Efficiency and Effectiveness of Gamma Rays and Sodium Azide in *Sesbania cannabina* Poir.

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Summary Mutagenic effectiveness and efficiency is an important factor for the selection of a mutagen for a mutation breeding program. Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit mutagen dose, while mutagenic efficiency is a measure of the proportion of mutations in relation to undesirable changes such as lethality, sterility, meiotic aberrations *etc.* The present study envisages the mutagenic effectiveness and efficiency of individual and combined treatments of chemical and physical mutagens *i.e.* sodium azide (individual), gamma rays (individual) and sodium azide+gamma ray (combined). For the individual treatment of sodium azide, the seeds of the *Sesbania cannabina* variety ND-1 were treated with 0.5% solution of sodium azide (SA) for four different time durations, *i.e.* 3, 5, 7, and 9 h, and for the individual treatment of gamma rays, dry and healthy seeds were treated with 20, 40, 60, and 80 Kr doses of gamma rays. For the combined treatment, the seeds were exposed to four different doses of gamma rays (20, 40, 60, and 80 Kr) and after irradiation seeds were treated with 0.5% solution of sodium azide for 3 h. After treatment, seeds subjected to individual and combined treatment were sown in randomized block design to raise the M₁ generation and a study was conducted on germination percentage, survival percentage, pollen fertility percentage, and chromosomal aberrations at different doses of the individual and combined treatments.

Key words *Sesbania cannabina*, Mutagens, Sodium azide, Gamma rays.

Mutations are the tools used by the geneticist to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for the genetic improvement of economic crops. Mutation breeding is expected to make a contribution primarily as an important adjunct to the conventional breeding approach. A large number of desirable varieties have been developed through mutation breeding in field and horticulture crops. The technology of mutation induction has become an established tool in plant breeding in order to supplement existing germplasms and to improve specific traits in cultivars. Varieties with better genotype and phenotype characters have been revealed to farmers as a result of induced mutations which have been used directly as new cultivars or they can be used in cross breeding programs (Gottschalk and Wolff 1983, Micke *et al.* 1987). For the *Sesbania cannabina*, thus far very limited work has been carried out using gamma rays alone or in combination with chemical mutagens for the improvement of quantitative and qualitative characters.

Sesbania cannabina is a quick growing succulent green manure crop and widely adaptable to adverse climatic conditions such as drought, waterlogging, high salinity, *etc.* It belongs to the family Fabaceae and its major agronomic use has been as green manure to improve the production of food crops, as it improves the soil fertility. In 1983, Bhatti *et al.* reported that *Sesbania* green ma-

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nuring substantially improves grain yield, by up to 72%. The induction of mutations is an important approach to creating genetic variability in plants. Genetic variability is fundamental to successful breeding programs in vegetatively and sexually propagated plants. The mutants so produced facilitate the isolation, identification, and cloning of genes used in designing crops with improved yields, increase stress tolerance, longer shelf life, and reduced agronomic input (Ahloowalia and Maluszynski 2001).

Physical and chemical mutagens have been successfully used to evolve new varieties with improved economic traits (Mahla *et al.* 1990, Rehman 1990, Kumar 1972). The combination of physical and chemical treatments used in many crops (Singh and Chaturvedi 1987, Chang and Gao 1988, Rakow and Raney 2003) has indicated there to be a higher frequency of mutations in combined treatments as compared with individual treatments.

Sodium azide has been reported to be mutagenic in a large number of crop species. Sodium azide (NaN_3) is a common laboratory chemical and is widely used in industry, agriculture, medical practice, and organic synthesis research. It is a common bactericide, pesticide, and industrial nitrogen gas generator, and known to be highly mutagenic in several organisms, including plants and animals (Rines 1985, Veleminsky and Anglis 1987, Raicu and Mixich 1992). Gamma rays are an energetic form of electromagnetic radiations produced by radioactivity or other nuclear or sub atomic processes with a wavelength of 10^{-11} to 10^{-7} cm. Gamma radiation has been reported to have beneficial effects on many crops. In African violets (*Saintpaulia ionantha* H. Wendl.), gamma radiation at 15 Gy improved the plant architecture of the mutants (Senevirante and Wijesundara 2007). In wheat, gamma radiation improved germination, plant height, grain per plant, and grain yield at 200 Gy (Jamil and Khan 2002) and at 300 to 400 Gy (Irfaq and Nawab 2001). The present study was undertaken to study the effect of physical and chemical mutagens on different parameters of *Sesbania cannabina*.

Materials and methods

Procurement of seeds

Seeds of *Sesbania cannabina* variety ND-1 were obtained from Sunn Hemp Research Station, Pratapgarh, India.

Treatment of sodium azide

Dry and healthy seeds were soaked in water for 14 h. Soaked seeds were then treated with 0.5% solution of sodium azide (SA) for four different time durations (3, 5, 7, and 9 h). After treatment, the seeds were washed in running water to remove the SA. After that, the treated seeds, together with the control seeds, were sown in triplicate to raise the M_1 generation.

Treatment of Gamma rays

Dry and healthy seeds were irradiated at four doses of gamma rays, viz. 20, 40, 60, and 80 Kr, using a ^{60}Co source at the National Botanical Research Institute (N.B.R.I.), Lucknow. The dose rate was 18 Krad/s with gamma chamber-900 model (the chamber was supplied by Bhabha Atomic Research Centre (BARC), Mumbai). After irradiation, the seeds were sown in their respective pots in triplicate to raise the M_1 generation. Unradiated seeds were also sown as a control set.

Combined treatment of gamma ray and sodium azide

For the combined treatment, the dry and healthy seeds were irradiated at four doses of gamma rays, viz. 20, 40, 60, and 80 Kr, using a ^{60}Co source at the National Botanical Research Institute (N.B.R.I.), Lucknow. The dose rate was 18 Krad/s with a gamma chamber-900 model. After irradiation, the seeds were treated with 0.5% solution of sodium azide for 3 h. After 3 h, the seeds were

washed in running water to remove the traces of SA and sown in their respective pots to raise the M_1 generation.

Field work

Data for germination and survival percentages were taken after 15 and 30d respectively. On the onset of budding, the young floral buds were fixed in Carnoy's fixative (1:3, glacial acetic acid:abs. alcohol) in their respective bottles for 24h and then stored in 70% ethyl alcohol in the refrigerator and were then used for cytogenetical analysis.

Cytological studies

Slides were prepared using the anther squash technique. Anthers were squashed in 2% aceto-carmine. Slides were observed under the microscope to observe different meiotic stages and various chromosomal aberrations and data were taken for chromosomal aberrations of each treated set as well as for each control set.

Results

Effect of individual and combined treatments of SA and gamma rays on germination, survival and pollen fertility percentages of Sesbania cannabina

From Table 1, it is clear that seed germination decreases with the increasing dose of treatment in the individual treatments as well as in the combined treatment, although the rate of reduction in germination percentage was higher in the individual treatment of gamma rays as compared to the combined treatment and the individual treatment of sodium azide. Control exhibited 100% germination and this was reduced to 30% at the highest dose of gamma rays (80 Kr), while it was 50% at the highest dose of the combined treatment. Unlike germination, survival percentages were 100% for all doses of the individual treatment (SA), while in case of the gamma rays individual treatment survival percentage was reduced to 25%, and in the combined treatment, survival percentage was 100% at 2 lower doses and at the highest dose *i.e.* 80 Kr+SA, it was reduced to 58.33%. Table 1 shows pollen fertility at different doses of treatment of the individual and combined treatments of SA and gamma rays. The control set exhibited the highest pollen fertility, *viz.* 94.16%, and fertility

Table 1. Effect of individual and combined treatment of SA and gamma rays on germination, survival, and pollen fertility percentages of *Sesbania cannabina*.

Mutagenic treatment	Germination percentage	Survival percentage	Pollen fertility (percentage)
Control	100	100	94.16
SA			
3 h	90	100	89.94
5 h	80	100	83.09
7 h	70	100	80.76
9 h	70	100	76.27
Gammas rays			
20 Kr	60	100	81.66
40 Kr	50	90	80.33
60 Kr	40	75	74.66
80 Kr	30	25	70.00
Gamma rays+SA (0.5%)			
20 Kr+SA	70	100	82.33
40 Kr+SA	70	100	77.50
60 Kr+SA	60	83.33	74.70
80 Kr+SA	50	58.33	70.34

Figs. 1-9. 1. Normal metaphase ($n=12$), 2. Normal anaphase (12:12), 3. Precocious movement at metaphase I with ring formation at the dose of 9h SA, 4. Precocious movement with Unorientation at metaphase I at the dose of 60Kr+SA, 5. Unorientation at anaphase II at the dose of 60Kr gamma rays, 6. Secondary association at metaphase I at the dose of 5h SA, 7. Multinucleate condition at the dose of 80Kr+SA, 8. Bridge at anaphase I at the dose of 80Kr gamma rays, 9. Scattering at metaphase I at the dose of 3h SA. Scale bar=10 μ m.

showed a decreasing trend in parallel with the dosage increase in individual and combined treatments. The lowest pollen fertility was observed at 80Kr, which was 70%, while at the highest dose of the combined treatment it was 70.34%.

Effect of SA on microsporogenesis of Sesbania cannabina

Analysis of pollen mother cells (PMCs) of the control set indicated 12 perfect bivalents ($n=12$) at metaphase (Fig. 1). At anaphase I (Fig. 2), the PMCs of the control plants exhibited normal 12:12 segregation. The spectrum of various meiotic anomalies and the total number of abnor-

Table 2. Effect of individual and combined treatments of SA and gamma rays on microsporogenesis of *Sesbania cannabina*

MUTAGEN	Doses of treatment	No. of PMCs observed	No. of abnormal PMCs	Metaphasic (I and II) abnormality (%)						Anaphasic (I and II) abnormalities (%)						Telophasic abnormalities (%)			Others (%)	Total abnormality (%)	
				Metaphasic (I and II) abnormality (%)						Anaphasic (I and II) abnormalities (%)						Telophasic abnormalities (%)					
				Uni	Un	Sc	St	Pr	Mul	Triv	St	Lg	Br	Un	Bin	Br	Dispol				
Control			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SA	3 h	185.5	24.5	2.12	1.21	2.77	0.91	1.80	–	–	–	2.03	0.67	0.82	–	–	–	–	0.92	13.30	
	5 h	132.75	22.5	1.57	1.55	3.02	1.09	2.38	–	–	–	2.29	1.69	1.65	–	–	–	–	1.71	17.01	
	7 h	159	30	2.36	1.41	4.72	1.41	2.81	–	–	–	2.34	1.24	1.54	–	–	–	–	0.92	18.83	
	9 h	165.75	34.25	3.49	1.36	4.23	1.67	4.02	–	–	–	1.95	1.65	1.49	–	–	–	–	0.75	20.68	
Gamma rays (GR)	20Kr	258.25	21	–	1.51	0.79	0.37	1.35	–	–	–	1.36	1.46	0.25	–	0.95	0.57	–	–	8.06	
	40Kr	275.75	31	2.16	1.52	0.7	0.78	1.37	0.45	–	0.45	0.75	1.24	0.38	–	0.95	0.45	–	–	11.24	
	60Kr	271.5	43.47	2.61	1.54	–	1.48	1.35	1.49	–	2.38	1.48	1.28	0.56	–	1.69	0.74	–	–	16.10	
	80Kr	264	58	1.67	2.66	–	2.53	2.27	1.51	–	2.74	1.9	2.86	1.77	–	1.7	1.61	–	–	22.01	
GR+SA (0.5 %)	20Kr+SA	322.5	40.25	–	1.28	–	0.90	1.28	2.5	1.41	1.07	–	0.58	0.84	1.27	–	–	–	1.01	12.22	
	40Kr+SA	297.5	52	–	1.66	–	1.50	1.33	2.58	1.58	1.57	–	1.59	1.40	1.91	–	–	–	2.1	17.52	
	60Kr+SA	262.5	53	–	1.99	–	2.22	1.99	3.28	1.81	2.01	–	2.0	1.61	1.59	–	–	–	1.61	20.20	
	80Kr+SA	311.75	76.5	–	1.86	–	2.39	1.56	3.02	2.95	2.69	–	2.09	2.24	2.59	–	–	–	3.02	24.48	

Uni-Univalent, Un-Unorientation, Sc-Scattering, St-Stickiness, Pr-Precocious movement, Mul-Multivalent, Triv-Trivalent, Lg-Laggard, Br-Bridge, Bipol-Bipolarity, Bin-Binucleate, Dis Pol-Disturbed polarity. Other abnormalities: Secondary association at metaphase, Forwards at anaphase, Cytomixis, Tripolarity, Scattering, Multinucleate condition, etc.

mal PMCs has been summarized in Table 2. The most frequent aberrations were: scattering at metaphase, precocious movement at metaphase, univalents at metaphase, and unorientation at anaphase (Fig. 5). Scattering at metaphase was found to be highest (4.72%) at the 7 h dose of treatment and the lowest (3.02%) at the 5 h dose of treatment. The highest frequency of precocious movement (Figs. 3, 4) was observed at 9 h, where it found to be 4.02%. Univalents were also found to be highest at 9 h, viz. 3.49%, and the lowest at the 5 h dose of treatment, viz. 1.57%. Among anaphasic aberrations, laggards were observed in higher frequency and were found to be the most frequent at 7 h (2.34%). The total abnormality percentage showed an exponential increase along with the increasing dosage of treatment and was observed to be 13.30 and 20.68% at the 3 h and 9 h doses of treatment, respectively.

Effect of gamma rays on microsporogenesis of Sesbania cannabina

The plants in the gamma irradiated sets exhibited various types of meiotic aberrations at each phase of meiosis. It is evident from Table 2 that different types of aberrations increased along with the increase in gamma ray dose. Gamma rays induced various types of chromosomal aberrations, such as univalents, precocious movement, multivalent, unorientation, laggards, bridges, stickiness, disturbed polarity, etc. The most prominent abnormalities were bridges at anaphase, stickiness at anaphase, unorientation at metaphase, stickiness at metaphase, and precocious movement at metaphase. Bridges at anaphase were found at the highest frequency at the 80 Kr dose, i.e. 2.86%. Bridges were observed at both anaphase and telophase but the frequency was highest at anaphase. Univalents and multivalents at metaphase were prominent at higher doses, while absent at 20 Kr. Scattering at metaphase I (Fig. 9) was observed only at the 2 lower doses and was absent at 60 Kr and 80 Kr. Stickiness at metaphase and anaphase was found to be most frequent at 80 Kr doses of treatment, at 2.53% and 2.74%, respectively. Among telophasic aberrations, bridges (Fig. 8) were more frequent than disturbed polarity (Table 2). The total abnormality percentage was observed to be highest at 80 Kr doses of treatment and was found to be 22.01% which was comparatively higher than that of the individual treatment of SA (Table 2).

Effect of gamma rays + SA on microsporogenesis of Sesbania cannabina

The spectrum of various meiotic anomalies induced by the combined treatment of SA and gamma rays has been summarized in Table 2. The total abnormality percentage showed an exponential increase along with the increase in treatment dosage. The most frequent chromosomal aberrations were multivalent formations at metaphase, trivalents at metaphase, and binucleates at telophase. Multivalents were found to be most frequent at 60 Kr+SA (3.28%), while at the highest dose of 80 Kr+SA the frequency was 3.02%. Binucleate cells were observed during telophase and were found to be most frequent (2.59%) at the 80 Kr+SA dose. Metaphasic aberrations were more prominent than anaphasic aberrations. Instances of stickiness at metaphase and anaphase increased in parallel with higher doses of treatment. The total abnormality percentage at the lowest dose of 20 Kr+SA was 12.22% and was 24.48% at the highest dose (80 Kr+SA), which was comparatively higher than the percentage seen in the individual treatments (Table 2).

Discussion

In the present experiment, the effect of individual treatments of SA and of gamma rays and of a combined treatment of SA and gamma rays on germination, survival, and pollen fertility has been studied. In all cases of treatment, as the treatment dose increases, the percentage value of these indices decreases. Among the treated sets, maximum germination was found at the 3 h dose of SA and this then decreased up to 50% at the highest dose of the combined treatment. These results are in line with the results of (Khan and Al-Qurainy 2009) who performed his experiment on *Eruca sa-*

tiva. The reduction in germination percentages may be attributed to the delay or inhibition of physiological and biological processes necessary for seed germination, which include enzyme activity (Chrispeeds and Varner 1976), hormonal imbalance (Khan and Al-Quainy 2009), and the inhibition of mitotic process (Ananthaswamy *et al.* 1971). The inhibitory effect of NaN_3 on germination could be azide anions which are strong inhibitors of cytochrome oxidase, which in turn inhibits oxidative phosphorylation (Kleinhofs *et al.* 1978). In addition, it is a potent inhibitor of the proton pump and alters the mitochondrial membrane potential (Zhang 2000). These effects together may hamper ATP biosynthesis resulting in the decreased availability of ATP, which may slow the germination rate and reduce the germination percentage. Various doses of treatment affected the survivability of seedlings in a dose dependent manner as shown in Table 1. According to Sato and Gaul (1967) and Natrajan and Shivshankar (1965), the reduction in seedling survival can be attributed to cytogenetic damage and physiological disturbances caused by mutagen treatment. Thus, the probable reason of the drop in seedling survivability may be the hindrance caused by the SA and gamma rays on different metabolic pathways of the cells. Similar findings have also been reported by Rachovska and Dimova (2000) in wheat, Akhaury *et al.* (1996) and Ilbas *et al.* (2005) in barley, Adamu and Aliyu (2000) in tomato, Khan *et al.* (2004) in mungbean, Al-Qurainy (2009) in *Eruca sativa*, and Mostafa (2011) in sunflower.

Pollen fertility is an index of meiotic behavior. The more frequent the chromosomal aberrations, the greater the pollen sterility, as in the case of the present experiment, in which a reduction in pollen fertility was observed in both cases, *i.e.* in the individual treatments as well as the combined treatment; this was found to be in a dose dependent manner. Similar results were also reported by Reddi (1977) and Bhat *et al.* (2007). They reported that pollen sterility was the result of interchanges of segments between non-homologous chromosomes and considered that the presence of laggards, univalents, micronuclei, and stickiness was closely associated with pollen sterility.

Analysis of microsporogenesis was done to study the chromosomal behavior at meiosis in order to correlate it with fertility of the species. The meiotic study of pollen mother cells of control exhibited a normal diploid status with chromosome number ($n=12$), *i.e.* 12 bivalents at metaphase I and normal separation (12:12) at anaphase I. Compared to the control sets, the treated sets showed various types of chromosomal aberrations. Different types of metaphasic, anaphasic, and telophasic aberrations were scored at all doses of the individual and combined treatments. Similar types of aberrations were also reported by many researchers in different plant materials after treatment with different mutagens. A dose dependent enhancement in the meiotic aberration due to the effect of different mutagens has also been studied by various investigators like Ahmad (1993), Kumar and Dubey (1998), Dharmayanthi and Reddy (2000), Ignacimuthu and Babu (1989), Ahmad and Yasmin (1992), Patil and Bhat (1992), Singh (1992), Misra *et al.* (2001). During the cytological observations of the individual and combined treatments of the present study, it was found that the combined treatment was more effective and efficient in inducing chromosomal aberrations.

Sinha and Godward (1972) suggested that paracentric inversions may lead to the formation of chromatin bridges at anaphase and telophase stages of meiosis. Bridge formation may occur due to the failure of chiasmata in a bivalent to terminalize and the chromosome stretched between the poles (Saylor and Smith 1996). Single and multiple chromosome bridges may be due to the occurrence of dicentric chromosomes formed as a result of breakage fusion bridge cycles (McClintock 1941). Chromosomal stickiness may be due to genetic or environmental factors (Consolaro *et al.* 1996) and several agents have been reported to cause stickiness of chromosomes (Souza and Pagliarini 1996). Laggards observed during the present cytological studies might have originated due to delayed terminalization, stickiness of chromosomal ends, or because of failure of the chromosomal movements (Jayabalan and Rao 1987, Soheir *et al.* 1989). Disturbed polarity observed during anaphase may be due to spindle disturbance (Bhat *et al.* 2007). Jayabalan and Rao (1987) suggested that stickiness might be due to disturbance in the cytochemically balanced reaction. Tarar

and Dnyansagar (1980) have reported that stickiness at meiosis is due to the depolymerisation of nucleic acids caused by mutagenic treatments; or, it may be due to chemically induced stickiness to direct action of mutagens on the histone proteins leading to improper folding of DNA (Gaulden 1987). Bridges are also induced by many other mutagens, such as methyl methane sulphonate (Khan and Al-Qurainy 2009), and SA (Srivastava and Kapoor 2008), *etc.* Precocious movement of chromosomes may be due to spindle dysfunction. Kumar and Rai (2007) reported that precocious chromosome migration to the poles may be due to the formation of univalent chromosomes at the end of prophase I or to precocious chiasma terminalization at diakinesis or metaphase. Unorientation at metaphase and scattering of chromosomes may be due to the inhibition of spindle formation or the destruction of spindle fibers formed (Kumar and Rai 2007).

Increasing mutation frequency is a very important aspect for bringing about improvements in the efficiency of mutation breeding programs. SA and gamma rays have been demonstrated to induce mutations by different mechanisms. Combinations of these mutagens in appropriate sequences have been shown to cause a synergistic increase in mutation frequency. Combined treatments induced a higher mutation frequency with relatively less injury, thus resulting in higher mutation efficiency, thereby offering more opportunities for selection in mutation breeding practice. Thus, in the present experiment, the combined treatment of gamma rays and SA appears to be most effective mutagenic treatment for the induction of mutations in *Sesbania cannabina* to increase yield component traits and to aid the selection of improved populations.

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