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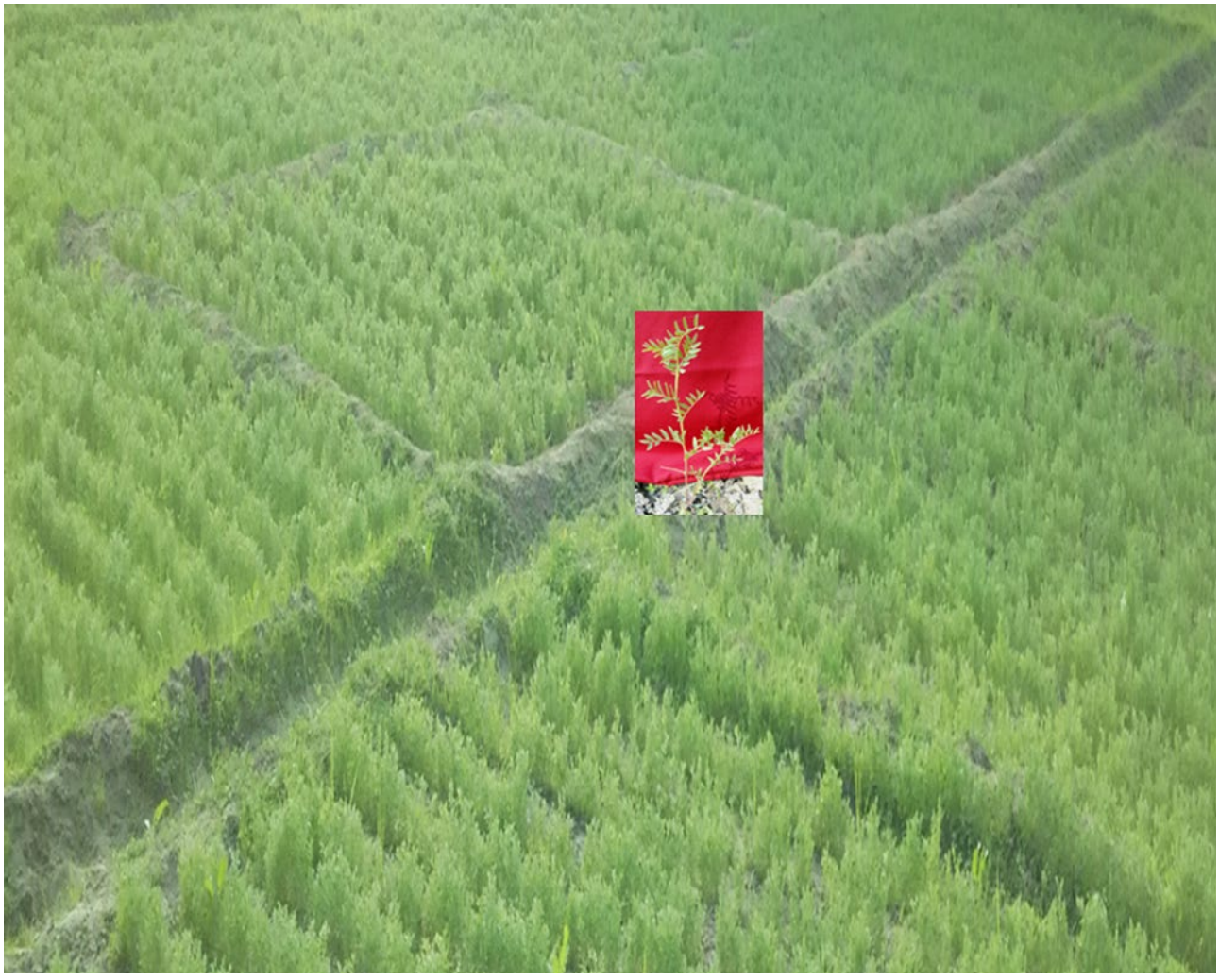

SOIL & CROP SCIENCES | RESEARCH ARTICLE

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Assessment of genetic response and character association for yield and yield components in Lentil (*Lens culinaris* L.) population developed through chemical mutagenesis

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Abstract: Genetic variation is imperative to any plant improvement program. Therefore, this study was primarily based on this aspect of inducing desirable genetic variation for enhancement of the available lentil genetic diversity. The lentil seeds were treated with methyl methanesulfonate (MMS) alone and in combination with dimethyl sulfoxide (DMSO) for inducing polygenic variation as well as determining the impact of DMSO on mutagenecity of MMS. Comparative observations were recorded for bio-physiological damages, morphological variation, and quantitative traits to assess the genetic response of the lentil cultivar L 4076 toward the different concentrations of chemicals. Significant statistics suggested that the DMSO interfere with the extent of mutagenecity of MMS in lentil which could be attributed to either synergistic action of both or variation in MMS uptake. The outcome of mutagenesis on the character association study revealed that mutagenic treatments can modify significantly the manner of association between any two traits in lentil. The moderate doses of MMS in combination with 2% DMSO showed notable diminution in the biological damages while accelerating the rate of desirable high-yielding mutants had proved to be economical. The segregate of the selected mutants in future generations will definitely contribute to the improvement of Lentil genotype.

Rafiul Amin Laskar

ABOUT THE AUTHOR

Rafiul Amin Laskar is a PhD research scholar at the Aligarh Muslim University of India. He obtained his MSc in Botany (specialization Genetics and Plant Breeding) and Post MSc Diploma in Plant Tissue Culture and Micropropagation from Aligarh Muslim University, India. His research laboratory works involve the genetic improvement of crops for qualitative and quantitative traits. He is actively engaged in the field of mutation breeding of pulses, by selecting effective and efficient mutagens and their doses for generating new genetic variability.

PUBLIC INTEREST STATEMENT

In the present era of mutable climatic conditions, consisting of temperature, amount of carbon dioxide (CO_2), and the frequency and intensity of extreme weather along with depleted arable lands, dwindling water resources, that have significant impacts on crop yields and food supply to the rapid increasing population. To establish food security for all, there is an urgent need of sustainable agricultural intensification and the only viable approach is to develop high-yielding crop varieties with wide adaptability. Lentil, a highly nutritious food legume with narrow genetic base, needs substantial research to boost the productivity. The coherent technique of targeted and accelerated evolution toward desirable characters through induce mutagenesis has appeared as the compelling supplement to the conventional plant breeding.

Subjects: Agriculture & Environmental Sciences; Soil Sciences; Bioscience; Biochemistry; Biotechnology

Keywords: Lentil (*Lens culinaris* Medik.); mutation breeding; methyl methanesulfonate (MMS); dimethyl sulfoxide (DMSO); quantitative traits; character association

1. Introduction

The diverse and important role played by pulse crops in the farming system, make them ideal crops for achieving the goal of global food security. Lentil (*Lens culinaris* Medik.) was first domesticated from *L. culinaris* subsp. *orientalis* (Boiss.) in about 9000 years ago (Zohary & Hopf, 2000). Seeds of lentil are the valuable source of high-quality plant proteins (26%) and rich in cholesterol-lowering soluble fiber. Lentil also plays an important role in crop rotations due to nitrogen fixing ability while plant residues can be used as livestock feed and fodder. According to FAO Statistics Division 2014, world lentil production in 2012 totalled to 4,557,972 tonnes from 4,206,024 hectares area harvested. India alone produces 20.84% of world production but yield is very low (0.5938 tonnes/hectare) compare to world average (1.0837 tonnes/hectare) which clearly indicates the lack of efficient agriculture practice and high-yielding varieties. The major constrains to yield are low-yielding cultivated varieties and the narrow genetic base (Asnake & Bejiga, 2003; Bejiga, Tsegaye, Tullu, & Erskine, 1996). Genetic variation is the currency of any crop improvement experiment and it is the long history of conventional legume breeding that causes the genetic bottlenecks which affects yield and quality. Lentil being a self-pollinated diploid ($2n$ = 14) (Alabboud, Szilagyi, & Roman, 2009) crop with a relatively large genome of 41063 Mbp (Arumuganathan & Earle, 1991), mutation breeding is the only feasible and sustainable technique to broaden their narrowing genetic bases to create a gene pool of numerous desirable traits of economic importance. Chemical mutagenesis is a coherent tool used in mutation breeding program for creating new alleles (Laskar & Khan, 2014a) and is relatively cheap to perform and equally usable on a small and large scale (Siddiqui & Khan, 1999). Records of the FAO/IAEA Mutant Variety Database reported 13 mutant lentil varieties (two from India) which appeared to be meager compared to 431 legume/pulses released mutant varieties. Most of these varieties were induced through physical sources of mutagenesis, therefore, work on chemical mutagenesis of lentil still needs to be exploited and standardize to develop reproducible protocols for mutation breeding. The range of induce mutation by different mutagenic doses varies according to the genotype used and the trait targeted. Mihov (1994) reported strong lethal effect of methyl methanesulfonate (MMS) than that of EMS and NaN_3 in lentil. Hakura et al. (2010) reported that the dimethyl sulfoxide (DMSO) has an inhibitory effect on metabolic activation of promutagen due to its inhibitory effect on the exogenous or endogenous drug-metabolizing enzymes involved in the parallel or sequential metabolic activation/detoxification pathways. Therefore, to explore only the phenomenal ability of DMSO as a biological tissue penetrant which increases the absorption of dissolved drugs and other chemicals (Leake, 1967; Leonard, 1967; Siddiq & Majid, 1969), the structurally simple direct chemical mutagen MMS was selected to get rid of the possible effects of DMSO on the metabolic activation or deactivation of mutagen. Since, long duration of treatments results in bio-physiological damages while providing higher mutation frequency, it would be important to develop ways to reduce treatment time while achieving relatively equal mutation rate. This investigation was carried out with the dual objectives of inducing genetic variability in morphological and yield contributing traits in a macrosperma cultivar of *L. culinaris* Medik. var. L 4076, using chemical mutagen MMS (direct alkylating mutagen) and to determine the effect of DMSO (reactive organic solvent) on the mutagenecity of MMS.

2. Material and methods

Dry (9–12% moisture) and healthy seeds of lentil (*L. culinaris* Medik. var. L 4076) were procured from Genetic Division of Genetics/Pulses, IARI, New Delhi. Fresh aqueous stock solutions of MMS and DMSO (1 and 2% v/v respectively) manufactured by Sissco Research Laboratories Pvt. Ltd, Mumbai, India,

were prepared in phosphate buffer at pH 7.0. The pH of the solution was maintained using buffer tablets (MERCK manufactures, Mumbai, India). From this stock, working solutions of 0.01, 0.02, 0.03, and 0.04% concentrations of MMS and combination of each MMS treatment with 2% DMSO were prepared. Seeds presoaked in distilled water for 9 h were subjected mutagenic treatments for 6 h with intermittent shaking at room temperature of 25 ± 2°C. Initially, an experiment was conducted to determine the lethal dose (LD 50) and suitable concentrations of the mutagens and duration of treatments for the crop was determined. After treatment, the seeds were thoroughly washed in running tap water for 30 min to remove the excess of mutagen. Thoroughly washed seeds from each treatment were grown on moist cotton in Petri plates in three replications five seeds each kept in the BOD incubator at 27 ± 1°C temperature to determine percentage of seed germination and seedling height i.e. root and shoot lengths and another five replications of five seeds each were sown in earthen pots filled with soil manure and kept in the Net House of the Department of Botany, Aligarh Muslim University during the Rabi season of the year 2012–2013. Seeds collected from M_1 generation were used to raise for further experimentation in M_2 generation. In M_2 generation, breeding behavior was observed and different agronomic traits viz., plant height, Number of primary branches/plant, pods/plant, seeds/pod, seeds/plant, 100 grain weight, and yield/plant were evaluated. The chlorophyll contents of leaves were estimated by the method of MacKinney (1941). To determine pollen fertility (%), the pollen grains from freshly dehisced anthers of 15 randomly selected plants per treatment were fixed in Carnoy's fluid (absolute alcohol: chloroform: acetic acid, 6:3:1 v/v) for 24 h after which they were stained with 1% acetocarmine through squashed technique and five slides per treatment were observed. The pollen grains stained as uniform deep red colors were counted as fertile and others as sterile. The treated as well as control populations were carefully screened for morphological mutations throughout the growth period in both the generations. Statistical analysis, namely, Mean ($\bar{X}$), Standard error (SE), Standard deviation (SD), Coefficient of variation (CV %), Least significance difference (LSD), and Pearson's correlation coefficient (r), were done using R 3.1.0 and IBM SPSS statistics 20 to assess the intra- and inter-population (mutagen) variations in different quantitative traits.

3. Result and discussion

This investigation showed that the action of chemical mutagen MMS alone and in combination with DMSO induces physiological, biochemical, metabolic, and genetic disturbances which results into significant bio-morphology and quantitative variations in *L. culinaris* Medik. L 4076. The data on the frequency of bio-morphological variants as well as on the yield contributing traits proved the DMSO effects mutagenecity of MMS significantly. Observations for identifying useful doses and desirable mutation for selection were made on Bio-physiological damages, morphological changes in plants, and quantitative parameters.

3.1. Bio-physiological damages

Biological damages caused by mutagen doses can be well assessed from effect on seed germination in any mutation breeding experiment (Figure 1). In this investigation, the seed germination and seedling heights generally decreased with increasing mutagen concentrations (Table 1). The minimum (66.66%) germination recorded in 0.04% MMS compared to (100%) in control which showed inhibitory effects on seed germination while DMSO reduces the inhibitory effect of MMS in all the concentrations. Similarly, seedling height decreases from 16.13 cm (control) to 5.63 cm at 0.04% MMS and 5.93 cm at 0.04% MMS + 2% DMSO. The reduction due to inhibitory effect of chemical mutagens. The reduction may be due to destruction of the activity of gibberellic acid, following the mutagenic treatment (Sideris, Nawar, & Nilan, 1971), and metabolic disturbances during germination (Ananthaswamy, Vakil, & Srinivasan, 1971). Griffiths and Johnston (1962) and Srivastava (1979) reported that reduction in germination percentage was due to weakening and disturbances of growth process, regulated in initial growing period. Krishna, Shivashankar, and Nath (1984) considered that the inhibition of germination may be due to interaction between mutagen and the seed cell system. It may also be due to toxicity of mutagens followed by mutational changes at genetic or chromosomal level because the reduction in germination corresponds with the increasing

Figure 1. (i) Germination pattern of mutagen treated seeds from lower to higher doses with MMS treated in first row and MMS + DMSO treated in second row and control on left, (ii) Isolated leaf morphological and chlorophyll variations, (iii) plant showing variations in the number of flowers and pods at the node.

chromosomal aberrations (Laskar & Khan, 2014b). The combination treatments increases the percentage of plant survival compared to the individual treatments of MMS. The highest plant survival at maturity was observed in the control (96%) which then decreases in highest dose of single and combination treatment to 72 and 76%, respectively. These finding were inconformity of the results by Ali and Shaikh (2007), Kumari and Singh (1996), Singh, Singh, Singh, Prasad, and Shahi (2007), and Verma, Srivastava, and Kumar (1999) in lentil and also by Vandana and Dubey (1988), Ashour and Abdou (1990), Kumar, Vandana, and Dubey (1993) in *Vicia faba*; Ansari and Siddiqui (1995) in *Ammi majus*; Mitra and Bhowmik (1999) in *Nigella sativa*. The degree of fertility of pollen grains was appreciably less in all MMS treatments (91.31–77.86) compared to their respective combination treatments (93.40–82.42%). The photosynthetic efficiency of a crop can be

Table 1. Comparative effect of mutagens (MH and MMS) on seed germination, seedling height, plant survival at maturity (%), pollen fertility, and total chlorophyll content in *L. culinaris* Medik. Var. L 4076

Treatments	Mutagens	Seed germination		Seedling height		Plant survival at maturity		Pollen fertility		Total chlorophyll	
		Actual (%)	% age inhibition	Actual (cm) ± SE Shift in mean	% age injury	Actual (%)	% age inhibition	Actual (%)	% age inhibition	Actual (mg/g) shift in mean	% age inhibition
	Control	100	–	16.13 ± 1.11 –	–	96		95.80	–	5.02 –	–
0.01%	MMS	93.33	6.67	12.37 ± 0.62, −3.76	22.50	92	4.16	91.31	4.68	4.85, −0.17*	03.38
	MMS + 2%DMSO	96.66	3.34	13.15 ± 0.88, −2.98	18.47	92	4.16	93.40	2.50	4.89, −0.13*	2.58
0.02%	MMS	76.66	23.34	9.77 ± 1.56, −6.36*	39.42	84	12.5	88.91	7.19	4.28, −0.74*	14.74
	MMS + 2%DMSO	83.33	16.67	10.53 ± 0.46, −5.60*	34.71	88	8.33	89.87	6.18	4.42, −0.60*	11.95
0.03%	MMS	83.33	16.67	12.93 ± 0.14, −3.20	19.83	80	16.66	81.49	14.93	3.64, −1.38*	27.49
	MMS + 2%DMSO	90	10	15.06 ± 1.56, −1.07	6.63	84	12.5	87.06	9.12	3.72, −1.30*	25.89
0.04%	MMS	66.66	33.34	5.63 ± 2.89, −10.5*	65.09	72	25.00	77.86	18.72	2.68, −2.34*	46.61
	MMS + 2%DMSO	73.33	26.67	5.93 ± 2.98, −10.2*	63.23	76	20.83	82.42	13.96	2.76, −2.26*	45.01
	LSD			5.40						0.02	

*The treatment mean differences from control is significant at the 0.05 level.

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well judged by estimating chlorophyll content. It was observed that total chlorophyll of leaves decreases from 5.02 mg/g in the control to 2.28 mg/g in 0.04% MMS and 2.76 mg/g in 0.04% MMS + 2% DMSO (Table 1). The chlorophyll mutation induction in lentil and other crops has been reported by several workers (Kousar, Suresh, Lavanya, & Kumari, 2013; Sharma & Sharma, 1986; Singh & Singh, 2001, 2003; Singh, Singh, Singh, & Prasad, 2006; Singh et al., 2007). The greatest chlorophyll content in plants occurs at the outset of the flowering phase, and chlorophyll is believed to take part in the process of organogenesis (Simova-Stoilova, Stoyanova, & Demirevska-Kepova, 2001). Concentration of nitrogen in crops is related to chlorophyll content, and therefore, indirectly to photosynthesis (Amaliotis, Therios, & Karatissiou, 2004; Cabrera, 2004; Haboudane, Miller, Tremblay, Zarco-Tejada, & Dextraze, 2002; Lelyveld, Smith, & Frazer, 2004). Nitrogen is a structural element of chlorophyll and protein molecules, and it thereby affects formation of chloroplasts and accumulation of chlorophyll in them (Daughtry, Walthall, Kim, Brown, & McMurtrey, 2000). Thus, estimation of chlorophyll content from early fresh leaves provides useful information about the physiological status of the growing plant. The diminution in the biological damages in combination treatments can be credited to the enhancement of germination due to DMSO as earlier observed in certain tree seeds (Smale, 1969) and in sprouting of potato tuber (Davidson, 1967).

3.2. Morphological variants

Different types of morphological variants affecting almost all parts of the plant were observed in moderate to higher doses of mutagens. Screening of morphological mutants was done based on variation in the plant height (tall and dwarf), and growth habits (erect, semi-erect and horizontal) importantly bushy and prostrate (Figure 2). The leaf morphological variations recorded were foliage color (light, medium, and dark) and leaflet size (small, medium, and large), and shape (narrow and broad). Observed dwarf- and small-leaved mutants showed small leaf, short internodes with few node, and reduction of leaf, pod, and ultimately low yield where as bushy and long broad-leaved mutants showed reduced height with significant increased primary branching, pod per plant, and yield (Figures 1 and 2). 0.03% + 2% MMS treatment provides high-yielding mutant due to enhanced branching and seed per pod compared to all other treatment. In addition to that in higher concentration, seedlings showed stunted growth bearing small, dark green, and thick leaflets. In 0.04% MMS, the entire leaflets were highly reduced in size, round, and rudimentary. The variation in length, width, rachis, and leaflet arrangement observed in different lentil leaf morphology mutants (Dixit & Dubey, 1983). The flower arrangement on the control plant was two per peduncle, however, in the treated population, single to triple flower per peduncle were noticed. In the high doses, the flower number gets reduced. Alteration in pod sizes viz. bold pod, narrow pods, and empty pods have also been recorded in the treated plants. Generally, in combination treatments, the frequencies of variants were more compared to single treatments of MMS in most of the cases. Similar abnormalities have also been reported in *Chloris guana* Kunth. (Krishna et al., 1984), *Vigna radiata* L. (Khan & Siddiqui, 1992), *V. faba* (Bhat et al., 2007; Kumar et al., 1993; Laskar & Khan, 2014b; Vandana, 1992; Vandana & Dubey, 1988). The easy induction of mutation in leguminous plants may also be the reasons of leaf abnormalities (Blixt, 1972) or due to chromosomal alterations (Grover & Virk, 1986).

3.3. Qunatitative parameters

The average height of mature plants had reduced with increasing concentrations of both the mutagens. The maximum reduction in average height was recorded in MMS treatments followed by MMS + DMSO (Table 2). Reduction in growth as seen in *L. culinaris* has also been observed by many workers such as Verma et al. (1999) in *L. culinaris,* Krishna et al. (1984) in Rhodes grass, Khan, Siddiqi, and Khan (1987) in mungbean, Vandana and Dubey (1988), and Kumar et al. (1993) in faba bean and Ansari and Siddiqui (1995) in *A. majus* and explained the causes of decreasing plant height due to mutagenic treatments. The structural changes in the constitution of chromosomes or chromosomal damage may be major factors in growth inhibitions (Arumugam, Reddy, Asir, Viswanathan, & Dhamodaran, 1997). On the other hand, growth inhibition may arise from interference of mutagens with the cell elongation (Sparrow & Sparrow, 1965) or injury caused to the meristematic cells (Ansari & Siddiqui, 1995). Some other aspects, such as auxin reduction (Krishna et al., 1984), physiological disorder (Gunckel, 1957), or

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Figure 2. (i) Variations in plant height compared to control showing semi-erect, high yielding, bushy and dwarf mutants respectively, (ii) variations in pods size and shape in control and mutagen treated plant placed increasing concentrations wise in upper row for MMS and lower for MMS + DMSO.

metabolic disturbances (Gupta & Sumata, 1967) may also play important role in the reduction in height in the treated plants. The variation in yield contributing characters (Table 2) due to different doses of mutagens as observed in *L. culinaris* in this study was also reported in *L. culinaris* (Ali & Shaikh, 2007; Khan, Wani, & Parveen, 2006; Kumar, Singh, & Singh, 1995; Rajput, Mutageneza, & Mutageneza, 2001; Singh et al., 2006; Sinha & Lal, 2007; Tripathi & Dubey, 1992), *V. faba* (Ismail, Heakal, & Fayed, 1977), mungbean (Tickoo & Chandra, 1999), *Oryza sativa* (Awan, Konzak, Rutger, & Nilan, 1980; Rao & Siddiq, 1977; Singh, Richharia, & Joshi, 1998), *Hordeum vulgare* (Gustafsson, 1963; Ramesh, Prasad, & Singh, 2001), *Triticum durum* (Sakin & Yildirim, 2004), *Cicer arietinum* (Shaikh, Yildirim, Majid, & Wadud, 1982; Sharma, Sood, & Malhotra, 1990), *Vigna unguiculata* (Mensah & Akomeah, 1992), *Cajanus cajan* (Srivastava & Singh, 1993, 1996), *Vigna radiate* (Wani & Khan, 2006), and *Vigna mungo* (Kundu & Singh, 1981; Meshram, Ali, Patil, & Meena, 2013; Singh & Singh, 2001).The decrease in yield attributing traits as observed in *L. culinaris* in this study was also reported by Verma et al. (1999) by gamma rays, Amer and Farah (1976) by carbamate pesticide in *V. faba*, Reddy and Rao (1982) in *Capsicum annum* by herbicides, Lakshmi, Prakash, and Harini (1988) in *C. annum* by insecticides, Vandana and Dubey (1988) in *V. faba* by EMS and DES, and Kumar et al. (1993) in *V. faba* by gamma rays and DES. The decrease in the number of pods and 100 seed weight occurred due to induced mutation in meiotic cycle which affected the frequency of normal microspores up to greater

Table 2. Statistical analysis of comparative effect of mutagens (MMS and MMS + DMSO) on various quantitative characters in M_2 generation of lentil (*L. culinaris* Medik.) var L4076

Treatments	Mutagens	Quantitative characters							
		Days to maturity Mean ± SD CV%, shift in mean	Plant height Mean ± SD CV%, shift in mean	Primary Branches/plant Mean ± SD CV%, shift in mean	Pods/plant Mean ± SD CV%, shift in mean	Seeds/pod Mean ± SD CV%, shift in mean	Seed/plant Mean ± SD CV%, shift in mean	100 Seed Weight (g)Mean ± SD CV%, shift in mean	Seed yield plant (g) Mean ± SD CV%, shift in mean
	Control	133.0 ± 0.710.53, –	43.0 ± 0.791.84, –	3.2 ± 0.4514.06, –	50.4 ± 2.615.18, –	2 ± 0.000.00, –	100.8 ± 5.225.19, –	3.06 ± 0.258.17, –	3.08 ± 0.216.82, –
0.01%	MMS	132.6 ± 0.550.41, −0.4	41.0 ± 0.791.93, −02.00*	2.0 ± 0.7135.50, −1.2*	36.8 ± 2.286.20, −13.6*	1.6 ± 0.5534.38, −0.40	59.6 ± 22.6538.00, −41.2*	2.76 ± 0.113.99, −0.30*	1.66 ± 0.6840.96, −1.42*
	MMS + 2% DMSO	132.4 ± 0.550.42, −0.6	38.8 ± 0.912.35, −04.20*	2.0 ± 0.7135.50, −1.2*	38.0 ± 1.413.71, −12.4*	1.4 ± 0.5539.29, −0.60	53.2 ± 20.8639.21, −47.6*	3.04 ± 0.113.62, −0.02	1.16 ± 0.6152.58, −1.92*
0.02%	MMS	135.0 ± 0.700.52, + 2.0	37.1 ± 0.741.99, −05.90*	2.2 ± 0.4520.46, −1.0	46.4 ± 1.673.60, −4.0*	1.6 ± 0.5534.38, −0.40	74.4 ± 26.0234.97, −26.4	2.72 ± 0.082.94, −0.34*	2.03 ± 0.7235.47, −1.05*
	MMS + 2% DMSO	134.2 ± 0.450.36, + 1.2	34.4 ± 0.651.89, −8.60*	2.2 ± 0.4520.46, −1.0	47.6 ± 1.673.51, −2.8*	1.6 ± 0.5534.38, −0.40	76.0 ± 25.6533.75, −24.8	2.84 ± 0.093.17, −0.22*	2.17 ± 0.7635.02, −0.91*
0.03%	MMS	135.8 ± 0.840.62, + 2.8	39.0 ± 0.501.28, −04.00*	2.6 ± 0.5521.15, −0.6	48.4 ± 1.673.45, −2.0	1.6 ± 0.5534.38, −0.40	77.2 ± 25.9133.56, −23.6	2.42 ± 0.135.37, −0.64*	1.85 ± 0.5831.35, −1.23*
	MMS + 2% DMSO	135.2 ± 0.450.33, + 2.2	36.8 ± 0.571.55, −6.20*	3.0 ± 1.2240.67, −0.2	49.2 ± 1.102.26, −1.2	1.8 ± 0.4525.00, −0.20	88.4 ± 21.5624.39, −12.4	2.68 ± 0.082.99, −0.38*	2.37 ± 0.5724.05, −0.71
0.04%	MMS	136.6 ± 0.550.40, + 3.6	31.3 ± 1.043.32, −11.70*	1.8 ± 0.4525.00, −1.4*	33.6 ± 1.674.97, −16.8*	1.8 ± 0.4525.00, −0.20	60.4 ± 15.1325.04, −40.4*	2.36 ± 0.114.66, −0.70*	1.43 ± 0.3927.27, −1.65*
	MMS + 2% DMSO	136.4 ± 1.141.05, + 3.4	32.0 ± 0.501.56, −11.00*	2.2 ± 0.8438.18, −1.0	35.2 ± 2.286.47, −15.2*	1.8 ± 0.4525.00, −0.20	63.2 ± 15.8525.08, −37.6*	2.48 ± 0.083.22, −0.58*	1.57 ± 0.4126.12, −1.51*
Total mean ± SE		134.58 ± 1.66	37.1 ± 3.89	2.36 ± 0.77	42.84 ± 6.67	1.69 ± 0.47	72.58 ± 23.85	2.71 ± 0.26	1.970.71
LSD		1.01	1.08	1.01	2.73	0.70	30.46	0.19	0.84

*The treatment mean differences from control is significant at the 0.05 level.

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Table 3. Phenotypic correlation coefficients among nine quantitative traits in MMS and MMS + DMSO treated M_2 populations of *L. culinaris* Medik. variety L 4076

Characters	Treatments	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) Days to maturity	Control	1.0000								
	0.01% MMS	1.0000								
	0.02% MMS	1.0000								
	0.03% MMS	1.0000								
	0.04% MMS	1.0000								
	0.01% MMS + 2% DMSO	1.0000								
	0.02% MMS + 2% DMSO	1.0000								
	0.03% MMS + 2% DMSO	1.0000								
	0.04% MMS + 2% DMSO	1.0000								
(2) Pollen fertility	Control	−0.729	1.0000							
	0.01% MMS	0.6424	1.0000							
	0.02% MMS	0.0665	1.0000							
	0.03% MMS	0.5227	1.0000							
	0.04% MMS	0.0880	1.0000							
	0.01% MMS + 2% DMSO	0.0940	1.0000							
	0.02% MMS + 2% DMSO	0.6779	1.0000							
	0.03% MMS + 2% DMSO	−.944*	1.0000							
	0.04% MMS + 2% DMSO	−0.874	1.0000							
(3) Chlorophyll content	Control	0.3162	−0.593	1.0000						
	0.01% MMS	−0.123	−0.717	1.0000						
	0.02% MMS	−0.310	−0.828	1.0000						
	0.03% MMS	0.3516	0.7962	1.0000						
	0.04% MMS	−0.244	−.887*	1.0000						
	0.01% MMS + 2% DMSO	−0.480	−.882*	1.0000						
	0.02% MMS + 2% DMSO	−0.196	−0.775	1.0000						
	0.03% MMS + 2% DMSO	0.7071	−0.525	1.0000						
	0.04% MMS + 2% DMSO	.983**	−0.839	1.0000						
(4) Plant height	Control	−0.447	0.5321	0.2828	1.0000					
	0.01% MMS	−0.866	−0.620	0.4264	1.0000					
	0.02% MMS	0.4767	−0.279	0.5026	1.0000					
	0.03% MMS	0.2988	−0.131	0.3922	1.0000					
	0.04% MMS	−0.176	−0.096	0.0000	1.0000					
	0.01% MMS + 2% DMSO	−0.553	−0.408	0.5069	1.0000					
	0.02% MMS + 2% DMSO	−0.772	−0.592	0.0673	1.0000					
	0.03% MMS + 2% DMSO	0.1961	−0.469	−0.416	1.0000					
	0.04% MMS + 2% DMSO	0.2193	0.0308	0.3297	1.0000					
(5) No. of primary branch	Control	0.0000	0.3242	0.0000	0.7071	1.0000				
	0.01% MMS	0.0000	−0.154	−0.238	−0.224	1.0000				
	0.02% MMS	−0.791	0.2907	0.1961	−0.075	1.0000				
	0.03% MMS	0.3273	0.3668	0.1790	0.0000	1.0000				
	0.04% MMS	0.6124	−0.111	−0.299	0.4313	1.0000				
	0.01% MMS + 2% DMSO	0.0000	0.7467	−0.620	0.1946	1.0000				
	0.02% MMS + 2% DMSO	1.000**	0.6779	−0.196	−0.772	1.0000				
	0.03% MMS + 2% DMSO	0.0000	0.1231	0.6455	−0.716	1.0000				
	0.04% MMS + 2% DMSO	0.4193	−0.691	0.2758	−0.598	1.0000				

(*Continued*)

Table 3. (*Continued*)

Characters	Treatments	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(6) Pods per plant	Control	−0.814	0.8617	−0.772	0.1213	−0.086	1.0000			
	0.01% MMS	0.7206	0.5509	−0.532	−.971**	0.3101	1.0000			
	0.02% MMS	0.4226	−0.351	0.3669	0.7655	−0.134	1.0000			
	0.03% MMS	0.0714	−0.374	0.1172	.896*	0.2182	1.0000			
	0.04% MMS	−0.218	0.3114	0.1597	−0.202	−0.802	1.0000			
	0.01% MMS + 2% DMSO	0.6455	0.6580	−.930*	−0.389	0.5000	1.0000			
	0.02% MMS + 2% DMSO	0.8018	.966**	−0.681	−0.733	0.8018	1.0000			
	0.03% MMS + 2% DMSO	−0.612	0.4798	−0.866	0.4804	−0.745	1.0000			
	0.04%MMS + 2%DMSO	0.3462	−0.126	0.3181	−0.439	0.1048	1.0000			
(7) Seeds per plant	Control	−0.814	0.8617	−0.772	0.1213	−0.086	1.000**	1.0000		
	0.01% MMS	0.2661	0.4668	−0.718	−0.670	0.0625	0.8017	1.0000		
	0.02% MMS	0.0544	0.0001	0.4787	0.7878	0.3782	0.2940	1.0000		
	0.03% MMS	.913*	0.7843	0.5602	0.0965	0.2185	−0.221	1.0000		
	0.04% MMS	−0.459	−0.845	.936*	−0.153	−0.429	0.0869	1.0000		
	0.01% MMS + 2% DMSO	0.2100	0.0662	−0.143	−0.829	−0.610	0.0678	1.0000		
	0.02% MMS + 2% DMSO	−0.567	−0.078	−0.547	0.5083	−0.567	−0.140	1.0000		
	0.03% MMS + 2% DMSO	0.1971	−0.171	−0.0880	−0.155	−0.076	−0.322	1.0000		
	0.04% MMS + 2% DMSO	−0.697	0.5902	−0.807	−0.694	0.1659	0.0885	1.0000		
(8) 100 seed weight	Control	0.0000	−0.638	0.6682	0.0000	−0.134	−0.504	−0.504	1.0000	
	0.01% MMS	0.4804	0.6234	−0.798	−0.832	0.3101	.923*	.922*	1.0000	
	0.02% MMS	0.0000	−.923*	.891*	0.5641	−0.134	0.6429	0.2481	1.0000	
	0.03% MMS	−0.642	−0.140	0.3760	0.3835	−0.210	0.4125	−0.542	1.0000	
	0.04% MMS	−0.320	−0.372	0.5860	0.6556	−0.196	0.4193	0.3885	1.0000	
	0.01% MMS + 2% DMSO	0.0801	0.1870	−0.423	0.3380	0.3101	0.6202	−0.278	1.0000	
	0.02% MMS + 2% DMSO	0.3750	0.8058	−.932*	−0.343	0.3750	0.8018	0.4794	1.0000	
	0.03% MMS + 2% DMSO	0.1336	−0.010	0.7559	−0.629	.976**	−0.764	−0.216	1.0000	
	0.04% MMS + 2% DMSO	−0.157	0.0054	−0.276	0.0000	0.4286	−0.629	0.3620	1.0000	
(9) Seed yield per plant	Control	−0.585	−0.109	0.1979	0.0812	−0.211	0.1467	0.1467	0.7802	1.0000
	0.01% MMS	0.2867	0.4712	−0.722	−0.693	0.0996	0.8226	.999**	.936*	1.0000
	0.02% MMS	0.0530	−0.069	0.5333	0.8058	0.3539	0.3169	.998**	0.3103	1.0000
	0.03% MMS	0.8703	0.8455	0.6760	0.1421	0.2275	−0.189	.989**	−0.417	1.0000
	0.04% MMS	−0.477	−0.838	.965**	−0.015	−0.431	0.1667	.984**	0.5486	1.0000
	0.01% MMS + 2% DMSO	0.2199	0.0810	−0.176	−0.820	−0.598	0.1128	.998**	−0.213	1.0000
	0.02% MMS + 2% DMSO	−0.529	−0.015	−0.595	0.4662	−0.529	−0.079	.998**	0.5316	1.0000
	0.03% MMS + 2% DMSO	0.2219	−0.178	0.0200	−0.248	0.0631	−0.439	.990**	−0.077	1.0000
	0.04% MMS + 2% DMSO	−0.672	0.5508	−0.792	−0.661	0.2216	0.0078	.992**	0.4739	1.0000

*Correlation is significant at the 0.05 level (two-tailed).

**Correlation is significant at the 0.01 level (two-tailed).

extent and the megaspore to a lesser extent and hence the fruit set was directly affected. The positive effect of DMSO treatment alone on yield of certain crops including flower bulbs, peas, potatoes, and soyebeans were observed (Smale, 1969). Reduction in mutation frequency in combination treatments with DMSO were observed in rice (Siddiq, Puri, & Singh, 1968), in soyebean (Pluenneke & Burson, 1973), barley (Khalatkar, 1976), and banana (Omar, Novak, & Brunner, 1989), whereas, increase effect was observed in Arabidopsis (Bhatia, 1967) and *Triticum monococcum* (Rana & Mathur, 1969).

3.4. Character association study

The degree of the linear relationship between two traits considered to be the function of selection, gene linkage, and pleiotropy (Sakai & Suzuki, 1964). Latief et al. (2011) and Yadav et al. (2007) reported presence of strong correlations between various quantitative traits in lentil. Selection for important polygenic trait like yield required the knowledge of existence relation between yield and other characters and also interrelationships among various characters, to develop effective selection criteria for the desired trait without compromising other equally essential characters in lentil mutation breeding. Existence of undesirable linkages involving yield attributes in the crop plants is common. The action of various mutagens in weakening, strengthening, or altering character association demonstrated in several crops (Borojevic, 1966; Patel, 1991; Ramanathan & Rathinam, 1983; Scossiroli, Palenzona, & Scossiroli-pelle-grini, 1966; Waghmare & Mehra, 2000), can be attributed to the result of its effect on gene linkage and/or altered pleiotropic effect of the newly mutated genes (Babariya, Vaddoria, Mehta, Madariya, & Monpara, 2008). Comparative results obtained from control and treated populations demonstrated mutable variations in degree of relationship between every pair of traits at different doses of treatments due to mutagenesis (Table 3). The results revealed that days to maturity showed negative correlation (r = −0.729) with pollen fertility in control but 0.03% MMS + 2% DMSO increased this negative correlation to a significant value (r = −0.944) and all other treatments converted it to positive correlation. Similarly, positive correlation (r = 0.316) between days to maturity and chlorophyll content become significant (r = 0.983) due to 0.04% MMS + 2% DMSO treatment. Pollen fertility and chlorophyll showed significant negative correlation at 0.04% MMS (r = −0.887) and 0.01% MMS + 2% DMSO (r = −0.882) and with 100 seed weight at 0.02% MMS (r = −0.923) while pollen fertility was positively correlated with pods/plant, significant at (r = 0.966 at 0.02% MMS + 2% DMSO). Significant correlation was induced by mutagenic treatments in pairs of chlorophyll content with pods/plant, seeds/plant, and 100 seed weight. Pods/plant was positively correlated with plant height (r = 0.318) which changed significantly at 0.01% MMS (r = −0.971) and at 0.03% MMS (r = 0.896). Similarly, pods/plant was negatively correlated with 100 seed weight (r = −0.504) and at 0.01% MMS relationship become significantly positive (r = 0.923). Seed yield per plant have positive correlation with chlorophyll content, plant height, pods per plant, seed per plant, and 100 seed weight. The mutagenic treatments significantly changed the relationship between yield and other characters. According to Scossiroli et al. (1966), the pleiotropic effect of induced mutation on related traits strengthened the correlation coefficients between characters, whereas of the correlative associations existed in the control populations leads to no change or diminution in correlation. Thus, the results suggested that the significant boost in the amount of correlation in pair of quantitative traits and breakage of undesirable relationship between characters can be achieved through mutation breeding in lentil which is unarguably of immense useful in selecting desirable attributes.

4. Conclusions

The findings of this mutation breeding study provide an extensive research on the MMS mutagenesis and effect of DMSO on the mutagenecity of MMS in lentil. The investigation posits that the DMSO as a useful organic solvent to create the required supplementary variation in the mutagenic treatments can be recommended to lentil mutation breeding program using MMS. Interactions of DMSO with the MMS in moderate concentrations resulted in the significant diminution of biological damages while increasing the frequency of desirable mutants and ultimately yield in the treated population of lentil.

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