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# Rice varietal identification by isozyme analysis

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## ABSTRACT

*The investigation was carried out involving characterization of ten rice varieties cultivated in the Kashmir valley on the basis of morpho-agronomic characters and biochemical marker system. Marked differences were developed among the ten varieties on the basis of plant morphology. The alkaline PAGE of three isozymes (esterase, peroxidase and malate dehydrogenase) showed considerable polymorphism whereas, one isozyme (alcohol dehydrogenase) showed little polymorphism. Dendrogram of isozyme data revealed the overall differences existing in the rice varieties. The possible application of these markers for differentiation and identification of closely related rice varieties is discussed.*

***Key words:*** *Isozymes, morphology, polymorphism, rice and varietal identification*

Accurate identification keys based on morphological parameters with clear cut features of distinctness are not always possible. Frequently morphological variations between cultivars within a plant species are so unclear that it is difficult to distinguish different cultivars (Kallo *et al*., 2001). Wilkinson and Beard (1972) reported that morphological description of plant cultivars often pose problems in clear cut identification because the phenotypic differences within plant species are too minute to discriminate. Thus, other characteristics obtained with the help of new laboratory based technologies, supplement the need for precise description. Gel electrophoresis of isozymes is a powerful tool to distinguish between genotypes of plant species. The proteins, being direct gene products, reflect the genomic composition of varieties and hence are good candidates for varietal distinctiveness.

To sustain the high production and productivity in rice, a good number of varieties have been developed and notified in recent past, out of which many varieties are now in seed production chain. However, there is lack of compilation reflecting the key diagnostic characters of these varieties that is very essential to carry out scientific seed production and certification, to enforce proper quality control and promote the seed trade in the emerging present scenario of private sector involvement.

An attempt was therefore, made with the aim to distinguish commercial rice varieties of Kashmir on the basis of morphoagronomic characters and isozymes and use the information to supplement the effort during variety identification programmes in seed production chain.

## MATERIALS AND METHODS

Ten rice genotypes (K-39, K-332, Jhelum, Chenab, Shalimar Rice-1, Kohsaar, Barkat, SKAU-337, SKAU-341 & SKAU-382) were studied in the investigation. All the genotypes were obtained from Rice Research and Regional Station, Khudwani (Anantnag), Kashmir.

These varieties were studied for sixteen plant morphological characters viz. Plant habit, Leaf colour, Leaf pigmentation , Leaf pubescence, Flag leaf angle, Leaf sheath colour, Collar colour, Ligule colour, Ligule shape, Auricle colour, Stigma colour, Panicle type, Secondary branching, Panicle exertion, Panicle axis and Apiculus colour as per the standard procedure suggested by IRRI (Annonymous, 1985). For generating data on each trait, a random sample of 10 plants in each replication was studied.

Employing the polyacrylamide gel electrophoresis technique using 7% gels, the isozyme banding patterns were studied. Extraction was done

by grinding seven day old etiolated seedlings (five seedlings randomly taken for each variety) with 0.1-0.2 ml of extraction solution (0.1M Tris-HCl buffer, PH 7.5) with the help of pestle and mortar. For ADH, the extract was prepared by crushing the presoaked seeds in 0.2 ml of extraction buffer and thereafter the crushed material was transferred to chilled eppendorf tubes which were subsequently spinned in a refrigerated centrifuge at 10,000 revolutions per minute (rpm) for 10 min at 4°C. The clear supernatant was used as sample for electropheretic analysis and 70µl of each sample was loaded. The electrophoresis was conducted using Tris-glycine buffer (pH 8.3) at a constant current of 45mA/gel (Shaw and Prasad, 1970).

The zymogram of isozymes were drawn for calculating the relative mobility (Rm) as the ratio of the distance of each band from the origin to the distance of the dye front. The Rm value for each band was computed from the mean of observations from four independent electrophoretic runs on two separate extractions. The dendrogram depicting the relationship of the varieties were constructed on the basis of presence/absence of bands.

## RESULTS AND DISCUSSION

The rice genotypes could be characterized into two different classes on the basis of plant habit (compact and open), three on the basis of leaf colour (green, pale green, and dark green), three on the basis of leaf pubescence (glabrous, medium and strong), two on the basis of flag leaf angle (Intermediate and erect), two on the basis of leaf sheath colour (green and dark green), three on the basis of ligule shape (cleft, 2-cleft and 3-cleft), two on the basis of auricle colour (green and pale green), three on the basis of stigma colour (white, light green and purple), two on the basis of panicle type (lax and compact), two on the basis of secondary branching (light and heavy), two on the basis of panicle exertion (moderately well exerted and well exerted) and two on the basis of apiculus colour (yellow and reddish brown) (Table 1). A number of morphological markers of the plant (leaf pigmentation, collar colour, ligule colour, and panicle axis) could not discriminate the genotypes and the possible reason for it may be attributed to increased number of genetically related releases by plant breeders that has made unique identification especially by morphological markers more difficult to achieve (Smith and Smith 1989). The problem of unique identification becomes more acute when convergent selection towards similar morphologies is practised.

An analysis of the alkaline polyacrylamide gel electrophoresis (PAGE) for three isozymes (esterase, peroxidase and malate dehydrogenase) extracted from seedlings and alcohol dehydrogenase isozyme extracted from seeds revealed moderate to low polymorphism among the ten rice varieties (Fig 1). Banding pattern for esterase revealed that the cultivar SKAU-337 could be identified by the presence of a specific band (no. 6) and absence of another band (no.5). Among other genotypes studied, Jhelum could be distinguished from Chenab and Shalimar Rice-1 by the absence of band no's. 2, 3 and 4. Likewise, Chenab could be distinguished from Shalimar Rice-1 by the absence of band no's. 1, 9 and 11 and presence of a specific band no. 14. Similarly, other varieties with respect to esterase isozyme could be identified on the basis of presence or absence of other bands. Similar results in rice have been reported by Yen (1987) and Habib *et al.* (2000). Peroxidase isozyme showed a total of nine bands differing in intensity from dark to very light. Barkat showed a characteristic band (no.8) for peroxidase. SKAU-332 and Kohsaar were distinguishable from other genotypes by the complete absence of band no.4. Likewise Shalimar Rice-1 could be distinguished from Jhelum and Chenab by the presence of band no. 3 and 5. The intervarietal zymogram variation of peroxidase could be used as an aid in varietal characterization as has been reported by Shahi *et al.* (1969), Srivastava *et al*. (2002) and Baishya *et al*. (2003). Isozyme polymorphism for malate dehydrogenase was comparatively less marked than that for peroxidase and esterase and frequency of electrophoretic variants was still lesser in the case of alcohol dehydrogenase. None of the varieties could be characterized for unique bands with respect to both the isozymes, though polymorphism was observed for several bands in malate dehydrogenase that could enable unique identification of these varieties. The banding pattern of alcohol dehydrogenase could not be found as an ideal discriminator among the genotypes as this isozyme did not reveal much significant variation in banding patterns of genotypes in the present investigation. Similar results were observed by other researchers like Habib *et al*. (2000) and Nasser (2002).

**Table 1. Showing plant morphological characters for ten rice genotypes.**

| Characters | Genotypes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Jhelum | SKAU-337 | SKAU-341 | Kohsaar | K-39 | SKAU-382 | Chenab | Shalimar Rice-1 | Barkat | K-332 |
| Plant habit | Compact | Compact | Compact | Compact | Compact | Compact | Compact | Compact | Compact | Open |
| Leaf colour | Green | Dark green | Pale green | Dark green | Dark green | Green | Dark green | Dark green | Dark green | Dark green |
| Leaf pigmentation | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| Leaf pubescence | Medium | Glabarous | Strong | Medium | Medium | Medium | Medium | Medium | Medium | Glabarous |
| Flag leaf angle | Erect | Erect | Erect | Intermediate | Erect | Erect | Intermediate | Erect | Intermediate | Intermediate |
| Leaf sheath colour | Green | Green | Green | Green | Green | Green | Green | Green | Green | Dark green |
| Collar colour | Green | Green | Green | Green | Green | Green | Green | Green | Green | Green |
| Ligule colour | White | White | White | White | White | White | White | White | White | White |
| Ligule shape | Cleft | 2-cleft | 2-cleft | 2-cleft | 2-cleft | 2-cleft | Cleft | 3-cleft | 2-cleft | 2-cleft |
| Auricle colour | Pale green | Pale green | Pale green | Pale green | Pale green | Pale green | Pale green | Green | Pale green | Pale green |
| Stigma colour | Light green | Whitish | Whitish | Whitish | Whitish | Whitish | Light green | Purple | Whitish | Whitish |
| Panicle type | Compact | Lax | Lax | Compact | Compact | Compact | Compact | Compact | Compact | Compact |
| Secondary branching | Heavy | Heavy | Heavy | Heavy | Heavy | Light | Heavy | Heavy | Heavy | Heavy |
| Panicle exertion | Well exerted | Well exerted | Well exerted | Well exerted | Well exerted | Well exerted | Well exerted | Well exerted | Moderately Well exerted | Well exerted |
| Panicle axis | Straight | Straight | Straight | Straight | Straight | Straight | Straight | Straight | Straight | Straight |
| Apiculus colour | Yellow | Yellow | Yellow | Yellow | Yellow | Yellow | Yellow | Yellow | Reddish brown | Reddish brown |

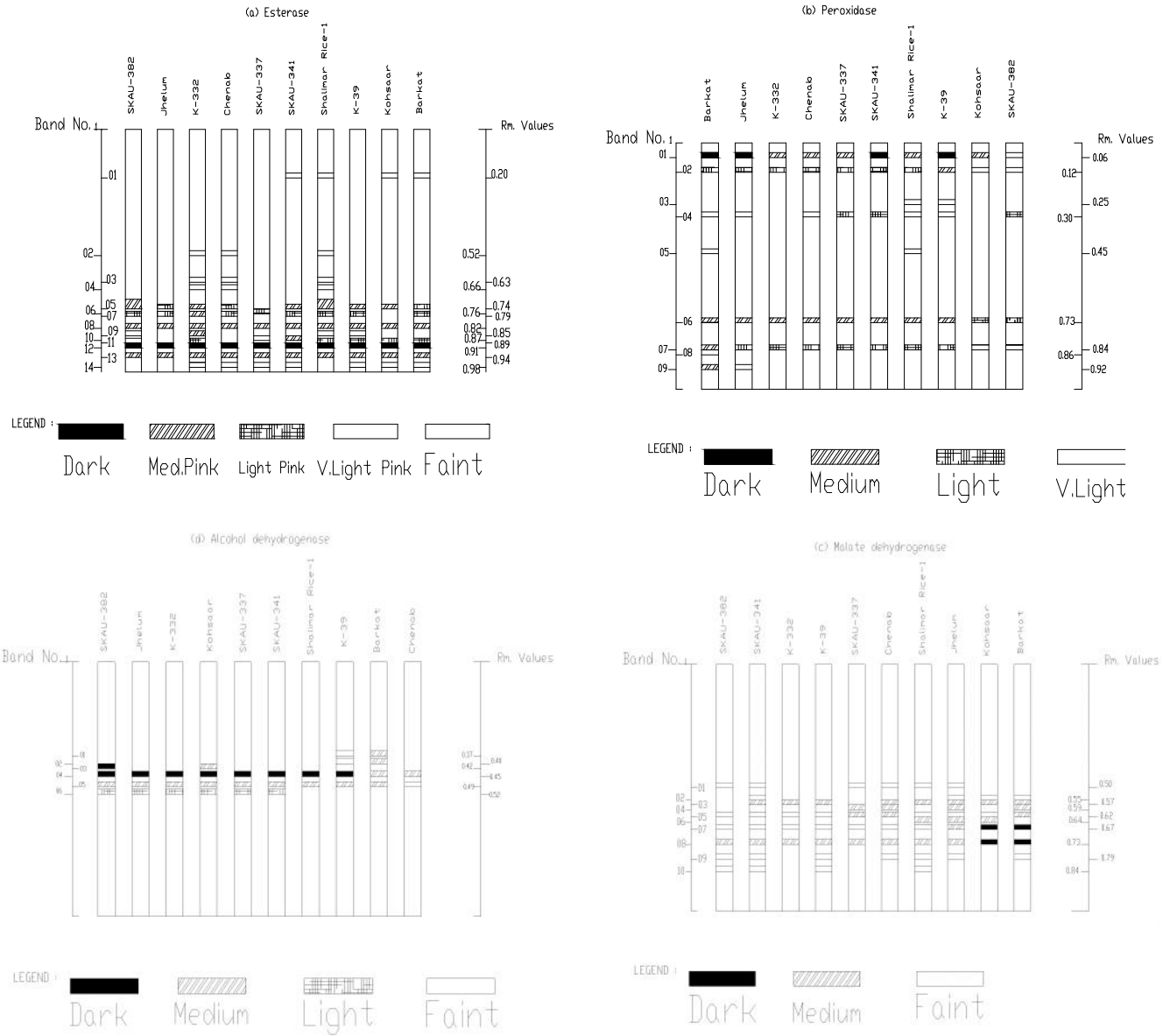


**Fig. 1.** The electrophoretic pattern of four isozymes in the representative varieties showing number and intensity of different isozyme bands; Esterase (a) , Peroxidase (b) , Malate dehydrogenase (c) and Alcohol dehydrogenase (d).

Cluster analysis using the combination of four isozymes revealed clear genetic relationship within the rice varieties (Fig 2). Rice variety Barkat, popular in high altitude regions of the valley was observed to be distinct from other varieties except Kohsaar, another variety suitable for high altitude regions. Intrestingly both the varieties share a common parent (IET-1444). Shalimar Rice-1, K-39 and Jhelum were also observed to be closely related probably because of their derivation from the same gene pool. These varieties as well as Chenab are popular in the lower altitude niches of the valley.

It can be concluded that in addition to morphological characters studied, the isozyme markers can be used for distinguishing varieties of rice through determination of polymorphism. It was also observed that a single isozyme by itself would not be useful to detect the full range of variability among the varieties. This study on four isozymes was probably optimum to bring out varietal differences besides reflecting the overall relationship among the varieties through

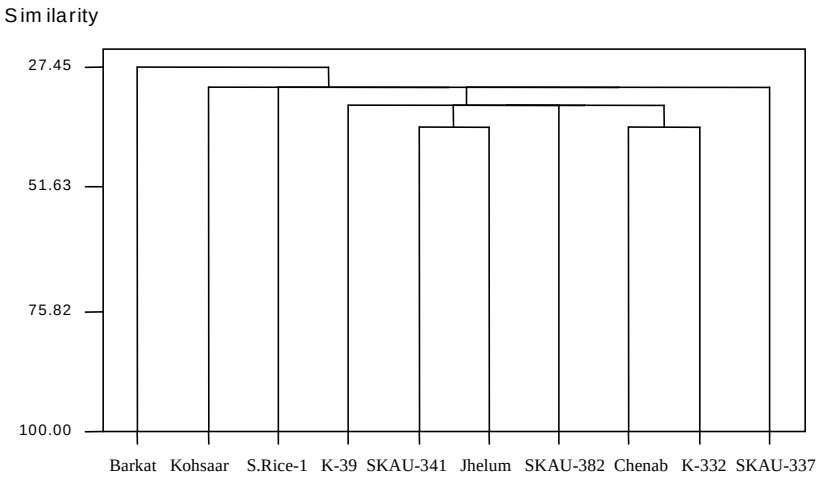


**Fig. 2.** Dendrogram showing the relationship of ten rice varieties

dendrogramic studies.

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