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EVALUATION OF SHORT STATURED MUTANTS OF BASMATI 370 FOR ENZYME ACTIVITY

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Eight short statured mutants viz. DM-16-5-1, DM-2, DM-107-4, DM-15-4, DM-15-11, DM-24, DM-38 and DM-179-1 and two standard varieties, Basmati 370 and IR 6 were studied for the activities of amylase and nitrate reductase (NR). Amylase (alpha and beta) was studied at seedling stage whereas nitrate reductase activity was estimated at 4 different growth stages; seedling, tillering, flowering and maturity. There was no significant difference between the mutants and the tall variety with respect to amylase activity. However, a clear cut difference was observed between all the mutants and varieties with respect to alpha and beta amylase activities. Alpha amylase activity was higher than beta amylase activity in Basmati 370, IR 6, DM-16-5-1, DM-15-4, DM-15-11, DM-24, DM-38 and DM-179-1 whereas beta amylase activity was slightly higher in DM-2 and DM-107-4. Nitrate reductase activity decreased from seedling stage with the progress of growth. Maximum activity of nitrate reducatse was observed in mutants DM-24 and minimum in DM-179-1. Semidwarf mutants DM-24 DM-2, DM-16-5-1 and DM-38 by virtue of their showing consistently high activity at all the stages seem to be promising and could be used in the hybridization programme for inducting nitrate reducatse activity.

INTRODUCTION

Although, plant breeders have been successful in developing high yielding cultivars by selecting for agronomic parameters of yield, there is a dire need for predictive selection criteria. The use of yield components and idiotypes are examples of selection criteria used in recent year to improve upon grain yield. Certain enzyme activities should also be logical criteria for the selection of superior cultivars because synthesis of all enzymes is under genetic control and some enzymes regulate the rate of metabolism. It has been shown that amount of enzyme activity varies widely within a species (Zeiesserl and Hageman, 1962) and that the level of activity of some enzymes is highly heritable (Warner *et al.*, 1969).

Amylases, alpha and beta, are very important from the germination/growth point of view in rice. These enzymes are synthesized *de novo* during germination and seedling development. Although, several studies on varietal differences in alpha amylase level have been reported in wheat, barley and triticale (King and Gale, 1980) but similar information is scanty in case of rice. However, some work on the enzyme starch metabolism has been reported in rice (Baun *et al.*, 1970).

Nitrate reducatse is another enzyme which is also considered to have a major role in regulating nitrogen metabolism in

cereals because it is the first enzyme in the pathway for reduction of nitrate (Hewitt and Afridi, 1959). It has been advocated that nitrate reducatse could be useful to the plant breeders as a selection criterion for selecting and developing high yielding varieties (Croy and Hageman, 1970). The objective of the present study was to find out any variation in the activities of amylases and nitrate reductase in the induced semidwarf mutants of rice varieties.

MATERIALS AND METHODS

a. Amylase: Thirty uniform seeds each of the semidwarf mutants DM-16-5-1, DM-2, DM-107-4, DM-15-4, DM-15-11, DM-24, DM-38, DM-179-1 and two commercial varieties. Basmati 370 and IR-6 stored at 20 ± 2°C for 30 days with 14% moisture content, were germinated in petridishes (10 seeds per petridish) containing wet filter paper at 27 ± 2°C in dark. Six days after germination, the seedlings were ground in a mortar with 10 ml of 0.3% calcium chloride in 0.2 N NaCl. The homogenate was used for the determination of amylase activity. Total amylase activity was measured by the method of Bernfeld (1955), whereby the reducing group liberated from starch were measured by reduction of 3, 5-dinitrosalicylic acid. For enzyme assay, 0.5 ml of extract was used to which 0.5 ml of starch reagent (1% starch in 0.016 M, acetate buffer pH, 4.8) was added. The mixture was incubated at 20°C for 3 minutes and after that 1 ml of 3, 5-dinitrosalicylic acid ($C_7H_4N_2O_7$) was added. The mixture was heated in boiling water bath for 5 minutes, cooled and 10 ml of water was added. The colour was read at 540 nm and enzyme units were calculated as:

$$\text{Units/mg} = \frac{\text{micro maltose liberated}}{\text{mg enzyme in reaction mixture} \times 3 \text{ minutes}}$$

Total proteins were determined by dye binding method of Bradford (1976). To 0.1 ml of the extrac' added 1.0 ml of dye reagent (100 mg Brilliant Blue G250 dissolved in 50 ml of 95% ethanol and added to 950 ml of 9% phosphoric acid). Absorbance was read at 595 nm after 5 minutes. Bovine serum albumin was used for preparing standard curve.

Alpha amylase activity was estimated in the same preparation after heating the diluted supernatant for 10 minutes at 70°C for inactivating beta amylase. Beta amylase was calculated as the difference between activity and that of alpha amylase.

b. Nitrate reductase: Nitrate reductase activity was estimated at the seedling, tillering, flowering and maturity stages of the plant growth. Thirty day-old seedlings of any mutant variety was transplanted in the main field with 3 replications. Plant parts (100 mg) at any given stage were cut into small pieces with a razor blade and vacuum infiltrated in 10 ml extractant solution containing 0.1 M potassium phosphate buffer (pH 7.5), 0.05 M KNO_3 and 1% n-propanol. All procedures up to incubation were carried out in a cold room (4°C) and glassware was kept in ice. Serum stoppered flasks were then flushed with nitrogen gas and incubated in dark at 30 C. Aliquots of one ml were taken at zero time and after 30 and 60 minutes. One ml of test sample was mixed with 1 ml of 1% (w/v) of sulphanilamide in 3 M HCl and one ml of 0.1% N (1-naphthyl) ethylenediamine dihydrochloride. Samples were incubated at 30°C for 30 minutes and colour intensity measured at 540 nm on a Beckman Model-25 Spectrophotometer. Concentration of nitrite was calculated from the standard curve of sodium nitrite solution. The activity of enzyme was expressed as micromoles of nitrite formed g^{-1} fresh weight $hour^{-1}$. The data were analyzed statistically.

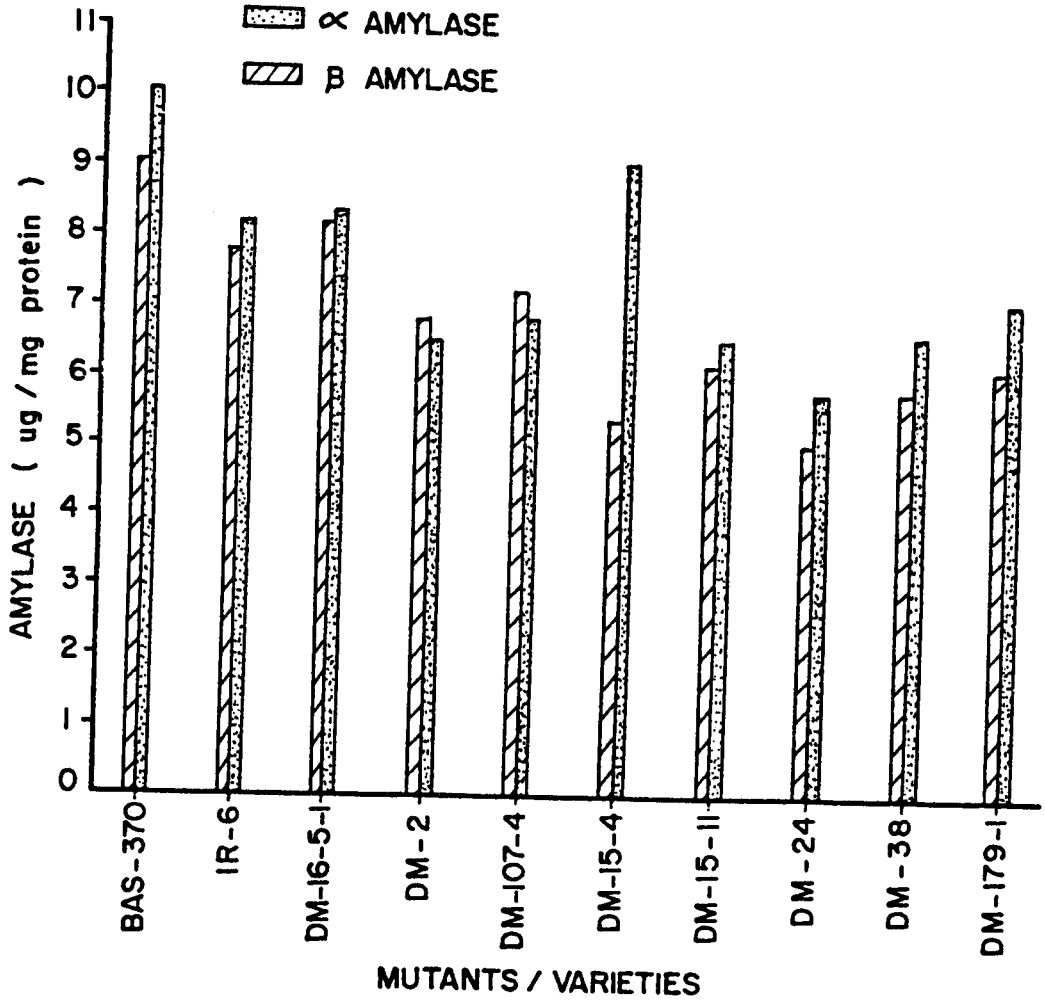

Fig. 1. Amylase contents in rice.

RESULTS AND DISCUSSION

The results of the present study did not show any clear cut difference between the semidwarf mutants and the tall variety Basmati 370 (Fig. 1). The highest amylase activity was found in Basmati 370 and lowest in semidwarf mutant DM-24. However, a noticeable difference was observed between the mutants with respect to alpha and beta amylase activity. Alpha amylase activity was higher than beta amylase in Basmati 370, IR-6, DM-16-5-1, DM-15-4, DM-15-11, DM-24, DM-38 and DM-179-1, whereas beta amylase activity was slightly higher in DM-2 and DM-24. Among the semidwarf

mutants, maximum difference between the activity of alpha and beta amylase was found in semidwarf mutant DM-107-4. High amylase activity is expected during germination when starch is broken down (Manners, 1974). The high amylase activity in the tall variety gives a clue that breakdown of starch takes place at a faster rate in tall varieties resulting in an increase in seedling height after 2 weeks of germination. The authors could not come across any published report on the difference in activities of alpha and beta amylase in rice. However, in other cereals, beta amylase has been reported to be higher than alpha amylase (Agarwal, 1977). In the present study, alpha amylase was higher than beta amylase in all the semidwarf mutants.

Nitrate reductase activity: Figure 2 shows that maximum activity of nitrate reductase was observed in DM-2 and minimum in DM-179-1. Basmati 370, IR 6 and dwarf mutants show the same pattern of enzyme activity. The activity decreases from seedling stage with the progression of growth. However, the genotypes differ in their level of activity at different stages. Beevers and Hageman (1980) reported that the net rate activity is affected by plant age and plant growth rate. The results of the present study on the extent of variation for NR activity are in agreement with earlier report (Reddy *et al.*, 1985). Semidwarf mutants DM-24, DM-2, DM-16-5-1 and DM-38 by virtue of their showing consistently high activity at all stages seem to be promising and can be used

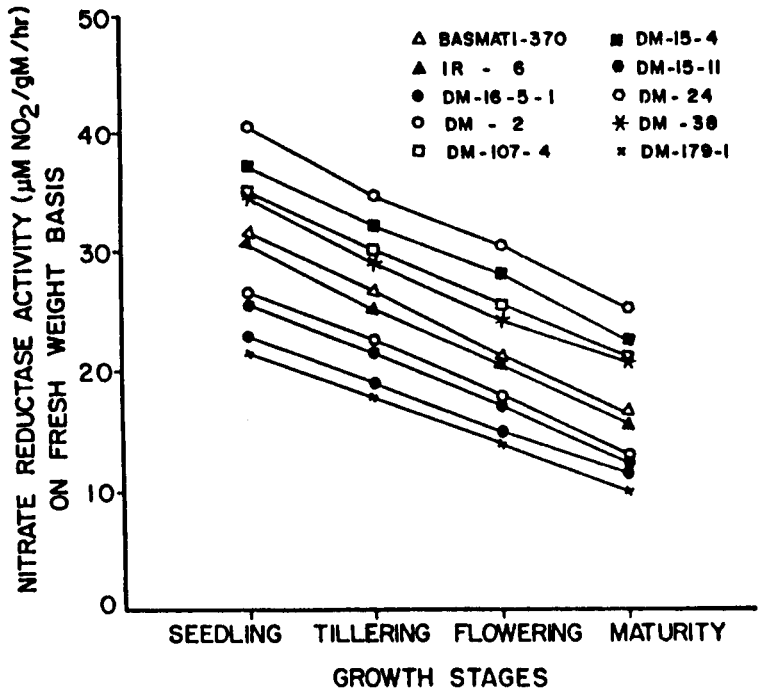

Fig. 2. Nitrate reductase activity in rice.

in hybridization programme for breeding of rice cultivars having NR to a desired level. The importance of NR as a selection criterion for high grain yield and high protein has been stressed (Croy and Hageman, 1970). The results of the present study are also in agreement with those of Siddiq and Reddy (1984). It can, therefore, be concluded that NR activity may be employed as an additional parameter in identifying superior genotypes in Basmati varieties.

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