ELECTRPPHORECTIC CHARACTERISATION OF EMBRYO PROTEINS IN RICE GERMPLASM

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Summary

Variations in the embryo proteins (salt soluble fraction) separated by SDS-PAGE have been observed in five accessions of *Oryza sativa* ssp indica and the densitographs of these embryo proteins is distinct and each profile is unique and showing considerable variations among themselves which emphasize that the systematic analysis of data based on electrophoretic variations of embryo proteins can provide reliable information for easy detection of varietal differences or testing the genetic purity of seeds in rice.

Key words : *Oryza sativa*, Embryo-proteins, Polyacrylamide Gel electrophoresis.

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Introduction

In a series of studies made earlier in the genus *Oryza,* the seed protein profiles based on polyacrylamide gel electrophoresis were recognized to be an important variable in detecting the genetic variations within and between the species. (1,2). The studies were mainly based on the salt soluble protein fractions, extracted from the whole kernel with the embryo intact. The protein fractions studied were found to be the products of the endosperm. The endosperm in rice grain constitutes the bulk of the kernel with a very minute embryo. The embryo protein fractions are found to be inadequate to develop any band in case of analysis based on single seed extract. There is no band yield when a single embryo is analysed following the same system applied for the whole kernel. A complete and highly resolved electrophoretic spectrum is developed, only when sample dose is increased by extracting proteins from four to six embryos taken together.

In the present study, thus by increasing the protein concentration, we have focused on the characteristics of embryo proteins (salt soluble fractions) in a number of rice accessions as recorded by polyacrylamide gel electrophoresis following the earlier system of analysis (1&2).

Materials and Methods

Plant Material

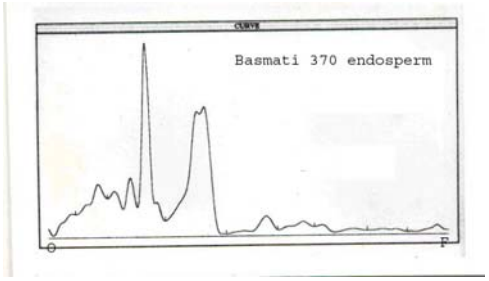
The materials comprised five accessions of *Oryza sativa* ssp indica (Basmati 370, Ratnagiri, Saket, PTB 29,EC 918) obtained from Central Rice Research Institute (CRRI), Cuttack, India. The voucher specimens are available in the germ plasm resource unit, CRRI. Individual accessions were analyzed taking five embryos per accession.

Protein extraction and electrophoresis

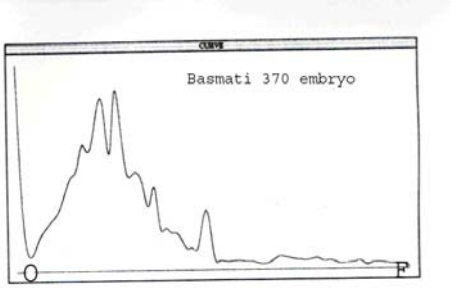
The protein extraction process and electrophoresis were carried out as described previously (1). Five embryos per accessesion were taken together and were homogenized in 0.3 ml of 0.5M Tris buffer (pH 7.6). For analysis of endosperm protein, a single kernel removing the embryo was analyzed (1). A cationic system of polyacrylamide gel electrophoresis was employed according to the method of Davis (3) and Ornstein (4) using 10% gel and β alanine buffer (pH 4.5). Gels were stained in 0.1% amido black and destained in 9% acetic acid. Gels were scanned in the densitometer at 570nm.

Results and Discussions

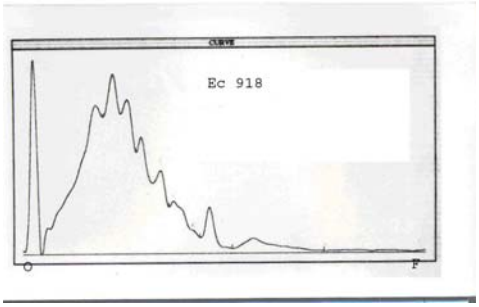
The respective protein profiles of endosperm and embryo tissue of Basmati 370 are shown in figure 1a & 1b. The profile of embryo (Fig.1b) appears to be distinct in contrast to that of the endosperm (Fig.1a). This tissue specific profile patterns corresponding to the embryo and endosperm were observed also in case of other accessions studied. From the densitographs of embryo proteins of five different accessions (Fig 1b, 2, 3, 4, 5), it is distinct that each profile is unique showing considerable variations among themselves. The profile character was found to be independent of environment (locations or years) similar to that of the endosperm proteins as reported earlier (1).



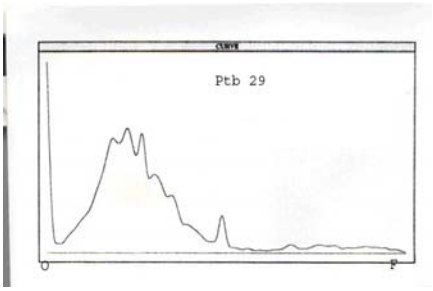
(Fig 1a)



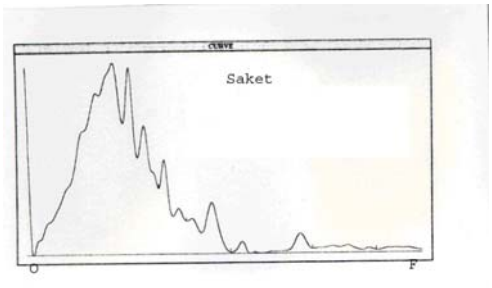
(Fig 1b)



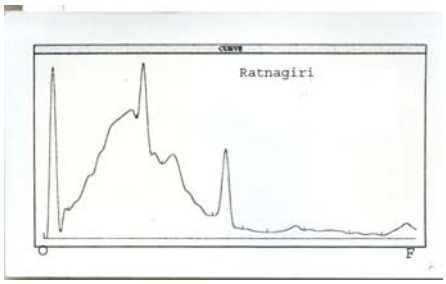
(Fig2)



(Fig 3)



(Fig 4)



(Fig 5)

Densitograph profiles of salt soluble proteins of endosperm (Fig.1a) and embryos (Fig.1b–5) of different rice accessions. *O- Origin, F- Front*

Earlier studies in *Oryza* genus, established that variations of endosperm proteins as revealed in electrophoregrams could be a useful marker in correct identification of varieties (Sarkar and Bose, 1984) as well as tracing the phylogeny within the genus (Sarkar and Raina, 1992). The basic pattern of banding was found to be essentially genome specific that helps assess genome relationships within the genus *Oryza* accurately. Further identification of the taxa at the species or subspecies level within the genome is possible based on discrete variations with regard to certain specific bands in an otherwise common profile. The present studies emphasize that the systematic analysis of data based on electrophoretic variations of embryo proteins too can provide additional and reliable information for easy detection of varietal differences or testing the genetic purity of seeds in rice.

Acknowledgements

The authors are thankful to the Council of Scientific and industrial Research (CSIR) – India for their financial support and the Central Rice Research Institute (CRRI), Cuttack, India for supply of seed samples.

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