Seed protein electrophoresis for varietal identification of rice

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ABSTRACT

Tris-HCl buffer soluble seed storage proteins from ten rice varieties were analysed using sodium dodecyl sulphate polyacrylamide gel electropheresis (SDS-PAGE) on 12% gels. The resultant banding patterns revealed moderate degree of polymorphism but enabled identification of all varieties on the basis of presence or absence of bands confirming the distinguishing power of this procedure for varietal identification of rice. The percentage similarities between all possible pairs of varieties were calculated and varied between 38.4 to 92.8 percent. The possible application of protein markers for DUS (distinctness, uniformity and stability) testing for the grant of the plant variety protection is discussed.

Key words: *DUS testing, polymorphism, Rice, SDS-PAGE, varietal identification*

Varietal identification has attained critical importance in India in view of the increasing multiplicity of varieties and imminent implementation of the protection of Plant Varieties and Farmers Rights Act, 2001. A breeder variety must fulfill the criteria of Distinctness, Uniformity and Stability (DUS) to be given protection under this Act. It is thus imperative to characterize all varieties of common knowledge and to prepare and maintain a comprehensive database. A good number of varieties have been developed and notified in recent past, out of which many are now in seed production chain. However, there is lack of compilation reflecting the key diagnostic characters of these varieties. Thus, there is a need of comprehensive characterization of rice varieties to protect the rights of breeders/breeding institutions.

Markers detected from seed protein electrophoresis are known to be quite stable and have been effectively employed for varietal identification in several crops including rice (Park and Stegemann 1979, Sarkar and Bose 1984, Aliaga-Morell *et al*. 1987, Hussain *et al*. 1989, Habib *et al*. 2000). Detection of protein markers involves relatively simple and inexpensive technique as compared to DNA markers. Electrophoresis of total seed proteins results in a large number of overlapping bands leading to confusion while scoring and interpretation. Analysis of individual fractions, however, could improve resolution thereby facilitating detection of distinct and reproducible banding patterns. Thus, the present study was conducted with the aim to distinguish commercial rice varieties of Kashmir using tris-soluble seed protein profiles and to assess their uniformity and stability.

Ten rice varieties (K-39, K-332, Jhelum, Chenab, Shalimar Rice-1, Kohsaar, Barkat, SKAU-337, SKAU-341and SKAU-382) obtained from the collection of the Rice Research and Regional Station, Khudwani (Anantnag), Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu and Kashmir for study. Sodium dodecyl sulphate polyacrylamide gel electropheresis (SDS-PAGE) of the total soluble proteins of the selected rice genotypes was done as per the method of Laemmli (1970). Proteins were extracted in 0.1M tris-HCl buffer, pH 7.5. Ten dehusked seeds of each variety were crushed in mortar and pestle and treated with 5ml of defattening solution (mixture of methanol , chloroform and acetone in the ratio of 2:1:1) to remove oil. 0.2 ml of the extraction buffer was added to the deoiled powder to make slurries which were transferred into polypropylene centrifuge tubes and centrifuged at 10,000 rpm at 10^0C for 10 minutes. The clear supernatant was used as sample for SDS-PAGE and 100 µl of each sample was loaded. The experiment was repeated, wherever

necessary, to get well stained gels with good resolution of bands. Electrophoresis was conducted under constant current (40mA) at 10^0C. The gels were fixed in 10% acetic acid before staining with Coomassie brilliant blue R 250. The relative mobility (Rm) values were calculated for each band. The similarity index value for each pair of the ten rice cultivars was calculated from their polypeptide banding patterns using the formula : Number of pairs of similar bands / Number of pairs of similar bands+ Number of different bands (Vaughan and Denford 1968).

The electrophoresis of tris-soluble seed proteins revealed a moderate degree of polymorphism among the rice varieties studied. The scoring was done for the presence or absence of bands, which were identified by their respective Rm and numbered in sequence from cathodal origin. A total of nineteen bands with Rm values ranging from 0.32 to 1.00 was observed. Five bands *i.e.*, number 1, 3, 8, 17 and 19 were common to all the varieties (Fig-1).

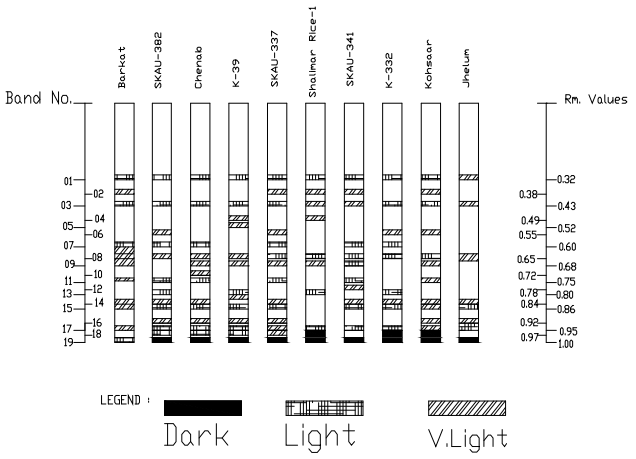

Fig 1. Zymogram displayed by soluble seed proteins of ten rice varieties

The soluble protein bands characteristic of only a particular variety included band number 5 (K-39), band number 10 (Chenab) and band number 12 (SKAU-341). Only band number 4 was distinctly found in two varieties in K-39 and Shalimar Rice-1. None of the bands was found common and distinct in three varieties whereas two bands (number 6 and 13) were distinctly marked in a group of four varieties that included SKAU-382, SKAU-337, K-332 and Kohsaar exhibiting band number 6 and SKAU-382, K-39, Shalimar Rice-1 and K-332 having band number 13 in common. Similarly the band number 2 was common and distinct in a group of five varieties (SKAU-337, Shalimar Rice-1, K-332, Kohsaar and Barkat); band number 7 common and distinct in a group of six rice varieties (SKAU-382, Chenab, SKAU-337, SKAU-341, K-332 and Barkat); band number 11 in six varieties (SKAU-382, Chenab, SKAU-337, SKAU-341, Kohsaar and Barkat) and band number 16 also in six varieties (SKAU-382, Chenab, SKAU-337, K-39, Kohsaar and Jhelum). The Similarity index (SI) values (Table 1) for each pair from ten rice cultivars under study revealed that the maximum similarity (92.8) exists between Kohsaar and SKAU-337 followed by Barkat and SKAU-341 (91.6). Least similarity (38.4) was observed between variety Shalimar Rice-1 and Jhelum. Variety specific banding patterns have also been found for salt soluble (Sarkar and Bose 1984) and alcohol soluble (Peruanskii and Savich 1990) seed proteins of rice.

SDS-PAGE is, thus, a simple but useful technique, which can be used for the identification and characterization

Table 1. Percentage similarities between the varieties based on the soluble protein component homologies

Genotypes		1	2	3	4	5	6	7	8	9	10
Jhelum	1	-	61.5	58.3	53.8	38.4	57.1	61.5	61.5	61.5	58.3
Kohsaar	2		-	60.0	66.6	53.5	92.8	62.5	73.3	73.3	71.4
K-332	3			-	53.3	50.0	66.6	60.0	60.0	84.6	57.1
SKAU-341	4				-	46.6	85.7	47.0	66.6	56.2	91.6
Shalimar Rice-1	5					-	50.0	64.2	43.7	43.7	50.0
SKAU-337	6						-	58.8	85.7	85.7	78.5
K-39	7							-	62.5	62.5	50.0
Chenab	8								-	73.3	71.4
SKAU-382	9									-	60.0
Barkat	10										-

of rice varieties and have been used in other crop species (Nagaraju *et al*. 2000). International Seed Testing Assocaition (ISTA 1999) has recommended SDS-PAGE as a standard method for verifying the identity of varieties of Tris-soluble proteins in *Pisum* and *Lolium.* (UPOV 1994) has recommended SDS-PAGE for high molecular weight glutenins in wheat. In case of rice, the technique appears to be useful for the grouping of varieties for further characterization.

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