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PROMISING SALT TOLERANT SOYBEAN MUTANTS DEVELOPED THROUGH GAMMA RAY IRRADIATION

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Abstract

An experiment was conducted to evaluate the performance of three promising lines along with two check varieties of soybean in respect of plant height, branches per plant, pods per plant, seeds per pod, days to maturity and seed yield, at five different locations during January to April 2022. Significant variations were observed both in individual location and combined over locations for all traits. Among the mutants, average days to maturity ranged from 109-114 days. It indicated that, all the mutant lines as well as check varieties had prolonged days to maturity, which eventually reflected significantly higher grain yield at all locations. Plant height ranged from 34-62 cm and number of branches plant^{-1} from 2.9 to 3.6. Mutant SBM-22 produced highest pods plant^{-1} (76); whereas, the check varieties Binasoybean-2 and Binasoybean-6 produced 43 Hundred seed weight was higher in Binasoybean-2 (13.4g) and lower hundred seed weight was obtained from SBM-26 (11.1g). Seed yield obtain from the mutants and checks significantly differs from each other. Mutant SBM-25 produced the highest seed yield of 2836 kg ha^{-1}. Among the locations, all mutants as well as check varieties gave the highest seed yield at Satkhira. Comparing with imposed salinity level and time, plant height as well as leaf was decreased. All the germinated genotypes were survived at initial salinity 4 dS m^{-1} (though 4 dS m^{-1} is not at all a problem for salinity) up to 21 days. All the mutants showed moderately tolerant at same treatment up to 7 days after sowing. Binasoybean-2, Binasoybean-6 and Lokon performed well with the advancement of time. Among the mutants SBM-22 and SBM-26 will be selected on the basis of yield contributed characters for further trials.

Key Words: Soybean, salinity, mutants, maturity, yield

Soybean [*Glycine max* (L.) Merr.] is an important leguminous crop producing the most valuable source of protein and vegetable oil closest to the optimum dietary essential amino acids profiles for human and animal nutrition (Lusas 2004). Soybean has a wide range of genetic variation in protein and oil contents. Domestication of the crop likely occurred between the 11th and 15th century BC, or perhaps earlier, from northeast China, and subsequently reached China's southern region and neighboring countries such as Korea, Japan, Thailand, the Philippines, etc. (Hymowitz 2004). It was not until the 18th century when soybean was introduced into Europe. In the western hemisphere, soybean was first brought into North America in 1765 and then into Central and South America during the mid-1900s (Hymowitz 2004). Since then, soybean has become a major economic crop for soybean producing areas. Worldwide, the expansion in soybean production is likely due to the increased value of protein and oil in soybean seeds. The range of protein content is from

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34.1 to 56.8% of dry seed mass, with a mean of 42.1%, while the range of oil content is 8.3 to 27.9%, with a mean of 19.5%. Between these two seed composition traits, there exists a widely known, strong negative correlation (Hurburgh *et al.*, 1990), indicating that efforts to improve protein content often results in decreased oil content, and vice versa. It is extremely rare to find germplasm in which the content for both protein and oil is relatively high (Wilson 2004). Thus, soybean workers who have extended great efforts to improve these important seed traits normally accept a sacrifice of one trait while attempting to improve the other. In recent years, efforts have been made to validate nutritional and pharmaceutical values of soybean protein. In nutrition studies, Birt *et al.* (2004) reported that consumption of soybean-based foods reduces cancer, blood serum cholesterol, osteoporosis and heart disease. As a valuable source of seed protein and vegetable oil for human and animal nutrient, soybean is the most economically important legume crop in many countries in the world. In addition, the values of soybean seed composition have received a sincere attention by soybean researchers to investigate for industrial applications and medical uses. Steadily increased world production and meal consumption of soybean for last ten years indicated that soybean crop continues to be an important leguminous crop plants significantly contributing to various aspects of agricultural production, animal industry, and in particular human health. Different abiotic stresses, drought, salinity, waterlogging/flooding and temperature stress (high and low) are the main factors reducing crop production worldwide and accounts for more than 50% yield loses. When plants are subjected to abiotic stresses, they activate different mechanisms to survive and sustain growth and yield. Thus, it is important to know what mechanisms exist to help plants surviving under severe stress. Plant traits linked to stress tolerance are multigenic, i.e. these traits are controlled by QTL. Molecular markers have been used to map the genomic location of genes/QTL for different traits related to drought, salinity and submergence tolerance in soybean. Salinity is a major environmental stress reducing soybean growth and yield. Cultivated soybean (*Glycine max*) is considered a salt sensitive crop whereas wild soybean (*Glycine soja*) is found to be tolerant; thus wild soybean (*G. soja*) can be used as resistance source for varietal improvement in soybean (*G. max*). Different authors have reported that yield can be reduced if soybean is subjected to salinity at the vegetative stage, but greater yield reduction occurs under waterlogging stress at the reproductive stage. Tolerance to salinity could increase yield. In Bangladesh maximum saline prone areas are not suitable for growing soybean and other legume and/or oil crops. BINA release two soybean varieties Binasoybean-2 and Binasoybean-6 were performed well in southern region than other cultivated varieties. Lokon is a highly salt tolerant variety but its yield is not acceptable. Day by day salinity increased in the seashore. For that reason, farmers are unable to cultivate those areas. If we can have developed a high yielding salt tolerant soybean mutant or variety for saline prone areas than it's a great value for plant breeding.

Seeds of popular soybean variety Binasoybean-2 and Binasoybean-6 were irradiated with 200, 350 and 400 Gy doses of gamma rays using a ^{60}Co source to induce new genetic variability for earliness, higher yield and yield atributes and planted M_1 generation at

Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh in 2018 for the selection of improved genotypes in the subsequent generations. Selection for the desired agronomic traits was carried out in M_{2-} M_6 generations based on maturity, higher pod and seed yield and salinity tolerance etc. (Reference from Annual Report). From the mutant (higher yield and yield related traits from four families) population, three M_6 mutant lines SBM-22 (Binasoybean-2-350Gy-Plant-5), SBM-25 (Binasoybean-6-200 Gy-Plant-1) and SBM-26 (Binasoybean-6-200Gy-Plant-4) were finally selected.

Experiments with three M_6 mutants: SBM-22, SBM-25, SBM-26 along with two check varieties (Binasoybean-2 & Binasoybean-6) were put into on-station trial at BINA sub-station farm at Noakhali, Barishal, Satkhira, Magura and Rangpur during Rabi (December 2021 to January 2022), 2022. The experiment was laid out in randomized complete block design with three replications. Spacing between rows was 30 cm, and 5-6 cm between plants in a row. Unit plot size was 12 m^2 (4m × 3m). Sowing was done within last week of December to first week of January, 2022 at different location.

Recommended production practices including application of fertilizers (20-25 kg urea, 60-70 kg TSP, 35-40 kg MoP, 35-45 kg gypsum per acre. Before final ploughing chemical fertilizers should be broadcasted followed by laddering) were followed to ensure normal plant growth and development. Data on various characters, such as plant height, branch number, number of seeds per pod were taken from 10 randomly selected plants. Maturity period was counted when the plant and pods of each plot turned into yellowish brown and all the leaves shed. Plot yield was converted into kg ha^{-1}. Appropriate statistical analyses were performed with the mean data of each character. Mean differences of different parameters were tested by Duncan's Multiple Range Test (Gomez and Gomez, 1984). Artificial salinity (crude salt mixed with distilled water and mixed it in the pot) was created with NaCl and maintained 4 dSm^{-1}, 6 dSm^{-1} and 8 dSm^{-1} in each pot (10 kg soil in each pot). Data on visual injury and pod setting rate was recorded from five randomly selected plants of each dose.

The genotypic variation in days to maturity, morphological parameters (plant height and no. of branch $plant^{-1}$), yield attributes (no. of pod $plant^{-1}$ & no. of seeds pod $^{-1}$) and seed yield in soybean was significant (Table 1). On an average, maturity period ranged from 109 days (Binasoybean-2) to 114 days (SBM-26). Plant height ranged from 34 cm (Binasoybean-2) to 62 cm (SBM-26) and branches $plant^{-1}$ ranged from 2.9 (Binasoybean-2) to 3.6 (SBM-22). Mutant SBM-22 produced highest pods $plant^{-1}$ (76); whereas, the check varieties Binasoybean-2 and Binasoybean-6 produced 43 and 53 pods $plant^{-1}$, respectively. Binasoybean-6, SBM-22 and SBM-26 produced the highest number of seeds pod^{-1} (3.9). Mutant SBM-25 had the highest pod length (2.9) followed by Binasoybean-2 (2.70) and mutants SBM-26 (2.6). Hundred seed weight was higher in Binasoybean-2 (13.4g) and lower hundred seed weight was obtained from SBM-26 (11.1g). Seed yield obtain from the mutants and checks significantly differs from each other. Mutant SBM-25 produced the highest seed yield of 2836 kg ha^{-1} followed by SBM-22 (2696 kg ha^{-1}). Among the locations the highest seed yield was obtained from BINA Sub-station farm Satkhira (2758 kg ha^{-1}) followed by the BINA sub-station farm at Barishal (2714 kg ha^{-1}).

Table 1. Mean performance of soybean mutants along with check varieties for yield and yield contributing characters

Locations	Mutants/ varieties	Days to maturity	Plant height (cm)	Branches $plant^{-1}$ (no.)	Pods $plant^{-1}$ (no.)	Seeds pod^{-1} (no.)	Pod length (cm)	100 seed weight (g)	Seed yield ($kgha^{-1}$)
BINA Sub-station, Noakhali	SBM-22	115a	52c	4.3b	55b	3.9b	2.3b	12.5c	2639b
	SBM-25	113b	62b	4.4a	64a	4.0a	2.6a	13.3a	2843a
	SBM-26	114b	73a	3.7c	46c	3.4d	2.2b	10.3e	2521d
	BINA-2	105c	25e	3.6d	44d	3.5c	2.2b	12.0d	2542c
	BINA-6	114b	47d	3.5e	47c	3.5c	2.0c	12.7b	2520d
BINA Sub-station, Barishal	SBM-22	111b	60c	3.3a	85a	4.0b	2.9a	12.5b	2781b
	SBM-25	115a	61c	3.3a	85a	4.4a	3.0a	13.7a	2874a
	SBM-26	114a	72a	3.0b	84a	3.2c	2.8b	10.5d	2537e
	BINA-2	112b	38d	2.0c	39c	3.0d	2.7b	12.0c	2668d
	BINA-6	111c	67b	2.2c	62b	3.1c	2.6c	12.1c	2710c
BINA Sub-station, Satkhira	SBM-22	112b	47c	3.6c	80b	3.9b	2.2b	12.8b	2806b
	SBM-25	112b	53b	4.4a	85a	4.0a	2.3a	13.0a	2940a
	SBM-26	114a	59a	3.3d	75c	3.2d	1.8d	11.2d	2547e
	BINA-2	111c	38d	3.3d	24d	3.5c	2.2b	12.4c	2715d
	BINA-6	113a	46c	3.8b	27d	3.5c	2.0c	12.2c	2783c
BINA Sub-station, Magura	SBM-22	112b	57b	4.3a	84a	4.2a	3.0a	12.9b	2574b
	SBM-25	115a	54c	3.2b	85a	4.3a	3.0a	13.5a	2637a
	SBM-26	114a	67a	2.9c	77b	3.6c	2.8c	12.3c	2492d
	BINA-2	110c	36d	2.5d	48d	3.7c	2.9b	12.8b	2510c
	BINA-6	112b	54c	3.1b	62c	4.0b	2.8c	12.1d	2550c
BINA Sub-station, Rangpur	SBM-22	110c	40c	2.3d	73b	4.1a	3.4b	12.9b	2681b
	SBM-25	113b	36d	2.6c	78a	4.2a	3.8a	13.9a	2887a
	SBM-26	115a	48b	2.0e	55e	3.3d	2.9d	11.2d	2537d
	BINA-2	108d	31e	3.0b	60d	4.0b	3.2c	12.1c	2668c
	BINA-6	112b	68a	3.4a	65c	3.8c	3.4b	12.3c	2669c
Combined mean over locations	SBM-22	112b	51b	3.6a	76a	3.9a	2.8a	13.2a	2696b
	SBM-25	113a	53b	3.5a	70b	3.8a	2.9a	13.4a	2836a
	SBM-26	114a	62a	3.0c	67c	2.9c	2.7b	11.1c	2527d
	BINA-2	109c	34c	2.9c	43e	3.6b	2.7b	12.5b	2620c
	BINA-6	112b	56b	3.2b	53d	3.7b	2.6c	12.4b	2646c
Location mean									
BINA Sub-station, Noakhali		112b	52b	3.9a	51c	3.7b	2.2c	12.2c	2613d
BINA Sub-station, Barishal		113a	60a	2.7c	71a	4.0a	2.9b	12.3c	2714b
BINA Sub-station, Satkhira		112b	49c	3.9a	51c	3.7b	2.1c	12.2c	2758a
BINA Sub-station, Magura		113a	54b	3.2b	70a	4.0a	2.9b	12.8a	2553e
BINA Sub-station, Rangpur		111c	45d	2.7c	66b	4.0a	3.3a	12.5b	2688c

N.B.: In a column, values with same letter do not differ significantly at 5% level. BINA-2 means Binasoybean-2 & BINA-6 means Binasoybean-6

From this experiment, it was observed that SBM-25 was the best performer among the mutants and checks (Table 1). Further trials will be needed to confirm the result.

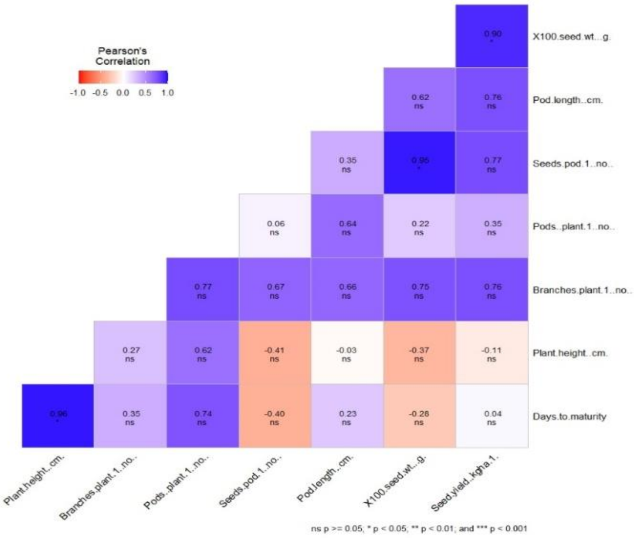

Fig. 1. The correlation coefficient of seven traits with significance levels in soybean mutants

From the correlation coefficient of seven traits with significance levels in soybean mutants (Fig. 1.), it was observed that plant height showed a significant positive correlation with days to maturity and a negative non-significant association with pod length (cm), 100 seed wt. (g) and Seed yield (kg ha^{-1}). Seeds pod^{-1} (no.) also showed a positive significant correlation with 100 seed wt. (g) and a non-significant association with pod length (cm) and Seed yield (kg ha^{-1}). 100 seed wt. (g) showed a positive significant correlation with seed yield (kg ha^{-1}). Seed yield (kg ha^{-1}) showed a significant correlation with 100 seed wt. (g).

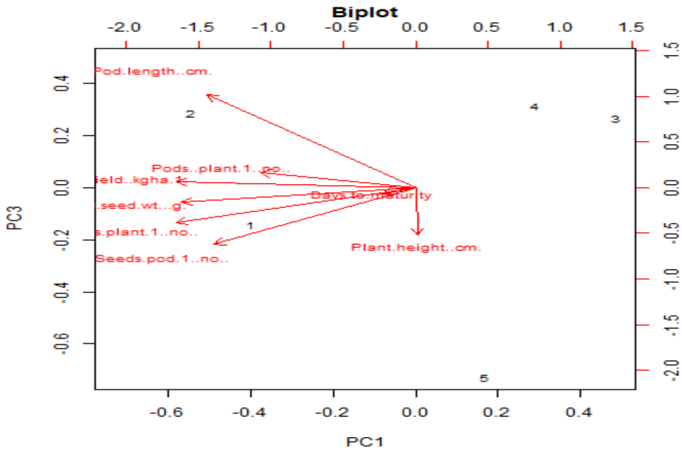

Fig. 2. Principal Components Analysis (PCA) ordination graph; PC1 vs PC2 biplot.

A biplot is a principal component that represents variables that are superimposed on a plot as vectors where the relative length of vectors represents the relative proportion of variability in each variable represented on the biplot (Fig. 2). If the angle between vectors of two traits is < 90° both are positively correlated whereas if the angle is > 90° there is a negative correlation and both vectors show no correlation if the angle is 90°. It is mostly used to determine the components where the effect is more to create the genotypic variation. The highest values indicate the highest influence of the trait on the total variation. Biplot analysis determines varietal stability in the multi-environmental trial. Plant height showed a significant positive correlation with days to maturity due to acute angle and a negative non-significant association with pod length (cm), 100 seed wt. (g) and Seed yield (kg ha^{-1}) due to obtuse angle. Seeds pod^{-1} (no.) also showed a positive significant correlation with 100 seed wt. (g) due to acute angle and a non-significant association with pod length (cm) and Seed yield (kg ha^{-1}). 100 seed wt. (g) showed a positive significant correlation with seed yield (kg ha^{-1}). Seed yield (kg ha^{-1}) showed a significant correlation with 100 seed wt. (g) due to acute angle.

Comparing with imposed salinity level and time, plant height as well as leaf was decreased. All the germinated genotypes were survived at 4 dS m^{-1} up to 21 days. The survival data are presented in Table 2. All the mutants showed moderately tolerant at 4 dS m^{-1} up to 7 days after sowing. Furthermore, Binasoybean-2, Binasoybean-6 and Lokon performed well with the advancement of time.

Table 2. Visual salt injury (survival) at seedling stage

Varieties/ mutant	7 days after seeding			14 days after seeding			21 days after seeding		
	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}
SBM-22	MT	NG	NG	MT	NG	NG	S	NG	NG
SBM-25	MT	NG	NG	MT	NG	NG	S	NG	NG
SBM-26	MT	NG	NG	MT	NG	NG	S	NG	NG
Binasoybean-2	HT	NG	NG	HT	NG	NG	MT	NG	NG
Binasoybean-6	HT	NG	NG	HT	NG	NG	MT	NG	NG
Lokon	HT	NG	NG	HT	NG	NG	HT	NG	NG

N.B.: HT= Highly tolerant, T= Tolerant, MT= Moderately tolerant, S= Susceptible, HS= highly susceptible and NG= Not germinated.

Total chlorophyll content was sharply decreased at saline condition (Fig. 3) compare to control. The decreasing rate was lower at Lokon than others indicating its salt tolerance potentiality. Total chlorophyll content was relatively higher for the mutants SBM-22 and lower for SBM-26. From the visual salt injury score and chlorophyll content it was concluded that the mutants SBM-25 and SBM-26 was susceptible for salinity whereas Binasoybean-2, Binasoybean-6 and Lokon performed well and could be selected for the parent of stress breeding program.

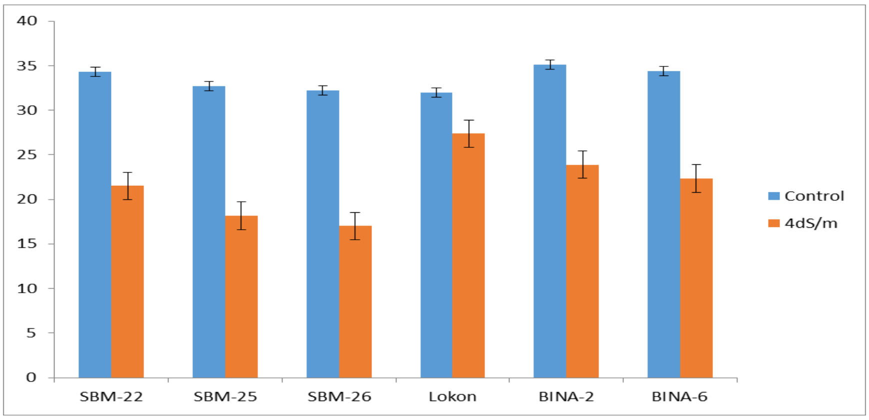

Fig. 3: Chlorophyll content of selected genotypes at saline (4 dS m^{-1}) and non-saline condition (Here, BINA-2 means Binasoybean-2 and BINA-6 means Binasoybean-6)

Hybridization is extensively used for creating variation. Emasculation is mandatory for self-pollinated crops, whereas for tiny type flowers like soybean it is too much laborious and time consuming. Mutation breeding is one of the best processes for creating variation in various crops including soybean (Ma *et al.*, 2021). Binasoybean-2 and Binasoybean-6 are two soybean varieties developed earlier used here as parent for irradiation due to the field performance in saline prone areas from previous different experiments. Environmental stresses including salinity, drought, waterlogging, toxic metals, extreme temperatures, etc. are nowadays the center of concern by the environmentalist and plant scientists worldwide as these are badly affecting food production (Hasanuzzaman *et al.*, 2022). Irradiation dosage variation can change the variability of important yield characteristics like as plant height, number of branches plant^{-1}, and number of pods plant^{-1} etc. (Hanafiah *et al.*, 2010). Salt stress is undoubtedly one of the worst conditions for plant growth as it creates both osmotic and ionic stress. Soybean is known to be partially sensitive to salt which may result in up to 40% yield loss depending upon salinity level. The presence of excess salt in the growing medium of soybean negatively affects the quality and quantity of seed, growth, and nodulation process (Khan *et al.*, 2019). Environmental adversities like salinity are making these tasks more challenging as it hampers soybean growth and productivity by inhibiting germination and other vital physiological processes. Soybean is known to be a moderately salt-sensitive crop, and therefore, to ensure the potential production of soybean we need to minimize the salt stress-induced damages in soybean (Hasanuzzaman *et al.*, 2016). The present investigation of M_6 generation selection procedure also supported by Shalini *et al.*, (2016) and Upadhyay *et al.*, (2019). The positive responses of mutation breeding for developing early maturity with higher yield salt tolerant soybean mutants was also found in Malek *et al.*, 2022; Nilahayati *et al.*, 2019 and Khan and Tyagi (2010) report from several experiments. We need to further trial in hydroponic culture solutions and also in the farmers filed for the appropriate evaluation. Based on agronomic performance studied mutant's overall performances indicated that these lines may use for advanced breeding trials.

In summary it was revealed that successfully deciphering of irradiated variations involving soybean to adapt to distinct environments will be able to help to break the bottle neck of soybean breeding. Mutation breeding has tremendous application for creating heritable genetic variability that alters the DNA structure of the plant species and able to generate unique traits. Based on the genetic variability and coefficient study it was found that plant height, maturity period and pods plant^{-1} was the most dominant yield contributing traits of soybean. Agronomic performances relation to yield and yield attributing characters SBM-25 and SBM-26 are the promising mutants. Furthermore, based on agronomic trait performance, studied mutants may use for high yielding soybean breeding program and further trials should be needed in saline prone areas.

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