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VARIETY IDENTIFICATION OF SOME RICE CULTIVARS BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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Abstract

Proteins of 5 rice cultivars grown in Hungary and 7 varieties grown in Vietnam were studied. After defatting of the samples with hexane or hexane and chloroform-methanol (1:1) mixture, the proteins were extracted with a TRIS-glycine buffer (pH=6.8) containing SDS and beta-mercaptoethanol. The protein extract was investigated by SDS-PAGE. Significant differences were found both in subunit distribution and intensities of the patterns making possible the identification of varieties.

Keywords: rice, protein, variety, identification, SDS-PAGE.

Introduction

The variety identification of cereals based on electrophoretic patterns of some protein fractions started with variety identification of wheat cultivars (BUSHUK – ZILLMAN, 1978). Later the electrophoretic variety identification was introduced also for investigation of other cereals. A review about methods used was published by WRIGLEY (1995). In Hungary, based on gliadin PAGE, a catalogue of electrophoretic spectra of 60 wheat varieties grown in Hungary, together with practical guidelines of variety identification was published (LÁSZTITY et al., 1985). Concerning other cereals grown in Hungary, including rice, there are no data at present whether the same technique might be useful in variety identification of this crop. Several authors reported about electrophoretic variety identification of rice. SARKAR – BOSE (1984) found that the single seed salt soluble rice proteins are suitable for electrophoretic variety identification. About the possible use of prolamins in rice variety identification GUO et al. (1986) and HUEBNER et al. (1991) published some data.

As it is generally known (LÁSZTITY 1996), the main protein fraction of rice is the glutelin, also called oryzanin (about 70 – 80% of total protein). In the experiments realized in our laboratory the possibility of using subunits of oryzanin for variety identification by electrophoresis was studied.

Materials and Methods

Materials

The investigated varieties and their codes are shown in *Table 1.* As seen, 5 Hungarian rice varieties and 7 varieties originating from Vietnam were studied. The latter samples were received from the Agricultural Research Institute of Vietnam. The varieties No. 1, 2 and 5 have been grown in Vietnam for a long time, other varieties originate from China and India and were imported 4 – 5 years ago. In *Table 2* some characteristics of the investigated rice varieties are listed.

Table 1
Investigated varieties

Code	Variety (cultivar)
1	CR-203
2	TAM THOM
3	TUONG HUONG
4	559
5	NEP THOM
6	NEP TK 79
7	NEP ANDO
8	Oryzella
9	Karmina
10	ÖKI -3-1-NÖMIS 5
11	HSC-2-SE 6
12	Sandora-4 NÖMIS 5

Table 2
Characteristics of some varieties grown in Vietnam

Characteristic	Rice variety			
	CR 203	Tam Thom	Tuong Huong	559
Vegetation period (days)	110 – 125	110 – 120	80 – 90	150 – 155
Period of growing	Jan. – Apr.	Jan. – Apr.	Jan. – Apr.	Jan. – Aug.
Kernel weight (g)	23 – 24	25 – 26	18 – 20	18 – 20
Height of plant (cm)	90 – 95	>100	>100	120 – 130
Yield (kg/360 m^2)	180 – 220	180 – 220	150 – 170	100 – 120
Quality	good	good	good, flavory	good, flavory

Methods

Extraction of proteins

Two procedures were used. In the first, the rice meal was defatted by hexane and chloroform methanol (1:1) mixture. After defatting, albumins, globulins and prolamins were stepwise extracted with distilled water, 5% sodium chloride and 70% ethanol, respectively. The remaining proteins (glutelins, oryzanin) were extracted with a TRIS-glycine buffer (pH=6.8) containing 4% sodium dodecylsulphate (SDS) and 1.5% beta-mercaptoethanol.

In the second procedure the defatted rice meal was directly extracted with the TRIS-glycine-SDS-mercaptoethanol solution mentioned above, and this extract was used for further investigations without additional fractionation.

Polyacrylamide gel electrophoresis (PAGE)

The SDS-PAGE method of LAWRENCE – SHEPHERD (1980) was used with slight modifications. An apparatus for vertical gel electrophoresis (Type ELTE, Hungary) was applied, using plates of 160×120×2.5 mm. The applied voltage was 100 V and the time of electrophoresis about 4 hours. A 10% gel concentration and TRIS-glycine buffer (pH 8.3) were applied. Protein bands were visualized with Coomassie Brillant Blue R-250 in a solution containing trichloroacetic acid, acetic acid and methanol.

Results and Discussion

The electropherograms of the investigated rice samples were evaluated by a combined densitometry-computer system. It was found that the use of oryzanin extracts produced with sequential solubilization has no significant advantages from the point of view of variety identification, in comparison to the one-step extraction of total protein. So the latter, simpler procedure may be recommended for electrophoretic variety identification.

In *Fig. 1* the computer-transformed electrophoretic patterns of the 12 varieties investigated are shown. Even at first look it is clear that the electrophoretic spectra are characteristic of different varieties. The protein bands occur in a wide range of molecular weights from 17 to 105 kD. On the basis of molecular weight distribution of protein (polypeptide) components,

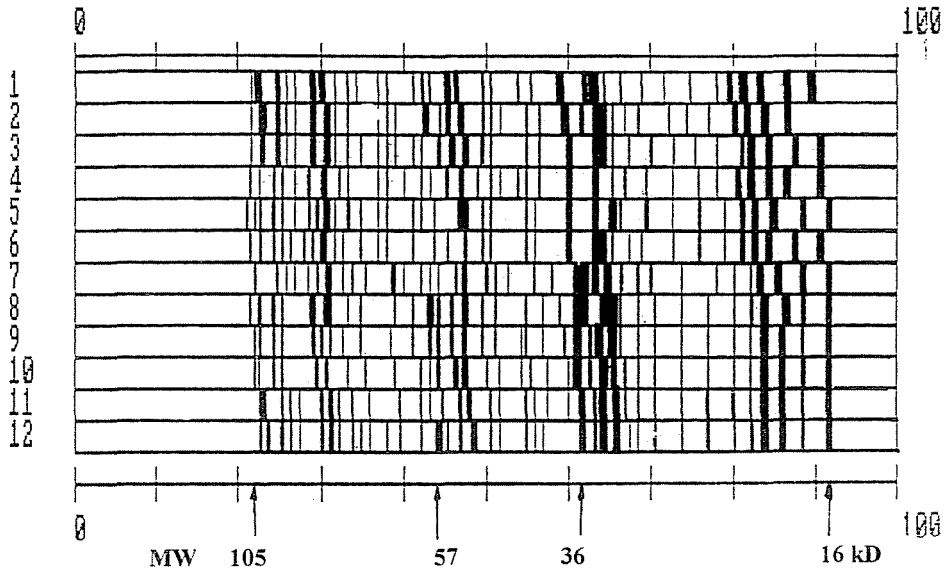

Fig. 1. Characteristic electropherograms of rice varieties 1. CR-203, 2. TAM THOM, 3. TUONG HUONG, 4. 559, 5. NEP THOM, 6. NEP TK 79, 7. NEP ANDO, 8. Oryzella, 9. Karmina, 10. ÖKI-3-1-NÖMIS 5, 11. HSC-2-SE 6, 12. Sandora-4 NÖMIS 5

the varieties may be divided into groups having similar distribution as follows (the numbers in parentheses are the codes of the samples):

I. Oryzella (8)
   Karmina (9)
   Sandora-4 NÖMIS 5 (12)
II. ÖKI-3-1-NÖMIS 5 (10)
   HSC-2-SE 6 (11)
III. CR-203 (1)
   TUONG HUONG (3)
   559 (4)
IV. TAM THOM (2)
   NEP TK 79 (6)
   NEP ANDO (7)
V. NEP THOM (5)

It was found that the differences in bands of Hungarian varieties are mainly in the molecular weight range 30 – 60 kD. In the other parts of

electropherograms great similarity was observed. For group I., the characteristic bands are as follows (in parentheses the intensities of bands on a scale from 1 to 10 are given): 58.8 kD (7), 57.2 kD (4), 54.2 kD (4), 52.6 kD (7). In group II., the band 58.8 kD is missing and a new band at 49 kD appears with low (1, 3) intensity. In group III., specific bands at 30.1 kD and 32.8 kD were found. The band at 60.4 kD occurs only in the variety TUONG HUONG. Some differences were observed within the group in intensities. In group IV., the bands of 31 kD and 32 kD are also present. The TAM THOM variety possesses a specific band of 59.6 kD (8). The remaining two varieties differ in the intensities of the bands 30 kD and 33.2 kD. Finally, the NEP THOM variety may be distinguished from all other varieties grown in Vietnam by bands 29.3 kD and 86.8 kD.

Conclusion

The results of investigations revealed that the subunit distribution of oryzanin may be the basis of electrophoretic variety identification of rice grains. The most suitable procedure includes defatting of meal by hexane and chloroform-methanol (1:1), extraction of proteins with TRIS-glycine-SDS-mercaptoethanol buffer and SDS-PAGE in TRIS-glycine buffer (pH=8.3).

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