Original Article

Journal of Phytology 2015, 7: 19-25
http:// scienceflora.org/journals/index.php/jp/
doi: 10.5455/jp.2015-09-016

Differential genotypic response of two varieties of *Hordeum vulgare* L. in response to hydrazine hydrate alone and in combination with dimethyl sulfoxide

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Received: 16.09.2015
Accepted: 19.10.2015
Published: 28.10.2015

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ABSTRACT

Induced mutation has been an important tool for a man in bringing desirable changes in plants since times immemorial. Cereals form an integral part of human diet along with pulses, especially in India. Cereals contain a high percentage of carbohydrates along with the considerable amount of proteins. The present experiment was carried out to induce the variability in *Hordeum vulgare* L. variety RD-2035 and BH-393. The mutagen used in this experiment was hydrazine hydrate (HZ). The mutagen was used singly and in combination with dimethyl sulfoxide (DMSO). Usually, the DMSO is considered as a carrier for a mutagen to enhance the mutagenic activity of that particular mutagen used in combination. The seeds of two varieties were treated with individual concentrations of HZ along with combination treatments with DMSO. The seeds were sown to raise the M_1 generation. Cytology of both control and treated plants was observed in the M_1 generation. The control plants showed normal meiosis, whereas, the treated plants showed different chromosomal abnormalities. The different chromosomal abnormalities were laggard, bridge, micronucleus, and univalents. The meiotic abnormalities were low at lower concentrations of both individual and combination treatments while as the percentage abnormalities increased with increasing concentrations of both individual and combination treatments. M_1 seeds were sown to raise the M_2 generation. The quantitative characters and frequency of morphological mutants were observed in M2 generation. The lower concentrations of HZ in combination with DMSO increased the yield and its attributing traits and the frequency of desirable morphological mutants more in variety BH-393 than in variety RD-2035. This shows the effectiveness of mutagen increased due to the use of carrier used. Moreover, the variety BH-393 responded more positively than variety RD-2035 towards lower concentrations of HZ alone and along with DMSO than higher concentrations.

KEY WORDS: *Hordeum vulgare* L., dimethyl sulfoxide, laggard, bridge, quantitative characters

INTRODUCTION

Cereals constitute an important source of food for modern man. Cereals form an integral part of human diet along with pulses, especially in India. Cereals are an important source of starch along with appreciable amount of proteins. Population explosion and malnutrition are posing severe threat to human civilization these days. Increase in production of pulses and cereals are of urgent need for the world to cope with the alarming increase of population and malnutrition rates. Scientists around the world are utilizing different approaches to induce desirable variations in cereals and pulses for increasing their yield. Out of these approaches, induced mutagenesis forms an important part. It induces the variability in a short period of time and overcome the limitations of variability (Kumar and Singh, 2003).

Barley, an important nutritional and medicinal plant is an ancient cereal grain. Barley originated in arid lands of North Africa, Western Asia and South Eastern Asia. About 1000 years back, barley arrived in India from the west and after that spread across the north regions and then southward (Harlan, 1979).

Barley, member of family Poaceae, is an important food crop. It has diploid chromosome number 14 (i.e., $2n = 14$). Barley contains 10-17% protein, 65-68% starch, 2-3% free lipids, 1.5-2.5% minerals, and 4-9% β-glucan (Quinde *et al*., 2004) while as the hull-less grains contain 11-14% insoluble dietary fiber, 3-10% soluble dietary fiber and 11-20% total dietary fiber (Marconi *et al*., 2000).

Barley has also medicinal properties used to treat hypertension as it contains the Unani drug Ajmaloon. The grains have also been found to have anticancer properties. It is predominantly autogamous. Hence, the variation in barley is found very low due to homozygosity of genes.

Therefore, the current experiment was conducted to study the effect of hydrazine hydrate (HZ) alone and in combination with DMSO on yield and its attributing traits.

MATERIALS AND METHODS

Fresh and healthy seeds of two varieties of *Hordeum vulgare* L. were used for this experiment. The two varieties used for this experiment were RD-2035 and BH-393. These two varieties were obtained at seed store Aligarh, India. 14 sets each containing 8 seeds were used for this experiment. Out of 14 sets, 2 sets were used as control (one set from each variety), the other three sets of each variety were treated with individual concentrations of HZ (0.01, 0.02, and 0.03%) while as the rest of three sets from each variety were treated with combination treatments (0.01% HZ + 0.01 DMSO, 0.02% HZ + 0.02 DMSO and 0.03% HZ + 0.03 DMSO). The mutagen used in this experiment was HZ and the carrier for this mutagen used was DMSO. The seeds of both varieties treated were put in different concentrations for 6 h, and the control seeds were put in distilled water. After that, the treated seeds were washed with running tap water to remove the residual effects of mutagen. The seeds were sown in 10″ × 15″ pots to raise the M_1 generation. Cytology of treated, as well as control plants, was observed in M_1 generation. Different quantitative characters and morphological variations were also were observed in M_2 plants.

RESULTS

Seed Germination and Inhibition (In Pots)

The control plants showed 100% seed germination in both the varieties. In the case of variety RD-2035, the percentage germination ranged from 79.16 to 95.18. The maximum germination was recorded in 0.01% HZ (95.83%) while as the lowest percentage germination was recorded in 0.03% HZ + 0.03% DMSO (79.16%). The control plants showed zero inhibition while as the percentage inhibition considerably increased with increasing concentrations of both individual and combination treatments. The maximum inhibition was recorded in 0.03% HZ + 0.03% DMSO (20.83%). In the case of variety BH-393, the control plants showed 100% seed germination, while as the percentage germination decreased with increasing concentrations of HZ and its combination with DMSO. The treatment plants showed variation in percentage germination from 87.50 to 95.83. The highest percentage in case of treated plants was shown by 0.01% HZ (100%) while the lowest percentage germination was shown by 0.03% HZ + 0.03% DMSO (87.5). The percentage inhibition also increased with increasing concentrations of mutagen and its combination with DMSO. The maximum inhibition was recorded in 0.03% HZ + 0.03% DMSO (12.50%) [Tables 1 and 2].

Seed Germination and Inhibition (In Petriplates)

Petriplate experiment was conducted to test the percentage germination of seeds in controlled conditions of the laboratory. In the case of variety RD-2035, the control showed 100% seed germination while as the treated plants showed percentage germination from 70 to 90. In case of inhibition, the control plants showed zero inhibition while as the treated plants showed percentage inhibition from 10 to 30. The maximum inhibition was observed in 0.03% HZ + 0.03% DMSO (30%). In variety BH-393,

Table 1: Effect of HZ alone and in combination with DMSO on seed germination and inhibition in *H. vulgare* L. variety RD-2035 (in pots)

Treatments	Percentage germination	Percentage inhibition
Control	100	0
0.01% HZ	95.83	4.16
0.02% HZ	91.66	8.33
0.03% HZ	87.5	12.5
0.01% HZ+0.01% DMSO	91.66	8.33
0.02% HZ+0.02% DMSO	87.5	12.5
0.03% HZ+0.03% DMSO	79.16	20.83

DMSO: Dimethyl sulfoxide, HZ: Hydrazine hydrate, *H. vulgare: Hordeum vulgare*

Table 2: Effect of HZ alone and in combination with DMSO on seed germination and inhibition in *H. vulgare* L. variety BH-393 (in pots)

Treatments	Percentage germination	Percentage inhibition
Control	100	0
0.01% HZ	100	0
0.02% HZ	95.83	4.16
0.03% HZ	91.66	8.33
0.01% HZ+0.01% DMSO	95.83	4.16
0.02% HZ+0.02% DMSO	91.66	8.33
0.03% HZ+0.03% DMSO	87.5	12.5

DMSO: Dimethyl sulfoxide, HZ: Hydrazine hydrate, *H. vulgare: Hordeum vulgare* the reduction in percentage germination and increase of percentage inhibition was observed less as compared to variety RD-2035. The percentage germination decreased with increasing concentrations of HZ along with combinations of DMSO. Similarly, the percentage inhibition was increased with increasing concentrations of both individual and combination treatments (Plate-II) [Tables 3 and 4].

Root and Shoot Length (Laboratory Experiment)

The root and shoot length was taken of 12-15 days plantlets during the petriplate experiment. In case of both the varieties, the lower concentrations of both the individual and combination treatments increased the root and shoot length. The root and shoot length decreased at higher concentrations of both individual and combination treatments [Tables 5 and 6].

Cytological Study

The cytology was observed in both control and treated plants. The normal meiosis was observed in control plants while as different abnormalities, like laggards, bridges, micronuclei and univalents, were observed in treated plants. From the cytological analysis, it was observed that both in varieties the frequency of meiotic abnormalities were increased with increasing concentrations of both individual and combination treatments. The variety RD-2035 showed more frequency of chromosomal abnormalities than variety BH-393 (Plate-I) [Tables 7 and 8].

Table 3: Effect of HZ alone and in combination with DMSO on seed germination and inhibition in *H. vulgare* L. variety RD-2035 (in petriplates)

Treatments	Percentage germination	Percentage inhibition
Control	100	-
0.01% HZ	90	10
0.02% HZ	90	10
0.03% HZ	80	20
0.01% HZ+0.01% DMSO	90	10
0.02% HZ+0.02% DMSO	80	20
0.03% HZ+0.03% DMSO	70	30

DMSO: Dimethyl sulfoxide, HZ: Hydrazine hydrate, *H. vulgare: Hordeum vulgare*

Table 4: Effect of HZ alone and in combination with DMSO on seed germination and inhibition in *H. vulgare* L. variety BH-393 (in petriplates)

Treatments	Percentage germination	Percentage inhibition
Control	100	-
0.01% HZ	100	-
0.02% HZ	90	10
0.03% HZ	90	10
0.01% HZ+0.01% DMSO	90	10
0.02% HZ+0.02% DMSO	90	10
0.03% HZ+0.03% DMSO	80	20

DMSO: Dimethyl sulfoxide, HZ: Hydrazine hydrate, *H. vulgare: Hordeum vulgare*

Quantitative Characters

Quantitative characters are important characters associated with yield. The quantitative characters were analyzed of both varieties. Variety BH-393 responded more positively than variety RD-2035 [Tables 9 and 10].

Variety RD-2035

Plant height

The control plants showed an average height of 66.20 cm while as the treated plants showed variations in plant height ranging from 60.80 to 67.53 cm. The maximum height was observed in 0.01% HZ + 0.01% DMSO (67.53 cm).

Number of spikes per plant

The lower concentrations of both individual and combination treatments showed increased number of spikes per plant while as the higher ones decreased the same. The control plants showed an average number of spikes per plant as 2.66. The maximum number of spikes per plant was observed in 0.01% HZ + 0.01% DMSO (4.33) followed by 0.02% HZ (4.23).

Table 5: Effect of HZ alone and in combination with DMSO on root and shoot length of *H. vulgare* L. var-RD-2035 (in petriplates)

Treatments	Root length (cm)	Shoot length (cm)	Total length (cm)
Control	4.26	9.50	13.76
0.01% HZ	4.54	10.30	14.84
0.02% HZ	4.92	10.50	15.42
0.03% HZ	4.10	9.20	13.30
0.01% HZ+0.01% DMSO	4.60	10.60	15.20
0.02% HZ+0.02% DMSO	3.8	8.80	12.60
0.03% HZ+0.03% DMSO	3.5	8.30	11.80

DMSO: Dimethyl sulfoxide, HZ: Hydrazine hydrate, *H. vulgare: Hordeum vulgare*

Table 6: Effect of HZ alone and in combination with DMSO on root and shoot length of *H. vulgare* L. var-BH-393 (in petriplates)

Treatments	Root length (cm)	Shoot length (cm)	Total length (cm)
Control	4.30	9.60	13.90
0.01% HZ	4.70	10.72	15.42
0.02% HZ	4.82	10.80	15.62
0.03% HZ	4	8.70	12.70
0.01% HZ+0.01% DMSO	4.90	10.80	15.70
0.02% HZ+0.02% DMSO	3.50	8.40	11.90
0.03% HZ+0.03% DMSO	3.20	8.10	11.30

DMSO: Dimethyl sulfoxide, HZ: Hydrazine hydrate, *H. vulgare: Hordeum vulgare*

Table 7: Effect of HZ alone and in combination with DMSO on the frequency of meiotic abnormalities in *H. vulgare* L. variety RD-2035

Treatments	Total number of PMC's analyzed	Percentage			
		Metaphase	Anaphase		Telophase
		U	Lg	Bg	Mc
Control	440	-	-	-	-
0.01% HZ	436	0.22	0.45	0.45	0.22
0.02% HZ	434	0.23	0.46	0.46	0.69
0.03% HZ	432	0.69	0.46	0.92	0.46
0.01% HZ+0.01% DMSO	432	0.69	0.46	0.69	0.46
0.02% HZ+0.02% DMSO	429	0.93	0.69	0.69	0.69
0.03% HZ+0.03% DMSO	426	0.70	0.46	0.93	1.17

U: Univalents, Lg: Laggard, Bg: Bridge, Mc: Micronucleus,
DMSO: Dimethyl sulfoxide, HZ: Hydrazine hydrate, *H. vulgare: Hordeum vulgare*

Table 8: Effect of HZ alone and in combination with DMSO on the frequency of meiotic abnormalities in *H. vulgare* L. var. variety BH-393

Treatments	Total number of PMC's analyzed	Percentage			
		Metaphase	Anaphase		Telophase
		U*	Lg*	Bg*	Mc*
Control	442	-	-	-	-
0.01% HZ	439	-	0.68	0.45	-
0.02% HZ	437	0.45	0.22	0.45	0.22
0.03% HZ	433	0.69	0.46	0.46	0.69
0.01% HZ+0.01% DMSO	436	0.22	0.91	0.22	0.45
0.02% HZ+0.02% DMSO	434	0.69	0.23	0.46	0.69
0.03% HZ+0.03% DMSO	432	0.92	0.46	0.69	0.69

U: Univalents, Lg: Laggard, Bg: Bridge, Mc: Micronucleus,
DMSO: Dimethyl sulfoxide, HZ: Hydrazine hydrate, *H. vulgare: Hordeum vulgare*

Spike length per plant

The control plants showed average spike length per plant as 6 while as the treated plants showed variations in spike length ranging from 4.50 to 7.13.

100 seeds weight

The average 100 seeds weight of control plants was observed as 4.66 g. The lower individual and combination treatments increased the yield considerably. The maximum yield was observed in 0.02% HZ (5.26 g) followed by 0.01% HZ + 0.01% DMSO (5.23 g).

Variety-393

Plant height

The control plants showed an average height of 71.76 cm while the height of treated plants ranged from 68.50 to 73.06 cm. The minimum height was observed in 0.03% HZ + 0.03% DMSO (68.50 cm) while the maximum height was observed in 0.01% HZ + 0.01% DMSO (73.06 cm).

Number of spikes per plant

The control plants showed an average spikes a plant as 3.50, whereas the treated plants showed variation in an average spikes per plant from 2.63 to 4.26. The maximum number of spikes per plant was observed in 0.01% HZ + 0.01% DMSO (4.26).

Spike length per plant

The control plants showed average spike length a plant as 6.80 cm while as the treated ones displayed an average spike length a plant ranged from 5.70 to 7.30 cm.

Table 9: Effect of HZ alone and in combination with DMSO on quantitative characters of *H. vulgare* L. variety RD-2035

Treatments	Mean±SE			
	Plant height (cm)	Number of spikes per plant	Spike length (cm)	100 seed weight (g)
Control	66.20±0.60	2.66±0.33	6±0.28	4.66±0.12
0.01% HZ	67.26±0.37	4.13±0.67	7.03±0.08	5.13±0.24
0.02% HZ	67.43±0.35	4.23±0.61	7.03±0.04	5.26±0.23
0.03% HZ	63.93±0.27	2±0.11	5.10±0.32	3.86±0.17
0.01% HZ+0.01% DMSO	67.53±0.37	4.33±0.67	7.13±0.08	5.23±0.24
0.02% HZ+0.02% DMSO	63.66±0.29	1.93±0.08	4.76±0.29	3.66±0.26
0.03% HZ+0.03% DMSO	62.80±0.61	1.70±0.15	4.50±0.28	3.40±0.30

DMSO: Dimethyl sulfoxide, HZ: Hydrazine hydrate, *H. vulgare: Hordeum vulgare*, SE: Standard error

Table 10: Effect of HZ alone and in combination with DMSO on quantitative characters of *H. vulgare* L. variety BH-393

Treatments	Mean±SE			
	Plant height (cm)	Number of spikes per plant	Spike length (cm)	100 seed weight (g)
Control	71.76±0.79	3.50±0.28	6.80±0.11	4.80±0.05
0.01% HZ	72.30±0.65	3.96±0.43	7.06±0.17	5.80±0.20
0.02% HZ	72.63±0.74	4.20±0.40	7.30±0.11	5.50±0.05
0.03% HZ	70.33±0.33	3.20±0.26	6.36±0.08	4.36±0.14
0.01% HZ+0.01% DMSO	73.06±0.63	4.26±0.37	7.30±0.20	5.90±0.14
0.02% HZ+0.02% DMSO	69.30±0.72	2.86±0.26	6.06±0.17	4.13±0.17
0.03% HZ+0.03% DMSO	68.50±0.46	2.63±0.26	5.70±0.23	3.80±0.25

DMSO: Dimethyl sulfoxide, HZ: Hydrazine hydrate, *H. vulgare: Hordeum vulgare*, SE: Standard error

100 seeds weight

The average yield (100 seeds weight) of control plants was observed as 4.80 g. The lower concentrations of both individual and combination treatments increased the yield. The maximum yield was observed in 0.01% HZ + 0.01% DMSO (5.90 g), while as the minimum yield was observed in 0.03% HZ + 0.03% DMSO (3.80 g).

Morphological Variations

The frequency of morphological variants is shown in Table 11. From the data, it is clear that the favorable mutation like bushy plants were observed mostly in lower concentrations of both individual and combination treatments of BH-393 indicating the more positive response of the variety toward lower concentrations of mutagens. The other non-favorable mutations like short/curved spikes were mostly observed in higher concentrations of both individual and combination treatments of RD-2035 (Plate-II).

DISCUSSION

The present experiment was carried out to study the effects of HZ alone and in combination with DMSO on both cytological, morphological, yield and its attributing traits of *H. vulgare* L. The two varieties used in this experiment were RD-2035 and BH-393. The cytological and morphological analysis was carried out in M_1 generation while as the analysis of yield and its attributing traits were carried out in M_2 generation. The lower individual concentrations of HZ and lower combination treatments with DMSO proved more effective in inducing desirable variations in both varieties. Variety BH-393 responded more positively toward mutagen than variety RD-2035.

Differential response of different varieties of the plant depends on the type of mutagen and on the genotype of plant. This differential response occurs due to the metabolic processes at the embryonic level which are affected by mutagens (Ashri and Herzog, 1972). The decrease in seed germination was observed with increasing concentrations of HZ and its combination with DMSO. It may occur due to Inhibition of physiological processes (Kurobane *et al*., 1979) and mitotic processes (Ananthaswamy *et al*., 1971). The maximum reduction was observed at higher combination treatments of HZ with DMSO. The reason for this inhibition is due to enhancement of mutagenicity of HZ as the DMSO acts as a carrier to the mutagen,

Table 11: Frequency of different morphological variants of two varieties of *Hordeum vulgare* L. in different concentrations of HZ alone and in combination with DMSO

Treatments	M_2 plants	Tall/bushy	Short spike	Curved and short spike
Control				
RD-2035	48	-	-	-
BH-393	49	-	-	-
0.01% HZ				
RD-2035	45	-	-	-
BH-393	46	2.17 (1)	-	-
0.02% HZ				
RD-2035	43	-	4.65 (2)	-
BH-393	44	6.18 (3)	-	-
0.03% HZ				
RD-2035	41	-	4.87 (2)	7.31 (3)
BH-393	42	-	-	2.38 (1)
0.01% HZ+0.01% DMSO				
RD-2035	43	2.32 (1)	-	-
BH-393	45	6.66 (3)	-	-
0.02% HZ+0.02% DMSO				
RD-2035	39	2.56 (1)	5.12 (2)	5.12 (2)
BH-393	41	-	-	-
0.03% HZ+0.03% DMSO				
RD-2035	37	-	8.10 (3)	5.40 (2)
BH-393	39	-	5.12 (2)	-

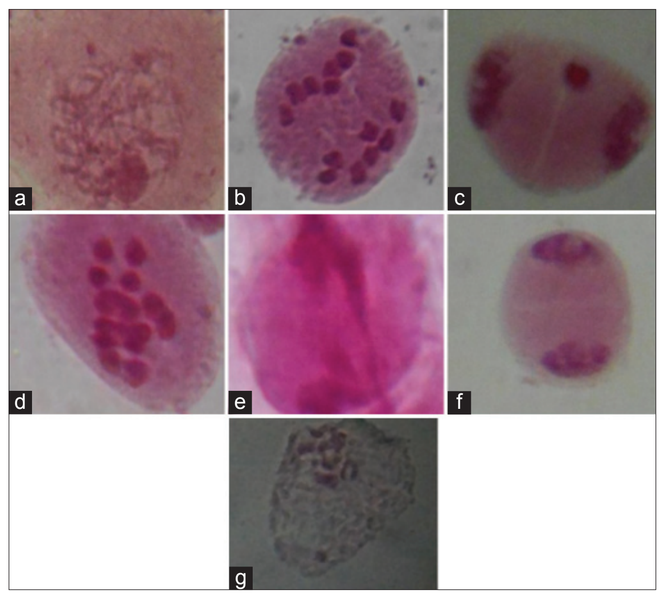

Figure 1: (a) Leptotene (Control), (b) Late Anaphase-I (Control), (c) Laggard at Anaphase-I, (d) Univalents at Metaphase-I, (e) Bridge at Anaphase-I, (f) Telophase-I (Control), (g) Micromucleus formation

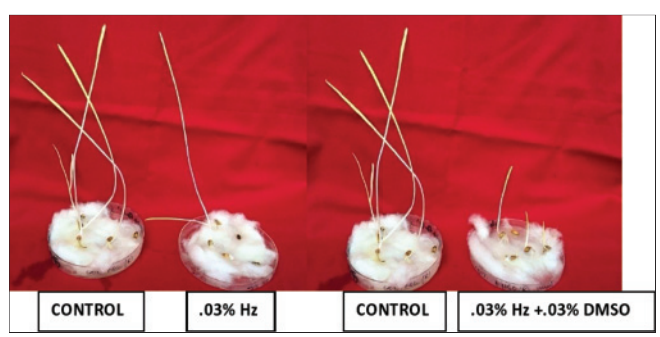

Figure 2: Seed germination and seedling height of control and treated population

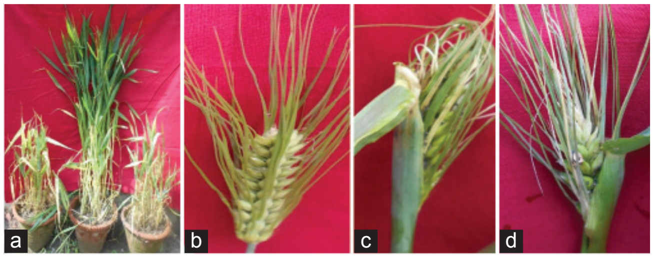

Figure 2: (a) Control plant (BH-393), Bushy/Tall, Control plant (RD-2035), (b) Spike of control plant, (c) Curved spike of variant, (d) Short spike of variant

thus increasing its activity. Some authors have reported the reduction in seed germination due to the destruction of gibberellic acid due to the mutagen thus halting the germination in GA-induced germinating seeds (Sideris *et al.*, 1971). Some authors have also documented the DNA synthesis inhibition as the main reason of reduction in seed germination (Hevesy, 1945).

In M_1 generation, cytology was also observed of PMC's of both control and treated plants. The control plants showed normal meiosis while as the treated plants showed different chromosomal abnormalities like univalents, micronucleus, laggard and bridge. Variety RD-2035 showed more chromosomal abnormalities toward mutagen of both individual and combination treatments at the same concentration than variety BH-393. The occurrence of univalents is due to the chromosomes which fail to pair at zygotene, or it can also occur due to the precocious separation of bivalent chromosomes at Anaphase-I (Sarbhoy, 1977). Many authors reported that laggards and univalents which fail to reach the poles form micronuclei (Azad, 2011; Zeerak and Zargar, 1998; Maurya *et al.*, 2006; Koduru and Rao, 1981). Chromosomal bridges have also been observed in this experiment. Different authors have given different explanations for bridge formation. It occurs due to the interlocking of bivalents (Bhattacharjee, 1953), dicentric chromosome, and unequal exchange (Tariq *et al.*, 2007), union of broken chromosome ends (Goyal and Khan, 2010).

Quantitative characters are important characters associated with yield. The current experiment revealed that lower concentrations of both individual and combination treatments increased the yield and its attributing traits in both varieties. Variety BH-393 responded more positively toward mutagen than variety RD-2035 at same concentrations. The lower doses promoting growth effects has already been reported by many (Khan and Wani, 2004; Khan and Wani, 2006). The polygenic mutation direction depends on genotypic background of material (Loesch, 1964). The direction of mutation in quantitative characters can be bidirectional, random, and it also depends on the type of mutagen and the genotype under study (Siddiqui *et al.*, 2009; Waxman and Peck, 2003). Induced variability in quantitative traits has already been reported by many workers in different plants such as in *Lathyrus sativus* (Waghmare and Mehra, 2000) and *Vicia faba* (Parveen *et al.*, 2012).

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