

Electrophoretic characterization of rice varieties using single seed (salt soluble) proteins

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Summary. Variation of salt soluble protein fractions of seeds has been observed in a number of rice varieties as recorded in their electrophoregram tracings: both qualitative and quantitative differences were present. Analysis of variance has been found to be useful in estimating the quantitative differences. These tracings or patterns appear to be unique for each of the varieties investigated and seem to be genetical in nature as they remain constant under different environmental conditions, and therefore could be conveniently used for variety identification.

Key words: *Oryza sativa* – Albumin – Globulin – Electrophoregram-tracings – Variance-analysis

Introduction

The correct identification of plant varieties is important for geneticists and plant breeders for their experimental work. Chromatography of phenolic compounds and electrophoresis of proteins have been extensively applied in the separation and identification of species and their hybrids (Jaworska and Nybom 1967; Bose and Fröst 1967; Johnson et al. 1967; Chu 1967). Electrophoretic separation has also been tried at the varietal level—mostly in crop plants—usually, based on qualitative differences (Siddiq et al. 1972; Bushuk and Zillman 1978; Gunzel 1978; Marchylo and LaBerge 1980; Inocencio et al. 1980).

In the present report an attempt has been made to characterize and to separate out varieties of rice using semiquantitative data obtained from densitograms of soluble protein band patterns of seeds. The intensity of staining of a particular band is proportional to the area it projects; variation of the area of a particular band in the different varieties can be measured and their

differences tested. In this way one could have a more sensitive method which could be effectively combined, where necessary, with any qualitative differences that might also be present.

Materials and methods

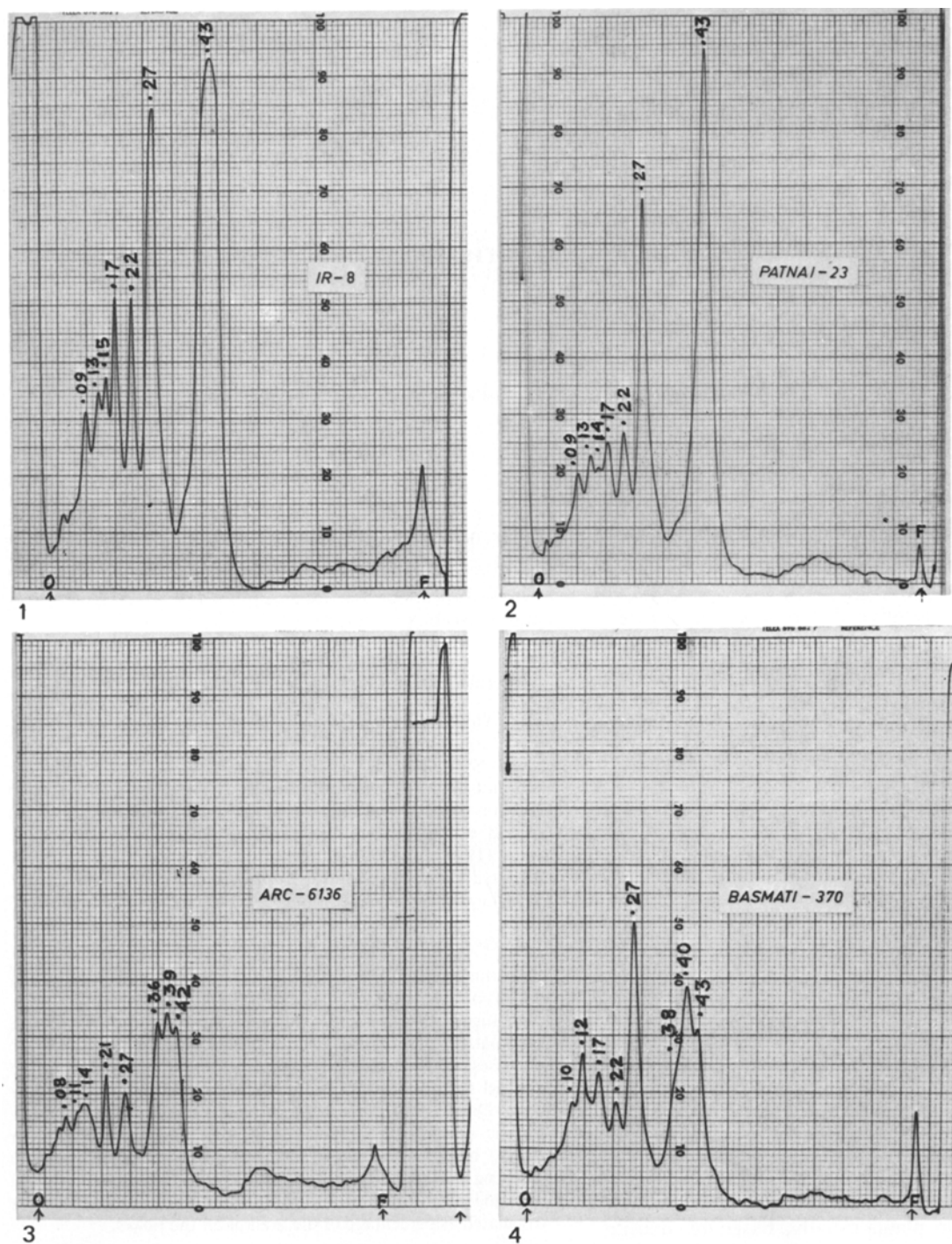
The following eight varieties were studied 1) 'IR-8'; 2) 'ARC-6136'; 3) 'Basmati 370'; 4) 'Randhunipagal' (RP); 5) 'Maharastra NCS.2272'; 6) 'AC-529 Kameji'; 7) 'AC.74-Hsinchu-4'; 8) 'Patnai-23'. In one experiment, varieties were grown in two locations (Falta and Shyamnagar) in the same year. In a second experiment varieties were grown for two consecutive years (1981 and 1982). Single grains from each variety were analysed taking three seeds from either each location or the year they were harvested.

Extraction and electrophoresis

Individual grains (dehusked) were weighed and crushed in 0.2 ml of 0.5 M Tris buffer (pH 7.6). The suspension was centrifuged at 4,000 g for 30 min: 20 μ l and 100 μ l of the supernatant were taken for densitometric and photographic purposes, respectively. A cationic system of polyacrylamide disc gel electrophoresis was employed according to the method of Davis (1964) and Ornstein (1964) using 10% gel and B-alanine buffer (pH 4.5) at 4 °C. Gels were stained in 0.1% Amido black and destained in 7% acetic acid. Densitometric scanning was done using a Gilson Holochrome with a gel scanning attachment at 570 nm. Individual peak areas were calculated as percentage of the area of all the peaks.

Fractional identification

Albumin and globulin fractions were extracted and purified following methods described by Cagampang et al. (1976). Their electrophoregrams were compared with the unknown spectra. The spectrum in all varieties studied indicated the presence of albumins and globulins. This was further confirmed by the differential staining of the bands obtained with Amido black (Minetti et al. 1973). Each specific globulin band in polyacrylamide gel was purified by elution of gel section, and run in SDS-PAGE. Its molecular weight was determined by the method of Weber and Osborn (1969).



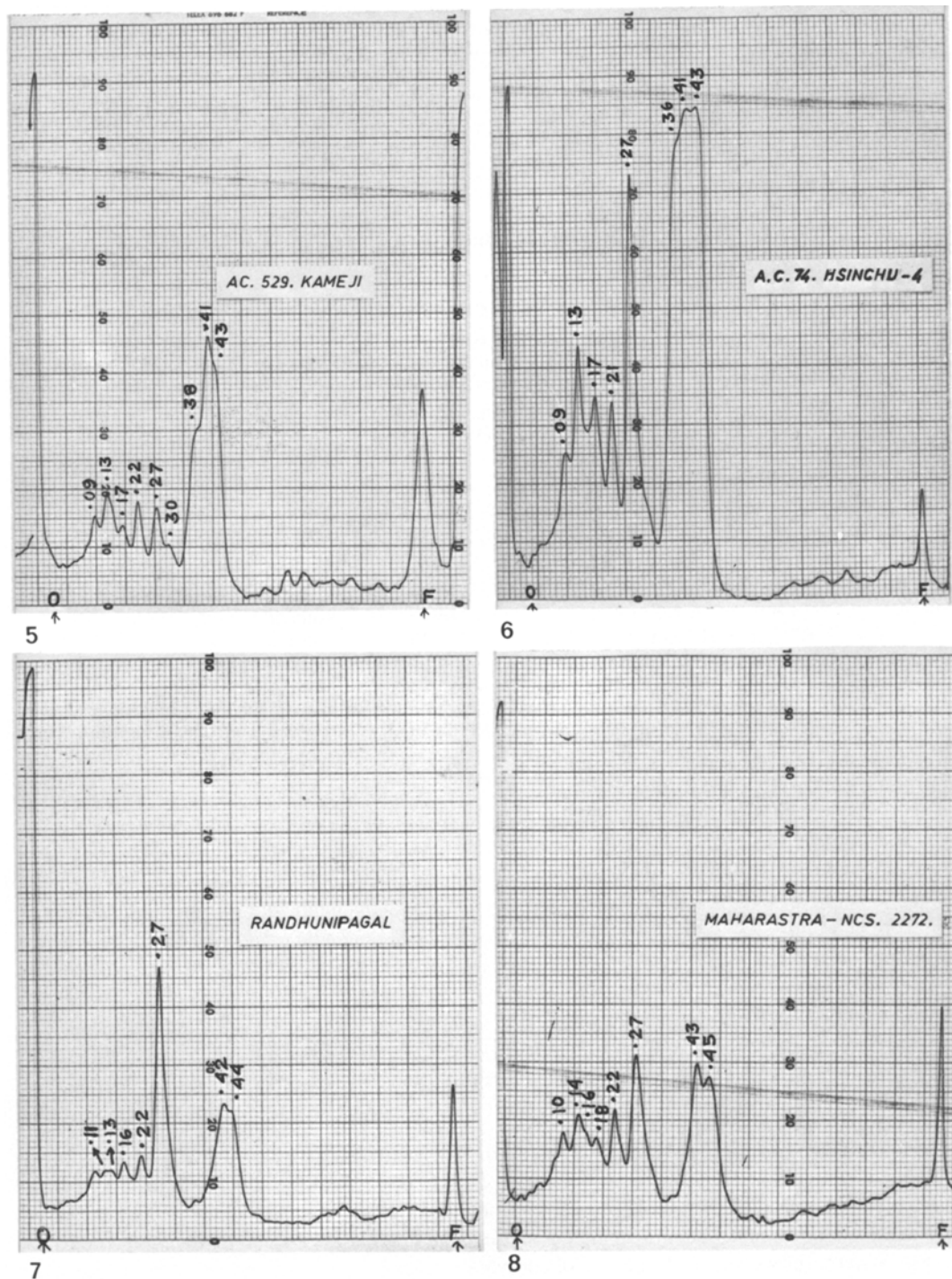
Figs. 1-8. Densitograph profiles of albumin and globulin fractions found in the varieties studied. Figures above the peaks refer to their Rf. values. Migration is from anode to cathode. *O* origin; *F* front

The albumin region containing different bands was eluted as a unit and the molecular weights of the bands were determined after runs were made in SDS-PAGE.

Results and discussions

Densitometric tracings of electrophoregrams of the salt soluble proteins of rice showed both qualitative and

quantitative differences among the varieties studied. Each of the eight varieties appeared to have a unique densitograph profile. Two to four large prominent fast moving bands have been identified as globulins and they usually give blue green colour when stained with Amido black. The peak at 0.27 (approximate mol.wt. 24,000) is present in every variety. This peak is noticeably



smaller in area in 'AC. 529 Kameji'. The other globulin component may vary, showing one peak at Rf. 0.43 (approximate mol.wt. 18,000) in IR-8 (Fig. 1) and 'Patnai 23' (Fig. 2) and sometimes three close peaks in the form of a trident (approximate mol.wt. ranging from 18,000 to 20,000) in 'ARC. 6136', 'Basmati 370', 'AC. 529 Kameji' and 'AC. 74 Hsinchu 4' having

different Rf. values (Figs. 3-6). In 'RP' and 'Maharashtra NCS 2272' (Figs. 7 and 8) two close peaks (approximate mol.wt. 18,000 and 17,000) were found in the same region, having Rf. values of 0.42 and 0.44; and 0.43 and 0.45, respectively (Fig. 9).

Albumins which stain blue black were present as minor but distinct peaks. A few inconspicuous notches

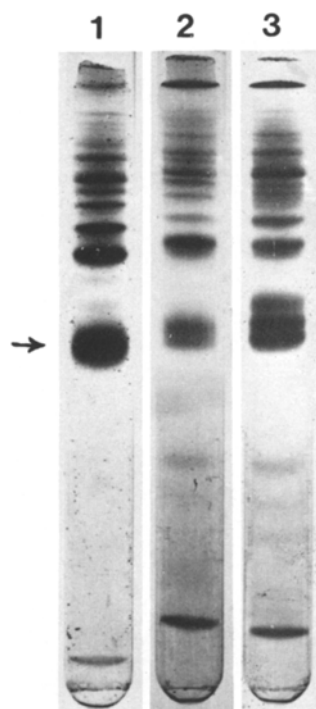


Fig. 9. Electrophoregrams of 1 'IR-8', 2 'RP' and 3 'Hsinchu-4'. The arrow indicates the fast moving globulin zone with single band, two bands and three bands, respectively

could also be detected. Rf values range from 0.01 to 0.22 and are variously present in the varieties studied. Of these, Rf. values of 0.01, 0.03, 0.09, 0.17 and 0.22 occur most frequently. The approximate molecular weights of the albumin components, as found by SDS polyacrylamide gel electrophoresis, lie between 41,000 and 69,000.

For an objective assessment of varietal differences, the relative areas of two globulin peaks at Rf. 0.27 and 0.43 were subjected to the analysis of variance. Varieties showed significant differences between themselves: environmental effects due to locations or years were found to be insignificant. By using Duncan's multiple range test, varieties could be separated from each other with respect to both or either of the peak areas (Tables 1 and 2).

In addition, the presence or absence of peaks (both albumins and globulins) can also be used to separate the varieties (Figs. 1–8). Where, however, a large number of varieties are involved, separation by the relative areas of selected peaks (albumins or globulins) will be a more reliable method. It may be mentioned that some 16 additional varieties were also successfully separated out using this method (unpublished data).

The densitograph profile of each individual variety was found to be independent of growing conditions, an observation similar to previous findings reported in wheat (Zillman et al. 1979) and in barley (Marchylo et

Table 1. Analysis of variance – relative areas of globulin peaks at Rf. 0.43 and 0.27 (varieties grown in Falta)

Source of variation	d.f.	Relative area of globulin peak – Rf. 0.43	d.f.	Relative area of globulin peak – Rf. 0.27
		M.S.		M.S.
Varieties	4	985.365*	7	581.145*
Replications	2	0.723	2	0.953
Error	8	2.346	14	0.712

* $P < 0.001$

Multiple Range Test (Duncan) – mean relative areas of globulin peaks – (a) Rf. 0.43 and (b) Rf. 0.27. Non-significant differences are underscored by a common line

(a)	'Patnai 23'	'IR-8'	'Hsinchu-4'		
	49.9	43.4	19.6		
			'Kameji'	'Maharashtra NCS. 2272'	
			11.5	11.2	
(b)	'RP'	'Basmati 370'	'IR-8'		
	42.1	33.4	26.2		
		'Patnai 23'	'Maharashtra NCS. 2272'	'Hsinchu-4'	
		21.2	20.8	19.4	
		'ARC. 6136'	'Kameji'		
		10.5	6.6		

Table 2. Analysis of variance – relative areas of globulin peaks at Rf. 0.43 and 0.27 (varieties grown in two locations/years)

Source of variation	d.f.	Relative area of globulin peak – Rf. 0.43		d.f.	Relative area of globulin peak – Rf. 0.27	
		Location Year			Location Year	
		M.S.	M.S.		M.S.	M.S.
Varieties	4	653.198*	579.799*	7	261.958*	258.504*
Locations/years	1	0.784	0.441	1	0.226	0.090
Error	4	0.161	1.361	7	0.137	0.771

* $P < 0.001$

al. 1980). This distinctiveness of the electrophoretic profile of each individual variety is therefore, essentially, under the control of genetic factors. These variations could be conveniently used in the identification of cultivars.

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