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Full Length Research Paper

Studies on Genetic Diversity of Mungbean Cultivars using Polypeptide Banding Pattern Polymorphism (PBPP) markers.

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Abstract
In the present investigation, water-soluble seed albumin and salt soluble seed globulin protein fractions of twenty four mungbean cultivars were electrophoresed to generate PBPP marker and access the genetic diversity. The PBPP markers generally exhibit a high level of polymorphism, some homology across taxa and a simple genetic control subject to minimal environmental influence.The approach of SDS-PAGE of seed proteins does not necessitate amplification as in case of Random amplified polymorphic DNA (RAPD) or radioisotope labeling as in case of Restriction fragment length polymorphism (RFLP) markers. Although the degree of intra specific variation exhibited by PBPPs is lower as compared to RAPDs and RFLPs, this simple and cheaper technique could be serve useful purpose in studies of genetic variation and genetic amelioration.

Key words: *Genetic diversity, Mungbean, Polypeptide Banding Pattern Polymorphism*

Introduction

Electrophoretic approach has been extensively used to analyze seed proteins, isozymes and DNA to generate banding patters for genome analysis and genetic improvement of several crop plants. Electrophoretic banding pattern using seed proteins, particularly derived from SDS-PAGE have effectively been in studies in evolution, domestication and genetic diversity in a large number of crop plants. This technique is relatively cheaper, simple and non-destructive. SDS-PAGE of seed proteins produces a profile composed of several polypeptide subunits which migrate in the gel according to their molecular weights. The number of such subunits indicates the number of multigene families involved (de Lumen, 1990). Mutation in these gene families or their regulatory gene leads to deletion of some or all the genes or production of new alleles which form the basis of variation in the polypeptide banding patterns which termed as PBPP markers. The PBPP markers could be used for characterization and categorization of genotypes, hybrid selection and also in marker assisted breeding. India leads the list of Mungbean growing countries with an area of 2.98million factors and production of 1.29million tones (Yadav, 1992).among the Mungbean growing states in India, Orissa ranks first interms of area and annual production (Ram & Singh, 1994).

Mungbean is a grain legume crop and contains 17.2 to 29.9% protein in seeds with an average of 22.83% (Naik, 1998). It should, therefore be envisaged as an ideal crop to employ SDS-PAGE. However, available literature reveals limited use of this approach in mungbean. A couple of reports elucidated only uniform banding pattern of the few genotypes investigated employing SDS-PAGE (Sahai and Rana, 1977; Thakare et al., 1988). However, recent investigations evidenced for successful categorization of genotypes or accessions on the basis of seed protein profile of mungbean (Tomooka et al., 1992; Naik, 1998). Naik (1998) also detected simple Mendelian inheritance and linkage inter se of the multigene families exhibiting polymorphism and also the stability of the banding patterns. However, no report is available on genotype specific polypeptide banding pattern that could be used as a fingerprint for genotype identification, therefore objectives are:

1. Assessment of genetic variation using electrophoretic banding patterns of seed albumin and globulin fractions in mungbean.

2. Categorization of mungbean genotypes into different protein types on the basis of polypeptide banding pattern polymorphism (PBPP) markers detected from albumin and globulin banding patterns.

3. Exploring the possibility to depict genotype specific PBPP finger printing in mungbean cultivars.

Materials and Methods

These include 24mungbean genotypes, 21 improved varieties and 3 local land races of Orissa.

The extractions of seed protein fractions, water soluble albumin and salt soluble globulin, from the seeds of 24 mungbean genotypes were carried out.0.05gm of seed flour was taken in 1.5 ml eppendorf tube and suspended in 350µl of pre-chilled ddH2O for 6 hours at 4ºC with intermittent mixing by tapping at an interval of 1 hour. The suspension was centrifuged at 12,000 rpm at 0ºC for 5 minutes and the supernatant containing albumin was stored at 0°C. the hypernatant was suspended in 800µl of 0.5M NaCl (pH 2.5) for 1 hour at room temperature with intermittent mixing by tapping at an interval of 10 min. the suspension was centrifuged at 12,000 rpm at 10º C for 5min. the supernatant containing globulin was stored at 0ºC.

Spectrophotometric estimation and equilibration

The albumin & globulin percentage in the seed protein were estimated according to UV method (Jayaraman, 1981) using the standard curve of Bovine serum albumin, fraction-V (Sigma). All samples were adjusted to a concentration of 2.0 µg/ µl concentration by dilution.

Denaturation of protein fractions

The protein samples containing albumins and globulins were well mixed with an equal volume of cracking buffer (0.125M Tris-Cl, pH 6.8; 4% SDS; 20% Glycerol; 10% B-mercaptoethanol and 0.01% Bromo-phenol blue) i.e. 100µl from each and were denatured by boiling in water bath at 95°C for 1 minute and 2 minutes respectively.

Electrophoresis of seed protein fractions

Electrophoresis was carried out in the modified discontinuous SDS-PAGE system of Laemmli (1970) using 10% acrylamide resolving gel (0.375M Tris-Cl, pH 8.8) and 4% stacking gel (0.125M Tris-HCl, pH 6.8). 20 µl of albumin & globulin loaded for each sample along with PMWM-2(B.G).

Staining of the gels

Staining of the gel was done in 0.2% (w/v) Coo-massie Brilliant Blue R-250 in 12.5% (w/v) TCA.

Scoring and molecular characterization of the polypeptide bands

The polypeptide bands in the electrophoregram were scored initially from the gels followed by verification from their photographs. The relative mobility (Rm) values of corresponding polypeptides were computed as the ratio of distance of polypeptide band migration to distance of tracking dye migration.

Proximity matrix studies and cluster analysis

Each polypeptide bands are considered as unit character and the data were organized into 0-1 matrix and analyzed using SPSS 8.0.1 software. The dendrogram or Hierarchical clustered analyses were carried out using between/within group linkage method and squared elucidation distance interval.

Molecular markers including polypeptide banding pattern polymorphism (PBPP) are efficiently used to elucidate genome relationship, evolutionary pathway, center of origin .and domestication in several crops including genus Vigna.

SDS-PAGE of seed albumins of 24genotypes led to detection of 31 polypeptide bands with molecular weight ranging from 157.345KDa to 14.3KDa (Fig.4.1 and Table-4.1). Out of these six polypeptides (MW 81.488, 54.259, 47.906, 21.331, 20.671, 14.3KDa) are universally present, while rest 25 polypeptide bands were varied in their expression. One polypeptide (MW 92.418KDa) was present only in the genotypes developed from OUAT and it might be due to the common source of origin or due to similar agro-climatic conditions.

SDS-PAGE of seed globulins of 24 genotypes led to detection of 21 polypeptide bands with molecular weight ranging from 126.474 KDa to 16.842 KDa (Fig.4.2 and Table-4.2). Out of these seven polypeptides (MW 12.474, 99.725, 85.916, 69.849, 43.178, 37.329, 32.240 KDa) were varied in their expression while others were universally present. The globulin composition is quite homogenous.

Proximity Matrix Analysis

Proximity matrix index values for each pair from 24 test genotypes under study (Table-3.1)revealed that the highest proximity matrix value (1.000) of combined albumin-globulin was obtained between K-851 and PUSA 9531, LGG407 and SML6678. The lowest proximity index (0.0) between PDM-84-139 and BPMR-145 was obtained. This higher extent of variation in proximity matrix revealed the greater extent of genetic diversity in mungbean germplasm.

Cluster Analysis using Dendrogram;

The cluster analysis (SPSS 8.0.1) of 24 accessions based on proximity matrix indices is represented by the dendrogram using nearest neighbour method and interval of single group linkage. Dendrogram analysis exhibited two major clusters and a series of minor clusters at various levels.

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