

## CHARACTERIZATION OF CMS LINES AND MAINTAINERS IN RICE BY HPLC OF SEED PROTEINS

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The morphological and histochemical properties have been used to identify or distinguish CMS lines and maintainers of rice. Based on these two properties, it could be possible to differentiate CMS (A) lines from that of maintainer (B) lines when they are at flowering stage. It would be convenient to discriminate CMS (A) lines from that of maintainer (B) lines when they are at flowering stage. It would be convenient to discriminate CMS lines on the basis of analysis of seed protein rather than characters of growing plant or the histochemistry of pollen.

In recent years, extensive research has been done in the application of HPLC for the differentiation of rice cultivars (Hussain et. al. (1989), Huebner et al (1990) and Su-Hwa Chen (1991). However, HPLC technique has not been used so far for the differentiation of male steriles and fertiles of rice based on seed protein pattern. So an attempt has been made to differentiate A and B lines of rice by using HPLC analysis.

The seeds of six male steriles, IR 54752 A, V20 A (WA source) Mangala A, Pushpa A, ES 18 A, Intan mutant A (MS 577A source) and their maintainers were used for protein analysis using HPLC equipment. Pharmacia, Sweden. This is based on the principle of size-exclusion chromatography. The sample preparation was done according to Bhowmik et. al. (1990) and the samples were analysed as per standard procedure.

Molecular weight of unknown proteins in the sample was estimated by making use of molecular weight of standard proteins. The standard proteins used here in this study with their molecular weight (MW) are given below :

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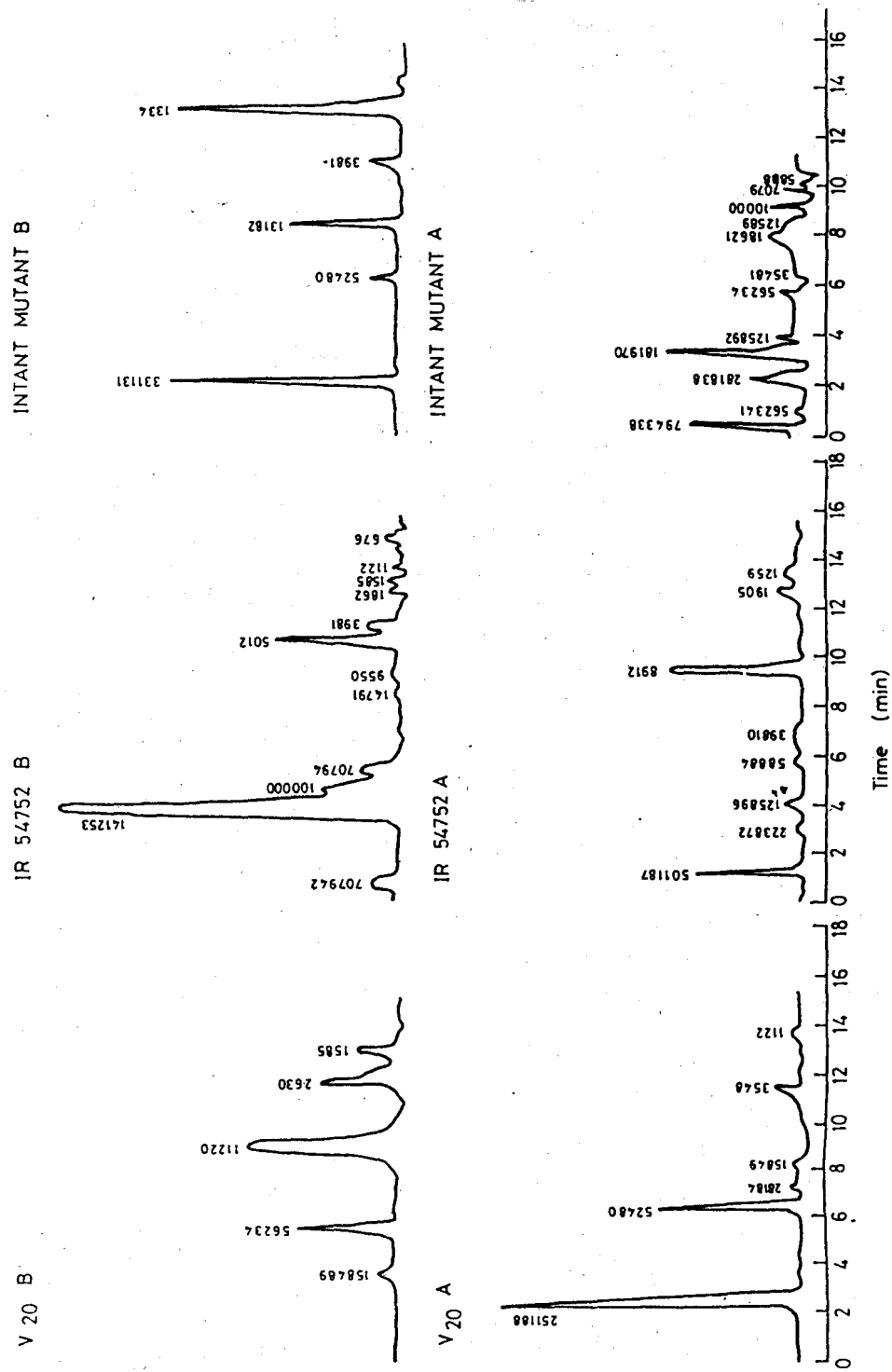


Fig. 1a. Chromatograms of seed proteins from three male steriles and their maintainers. Estimated molecular weights are indicated for major chromatographic peaks

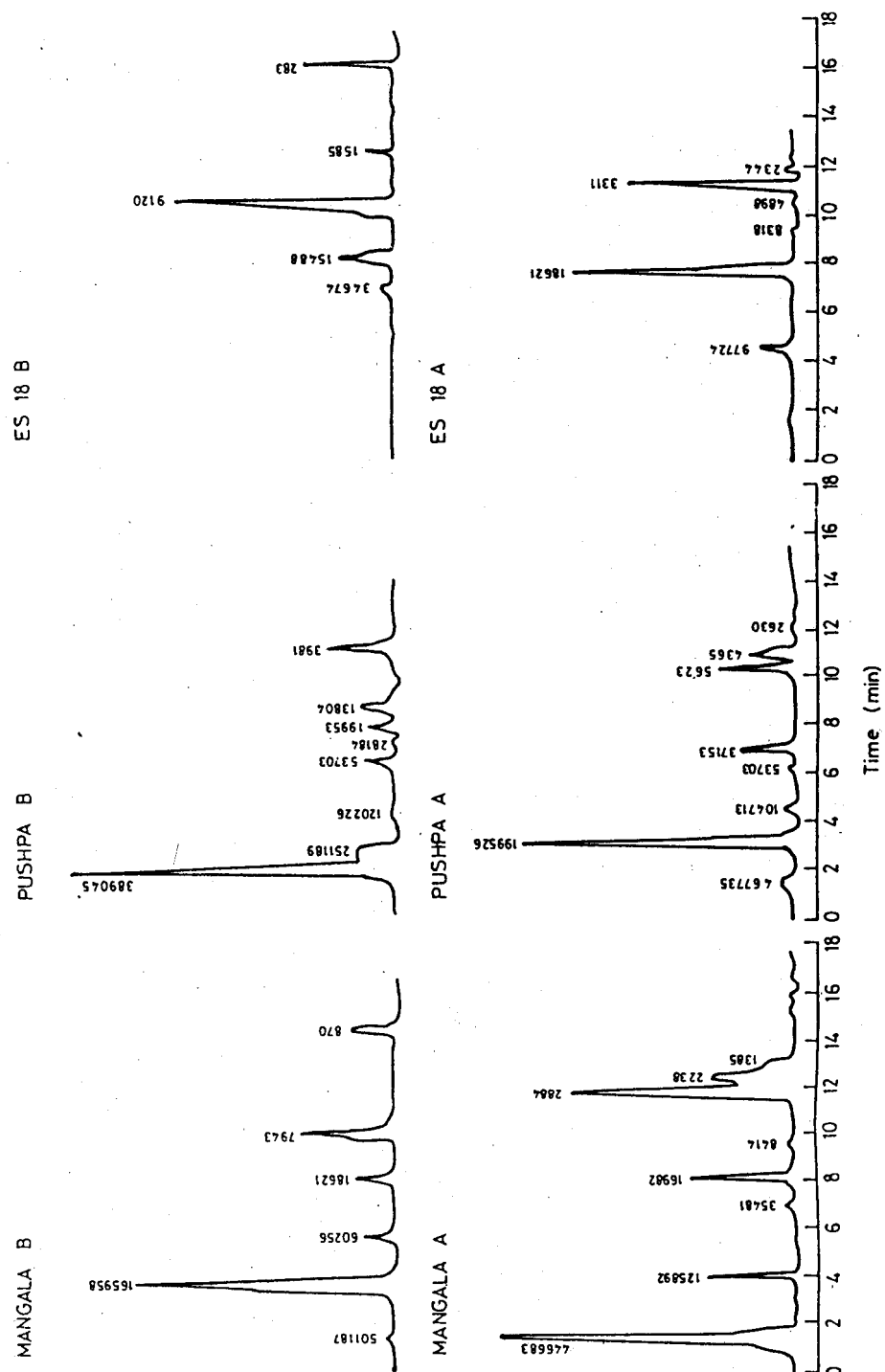


Fig. 1b. Chromatograms of seed proteins from three male steriles and their maintainers. Estimated molecular weights are indicated for major chromatographic peaks

Compound	MW (daltons)
Macroglobulin	340,000
Phosphorylase B	97,400
Glutamate dehydrogenase	55,400
Lactate dehydrogenase	34,500
Trypsin inhibitor	20,000

The chromatograms obtained by HPLC showed that each male sterile line had its own characteristic peak pattern and it varied with that of corresponding maintainer line. All the six cytoplasmic male sterile (A) lines and their maintainers (B lines) differed from each other qualitatively as well as quantitatively. Though the CMS lines IR 54752A and V20A originated from the same cytoplasmic source (WA), they differed from each other in number of protein peaks and also their MW. Even the isogenic lines i.e., V20A and V20B did not have similar kind of protein peaks (Fig. 1a and 1b).

Molecular weight of proteins estimated by using standard proteins with known MW also revealed both qualitative and quantitative differences in protein patterns of CMS lines and their maintainers (Fig. 1a and 1b). In A lines, the MW of proteins ranged from 794328 to 1122 daltons. Whereas in B lines, the protein peaks varied with a MW ranging from 707946 to 282 daltons.

In general, the number of protein peaks i.e., different protein fractions were more in B lines compared to A lines, except in IR 54752B and Mangala B. It was also observed that high MW proteins were more in A lines except IR 54752A and Mangala A, while low MW proteins were more in B lines. The differences in accumulation of proteins with different MW may be a cause of sterility in CMS lines. This can be confirmed by studying pollen protein pattern through HPLC analysis.

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#### REFERENCES

- Bhowmik, A., T. Omura and T. Kumamaru. 1990. Screening of rice varieties for endosperm storage proteins. *Plant Breeding*, 105 : 101-105
- Huebner, F.R., J.A. Bietz, B.P. Webb and B.O. Juliano. 1990. Rice cultivar identification by high-performance liquid chromatography of endosperm proteins. *Cereal Chem.* 67 : 129-135
- Hussain, A., M.G. Scanlan, B. Juliano and W. Bushuk. 1989. Discrimination of rice cultivars by polyacrylamide gel electrophoresis and high-performance liquid chromatography. *Cereal Chem.* 66 : 353-356.
- Su-Hwa Chen. 1991. Comparative study of pollen proteins of rice by Isoelectric focusing and high-performance liquid chromatography. *Cereal Chem.* 68 : 91-94.