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BIOCHEMICAL BASIS OF GRAIN DORMANCY IN RICE (*ORYZA SATIVA* L.)[1]

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Summary. Three dormant varieties, Basmati 370, Tilakchandan and Thapachini, three nondormant varieties, Bala, Cauvery and Govind were used for the biochemical analysis. The embryo and endosperm of presoaked seeds, leaf and root after seven days of germination were electrophoretically analysed for RNases. A total of six bands (isozymes) of RNase I and three bands of RNase II were observed in different tissues. Not all the bands were present together in any tissue. The isozymic variations of RNase I and II were observed only in the embryo. Bands 3 and 4 were specific and associated with the varieties showing grain dormancy. Similarly band 3 of RNase II found in the embryo, was also specific of varieties with grain dormancy. No isozymic variations was observed in the endosperm, leaf and root. The embryo appeared to be the seat of grain dormancy in rice. Moreover, some isozymes were present in two or more tissues, whereas some isozymes were unique which indicated the tissue specificity of isozymes.

The conventional methods for identifying plants are based on phenotypic expressions. These expressions are highly influenced by the environment. So these conventional methods are being replaced by chemical methods. One of these main methods is isozyme electrophoresis, owing to which chances of correct cultivar identification are highly improved. Since isozymes are expressions almost exclusively of the genetic make up of the plant or seed and are therefore little affected by the environmental conditions (Lee and Ronalds 1967, Schwartz 1960), their patterns are highly repeatable. So, the use of electrophoretic procedures is increasing in genetic research for the assessment of evolutionary pathways, determination of genetic similarities and identification of genomes, species and cultivar of crop plants (Johnson et al. 1967, Johnson 1972, Gupta, Malik 1978).

RNase I is a RNA specific endonuclease that releases purine-3 nucleotides. It has optimum pH 5.0 and molecular weight 23000. It was first isolated from the corn endosperm (Wilson 1967). RNase II acts on both purine and pyrimidine cyclic nucleotides. It has optimum pH 5.4 to 7.0 and molecular weight 17000 (Wilson

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1968). It was isolated from wheat germs (Torti et al. 1973). It forms an integral part of ribosomal particles in homogenates (Wilson 1975). Davies and Larkin (1973) reported that polysome is extremely sensitive to the traces of RNase and that polysome is degraded by the rupture of interribosomal bonds in mRNA.

The present study was planned to reveal isozymic variation of RNase I and II in the endosperm and embryo, isozymic variation of RNase I in the root and shoot of dormant and nondormant rice cultivars, if any, and tissue specificity of isozymes.

MATERIALS AND METHODS

The germination experiment to determine grain dormancy in different rice cultivars was made in freshly harvested grains. The grains were germinated at room temperature (32 ± 2°C) in Petri dishes containing filter paper and 10 ml distilled water. There were three replications with 100 grains each. The number of germinated seeds was recorded daily. The final counting was done after seven days of seeding. Varieties showing less than 20 per cent germination were classified as dormant and these showing more than 75 per cent germination were recognized as nondormant.

Six varieties including three dormant: Basmati 370, Tilakchandan, Thapachini and three nondormant: Bala, Cauvery and Govind were taken for an electrophoretic study. Grains of the six cultivars were soaked for 18 hours. The endosperm and embryo were separated for electrophoresis. The leaf and root of each variety were taken after seven days of seeding in Petri dishes. A sample of 500 mg of each endosperm, embryo, leaf and root of each variety was ground with chilled pestle and mortar. 0.5 ml of 0.9 M sodium chloride was added at the time of grinding. The ground material was left in the refrigerator for 12 hrs and then centrifuged at 14000 rpm for 30 minutes in the refrigerated centrifuge. Each sample was centrifuged twice. The light yellow supernatant was dialyzed in 0.2 ml phosphate buffer pH 7.0 and collected in separate vials for electrophoretic study. The polyacrylamide gel electrophoresis was conducted according to the procedures of Davis (1964). The electrophoresis was performed in tris-glycine electrode buffer (pH 8.5). The current was applied at 3.5 mA/tube for 30-35 minutes. Bromophenol blue was used as a tracking dye.

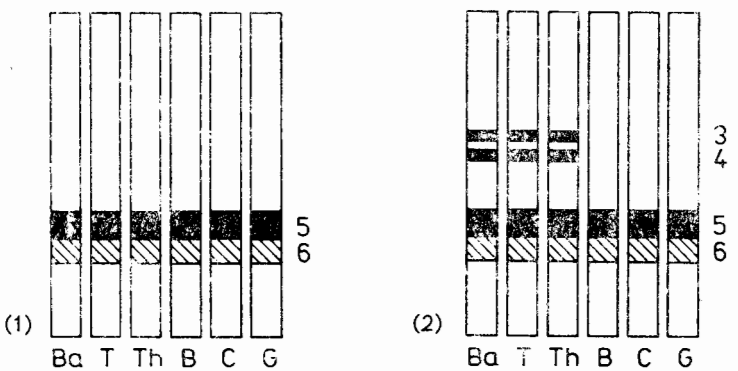
STAINING

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 solution was replaced with distilled water for two minutes. The gels were transferred to a plastic screen and dipped into the solution of 0.2 per cent toluidine blue in 0.1 per cent acetic acid adjusted to pH 3.0. After 30 seconds, the gels were

briefly rinsed in running tap water and returned to the test tube with 5 per cent acetic acid pH 3.0 for destaining. Bands were clearly visible on the gels where ribonucleases acted.

RESULTS AND DISCUSSION

The total number of six bands of RNase I were observed in different tissues of rice plant. Bands 5 and 6 were present in the endosperm in all the rice cultivars under study. So no isozymic variation of RNase I was found in the endosperm of any cultivar (Fig. 1). Bands 3, 4, 5 and 6 were present in the embryo. Bands 3 and 4 were



Figs. 1 - 2. Electrophoretic patterns of RNase I activity in six cultivars of rice

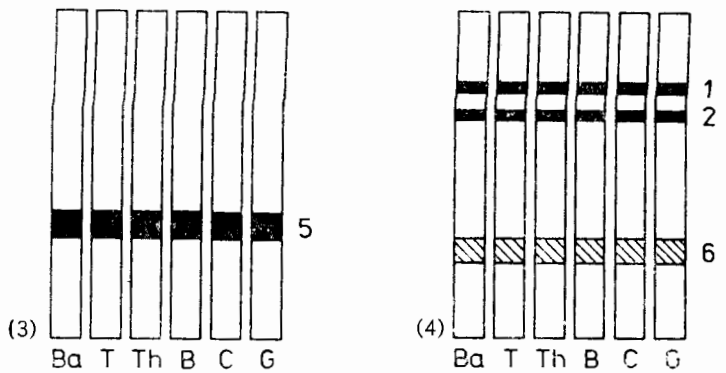
1 — Endosperm, 2 — Embryo (Ba — Basmati 370, T — Tilakchandan, Th — Thapachini, B — Bala, C — Cauvery, G — Govind)

specific of Basmati 370, Tilakchandan and Thapachini, being absent in Bala, Cauvery and Govind. Bands 3 and 4 of RNase I found in the embryo of dormant varieties may be related to the grain dormancy (Fig. 2). Bands 3 and 4 were not observed in other plant parts. Only varieties with grain dormancy showed these two bands, but they were not present in the varieties with non-dormant grains. Bands 3 and 4 were characteristic of the varieties having grain dormancy and may be related to this trait. These bands can be used as a marker in screening varieties for this trait.

In the leaf only band 5 was present in all the six varieties (Fig. 3). No other band was observed. Bands 1, 2 and 6 were found to be present in the root of all the varieties (Fig. 4). No isozymic variation was observed in the tissues, leaf and root. Therefore, root and shoot cannot be used for screening varieties for the grain dormancy.

The isozymic pattern of RNase II was also observed in both the endosperm and embryo. A total of three bands were found to be present. Only band 1 was present in the endosperm of all the varieties (Fig. 5), whereas bands 2 and 3 were present in the embryo. Band 2 was present in all the varieties but band 3 was found to be present in Basmati 370, Tilakchandan and Thapachini and absent in Bala, Cauvery

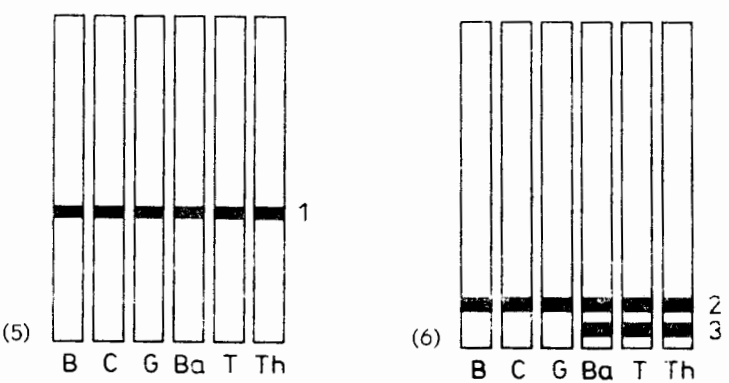
and Govind (Fig. 6). Band 3 of RNase II was found specific of the varieties showing grain dormancy. Hence, this band can also be used as a genetic marker. The activity of ribonucleases in a dormant embryo was higher than that of a nondormant embryo. Jacobsen et al. (1966) found very few peroxidase isozymes in the barley seed embryo and more in the endosperm. From the isozymic pattern of RNase I and II, it appears



Figs. 3 - 4. Electrophoretic patterns of RNase I activity in six cultivars of rice

3 — Leaft, 4 — Root (Ba — Basmati 370, T — Tilakchandan, Th — Thapachini, B — Bala, C — Cauvery, G — Govind)

that embryo genotypes are responsible for the grain dormancy, since isozymes are expressions of the genetic make up of plants (Schwartz 1960). Ribonucleases may be one of the factors responsible for it, because only the embryo showed isozymic variations. It further appears that RNase activity checks gibberellin synthesis in the dormant grains of rice cultivars.



Figs. 5 - 6. Electrophoretic patterns of RNase II activity in six cultivars of rice

5 — Endosperm, 6 — Embryo (Ba — Basmati 370, T — Tilakchandan, Th — Thapachini, B — Bala, C — Cauvery, G — Govind)

Plant tissues differ not only in different enzyme content but also in the forms of specific isozymes and in the time of the occurrence of various proteins, enzymes and isozymes (Scandalios 1974). Data presented in this paper clearly demonstrated that some isozymes were characteristic of certain tissue while others were present in two or more tissues. Band 5 and 6 were present in both the endosperm and embryo but band 6 was absent in the leaf and band 5 in the root. Bands 1 and 2 were present only in the root. Bands 3 and 4 were present in the embryo of the dormant grain. Similarly band 1 of RNase II was present only in the endosperm and absent in the embryo. Bands 2 and 3 were present in the embryo, whereas band 3 was specific only of the varieties showing grain dormancy. These results support the isozyme tissue specificity observed in other plant species (Evans, Alldrige 1965, Hess 1967, Scandalios 1968). Tissue specificity of isozymes can be interpreted in terms of genetic regulatory control mechanism as proposed by Jacob and Monod (1961). The presence and absence of the band(s) in a particular tissue is the reflection of the activation or inactivation of gene(s) synthesizing the enzyme. The synthesis depends upon the need of a particular tissue.

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BIOCHEMICZNE PODSTAWY SPOCZYNKU ZIARN RYŻU (*ORYZA SATIVA* L.)

Streszczenie

Przedmiotem badań było sześć odmian ryżu: Basmati 370, Tilakchandan i Thapachini (odmiany spoczynkowe) oraz Bala, Cauvery i Govind (odmiany niespoczynkowe). Przeprowadzono elektroforetyczną analizę aktywności rybonukleazy I i II w zarodkach, endospermach, liściach i korzeniach. Stwierdzono występowanie łącznie sześciu prążków enzymatycznych reprezentujących rybonukleazę I i trzy prążki reprezentujące rybonukleazę II. Zróżnicowanie aktywności rybonukleazy I i II między odmianami obserwowano tylko w zarodkach. Zróżnicowanie to związane było ze spoczynkiem ziarniaków. W ziarnach spoczynkowych zaobserwowano obecność dwóch dodatkowych prążków (3 i 4) dla rybonukleazy I i jednego dodatkowego prążka (3) dla rybonukleazy II. Nie stwierdzono różnic między odmianami w polimorfizmie RNazy I i II w bielmach, liściach i korzeniach.

БИОХИМИЧЕСКАЯ ОСНОВА ПОКОЯ ЗЕРНА У РИСА (*ORYZA SATIVA* L.)

Резюме

Три сорта, Басмати 370, Тилакчандан и Тапачини, в состоянии покоя и три сорта, Бала, Каувери и Говинд, не в состоянии покоя, были использованы для биохимического анализа. Зародыш и эндосперма предварительного намоченных семян, лист и корень после 7 дней прорастания были подвергнуты электрофоретическому анализу на рибонуклеазы. (RNases). В различных тканях наблюдалось всего 6 полос (изозимов) RNase I и 3 полосы RNase II. Не все полосы появлялись вместе в какой-нибудь ткани. Изозимическая изменчивость RNase I и II наблюдалась только у зародыша. Полосы 3 и 4 были специфичными и появлялись у сортов, у которых зерно было в состоянии покоя. Подобным образом полоса 3 RNase II, обнаруженная у зародыша, была также специфична для сортов с зерном в состоянии покоя. В эндосперме, листе и корне не наблюдалось изозимической изменчивости. Оказалось, что зародыш является местом спячки зерна и риса. Кроме того, некоторые изозимы находились в двух или более тканях, в то время как другие были уникальны, что указывало на специфичность ткани относительно изозимов.