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Radiation induced variability of seed storage proteins in soybean [*Glycine max* (L.) Merrill]

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Abstract

Soybean variety VLSoy-2 was irradiated with 250 Gy gamma rays to induce variability. A large number of mutants affecting morphological characters were identified and characterized. True breeding mutants obtained were used for studying the variation in seed storage proteins. The mutants M-231, M-17 and M-291 lacked the A_3 subunit of glycinin (11S) protein. Among the three, two mutants M-231 and M-17 were also characterized by the lack of α and α′-subunits of β-conglycinin (7S). In addition, the mutant M-291 also showed low levels of trypsin inhibitor activity (TIA) and low levels of α and α′-subunits of 7S protein.

Keywords: γ-Ray; Glycine max; Glycinin; β-Conglycinin; Mutant; Soybean; Trypsin inhibitor

1. Introduction

Soybean is a protein rich oilseed crop, which is presently the leading edible oil source through out the world. Though soy protein ranks high compared to other vegetable proteins, it is still of poor nutritional quality compared to animal proteins. Sulphur containing amino acids like cysteine and methionine are the most nutritionally limiting amino acids in soybean protein. Soybean protein mainly consists of globulins (Osborne & Campbell, 1898) made up of two sub-groups known as glycinin and β-conglycinin. Danielsson (1949) demonstrated that the glycinin and β-conglycinin had sedimentation coefficients of about 11S and 7S, respectively. They are multi-subunit proteins and account for approximately 70% of total storage proteins (Hill & Breidenbach, 1974). Glycinin is a hexameric molecule with a molecular weight of about 360,000 Da. Each subunit consists of an acidic and a basic polypeptide connected by disulphide linkages (Staswick, Hermandian, & Nielsen, 1981). On the other hand, β-conglycinin (molecular weight 180,000 Da) is composed of α, α′ and β-subunits (Shattuck-Eidens & Beachy, 1985) and lacks disulphide linkages. Glycinin contains 3–4 times more sulphur containing amino acids than that of β-conglycinin per unit protein (Kitamura, 1995). The processing properties of texurized and filmed soy foods as well as tofu gels are improved by increased glycinin content (Fukushima, 1991). Furthermore, the α-subunit of the β-conglycinin is identified as one of the major allergenic proteins in soybean seed (Ogawa et al., 1991). Thus, the development of soybean lines in which it is either absent or reduced amount of α and α′ subunits of β-conglycinin will help to improve the soybean protein quality.

The absence of α′-subunit and decrease in the levels of α and β-subunits of β-conglycinin are controlled by three recessive alleles (Kitamura, Davies, & Nielsen, 1984; Tsukada, Kitamura, Harada, & Kaizuma, 1986). The α-subunit deficiency of β-conglycinin is reported to be controlled by a single recessive allele (Takahashi, Mizuno, Yumoto, Kitamura, & Nakamura, 1996) and α and β-subunits deficiency of β-conglycinin by a single recessive gene (Phan, Kaizuma, Odawaka, & Takahata, 1996). Since the absence of these subunits is controlled by recessive genes, there is enough scope to improve the soybean protein quality by

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mutation breeding. Several mutant lines lacking α, α′ and β-subunits of β-conglycinin in soybean have been identified (Nakamura, Odonaka, & Kaizuma, 1989; Phan et al., 1996; Takahashi, Banba, Kikuchi, Ito, & Nakamura, 1994; Yang & Burton, 1994). The present paper describes the identification of soybean mutants, which lacks certain subunits of storage proteins.

2. Materials and methods

2.1. Plant material and mutation studies

Soybean variety VLSoy-2 was irradiated with 250 Gy gamma rays using a ^{60}Co source in a Gamma cell 200. The irradiated seeds, along with the control were sown in the field and the M_1 plants were harvested individually and carried forward to M_2 generation as plant-to-row progenies. Nearly 14,000 M_2 plants were screened for morphological mutants. A large number of mutants affecting plant height, flower colour, sterility, leaf shape, early and late maturity were identified and characterized. The progeny of all the mutants were raised in M_3 and subsequent generations along with the parent. The progeny of 24 true breeding mutants were sown in M_5 generation for studying morphological characters and breeding behaviour. The seeds obtained from M_5 generation were further used for protein studies.

2.2. Protein profile studies

The buffer soluble proteins were extracted in 50 mM Tris–HCl buffer, pH 8.0 containing 0.1% SDS, 10% glycerol and 1 mM EDTA. The tissue to buffer ratio was maintained 1:5 (w/v) for all the samples. The slurry was centrifuged at 17,000*g* for 30 min at 4 °C in a Kubota centrifuge. The clear supernatant was used for further analyses. The protein was estimated using the biuret method (Layne, 1957). The samples were electrophoresed in a slab gel using 12% SDS–PAGE following the method of Laemmli (1970). Equal protein (about 100 μg) was loaded in the well for all the samples. The electrophoresis was carried out at a constant voltage (100 V) using a vertical slab gel apparatus (GE 2/4, Pharmacia). After electrophoresis the gel was stained with 0.2% coomassie brilliant blue prepared in destaining solution (acetic acid:methanol:water, 1:3:6 v/v) and the gel was destained in the same solution. The molecular weights of the polypeptides were calculated from the standard graph plotted for R_f vs. Log. Mol.Wt. of marker proteins electrophoresed along with the samples.

2.3. Estimation of oil and crude protein content

Oil content of seed samples was estimated by solvent extraction method (AACC, 1976) using Soxhlet apparatus [Soxtec system – HT (1043)]. The nitrogen content of the seed was determined by the Micro-Kjeldahl method (AOAC, 1984) and the amount of total protein was calculated from percent nitrogen content using a conversion factor of 6.25. Analysis of variance and *F*-test was done as per standard statistical procedure (Panse & Sukhatme, 1967).

2.4. Trypsin inhibitor assay

Dry seeds were soaked in water overnight at room temperature and 1 g of cotyledons was extracted in 5 ml of 50 mM Tris–HCl buffer, pH 8. The suspension was centrifuged at 17,000*g* for 30 min in a Kubota Centrifuge 6800. The clear supernatant was collected and used for trypsin inhibitor assay. For measuring the trypsin inhibitor activity (TIA), 20 μg of trypsin was mixed with appropriate quantity of seed extract containing inhibitors (so as to inhibit 50–60% trypsin), 1 ml of 1 mM BAPNA (*N*-α-benzoyl DL-arginine *p*-nitroanilide) and incubated at room temperature for 10 min (Erlanger, Kokowsky, & Cohen, 1961). The reaction was stopped after 10 min by adding 200 μl of 30% acetic acid. The liberated *p*-nitroanilide was measured at 410 nm using a spectrophotometer.

3. Results

The analysis of protein profiles of 24 mutants of cultivar VLSoy-2 by SDS-PAGE, showed variability in 3 mutants (Fig. 1). The parent cultivar VLSoy-2 (Lane 2) showed six polypeptide bands of molecular weights ranging from 83 to 22 kDa. Three mutants, M-291 (Lane 3), M-17 (Lane 9) and M-231 (Lane 13), were characterized by the absence of A_3 subunit of glycinin. The mutants M-17 (Lane 9) and M-231 (Lane 13) were also characterized by the absence of α′ and α-subunits of β-conglycinin. The mutant M-291 (Lane 3) showed low levels of α′ and α-subunits of the 7S protein.

In M_5 generation the germination percentage of all the three mutants was normal and they showed normal plant

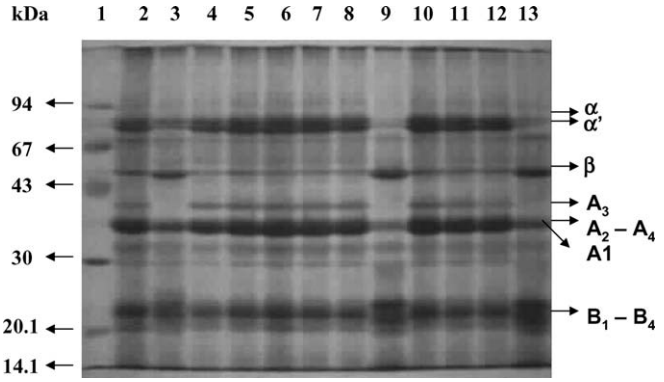

Fig. 1. Protein profiles of VLSoy-2 mutants by 12% SDS-PAGE: Lane 1, molecular weight marker; Lane 2, parent VLSoy-2; Lane 3, mutant M-291 (showing low levels of α and α′ of 7S protein and absence of A_3 subunit of 7S protein); Lane 9, mutant M-17 (showing absence α and α′ of 7S protein and absence of A_3 subunit of 11S protein); Lane 13, mutant M-231 (showing absence α and α′ of 7S protein and absence of A_3 subunit of 11S protein). Mutants in remaining lane showed similar protein profile as that of parent.

Table 1
Morphological and quality characters of mutants of VLSoy-2 in M_5 generation

Genotypes	Days to		Plant height (cm)	100 seed weight (g)	Yield/plant (g)	Oil content (%)	Protein content (%)	Trypsin inhibitor (TIU mg^{-1}) seed meal
	Flowering	Maturity						
M-17	32	95	17.9	16.0	13.1	19.0	39.7	20.3
M-231	32	96	15.8	15.5	3.6	17.9	40.5	20.6
M-291	31	96	17.3	15.4	3.9	18.9	38.9	15.7**
VLS-2	31	95	22.5	15.6	8.3	19.7	39.7	22.4
SE			0.4	0.3	0.8	0.3	0.5	1.5
CD 5%			1.3	1.0	2.4	0.8	1.5	1.6
CD 1%			1.8	1.4	3.2	1.1	2.0	2.1
CV%			3.8	9.5	3.3	2.1	1.9	4.0

** Significant at 1%.

growth. The morphological characters of the mutants are shown in Table 1. The maturity period of mutants was similar to that of parent VL Soy-2 (95 days). The mutants M-291 (17.3 cm), M-17 (17.9 cm) and M-231 (15.8 cm) showed reduced plant height as compared to the parent VLSoy-2 (22.5 cm). The mutant M-17 showed significantly higher yield (13.1 g) while, the mutants M-291 (3.9 g) and mutant M-231 (3.6 g) showed lower yields as compared to parent VLSoy-2 (8.3 g) when calculated on per plant basis. The oil percent of mutants M-291 (19.9%) and M-17 (19.0%) were found to be on par with the parent VLSoy-2 (19.7%) while mutant M-231 (17.9%) showed less oil percentage. The protein content of all the three mutants was similar to that of parent. The mutant M-291 showed significantly lower TIA (15.7 TIU mg^{-1} seed meal) as compared to parent VLSoy-2 (22.4 TIU mg^{-1} seed meal).

4. Discussion

Due to differences in amino acid composition and structure, glycinin and β-conglycinin exhibit differences in both nutritional quality as well as functional properties (Kitamura, 1995). In general, the glycinin (11S globulin) contains 3–4 times more methionine per unit of protein than that of β-conglycinin, the 7S globulin (Kitamura, 1995). As soybean protein is deficient in sulphur amino acids, the increase in glycinin content is more valuable from nutritional point of view. Three mutants, M-291, M-17 and M-231 of the cultivar VLSoy-2 were characterized by the lack of A_3 subunit of glycinin. The mutant M-291 showed low levels of α′, α-subunits of the 7S protein and low levels of acidic subunit of 11S protein. The mutants M-17 and M-231 were identified by the lack of α′ and α-subunits of β-conglycinin and increased levels of β -subunits of β-conglycinin. Nakamura et al. (1989) obtained a soybean mutant which lacked the α and β-subunits of β-conglycinin by gamma ray irradiation. Yang and Burton (1994) reported three soybean mutants with low levels of α, α′ and β subunits of the 7S protein. Phan et al. (1996) isolated a mutant, which lacked α-and β-subunits of β-conglycinin from the population of irradiated seeds with 100–150 Gy gamma rays.

Trypsin inhibitors are widely distributed among plants species, particularly in legumes and their role is known to serve as storage proteins and as regulator of endogenous proteases (Liener & Kakde, 1980). Trypsin inhibitor is one of the major anti-nutritional factors that exert negative effect by causing pancreatic hypertrophy, hyperplasia that ultimately results in the inhibition of growth. Growth inhibition was observed in rats, chicks and mice when fed with purified extracts from soybean rich in trypsin inhibitors (TI) (Liener & Kakde, 1980). Hence, development of cultivars with low trypsin inhibitors will help to improve nutritional quality of soybean. Mutant M-291 has showed significantly lower TIA and can be a useful genetic stock for breeding low TI lines (Table 1).

Methionine is the primary limiting amino acid in soybeans and its average concentration ranges from 10.7 to 12.6 g/kg of crude protein (Serretti, Schapaugh, & Leffel, 1994). The poultry and pig industry is overcoming this problem by supplementing methionine to cereal–soybean meal based animal feed. However, this causes increase in the cost of meal and also during the processing of the meal there is leaching of methionine and bacterial degradation with the formation of undesirable, volatile sulphides (Clarke & Wiseman, 2000). The development of a soybean variety with increased methionine content will overcome the above problem to a greater extent. In the present study, the changes in protein profiles of all the three mutants indicate genetic variability and probably, these mutants have increased amount of sulphur containing amino acids. All the three mutants showed normal plant growth in M_5 generation without any physiological abnormalities. This implies that the change in protein profile does not have any negative impact on the plant growth in the case of soybean. Although the mutants showed absence of α and α′-subunits of the 7S protein, there was no change in total seed protein content. Detailed studies on inheritance of all these mutants are essential, since these mutants show variation for glycinin subunit also.

Presence of allergens like Gly m Bd 30 K (Ogawa et al., 1991) or its homologue P34 makes the deterioration of the soybean protein quality. The two additional proteins namely, β-subunit of β-conglycinin (Gly m Bd 60 K) and

a vicilin-like glycoprotein (Gly m Bd 28 K) were also considered to be equally allergenic (Ogawa et al., 1993; Tsuji et al., 1997). The other major allergens include the members of the glycinin protein family (Helm et al., 2000). Preliminary studies using the soybean proteins isolated from the mutants devoid of these allergens have shown promising results (Ogawa, Samoto, & Takahashi, 2000). We have also identified similar mutants in our studies with genetic variability in both β-conglycinin as well as glycinin proteins. Confirmation of the absence of allergens in these mutants can make a great impact on the soybean protein quality.

5. Conclusion

The result of protein profile studies showed changes in protein profiles of all the three mutants and one mutant also showed low TIA. All the three mutants showed normal plant growth in M_5 generation without any physiological abnormalities. This implies that alteration in the protein structure does not have any negative impact on the plant growth in case of soybean. Detailed studies on inheritance of all these mutants are essential, since these mutants show variation for glycinin subunit also.

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