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SDS – PAGE Protein Profiling of Mutants of Winged Bean *Psophocarpus tetragonolobus* (L.) DC

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Abstract:

The induced morphological mutants of winged bean *(Psophocarpus tetragonolobus* (L.) DC) like high yielding, long pod, large leaf, flat pod, early maturing and dwarf mutant lines were assessed for their protein profiling through Native and SDS –Polyacrylamide gel electrophoresis. From the literature, an indication became available that the polyacrylamide gel electrophoretic analysis of seed storage proteins is quite suitable to help identify plant genetic diversity and polymorphism. In present investigation the protein profiles and polypeptide profiles of some mutant lines of winged bean have been analyzed on Native and SDS-PAGE to understand genetic variability prevailing in them. The seed protein profiles showed polymorphism in early maturing-1, dark green/flat pod-1, FP/La.L.-2 and anthostem-3 especially in regard to additional polymorphic bands. FP/wingless-1, FP/Li.L.-1 and LP/black seed-1 mutant lines indicated the missing of some protein bands in their profiles. Most of the mutant lines showed variation in their band number besides mobility. In all the mutant lines the molecular weight of polypeptides exhibited a good amount of variation which indicated genetic alteration in different mutant lines of winged bean with respect to proteins.

Keywords: SDS-PAGE, Winged bean, protein profiling.

Introduction:

The two plant families having greatest impact on world agriculture comprise the Poaceae and Leguminosae. The legume family contains about 650 genera and 18000 species. The scenario of world production of grain legumes indicated a figure of 240 million tonnes/year while for the cereals the same was 2110 million tonnes/year. Soybean has been a major grain legume constituting 75 % (182 Mt/Y) of grain legume world production. The remaining 25 % production is attained by several other grain legume species. India, Pakistan, Bangladesh, Nepal and Bhutan together comprise 25 % of the grain legume/ pulses world production excluding soybean [1].

A large number of legume species hitherto unexploited possess a great nutritional potential for contributing to not only protein rich food for humans but also an excellent quality feed and forage for animals. Among such novel legumes the winged bean (*Psophocarpus tetragonolobus (L.) DC*) stands outstanding and hence is receiving the worldwide attention of nutritionists and developmental agencies. Winged bean (*Psophocarpus tetragonolobus* (L) DC.) is one of the unknown plant systems, which has demonstrated an exceptionally fast rate of dispersal, development and acceptance as a new legume food crop throughout the tropical regions of the world. It has large potential to fulfil the need of staple food that is rich in protein and oil for man and as a fodder for animals. Masefield (1973) was the first person who could highlight the potential utility of this plant [2].

The worldwide interest in this plant got triggered subsequently through the publication entitled "Winged bean- A High Protein Crop for the Tropics" brought out by the National Academy of Sciences, United States [3].

The nutritional value of winged bean is mainly due to its mature seeds. The seeds contain 29 % to 42% proteins and are comparable to those of soybean [4]. The seeds mimic soybean in composition and nutritive value and they can be promoted as a substitute for soybean for the tropics. The amino acid profile of winged bean seed protein is similar to that of soybean [5] [6] [7].

By keeping this end in view the present study was organized to assess the proteins and polypeptide from the mutants of winged bean developed through earlier mutation breeding programme. It was believed that such efforts would lead to an understanding of the exact quantum of improvement in the protein profiling of different morphologically desirable mutant lines of winged bean.

REVIEW OF LITRATURE

The present review comprises the earlier published information on biochemical and induced mutational aspects of winged bean besides taxonomy and other related aspects. The winged bean is classified under family Leguminosae. Botanically it is known as *Psophocarpus tetragonolobus* (L.) DC. The genus *Psophocarpus* was first proposed by Neckner in 1790 [8]. It is commonly known as Goa bean, Asparagus bean and four angled bean. Winged bean was introduced in India during 1799 and now it is growing in Assam, Tripura, Meghalaya, West Bengal, Orissa and other Southern states [9].

Mutation breeding in pulses

The "Green Revolution" has not increased the yield of pulses. On the contrary its excessive emphasis on cereals has often led to decreased production of legumes/ pulses. The research workers in past have employed different methods of plant breeding like hybridization, selection and induced mutagenesis. The high yielding and different economically important mutants have been developed through this programme and the production of pulses has been increased to some extent.

Different types of mutant lines have been developed in pulses to increase their quantity and quality. Such lines have comprised dwarf, high yielding and early maturing mutants in cowpea [10], flower mutant and high yielding mutant in pigeon pea [11], high pod yield in faba bean [12], besides the compact bushy, dwarf and early maturing mutant lines in lentil [13]. The seed coat colour mutant in grass pea [14], disease

resistant, early maturing and high yielding mutant lines in soybean [15] and evolution of kabuli chickpea in *Cicer reticulatum* [16] have been some of the other notable achievements of recent past.

Biochemical mutants in crop plants

In day to day life, human beings and different animals are suffering from different types of health problems. This is happening due to the quality of food they are consuming especially the low quality proteins in the food and the high antinutritional factors carried by that food.

Many of the scientists and plant breeders have contributed and developed high yielding varieties in different crop plants. This study mainly revolved around the quantitative as well as qualitative characters of the proteins, oils, carbohydrates and vitamins in plant products. The initial approach was to look for quantitative aspect. This was followed by qualitative consideration of food materials. Hence now a days the quality rich protein and oil food items are in demand. To obtain the quality protein and oil from plants sources, mutagenesis has been considered an important tool which can alter the biochemical composition of plants.

In *Lathyrus sativus* L. have suppressed the neurotoxic amino acid β-N-oxalys-L-α diaminopropionic acid (ODAP/ BOASA) in seed storage protein through induced mutation by using EMS and gamma rays [17].

In *Brassica napus* a double zero line has been developed with significant increase in the oleic acid content in these mutants, by using gamma rays at Bhabha Atomic Research Center, Mumbai, India [18]. Novak and Hados (2003) noted high oil and high protein mutant in soybean at 100 GY and 250 GY of gamma irradiation [19]. Wang et .al., (2003) have screened high oil and protein mutant lines developed through ^{60}Co irradiation [20].

Ethyl methanesulphonate (EMS) was used to induce mutation in flax altering linolenic and palmitic acid levels [21]. high oleic and low linolenic acid mutants achieved in soybean oil by the treatment of gamma radiation and EMS [22]

George (2006) succeeded in developing high protein and high oil mutant in soybean through the combined treatment of gamma rays, EMS and sodium azide. She also noted the improved biochemical and nutritional content in mutant lines of soybean [23].

Recently, Savant (2008) has gained success in altering the oil and protein content in sesame through induced mutagenesis. He also demonstrated the improved oil quality in selected sesame mutant lines [24].

Biochemical studies in winged bean

The winged bean is a high protein crop, comparable to soybean. It also contains good amount of edible oil in its seeds. Its leaves are edible and contain significant percentage of β-carotene. The additional feature of winged bean is that its root system possesses nutritional tubers which contain high amount of proteins as compared to other tuber crops. All such biochemical features have made winged bean a nutritional crop of great significance.

The winged bean has been rather a neglected crop and hence the research work on this plant is rather limited.

MATERIALS AND METHODS

Fourteen true breeding M_6, M_7 and M_8 mutant lines of variety EC 38955-A of winged bean obtained from the earlier mutation breeding programme [25] were taken for the SDS-PAGE protein profiling study. The list of mutants of winged bean used in the present study is as follows:

1. Long pod
2. Early maturing
3. Flat pod/wingless
4. Large leaf/high yield
5. Flat pod/linear leaf
6. Flat pod/large leaf
7. Anthostem
8. Long pod/large leaf
9. Long pod/black seed
10. Flat pod/long pod
11. Dwarf
12. Wingless/small pod
13. Dark green/flat pod
14. Large Leaf/stiff stem

SDS-PAGE

In SDS-PAGE, polypeptides were separated according to their molecular weight, not by intrinsic electrical charge. Sodium dodecyl sulphate (SDS) is an ionic detergent that denatures proteins by wrapping around the polypeptide backbone. In doing so, the SDS confers a negative charge to the polypeptide in proportion to its length. When proteins are treated with SDS and reducing agent 2ME (2-Mercaptoethanol), the polypeptides become rods of negative charges with equal charge unit per length. Here, denaturing discontinuous PAGE system was used as described by Laemmli [26]. This system is almost similar to the native PAGE [27] except for the presence of SDS.

1) Stock solutions

All stock solutions for resolving, stacking gel and electrode buffer were prepared according to Davis method, except the use of SDS and 2-ME.

A) Sodium Dodecyl Sulphate (10 %): This was prepared by dissolving 20 g of SDS (anionic detergent) in 150 ml of distilled water and the final volume (200 ml) was adjusted with distilled water.

2) Preparation of resolving gel and stacking gel: All the composition of resolving gel was similar as described earlier (please refer to section 18.I.2&3) except use of 300 µl of 10% SDS and 100 µl of 10% SDS solution used for stacking gel while remaining were similar to above.

3) Application of sample:

The 10 µl 10% SDS added to protein sample (90 µl) and mixed with 100 µl loading dye (containing 1% SDS, 25% glycerol, 0.1% 2-mercaptoethanol and 100mg bromophenol blue). This sample was heated in boiling water for 5 minutes and then used for electrophoresis. Electrophoresis was performed at constant voltage (150) for 4.5 hours. The gels were fixed in 10 % TCA for 30 minutes. Then the gels were stained with staining solution for 5 hrs and washed briefly in destaining solution till the background became clear. The gels were photographed and molecular weight of every band was determined using gel documentation system. The Gene snap software was used for Rf value and molecular weight determination.

Results And Discussion:

Seed polypeptide (SDS-PAGE) analysis

Twenty-one-winged bean mutant lines were analysed electrophoretically on SDS-PAGE. The 29 mutant lines showed 24 resolving bands and the relative molecular weight mainly ranging from 11.73 to 1501.69 KD. The number of bands varied from 10 to 24. The lowest number of bands (10 bands) was seen in LP/black seed-1 and La.L./high yield-2 mutant lines while in control (EC-38955-A) 16 bands were found. Out of the 29 mutant lines 17 lines exhibited 11 to 15 polypeptides and 11 mutant lines have shown 16 to 20 polypeptide bands. In very few mutant lines more than 20 polypeptide bands were found, like early maturing-1 (24) FP/wingless-1 (21) and La.L./ high yield -1 (22) mutant lines.

The Rf values in every mutant line were variable and the molecular weight also exhibited lot of variation in mutant lines. This indicated the polymorphism in seed polypeptide profiles.

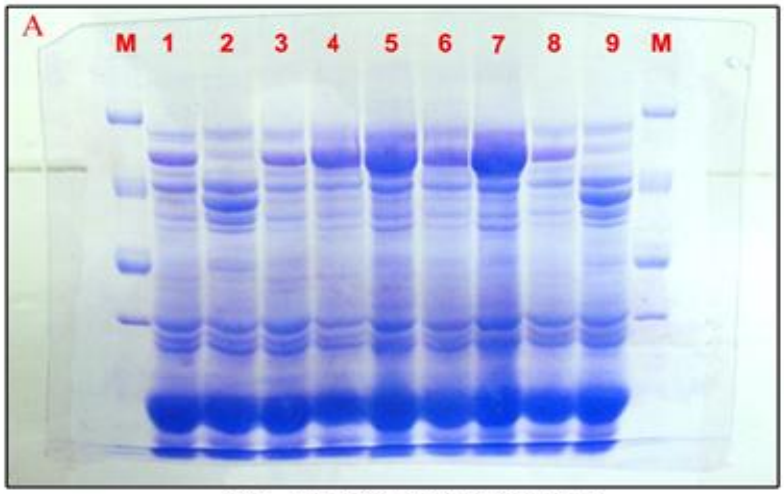
The seeds are the storehouse of different biomolecules, especially the proteins which are of much importance. The high protein content of seeds especially in legumes has offered scope to the geneticists for undertaking detailed biochemical characterization of that material and understanding the genetic control involved in their synthesis.

Seed storage proteins are the products of gene expression with genetic stability and are not affected by environment. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technology is widely used to detect the seed polypeptides and to study plant taxonomy, affinities and genetic diversity.

In present investigation, protein profiles and polypeptide profiles of some mutant lines have been analysed on Native and SDS-PAGE to understand genetic variability in winged bean mutant lines. The protein bands ranged from 8 to 10 in different mutant lines. The mutant lines early maturing-1, dark green/flat pod-1, FP/La.L.-2 and anthostem-2 exhibited polymorphic band ‘C’. FP/wingless-1 showed absence of band ‘D’ while in FP/Li.L.-1 and LP/black seed-1, absence of band F and G could be noted. Most of the mutant lines showed J and K bands but the K band in FP/La.L.-1 mutant line could be noted as missing.

The presence and absence of protein bands indicate the genetic variation in different mutant lines. Through this technique several researchers have proved the genetic variation in different mutants, cultivars and germplasm lines. Such workers comprise in winged bean [28], in moth bean and in mung bean.

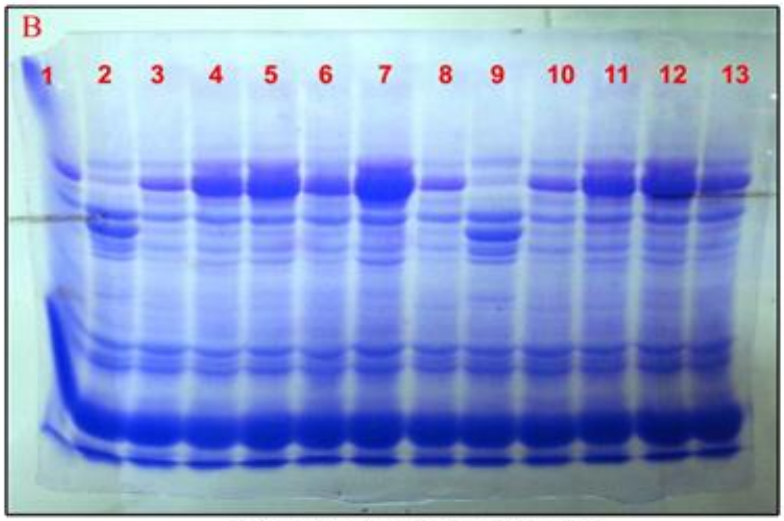
It is believed that the SDS-PAGE analysis of seed storage proteins is suitable to identify plant genetic diversity and polymorphism. The results pertaining to SDS-PAGE revealed great genetic variability in their protein molecular weight. The LP/black seed-1 and La.L./high yield-2 exhibited lowest (10) number of polypeptide bands than the control (16) population. The highest (24) number of polypeptides was noted in early maturing-1, La.L./high yield-1 (22) and FP/wingless-1 mutant line (21).



SDS - PAGE of muntants seed proteins

1. LP/black seed-1
2. FP/LP-1
3. Dwarf-1
4. Wingless/small pod-1
5. Dark green/flat pod-1
6. La.L./stiff stem-1
7. Long pod -2
8. Early maturing -2
9. FP/wingless-2

M= Standard protein molecular weight markers.



SDS - PAGE of muntants seed proteins

1. La.L./high yield-2
2. FP/Li.L.-2
3. FP/La.L-2
4 . Anthostem-2
5. LP/La.L.-2
6. LP/black seed-2
7. FP/LP-2
8. Dwarf-2
9. Wingless/small pod-2
10 Dark green/flat pod-2
11 La.L./stiff stem-2
12 Long pod-3

Fig.-1 [A & B]: SDS-PAGE seed protein profiling of winged bean mutant lines.

The SDS-PAGE polypeptide profiles have been studied in different legumes, grasses and forage legumes, soybean [23], *Vigna* and its cultivars [29].

The SDS-PAGE of seed polypeptide profiles exhibited a good amount of variation in different winged bean mutant lines.

The LP/black seed-1 and La.L./high yield-2 mutant lines have shown missing of 6 polypeptides as compared with control. The highly polymorphic lines were the early maturing-1, La.L./high yielding-1 and FP/wingless-1 which showed additional 8, 6 and 5 polypeptides, respectively. In all the mutant lines the molecular weight of polypeptides exhibited a good amount of variation which indicated genetic alteration in different mutant lines of winged bean.

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