Induced Mutagenesis-A Reliable Technology to Overcome the Limitations of Low Genetic Variability in Lentils

Mohammad Rafiq Wani, Aamir Raina, Nasya Tomlekova, Rafiul Amin Laskar, Mohammad Feroz, and Samiullah Khan

Abstract Practices of agriculture and plant breeding approaches are indispensable for feeding the populaces of the world. In agriculture, the grain legumes occupy a unique position for their value as food and fodder, their role in biological nitrogen fixation, and as industrial raw materials. There are several reasons for the low productivity of pulses, which include a lack of high yielding genotypes, the vagaries of the monsoon, sowing on marginal lands under rain-fed conditions, negligence of plant protection, and imbalances of plant nutrients. Lack of genetic variability limits the scope of selection for better genotypes. For improvement in seed yield, genetic reconstitution of such crops is required to evolve better plant types. Mutation breeding has proven beneficial to upsurge the existing germplasm variability for improving certain specific traits of the varieties. By integrating molecular high throughput

M. R. Wani (✉)
Department of Botany, Abdul Ahad Azad Memorial Degree College Bemina, Cluster University, Srinagar, Jammu and Kashmir, India

A. Raina
Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Botany Section, Women's College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

N. Tomlekova
Molecular Biology Laboratory, Maritsa Vegetable Crops Research Institute, Agricultural Academy, Plovdiv, Bulgaria

R. A. Laskar
Department of Botany, Pandit Deendayal Upadhyaya Adarsha Mahavidyalaya (PDUAM), Eraligool, Karimganj, Assam, India

M. Feroz
Department of Zoology, Abdul Ahad Azad Memorial Degree College Bemina, Cluster University, Srinagar, Jammu and Kashmir, India

S. Khan
Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

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mutation screening techniques, induced mutations could increase the required genetic diversity for the improvement of pulses, particularly lentils.

Keywords Food security · Mutagenesis · Mutagens · In vitro technologies · Soma clonal variation · Tissue culture

1 Introduction

Pulses, or grain legumes, are important crops that provide high-quality proteins in developing countries, including India. Pulses, designated as the chief components of agricultural food crops, are consumed by the predominantly substantial vegetarian population of India. In dietetic terms, pulses match cereals in terms of protein and minerals, besides serving as a rotation crop with cereals, thereby lessening the soil pathogens and improving the physical properties of the soil. Pulses build up a mechanism for fixing atmospheric nitrogen to meet their nitrogen requirements (Wani et al., 2021; Raina et al., 2022a). In India, pulses are generally grown with minimum resource inputs, so they are less pricey than animal proteins.

Cultivated in rain-fed environments, pulses usually do not require rigorous irrigation facilities which qualifies them to grow even in such soils that are not favorable for the cultivation of cereals and cash crops. Moreover, pulses possess several other useful qualities, such as improving soil fertility, fitting in mixed and intercropping systems, and providing green pods as vegetables for humans and feedstuff for livestock. Despite limitations like an unfavorable environment, a dearth of superior seeds, a lack of proper post-harvest management, and deficient marketability, India has been successful to raise the annual pulse production from 8.41 to 23.02 million tonnes owing to an area expansion from 19.09 million hectares in 1950–51 to 27.87 million hectares in 2019–20 and filling a yield gap from 441 to 826 kg/ha (Table 1). In world agriculture, legumes are specially cultivated for food proteins (Khadke & Kothekar, 2011; Raina et al., 2022b; Rasik et al., 2022).

A large number of legume species, hitherto unexplored, have great potential for not only contributing as a major source of dietary protein for humans but also providing excellent fodder for livestock. The Food and Agriculture Organization (FAO) of the United Nations recognizes 10 primary and 5 minor pulse crops cultivated globally in over 105 countries. From the production perspective, dry beans (26.8 mt), dry pea (14.3 mt), chickpea (12.0 mt), cowpea (7.69 mt), lentil (6.3 mt), and pigeon pea (4.4 mt) are of utmost importance (FAO, 2017). Among the ten primary pulse crops recognized by the FAO, lentil is indispensable. In 1950–51, the percent share of pulses in the total food-grain basket in India, vis-à-vis area, production, and productivity, was 19.62, 16.55, and 84.48, respectively. This trend continued till 1960–61 and started dwindling from 1970–71 due to non-advancement in the production technologies of pulses as compared to other food grains. In 2019–20, the

Table 1 Share of pulses to total food grains in India

Year	Pulses			Food grains			Pulses % to food grains		
	A	P	Y	A	P	Y	A	P	Y
1950–51	19.09	8.41	441	97.32	50.82	522	19.62	16.55	84.48
1960–61	23.56	12.70	539	115.58	82.02	710	20.38	15.48	75.92
1970–71	22.54	11.82	524	124.32	108.42	872	18.13	10.90	60.09
1980–81	22.46	10.63	473	126.67	129.59	1023	17.73	8.20	46.24
1990–91	24.66	14.26	578	127.84	176.39	1380	19.29	8.08	41.88
1995–96	22.28	12.31	552	121.01	180.42	1491	18.41	6.82	37.02
2000–01	20.35	11.08	544	121.05	196.81	1626	16.81	5.63	33.46
2001–02	22.01	13.37	607	122.78	212.85	1734	17.93	6.28	35.01
2002–03	20.50	11.13	543	113.86	174.77	1535	18.00	6.37	35.37
2003–04	23.46	14.91	635	123.45	213.19	1727	19.00	6.99	36.77
2004–05	22.76	13.13	577	120.00	198.36	1652	18.97	6.62	34.93
2005–06	23.39	13.39	598	121.60	208.60	1715	18.41	6.42	34.87
2006–07	23.76	14.11	594	124.07	211.78	1707	19.15	6.66	34.80
2007–08	23.63	14.76	625	124.07	230.78	1860	19.05	6.40	33.58
2008–09	22.09	14.57	660	122.83	234.47	1909	17.98	6.21	34.55
2009–10	23.28	14.66	630	121.33	218.11	1798	19.19	6.72	35.03
2010–11	26.40	18.24	691	126.67	244.49	1930	20.84	7.46	35.80
2011–12	24.46	17.09	699	124.76	259.32	2079	19.61	6.59	33.61
2012–13	23.25	18.34	789	120.77	257.12	2129	19.25	7.13	37.06
2013–14	25.21	19.25	764	125.04	265.04	2120	20.16	7.26	36.03
2014–15	23.10	17.16	743	122.07	252.67	2069	18.92	6.79	35.91
2015–16	24.91	16.35	656	123.22	251.57	2042	20	7	32
2016–17	29.45	23.13	786	129.23	275.11	2129	23	8	–
2017–18	29.81	25.42	853	127.52	285.01	2234	23	9	–
2018–19	29.16	22.08	757	124.78	285.21	2286	23	8	–
2019–20*	27.87	23.02	826	124.77	291.95	2340	22	8	–

Source: http://dpd.dacnet.nic.in; DES, Ministry of Agri. & FW (DAC&FW), Govt. of India
*III Advance Estimate
A million hectares, *P* million tonnes, *Y* kg/ha

production has gone down to 8% as compared to other food grains (Table 1). Even though this crop group is imperative from a nutritional perspective, there has been no significant rise in area and production recorded from 1950–51 to 2009–10. Nevertheless, substantial progress in area and production was recorded from 2010–11 to 2018–19. Due to the progression in infrastructural and irrigation amenities, the pulse crops get sidelined treatment, which pushes them to nutrient deficient and marginal land pieces (http://dpd.dacnet.nic.in), thereby leading to the emergence of poor crops with deficient productivity and poor seed quality.

2 Origin, Area, Production, and Productivity of Lentil

Lentil (*Lens culinaris* Medik) is an annual herb, erect in growth, light green in color, freely branched, with a slender stem and soft, hairy foliage (Fig. 1). Being one of the oldest cultivated legume crops, lentil originated in the Fertile Crescent of the Near East and then spread to Europe, the Middle East, Northern Africa, and the Indo-Gangetic plains (Ford et al., 2007); and it was domesticated in the Near East arc in early Neolithic times (Ladizinsky, 1979a). Lentil is an important crop of dry-land agriculture and a valuable human food, mostly consumed as dry seeds. The straw and pod walls have a high food value, and the husk is used as livestock feed.

India stood first in the area and second in production of lentils with 43% and 23% of the global area and production, respectively. New Zealand recorded the highest yield of 2667 kg/ha followed by China with a yield of 2239 kg/ha. Canada ranked first in production (38%) producing a yield of 1971 kg/ha followed by India (23%) producing a yield of 600 kg/ha (Table 2). However, as per the average data of 2014–18 (Table 3), India ranked second in area and production, with 30% and 20% of world area and production, respectively.

Lentil (2n = 14), having a large genome of 41,063 Mbp (Arumuganathan & Earle, 1991), is an important pulse crop of the winter season and grows in nearly all parts of India as an intercrop or pure crop. The large chromosome size and small chromosome number make lentils suitable material for cytogenetic studies. In India, the area, production, and productivity of lentils during 2019–20* were 1.32 million hectares, 1.18 million tonnes, and 894 kg/ha, respectively (Table 4). Being a cool-season crop, lentil production is mostly narrowed to northern and central India. Uttar Pradesh, Madhya Pradesh, West Bengal, Bihar, and Jharkhand are the major lentil-producing states of India on the basis of their percentage share of production during 2019–20* (Table 5; Fig. 2).

Fig. 1 Lentil sprout buds. (Source: pixabay.com)

Table 2 Global ranking in area, production, and yield of lentil: major countries

	Area (Lakh ha)			Production (Lakh tonnes)			Yield (kg/ha)	
Rank	Country	Area	% to world	Country	Production	% to world	Country	Yield
1	India	18.90	43.50	Canada	18.805	37.98	New Zealand	2667
2	Canada	9.542	21.96	India	11.340	22.90	China	2239
3	Turkey	2.812	6.47	Turkey	4.170	8.42	Australia	2237
4	Nepal	2.065	4.75	Australia	3.241	6.55	Egypt	2167
5	Australia	1.449	3.34	USA	2.277	4.60	Canada	1971
6	USA	1.404	3.23	Nepal	2.269	4.58	USA	1621
7	Syrian Arab	1.280	2.95	China	1.500	3.03	France	1613
8	Iran	1.200	2.76	Ethiopia	1.298	2.62	Turkey	1483
9	Ethiopia	1.081	2.49	Syrian Arab	1.250	2.52	Armenia	1308
10	Bangladesh	0.898	2.07	Bangladesh	0.930	1.88	Argentina	1250
11	Australia	1.449	3.34	USA	2.277	4.60	Canada	1971
							India	600
	World	**43.447**		**World**	**49.517**		**World**	**1140**

Source: FAO Statistics 2013

Table 3 Area (lakh hectares), production (lakh tonnes), and yield (kg/ha) of lentils in major countries (Average: 2014–18)

Country	Area	% Contribution	Production	% Contribution	Yield
Canada	17.98	34	27.03	46	1503
India	15.92	30	11.50	20	722
Turkey	2.53	5	3.65	6	1441
USA	2.74	5	3.33	6	1218
Nepal	2.04	4	2.31	4	1131
All above	**41.21**	**(79%)**	**47.82**	**(81%)**	**1160**
World	**52.23**		**58.84**		**1127**

Source: Lentil.cdr (dacnet.nic.in); Ministry of Agriculture and Farmers Welfare, Department of Agriculture, Cooperation and Farmers Welfare, Directorate of Pulses Development, Vindhyachal Bhavan, Bhopal, Madhya Pradesh

The country's area under lentil cultivation was 13.90 lakh hectares, with a production of 10.93 lakh tonnes (twelfth plan 2012–15; Fig. 3, Table 6). Madhya Pradesh was ranked first with respect to acreage at 39.59% (5.50 lakh hectares), followed by Uttar Pradesh at 33.95% and Bihar at 11.29%. Regarding production, Uttar Pradesh stood first with 34.36% (3.76 lakh tonnes) followed by Madhya Pradesh with 30.73% (3.36 lakh tonnes) and Bihar with 17.35% (1.90 lakh tonnes). The state of Bihar recorded the highest yield of 1209 kg/ha followed by Rajasthan and West Bengal with 962 kg/ha and 960 kg/ha, respectively. The national yield average was 786 kg/ha. The lowest yield of 327 kg/ha was recorded in the state of Chhattisgarh, followed by Maharashtra (400 kg/ha) and Madhya Pradesh (610 kg/ha).

Table 4 All-India area, production, and yield of lentil

Year	Area	Production	Yield
2000–01	1.48	0.92	619
2001–02	1.47	0.97	664
2002–03	1.38	0.87	634
2003–04	1.40	1.04	743
2004–05	1.47	0.99	675
2005–06	1.51	0.95	629
2006–07	1.47	0.91	621
2007–08	1.31	0.81	622
2008–09	1.38	0.95	693
2009–10	1.48	1.03	697
2010–11	1.60	0.94	591
2011–12	1.56	1.06	678
2012–13	1.42	1.13	797
2013–14	1.34	1.02	761
2014–15	1.47	1.04	705
2015–16	1.28	0.98	765
2016–17	1.46	1.22	838
2017–18	1.55	1.62	1047
2018–19	1.36	1.23	901
2019–20*	1.32	1.18	894

Source: Directorate of Economics & Statistics, DAC&FW; Agricultural Statistics at a Glance – 2020 (English version).pdf (dacnet.nic.in)
*4th Advance Estimates
Area – million hectares, Production – million tonnes, Yield – kg/ha

3 Nutrient Composition and Growth Habit of Lentil

Lentil is a valuable protein-rich human food. The protein content ranges from 24 to 100 g. It is an excellent dietary supplement due to its high protein content and nutrient density, which stabilize the nutritive insufficiencies of a cereal-based diet. The pulse plants, during their cultivation, augment nutrient status by accumulating nitrogen, carbon, and organic matter in the soil besides increasing the farmer's revenue with high market returns. It also has high levels of dietary fiber, vitamins, and carbohydrates (Erskine et al., 1990). The young pods are eaten as vegetables and ground into flour to make a variety of preparations. In the Indian subcontinent, lentil is commonly consumed as "dal." Moreover, bold and attractive-looking lentil grains have a high demand for exportation at premium prices. Lentils are supposed to prevent constipation and represent a major source of lectins, which are used for treating the prophylaxis of retroviral infections, including HIV. Also, lentils have anti-carcinogenic, blood pressure-lowering, hypocholesterolemic and hypoglycemic

Table 5 Area, production, and yield in the major lentil producing states of India during 2018–19 and 2019–20*

2019–20*						2018–19				
State	Area	% to All-India	Production	% to All-India	Yield	Area	% to All-India	Production	% to All-India	Yield
Uttar Pradesh	0.46	35.17	0.45	38.47	978	0.48	34.93	0.49	39.78	1026
Madhya Pradesh	0.38	28.79	0.32	26.95	837	0.42	30.89	0.33	26.85	783
West Bengal	0.17	13.07	0.16	13.88	950	0.17	12.49	0.14	11.52	831
Bihar	0.15	11.57	0.12	10.26	793	0.15	10.85	0.15	12.06	1001
Jharkhand	0.06	4.73	0.05	4.59	867	0.06	4.20	0.05	4.08	875
Others	0.09	6.67	0.07	5.84	782	0.09	6.63	0.07	5.72	777
All-India	**1.32**	100.00	**1.18**	100.00	**894**	**1.36**	100.00	**1.23**	100.00	**901**

Source: Directorate of Economics & Statistics, DAC&FW; Agricultural Statistics at a Glance – 2020 (English version).pdf (dacnet.nic.in); *4th Advance Estimate

Area – million hectares, Production – million tonnes, Yield – kg/ha

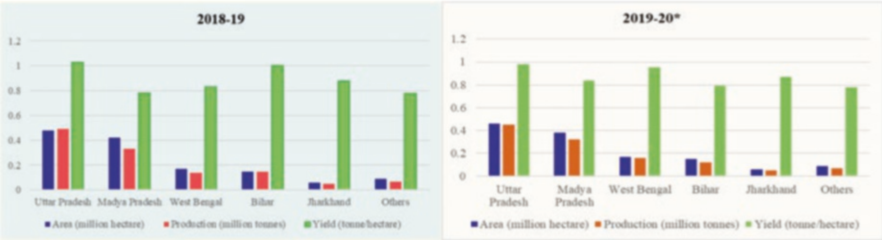

Fig. 2 Comparative area (million hectares), production (million tonnes), and yield (tonnes/ha) in major lentil producing states of India during 2018–19 and 2019–20*. (Source: Directorate of Economics & Statistics, DAC&FW; *4th Advance Estimate)

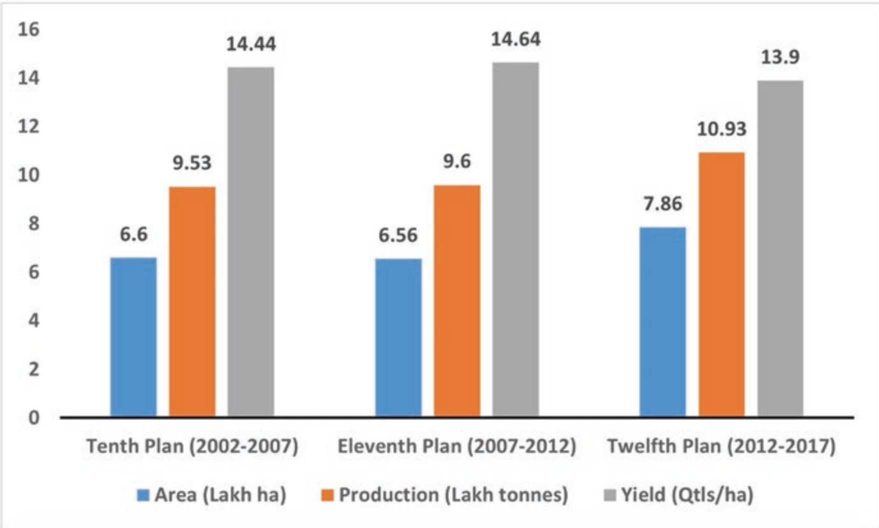

Fig. 3 Plan wise national scenario of lentil. (Source: http://dpd.dacnet.nic.in)

effects (Faris et al., 2012). The comprehensive nutritional composition of lentils is given in Tables 7 and 8.

Lentil plants are slender, semi-erect, with compound leaves (4–7 pairs of leaflets) that terminate at apices with a tendril. Plants normally range from 20–30 cm tall. Flowering begins from lower to upper branches and continues till harvest. Pods are oblong and smooth, about 1.3 cm long, and contain one or two lens-shaped seeds. Flowers are self-pollinated and are white or pale blue in color. Plants tend to lodge at maturity due to their weak stems. Germination is hypogeal.

Table 6 Plan-wise lentil scenario – states

State	Xth Plan	% to AI	XIth Plan	% to AI	XIIth Plan	% to AI	State
Assam	A	0.20	1.39	0.22	1.50	0.29	2.12
	P	0.11	1.15	0.11	1.15	0.20	1.80
	Y	548		511		668	
Bihar	A	1.72	11.91	1.81	12.36	1.57	11.29
	P	1.35	14.17	1.59	16.56	1.90	17.35
	Y	787		878		1209	
Chhattisgarh	A	0.17	1.18	0.16	1.09	0.14	1.00
	P	0.05	0.52	0.05	0.52	0.05	0.42
	Y	212		322		327	
Haryana	A	0.06	0.42	0.04	0.27	0.05	0.39
	P	0.05	0.52	0.03	0.31	0.05	0.46
	Y	900		783		935	
Madhya Pradesh	A	5.06	35.04	5.5	37.57	5.50	39.59
	P	2.43	25.50	2.33	24.27	3.36	30.73
	Y	481		424		610	
Maharashtra	A	0.07	0.48	0.07	0.48	0.04	0.26
	P	0.03	0.31	0.03	0.31	0.01	0.13
	Y	368		431		400	
Punjab	A	0.03	0.21	0.01	0.07	0.01	0.061
	P	0.02	0.21	0.01	0.10	0.01	0.050
	Y	560		673		647	
Rajasthan	A	0.19	1.32	0.28	1.91	0.31	2.23
	P	0.19	1.99	0.25	2.60	0.30	2.72
	Y	995		917		962	
Uttar Pradesh	A	5.96	41.27	5.56	37.98	4.72	33.95
	P	4.65	48.79	4.44	46.25	3.76	34.36
	Y	781		799		796	
Uttarakhand	A	0.16	1.11	0.15	1.02	0.11	0.82
	P	0.08	0.84	0.09	0.94	0.10	0.89
	Y	494		605		847	
West Bengal	A	0.65	4.50	0.55	3.76	0.65	4.66
	P	0.45	4.72	0.44	4.58	0.62	5.68
	Y	686		791		960	
All-India	A	14.44		14.64		13.90	
	P	9.53		9.60		10.93	
	Y	660		656		786	

Source: http://dpd.dacnet.nic.in
AI All-India, *A* lakh hectares, *P* lakh tonnes, *Y* kg/ha

Table 7 Nutrient composition of lentil (raw seeds)

Nutrient	Unit	Value per 100 g
Proximates		
Water	g	8.26
Energy	kcal	352
Total lipid (fat)	g	1.06
Carbohydrate	g	63.35
Fiber, total dietary	g	10.7
Sugars, total	g	2.03
Minerals		
Calcium, Ca	mg	35
Iron, Fe	mg	6.51
Magnesium, Mg	mg	47
Phosphorus, P	mg	281
Potassium, K	mg	677
Sodium, Na	mg	6
Zinc, Zn	mg	3.27
Vitamins		
Vitamin C, total ascorbic acid	mg	4.5
Thiamin	mg	0.873
Riboflavin	mg	0.211
Niacin	mg	2.605
Vitamin B-6	mg	0.540
Folate, DFE	µg	479
Vitamin B-12	µg	0.00
Vitamin A, RAE	µg	2
Vitamin A, IU	IU	39
Vitamin E (alpha-tocopherol)	mg	0.49
Vitamin D (D2 + D3)	µg	0.0
Vitamin D	IU	0
Vitamin K (phylloquinone)	µg	5.0
Lipids		
Fatty acids, total saturated	g	0.154
Fatty acids, total monounsaturated	g	0.193
Fatty acids, total polyunsaturated	g	0.526
Fatty acids, total trans	g	0.000
Cholesterol	mg	0
Others		
Caffeine	mg	0

Nutrient values and weights are for edible portions
Source: https://www.nutritionvalue.org/Lentils%2C_raw_nutritional_value.html

Table 8 Amino acid composition of lentil (g/100-g protein)

Amino acid	Value per 100 g
Protein	24.63 g
Alanine	1.029 g
Arginine	1.903 g
Aspartic acid	2.725 g
Cysteine	0.322 g
Glutamic acid	3.819 g
Glycine	1.002 g
Histidine	0.693 g
Isoleucine	1.065 g
Leucine	1.786 g
Lysine	1.720 g
Methionine	0.210 g
Phenylalanine	1.215 g
Proline	1.029 g
Serine	1.136 g
Threonine	0.882 g
Tryptophan	0.221 g
Tyrosine	0.658 g
Valine	1.223 g

Source: https://www.nutritionvalue.org/Lentils%2C_raw_nutritional_value.html

4 Varieties, Climatic Conditions, Insect Pests, and Diseases of Lentil

The varieties of lentils are broadly classified as *microsperma* and *macrosperma* types. *Microsperma* is small, with round seeds about 2–6 mm in diameter, cotyledons are yellow or orange, and the testa ranges from pale yellow to black in color. *Macrosperma,* on the other hand, has large, flattened seeds, 6–9 mm in diameter, having yellow cotyledons and pale green testa. In India, large seeded (*macrosperma*) types are mostly cultivated in the central zone, such as the Bundelkhand regions of Uttar Pradesh, Madhya Pradesh, and Maharashtra, whereas small seeded (*microsperma*) types are grown in Indo-Gangetic plains such as Bihar, Eastern Uttar Pradesh, West Bengal, and Assam. Bold seeded types are generally poor yielders.

Lentil is adapted to cool growing conditions. It is a resilient crop, tolerating frost and severe winters to a greater extent. Well-drained loam soils are best suited for lentil cultivation. Extreme drought and high temperatures during the flowering and pod-filling stages reduce the yield. The world is expecting 30% population growth by 2050, which puts an unprecedented demand on already climate threatened agricultural production. Pulses, especially lentils, have great potential to solve global food insecurity in changing climates. Lentil is infected by many insect pests and aphids. *Aphis craccivora* is dominant among aphid species that attack and damage the crop. Spiny pod borer (*Etiella zinckenella*) causes minor to moderate damage to lentil pods. The major diseases of lentils are wilt (*Fusarium oxysporum*) and rust (*Uromyces fabae*). However, the occasional incidence of stem root rot, powdery mildew, and *Alternaria* blight are also reported, particularly under humid climatic conditions.

5 Limitations and Scope of Traditional and Modern Plant Breeding

In the late ’70 s and early ’80 s, inheritance studies of several morphological and agronomical traits, viz., seed coat, epicotyl and flower color, dehiscence of the pod, etc., showed that most of the traits are monogenic and useful as morphological markers (Haddad et al., 1978; Ladizinsky, 1979b; Muehlbauer & Slinkard, 1981). In lentil, Zamir and Ladizinsky (1984) reported the first genetic linkage analysis. The first use of recombinant inbred lines (RILs) for mapping lentil markers was reported by Tahir et al. (1994), who determined six linkage groups in which they mapped four morphological and 17 isozyme markers. The use of intraspecific crosses was rare in the past for the development of linkage maps due to the limited availability of variation in cultivated species. However, segregating populations of lentils from intraspecific crosses has been utilized in several classical mapping experiments to establish linkage groups among the morphological markers. Linkage relationships among seed coat pattern, pod pubescence, and flowering time (Sarker et al., 1999), leaf color, and plant pubescence (Hoque et al., 2002) were demonstrated in lentil. Kumar et al. (2004, a, b) found two linkage groups among different morphological markers in lentils, namely, leaf pigmentation, stem pigmentation, pod pigmentation, erect growth habit and color of the leaf, pubescence of the plant, number of leaflets per leaf, and plant height.

Breeding objectives of lentils usually differ depending on the difficulties and primacies of farmers and consumers of the specific regions. Higher stable seed yield, disease resistance, and better seed quality are the main breeding goals of the key exporting countries; however, for import-dependent countries like India, increased yield per hectare remains the key resolve (Muehlbauer et al., 1995). Local factors and several biotic and abiotic stresses are the main impediments toward the global yield improvement of lentils, particularly in resource-deprived countries (Tivoli et al., 2006; Muehlbauer et al., 2006; Sinclair & Vadez, 2012). Though in lentils, traditional breeding has been efficacious over the past in addressing main production constraints and developing varieties resistant to major biotic or abiotic stresses (Muehlbauer et al., 2006; Materne & McNeil, 2007), its scope is limited attributable to low genetic variability, scarceness of genetic information, and accurate selection methods.

Molecular breeding has provided plant breeders with a reliable means to overcome these limitations for rapidly improving the crop. PCR-based markers have been proven to be a useful tool for indirect selection of desirable traits with high accuracy that would otherwise be difficult or time-consuming using conventional methods. Currently, the regular use of markers in lentil breeding programs is very limited. In the near future, the sustainable implementation of advanced molecular techniques to develop novel markers for highly sought-after traits would accelerate the lentil improvement programs.

Being a self-pollinated crop, lentil predominantly has a low rate of outcrossing, which results in low genetic variability and restricts the trait improvement programs. Due to the dearth of sufficient natural variability, conventional methods of plant breeding had a limited scope for the improvement of lentils. In these circumstances, mutation breeding, a well-functioning branch of plant breeding, supplements the conventional methods in a favorable manner (Gottschalk, 1986). Mutation breeding is widely exploited to modify one or a few traits in an otherwise outstanding variety without altering its original genetic makeup and other phenotypic traits (Raina & Khan, 2020; Raina & Danish, 2018). In that sense, it provides a rapid method to improve indigenous crop varieties without going through exhaustive procedures of hybridization and backcrossing (Raina et al., 2019; Sellapillaibanumathi et al., 2022). Induced mutagenesis is a powerful tool to generate new genetic variability in the traits of interest for boosting the breeding programs has already been established in different crop plants (Sharma, 1990; Reddy & Annadurai, 1992; Wani & Khan, 2003; Solanki, 2005; Wani et al., 2017; 2021; Wani, 2018, 2020, 2021; Amin et al., 2016, 2019; Goyal et al., 2019a, b, 2020a, b, 2021a, b; Raina et al., 2020a, b, 2021, 2022d) and with the advent of marker-based selection techniques; the possibilities of improving the crop plants in general, and lentils in particular, have tremendously increased.

6 Mutagenesis in Lentil

6.1 *Mutagenesis and Biological Damage*

In biological material, mutagens induce biological damage, gene mutations, and chromosomal aberrations in the M_1 generation. Out of these, gene and chromosomal mutations may pass on to the subsequent generations, while biological damage may remain confined to only the M_1 generation. The study of biological damage in the M_1 generation is normally used to appraise the mutagenic potency and sensitivity of the biological material. Biological effects represent injuries that can be determined cytologically and measured by growth reduction and death of the plant. The induction of mutations and their use in the development of mutant varieties in lentils are well documented by Sharma (1997) with different doses of gamma rays and NMU in *microsperma* and *macrosperma* lentils. A progressive reduction in seed germination, pollen fertility, seedling growth, and plant survival was reported with increasing doses of mutagens in the M_1 generation. Root length was more affected than shoot length concerning mutagenic treatments.

Based on M_1 biological parameters, Sinha and Godward (1972) found *macrosperma* lentils to be more sensitive to mutagens than *microsperma* lentils.

Sarker and Sharma (1989) reported that mutagenic treatment induced significant biological damage in M_1 parameters. However, the trend of mutagenic damage varied with different doses and durations of mutagenic treatments. Gamma rays drastically affected fertility and seedling height, while EMS and NEU severely impacted fertility, germination, and plant survival.

Different mutagenic treatments revealed differential trends vis-à-vis plant survival, plant height, and seeds per pod in variety K-85 of lentils (Tripathi & Dubey, 1992). Tufail et al. (1998) reported that the proportion of plant emergence and plant survival at maturity in varieties Pant L406, Masoor 85, Precoz, and L605 decreased with a corresponding increase in radiation doses. Variety L605 appeared to be the most sensitive, followed by Pant L406, Masoor 85, and Precoz. Sharma and Sharma (1986), Sinha and Chaudhary (1987), and Kalia and Gupta (1988) reported greater radiosensitivity of *macrosperma* lentil, whereas Singh et al. (1989) reported that the *microsperma* variety Pant L639 was more sensitive than the *macrosperma* variety RAU101 to 5 to 25 kR doses of gamma rays. The difference in the radiosensitivity of varieties from both groups was attributed to the difference in their genetic backgrounds (Rajput & Siddiqui, 1981; Malik et al., 1988). Tyagi and Sharma (1981) reported that differences in radiosensitivity exist within and between *microsperma* and *macrosperma* groups and concluded that the varietal differences were more conspicuous than the inter-group ones.

6.2 Cytological Effects

The diploid chromosome number of lentils is $2n = 2\times = 14$. Two pairs of chromosomes are metacentric, two pairs are submetacentric, and three pairs are acrocentric. Cytological effects of physical and chemical mutagens were studied by Dixit (1985) in a variety of T-36 of lentils and reported direct linking of mitotic abnormalities with mutagenic dose. In comparison to gamma rays, NMU induced a lesser proportion of anomalous cells. Combination treatments of gamma rays and NMU showed the direct additive effect. Similarly, NMU induced the highest percentage of abnormal cells, as reported by Dixit and Dubey (1984). There was a direct association between the anomalies induced and the concentrations of mutagens applied. This statement agrees with Sinha and Godward's (1968) observation of lentils. Mitotic anomalies were directly proportional with increasing mutagen doses in variety K-333 (Tripathi, 1995). The anomalies detected included clumping, fragmentation, bridges, laggards, and an unequal distribution of chromosomes at anaphase. Chromosomal aberrations play an important role in inducing sterility, thereby influencing the recovery of mutations. Meiotic anomalies increased with increasing irradiation doses of gamma rays in variety T-36 of lentils. However, gamma rays and NMU in combination did not exhibit a synergistic effect on inducing meiotic abnormalities (Dixit & Dubey, 1983a).

6.3 Chlorophyll and Morphological Mutations

The proficiency of different mutagens in bringing genetic variability for crop improvement is evaluated with the help of chlorophyll mutations, which are utilized as genetic markers in elementary and applied research. In NEU treatments, the incidence of chlorophyll mutants such as viridis, xantha, and chlorina was reported to be higher as compared to EI and gamma rays (Solanki & Sharma, 2001; Sarker & Sharma, 1989). Gamma rays at a dose of 15 kR, induced xantha, albo-xantha, and tigrina types of chlorophyll mutants (Paul & Singh, 2002). Chemical mutagens induced a higher frequency of chlorophyll mutations than radiation (Sharma & Sharma, 1981b; Tripathi & Dubey, 1992; Reddy et al., 1993; Vandana et al., 1994). Varied effectiveness and efficiency of mutagens in inducing chlorophyll mutations were reported by Dixit and Dubey (1986) in lentils. Sharma and Sharma (1979) compared the effectiveness and efficiency of NMU using *microsperma* genotypes as test symbols. In all the varieties, the mutation rate per unit dose of NMU was approximately three times higher than that of gamma rays.

Singh and Singh (1989) confirmed three categories of chlorophyll mutations, viz., albina, xantha, and viridis, in *microsperma* and *macrosperma* varieties of lentils following mutagenic treatments with gamma rays, EMS, and hydroxylamine (HA). As compared to gamma rays and HA, EMS was found to be the most efficient mutagen. Moreover, *microsperma* variety appeared more sensitive toward mutagenic agents than *macrosperma* variety indicating a possible role of seed size in the mutagenesis. Laskar and Khan (2017) studied the mutagenic effects of gamma rays and HZ in DPL-62 and Pant L-406 varieties of lentils, which resulted in the isolation of several kinds of mutants with altered phenotypes. Gamma rays and HZ at moderate doses showed higher effectiveness and efficiency, whereas, for combination treatments with some inter-varietal exceptions, lower doses were found to be most effective and efficient. The frequency of induced mutations in the M_2 generation appeared to have a direct association with mutagen-sensitive parameters in the M_1 generation (Dixit, 1985; Tripathi, 1995). Hence, the extent of induced mutagen damage through the reduction in germination, seedling growth, plant survival, chromosomal anomalies, and pollen and ovule sterility could be interconnected with mutational efficiency.

Induction of morphological mutations by physical or chemical mutagens in lentils was reported by various workers (Ramesh & Dhananjay, 1996; Solanki & Sharma, 1999; Laskar et al., 2018a, b; Wani et al., 2021). Sharma and Sharma (1979) studied the leaf mutation by treating the dry seeds of lentil with NMU and gamma rays. Leaf mutants isolated in M_2 included the boat leaf mutant (3–4 boat-shaped leaflets per leaf) and the crinkled leaf mutant (short leaf having 6–8 small, overlapping, and irregularly shaped leaflets). The segregation pattern showed that the crinkled leaf mutation was controlled by a single recessive gene designated as "*crl*." Sarker and Sharma (1986) studied the chlorophyll and morphological mutations in lentils after treatments with gamma rays, EMS, NEU, and SA. Of all the four mutagens, NEU was found to be more effective in inducing chlorophyll as well as morphological mutations. Among the morphological mutations, narrow leaf and tendrilled mutations were induced more frequently by NEU and EMS, whereas broadleaf and bushy dwarf mutants were higher in gamma rays and SA treatments.

As compared to gamma rays in the M_2 and M_3 generations, the frequency of macromutations was higher in EMS treatments (Tyagi & Gupta, 1991). Mutants for growth habit and foliage types were induced by EMS treatments, whereas mutants for flowering behavior, maturity, duration, and plant height were induced by SA treatments (Solanki et al., 2004; Khan et al., 2006; Solanki, 2005; Solanki & Phogat, 2005). NMU induced sterile mutants and the mutants with tendrils instead of terminal two to three leaflets (Sharma & Sharma, 1978a). Sterile plants with elongated peduncles and multi-floret inflorescences were also reported by Sharma and Sharma (1981a).

In lentil, mutations for plant height, growth habit, branching, stem structure, leaf morphology, inflorescence, calyx, flower, pod, fertility, and seed colour were reported by different workers (Sharma & Sharma, 1983; Sinha et al., 1987; Tyagi & Gupta, 1991; Ashutosh & Dubey, 1992; Vandana et al., 1994; Ramesh & Dhananjay, 1996; Tyagi & Ramesh, 1998; Solanki & Sharma, 1999; Jeena & Singh, 2000). Based on the extent of height reduction, plant mutants were classified into dwarf, semidwarf, and bushy-dwarf types (Sharma & Sharma, 1982; Dixit & Dubey, 1983b; Vandana et al., 1994). At places of emergence, the branches were fused with the main stem, and the plant looked like a bunch of closely merged branches, giving it a "bunchy top" appearance. The mutation was controlled by a single recessive gene, *"fa,"* besides inducing disease resistance in mutant lines (Bravo, 1983).

6.4 Induced Variability for Quantitative Traits

Due to the absence of adequate natural variability, conventional methods of plant breeding, i.e., introduction, selection, and hybridization, had a limited scope for crop improvement, particularly in pulses. New genetic variability demands could be achieved by crossing landraces with exotic material and/or through mutation breeding. Mutation breeding is a potent tool for creating genetic variability, particularly in species where hybridization is difficult or naturally existing variability has been exhausted (Raina et al., 2016, 2022c; Khursheed et al., 2016; Laskar et al., 2015; Tantray et al., 2017; Sellapillai et al., 2022). It is a sustainable technique available with plant breeders to broaden the genetic bases of crop plants and to create a gene pool of numerous desirable agro-economic traits (Raina et al., 2017) and is relatively cheaper to perform at a large scale (Siddiqui & Khan, 1999). The conventional mutagenesis technique for crop improvement is undergoing a renaissance due to progressions in contemporary cutting-edge technologies. Under a changing climate, mutation induction is a recognized technique to create diversity in existing crop varieties to expand the degree of adaptability for crop biomass enhancement (Laskar et al., 2019).

The mutation technique is considered better than other methods of crop improvement because it requires the least investment of land and labor (Gustaffson, 1947). In recent times, a lot of work has been done on induced mutagenesis in various crop plants. In these experimental crops, the mutational effect varied with varying mutagens and mutagenic doses. Thus, selecting an optimum dose of a mutagen for a genotype is an important step in mutation breeding programs (Khursheed et al., 2015, 2018a, b, c). Improvement of high yielding varieties is the basic necessity of the time. Plant breeders over and across the country have adopted various crop improvement strategies for generating variability and designing genotypes with high yield potential. Among the various breeding methodologies adopted, mutation has been considered a potent tool in the generation of requisite variability. The use of mutations resulting from irradiation or chemical mutagens has not received much attention as a breeding method in lentils. However, genetic variability has been created for many qualitative and quantitative characters such as pod and seed size, plant height, number of branches per plant, number of pods per plant, number of seeds per pod, dwarfing, early maturity, seed yield, days to flowering, and plant type (Sharma & Sharma, 1978b, 1981c; Dixit & Dubey, 1986).

Complex traits such as yield or seed size may be influenced by several genes, each with a very small effect, as suggested by classical quantitative genetic theory. These genes are commonly known as polygenes (Mather & Jinks, 1971). The mutation in polygenes is known as micromutation, and its usefulness in crop breeding has been emphasized by several workers (Lawrence, 1965; Scossiroli, 1966; Sindhu & Slinkard, 1983; Sinha & Chowdhary, 1984; Sarker & Sharma, 1988; Kalia & Gupta, 1989; Swarup et al., 1991; Ashutosh & Dubey, 1992; Khan et al., 2004; Khan & Wani, 2005; Khan & Wani, 2006; Khursheed et al., 2019). Experiments demonstrated that random mutations in quantitative traits could be induced in both positive and negative directions with the increase in variances. Such changes are due to increased genetic variation in the population (Yamaguchi, 2005). There are, however, conflicting reports as to whether mutations are induced equally in plus and minus directions or are unidirectional. Jalil and Yamaguchi (1964) observed in gamma-irradiated progenies of rice that, without selection, the mean values for seed size decreased due to successive irradiation. Moreover, subsequent irradiation with selection shifted the mean values toward the desired direction.

Physical and chemical mutagens in lentils have been used in the past, and as a result, varietal development has come up. Globally, up to the thirtieth of January 2022 (https://mvd.iaea.org accessed on 30 January 2022), mutation breeding has been successful in developing 3348 mutant varieties of crop plants (Fig. 4), including 466 varieties of pulses and 18 varieties of lentil. The principal contribution is from cereals (1596), followed by ornamental flowers (666), pulses (466), and edible oil crops (103). Among the 18 released mutant varieties of lentils, two varieties, namely, Ranjan and Rajendra Masoor 1 have been developed in India for various improved traits, particularly high yield, resistance to diseases, early maturity, and tolerance to cold. The description of lentil varieties released globally through mutagenesis is depicted in Table 9.

This study concludes that although there are numerous ways of improving the varieties of lentil and other pulse crops through conventional and contemporary breeding methods, the methods need to be improved in such directions to accomplish better success in breeding programs for these nutritionally important crops.

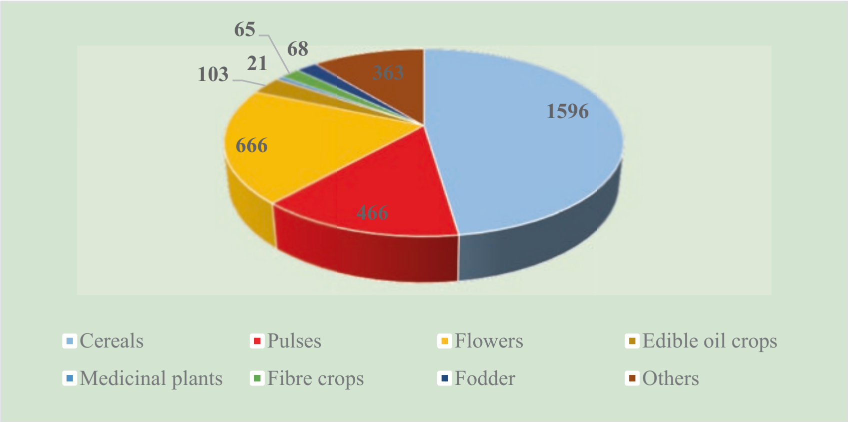

Fig. 4 Number of mutant varieties of crop plants released in the world (Source: Joint FAO/IAEA, Vienna Mutant Variety Database (MVD); http://mvgs.iaea.org accessed on 30 January 2022)

Table 9 Details of lentil varieties developed through mutation breeding

Mutant variety name	Country	Year of registration	Developed by	Main improved attributes
S-256 (Ranjan)	India	1981	Irradiation	High yield, spreading type
Rajendra Masoor 1	India	1996	100 Gy gamma rays	Low-temperature tolerance, early maturity, good for late sowing
Mutant 17 MM	Bulgaria	1999	40 Gy gamma rays	Vigorous growth habit, large leaflet, pods, and seeds, resistance to anthracnose, *Stemohylium,* and viruses, high yield, drought tolerance, improved cooking quality
Zornitsa	Bulgaria	2000	0.1% EMS	High yield, high protein content (28.7%), good culinary and organoleptic quality, and resistance to anthracnose, viruses, and *Ascochyta* blight
Djudje	Bulgaria	2000	30 Gy gamma rays	High yield, dwarf bushy habit, non-shattering, resistance to *Fusarium* and *Botrytis*, high protein content (27.9%), good culinary and organoleptic quality, suitable for mechanized harvesting
Binamasur-1	Bangladesh	2001	Chemical mutagen	High yield, tolerant to rust and blight, black seed coat
Elitsa	Bulgaria	2001	40 Gy gamma rays	High yield (34.4%), resistance to major disease

(continued)

Table 9 (continued)

Mutant variety name	Country	Year of registration	Developed by	Main improved attributes
NIAB Masoor-2002	Pakistan	2002	Irradiation	Erect growth habit, early maturity (120 days), black seed coat color, high grain yield, disease resistance, synchronous pod maturity
Verzuie	Moldova, Republic	2004	250 Gy gamma rays	The main improved attributes are drought resistance, vegetative period, proteins, oils, fructose, glucose, starch, and cellulose.
Aurie	Moldova, Republic	2005	250 Gy gamma rays	Drought resistance, high yield, early maturity, high protein content
Binamasur-2	Bangladesh	2005	200 Gy gamma rays	High yield, early maturity, and tolerance to rust and blight
Binamasur-3	Bangladesh	2005	0.5% EMS	High yield, early maturity, rust, and blight tolerance
NIAB Masoor-2006	Pakistan	2006	200 Gy gamma rays	A higher number of pods, resistance to lodging, blight, and rust, and 20–60% higher seed yield
Binamasur-5	Bangladesh	2011	200 Gy gamma rays	Early maturity, high yield
Binamasur-6	Bangladesh	2011	250 Gy gamma rays	Early maturity, high yield
Binamasur-8	Bangladesh	2014	200 Gy gamma rays	Early maturity, high yield
Binamasur-9	Bangladesh	2014	200 Gy gamma rays	Early maturity, high yield
Binamasur-11	Bangladesh	2017	200 Gy gamma rays	Early maturity, high yield, and plant architecture

Source: Joint FAO/IAEA, Vienna Mutant Variety Database (MVD); https://mvd.iaea.org accessed on 30 January 2022

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