sources of creating genetic variability are not well documented. In this technique, F_1M_0 seeds are mutated with different doses of mutagens (EMS or Gamma rays) and are planted in the field to get F_2M_1 generation and then F_2M_1 seeds are planted in the field to get F_2M_3 generation. Segregating generation (F_2M_3) creates a large amount of genetic variation desired by the plant breeders to develop high-yielding and disease resistant varieties. Up till now, little work has done on mutations induced in hybrids as a combination of both hybridization and mutation breeding. However, few pieces of evidence of comparison of irradiation, EMS and hybridization have been documented (Khadr and Shukry, 1972). Both the mutagens produced substantial genetic variation in two wheat varieties, but none of them was found efficient in enhancing the genetic variability in their hybrid context. The comparative degree of induced variability as compared to hybridization, determined by specific mutagen and its quality, on an average was less than 50% from hybridization. It has been found that mutated populations had higher genetic advance and heritabilities than hybrid populations (Khadr and Shukry, 1972). Sangsiri *et al*. (2005) irradiated F_1 and F_2 seeds of two mungbean varieties with gamma rays (500 Gy) and indicated that irradiated F_1 population had the highest frequency of mutants followed by F_2. Akhtar *et al*. (2015) first time used F_1 seed mutagenesis to create genetic variation in three distinct set of eight variable populations of lentil and found a large amount of genetic variation in F_1 mutated plants for all morphological traits included in the study. Up till now no lentil variety developed through F_1 seed mutagenesis.

Conclusions

From above discussion, it is clear that hybridization is used widely for the creation of genetic variability in lentil as well as other crops. Induced mutations are also a good source of creating genetic variability. We have recommended that combination of both these approaches i.e. hybridization and induced mutations (F_1 seed mutagenesis) will create a large amount of genetic

variation in lentil. Breeding scheme for F_1 seed mutagenesis was also given. Breeders should use this novel strategy for creating genetic variation in lentil as well as in other crops.

Breeding scheme for F_1 seed mutagenesis is as follows:

F_0 generation	Cross the parents possessing desirable genes.
F_1M_0 generation	Irradiate/Induce mutations in air-dried seeds of lentil F_1 hybrids with different doses of mutagen (s).
F_2M_1generation	Grow M_1 generation of mutated seeds of F_1 hybrids along with non-treated control (s) for the comparison of germination, growth, survival, M_1 injury, and sterility, and to assess any dominant mutation if appears. Phenotype/visually select desirable recombinant mutants.
F_3M_2 generation	Identify the superior single plants among space-planted progeny rows.
F_4M_3–F_5 M_4 Generations	Continue selection of single plants/lines. Also, select promising true breeding lines if found.
F_6M_5 generation	The final selection of true breeding lentil lines.
F_7M_6 generation	Evaluate the yield potential of selected true breeding lines in yield screening nursery.
F_8M_7 generation	Carry out replicated preliminary yield trial(s) and select top yielding lines.
F_9M_8–$F_{10}M_9$ generation	Carry out replicated advance line yield trial (s).
$F_{11}M_{10}$ generation	Carry out Multi-locational, replicated yield trials and disease reaction tests to make the final selection of the lines to be approved for commercial use.
$F_{12}M_{11}$ generation	Contribute best lines (Maximum 5) in MNUYT. Carry out Multi-locational, replicated yield trials and disease reaction tests to make the final selection of the lines to be approved for commercial use.
$F_{13}M_{12}$ generation	Contribute line(s) securing top positions in previous year MNUYT if any again in MNUYT. Seed Multiplication, DUS, Agronomic and other studies, DNA Fingerprinting and Spot examination.
$F_{14}M_{13}$ generation	Seed Multiplication, DUS studies, Meetings of Expert Sub-Committee and Punjab Seed Council. The release of the new variety.

MNUYT= Multilocation National Uniform Yield Trial
DUS= Distinct Uniform Stability.

For the study of genetic variation, only F_3M_2 generation is required. For new variety release, the whole breeding scheme should be followed.

Table 1 Latest lentil varieties developed through hybridization in Pakistan and India

Sr. No.	Variety	Year	Country	Traits improved
1	Mas00r-93	1993	Pakistan	Yield, disease resistance
2	Masoor-85	1985	Pakistan	Yield, disease resistance
3	NIAB Masoor-2001	2001	Pakistan	Yield, disease resistance
4	Markaz-2009	2009	Pakistan	Yield, disease resistance
5	Punjab Masoor-2009	2009	Pakistan	Yield, disease resistance
6	Chakwal Masoor	2011	Pakistan	Yield, disease & drought resistance
7	VL-Masoor-125	2006	India	Grain yield, plant height, maturity
8	VL-Masoor-126	2006	India	Grain yield, plant height, maturity
9	VL-Masoor-507	2006	India	Grain yield, plant height, maturity
10	VL-Masoor-129	2010	India	Grain yield, plant height, maturity
11	VL-Masoor-133	2011	India	Grain yield, plant height, maturity
12	VL-Masoor-514	2011	India	Grain yield, plant height, maturity
13	VL-Masoor-516	2016	India	Grain yield, disease resistance

Source: http://www.vpkas.nic.in/varchar/ln126.htm;
https://www.icarda.org/blog/%5Bnode%3ABlog%20type%5Dnew-lentil-variety-released-northern-india

Table 2 Lentil varieties developed through mutation breeding

Sr. No.	Variety	Year	Country	Traits improved
1	NIAB Masoor 2006	2006	Pakistan	Yield (1800 Kg/ ha), medium maturing
2	Mutant 17 MM	1999	Bulgaria	Seed Size
3	S-256	1981	India	Spreading Type

Source: (Maluszynski et al., 2000)

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