RESEARCH ARTICLE Legume Research- An International Journal, Volume 46 Issue 9: 1162-1167 (September 2023)

Qualitative and Quantitative Traits Associate Genetic Variability of Soybean (*Glycine max*) Mutants for Expedited Varietal Improvement Program

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10.18805/LRF-735

ABSTRACT

Background: Soybean is an excellent source of protein, also richer in oil than most legumes, making them a good source for vegetable oil and biofuels. Among various difficulties the maturity period of existing soybean varieties is the main hindrance of utilizing this for the existing cropping system. The narrow genetic base of cultivated soybean varieties and germplasm limit the scope to utilize directly in the breeding program.

Methods: Mutation breeding is one of the techniques that provide large genetic diversity from a single source. To broaden the genetic diversity Binasoybean-3 and Binasoybean-4 were imposed to different doses of gamma radiation. The mutants were selected based on their agronomic performance and grouped at five different clusters at M_5 generations. Maximum selection pressure was done during maturity period with protein and oil content.

Result: Finally, eight mutants were selected for the advance breeding program, whereas mutants SM-03-15-5 mature within 90 days, containing 38% protein and 18.4% oil content will be considered directly for further steps of varietal release system.

Key words: Correlation coefficients, Mutation breeding, Oil content, Protein, Soybean, Trait selection.

INTRODUCTION

Sharply increasing trend of the world population with limiting cultivable land and changing climates creates continuous pressure on food security (Brivery *et al*., 2021). It provides additional pressure to ensure SDGs (Sustainable Development Goals) second goal (United Nation, 2015) whereas by the end of 2050 earth's population is predicted to reach 9.8 billion demands for major cereals will increase three-fold with doubling the requirements of animal-sourced food (ASF) (Ittersum *et al*., 2016). Considering various uses of soybean products, the projected demand for this grain will be 371.3 million metric tons in 2030 with a 1.8% annual growth rate (Siamabele, 2021). The development of higher yield potential soybean variety is the main focus for soybean breeders to ensure food security of this century (Kuchlan and Kuchlan, 2023). A narrow genetic base is the major threat to varietal improvements of soybean. Using pedigree relationships, Gizlice *et al*. (1994) showed that 35 ancestors contributed to 95% of genes of mostly cultivated soybean in North America. For crops with a limited genetic base like soybean, an efficient strategy is using mutation breeding that enhances the modification of a few traits without altering the remaining genotype. Mutation breeding techniques have been used widely to improve desirable traits like as seedcoat color (Tsuda *et al*., 2015), reduced Lipoxygenase (Lee *et al*., 2013) seed yield and oil quality (Lakhssassi *et al*., 2017) of soybean. Considering the remarkable success of the mutation technique in soybean trait development, reported research was conducted to focus mutation induced genetic variability for various genetic parameters of soybean and their utilization in the future varietal development process.

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How to cite this article: Alam, M.A., Bhuiyan, M.S.H., Malek, M.A., Emon, R.M., Khatun, K. and Kobir, H. (2023). Qualitative and Quantitative Traits Associate Genetic Variability of Soybean (*Glycine max*) Mutants for Expedited Varietal Improvement Program. Legume Research. 46(9): 1162-1167. doi: 10.18805/LRF-735.

Submitted: 03-01-2023 **Accepted:** 27-05-2023 **Online:** 13-06-2023

MATERIALS AND METHODS

Binasoybean-3 and Binasoybean-4 are two soybean varieties developed by the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh was subjected to 150 Gy, 250 Gy, 350 Gy and 450 Gy doses of gamma radiation in 2017. Continuous selection was done from M_2 to M_4

generation. Twenty-three genotypes have been selected from the M_4 generation, which was considered as material for this experiment (Table 1).

The experimental site, design and management

The experimental was conducted at BINA, Mymensingh following randomized complete block design with three replications. Sowing was done within the second week of January 2021 *Rabi* season. Spacing between plants in a row was 8-10 cm, whereas 25 cm for each row. Unit plot size was 16 m^2 (4 m×4 m). Recommended management were followed to ensure proper growth and development of plants.

Data analyses

From each experimental unit randomly selected 15 plants were considered as a sample. Data on various quantitative characters such as days to maturity (days), plant height (cm), number of branches $plant^{-1}$ (no.), pods $plant^{-1}$ (no.), pod length (cm), seeds pod^{-1} (no.) were taken from selected sample of each experimental unit. Genotypic and phenotypic variability with coefficient of genotypic and phenotypic variation, heritability and genetic progress was calculated using the formula developed by Steel *et al.* (1997). The heat maps were created using online heatmapper tool (www2.heatmapper.ca) whereas box plots were designed by RStudio. Protein content was determined by the formula of Beljkas *et al.* (2010) and oil content by Arnoid *et al.* (1944).

RESULTS AND DISCUSSION

Genotypic mean sum of square for days to maturity, plant height, pods $plant^{-1}$, thousand seed weight and seed yield showed highly significant ($P<0.01$) variations among the mutant and parents (Table 2). The CV range varied from 1.2 to 22.47. Maximum CV values were obtained from seed yield followed by thousand seed weight, whereas a minimum CV value was carried out by seeds pod^{-1} followed by branches $plant^{-1}$. Genotypic (σ^2g) and phenotypic variance (σ^2p) with phenotypic (PCV) and genotypic (GCV) coefficients of variation follow similar patterns.

The genotypic (σ^2g) variance ranges from 0.06 to 5956.18 and phenotypic (σ^2g) variance ranges from 0.18 to 6028.46. The maximum genotypic (σ^2g) and phenotypic variance (σ^2p) were obtained from seed yield followed by pods $plant^{-1}$ and days to maturity. The mode of expression of genotypic (GCV) and phenotypic (PCV) coefficients were like genotypic (σ^2g) and phenotypic variance (σ^2p) that indicate higher phenotypic variance and coefficients than genotypic variance and coefficients. Although the CV% was higher for the seed yield (kg/ha), it might be due to the diversity of the genotypes studied here. Another supremacy of mutation

Table 1: List of genotypes considered in this research selected from M_4 generations.

Name of the mutants	Parent	Dose (Gy)	Name of the mutants	Parent	Dose (Gy)
SB-03-15-27	Binasoybean-3	150	SB-04-15-21	Binasoybean-4	150
SB-03-15-67	Binasoybean-3	150	SB-04-15-4	Binasoybean-4	150
SB-03-15-37	Binasoybean-3	150	SB-04-20-9	Binasoybean-4	200
SB-03-15-5	Binasoybean-3	150	SB-04-20-21	Binasoybean-4	200
SGB-03-20-7	Binasoybean-3	200	SB-04-20-11	Binasoybean-4	200
SB-03-25-41	Binasoybean-3	250	SB-04-20-3	Binasoybean-4	200
SB-03-25-5	Binasoybean-3	250	SB-04-25-3	Binasoybean-4	250
SB-03-25-15	Binasoybean-3	250	SB-04-35-2	Binasoybean-4	350
SB-03-30-9	Binasoybean-3	300	Binasoybean-3	Wield Parent	-
SB-03-30-10	Binasoybean-3	300	Binasoybean-4	Wield Parent	-
SB-03-35-20	Binasoybean-3	350	BARI Soybean-5	Check Variety	-
SB-04-15-19	Binasoybean-4	150	Total		23

Table 2: Estimates of genetics parameters for different quantitative and qualitative characters.

Trait	GMS	CV (%)	σ^2g	σ^2p	(GCV)	(PCV)	h^2b	GA	GA (%)
Days to maturity	458.53	7.51	145.89	166.75	10.47	11.20	87.49	23.27	20.18
Plant height	378.18	9.18	115.68	146.80	17.87	20.13	78.80	19.66	32.68
Branch/plant	1.24	2.4	0.42	0.41	26.43	26.29	78.58	1.32	14.45
Pods/plant	439.79	3.4	146.17	146.71	23.13	23.48	79.84	14.93	34.64
Seeds/pod	0.54	1.2	0.06	0.18	17.79	17.11	82.32	4.4	15.26
Pod length	0.71	8.9	0.14	0.89	10.46	17.72	34.90	6.53	12.74
Thousand seed wt.	175.09	13.95	34.39	86.30	6.77	11.90	32.35	6.12	7.49
Seed yield (kg/ha)	79353.21	22.47	5956.18	6028.46	25.46	25.75	67.54	48.16	52.76
Oil (%)	11.49	3.37	2.43	6.62	8.64	14.26	38.34	3.94	10.79
Protein (%)	1.25	6.54	1.25	6.13	3.06	7.00	19.78	1.67	2.76

breeding is its ability to crate narrow genetic difference with higher heritability, that was reflected in this study.

The genotypic coefficient of variation ranged from 6.77 to 26.29 which indicates considerable variation among the character studied. Out of eight traits higher genotypic coefficient of variation was observed on branches plant^{-1}, seed yields and pods per plant (26.29, 25.46 and 23.13, respectively). Highest PCV (28.48) were shown for pods plant^{-1} followed by branches plant^{-1} (26.43) and seed yield (25.75). The difference between GCV and PCV was higher for pod length, thousand seed weight and pods plant^{-1}. On the others the lowest difference was performed by the trait's days to maturity and plant height, whereas it was minimum for branches plant^{-1}, seeds pod^{-1} and seed yield (Table 2). Estimates ranged of broad sense heritability were 32.35 to 87.49. More than 80% heritability was obtained from days to maturity, branches plant^{-1}, pods plant^{-1}, seeds pod^{-1} and it was above 60% for plant height and seed yield. Heritability combined with genetic advance guided more accurate selection efficiency than heritability alone. Genetic advance was maximum for seed yield (48.16) whereas the maximum genetic advanced over mean was also higher for seed yield followed by pods plant^{-1}, plant height and lowest for thousand seed weight.

The genotypic correlations were higher than phenotypic correlations with some exceptions (Table 3). For the number of branches plant^{-1} phenotypic correlations were higher with number of pods plant^{-1} (0.61), seeds pod^{-1} (0.43) and pod length (0.80). Plant height has a significant positive relation with number of pods plant^{-1} and negative relation with pod length and thousand seed weight for both genotypic and phenotypic correlations with higher genotypic values. A total number of pods plant^{-1} has a strong relation with branches plant^{-1}, plant height and reveres relation with thousand seed weight. The yield had a highly significant positive correlation with number of pods plant^{-1}, seeds pod^{-1} and thousand seed weight. In case of yield maximum genotypic correlations were obtained from number of pods plant^{-1} (0.87), thousand seed weight (0.46) followed by number of seeds pod^{-1}.

Selection of superior genotypes

The first three traits accounted eigen values were more than one and it was maximum for days to maturity (2.595) and minimum eigen values was obtained from seed yield (0.255, Table 4). Similarly, days to maturity contributed 32.4% of the total variation followed by plant height (19.5). The lowest percentage of variation was obtained from seed yield (3.2%, Table 4).

Considering the performance eight traits of studied soybean genotypes were grouped into five clusters (Table 5 and Fig 1). The number of genotypes ranged from 2 to 10 in

Table 3: Correlation coefficients of pair's genetic parameters for yield associated quantitative characters of soybean.

Traits		PH	NBP	NPP	NSP	PL	TW	Yield
DM	r_g	0.22	-0.41	0.12	-0.41*	0.05	-0.04	0.09
	r_p	0.18	-0.38**	0.39**	-0.11	0.43	0.06	0.08
PH	r_g		-0.24	0.67**	0.30	-0.43*	-0.58**	-0.33
	r_p		-0.21	0.59**	0.27*	-0.21	-0.32**	-0.29*
NBP	r_g			0.41*	0.14	0.01	0.18	-0.14
	r_p			0.61**	0.43	0.96	0.10	-0.14
NPP	r_g				0.20**	-0.15	-0.14*	-0.07
	r_p				0.02	-0.09	-0.08*	-0.01
NSP	r_g					0.55**	0.82**	0.20*
	r_p					0.32**	0.47**	0.73**
PL	r_g						0.80**	0.24
	r_p						0.58**	0.14
TW	r_g							0.46*
	r_p							0.27*

**Significant at P≤0.01 and *Significant at P≤0.05.

Table 4: Eigen values, per cent of variation and total variation contribution of 8 characters of 23 genotypes of soybean.

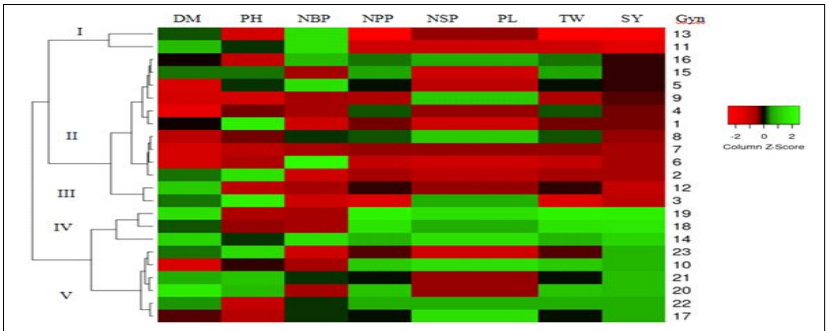
Principal component characters	Eigen values	Percent of total variation	Cumulative variation
Days to maturity	2.595	32.4	32.4
Plant height (cm)	1.560	19.5	52.0
Branch/plant	1.118	14.0	65.9
pods/plant	0.956	12.0	77.9
Seeds/pod	0.567	7.1	85.0
Pod length	0.553	6.9	91.9
Thousand seed wt	0.392	4.9	96.8
Seed yield (kg/ha)	0.255	3.2	100.0

different clusters. The distribution pattern indicated that the maximum number of test genotype 10 was grouped into cluster II (43.48%), 6 genotypes in cluster V (26.08%) and V (6) followed by 3 genotypes in cluster IV (13.04%). Cluster I and cluster III contained the lowest (2) number of genotypes and that was 8.70% of the total population. Seed yield and maturity period were a main focus for the present circumstance of Bangladesh. Based on yield (about 3000 kg ha^{-1}) and maturity period (around 100 days), eight genotypes were selected. SB-04-15-4 (3380 kg ha^{-1}) ensures maximum seed yield, whereas SB-03-15-5 (93 days) matures earlier than others (Fig 2).

Varietal response against radiation was not uniform because that mutation can cause genomic instability in cells by altering the number of copies of the genome, amplification, rearrangement and deletion of genes (Morgan *et al*., 2003). Mutants obtained from different parents with different does place in one group. In group II, there were ten genotypes; out of them, eight were obtained from Binasoybean-3 and two from Binasoybean-4. Two parents and check variety BARI soybean-5 was in the same group (V) indicating they originated from the similar genetic background. Presence of the other three mutants in group V (SB-04-20-11, SB-04-35-2 and SB-03-30-10) indicates the narrow genetic distance with the existing soybean variety of Bangladesh. Continuous selection pressure from existing germplasm is responsible for narrowing the genetic background of existing soybean cultivars. Considering the pattern-based cropping system, soybean breeder needs to consider maximum yield with a minimum maturity period. Soybean mutants SB-03-15-5, SB-03-15-7, SB-03-25-5, SB-03-25-15, SB-03-30-10, SB-04-15-19, SB-04-15-4 and SB-04-20-3 provide maximum yield and the mature around 100 days.

Table 5: List of distributed genotypes in different clusters with their percentage over total genotypes.

Cluster number	Number of genotypes	Percentage of genotype	Name of genotype
I	2	8.70	SB-03-35-20, SB-04-15-21
II	10	43.48	SB-03-15-27, SB-03-15-67, SB-03-15-5, SB-03-20-7, SB-03-25-41, SB-03-25-5, SB-03-25-15, SB-03-30-9, SB-04-20-9, SB-04-20-21
III	2	8.70	SB-03-15-37, SB-04-15-19
IV	3	13.04	SB-04-15-4, SB-04-20-3, SB-04-25-3
V	6	26.08	SB-04-20-11, SB-04-35-2, SB-03-30-10, Binasoybean-3, Binasoybean-4, BARI Soybean-5



The red and blues colors in the legend indicate highest and lowest values, respectively. DM=Days to maturity, PH=Plant height (cm), NPB=Number of primary branches plant^{-1}, NP=Number of pods plant^{-1}, NSP=Number of seed pod^{-1}, TW=Thousand seed weight (g) and SY= Seed yield.

Fig 1: Heatmap representations of 23 soybean genotypes into different clusters.

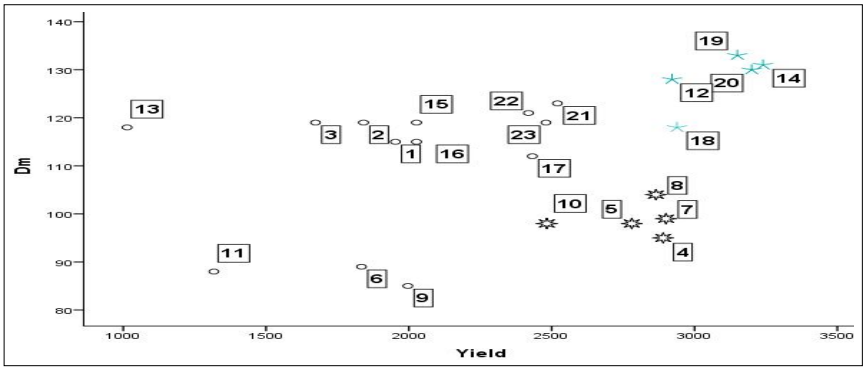
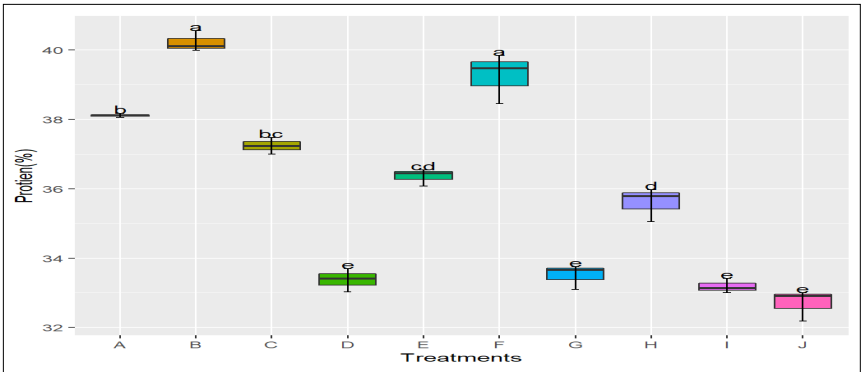
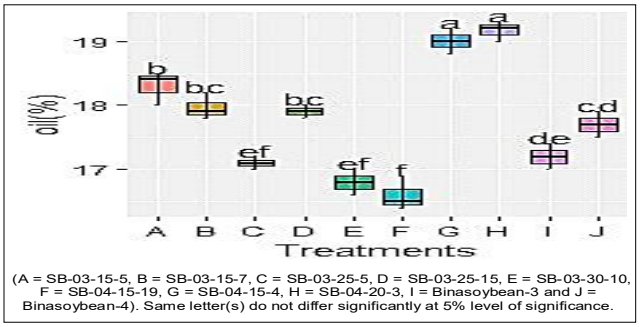


Fig 2: Performance of 23 soybean genotypes considering seed yields and maturity period.



(A = SB-03-15-5, B = SB-03-15-7, C = SB-03-25-5, D = SB-03-25-15, E = SB-03-30-10, F = SB-04-15-19, G = SB-04-15-4, H = SB-04-20-3, I = Binasoybean-3 and J = Binasoybean-4). Same letter(s) do not differ significantly at 5% level of significance.

Fig 3: Protein content (%) of selected soybean mutant with their parents.



(A = SB-03-15-5, B = SB-03-15-7, C = SB-03-25-5, D = SB-03-25-15, E = SB-03-30-10, F = SB-04-15-19, G = SB-04-15-4, H = SB-04-20-3, I = Binasoybean-3 and J = Binasoybean-4). Same letter(s) do not differ significantly at 5% level of significance.

Fig 4: Oil content (%) of selected soybean mutant with their parents.

The protein and oil content of these eight genotypes were selected based on yield potential and maturity period with two parents (SB-03-15-5, SB-03-15-7, SB-03-25-5, SB-03-25-15, SB-03-30-10, SB-04-15-19, SB-04-15-4, SB-04-20-3, Binasoybean-3 and Binasoybean-4) showed significant different (Fig 3 and 4). Maximum protein content was obtained from SB-03-15-7 (40%) which was statistically identical with SB-04-15-19 (39.2%) followed by SB-03-15-5 (38%), on the others lowest protein content was obtained from the parent Binasoybean-4 (32.8%). Accordingly maximum oil content was obtained from SB-04-15-4 (19%) and SB-04-20-3 (19.2%) followed by SB-03-15-5 (18.4%).

The success of mutation breeding for developing early maturity with higher yield soybean mutants was supported by Malek *et al.* (2022) and Nilahayati *et al.* (2019). The positive responses of mutation breeding regarding maturity were also found in sesame (Bhuiyan *et al*., 2019), lentil (Laskar *et al*., 2017) and in linseed (Terfa *et al*., 2020) that support the effectiveness of mutation breeding towards specific trait improve. KEX-2 and Bangsakong are the two mutant soybean varieties developed by Korea Atomic Energy Research Institute that ensures 30% and higher, whereas Josaengseori (mutant of soybean) led to 2.4 times higher seed yield than their parent. Among this KEX-2 also matured 11 days earlier than the original cultivar (Ha *et al*., 2014). Both protein and oil content were higher at the mutant SB-03-15-5, moderate at SB-03-15-7 and higher protein and low oil content at SB-04-15-19. Generally, there was a negative relation between oil and protein content that was contradictory to this result but fully support by Deswal *et al.* (2015), where they found mutation increased protein and oil content of the same genotypes. This is the beauty of mutation, which has a tremendous ability to change alleles without affecting the linked gene (Yao *et al*., 2023). Patil *et al.* (2017) finds out that *Glycine max* is the main source of various high-protein alleles. Two parents of this study were also from *Glycine max.* Prenger *et al.* (2019) mention that deletion on chromosome 12 was associated with increased protein content using mutation on two soybean genotypes (G00-3213 and G00-3880) and their work fully supports our findings.

CONCLUSION

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. Based on agronomic trait, performance studied genotypes were grouped into five clusters. Considering overall performance and protein contain SB-03-15-5, SB-03-15-7, SB-03-25-5, SB-03-25-15, SB-03-30-10, SB-04-15-19, SB-04-15-4 and SB-04-20-3 have been selected for future advancement. Among them SB-03-15-5, SB-03-25-5 and SB-03-25-15 will be used for advanced breeding trials, whereas SB-04-15-19, SB-04-15-4, SB-04-25-3 and SB-04-35-2 will be used as parent material for varietal improvement program.

ACKNOWLEDGEMENT

The authors sincerely acknowledge Bangladesh Institute of Nuclear Agriculture (BINA) and the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah for all the supports for conducting the study and publishing the research findings.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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