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Article : Induced Variation in Quantitative traits due to Chemical mutagen (Hydrazine Hydrate) treatment in Lentil (Lens culinaris Medik.).

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Abstract:

Effect of chemical mutagen in lentil var. K-75 and L-4076 were exposed to hydrazine hydrate (HZ). Seeds of both varieties of lentil were subjected to different concentrations 0.01%, 0.02%, 0.03% and 0.04% of HZ. Both varieties were evaluated for days to flowering, days to maturity, plant height, pods per plant, 100-seeds weight and total plant yield. Both the parental varieties (K-75 and L-4076) differed significantly between themselves for both percentage seed germination, seedling height and all the characters for HZ treatments. Variances among treatments (four doses of mutagen + one control) were also significantly high.

Key Words: *Lens culinaris*, HZ, quantitative traits.

Introduction:

Lentil (*Lens culinaris* Medik.), cereal-based diet of common man, plays an important role to meet the protein requirements. It is most important pulse crop in India as it has 25% protein (Gupta, 1982). The seeds of lentil are an important protein and carbohydrate- rich food in many developing regions and are becoming increasingly popular in developed countries, where they are perceived as a healthy component of the diet (Sevage, 1991).

A series of experiments carried out with various crops have established that chemical mutagens induce polygenic variability (Kharkwal, 1999; Rajput et al., 2001; Singh & Singh, 2001; Khan etal., 2004). HZ (Hydrazine hydrate) has been reported to induce a variety of morphological, physiological and chlorophyll mutants in several crop plants (Wani and Khan, 2006). It reacts with the pyrimidines in DNA to saturate the 5,6 double bond, especially of thymine to form N^4-amino-cytosine and to open up the pyrimidines from DNA or through

intermediate radical reactions including the formation of hydrogen peroxide depending upon the hydrazine derivatives involved (Kimball, 1977).

Therefore the effects of HZ in two lentil varieties K-75 and L-4076 has been studied in order to induce genetic diversity and selection of mutants.

Materials and Methods:

Certified, healthy seeds of two varieties of lentil (*Lens culinaris* Medik) viz., K-75 and L-4076 were procured from Division of Genetics, Indian Agriculture Research Institute (IARI), New Delhi. The seeds were presoaked in distilled water for 24 h and then treated with four different concentrations of hydrazine hydrate (HZ), prepared in sodium phosphate buffer at 7.0 pH for 24 h with constant intermittent shaking. The treated seeds were washed in running tap water for 2 to 3 times to remove the residual effect of the mutagen sticking to the seed coat. One set of seeds was kept untreated in buffer solution to act as control for comparison. The seedlings were transplant in the field in randomized block design in 2 replicates in each dose along with control.

Result:

Both varieties of *Lens culinaris* (lentil) differed in their response to mutagenic treatments with regard to germination as the maximum germination was attained on 10^{th} day in control and 20^{th} day in the highest dose of HZ. The percentage of seed germination in var. K-75 and L-4076 decreased gradually from 93.00 and 93.75% (control) to 21.25 and 18.75% in 0.04% HZ respectively (Table-1). Percent inhibition in germination as compared to control increased linearly from 12.63 to 76.61 and from 13.33 to 80.00 in var. K-75 and L-4076 respectively due to decrease in the germination percentage. HZ has induced many variations in the plant survival in both varieties. The plant survival decreased from 87.50 (control) to 69.33% in var. K-75 and from 89.72 (control) to 32.44% in var. L-4076 in 0.04% HZ (Table-1). The survival decreased due to the increasing lethality from 17.24 to 25.60% in var. K-75 and from 22.47 to 64.04% in var. L-4076 from 0.01 to 0.04% of HZ. The variation was nil in control but increased from 1.52 to 6.33% in var. K-75 and from 1.78 to 6.97% in var. L-4076 from 0.01 to 0.04% HZ (Table-1).

The flowering was delayed from control (59.20 days) to 0.03% HZ insignificantly but it delayed significantly to 60.60 days in 0.04% HZ in var. K-75. Whereas in var. L-4076, although delaying effect was recorded but it was insignificant as compared to control population. The days taken for maturity (ripening of fruits) increased in both varieties from control 69.20 and 76.86 to 70.06 and 78.26 whereas in still higher doses, life cycle (till ripening of fruits) decreased significantly at 1% level in both varieties from control to 67.86 and 75.93 days respectively (Table-2). Plant height decreased significantly from

control (54.06 cms, fig. A) to 33.13 cms (in 0.04% HZ) in var. K-75 (fig. B) whereas in var. L-4076 plant height increased from 50.86 cms in control to 53.10 cms in 0.01% HZ (fig. C) but decreased significantly in all higher doses of HZ (Table-2, fig. D). This resulted in induction of dwarf mutants.

The number of fertile branches increased significantly from 12.00 (control) to 15.86 (at 1% level) in 0.03% HZ in var. K-75 (Fig. E) and from 10.06 (control) to 14.00 in 0.02% HZ (at 1% level) in var. L-4076 (fig. F) whereas in still higher doses they decreased significantly in both varieties to 5.40 per plant (at 1% level) in K-75 and to 7.06 (at 5% level) in var. L-4076. Number of pods per plant in var. K-75 showed positive effect in 0.01% HZ where in the number increased from 153.66 (control) to 169.00 (0.01% HZ) but still in higher concentration it decreased significantly whereas in var. L-4076, the number increased significantly from 76.33 (in control) to 373.33, 184.80 and 101.86 pods per plant 0.01 to 0.03% HZ but decreased in higher dose showing that the var. L-4076 is more responsive to HZ treatment.

The number of seeds per pod and 100 seeds weight, were not much affected as their values decreased/ increased over control insignificantly in all doses. There was positive relationship between number of pod and yield per plant in 0.01 % HZ in var. K-75 as the yield increased in this concentration from 4.25 gm to 5.15 gm but in still higher doses there was significant decreased. In var. L-4076 there was positive relationship between number of fertile branches per plant and number of pods per plant resulting into the significant increase in yield from 2.43 to 10.28, 5.27 and 3.11 gm per plant in 0.01, 0.02 and 0.03% HZ respectively (Table-2).

Discussion:

Induced mutagenesis has been accepted as a significant tool to break through the limitations of variability and to create variability in a short period of time. These two varieties of lentil K-75 and L-4076 differed in response to HZ treatments regarding the induction of variability in seed germination, plant survival, growth, morphology, pollen fertility and yield.

The seed germination decreased in both varieties due to increasing inhibiting effect of HZ but L-4076 was more adversely affected. Resultantly the plant survival decreased particularly in the var. L-4076. The reduction and inhibition in seed germination and plant survival were found to be dose dependent and decreased linearly with an increasing lethal effect of HZ concentrations. Similar observations were recorded in*Vigna radiata* by many workers (Khan and Ali, 1987; Khan et al., 1998; Padavai and Dhanavel, 2004; Khan and Wani 2005; Singh and Kole, 2005; Parveen, 2006) Inhibition in seed germination after mutagenic treatment has been attributed to changes in biochemical and physiological system

(Sparrow and Woodwel, 1962), and inhibitory effect of mutagen (Sahai, 1974). Moreover, the var. L-4076 was highly affected as compared to var. K-75. It may also be interpreted that var. L-4076 was more sensitive to the mutagen. Although most of the seedlings were eliminated due to the lethal effect of HZ even then the variations in treated population ranges between 1.52% to 6.33% in var. K-75 and 1.78% to 6.97% in var. L-4076, whereas no variation was observed in control population. Some of the morphological variations such as dwarf, tall, bushy and prostrate appeared more frequently. Similar results have been reported in lentil (Tripathi and Dubey, 1992; Vandana et al., 1994; Ramesh and Dhananjay, 1996; Tyagi and Ramesh, 1998; Solanki and Sharma, 1999; Khan et al., 2006), black gram (Rahman etal., 2001), and chick pea (Atta et al., 2003; Khan etal., 2004a, 2004b).

A depression in pollen fertility was also reported in *Vigna radiata* (Khan and Hashim, 1978; Ganguli and Bhaduri, 1980; Khan and Siddiqui, 1992 and Wani, 2007) and in *Lens culinaris* (Sinha and Godward, 1972; Reddy and Annadurai, 1991; Wani and Khan, 2003).

The increasing doses of gamma-rays and N-nitroso-N-methyl urea (NMU) caused a progressive increase in the biological damage measured in terms of reduction in seed germination, plant survival and pollen sterility in M_1 generation (Sharma, 1977). Days taken for flowering and fruiting were delayed significantly in var. K-75 but in var. L-4076, the life span decreased in higher concentrations of HZ.

Reduction in mean number of days to flowering in var. L-4076 indicates the possibility of isolating early maturity type in later generations. The effect of higher doses of HZ at maturity is more significant as the life span in both varieties decreased significantly. Similar observations were recorded in peas by other workers (Kaul, 1980; Thurling and Depittayanan, 1992). The induction of short duration varieties is an important achievement.

Plant height reduced significantly in increasing doses of HZ in var. K-75, whereas the 0.01% HZ exhibited enhancing effect on height in var. L-4076 but still higher concentrations had adverse effect. Reduced growth due to higher doses has been explained differently by different workers. It may be attributed to one or more of the following reasons. (i) Increase in the destruction of growth inhibitors (ii) due to the induced chromosomal aberrations. The enhancing effect may be due to sudden increase in the metabolic status of seedlings and increase in the activity of growth promoters. These findings are in close agreement with the earlier reports of Wang and Yu (1988), Solanki and Sharma (1999, 2002), Kumar and Selvaraj (2003), Solanki and Phogat (2005).Moreover, the dwarfness in plant height as

observed in higher concentrations is associated with earliness in maturity (Olejniczak and Adamska, 1999), which is a desirable character in crop plants.

Number of branches increased significantly in all concentrations of HZ in var. K-75 and in var. L-4076 but decreased in still higher concentrations of HZ because the loss of apical dominance resulted in lateral distribution of growth hormone and hence the increasing branching. Number of pods per plant increased insignificantly in lower doses but decreased significantly in higher dose in var. K-75, whereas it increased significantly in most of doses in var. L-4076, however the highest doses in both the varieties caused negative effect. Number of seed per pod and 100-seeds weight was not much affected in both varieties as their value did not follow any definite trend. Moreover the total yield per plant was directly related with number of pods per plant because whenever the number of pods per plant increased, the yield also increased and this occurred in those concentrations of HZ which exhibited enhancing effect.

Increased seed yield following increase in leaf, flower, pod and seed size has been reported in other pulse crops also (Swaminathan, 1972; Prasad, 1976; Sharma and Sharma, 1981).

It is considered that lower concentration of HZ has induced positive effect which produces more variants such as shortening of life cycle, increase of fertile branches bearing pods and ultimately increasing yield. However the higher doses of HZ exhibited negative results in these characteristics.

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Table1, Effect of mutagens on seed germination, plant survival and pollen fertility in *Lens culinaris* Medik. Var. K-75 and L-4076

Concentrations	Seed germination (%)/ (Days taken for maximum germination)	Percentage inhibition	Plant survival at maturity (%)	Lethality (%)	Variation frequency (%)	Pollen fertility (%)
Var. K-75						
Control	93.00 (10)	-	87.50	-	-	98.71
0.01% HZ	81.25 (10)	12.63	69.75	20.68	1.52	96.19
0.02% HZ	68.75 (15)	26.00	72.20	17.24	2.20	93.59
0.03% HZ	50.00 (18)	46.33	70.85	19.54	1.70	90.42
0.04% HZ	21.75 (20)	76.61	69.33	20.68	6.33	84.27
Var. L-4076						
Control	93.75 (10)	-	89.72	-	-	97.86
0.01% HZ	81.25 (12)	13.33	67.55	24.72	1.78	94.16
0.02% HZ	62.50 (16)	33.33	69.30	22.47	3.50	91.43
0.03% HZ	43.75 (19)	53.33	58.42	31.00	5.75	87.95
0.04% HZ	18.75 (21)	80.00	32.44	64.04	6.97	82.82

Table 2, Mean, SD, CV and shift in mean, of various quantitative characters in *Lens culinaris* Medik. Var. K-75 and L-4076

Days to Flowering Mean±SD CV, Shift in mean	Days to maturity Mean±SD CV, Shift in mean	Plant height Mean±SD CV, Shift in mean	No.of Fertile branches/plant Mean±SD CV, Shift in mean	No.of Pods/plant Mean±SD CV, Shift in mean	No. of seeds/pod Mean±SD CV, Shift in mean	100 Seeds weight (g) Mean±SD CV, Shift in mean
Var. K-75						
59.20±0.86 1.45, -	69.20±0.77 1.11, -	54.06±8.96 16.61, -	12.00±2.82 23.50, -	153.66±45.57 29.65, -	1.73±0.45 26.01, -	2.68±0.18 6.72, -
59.33±0.72	69.40±0.63	42.80**±3.29	16.13**±2.66	169.00±83.98	1.73±0.45	2.63±0.14

1.21, +0.31	0.91, +0.20	7.68, -11.26	16.49, +4.13	49.69, +15.34	26.01, 0.00	5.32, -0.05
59.40±0.63 1.06,+0.20	70.06**±0.79 1.13, +0.86	43.13**±5.23 12.12, -10.93	16.46**±4.91 29.83, +4.46	102.33**±87.76 85.76, -51.33	1.73±0.45 26.01, 0.00	2.69±0.16 5.95, +0.01
59.53±0.83 1.39, +0.33	67.33**±0.72 1.06, -1.87	40.46**±5.02 12.40, -13.60	15.86**±4.56 28.75, +3.86	74.33**±34.27 46.11, -79.33	1.80±0.41 26.01, +0.07	2.67±0.16 5.99, -0.01
60.60**±1.05 1.73, +1.40	67.86**±0.63 0.93, -1.34	33.13**±9.58 28.92, -20.93	5.40**±0.98 18.15, -6.60	64.73**±16.22 25.05, -88.93	1.73±0.45 26.01, 0.00	2.68±0.17 6.34, 0.00
0.68	**0.57**	**4.66**	**2.88**	**37.98**	**0.35**	**0.12**
0.91	**0.76**	**6.21**	**3.83**	**50.52**	**0.46**	**0.16**
			Var. L-4076			
62.06±0.79 1.27, -	76.86±0.91 1.18, -	50.86±8.91 17.52, -	10.06±1.94 19.28, -	76.33±15.72 20.59, -	1.73±0.45 26.01, -	2.71±0.15 5.54, -
63.06±0.88 1.39, +1.00	77.13±0.83 1.07, +0.27	53.10±6.31 11.88, +2.24	17.00**±2.20 12.94, +6.94	373.33**±101.53 27.19, +297.00	1.80±0.41 22.77, 0.00	2.69±0.16 5.95, -0.02
64.00±0.84 1.31, +1.94	78.26**±0.79 1.01, +1.40	44.60*±6.24 13.99, -6.26	14.00**±1.46 10.43, +3.94	184.80**±89.40 48.37, +108.47	1.73±0.45 26.01, -0.07	2.67±0.17 6.37, -0.04
60.86±0.83 1.36, -1.20	75.06**±0.79 1.05, -1.80	36.33**±4.46 12.27, -14.53	11.33±2.19 19.33, +1.27	101.86±43.83 43.03, +25.53	1.66±0.48 28.91, -0.14	2.68±0.17 6.34, -0.03
61.80±0.77 1.24, -0.26	75.93**±0.79 1.04, -0.93	23.33**±3.13 13.42, -27.53	7.06*±2.73 38.67, -3.00	48.86±22.22 45.47, -27.47	1.73±0.45 26.01, -0.07	2.61±0.15 5.75, -0.10
17.46	**0.60**	**5.82**	**2.32**	**50.02**	**0.30**	**0.11**
23.23	**0.80**	**7.74**	**3.10**	**66.53**	**0.42**	**0.15**

SD= Standard Deviation, CV=Coefficient of Variation

Explanation of figures:

A: Control plant of var. K-75.
B: Dwarf and low yielding variant of var. K-75 (0.04% HZ).
C: Tall variant of var. L-4076 (0.01% HZ).
D: Dwarf and low yielding variant of var. L-4076 (0.04% HZ).
E: Bushy and high yielding variant of var. K-75 (0.02% HZ).
F: Bushy and high yielding variant of var. L-4076 (0.03% HZ).