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RIBONUCLEASES ACTIVITY IN BACTERIAL LEAF BLIGHT RESISTANT AND SUSCEPTIBLE VARIETIES OF RICE (*Oryza Sativa* L.)

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Electrophoretic study was conducted to find out the isozymic differences of ribonucleases (RNases) between bacterial leaf blight (BLB) resistant and susceptible varieties of rice. Three susceptible varieties; T(N) 1, Jamuna and Varuna and three resistant varieties; IR 20, IR 22 and UPRI 71-12 were used in the investigation. Maximum of eight bands (isozymes) of RNase I and five bands of RNase II were observed at the various stages of development. The activity of RNase I decreased whereas RNase II activity increased from zero-hour to 96-hour stages of germination. The activity of RNase I was higher in the susceptible varieties than the resistant varieties while RNase II activity was higher in the resistant varieties throughout the different stages of germination. The specific bands 7 and 8 of RNase I were associated with BLB susceptiblity. The band 5 of RNase II was specific to resistant varieties.

The conventional methods for identifying the different cultivars of crop plants are being replaced by the chemical methods which are more reliable, The Polyacrylamide gel electrophoresis enhances the chances of correct cultivar identification and are highly improved techniques. Since isozymes are the expressions almost exclusively of the genetic make up of the plant or seed they little affected by the environ mental conditions (Lee and Ronalds, 1967). The electrophoretic procedures are increasingly used in genetic research for the assessment of evolutionary pathways, determination of genetic similarities and identification of genomes, species and cultivars of crop plants (Johnson *et al*, 1967; Johnson, 1972 and Gupta and Malik, 1978).

The present study was planned to find out (i) isozymic variation of RNase I and II at the various stages of germination, and (ii) genetic marker(s) for screening the varieties resistant to bacterial leaf blight, if any.

MATERIALS AND METHODS

Three susceptible viz., T (N) 1, Jamuna and Varuna and three resistant *viz.*, IR 20, IR 22 and UPRI 71-12 rice varieties to BLB were used. Seeds of these varieties were allowed to germinate in petridishes which were layered with filter paper and wetted with distilled water. The germinating seeds were used at 0, 24, 48 and 96 hour stages of germination for electrophoretic study. Two grams seed of each variety was taken, washed twice with distilled water and then dried with filter paper. The seeds were ground in a chilled pestle and mortar in 2 ml of 0.9 M sodium chloride solution. The ground material was put in the refrigerator for 16 hrs for extraction. The homogenates were then centrifuged at 13000 rpm for 20 minutes in the refrigerated centrifuge, Each sample was contrifuged twice. The light yellow supernatant was dialysed in 0.2 ml phosphate buffer (pH 7.0) and collected in seperate vials for electrophoresis.

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Polyacrylamide gels were prepared as described by Davis (1964). The electrophoresis was performed in tris-glycine electrode buffer (pH 8.5). The current was applied at 4 mA/tube for 30-45 minutes. Bromophenol blue was used as an indicator.

The gels were removed from the tubes and dipped into the test tube containing freshly prepared yeast RNA in acetate buffer (pH 5.0) for RNase I and 6.2 for RNase II. The gels were incubated in RNA buffer solution for 10-20 minutes at 37°C. The RNA buffer was replaced with distilled water for two minutes. The gels were dipped into a solution of 0.2% toluidine blue in 0.1% acetic acid adjusted at pH 3.0. After 40 seconds, the gels were rinsed in running tap water and returned to test tube with 5 per cent acetic ecid pH 3.0 for destaining. The bands were clearly visible on the gels where Ribonucleases has acted. The gels were preserved in 5 per cent perchloric acid.

RESULTS

Typical enzyme activity of ribonuclease was recorded from zero-hour to 96-hour stages of germination. Total number of 3 bands (isozymes) of RNase I were found during the different developmental stages. At zero-hour stage, bands 1 to 8 were present in T(N) 1, Jamuna and Varuna. The bands 1 to 6 were present in IR 20, IR 22 and UPRI 71-12. At 24-hour stage of germination bands 4 and 6 disappeared in all the varieties. Band 1 also disappeared in IR 20, IR 22 and UPRI 71-12 but no change in the banding pattern was recorded in T(N)1, Jamuna and Varuna at 48-hour stage. At 96 hour stage band 1 disappeared in the varieties T(N)1, Jamuna and Varuna. Bands 2, 3, 5, 7 and 8 were present in T(N) 1, Jamuna and Varuna whereas bands 2, 3 and 5 were present in IR 20, IR 22 and UPRI 71-12

A total number of 5 bands of RNase II were seen at the various germinating stages of rice. Band 1, 2 and 5 were present in IR 20, IR 22 and UPRI 71-12 while band 1 and 2 were present in T (N) 1, Jamuna and Varuna. Band 3 appeared at 24-hr stage in all the six varieties. At 48-hr stage, one more new band appeared. All the rice varieties showed the disappearance of band 1 at 96-hr stage

DISCUSSION

The differentiation and development of multicellular organism is due to the differential gene action. Certain genes are active or inactive at a particular stage of development. The enzyme or isozyme are the product of gene(s). The plant tissues differ not only in the enzyme content but also in the forms of specific isozymes and in the timing at which various isozymes appear (Scandalios, 1969). Isozymes provide a natural 'built in' marker system for biochemical, developmental and genetic studies (Scandalios, 1974). In the present study appearance and disappearance of bands were recorded as germination advanced. Some bands disappeared only after 24-hr of germination and others after 48-hr, and 96-hr of germination. At the same time some new bands also appeared during the different stages of germina-

tion. Some other bands were found unique or specific for some varieties and were stable.

The results showed the variation in the isozymic pattern of RNase I and II. At zero-hr stage, 8 bands of RNase I were observed in BLB susceptible varieties, T(N)1, Jamuna and Varuna whereas resistant varieties, IR 20, IR 22 and UPRI 71-12 had six bands.

The activity of RNase I decreased from zero-hr to 96-hr stages of germination. At 24-hr of germination, band 4 and 6 disappeared in both the susceptible as well as resistant varieties. The disappearance of bands indicated that gene(s) controlling the activity or synthesis of these isozymes turned inactive. As the development advanced, the activity of RNase I continuously decreased. At 48-hr stage band 1 disappeared in the resistant varieties. Band 1 also disappeared from the susceptible varieties at 96-hr stage of develpoment. The activity of RNase I in BLB susceptible varieties remained higher than the resistant varieties throughout the growth period. The specific band 7 and 8 af RNase I were associated with BLB susceptibility These specific bands can be used as a genetic marker for screening the BLB resistant and susceptible varieties of rice.

The activity of RNase II first increased and then decreased at 96-hr of germination but its activity remained higher in the resistant varieties than the susceptible varieties throughout the stages of germination. Band 1 and 2 were present in the susceptible varieties. Band 1, 2 and 5 were observed in BLB resistant varieties. At 24-hr stage of germination, band 3 and at 48-hr band 4 appeared in both the susceptible as well as resistaat varieties. Band 1 disappeared at 96-hr in all the varieties. The band 5 of RNase II was specific to the resistant varieties whereas band 7 and 8 of RNase I were the unique or specific for the susceptible varieties. These specific bands may be related to the some physiological process of disease reaction and can be used as a genetic markers.

Differential gene activity was observed in the different varieties of rice during their germinating stages. RNase I activity progressively decreased from zero to 96-hour stage. RNase II activity first increased and then decreased at 96-hour stage of germination. Since isozymes are the product of gene (s), the disappearance or appearance of bands was the reflection of the inactivation or activation of gene(s) synthesizing the isozymes. Ribonuclease degrade the RNA which is the primary source in protein synthesis. RNase II is associated with ribosome so it may regulate the translation. The stage which contains more Ribonuclease activity has less enzyme synthesis and *vice versa* (Kessler and Engelberg, 1962) Bands 7 and 8 of RNase I were specific for BLB susceptible varieties which were not observed in the resistant varieties at any stage of development. Similarly band 5 of RNase II was specific to resistant varieties which was not seen in susceptible varieties. This study is very useful in plant breeding programme for screening BLB resistant varieties in laboratory. Ribonu-

clease enzyme may be one of the factors responsible for BLB susceptibility or resistance through their increased or decreased activity in rice cultivars.

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STUDIES ON COMBINING ABILITY FOR YIELD COMPONENTS IN RICE*

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A diallel technique was employed in which ten varieties of rice were crossed among themselves in all possible combinations. A total of 90 hybrids and ten parents was studied. The analysis for combining ability was significant for all the characters which indicated the presence of both additive and non-additive gene actions. The GCA variances were higher than SCA variances which revealed the predominance of additive gene actions for all the characters. Significant gca effects for plant height, panicle length, number of grains per panicle and straw yield per plant were shown by Co.20 whereas TKM 6 showed significant effects for earbearing tillers per plant, Jaya for 100 grain weight, Dee-geo- Woo-gen (Dg Wg) for grain yield per plant and grain-straw ratio, Basmati 370 for grain length, I-geo-tze (Igt) for grain width and SLO 16 for length-width ratio of grain. No specific cross combination was found desirable for all the characters as indicated by their sca effect but the combinations involving Jaya and Dg Wg with Basmati 370 and TKM 4 were found generally good for grain yield, short stature and fine quality of grain.

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