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Investigations on Chemical Mutagen Sensitivity in Onion (*Allium cepa* L.)

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Abstract: Genetic improvement of onion (*Allium cepa* L.) by conventional methods of hybridization is slow due to its highly heterozygous, outcrossing and biennial nature. To augment this process induced mutagenesis could serve as a useful tool. Keeping this in view, the present study was conducted to test the efficacy of Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) in inducing variability in onion. For this purpose, seeds of six onion varieties were soaked in solutions of different concentrations of EMS (0.1, 0.15, 0.2, 0.25%) and SA (0.1, 0.3, 0.5, 0.7%) for 4 h and their sensitivity towards these mutagens was assessed in terms of seed germination and seedling growth. Significant differences ($p < 0.01$) were observed in the varieties and treatments with respect to the traits studied. Lower dose (0.1%) of both SA and EMS showed stimulatory effect on germination potential of seeds of all except variety-4 and variety-5. Shoot length was enhanced over control in variety-3 and variety-5 at 0.1% SA and variety-4 at 0.1% EMS. Root length was slightly higher than control in variety-3 and variety-4 at 0.1% SA and 0.1% EMS, respectively. Reduction in all the three traits was observed at higher doses of EMS and SA in all the varieties. The reduction in shoot and root length was more pronounced under SA treatment than EMS. Also, germination process was more sensitive to mutagens than the seedling growth. The present study demonstrated that both mutagens at low dose could be suitable for the creation of variability in onion.

Key words: Genetic variation, induced mutagenesis, mass selection, crop improvement, germination potential

INTRODUCTION

Induced mutations serve as a complementary approach in genetic improvement of crops (Mahandjiev *et al.*, 2001). Various physical and chemical mutagenic agents are used to induce favourable mutations at high frequency in plants (Ahloowalia and Maluszynski, 2001; Goyal and Khan, 2010). Among the chemical mutagens, ethyl methane sulphonate (Jabeen and Mirza, 2002; Sheeba *et al.*, 2005; Jayakumar and Selvaraj, 2003; Karthika and Lakshmi, 2006; Kozgar *et al.*, 2011), sodium azide (Aliyu and Adamu, 2007; Al-Qurainy, 2009; Mostafa, 2011), diethyl sulphate (Samiullah and Wani, 2005; Khan and Wani, 2005; Aruna *et al.*, 2010), N-nitroso methylurea (Gupta *et al.*, 2000; Alghamdi *et al.*, 2010) have been widely used for introducing variability in higher plants. The variants/mutants can be identified using molecular markers (Mostafa and Alfirmawy, 2011). Such mutants could facilitate the isolation and cloning of genes used in designing crops with improved yields, increased stress tolerance, longer shelf life and reduced agronomic input (Ahloowalia and Maluszynski, 2001).

Onion (*Allium cepa* L.) is an outcrossing and highly heterozygous crop (Eady, 1995). It is propagated by seeds, bulbs or sets (small bulbs). Being a biennial species it takes more time to improve this crop by conventional methods such as hybridization, recombination and selection (Lawande *et al.*, 2009). In addition, the lack of inbred lines makes it difficult to perform genetic linkage analysis in onion (Cramer and Havey, 1999). To explore the possibility of using induced mutagenesis as a tool for improvement of onion, several workers (Al-Oudat, 1990; Kirtane *et al.*, 2000; Hao and Cheng-Zuo, 2006; Hassan and Ahmad, 2000; Amjad and Anjum, 2002; Kirtane and Dhumal, 2004; Asita and Matobole, 2010) have tested the efficiency of various mutagens. Most of these studies used radiations and a perusal of literature clearly indicates the need of chemical mutagen sensitivity investigations in onion.

For improvement of crop by mutation breeding, it is very important to determine a suitable mutagen dose. Higher doses produce undesirable effects such as poor growth and genetic damage. A relatively low dose usually results in altered growth characteristics whereas very low doses have been shown to stimulate plant growth

(Fowler and MacQueen, 1972). Seedling growth and cytological characteristics are generally evaluated for testing mutagen sensitivity in Plants (Amjad and Anjum, 2002). The present investigation was carried out to obtain practical knowledge about the effectiveness of SA (Sodium azide) and EMS (chemical mutagen) as a mutagen in onion by studying their influence on seed germination and seedling growth.

MATERIALS AND METHODS

The present study was performed during the period January-August, 2010 and the work was started on 5th January-2010.

Plant material: The seeds of six Indian varieties of onion (*Allium cepa* L.) viz. N-2-4-1 (variety-1), B-780 (Variety-2), L-28 (variety-3), Phule Samarth (variety-4), Bhima Red (variety-5) and Bhima Raj (variety-6) were obtained from the National Research Centre for Onion and Garlic, Rajgurunagar, Pune, India.

Mutagenesis: Seeds of each of the six variety were treated with different concentrations each of sodium azide (0.1, 0.3, 0.5, 0.7%) and EMS (0.1, 0.15, 0.2, 0.25%) for 4 h followed by washing under running tap water for 1 h. Subsequently, treated and control (those which were not given mutagen treatment) seeds were placed over Whatman filter paper No. 1 moistened with distilled water in Petri plates for germination. The Petri dishes were sealed with parafilm M to prevent drying of seeds and were placed under standard growth chamber conditions of temperature $28 \pm 2^\circ\text{C}$, photoperiod of 16 h having $45 \mu\text{mole m}^{-2} \text{s}^{-1}$ illumination provided by cool white fluorescent tubes. The experiments were performed twice and three replicates of thirty seeds each were used for all the treatments.

Germination and seedling growth: Germination in terms of radicle emergence (at least 2 mm) was assessed after every 2 days till 15 days by visually counting the number of germinated seeds for each treatment. Final germination (%) was expressed as percentage of all seeds with fully emerged radicle (greater than 2 mm) in a given batch. Seedling root and shoot lengths were measured after 15 days of sowing for both control and treated seeds. Root length and shoot length were calculated by measuring the length (cm) from root tip to the root-shoot junction and shoot tip to the root-shoot junction, respectively.

Statistical analysis: The experiment was arranged in a completely randomized design with three replications of

30 seeds each. Data were subjected to Analysis of Variance (ANOVA) using SPSS (ver. 14, SPSS Inc., Irvine, Calif.). Means of different treatments were separated with LSD at 5% level of probability.

RESULTS AND DISCUSSION

Determination of mutagen efficiency is necessary for its use in mutation breeding (Makeen and Babu, 2010). In the present study, both EMS and SA showed influence on seed germination and early seedling growth in six varieties of onion. Analysis of variance presented in Table 1 indicated that variety and mutagen concentration had significant ($p < 0.01$) effect on all the traits studied. Mutagen X variety interactions were also significant ($p < 0.01$) for all the traits, indicating that differences exist between varieties regarding sensitivity to SA and EMS treatment. As observed in the present study, the existence of varietal differences in sensitivity to chemical mutagens is well known and has been reported by Wani and Khan (2006) and Khan and Goyal (2009).

Seed germination: Seed germination is an important parameter used to measure the response of plant to mutagenic treatments (Shah *et al.*, 2008). Germination potential of seeds of different varieties of onion varied from 100% (variety-4) to 57.14% (variety-1) under control conditions. Seed germination was stimulated by lower dose of SA in some varieties of onion. It was higher than control in variety-1, variety-3 and variety-6 by 30%, 20% and 85, respectively, at 0.1% SA but decreased linearly with further increase in dose (Table 2). Similarly, lower dose (0.1%) of EMS also improved seed germination potential over control in variety-1, variety-2, variety-3 and variety-6 by 40, 10, 30 and 8%, respectively but reduced with further increase in dose (Table 3). As observed in present study, promoting effects of low doses of EMS and SA on biological parameters have also been reported earlier in *Vicia faba* (Dubey, 1988). In contrast to our results, a reduction in seed germination at all the doses of

Table 1: Analysis of variance summaries (mean squares) of data on effect of different doses of sodium azide and EMS on germination and early seedling growth parameters of *Allium cepa* L.

Source of Variation	DF	(%) germination	Shoot length	Root length
Variety	5	1152.14**	4.24**	1.55**
SA	4	2443.63**	11.46**	8.55**
Variety×SA	20	642.69**	2.33**	1.05**
Error	60	47.5800	0.0900	0.0700
Variety	5	2194.62**	5.47**	1.65**
EMS	4	774.75**	3.17**	1.99**
Variety×EMS	20	535.93**	1.38**	0.72**
Error	60	14.6500	0.1600	0.1000

***: Significant at 5 and 1% level of probability, respectively, ANOVA

Table 2: Effect of different concentrations of sodium azide on seed germination and seedling growth of *Allium cepa* L.

Concentration of SA (%)	Germination potential (%)						Shoot length (cm)						Root length (cm)					
	V-1	V-2	V-3	V-4	V-5	V-6	V-1	V-2	V-3	V-4	V-5	V-6	V-1	V-2	V-3	V-4	V-5	V-6
0.0 (Control)	57.14 ^a	82.35 ^a	66.67 ^c	100 ^a	93.0 ^a	80.0 ^a	3.53 ^a	2.44 ^a	3.65 ^a	2.4 ^a	2.31 ^{bc}	3.51 ^a	3.25 ^a	2.06 ^a	2.26 ^a	1.59 ^a	3.34 ^a	0.66 ^a
0.1%	86.67 ^a	70 ^b	86.67 ^b	93.33 ^a	83.33 ^b	87.77 ^a	1.92 ^b	1.38 ^b	3.72 ^a	2.33 ^a	2.56 ^b	1.91 ^b	0.96 ^b	0.84 ^b	2.31 ^a	1.57 ^a	1.83 ^b	0.58 ^b
0.3%	66.67 ^b	69.23 ^c	100 ^a	66.67 ^b	78.63 ^b	81.76 ^a	1.82 ^b	1.18 ^b	2.73 ^b	1.04 ^b	3.23 ^a	0.31 ^c	0.59 ^{bc}	0.82 ^b	1.12 ^b	0.92 ^{ab}	1.18 ^a	0.31 ^{ab}
0.5%	53.22 ^c	69.23 ^c	60.0 ^d	60.0 ^b	63.07 ^c	61.02 ^b	0.9 ^c	0.99 ^b	1.84 ^c	0.97 ^b	2.07 ^c	0.17 ^c	0.46 ^c	0.62 ^{bc}	1.04 ^b	0.41 ^{bc}	0.88 ^{cd}	0.26 ^b
0.7%	36.36 ^d	60 ^d	33.33 ^c	58 ^b	50.44 ^d	47.25 ^c	0.27 ^d	0.27 ^c	0.53 ^d	0.36 ^c	0.87 ^d	0.12 ^c	0.15 ^c	0.25 ^c	0.28 ^c	0.38 ^c	0.59 ^d	0.17 ^b

*Means followed by different letters differ significantly at 5% level of probability using LSD test, V: Variety

Table 3: Effect of different concentrations of EMS on seed germination and seedling growth of *Allium cepa* L.

Concentration of EMS (%)	Germination potential (%)						Shoot length (cm)						Root length (cm)					
	V-1	V-2	V-3	V-4	V-5	V-6	V-1	V-2	V-3	V-4	V-5	V-6	V-1	V-2	V-3	V-4	V-5	V-6
0.0 (Control)	57.14 ^a	82.35 ^b	66.67 ^c	100 ^a	93.0 ^a	80.0 ^b	3.53 ^a	2.44 ^a	3.65 ^a	2.4 ^{ab}	2.31 ^a	3.51 ^a	3.25 ^a	2.06 ^a	2.26 ^a	1.59 ^b	3.34 ^a	0.66 ^a
0.1	97.98 ^a	91.89 ^a	91.3 ^a	91.3 ^b	92.0 ^a	88.0 ^a	3.02 ^a	1.4 ^b	2.33 ^b	3.02 ^a	2.48 ^a	1.77 ^b	1.4 ^b	1.36 ^b	1.53 ^b	2.97 ^a	1.55 ^b	0.56 ^a
0.15	93.51 ^a	73.07 ^c	75.0 ^b	85.0 ^c	82.63 ^b	51.61 ^c	3.21 ^a	1.28 ^{bc}	1.39 ^c	2.97 ^{ab}	2.01 ^b	1.42 ^b	0.9 ^b	1.13 ^b	1.45 ^b	1.28 ^a	1.18 ^b	0.48 ^a
0.2	76.19 ^b	67.92 ^c	65.0 ^c	80.0 ^d	81.81 ^b	40.9 ^d	1.47 ^b	0.73 ^{cd}	1.19 ^c	2.32 ^b	1.82 ^{bc}	1.07 ^d	0.83 ^c	0.99 ^c	1.17 ^c	0.85 ^c	0.99 ^c	0.47 ^a
0.25	57.89 ^c	54.54 ^d	63.0 ^c	76.19 ^d	70.0 ^c	37.5 ^d	1.38 ^c	0.44 ^d	1.13 ^c	1.43 ^c	1.46 ^c	0.40 ^d	0.27 ^c	0.84 ^c	0.42 ^c	0.43 ^c	0.88 ^c	0.13 ^b

*Means followed by different letters differ significantly at 5% level of probability using LSD test, V: Variety

sodium azide has been observed in *Eruca sativa* L. (Khan and Al-Qurainy, 2009) and *Helianthus annuum* (Mostafa, 2011).

In variety-4 of onion, the percent germination was lower than control at all the doses of both the mutagens and it decreased with increase in mutagen concentration. At 0.1% EMS and SA, seed germination of variety-4 was reduced by 7-9% as compared to control (Table 2, 3). In variety-5, germination potential remained unaffected at low dose (0.1%) of both SA and EMS but decreased with further increase in mutagen concentration. Similar to our observations a linear relationship between increasing doses of mutagen (EMS and SA) and reduction in seed germination have been reported in chickpea barley rice bean (Prakash and Shambhulingappa, 2000), durum wheat (Kalia *et al.*, 2001), red gram (Potdukhe, 2004), Soybean (Padavai and Dhanavel, 2004), okra (Singh *et al.*, 2000), Mung beans (Singh and Kole, 2005), groundnut (Adamu *et al.*, 2002; Mensah and Obadoni, 2007), Horse gram (Kulkarni, 2011). In the present study maximum reduction (39%) of seed germination was recorded in variety-5 at 0.7%SA. Reduction in germination due to mutagenic treatments has been explained due to delay or inhibition in physiological and biological processes necessary for seed germination which include enzyme activity, hormonal imbalance and inhibition of mitotic activity (Khan and Al-Qurainy, 2009; Kulkarni, 2011).

Shoot length: Seedling shoot length is widely used as an index in determining the biological effects of various mutagens (Konzak *et al.*, 1972). In the present study at control conditions, variety-3 and variety-4 exhibited highest (3.65 cm) and lowest (2.4 cm) shoot length, respectively (Table 2). Low dose of both the mutagens

elicited a stimulatory effect on seedling shoot length in few varieties of onion. Shoot length in variety-3 and variety-5 increased slightly over control at lower dose (0.1% and 0.3%, respectively) of SA but decreased with further increase in dose (Table 2). Similarly, shoot length of variety-4 was higher (3.02 cm) than control (2.4 cm) and nearly equal to control in variety-5 at 0.1%EMS (Table 3). Stimulation of seedling height by low dose of mutagen has also been noticed in tomato (Aliyu and Adamu, 2007). Contrasting results wherein the seedling shoot length was inhibited even at low dose of EMS has been reported in chickpea (Shah *et al.*, 2008) and durum wheat (Kalia *et al.*, 2001). The hypothetic origin of these stimulations by irradiation and EMS treatments was due to cell division rates as well as an activation of growth hormone, e.g., auxin (Gunckel and Sparrow, 1961; Zaka *et al.*, 2004).

Few varieties of onion displayed a reduction in shoot length with increase in dose of both the mutagens. The maximum and minimum overall reduction in shoot length was observed in variety-6 (96.58%) and variety-4 (85%), respectively at highest dose (0.7%) of SA (Table 2). For EMS, maximum and minimum overall reduction was recorded for variety-6 (88.6%) and variety-1 (60.9%), respectively (Table 3). Similar to our results, decline in shoot length with increasing dose of EMS and SA has been reported in chickpea (Haq *et al.*, 1992; Toker *et al.*, 2005) and *Vigna radiata* (Rakshit and Singh, 2001). Reduced growth has been attributed to auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances (Gunckel and Sparrow, 1954; Gordon, 1957; Singh, 1974; Ussuf and Nair, 1974). The reduction in seedling shoot length with increasing doses of the mutagens has been established in the past by many

workers, including Singh and Yadav (1987), Uma and Salimath (2001) and Jayakumar and Selvaraj (2003).

Root length: Root length is another important parameter used to test mutagen sensitivity. In the present study, varietal variation was observed in root length of seedlings grown under control conditions with maximum and minimum root length exhibited by variety-5 (3.34 cm) and variety-6 (0.66 cm), respectively (Table 2). Low dose of both the mutagens elicited a stimulatory effect on seedling root length in few varieties of onion. Root length was slightly higher than control in variety-3 at 0.1% SA (Table 2) whereas in variety-4 it was two times higher than control at 0.1% EMS (Table 3). Further increase in both the mutagen dose resulted in decline in root length. Stimulation of root length on exposure to low dose of chemical mutagen has also been reported in *Jatropha carcus* (Dhakshanamoorthy *et al.*, 2010). In contrast to our observations, Haq *et al.* (1992) and Shah *et al.* (2008) observed an inhibitory effect of low dose of EMS on root length in chickpea.

In few varieties of onion, root length was low as compared to control at all doses and it reduced with increase in dose of both the mutagens. The overall reduction was maximum and minimum in variety-1 (95.38%) and variety-6 (74.24%), respectively in case of SA treatment (Table 2). For EMS, the overall reduction in root length was maximum and minimum in variety-2 (59.22%) and variety-1 (91.69%), respectively (Table 3). Similar observation of decline in root length with increase in mutagen dose has been reported in red gram (Potdukhe, 2004), sunflower (Jayakumar and Selvaraj, 2003), black gram (Lal *et al.*, 2009). According to Datta *et al.* (1992), the reduction in root length may be the result of marked suppression of mitotic division, affected nuclear condensation causing irregular distribution of chromosomes, bridges and fragmentation.

CONCLUSION

From the present study, it is quite evident that low concentration of both SA and EMS could be suitable for inducing genetic variability in the natural gene pool of *Allium cepa* L. Further studies on evaluation of yield and other agronomic characters needs to be conducted.

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