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Inheritance of α-amylase isozyme patterns and their association with early vigour-related traits in rainfed upland rice

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Early vigour is an important component of weed competitiveness in rainfed upland rice (*Oryza sativa* L.). The α-amylase enzyme is involved in the conversion of starch into sugar, liberating energy which is utilized for the germination process. The α-amylase isozyme pattern on PAGE showed high mobility in high-vigour parent IR64 and low mobility in low-vigour parent Amrut. The straight and reciprocal F_1s exhibit high mobility, while F_2s show 3 (high): 1 (low) mobility pattern, indicating monogenic control of the trait. Association was observed between the shoot length, the key component of early vigour in rice and high mobility pattern of the isozyme and hence, α-amylase isozyme high mobility pattern could be useful in marker-aided selection (MAS) for high early vigour in rice.

RICE is one of the predominant food crops of the world. The rice consumers world over are likely to increase from 2.4 billion to 4.0 billion in the next two decades. There is a need to increase rice production from 5.8 billion tonnes to 8.7 billion tonnes by the year 2025, which is possible by increasing productivity through crop improvement[1].

Under upland conditions, rice productivity is low due to heavy weed infestation, moisture stress, ill-distributed rainfall and heavy labour costs. The genotypes with high early vigour are preferred under upland condition, as vigour influences rapidity of seedling emergence, early vegetative growth, agronomic traits and grain yield[2,3]. The early vigour in rice is measured through root and shoot length of seedlings. The information on α-amylase isozymes and their association with early vigour-related traits would be helpful in planning breeding programmes focused at development of rice varieties with good early vigour and suitable for rainfed upland conditions.

The present work has been carried out to study the inheritance of α-amylase isozyme patterns and their association with early vigour-related traits, i.e. root and shoot length of seedlings in rainfed upland rice genotypes.

The material used for the study included high-vigour parent IR64 (H) and low-vigour parent Amrut (L). IR64 shows high mobility and Amrut shows low mobility pattern of α-amylase isozymes. Both the parents were crossed in straight and reciprocal combination to obtain F_1s which were subsequently selfed to obtain F_2s. The seeds of parents, F_1 and F_2 were germinated and the 14-day-old seedlings were taken for α-amylase isozyme analysis. The α-amylase (1,4-α-D-glucan; glucono hydrolase, EC 3.2.1.1) pattern was determined by native polyacrylamide gel electrophoresis (PAGE) technique, followed with α-amylase enzyme-specific staining[4]. The enzyme extraction was done by grinding seedlings with Tris-HCl buffer 0.05 M (pH 8.0). The tissue:extraction buffer ratio was 1:2 (w/v). The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant so obtained was carefully transferred to another centrifuge tube and used as source of enzyme.

The gels were prepared according to the standard method[5] and run under constant voltage of 70 V. After the run was over, the gels were stained with α-amylase isozyme-specific staining solution[4]. The pattern of staining was photographed.

IR64 showed higher electrophoretic mobility pattern, while Amrut showed lower mobility pattern (Figure 1). In straight and reciprocal crosses, F_1s exhibited the higher mobility isozyme pattern (Figures 1 and 2), indicating dominance of high mobility pattern over the

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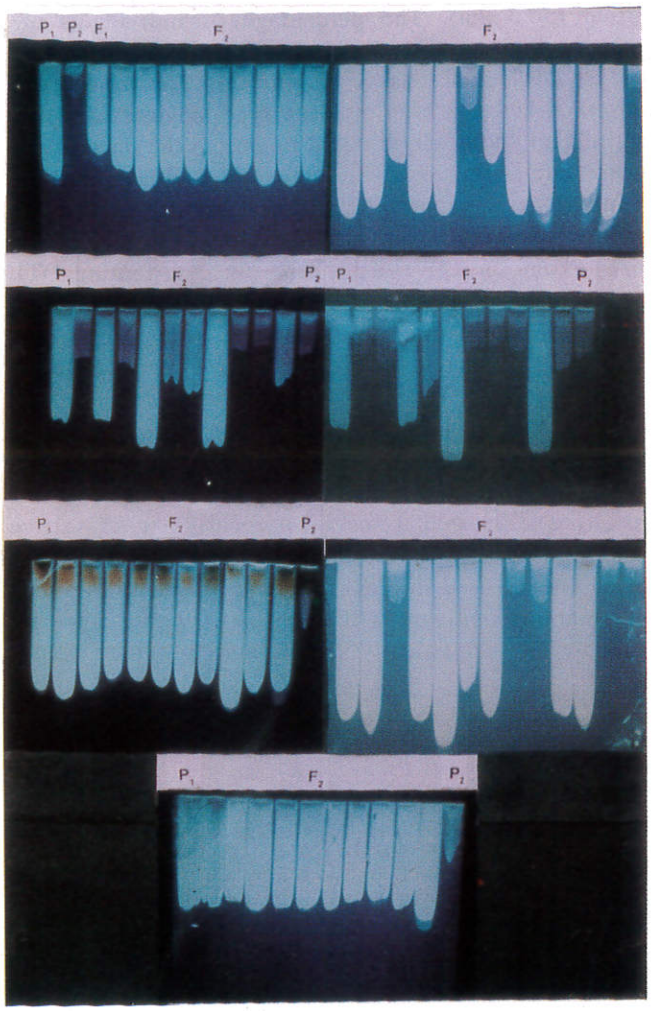

Figure 1. α-amylase isozyme patterns of the parents, F_1 and F_2 seedlings of the cross IR64/Amrut (P_1 = IR64; P_2 = Amrut).

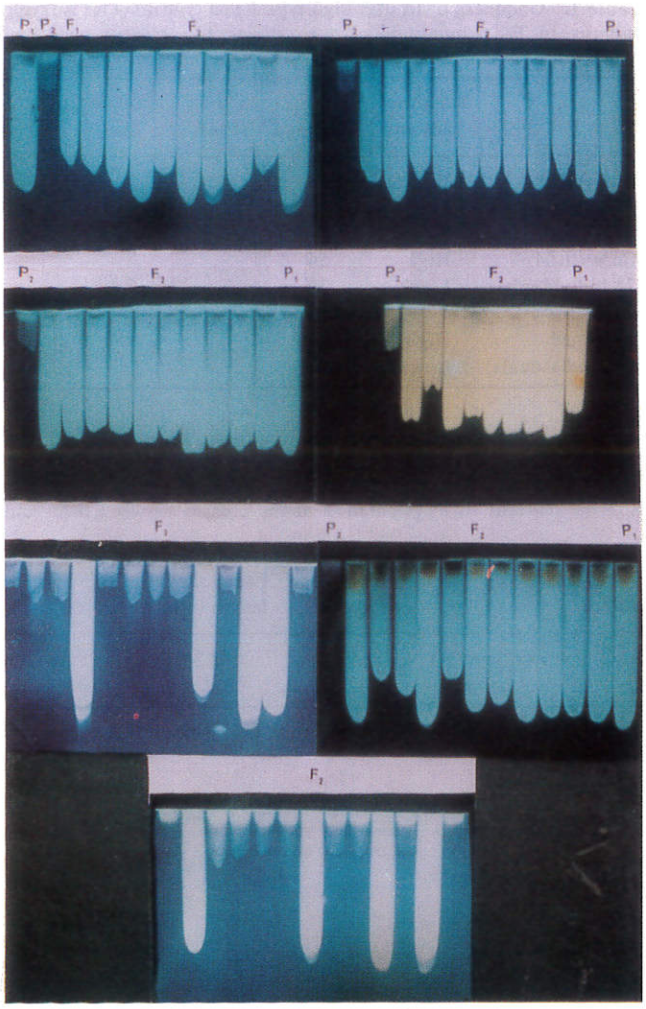

Figure 2. α-amylase isozyme patterns of the parents, F_1 and F_2 seedlings of the cross Amrut/IR64 (P_1 = IR64; P_2 = Amrut).

lower one and absence of reciprocal difference. In F_2, segregation was observed for the isozyme mobility patterns, which fit well into 3 high type:1 low type (Table 1). In F_2 of IR64/Amrut (Figure 1), out of 75 individuals studied, high mobility type was observed in 58 individuals while low mobility type was observed in 17 individuals. The chi-square test indicated goodness of fit with the expected 3 high:1 low ratio (Table 1). In F_2 of Amrut/IR64 (Figure 2) out of 74 individuals studied, high mobility type was observed in 56 individuals while low mobility type was observed in 18 genotypes. The indicated goodness of fit with the expected 3 high:1 low (Table 1). As there was no reciprocal difference observed, F_2s of both the crosses were combined and out of the total F_2 population of 149 individuals, 114 individuals expressed high mobility type and 35 individuals low mobility type, which also fit well into the expected 3:1 (high:low) ratio (Table 1).

These results can be interpreted as indicative of involvement of dominant allele of a single gene controlling the α-amylase higher mobility pattern in the high vigour parent IR64 and recessive allele regulating lower mobility pattern in the low vigour parent Amrut. The absence of reciprocal difference, presence of higher mobility pattern in F_1 and segregation in F_2 indicate location of genes in the nucleus[6], implying monogenic Mendelian inheritance for α-amylase isozyme patterns observed. The monogenic pattern observed for amylases may be due to detection of only Group-I α-amylase isozymes in the gel containing starch, similar to the reports in rye[7].

In F_2, segregation was also observed for two of the early vigour-related parameters, viz. root length and shoot length. The influence of α-amylase pattern on only the root length and shoot length traits could be studied, as only these two parameters could be assessed on individual seedling basis (in F_2, each seedling may be a different genotype).

The comparison of mean values (Table 2) for the shoot length and root length between the high amylase

Table 1. Goodness of fit of 3 high type:1 low type amylase isozyme patterns in F_2 populations of the crosses IR64/Amrut and Amrut/IR64

Cross	Amylase pattern	Observed	χ^2 for 3H:1L	Probability for χ^2
IR64/Amrut (75 individuals)	High	58	0.11	$P_{0.8} < 0.11 > P_{0.7}$
	Low	17		
Amrut/IR64 (74 individuals)	High	56	0.00	$< P_{0.001}$
	Low	18		
Combined (149 individuals)	High	114	0.11	$P_{0.8} < 0.11 > P_{0.7}$
	Low	35		

Table 2. Comparison of mean of high and low groups of amylase isozyme patterns in F_2 population of the crosses between IR64 and Amrut

α-amylase pattern	No. of genotypes	Mean shoot length (mm)	Root length (mm)
High mobility type (H)	114	138.3*	93.5
Low mobility type (L)	35	102.0	90.3

*Significantly higher at 5% level of probability by *t* test.

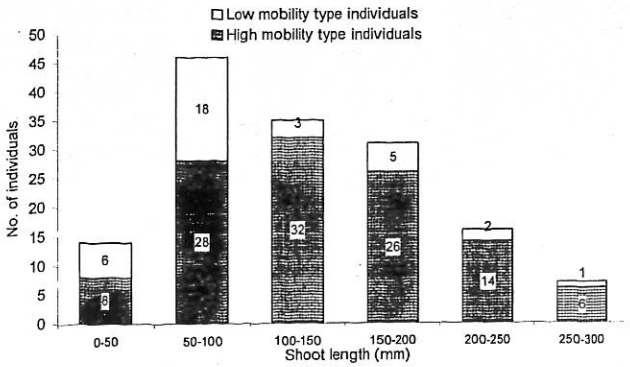

Figure 3. Distribution of high and low mobility type individuals across different shoot length classes in F_2 of a cross between IR64 and Amrut.

type group and low amylase type group by *t* test revealed that the mean of high mobility group for shoot length (138.3 mm) was significantly higher than the mean of low mobility group (102.0 mm), while the mean root length of high mobility group (93.5 mm) did not differ significantly from the mean of low mobility group (90.3 mm). The percentage of individuals expressing high mobility isozyme pattern is higher in upper shoot length classes (Figure 3). The association observed between high mobility type amylase isozymes and higher shoot length could be due to the presence of genes controlling both the traits on a single chromosome. This is supported by the observations that the genes governing α-amylase isozymes are located on rice chromosome 1 and a positively contributing QTL for shoot length is also on chromosome 1 (ref. 8). With its significant positive influence on seedling length, and a seedling parameter very closely associated with early vigour in rice, the high mobility type profile of α-amylase can be used as a molecular marker for high early vigour in rice.

The information on inheritance of α-amylase isozyme pattern and molecular marker for early vigour in rice can be thus useful in selecting genotypes with high early vigour, used in plant breeding programmes aimed at obtaining cultivars with high early vigour, that are suitable for ecologically harsh rainfed upland conditions.

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