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Short communication

Studies on mutagenesis in garlic using chemical mutagens to determine lethal dose (LD_{50}) and create variability

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ABSTRACT

A study was undertaken to determine the LD_{50} in garlic variety G-41, to create variability for desirable traits using sodium azide (SA), colchicine and ethyl methane sulphonate (EMS). Half cut garlic cloves after treating for 6 and 12 h, were planted in randomized block design. Among the durations of treatments, concentrations required to attain LD_{50} was higher when treated for 6 hours as compared to 12 h in all the three mutagens. LD_{50} in SA can be obtained at 0.021 and 0.024%, in colchicine at 0.058 and 0.097% and in EMS at 0.674 and 1.222% concentration in 12 h treatment, whereas, it can be obtained at 0.095 and 0.108% in SA, 0.124 and 0.145% in colchicine and 0.826 and 1.222% in EMS in 6 h treatment, respectively at the time of harvesting and at 45 days after planting. Same concentrations can be tried in other varieties or lines of garlic to create variability for further selection.

Key words: Colchicine, ethyl methane sulphonate, garlic, mutation, LD_{50}, sodium azide.

Garlic (*Allium sativum* L.) is one of the most important vegetable crops. India ranks second to China in area (2.45 lakh hectares) with a production of 12.26 lakh tonnes but the productivity of garlic is 5 t/ha in India, which is quite less as compared to average productivity of 16.71 t/ha in the world (Anon, 2). Among various reasons for low productivity, non-availability of high yielding genotypes is one of the major factors, which needs to be addressed at the earliest. Garlic being a vegetatively propagated crop, posse's low variability. Clonal selection is one of the main methods for garlic improvement (Agrawal *et al*., 1), hence it is difficult to create variability for the breeders for selecting the promising genotypes. Induced mutations have successfully been utilized in the genetic improvement of many crops (Mishra and Raja, 8). Sensitivity towards EMS and SA for four hours was assessed for shoot and root length in onion varieties and demonstrated that both mutagens at low dose could be suitable for the creation of variability in onion (Joshi *et al*., 5). There are no reports available in garlic mutation and the only way to create variability in garlic is through induced mutation or somaclonal variations for desirable traits. Therefore, as a preliminary step towards mutation breeding in garlic, an experiment was undertaken to determine the LD_{50} in field conditions to create variability for desirable traits.

Variety G-41 was taken for the study as it performs best under Rajgurunagar conditions. Half cut cloves of variety G-41 were treated with different chemical mutagens, *viz*. sodium azide (SA), ethyl methane sulphonate (EMS) and colchicine. The physical mutagen gamma radiations were also tried from 3 to 21 Kr at interval of 3 Kr. To obtain different concentrations of sodium azide, EMS and SA, methods given in Anon (3) and Harten (4) was followed. Six concentrations of SA and EMS each and eight concentrations of colchicine were used in the range of 0.01-0.6, 0.05-0.55 and 0.05-0.55, respectively (Tables 1, 2 & 3). About 120 half-cut cloves were treated for 6 and 12 h in each treatment, which were gently shaken on a shaker. After the treatment, the cloves were washed with water and planted in two replications in a randomized block design in the field at 10 cm × 15 cm spacing and plot size of 1 m × 1 m along with the control (untreated) during fourth week of October. Recommended cultural practices were followed for raising the crop. Observations were recorded in the field for survival after 45 days of planting (DAP) & final observations were recorded at the time of harvest. Number of plants surviving in each treatment was counted and mortality rate was calculated for different doses of chemical mutagens. LD_{50} was calculated at 45 days after planting (DAP) and at the time of harvest. Observations on other characters like plant height and number of leaves was also recorded 100 days after planting from 10 plants and yield was recorded at the time of harvesting on plot basis. The data was analyzed in randomized block design using Indostat software for plant height and yield characters.

The LD_{50} for different chemical mutagens, *viz*., sodium azide, EMS and colchicine were worked out.

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Table 1. Effect of sodium azide on growth and yield of garlic variety G-41.

Sodium azide conc. (%)	Plant height (cm)	No. of leaves	Mortality (%) in field		Yield (kg/ plot)
			45 DAP	137 DAP	
12 h dip					
Control	49.7	13.1	8.3	12.6	0.843
0.01	47.7	10.7	11.8	28.3	0.560
0.02	44.6	10.4	42.1	47.4	0.497
0.04	35.8	10.4	84.0	86.3	0.087
0.08	33.2	8.5	96.9	98.3	0.038
0.10	24.0	4.4	98.4	100.0	0.000
0.20	0.0	0.0	100.0	100.0	0.000
0.40	0.0	0.0	100.0	100.0	0.000
6 h dip					
Control	48.7	10.1	11.1	14.9	0.910
0.01	44.4	9.4	14.2	16.1	0.871
0.04	41.9	9.0	15.8	25.0	0.720
0.10	41.3	8.4	46.5	52.6	0.504
0.20	30.5	7.8	70.5	79.0	0.239
0.40	16.5	5.5	90.3	95.8	0.020
CD at 5%	5.9	1.1	5.4	8.4	0.119

DAP = Days after planting.

Table 2. Effect of colchicine on growth and yield of garlic variety G-41.

Colchicine conc. (%)	Plant height (cm)	No. of leaves	Mortality (%) in field		Yield (kg/ plot)
			45 DAP	137 DAP	
12 h dip					
Control	46.5	11.7	1.7	16.8	0.782
0.05	42.3	10.5	36.7	43.0	0.311
0.08	39.5	9.5	40.9	70.4	0.056
0.10	34.7	9.2	51.4	78.7	0.047
0.15	32.7	7.9	86.7	93.5	0.024
0.25	30.4	7.2	91.7	96.3	0.020
0.35	24.4	6.7	92.5	95.3	0.014
0.45	9.0	2.8	97.5	99.1	0.003
0.55	0.0	0.0	100.0	100.0	0.000
6 h dip					
Control	42.1	10.9	3.3	15.8	0.803
0.05	41.7	10.2	15.0	15.8	0.699
0.08	39.8	9.3	18.8	19.7	0.451
0.10	38.8	8.9	28.8	39.1	0.384
0.15	36.0	8.5	51.7	61.9	0.157
0.25	31.3	8.3	86.7	98.2	0.016
0.35	28.5	7.7	94.2	100.0	0.000
0.45	12.8	3.3	95.8	100.0	0.000
0.55	0.0	0.0	100.0	100.0	0.000
CD at 5%	12.85	3.20	11.72	11.06	0.09

DAP = Days after planting.

Sodium azide is the only chemical mutagen that, in addition to the alkylating agents has been used on a relatively large scale and has been described as a “Super mutagen” due to very low toxicity of the compound and hence mutagenic effectiveness is close to 100% as reported by Nilan *et al*. (10). Mutagen EMS and SA for 4 h was studied for their sensitivity towards these mutagens for shoot and root length in onion varieties (Joshi *et al*., 5). Thus, SA was used as one of the mutagen to create variability in garlic with concentrations from 0.01 to 0.4%. Plant height and number of leaves in case of SA was not affected at lower concentrations, *i.e.* up to 0.02%, but it was reduced at increasing concentrations with 24 cm at 0.1% in 12 h treatment and 16.5 cm at 0.4% in 6 h of treatment against the check which had 49.7 and 48.7 cm plant height, respectively. The reduction in shoot and root length in onion varieties was also observed under SA treatment by Joshi *et al.* (5). The number of plants at 0.08% (12 h) and 0.4% (6 h) treatments were less than five. Number of leaves was affected due to SA treatments, which ranged from 4.4 to 10.7 per plant. In 0.10% (12 h) treatment, 4.4 leaves were present at 100 days after planting but the plants did not survive till harvest, whereas, it was 8.5 in number at 0.08% concentration. Number of leaves in 6 h treatment at 0.4% was 5.5 and at 0.2% it was 7.4 against control, which had 10.1 leaves/plant. Yield was significantly reduced to about 50% in 12 h of treatment even at lower concentration of 0.015%. Similarly, in 6 h of treatment the yield was significantly reduced to 0.720 kg per plot at 0.04 per cent against the check (0.910 kg per plot) (Table 1).

Mortality in different SA treatments at the time of harvest was not affected statistically up to 0.01% concentration in both 12 and 6 h of treatment. It was 47.3 per cent at 0.02% and 52.5 at 0.10% concentration in 12 and 6 h, respectively, at the time of harvest. LD_{50} was calculated 45 days after planting and at the time of harvest as per the field conditions. LD_{50} in SA at the time of harvesting in 6 h treatment was attained at 0.095% concentration and in 12 h of treatment was attained at 0.021%, whereas at 45 days after planting it was attained at 0.108% concentration in 6 h of treatment and at

0.024% concentration in 12 h of treatment as per field condition (Table 4).

In colchicine, different parts of the plant can be treated with 0.005 to 1.5% concentrations for 1 to 24 h or even up to 10 days in other crops as reported by Harten (4). Besides polyploidy, colchicine also induces mutations may be due to mutatic irregularities and quantative changes and induced mutations in crops like red clover, ryegrass, turnip, chrysanthemum (Maluszynski *et al*., 7). Hence, colchicine was used in different concentrations to create variability through induce mutation in garlic. Like sodium azide, the mortality was very high in higher concentrations of colchicine, above 0.08% at 12 h and 0.15% at 6 h of treatment, hence lower concentrations were used. Growth parameters were studied and it was found that plant height was not affected up to 0.05% and 0.1% colchicine concentrations at 12 and 6 h of treatment, respectively but at higher concentrations it was reduced. The plants were less than 10 in number at and above 0.15% for 12 h and 0.25% for 6 h of treatment. The number of leaves was also not affected up to 0.1% for 12 h and 0.25% for 6 h treatments. Yield per plot was reduced to 0.311 kg/plot at 0.05% concentration in 12 h treatment against the check (0.782 kg/plot). Whereas, in 6 h of treatment, it was 0.699 kg against check 0.803 kg/plot (Table 2). At higher concentrations, the yield was drastically reduced to less than 50 g per plot above 0.08% in 12 h and 0.25% in 6 h of treatment. LD_{50} after 45 days of planting in colchicine treatment was attained at 0.145% in 6 h of treatment, while it was attained at 0.097% in 12 h of treatment, whereas, at the time of harvest it was attained at 0.124 in 6 h treatment and at 0.058% in 12 h treatment as per the field conditions (Table 4).

The concentrations of EMS used in different crops ranged from 0.05 to 0.3% in Anon (3), but dosages well outside these have been mentioned in literature (Harten, 4). Hence, six concentrations, *viz*., 0.05, 0.15, 0.25, 0.35, 0.45 and 0.55% were used for 6 and 12 h of treatments. Mortality at the time of harvesting at 0.55% was 33.3 and 40.8% under field conditions in 6 and 12 h of EMS treatments, respectively, whereas it was hardly up to 22.5% at 0.55% concentrations at 45 days of planting (Table 3). LD_{50} in EMS was calculated at the time of harvesting and in 6 h treatment it was attained at 0.826% concentration and in 12 h of treatment it was attained at 0.674%, whereas, at 45 days after planting it was attained at 1.375% concentration in 6 h of treatment and at 1.222% concentration in 12 h of treatment as per field condition (Table 4). Nilan (9) concluded that EMS induces higher proportions of mutations to chromosome aberrations. It also induces high frequencies of base-pair substitutions exclusively of transition type at G_C site and little lethality.

Table 3. Effect of EMS on growth and yield of garlic variety G-41.

EMS conc. (%)	Plant height (cm)	No. of leaves	Mortality (%) in field		Yield (kg/ plot)
			45 DAP	137 DAP	
12 h dip					
Control	43.8	11.6	0.8	6.7	0.883
0.05	42.5	11.4	4.2	10.8	0.670
0.15	39.6	11.3	9.2	30.0	0.625
0.25	27.5	8.4	9.2	33.3	0.315
0.35	27.7	7.7	10.8	35.0	0.273
0.45	23.8	7.2	16.7	35.8	0.215
0.55	21.8	7.1	22.5	40.8	0.145
6 h dip					
Control	44.2	10.4	6.7	9.2	0.920
0.05	43.3	10.3	6.7	14.2	0.907
0.15	45.7	10.2	6.7	19.7	0.905
0.25	42.9	9.7	8.3	19.2	0.828
0.35	42.4	9.3	10.0	24.2	0.680
0.45	38.4	9.4	11.7	31.7	0.460
0.55	36.9	9.4	20.0	33.3	0.428
CD 5%	5.1	1.5	8.0	12.9	0.317

DAP = Days after planting.

Table 4. Determination of LD_{50} in garlic for different chemical mutagens treated for 6 and 12 hours duration.

Time of observation/ duration of treatment	Sodium azide (%)	Colchicine (%)	EMS (%)
Treated for 6 h			
45 days after planting	0.108	0.145	1.375
At the time of harvesting	0.095	0.124	0.826
Treated for 12 h			
45 days after planting	0.024	0.097	1.222
At the time of harvesting	0.021	0.058	0.674

Growth parameters like plant height, number of leaves and yield per plot were also studied. There was significant difference in these characters. Average plant height in control was 44.1 and 43.7 in 6 and 12 h of treatment, respectively. It was at par in 0.05 and 0.15% treatments of EMS for 6 h and 0.05% treatment for 12 h. Plant height decreased with the increase in the concentrations. Joshi *et al*. (5), recorded similar

results in onion. Number of leaves varied from 11.3 in control to 7.0 in 0.55% 12 h treatment. The number of leaves was not affected up to 0.15% concentrations in 12 h treatment, while, it was at par up to 0.45% EMS concentration in 6 h of treatment. The effect on number of leaves was more in 12 h of treatment as compared with 6 h of treatment. Yield per plot was also affected due to EMS, which was at par with control up to 0.15% for 12 h of EMS treatment, which ranged between 0.670 to 0.882 kg per plot and up to 0.35% for 6 h treatment (0.680 to 0.905 kg/plot). Yield per plot was 0.145 and 0.460 kg in 12 and 6 h of treatment with 0.55% of EMS, respectively (Table 1). In case of onion, increasing dose of EMS and other mutagens resulted in decrease in germination, seedling height, survival and bulb weight (Kataria and Singh, 6).

Among the duration of treatments, concentrations required to attain LD_{50} was higher when treated for 6 h as compared to 12 h in all the three mutagens in garlic variety G-41. The concentration of SA to achieve LD_{50} was comparatively less as compared to colchicines, while the concentration of EMS required was quite high compared to both. Thus, LD_{50} in SA can be obtained at 0.021% concentration at the time of harvesting and at 0.024% after 45 days of planting in 12 h treatment, whereas it can be obtained at 0.095% at the time of harvesting and at 0.108% after 45 days of planting in 6 hours treatment. In colchicine LD_{50} can be obtained at 0.058% concentration at the time of harvesting and at 0.097% after 45 days of planting in 12 h treatment, whereas it can be obtained at 0.124% at the time of harvesting and at 0.145% after 45 days of planting in 6 hours treatment. In case of EMS, concentrations LD_{50} in 12 h treatment can be attained at 0.674% at the time of harvesting and at 1.222% after 45 days of planting and in 6 h of treatment at 0.826% at the time of harvesting and at 1.375% after 45 days of planting. Hence, these concentrations can be tried in other varieties or lines of garlic to create variability for improvement.

REFERENCES

1. Agrawal, M.K., Fageria, M.S. and Dhake, R.S. 2003. Garlic breeding – a review. *Agric. Rev.* **24**: 70-74.
2. Anonymous 2013. *Statistical Database*, FAO Stat-Agriculture website, 2013.
3. Anonymous 1977. *Manual on Mutation Breeding* (2nd Edn), IAEA, Vienna.
4. Harten, A.M. 1998. *Mutation Breeding, Theory and Practical Applications*, Cambridge University Press, 353 p.
5. Joshi, N., Ravindran, A. and Mahajan, V. 2011. Investigations on chemical mutagen sensitivity in onion (*Allium cepa* L.). *Int. J. Bot.* **7**: 243-48.
6. Kataria, A.S. and Singh, N. 1989. Mutation studies in onion (*Allium cepa*). III. Types and frequency of macro-mutations. *Indian J. Hort.* **46**: 395-400.
7. Maluszynski, M., Ahloowalia, B.S. and Sigurbjovnsson, B. 1995. Application of *in-vivo* and *in-vitro* mutation techniques for crop improvement. *Euphytica* (Selected papers, meeting EUCARPIA, Section genetic manupulation in plant breeding, Cork. Ireland). **85**: 303-15.
8. Mishra, S.K. and Raja, R. 2000. Exploitation of induced mutagenesis in crop improvement. *Ann. Agric. Res*. **21**: 1-10.
9. Nilan, R.A. 1981. Induced gene and chromosome mutants. *Philos. Trans. Royal Soc. London*, **292**: 457-66.
10. Nilan, R.A., Sideris, E.G., Kleinhofs, A., Sander, C. and Konzac, C.F. 1973. Azide – a potent mutagen. *Mutation Res.* **17**: 142-44.

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