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# Identification of parental lines and rice hybrid (KRH-4) using protein and isozyme electrophoresis

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***Abstract****: Identification and characterization of parental lines/hybrid are important for seed trade. The applicability of protein and isozyme markers was studied for identification of rice hybrid (KRH-4) and its parental lines (CRMS32A and MSN36). The electrophoretic banding pattern of total soluble seed protein profile and isozymes revealed that there was no significant difference between parental lines and rice hybrid. Both parental lines and hybrid exhibited similar number of bands and relative mobility. The parental lines can be distinguished from hybrid based on relative intensity in the region E, F and G. In region E and F, KRH-4 (H) exhibited dark intensity band, while CRMS32A (A) exhibited medium intensity band and light intensity band was observed in MSN36 (R). Further, MSN36 (R) can be differentiated from CRMS32A (A) and KRH4 (H) based on relative intensity in the banding pattern of the region G. In region G, CRMS32A (A) and KRH4 (H) exhibited a medium intensity band as where in MSN36 (R) light intensity band was observed. Further in the isozyme banding pattern of alcohol dehydrogenase, hybrid and its parental lines differed in terms of intensity.KRH-4 exhibited dark intensity band where as CRMS32A (A) and MSN36 (R) exhibited light intensity band.*

## I. Introduction

Rice is the principal food crop grown across 18 different countries feeding more than half of the world's population. India is the second largest rice producer. The estimated area under rice in India is over 44.4 million hectare, with an annual production of 80.65 million tonnes with productivity of 2177 kg/ha. Tropical rice growing countries including India need to set up their rice production, because of increasing population and decreasing land, water resources. Hybrid rice cultivation offers an opportunity to increase seed yield and there by ensure a steady supply of rice (Virmani and Ish Kumar, 2004). Large number of high yielding hybrids has been released by public and private sector hence unambiguous identification of hybrids is essential for their protection and prevention of unauthorized commercial use. Beside india being a signatory to the GATT (General Agreement on tariff and trade) agreement. With the introduction of IPR at the global level, it has become imperative to register, characterize and prepare documentation of hybrids/varieties in seed production chain. For registration of variety /hybrid, the government of india has enacted its 'sui generic 'system called Protection of Plant Varieties and Farmers Rights Act 2001. In view of the above fact the identification of hybrid gained more importance and more over the ability to distinguish and identify the crop varieties and hybrids is a fundamental operation in seed trade (Cooke, 1984).

The traditional method usually record expression of morphological characters at different stages of the crop. The expression of these morphological characters is not stable over the year and location due to high interaction with environment. Moreover, it also becomes difficult to distinguish between parental lines and hybrid by morphological characters alone (Kanchan Singh *et al*., 2006). Ideally the difference between parental lines and hybrid should be based on the gene differences but direct comparison of gene is difficult and time consuming. However the differences can be measured by comparing the product of gene activity i.e. by using protein or isozyme (Kalloo *et al*., 2001).

Many workers reported that qualitative and quantitative variations among the different varieties, hybrids and its parental lines can be well-known by presence or absence of specific bands, $R_m$ and intensity of bands value in SDS-PAGE of total soluble seed proteins (Rao *et al* 2012 ; Nethra *et al.* 2007)

Therefore, the present investigation was undertaken to study the possibility of applying an alternative method protein electrophoresis to identify the parental lines and hybrid. KRH-4 is a newly released hybrid, which is non-scented, non- shattering, with a less dormancy period (10-15days) and grain yield of 8 to 8.5t/h. It contains higher amount of protein (8.52%), fat (1.38%), and carbohydrate (76.88%) compared to KRH-2.

## II. Material and Methods

Pure seeds of parental lines and rice hybrid (KRH-4) were obtained from ZARS, VC Farm, Mandya. SDS-PAGE of total soluble seed protein was carried out by using 15 per cent gel according to the method prescribed

by Laemmli (1970) with slight modifications. Protein was extracted from single seed of each genotype by adding 0.2 ml Tris glycine extraction buffer (25mM, pH 8.5). Incubated for overnight at -20$^0$c. The suspension was centrifuged at 10,000 rpm for 15 minutes. The extract was dissolved in equal amount of working buffer (Tris-HCl 0.0625 M, pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 15% glycerol and 0.001% bromophenol blue) and kept in boiling water for 2 minutes, again centrifuged and the supernatant was used for loading in 15 per cent gel. Gel stained with coomassie brilliant blue was scored for the presence or absence of bands in both the parental lines and hybrid. Relative mobility (RM) and similarity index (SI) between parental lines and hybrid were measured. Two isozymes *viz.,* Alcohol Dehydrogenase (ADH) and Malate Dehydrogenase (MDH) were analyzed for isozymic pattern as described by Glaszman *et al.* (1988) with slight modifications. Four to six day old single seedlings was ground thoroughly in pestle and mortar with liquid nitrogen and then a pinch of PVP was added along with 200 µl of extraction buffer. The extract was taken in 2 ml centrifuge tube and incubated at 4$^0$C for extraction of enzyme for two hours. After two hours, sample was centrifuged at 12,000 rpm for 15 minutes at 4$^0$C. The supernatant was collected and 10 µl of tracking dye (1% bromophenol blue and drop of glycerol) was added and again centrifuged at 12,000 rpm for 5 minutes at 4$^0$C. 50 µl supernatant was loaded in to the gel for electrophoresis. The gels were incubated for different isozyme activity staining systems by adding suitable substrate. When the isozyme bands developed, the reaction was stopped with 7% acetic acid and photographed immediately.

## III. Results and Discussion

Electrophoretic protein profiles could be efficiently used for distinguishing varieties, hybrids and its parental lines. Since proteins are the direct products of genes, many workers have attempted to classify rice genotypes on the basis of electrophoretic pattern of protein profiles (Nandita Patra and Chawla, 2010; Rao *et al*., 2012). Hence, an attempt was made to reveal the variations in total soluble seed protein profiles of parental lines (CRMS32A and MSN36) and rice hybrid (KRH-4). The proteins separated on 15 per cent gel could be distinguished and grouped based on the standard molecular weight marker (100 kD). Total soluble seed protein profile of parental lines and rice hybrid (KRH-4) was fractionated into 11 bands (Figure 1). Both parental lines and hybrid exhibited similar number of bands and relative mobility (Table 1 and Table 2). The highest number of bands was observed in the region D (51 to 30 kD) and region E (30 to 18 kD). The parental lines can be differentiated from hybrid based on intensity in the region E, F and G. In region E and F (Table 3), KRH-4 (H) exhibited dark intensity band, while CRMS32A (A) exhibited medium intensity band and light intensity band was observed in MSN36 (R). Further, MSN36 (R) can be differentiated from CRMS32A (A) and KRH4 (H) based on relative intensity in the banding pattern of the region G. In region G, CRMS32A (A) and KRH4 (H) exhibited a medium intensity band as where in MSN36 (R) light intensity band was observed. Hence the parental lines and rice hybrid (KRH-4) can be classified based on relative intensities but not on the number of bands and relative mobility. Similar observation was also made by Nethra *et al.* (2007).

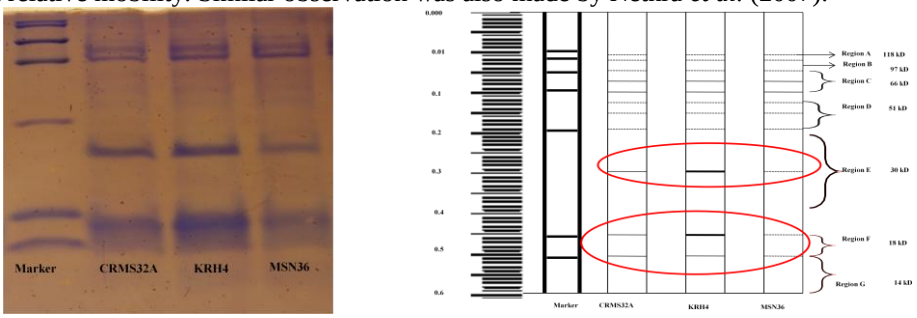


**Figure 1 Total soluble seed protein profileand an electrophoregram of parental lines and rice hybrid**

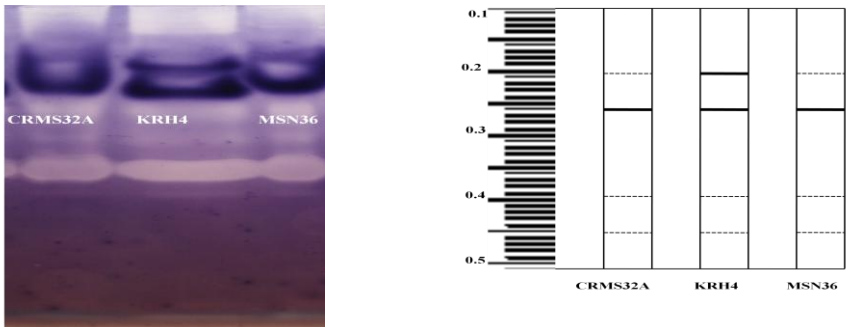


**Figure 2 Isozyme banding pattern and Zymogram of alcohol dehydrogenase of parental lines and rice hybrid (KRH-4)**

**Table 1. Intensity and relative mobility of total soluble seed proteins of parental lines and rice hybrid (KRH-4)**

| Band Number | $R_m$ Value | Female Parent (CRMS32A) | Hybrid (KRH-4) | Male Parent (MSN36) |
|---|---|---|---|---|
| 1 | 0.02 | + | + | + |
| 2 | 0.03 | + | + | + |
| 3 | 0.06 | + | + | + |
| 4 | 0.08 | ++ | ++ | ++ |
| 5 | 0.11 | ++ | ++ | ++ |
| 6 | 0.14 | + | + | + |
| 7 | 0.16 | + | + | + |
| 8 | 0.2 | + | + | + |
| 9 | 0.3 | ++ | +++ | + |
| 10 | 0.46 | ++ | +++ | + |
| 11 | 0.55 | ++ | ++ | + |

+ Light Intensity; ++ Medium Intensity; +++ Dark Intensity

**Table 2. Number of total soluble seed protein bands observed in parental lines and rice hybrid (KRH-4)**

| Genotype | Light intensity | Medium intensity | Dark intensity | Total number of bands |
|---|---|---|---|---|
| CRMS32A | 3 | - | 1 | 4 |
| MSN36 | 3 | - | 1 | 4 |
| KRH-4 | 2 | - | 2 | 4 |

**Table 3. Region wise classification of protein bands in parental lines and rice hybrid (KRH-4)**

| Genotypes | Region A | | | Region B | | | Region C | | | Region D | | | Region E | | | Region F | | | Region G | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Intensity | L | M | D | L | M | D | L | M | D | L | M | D | L | M | D | L | M | D | L | M | D |
| CRMS32A | 2 | - | - | - | - | - | 1 | 2 | - | 3 | - | - | - | 1 | - | - | 1 | - | - | 1 | - |
| MSN36 | 2 | - | - | - | - | - | 1 | 2 | | 3 | - | - | 1 | - | - | 1 | - | - | 1 | - | - |
| KRH4 | 2 | - | - | - | - | - | 1 | - | 2 | 3 | - | - | - | - | 1 | - | - | 1 | - | 1 | - |

**Table 4. Similarity index (%) of protein bands of parental lines and rice hybrid (KRH-4)**

| Parental lines and hybrid | CRMS32A | MSN36 | KRH4 |
|---|---|---|---|
| CRMS32A | - | 72.7% | 81.8% |
| MSN36 | 72.7% | - | 72.7% |
| KRH-4 | 81.8% | 72.7% | |

**Table 5. Intensity and relative mobility of isozyme alcohol dehydrogenase (ADH) in parental lines and rice hybrid (KRH-4)**

| Band number | $R_m$ value | CRMS32A | KRH4 | MSN36 |
|---|---|---|---|---|
| 1 | 0.20 | + | +++ | + |
| 2 | 0.26 | +++ | +++ | +++ |
| 3 | 0.38 | + | + | + |
| 4 | 0.46 | + | + | + |

**Table 6. Number of isozyme alcohol dehydrogenase (ADH) bands observed in parental lines and rice hybrid (KRH-4)**

| Genotype | Light intensity | Medium intensity | Dark intensity | Total number of bands |
|---|---|---|---|---|
| CRMS32A | 3 | - | 1 | 4 |
| MSN36 | 3 | - | 1 | 4 |
| KRH-4 | 2 | - | 2 | 4 |

**Table 7. Intensity and relative mobility of isozyme malate dehydrogenase (MDH) in parental lines and rice hybrid (KRH-4)**

| Band number | $R_m$ value | CRMS32A | KRH-4 | MSN36 |
|---|---|---|---|---|
| 1 | 0.58 | + | + | + |

Similarity index expressed in per cent varied from 72.7 to 81.8 which showed that there is a less variability (narrow genetic background) among the parental lines and hybrid studied for protein polymorphism. The parental line CRMS32A (A) recorded highest similarity of 81.8 per cent (Table 4) with its hybrid KRH-4 (H) where as MSN36 (R) exhibited common similarity of 72.7 per cent for both with CRMS32A (A) and KRH-4 (H) Thus from the study, it is revealed that the parental lines and hybrid can be characterized based on similarity index

The identification of parental lines and hybrid based on the isozyme banding pattern of alcohol dehydrogenase and malate dehydrogenase was also attempted in the study. The variability observed among the parental lines and hybrid for alcohol dehydrogenase (ADH) isozyme banding pattern would be the indication of potentiality of this technique for characterization of these parental lines and hybrid.

Alcohol dehydrogenase isozyme banding pattern revealed significant variations among the parental lines and hybrid in terms of intensity but they exhibited similar number of bands and relative mobility (Table 5 and Table 6). The intensity of banding pattern differed among the parental lines and hybrid in band number 1 with an $R_m$ value of 0.20. KRH-4 which exhibited dark intensity band where as CRMS32A (A) and MSN36 (R) exhibited light intensity band in the band number 1 (Figure 2) and thus based on the intensity the parental lines can be distinguished from hybrid. However, there was no significant difference between CRMS32A (A) and MSN36 (R) in the isozyme banding pattern of alcohol dehydrogenase. Further, significant difference was not observed in the banding pattern of isozyme malate dehydrogenase among the parental lines and hybrid. Both parental lines and hybrid expressed a single band of light intensity with an $R_m$ value of 0.58 (Table 7).

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