CHAPTER 13

MUTATION BREEDING

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Abstract: When genetic variability is narrowed using traditional breeding methods for a long period, induced mutations are one of the most important approaches for broadening the genetic variation in lentil to circumvent the bottleneck conditions. The aim of this chapter is to review lentil breeding using induced mutations from the beginning of mutation breeding work to the present and to list the outcomes of mutagenesis works on lentils. The number of mutant varieties of all species officially released and recorded in the Food and Agricultural Organization/International Atomic Energy Agency (FAO/IAEA) Mutant Varieties Database is over 2300. From these mutant varieties, more than 265 grain legume cultivars have been developed using induced mutations and have subsequently been released. Gamma rays were the most frequently used technique to alter genes. Many mutant lentils have been mentioned in the available literature while seven mutants have been released for commercial production so far. Mutant lentils have now contributed several million dollars annually to global agriculture. Several specific regional problems in lentil production areas have been coped with using mutant lentil cultivars. Fundamental genetics, physiological and molecular studies will also be come to light using mutant lentils

1. INTRODUCTION

The genus *Lens* Mill. includes seven taxa including the cultivated species *Lens culinaris* Medikus and its wild relatives [*L. orientalis* (Boiss.) Ponert., *L. odemensis* Ladiz., *L. tomentosus* Ladiz., *L. lamottei* Czefr., *L. nigricans* (M. Bieb.) Godr., *L. ervoides* (Brign.) Grande] (van Oss et al. 1997, Ferguson et al. 2000, Ferguson and Erskine 2001, Sarker and Erskine 2006). The cultigen (*Lens culinaris* Medik.) is divided into two subspecies, ssp. *macrosperma* and ssp. *microsperma*, on the basis of seed size by Barulina (see Cubero 1981). This approach has been renewed by Cubero (1981) as race *macrosperma* and race *microsperma*. The breeding methods

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in lentil are similar to those utilized in breeding other self-pollinated crops, *i.e.* pure line selection or hybridisation followed by the bulk method, the pedigree method, the single seed descent, or modification of these methods supplemented by mutation breeding and polyploid breeding (Muehlbauer and Slinkard 1981, Muehlbauer et al. 1993, 1996). The use of these traditional breeding methods for a long period may have narrowed available genetic variability. Mutation breeding is one of the most important possible routes to broadening the genetic variation in lentil under bottleneck conditions (Erskine et al. 1998). Other options include use of wild relatives, and molecular genetic approaches which are discussed elsewhere in this book. This chapter summarises the efforts that have gone into creating induced mutations from the pioneering experiments to the release of the most recent lentil cultivar. The chapter also reviews current knowledge on how induced mutation works on lentil.

2. HISTORY OF MUTATION BREEDING

The history of mutation breeding has been well reviewed by van Harten (1998). The earliest description of natural or spontaneous mutants was presented for cereal crops in an ancient book, "*Lulan*". This appeared around 300 BC in China. After this, many aberrant plant forms or variations in plants were discovered from 1590 to C. Darwin's bud variations in 1868. The period between these dates is called the first period of mutations. The second period encompasses the time between the discovery of *X-rays* by W.K. Rontgen in 1895 and application of mutagens from 1897 (the first mutagen treatment) to 1920 (N.I. Vavilov's "law of homologous series of variations" (van Harten 1998). The experiment of L. J. Stadler in the 1920s which used radiation to generate genetic changes in plants (Stadler 1928) initiated "mutation breeding" (Maluszynski et al. 2004). The third period ranges from induction of mutations to the first commercial mutant cultivar, the so called "chlorine-type" in *Nicotiana tabacum.* The fourth period starts with the development of international coordination and some financial assistance by the Food and Agricultural Organization/International Atomic Energy Agency (FAO/IAEA) from 1964 to onwards (van Harten 1998). Plant breeders have therefore been encouraged to use mutation breeding as one of the "peaceful uses of atomic energy". Recently, mutagenesis has received considerable attention for its use in a promising new technique known as "targeted induced local lesions in genomes" (TILLING) (Muehlbauer et al. 2006).

3. NATURE AND TYPES OF MUTATIONS

Mutations are phenotypically classified into two groups (Gaul 1964); (i) macro-mutations: These are easily detectable in individual plants, phenotypically visible and morphologically distinct and they are qualitatively inherited genetic changes, and occur in major genes or oligogenes, and (ii) micromutations: These result in a small effect that, in general, can be detected only by help of statistical methods and quantitatively inherited genetic changes, and occur in minor genes or polygenes.

Mutations are also divided into groups such as chlorophyll mutations and many other grouped morphological mutations (Table 3).

3.1. Gene Mutations

Mutations are theoretically all changes which occur in DNA sequence and result in changes in the genetic code. A gene mutation or point mutation is the group of all heritable changes which occur within the limits of a single gene. The majority of gene mutations show recessive inheritance but dominant gene mutations occur at a very low frequency (Micke 1999). A frameshift mutation is a deletion and insertion for any number of nucleotides other than three. Paramutations, transmutations and transposable genetic elements or transposons are mutation like effects (van Harten 1998) and widely used in molecular breeding.

3.2. Chromosome Aberrations

Chromosomal mutations span a number of genes (and intergenic regions) and often have a multiplicity of effects. Four types of chromosomal mutations within chromosomes are commonly distinguished: (i) deletions or deficiency, (ii) duplications, (iii) inversions and (iv) translocations. These types of mutations are also called chromosome rearrangements or structural mutations, and are generally not as valuable for plant breeding as gene mutations.

3.3. Chromosome and Genome Alterations

The basic chromosome number of a plant species is indicated by the symbol x. For examples in lentil and tetraploid wheat (*Triticum* sp.), $x = 7$. However, the number of chromosome pairs is seven ($n = 7$) in lentil, while $n = 14$ for tetraploid wheat. The term ploidy refers to the number of sets (each containing x chromosomes) of chromosomes or genomes in a cell, tissue and plant. Possible levels of ploidy include; haploid (n), diploid (2n), triploid (3n) and tetraploid (4n). The subject of ploidy level such as haploidy, polyploidy and allopoliploidy are of considerable importance for fundamental genetics, plant physiology and plant breeding. There may also be additional or B chromosomes that are unstable components of the plant and vary in number (Perfectti and Werren 2001).

3.4. Extra Chromosomal Mutations

Mutations may occur both in chromosomes in the nucleus and outside the nucleus. In a plant cell there are two extranuclear (extra chromosomal) genetic systems, the chloroplast and mitochondrion. Extra-chromosomal mutations bring about leaf variegation, dwarf growth and extrachromosomal genes induce tolerance to herbicides and also cause cytoplasmic male sterility, which is encoded by mitochondrial genome (Lonsdale 1987). The extra chromosomal mutations have considerably importance in practical application of plant breeding.

4. CONSIDERATIONS FOR INDUCED MUTATIONS

The following considerations should be taken into account before starting mutation breeding programmes: (i) Mutations are mostly recessive and they cannot be selected for until the second generation, M_2. Unlike recessive mutations, dominate mutations occur at low frequencies and they can be selected for in the M_1 generation (Micke and Donini 1993). Muehlbauer and Slinkard (1981) reported that mutation breeding is more adaptable for inducing recessive genes than dominant genes. Selection for polygenic traits should be started in individual plant progenies of the M_3 generation after some fixing of multiple homozygotes has commenced (Micke and Donini 1993, van Harten 1998); (ii) Mutations are beneficial with very low frequencies, while the treatments themselves can be detrimental reducing germination, growth rate, vigour and pollen and ovule fertility in the living organisms; (iii) Mutations are randomly induced and they might occur in any gene(s). However, some gene(s) can be more frequently induced to mutate than others; (iv) Mutations can be recurrent. The same gene(s) in a crop plant species may be induced to mutate again and again with different versions potentially having different effects; and (v) Mutations generally have pleiotropic effects due to closely linked gene(s) (Singh 2005).

5. SELECTION OF VARIETY, MUTAGEN AND DOSE

5.1. Which Varieties?

Mutation breeding programmes should be clearly planned and well defined, and large enough to select desirable mutations at the low frequencies likely to be encountered. The variety selected for mutagenesis should in particular be one of the best varieties released recently. At least, two varieties should be used because response to mutagens is different from a variety to another variety. It will be useful in improving specific characters of well adapted and high yielding varieties, which are deficient one or two traits (Anonymous 1977). The varietal group “macrosperma” has been found to be more sensitive to both the mutagen types used than the “microsperma” group (Sharma and Sharma 1986, Reddy and Viswanathan 1993). Sharma and Kharkwal (1982) found that the genotypes in macrosperma group were more responsive to mutagenic treatment and gave a higher frequency of mutated progenies in the M_2. Genotypic response to mutagens has been found to differ within the same group (Sharma and Sharma 1979c).

5.2. Which Mutagens?

The agents that induced mutations are called mutagens and mutagens mainly consist of two different kinds; (i) radiation (physical) and (ii) certain chemical mutagens (Table 1). Mutagens are not only beneficial to create genetic variability in a crop species, but also useful for the effective control of pests during post-harvest storage (Chaudhuri 2002). In addition to the use of induced mutations in plant breeding, there is a tremendous use of induced lentil mutations in fundamental

Table 1. Common mutagens and action mode

1. Physical Mutagens (Radiation)	Action mode	2. Chemical mutagens	Action mode
1.1. Ionising radiation	Breakage of hydrogen bonds and sugar phosphate moiety, cross-linking DNA strands.	2.1. Alkylating agents, i.e. sulphur and nitrogen mustards, ethylene amine (EI), ethylene oxide (EO), ethyl methane sulphonate (EMS), ethyl ethane sulphonate (EES), diethyl sulphate (DES), N-nitroso-N-ethyl urea (NEU) and N-nitroso-N-methyl urea (NMU), N'-methyl-N-nitro-N-nitroso-guanidine (MNNG)	Alkylate phosphate groups, purine and pyrimidine bases. Leads to mis-pairing or loss of bases.
1.1.1. Particulate radiation, i.e. α-rays (Alfa-rays), β-rays (Beta-rays), fast and thermal neutrons		2.2. Acridines, i.e. acriflavine, proflavine, acridine orange, acridine yellow, ethidium bromide	Intercaletes between bases disrupting their alignment and pairing. Results in deletion or addition of bases.
1.1.2. Non-particulate radiation, i.e. X-rays and γ-rays (Gamma-rays)		2.3. Base analogues, i.e. 5-Azocytidine, 5-bromo-deoxyuridine, 2-Aminopurine, Hypoxanthine, Maleic hydrazide, 6-Mercapto purine	Base pair substitution.
1.2. Non-ionising radiation, i.e. UV radiation	Induction of purine or pyrimidine dimers.	2.4. Others, i.e. nitrous acid, hydroxyl amine, sodium azide	Replacement of amino group with a hydroxyl group. Conversion of cytosine to a modified base.

genetics and plant physiology (Sharma and Sharma 1978ab, 1979b, 1981abc, Wilson and Hudson 1978, Miller et al. 1984; Vandenberg and Slinkard 1987, 1989ab, Sinha 1988, 1989ab, Tyagi and Gupta 1991, Sinha and Chowdhury 1991).

Sharma and Kant (1975) treated lentil by gamma rays and N-nitroso N-ethyl urea (NEU) to induce mutations and found that chemical mutagenesis was more successful. Generally chemical mutagens were more efficient than physical mutations for inducing mutations in lentil (Sharma and Kant 1975, Sharma and Sharma 1979ac, 1981, Ravi and Minocha 1987, Sarker and Sharma 1989, Solanki and Sharma 1994, 1999). Among the chemicals, morphological mutation frequency was obtained higher with ethyl methane sulphonate (EMS) than sodium azide

(Gaikwad and Kothekar 2004, Solanki et al. 2004; Solanki 2005, Solanki and Phogat 2005). EMS was observed to be more efficient than sodium azide (Gaikwad and Kothekar 2004) and gamma rays and hydroxylamine (Singh et al. 1989). N-nitroso-N-ethyl urea (NEU) or N-nitroso-N-methyl urea (NMU) was the higher potent than ethyleneimine (EI) and gamma rays based on the frequency of morphological mutation (Sharma and Sharma 1979c, 1981ab, Solanki and Sharma 2000, 2001). Similar findings have been made for other legume species (e.g. soybeans, Carroll et al. 1985).

5.3. Which Doses?

The dose of a chemical mutagen mainly depends on (i) concentration, (ii) duration of treatment, (iii) temperature during treatment (Anonymous 1977). Modifying factors are: (i) pre-soaking, (ii) pH of the solution, (iii) metallic ions, (iv) carrier agents, (v) subsequent washing of seeds (post-washing), (vi) post drying and (vii) storage of treated seeds. To change gene(s) for inducing morphological mutations, EMS doses are between 0.01 and 0.8%. The dose to use in the treatment varies from species to species with very small differences (Siddiqui 1999).

Malik et al. (1998) found that effective dose ranged from 214 to 218 Gy gamma rays for chlorophyll and morphologic mutations, and they found that the 50% lethal dose (LD_{50}) for survival was 250 Gy and radiation sensitivity varied among eight diverse lentil genotypes (Malik et al. 1998). On the other hand, Rajput et al. (1996) found that the lowest chlorophyll mutation frequency occurred at 200 Gy, and the highest occurred at 600 Gy. Paul and Singh (2002) observed that the highest frequency was observed in E 258 at the 150 Gy dose, while the lowest frequency was observed in Pant L 406 at the same dose. The 50% growth reduction (GR_{50}) of primary shoots and useful dose range for mutation breeding in lentil were given as 160–250 Gy and 100–170 Gy for gamma rays, and 9–14 Gy and 50–10 Gy for fast neutrons (N_f), respectively (Anonymous 1977). GR_{50} and a dose close to GR_{50} are considered the optimum dose for lentil by many researchers. Optimum dose produces the maximum frequency of mutations with minimum hazard. An optimum dose can be determined with a preliminary treatment. Overdoses of mutagens will kill too many plants, while under dosing will produce low mutation frequencies. However, lower frequencies may give an advantage of having fewer undesirable background mutations being induced in addition to the mutation being sought. Some factors that influence a mutagen's effects are biological (nuclear volume, chromosome volume and DNA content of variety, and genetic and varietal differences), environmental (oxygen, water status and temperature), and chemical (Anonymous 1977, Sigurbjornsson 1983).

Gamma rays were the most used mutagen to change gene(s) in lentil (Table 2 and 3) due to their easy application. However, as mentioned in the previous section they are not necessarily the most effective. The use of a chemical mutagens requires several procedures such as (i) preparation of seeds, (ii) pre-soaking, (iii) mutagen treatment considering suitable concentration, treatment temperature and

time, (iv) post-washing and (v) post-drying; while treatment with physical mutagens includes only two steps which are (i) preparation of seeds and (ii) mutagen treatment. Chemical mutation treatments also require the disposal of left over mutagen which can be highly toxic.

6. PARTS OF LENTIL TO BE TREATED

Although whole plants, seeds, pollen grains, meristems, cells or tissue in culture in crop plants are used (Anonymous 1977, van Harten 1998, Kaul and Nirmala 1999), air dried seeds are the most frequently used part of lentil for mutagenesis (Table 2 and 3). Pollen grains may be used directly such as has been done with pea (*Pisum sativum* L.) (Davies 1984, Saccordo et al. 1993) and maize (*Zea mays* L.) (Neuffer and Chang 1989), and vegetative organs for *in vitro* mutagenesis may be used as well. However, pollen grains are used infrequently because emasculation and pollination are very difficult, time consuming, and pollen survival is short.

Abbo and Ladizinsky (1994) studied genetic aspects of embryo abortion in the genus *Lens*. They found that embryo abortion was not associated with chromosomal aberrations. Irradiation of pollen grains can be beneficial to overcome pre- and post-fertilization problems especially in inter-specific hybridizations. In addition to this, mutations do not induce chimeras when pollens are irradiated (Micke and Donini 1993, Saccardo et al. 1993) whereas in seeds only some of the cell lines may be affected giving a genetically effective cell number of greater than one (Carroll et al. 1988) necessitating delaying selection and requiring a greater number of plants to be screened.

Table 2. Mutant lentils released for commercial production

Mutant variety	Parent variety	Mutagen(s)	Main characters induced	Released	
				Country	Year
S-256 (Ranjan)	B 77	Radiation	Spreading type, high yielding	India	1982
PL 77-2*	BR 25	–	Tolerant to wilt and ascochyta blight	India	1984
Rajendra Masoor 1	–	Gamma rays	Cold tolerant	India	1996
Mutant 17 MM	–	Gamma rays 40 Gy	Seed size	Bulgaria	1999
RH44**	–	EMS	Herbicide (Imidazolinone)-Tolerant	Canada	2006

* Yadav 2005. ** RH44 is one of three mutant varieties (Dr. Vandenberg, pers. comm.).

Table 3. Induced mutant lentils recorded in the available literature

Mutant	Parent variety	Mutagen(s)	Characters induced	Sources
Pod and seed size mutants	L235	Gamma rays 60 Gy	Larger and longer pod and seeds	Sharma and Sharma 1978a
Tendril	L235	Gamma rays 60 Gy or NMU 0.01%	Tendril leaflets	Sharma and Sharma 1978b
Crumpled petal	L235	Gamma rays 100 Gy	Sterile	Sharma and Sharma 1981a
Boat-shaped leaflet and	L258	NMU 0.01%	Boat leaf	Sharma and Sharma 1981b
crinkled leaf	L258	Gamma rays 100 Gy	Crinkle leaf	
Long peduncles	L258	NMU 0.01%	Elongated peduncles	Sharma and Sharma 1981c
Multi-flowers	L235	NMU 0.005%	Sterile	
SKL 2659, HR 73–76, HR 32–35, HR 28–31	–	–	High yield, Earliness	Sharma and Kharkwal 1983a
LM 1, LM 4	–	–	High nodule weight	Rai and Prasad 1983
Compact	T36	Gamma rays and/or NMU	Compact branching	Dixit and Dubey 1986
Dwarf	T36		Dwarf (8–12 cm)	
Staggering	T36		Long branches	
115-1-78, 218–78, 318–78, 514-2-78	–	Gamma rays	High amino acid	Tirdea and Mancas 1986
Dwarf	LL78	Gamma rays 200 Gy	Dwarf (16 cm)	Sinha 1988
Shy mutant	Sehore 74–7	Gamma rays 100 Gy	Dwarf	Sinha 1989a
Male-sterility	LL78	Gamma rays 50–200 Gy	Male-sterile	Sinhac 1989bc
Male sterile lentil	–	Gamma rays 100 Gy	Male sterile	Srivastava and Yadav 2001
A semi-dwarfism	LL78	Gamma rays 50–200 Gy	Plant height = 8.5 cm	Sinha and Chowdhury 1991
Fasciation	L830	Gamma rays 200 Gy + 0.1% EMS	Fasciation	Tyagi and Gupta 1991
M1-30, M1-596	L112	–	Drought tolerant	Salam and Islam 1994
ML-9,	Utfala	Gamma rays 150 Gy	Erect and bush type	Begum et al. 1995

ML-27,	Utfala	Gamma rays 250 Gy	Erect and bush type	
ML-40; ML-42	Utfala	Gamma rays 250 Gy	Erect and synchronous flowering	
Semi-dwarf	Pant L-639	–	Dwarf	Ramesh and Dhananjay 1996
Stunted	Pant L-639		Reduced leaves and pods	
ML-438/8	L-5	–	Lower nitrate reduction	Dutta et al. 1998
Dwarf	P38	–	Dwarf	Tyagi and Ramesh 1998
Bushy dwarf	P38		Bushy dwarf	
AM1-AM40 (40 mutants)	T-36	Gamma rays 50–150 Gy +	Yield criteria	Dubey and Kumar 1999
AML1-AML20 (20 mutants)	K-333	EMS, NMU, DES		
Earliness	P38	Gamma rays	Early maturing	Ramesh and Tyagi 1999
Faciated	P38		Faciation on stem and upper branches	
High yielding	P38		High yielding	
Macrosperma mutants (Six mutants)	HPL 4	–	Earliness, high yield	Sharma and Chahota 1999
AEL 12/30/91	ICARDA-8	Gamma rays 300 Gy	High yielding, earliness	Rajput et al. 2001
AEL 49/20/91	Mansoor-85	Gamma rays 200 Gy		

7. MUTATION BREEDING

7.1. Advantages

Mutation breeding not only creates variability in a crop species, but also shortens the time taken for the development of cultivars via induced mutation compared to those via hybridizations. The average time elapsed from initial mutation treatment to the release of the mutant cultivars was approximately 9 years (Figure 7.1), while this time was more than 9 years for cultivar arising from crossing programmes (Brock, 1977). Moreover mutations induced both qualitative and quantitative characters in a short time altering new alleles of known and previously unknown genes, and modify linkage (Konzak et al. 1977). Further desirable variability could be brought about as new variability in the families *Leguminosae* or *Fabaceae* through induced mutations (Toker and Cagirgan, 2004), whereas variability in hybridization programs is limited to that present in the

genotypes/phenotypes of the parents crossed. The existence of mutations is interpreted as supporting N.I. Vavilov's concept of homologous series in heritable variation (Gottschalk 1988). That is, theoretically mutagenesis may create all types of variation that are present in another member of a plant family if the gene exists in the plant treated. It was reported that many mutant lentils were resistant to *uromyces fabae* (Bravo 1983) and out-yielded their parents (Sen 1982, Sharma and Kharkwal 1983b, Salam and Islam 1994, Begum et al. 1995; Ramesh and Tyagi 1999, Sharma and Chahota 1999, Tonev et al. 1999, Mihov et al. 2001). Furthermore mutations are one of the three components of evolution (Sigurbjornsson 1983).

7.2. Disadvantages

The frequency of desirable mutations is very low at about 0.01%. However, mutation frequency will vary for different plant species. Even within a species, cultivars respond differently to mutagen treatment (Sigurbjornsson 1983). Success in mutation breeding depends on methods used handled, effective screening techniques and population grown in M_1 and successive generations. The larger the population in the M_1 is the more success in selection of desirable mutants. Breeders have to screen large populations for desirable mutations. The screening procedures in large populations will require considerable time, labour and other resources. Some mutations have pleiotropic effects due to linked gene(s), other mutations, chromosomal aberrations and deletions. These mutants often have to be backcrossed to parents or adapted varieties. Backcrossing is time consuming work and linkages between genes cannot be broken down easily.

8. MUTANT LENTILS

According to FAO/IAEA Mutant Varieties Database, the number of mutant varieties officially released and recorded is more than 2300 (Jain, 2005). From these mutant varieties, over 265 grain legume cultivars have been released (Bhatia et al. 2001; Maluszynski 2003, Ahloowalia et al. 2004). A variety of common bean (*Phaseolus vulgaris* L.) as a legume, Sanilac, was the first released mutant in Michigan in 1956 (Micke 1988; van Harten, 1998).

Varietal improvement of lentil was initiated as early as 1924 in India (Jeswani, 1988). Similarly, mutation induction work for lentil was probably first initiated in the Indian sub-continent. The first mutant lentil (Table 2) being released there. The Indian Agricultural Research Institute (IARI) has been a pioneer institution for research on induced mutations since 1957, and has released many mutant varieties of legume crops (Ahloowalia et al. 2004; Chopra 2005). The works have been encouraged in order to create useful variation by FAO/IAEA projects (Khan and Shakoor 1977, Ramanujan 1977, Shaikh 1977, Sarma and Kharkwal, 1982, Shaikh et al., 1983, Sarma and Kharkwal, 1983). These mutation breeding efforts created some unique mutations for use in plant breeding programmes. Some mutant

Year	Generation	Application	Progress
1	M_0	• Mutagenic application: Physical or chemical mutagens.	Seeds
1	M_1	• Growing the plants in isolation. • Selection for dominant mutations. • Single plant or bulk harvest.	Chimeric plants
2	M_2	• Growing the plants in single-plant-row or bulk rows • Selection for recessive mutations. • Harvest putative mutants individually. • Single seed descent (SSD) at least two sets. • Bulk harvest the remaining plants.	Segregation for recessive gene(s)
3	M_3	• Growing the plants in traditional sowing density. • Confirmation of the putative mutants. • Continue selection.	Further segregation
4	M_4	• Agronomic evaluation in mini-plots. • Propagation of promising mutants. • Use of mutants in crosses.	Evaluation of genetic stability
5–8	$M_5 - M_8$	• Agronomic evaluation in large plots. • Agronomic evaluation at different locations. • Evaluation of mutants in crosses.	Direct and indirect use of mutants
9	M_9	• Official testing of mutant lines.	Releasing of mutant varieties

Figure 1. Mutation breeding scheme for the improvement of lentil (SSD sets could independently be evaluated for any stress at the target environment)

lentils have been released for commercial production (Table 2). However, in spite of many useful mutant lentils being recorded in the available literature (Table 3) only seven mutant lentil varieties have been released. A comparatively poor figure in comparison to soybean (*Glycine max* L.), groundnut (*Arachis hypogea* L.), pea, common bean, faba bean (*Vicia faba* L.), mung bean [*Vigna radiata* (L.) Wilczek] and chickpea (*Cicer arietinum* L.). On the other hand, special problems in production of lentil will be solved via mutation breeding, i.e. Imidazolinone-Tolerant Lentil Line RH44 (Dr. A. Vandenberg pers. comm.). Herbicide tolerant lentils will act

crucial role to overcome weeds in lentil fields in the future. In India, 4 mutant cultivars of blackgram [*Vigna mungo* (L.) Hepper] 8 mutant cultivars of mungbean and 3 of lentil with high yielding capacity have contributed several million dollars annually to the country's agricultural production, *i.e.* for mungbean with an annual value of 64.7 million US$ (Ahloowalia et al. 2004, Chopra 2005).

9. CONCLUSIONS

Genetic variation in available germplasm collections of lentils has been widely used to combat biotic and abiotic stresses. Indigenous lentils are specific ecotypes in the most important lentil production regions of the world and exhibit a marked lack of variability (Erskine et al. 1994). Breeding progress in the cultigen may be limited by this bottleneck which has reduced genetic variability. Although some desirable sources of resistance have been found in the wild species (Erskine et al. 1994, Erskine and Muehlbauer 1995, Tullu et al. 2006; Sarker and Erskine 2006)), there is a difficulty with crosses involving some wild taxa because of post-fertilisation barriers (Muehlbauer et al. 1993). Even if crosses between cultigen and wild relatives are successful, (which is often difficult or even impossible), in addition to the desired gene(s) from wild relative, many undesired gene(s) may be introduced as well. Under this circumstance, a backcrossing programme will be necessary to get rid of undesired gene(s). Therefore, a common and efficient tool to create new and desirable genetic variability in lentil is mutagenesis. Mutant lentils have contributed millions of dollars annually to global agriculture because specific regional production obstacles in lentil have been dealt with using mutant lentil cultivars. Mutations in lentil have also been used to clarify fundamental genetics and physiological processes in lentils.

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