Journal of Plant Molecular Breeding (JPMB)
Vol.1/No.1/ December 2012/ 10-15

Improvement of qualitative and quantitative traits in soybean [*Glycine Max* (L.) Merrill] through gamma irradiation

M. Arefrad[1], G. Nematzadeh[2,*], N. Babaian Jelodar[2] and S.K. Kazemitabar[2]

1. Genetics and Agricultural Biotechnology Institute of Taberestan (GABIT) Sari, Iran. P.O.Box: 578, Tel: +98 151 382578
2. Department of Agronomy and Plant Breeding, Agricultural Sciences and Natural Resources University of Sari. Iran.

*corresponding author: Gh.nematzadeh@sanru.ac.ir

Received: November 2011 Accepted: May 2012

Abstract

Gamma irradiation was used at different doses (80, 160 and 240 Gy) on *Glycine Max* (L.) Merrill cv. Hill homogenous seeds. A single suitable M_2 plant was selected and evaluated at M_3 and M_4 along with its parent and three other varieties as control in RCBD experiment in Sari Agricultural Sciences and Natural Resources University's experimental field. M-80-709 and M-160-3429 mutant lines were significantly shorter (55.05 and 72.04 cm respectively). Their branch numbers were however significantly more (8.70 and 11.53 respectively) compared to the parent cultivar (80.82 cm and 6.10 respectively) and other genotypes ($p \leq 0.05$), when calculated on per plant basis. Besides, the M-160-3429 was characterized by highest grain yield and oil content (38.25 g and 19.22% respectively), in compare with its parent (12.73 g and 19.09% respectively) and all others control cultivars ($p \leq 0.05$). The M-160-3429 mutant line with high grain yield and oil content accompanied with some other favorite morphological traits can be considered as a new promising line of soybean for future studies. Results from this study suggested that mutation breeding procedures at the ranges of 80 to 160 Gy is a capable method for breeding higher grain yield including increasing the oil content as well.

Keywords: Soybean, Mutation breeding, Seed yield, Oil content, Protein content.

Introduction

Soybean is one of the most important oilseed crop plants worldwide. A soybean seed have high nutritional value and is rich in proteins and oil with an average of 40% protein and 20% oil respectively. Considering enormous annual soybean imported by Iran in different forms of whole grain, crashed grain, crude and purified edible oil, increasing soybean production is of paramount significance for achieving the goals of self sufficiency and food security of the country. Development of new genotypes of soybean with high grain yield will therefore be important. So far, different breeding approaches have been employed to make some genetic improvements to meet the industrial and agricultural requirements.

Mutation breeding using Gamma irradiation is an effective procedure in plant breeding which can be widely used to induce genetic recombination. Till now more than 2600 mutant varieties, with desirable quantitative and qualitative traits have been introduced (Hajos-Novak 2009). Katoh *et al.,* (1992; 1993; 1994; 1995); Byun *et al.,* (1993); Khan and Tyagi (2010) and Pavadai *et al.,* (2010) obtained favorite traits using a number of chemical and physical mutagens in soybean. Likewise Sagel *et al.,* (1995) registered a mutant variety of soybean with high oil (25.5%) and high protein content (39.2%). Hajos-Novak and Hodos (2003) also released a mutant high yielding soybean variety.

The aim of this study was to obtain promising lines of soybean with favorite quantitative and qualitative characteristics through Gamma irradiation.

Materials and Methods

The seeds of Hill variety were obtained from Mazandaran Agricultural and Natural Resources Research Center, and were irradiated with Gamma (Co^{60}) doses of 80, 160 and 240 Gy at the Nuclear Agriculture Center affiliated to Iran Atomic Energy Agency. The irradiated seeds along with the parent cultivar were grown in the experimental field of Genetics and Agriculture Biotechnology Institute of Tabarestan (GABIT). All the M_1 plants were harvested individually and carried forward to M_2 generation as plant-to-row progenies in the following year (2008). Up to 3000 M_2 plants were screened for favorite agronomic traits including plant height, branching, grain yield and plant lodging. Among these M_2 plants, two mutant lines (M-80-709 and M-160-3429) were selected and were evaluated along with the original parent genotype and three other cultivars as control (Sahar, 032 and 033). RCBD with three replicates was used at two consecutive years (2009-2010) in this experiment. Each plot included five rows. The distance between rows and between plants was 50 and 10 cm respectively. After complete growth and elimination of marginal effect, ten plants were randomly selected in each plot and were evaluated for agronomic traits; day to maturity, plant height and number of branches, 1000-grain weight, grain yield, protein content and oil content. In addition, three interior rows from each plot were used to estimate grain and oil yield per unit area.

Oil content was estimated using a Soxhlet apparatus (AACC, 1976) and nitrogen content by Kjeldahl method (AOAC, 1984). The amount of total protein was calculated from percent of nitrogen content using a conversion factor of 6.25. The data were analyzed statistically according to Gomez and Gomez (1984) method and the mean values were compared by DMR test at %5 level of significance.

Results and Discussion

Quantitative traits

The two mutant lines (M-80-709 and M-160-3429) didn't show any differences in day to

Table 1. Characterization of qualitative and quantitative traits in four cultivars and two promising mutants in 2009.

Genotype	Days to maturity	Plant height (cm)	Number of Branches	Grain yield/plant (g)	1000 grain weight (g)	Grain yield (kg ha^{-1})	Protein content (%)	Oil content (%)	Oil yield (kg ha^{-1})
Hill	122.00^{c}	80.40^{bc}	5.30^{c}	12.13^{e}	127.43^{c}	2421.33^{e}	39.10^{ab}	19.08^{ab}	471.33^{e}
Sahar	116.00^{d}	75.00^{bc}	5.10^{c}	21.50^{d}	137.90^{b}	4227.20^{d}	39.11^{ab}	19.09^{ab}	811.35^{d}
032	133.00^{a}	89.32^{a}	7.20^{b}	30.11^{c}	143.72^{a}	6050.13^{c}	39.92^{a}	18.90^{ab}	1140.50^{c}
033	130.00^{b}	89.90^{a}	5.00^{c}	32.51^{b}	144.50^{a}	6487.47^{b}	40.05^{a}	18.98^{ab}	1228.10^{b}
M-80-709	122.00^{c}	55.00^{c}	8.20^{b}	32.88^{b}	126.81^{c}	6585.51^{b}	38.50^{b}	19.10^{ab}	1260.37^{b}
M-160-3429	122.00^{c}	70.71^{c}	11.20^{a}	38.15^{a}	122.20^{d}	7593.08^{a}	38.41^{b}	19.21^{a}	1399.33^{a}

Means followed by the same letters are not significantly different from each other at 0.05%.

maturity (122 day) compared to the parent genotype, but had significant difference ($P\leq0.05$) with others control varieties (Table 1). The mutant line M-80-709 was the shortest line flowed by M-160-3429 (55.00 and 70.71 cm) respectively (Table 1). The maximum branch numbers was obtained from line M-160-3429 followed by the line M-80-709 and cultivar 032 (11.20, 8.20 and 7.20 respectively) (Table 1). Cultivars 033 and 032 showed highest 1000-grain weight (144.50 and 143.72 g respectively), and the line M-160-3429 mutant showed the lowest (122.20 g) (Table 1). The same results also were also obtained in the following year 2010 (Table 2) and the pooled data over the two years (Table 3).

Our primary aim was to select semi dwarf plants with other suitable characteristics. There are several reports for development of short plant height through mutation breeding (Larik, 1975; Shah *et al.,* 1990). As is shown in Table 1, increased branch number and reduced plant height were correlated with grain yield increase in mutant lines M-80-709 and M-160-3429 (32.88 and 38.15 g respectively) compared to the yield increase than the parent genotype when calculated on per plant basis ($p \leq 0.05$). These data were constant with the data obtained in 2010 (34.87 and 38.36 g respectively) (Table 2), and the pooled data over the two years (33.87 and 38.25 g respectively) (Table 3). Igbal *et al.,* (2010) also reported that increasing the branch numbers in soybean, results in the increased grain yield. The mutant M-160-3429 had significantly ($p\leq0.05$) higher grain yield per unit area than its parent and the other control varieties (7593.08 kg ha^{-1}) in 2009, (Table 1), 2010 (7658.81 kg ha^{-1}) (Table 2) and pooled data over the two years (7625.94 kg ha^{-1}).

In the other words, the grain yield of the mutant was about 15% more compared to the best adopted variety (033) (Table 3).

Qualitative traits

Among all evaluated genotypes in this study, the highest protein content was obtained from cultivars 033 and 032 (40.05 and 39.92% respectively). Both mutant lines had less protein (38.41 and 38.50% respectively) (Table 1). The oil content of M-160-3429 mutant (19.21%) however, significantly more than that obtained from its parent (19.08%) and the other cultivars (Table 1). Although the mutant line H-80-3429 mutant did not have significantly higher oil content than the other genotypes, it still produced more oil per unit area due to increased grain yield (1277.83 kg ha-1) (Table 3). However, the

Table 2. Characterization of qualitative and quantitative traits in four cultivars and two promising mutants in 2010.

Genotype	Days to maturity	Plant height (cm)	Number of Branches	Grain yield/plant (g)	1000 grain weight (g)	Grain yield (kg ha^{-1})	Protein content (%)	Oil conte nt (%)	Oil yield (kg ha^{-1})
Hill	137.00^{c}	81.24^{bc}	6.90^{c}	13.33^{d}	126.83^{c}	2566.50^{e}	38.52^{ab}	19.11^{ab}	486.69^{e}
Sahar	132.00^{d}	77.15^{bc}	5.70^{d}	22.60^{c}	137.27^{b}	4502.44^{d}	38.55^{ab}	19.02^{ab}	855.34^{d}
032	147.00^{a}	91.70^{a}	8.70^{b}	31.79^{bc}	142.60^{a}	6318.24^{c}	39.77^{a}	18.93^{ab}	1190.89^{c}
033	143.00^{b}	90.22^{a}	6.20^{c}	33.78^{b}	143.50^{a}	6699.20^{b}	39.78^{a}	18.78^{ab}	1250.27^{b}
M-80-709	137.00^{c}	55.11^{d}	9.20^{b}	34.87^{b}	125.40^{c}	6784.09^{b}	38.14^{b}	19.12^{ab}	1295.30^{b}
M-160-3429	137.00^{c}	73.37^{c}	11.50^{a}	38.36^{a}	119.80^{d}	7658.81^{a}	38.07^{b}	19.23^{a}	1475.09^{a}

Means followed by the same letters are not significantly different from each other at 0.05%.

Table 3. Pooled of qualitative and quantitative traits in four cultivars and two promising mutants in 2009 - 2010.

Genotype	Days to maturity	Plant height (cm)	Number of Branches	Grain yield/plant (g)	1000 grain weight (g)	Grain yield (kg ha^{-1})	Protein content (%)	Oil content (%)	Oil yield (kg ha^{-1})
Hill	129.50^{c}	80.82^{bc}	6.10^{c}	12.73^{d}	127.14^{c}	2493.91^{e}	38.82^{ab}	19.09^{ab}	479.01^{e}
Sahar	124.00^{d}	76.07^{bc}	5.40^{c}	22.05^{c}	137.58^{b}	4364.82^{d}	38.83^{ab}	19.05^{ab}	833.34^{d}
032	140.00^{a}	89.01^{a}	7.95^{b}	31.04^{bc}	143.16^{a}	6184.18^{c}	39.84^{a}	18.91^{ab}	1165.69^{c}
033	136.50^{a}	90.06^{a}	5.60^{c}	33.14^{b}	144.00^{a}	6595.33^{b}	39.91^{a}	18.88^{ab}	1239.18^{b}
M-80-709	129.50^{c}	55.05^{d}	8.70^{b}	33.87^{b}	126.10^{c}	6648.80^{b}	38.32^{b}	19.11^{ab}	1277.83^{b}
M-160-3429	129.50^{c}	72.04^{c}	11.35^{a}	38.25^{a}	121.00^{d}	7625.94^{a}	38.24^{b}	19.22^{a}	1437.21^{a}

Means followed by the same letters are not significantly different from each other at 0.05%, based on DMS test

Table 4. Correlation coefficient of characters among 7 lines of soybean.

Traits	Plant height (cm)	Number of Branches	Grain yield/plant (g)	1000 grain weight (g)	Protein content (%)	Oil content (%)
Days to maturity	0.60^{**}	-0.09^{ns}	0.33^{**}	0.55^{**}	0.60^{**}	-0.51^{**}
Plant height (cm)		-0.38^{**}	-0.20^{*}	0.71^{**}	0.68^{**}	-0.50^{**}
Number of Branches			0.58^{**}	-0.55^{**}	-0.42^{**}	0.39^{**}
Grain yield/plant (g)				-0.36^{**}	0.09^{ns}	0.45^{ns}
1000 grain weight (g)					0.71^{**}	-0.67^{**}
Protein content (%)						-0.51^{**}

*, ** and ns, indicate significant, highly significant and non significant at 5% level respectively.

M-160-3429 mutant had the highest grain yield ($p \leq 0.05$) (38.25 g) and oil content (19.22%) compared to all other tested cultivars when calculated on per plant basis (Table 3).

Correlation studies

The correlation coefficient is a proper index to determinate the relationship between the grain yield and the other traits. The grain yield showed positive correlation with day to maturity (0.33^{**}) and number of branches (0.58^{**}) and negative correlation with plant height (-0.20^{*}) and 1000-grain weight (-0.36^{**}) (Table 4). These results are in agreement with those reported by Liu *et al.,* (2005) and Bangar *et al.,* (2003). Furthermore, Faisal *et al.,* (2007) and Igbal *et al.,* (2010) observed significantly positive correlation between grain yield and day to maturity and number of branches per plant. Despite the high grain yield of mutants M-160-3429 and M-80-709, these two lines had less 1000-grain weight compared to its parent and the other cultivars. Jyoti and Tyagi (2005) and Basavaraja *et al.,* (2005) however, reported the significantly positive correlation for grain yield and 1000-grain weight. The mutant M-160-3429 with higher grain yield had smaller seed size, but the mutant M-80-709 with less grain yield had larger seed size (Table 3).

The oil content is also the main breeding objective of oilseed crops improvement. It showed significantly negative correlation with day to maturity (-0.51**), plant height (-.050**), 1000-grain weight (-0.67**) and protein content (-0.51**), but positive correlation with number of branches (0.39**) and higher grain yield (0.45) was obtained (Table 4) in our study. Igbal *et al.,* (2010) also reported that the oil content was negatively correlated with day to maturity and plant height, but was positively correlated with grain yield per plant. Panthee *et al.,* (2005) also found a negative correlation between oil content from one hand and protein content and grain weight on the other hand in soybean.

The correlation of oil content with others evaluated traits revealed that the mutation is a capable method for breeding higher grain yield including increasing the oil content as well.

Conclusion

We conclude that the mutant line M-160-3429 is a promising line with the objective of commercialization for its higher yield as well as oil content. We also conclude that the Gamma ray irradiation at the ranges of 80 to 160 Gy is effective for generating genetic variation and selection for new high grain yield and oil content for soybean breeding programs.

Acknowledgment

The authors are highly appreciated the GABIT staffs and financial support including lab and preparing facilitates for conducting this research.

References

AACC. 1976. Approved Methods: American Association of Cereal Chemists. MN USA: St Paul.

AOAC. 1984. Official Methods of Analysis: Association of Official Agricultural Chemistry. Washington DC.

Bangar, N.D., Mukhekar, G.D., Lad, D.B. and Mukhekar, D.G. 2003. Genetic variability, correlation and regression studies in soybean. J Maharashtra Agric Uni, 28: 320-321.

Basavaraja, G.T., Naidu, G.K. and Salimath, P.M. 2005. Evaluation of vegetable soybean genotypes for yield and component traits. Karnataka J Agric Sci, 18: 27-31.

Byun, M.W., Kwon, J.H., and Mori, T. 1993. Improvement of physical properties of soybeans by gamma irradiation. Radiat Phys Chem, 42: 313-317.

Faisal, M.A.M., Ashraf, M., Qureshi, A. and Ghafoor, A. 2007. Assessment of genetic variability, correlation and path analysis for yield and its components in soybean. Pakistan J Bot, 39: 405-413.

Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedures for Agricultural Research. 2nd Edition. John Wiley and Sons USA.

Hajos-Novak, M. 2009. Results of mutation induction in corn, pea and soybean at the department of genetics and plant breeding between 1958 and 2008. Bull. Szent Istvan Uni. pp. 50-57. Godollo, Hungry.

Hajos-Novak, M. and Hodos, G.K. 2003. Soybean breeding for earliness and seed quality by induced mutations. International Atomic Energy Agency. Plant Breeding and Genetics Section. Improvement of new and traditional industrial crop by induced mutations and related biotechnology. (pp. 95-105). IAEA VIENNA 2003 IAEA-TECDOC-1369.

Iqbal, Z., Arshad, M., Ashraf, M., Naeem, R., Malik, M.F. and Waheed, A. 2010. Genetic divergence and correlation studies of soybean [*Glycine Max* (L.) Merrill.] Genotypes. Pakistan J Bot, 42: 971-976.

Jyoti, S. and Tyagi, S.D. 2005. Direct and indirect contribution of different traits towards maximization of grain. Adv Plant Sci, 18: 331-335.

Katoh, Y., Maekawa, M. and Sano, Y. 1992. Effects of 2-amino-3-methylimidazo [4,5-f] quinoline (IQ) on somatic mutation in soybean test system. Mutation Res, 279: 239-243.

Katoh, Y., Maekawa, M. and Sano, Y. 1993. Effects of 5-azacytidine on somatic mutation in soybean test system. Mutation Res, 300: 49-55.

Katoh, Y., Maekawa, M. and Sano, Y. 1994. Mutagenic effects of nitropyrenes in a soybean test system. Mutation Res, 320: 59-68.

Katoh, Y., Maekawa, M. and Sano, Y. 1995. Mutagenicity of O-diazoacetyl-L-serine (azaserine) and 6-diazo-5-oxo-L-notleucine (DON) in a soybean test system. Mutation Res, 342: 37-41.

Khan, M.H. and Tyagi, S.D. 2010. Induced polygenic variability in soybean (*Glycine max* L. Merrill). Res J Agric Sci, 1: 93-101.

Larik, A.S. 1975. Induced mutations in quantitative characters of *Triticum aestivum*. Genet Agraria, 24: 241-250.

Liu, X., Jian, J., Herbert, S.J., Zhang, Q. and Wang, G. 2005. Yield components, dry matter, LAI and LAD of soybeans in Northeast China. Field Crops Res, 93: 85-93.

Pavadai, P., Girija, M. and Dhanavel, D. 2010. Effect of Gamma rays on some yield parameters and protein content of soybean in M_2, M_3 and M_4 generation. J . Exp Sci, 1: 8-11.

Panthee, D.R., Pantalone, V.R., West, D.R., Saxton, A.M. and Sams, C.E. 2005. Quantitative trait loci for seed protein and oil concentration, and seed size in soybean. Crop Sci, 45: 2015-2022.

Sagel, Z., Atila, A.S. and Tutluer, M.I. 1995. Characteristics of improved mutant varieties in soybean (*Glycine max* (L.) Merill). FAO/IAEA Int Symp Use of Induced Mutations and Molecular Techniques for Crop Improvement. (pp. 160-164) IAEA Vienna.

Shah, S.A., Ali, I. and Rahman, K. 1990. Induction and selection of superior genetic variables of oilseed rape (*Brassica napus* L.). The Nucleus, 27: 37-40.