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Research Article

CHARACTERIZATION OF CULTIVARS BASED ON ELECTROPHORETIC ANALYSIS OF SEED PROTEINS, ISOZYMES AND DNA MARKERS IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT
Studies have been carried out for the identification of the genuiness and purity of eighteen rice genotypes using electrophoretic analyses of total soluble seed proteins, isozymes and DNA. Region A, B and D were found useful to identify most of the genotypes studied by protein bands. Malate dehydrogenase isozyme banding pattern showed variation but, Alcohol dehydrogenase banding pattern was similar among the genoypes. RAPD primers showed 73.43 per cent polymorphism between genotypes studied. Variations were observed between these biochemical markers in terms of number of bands, their relative mobility and intensity of bands.

Keywords: *Electrophoresis, Gel, Genotypes, Identification, Malate Dehydrogenase, Isozymes, Protein, Seed, Rice*

INTRODUCTION
Rice is a major crop in Asia. Genetically divergent rice genotypes are available due to diverse conditions of their cultivation and hence it is very difficult to visually identify cultivars on the basis of phenotypic characters. Uses of biochemical and molecular markers have proved to be of immense help to breeders in improving important agronomic traits in rice. The present investigation was undertaken to characterize the 18 rice genotypes based on the banding pattern of total soluble seed proteins, malate dehydrogenase isozyme and DNA markers as their expression and detection were unaffected by environmental interactions and could be conveniently used as markers in identifying rice genotypes (Cooke, 1987).

MATERIALS AND METHODS
Seed proteins: SDS-PAGE of total soluble seed proteins was carried out by using 15 per cent gels according to the methods prescribed by Laemmli (1970) with slight modifications.
Protein was extracted from single seed by adding 0.2 ml Tris glycine extraction buffer (25 mM, pH 8.5). The suspension was centrifuged at 10000 rpm for 15 minutes. The extract was dissolved in equal amount of sample buffer and kept in boiling water for 2 minutes for denaturation of proteins. Then centrifuged at 10000 rpm for 15 minutes and the supernatant was used for loading on to the gel. A current of 1.5 mA per well with a voltage of 80 volts was applied until the tracking dye crossed the stacking gel. Later the current was increased to 2 mA per well and voltage up to 120 volts. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel. Then the gel was stained using coomassie blue solution overnight and destained using a mixture of 227 ml of methanol, 46 ml of acetic acid and 227 ml of distilled water until the bands were clearly visible.
Isozymes: Seven day old seedling was ground in 200 µl of extraction buffer (0.1 M Tris-HCl, pH 7.5) and centrifuged at 10000 rpm for 15 minutes and the supernatant was used for loading as prescribed by Glaszman *et al.*, (1988). The gels were stained using Malic acid 15 ml, 15 ml Tris buffer, 50 mg NAD, 50 mg NBT, 25 mg PMS, 75 ml distilled water under dark.
DNA analysis: Sixteen days old seedlings were used for DNA extraction. DNA was prepared as per modified CTAB (cetyl trimethyl ammonium bromide) method (Cao and Oard, 1997). The PCR reaction mixture consists of 1.0 µl of template DNA, 22.0 µl primer, 2.0 µl dNTPs, 2.0 µl Tag, 2.0 µl of 1X PCR buffer and 12.6 µl of sterile water in an volume of 20µl and the amplification was carried out as follows:

Initial denaturation temperature : 94°C- 4 minutes
Denaturation : 94 °C - 1minute
Primer annealing : 36 °C- 1 minute
Primer extension : 72 °C -2 minutes
Later three stages were repeated 35 times
Complete primer extension : 72 °C - 5minutes
Soak temperature : 4 °C – until removed

The electrophoresis was carried out on 1.4 per cent agarose gel. Ethidium bromide was added at 0.5 µg/ml of gel. A voltage of 1.5 v/cm was given. After completion of electrophoresis the gel was viewed under UV light for the presence of bands and photographed.

RESULTS AND DISCUSSION

Proteins: Total soluble seed proteins could be fractionated into 31 bands, which showed heterogenity among the genotypes. The highest number of bands (17) was observed in Rasi. Regions A (7), B (4) and D (6) were found useful to identify most of the genotypes studied as the banding pattern was distinct for each genotypes in these regions. The cultivars differed in the number of bands, their mobility (position) and intensity. The results on the banding pattern of the proteins profiles suggested that the specific genotype could be differentiated by either based on the position or intensity of bands but not on number as some of the genotypes expressed similar number of bands. Similar observations were noticed by Rohini Devi (2000) and Dhanaraj (2001).

Malate dehydrogenase isozyme showed some variation for the banding pattern. Cvs. Red Triveni and Pusa Basmati-1 showed only one band and were found to be similar in banding pattern. The intensity of banding pattern differed among the genotypes and could be used for characterization and varietal distinction. Alcohol dehydrogenase banding pattern could not be used for characterization, as it was recorded only one band with similar intensity as well as at same Rm value in all the genotypes.

The evaluation of genotypes at molecular level for polymorphism by using seven RAPD primers revealed 73.43 per cent polymorphism between the genotypes. Among the primers studied OPAS-15 was found useful in identifying most of the genotypes as it generated nine polymorphic bands. Cv. Jyothi showed specific band for the primer OPAS-15 and could be easily distinguished from other genotypes. However, cv. Swarna differed from other genotypes for one specific band generated by OPAS-17

Thus present study suggested that any number of rice genotypes could be identified based on the total soluble seed proteins, isozymes and DNA markers as the banding pattern and intensity were different for each genotype.

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