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SEED STORAGE PROTEIN OF MUNGBEAN MUTANTS

Sanjay Auti*[1] and Digambar Ahire[2]
[1]Department of Botany, H.PT. Arts and R.Y.K Science College, Nashik-422005, India
[2]Department of Botany, New Arts, Commerce and Science College, Ahmednagar, India

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Abstract: Mungbean is a popular legume in Asian countries. It contains 17-16% proteins and is considered to be a major source of protein. Globulins and albumins are the dominant storage proteins in legume seeds and account for 50-90% of seed proteins. In this studies seeds of the mungbean cultivar Vaibhav and Kopargaon-1 were irradiated with different doses of gamma radiations (30, 40 and 50 kR) and concentrations of EMS (0.01, 0.02, 0.03, and 0.04M) to induced mutations. Obtained induced mutants were analysed for its globulin and albumin content quantitatively by following Lowery's method and qualitatively by following SDS- PAGE analysis. Obtained results shows that high yielding mutant contain globulin (16.1%) and albumin (13.7%) over to their control (12.7% and 10.2%) and other mutants. SDS-PAGE result revealed minor differences in the globulin and albumin profile among the mutants and control.

Key Words: Mungbean, Globulin, Albumin, SDS-PAGE analysis

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is one of the most important pulse crops due to its nutritive value and property of maintaining and restoring soil fertility through biological nitrogen fixation. Albumins and globulins comprise the storage proteins of dicots e.g. pulses, whereas prolamins and glutelins are major proteins in monocots e.g. cereals (Derbyshire *et al.*, 1976). Storage proteins are a group that comprises proteins generated mainly during seed production and stored in the seed that serve as nitrogen sources for the developing embryo during germination. It is obviously more effective for the plant to use proteins instead of secondary plant products for this purpose. The seed storage proteins are nonenzymatic and have the sole purpose of providing proteins (nitrogen and sulphur source) required during germination and establishment of a new plant. Seed proteins were empirically classified by Osborne (1924) based on their solubility. The average protein content of cereal grains is 10-15 % of their dry weight that of leguminose seeds 20-25%, while it is only 3-5 % in normal leaves. Besides seeds, storage proteins can also be found in root and shoot tubers, like in potatoes. Most of the seed proteins have high molecular weights and their water solubility is poor. Seed protein electrophoresis technique is a reliable yet, relatively inexpensive way of developing genetic markers for the identification and genetic analysis of several important agriculture commodities (Bushuk & Zillman, 1978; Wrigley *et al.*, 1982; Kreis *et al.*, 1983; Ferguson & Grabe, 1986).

MATERIALS AND METHODS

Obtained Mungbean mutants induced by Gamma radiation (30, 40 and 50 kR) and EMS (0.01, 0.02, 0.03, and 0.04M) were used for seed storage protein profiling. These mutants are high yielding, tall, dwarf, wrinkled seeds, smooth seeds, small and bold seeded, and black and green seed coat colour.

Healthy, mature seeds of control and isolated mutant plants were used for extraction of proteins. Seed sample (3 gram) was weighed and ground to a powder and was sieved through a fine mesh to get fine powder. This powder was transferred to a glass column packed with glass wool and sodium sulphate and defatted with sufficient acetone (100 ml), then with a mixture of acetone and ether in equal proportions and finally with pure ether. The defatted meal was then baked at 60°C in an oven for 1-2 hours to evaporate and remove the excess ether. After defatting the powder was dissolved in 2.5 ml of 0.1 molar phosphate buffer (pH 7.0). The extract was centrifuged at 5000 rpm for 15 minutes at 4°C. The clear supernatant was collected and used as the source of protein.

One gram defatted seed meal was extracted in 10 ml extraction buffer for 1 hour at 4°C on magnetic stirrer. The extract was then centrifuged at 10,000 rpm for 15 minutes. The supernatant was saved as source of albumins and globulins. For purification of albumins and globulins supernatant was taken in dialysis bags and dialyzed against sodium acetate buffer (pH 4.75) at 4°C overnight. Globulins usually get precipitated because of decrease in ionic strength of the extract and alteration in pH by sodium acetate buffer. The precipitate of globulin was removed by centrifuging the contents of dialysis bag at 10000 rpm for 10 minutes. The supernatant contained albumins, as albumins are soluble in water as well as in salt solution with low ionic strength. The globulin fraction was dissolved in 5 ml of Tris-HCl buffer.

***Corresponding Author:**
Prof. Sanjay Auti,
Department of Botany,
H.PT. Arts and R.Y.K Science College,
Nashik-422005, India.

Proteins, albumins and globulins thus extracted were estimated using Lowry *et al.,* (1951). SDS-PAGE was carried out by the method of Davis (1964), Laemmli (1970) and Dadlani and Varier (1993). Equal quantities of samples along with protein molecular weight marker (108, 78.6, 50.6, 35.9, 27.1, 19.2 kDa bands) were loaded into 10% gels.

RESULTS AND DISCUSSIONS

Results of quantitative analysis shows variation in albumin and globulin content (Table 1). Among the obtained mutants high yielding mutant shows significantly higher content of albumin (13.7%) and globulin (16%) over to control (12.7% and 10.2%)and other mutants. Remaining mutants showed significantly low protein, globulin and albumin content over to control (Table 1).

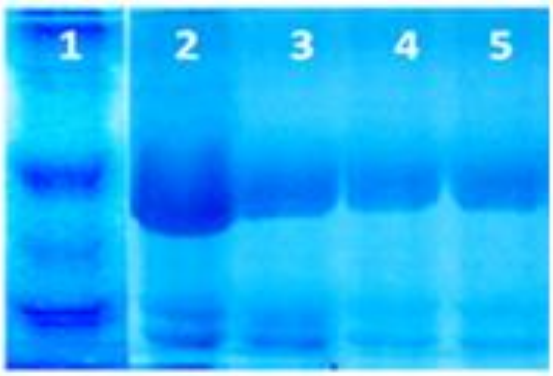
Table 1: Seed storage proteins of Mungbean mutant

Mutant	Protein %	Globulin%	Albumin%
Control	22.2	12.7	10.2
Tall Mutant	22.3	13.3*	10 #
Dwarf	24.7*	13.1*	9.2 #
High Yielding	29.3*	16.1*	13.7*
Dissected	19.5#	10.4 #	7.2#
Early maturing	23.4	11.7 #	9.8 #
Late maturing	17.5 #	9.3 #	5.7 #
Varegated	14.7 #	7.3 #	5.1 #
Lhb	18.9 #	10.5 #	6.4#

Significantly higher (*) and lower (#) than control at probability of 0.01%.

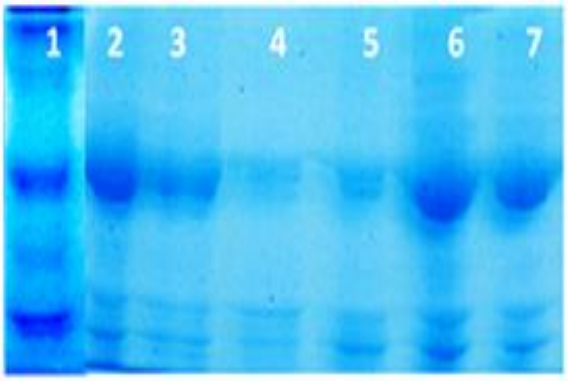
Eletrophoretic analysis has revealed variation in banding pattern of albumins in macromutants of Vaibhav (high yielding mutant, dwarf mutant and lhb mutant) and their control. Eletrophoregram of dwarf & lhb mutants and control exhibited 6 polypeptide bands for albumins (Fig.3). Molecular weight of these bands ranged from 29,854 to 11,220 Daltons. Out of these six bands, bands 1 and 4 are very distinct. They had a molecular weight of 29,854 and 13,335 Daltons.

Figure 1: Eletrophoregram of globulins of Micromutants of Kopargaon-1



Lane 1.Marker, 2. Dwarf, 3. Tall, 4. High yielding, 5. Early maturity

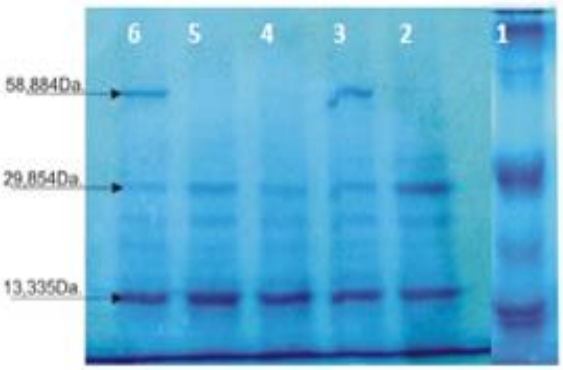
Figure 2: Eletrophoregram of globulins of Micromutants of Vaibhav



Lane 1. Marker, 2. Vaibhav, 3. Wrinkled seed, 4. Black seed, 5. Small rough seed, 6. Dark green bold, 7. Large seeds with rough coat

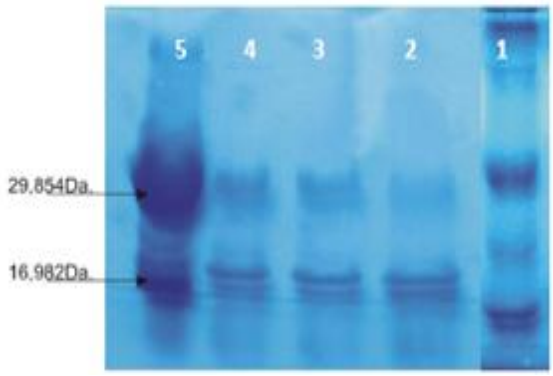
High yielding mutants differed from the above in their banding pattern for albumins (Fig. 3 and 4). They showed nine bands. Six bands were similar to those of dwarf & lhb mutants and control plants. The remaining three were exclusively present only in the high yielding mutants. Out of these three extra bands, the uppermost band is distinct with molecular weight 58,884 Daltons. Next to this band were the other two bands. They appeared as narrow and faint. They had a molecular weight of 39,811 and 35,481 Daltons. Thus the molecular weight of the nine polypeptide bands of high yielding mutants ranged from 58,884 to 11,220 Daltons. The high yielding mutants differed from other mutants and control in having three extra bands with molecular weights 58,884, 39,811 and 35,481 Daltons.

Figure 3: Eletrophoregram of albumins of Micromutants of Vaibhav



Lane 1. Marker, 2. Vaibhav(C), 3. High yielding, 4. Dwarf, 5. Lhb mutant, 6. High Yielding mutant

Figure 4: Eletrophoregram of albumins of Micromutants of Kopargaon-1



Lane 1. Marker, 2. Kopargaon-1(C), 3. Rough seed coat, 4. Wrinkled seeds, 5. Dark green bold seed mutant

Micromutants of Kopargaon-1 (seeds with rough seed coats, wrinkled seeds and dark green bold seeds) and their control were analysed on SDS polyacrylamide gels for albumin profiles. Control plants and two micromutants (seeds with rough seed coats and wrinkled seeds) resembled one another in albumin profiles and exhibited the presence of five light and narrow bands. Molecular weight of these polypeptide bands ranged from 58,884 to 14,962 Daltons.

The dark green bold seeded micromutant, on the other hand showed 8 polypeptide bands in its eletrophoregram. Bands of this mutant are dark, broad and had molecular weight in the range of 63,096 to 14,962 Daltons. Band number 5 is very broad and distinct with molecular weight 29,854. Band number 7 is also distinct but narrow. It had a molecular weight of 16,982 Daltons. Thus dark green bold seeds showed increase in the number of albumin polypeptides due to the mutagenic action.

Control plants of Vaibhav exhibited 8 bands and Kopargaon-1 exhibited 7 globulin polypeptide bands that differed with the variety (Fig.1 and Fig.2). The molecular weight of these polypeptides ranged from 58,884 to 10,470 Daltons in Vaibhav (58884, 25119, 18621, 16982, 14125, 11748, 11885 and 10470 Daltons), while in Kopargaon-1 they ranged from 63,096 to 10,470 Daltons (63096, 28184, 18621, 14125, 11748, 11885 and 10470 Daltons).

Some bands were similar in both the varieties. Band number 3 in both the varieties was broad and has a molecular weight of 18,621 Daltons. Similarly, bands 7 and 8 in both the varieties are narrow and distinct. They had similar molecular weights, i.e., 11,885 and 10,470 Daltons respectively.

Eletrophoregram of globulins of control and micromutants of Vaibhav (wrinkled bold seeds, black seeds, small seeds with rough coats, dark green bold seeds and large seeds with rough coats) exhibited similarity in banding pattern with minor differences (Plate 4.17 A). All of them except two micromutants (the dark green bold seeded and large seed with rough coats) showed 9 polypeptide bands. Molecular weight of these bands ranged from 58,884 to 9,440 Daltons.

The dark green bold seeded micromutants showed 12 bands. These bands were broad and distinct having molecular weights in the range of 10, 4713 to 11,748 Daltons. Large seeds with rough coats exhibited 10 bands. These bands were also distinct but the molecular weight ranged from 94,406 to 8913 Daltons. Dark green bold seeds showed 3 extra bands while large seeds with rough coats showed 1 extra band for globulins.

Eletrophoregram of globulins of macromutants of Kopargaon-1 (dwarf mutant, high yielding mutant and early maturing mutant) showed resemblances in their banding patterns (Plate 4.17 b). All these mutants showed 11 bands. Molecular weight of these 11 bands ranged from 74,989 to 10,715 Daltons. The bands of the dwarf mutants were distinct as compared to rest of the mutants.

Tecson-Mendoza *et al.,* (2001) studied in seed storage proteins in mungbean and found that major seed proteins of mungbean are storage globulins of the basic 7S globulins (~3.4%), vicilin type (8S~89%) and legumin type (11S~7.6%). Legumes seed storage proteins are mostly (7-11S globulins), which tend to be deficient in sulphur containing amino acids. Mungbean seed storage proteins can be characterized on the basis of physiochemical and structural properties on seed protein fractions into 8S and 11S globulins (Tang *et al.,* 2010). Seed storage protein in mung bean (*Vigna radiata*) can be classified on the bases of Vicilin type (8S) and basic 7S globulins and legumin type (11S) globulins.

In dicotyledonous plants, seed storage proteins have been classified as either albumin or globulin types. Globulins are the major storage proteins in most dicot seeds, especially legumes, although a low-molecular-mass sulfur-rich albumin (one of the 2S albumins, named for their sedimentation coefficient) also is present in these and other species. The globulins account for up to 70% of the total seed nitrogen (Croy and Gatehouse, 1995), and in the legumes they consist of two major protein families, the 7S vicillin/ convicilin and the 11S legumin groups, which are differentiated by molecular mass. The globulins are further divided into two subgroups according to their sedimentation coefficients: 7S vicilin-type and 11S legumin-type globulins (Danielsson, 1949).

Mendoza *et al.,* (2001) and Amjad Hamed *et al.,* (2012) isolated protein fraction from mungbean, that were globulin, legumin and vicilin detected globulin and vicilin subunit on native and SDS-PAGE. Malviya *et al.,* (2008) identified 11S and 2S globulins as seed storage protein having molecular weights of 17 kDa, and 14 kDa in green gram.

Uppal & Matta (1996) working with black gram or urad bean (*Vignamungo*) and Krishna & Bhatia (1985) with pigeon pea (*Cajanus cajan*) found the major globulin fraction as 7S vicilin, a trimeric protein composed of one large (70–72 kDa) and two small (40–57 kDa) subunits not linked by disulphide bridges.

In mungbeans, SDS-PAGE cannot be used to identify genotypes on the basis of intraspecific variation because similar banding patterns were observed in some accessions that differed on the basis of characterization and evaluation; therefore, this technique might be more suitable to identify inter rather than intraspecific variation in *Vigna* spp., (Ghafoor *et al.,* 2002). Electrophoresis (SDS-PAGE) of seed storage proteins can be economically used to assess genetic variation and relation in germplasm and also to differentiate mutants from their parent genotypes (Hameed *et al.,* 2009).

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CONCLUSION

SDS-PAGE profiling of seed storage proteins proved to be an economical and simple technique for analysis of genetic variation in mungbean mutants. Mungbean mutnats shows narrow or less variation albumin and globulin profile. High yielding mutant shows higher amount of globulin and albumin over to control and other mutants.

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