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Full Length Research Paper

Germination potential index of Sindh rice cultivars on biochemical basis, using amylase as an indicator

Sadia Galani, Afsheen Aman and Shah Ali Ul Qader*
The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi, Karachi-75270, Pakistan.

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Time sequence analysis of germination and vigor of five rice cultivars were carried out by investigating the associated biochemical changes. The experiments were conducted with varying periods of incubation for germination and varietal differences were observed for different parameters. Alpha amylase activities were found to be directly correlated with germination percentage. Gradual increase in reducing sugars along with α-amylase activity was observed, while total carbohydrates decreased as germination proceeded. The genetic variability of seed vigor of rice cultivars may be attributed to varietal differences of α-amylase activity at different stages of germination. Simultaneous increase in reducing sugars due to degradation of reserve carbohydrates was observed. The results indicated that α-amylase activity is a biochemical indicator showing different germination abilities of rice varieties, leading to different seed vigor. Among all five seed cultivars, SADA HAYAT demonstrated maximum seed vigor and alpha amylase activity along with germination period. This cultivar shows a potential for maximum production for successful breeding program.

Key words: Germination potential, α-amylase, seed vigor, rice, cultivars.

INTRODUCTION

Rice is the second most staple food after wheat and second major foreign exchange earning commodity after cotton in Pakistan. It contributes approximately 17% of the total foreign exchange earnings. But the low production of this foreign commodity may be due to many constraints such as poor seed quality, seedling age, improper agronomic practices, poor pest and weed control. Among these factors, seed quality is considered as a major uptake barrier of improved rice production (Alam et al., 2009) and it is genetically controlled (Perry, 1972). Successful crop establishment requires rapid germination, high seed vigor and seedling vigor (Karrer et al., 1993; Mackill and Redona, 1997). Regarding germination processes, key enzyme, α-amylase, is involved in the conversion of starch to simple sugars for growing seedlings (Sun and Henson, 1991). Amylase mainly carries out this process until the seedling becomes photosynthetically self sufficient (Nandi et al., 1995). Germination ability of cereal seeds probably depend on the extent of α-amylase activity (Nandi et al., 1995), while in seeds of reduced viability, low α-amylase activities have been reported (Livesley and Bray, 1991). In this way, improved α-amylase activity increased the level of soluble sugars which triggered the faster and uniform emergence of seedlings (Lee and Kim, 2000; Farooq et al., 2006).

Plant breeders have been successful in developing high yielding cultivars by selecting agronomic parameters. There is still great need for predictive selection criteria on biochemical basis for seed and seedling vigor in order to attain maximum production and successful breeding programs. Therefore, it is important to assess the relative genetic variances and type of gene action involved in seed vigor, so that genetic differences may be exploited appropriately among rice cultivars. Keeping in view the above facts, the present study was designed to estimate the extent of genetic variability among the rice cultivars for seed vigor traits on biochemical basis. For this purpose, initial five different local rice varieties were tested for different biochemical analysis.

*Corresponding author. E-mail: madar_chem@yahoo.com.

Table 1. Rice cultivars used in the current study.

Genotype	100 grains weight (g)	Seed size
SADA HAYAT	2.45	Medium
DR-82	2.15	Medium
SAHAKAR	2.37	Medium
IR-6	2.80	Small
IR-8	2.90	Large

MATERIALS AND METHODS

Plant material

Seeds of five rice cultivars including DR-82, IR-8, IR-6, SADA HAYAT and SAHAKAR were provided by Rice Research Center, Dokri, Sindh, Pakistan (Table 1). The seeds were surface sterilized using 3% commercial bleach for 10 min and rinsed several times with distilled water.

Germination

Twenty seeds of each cultivar of rice were soaked for 1 h in distilled water at room temperature and placed in petri-dishes furnished with 2 sheets of filter paper (Whatman No. 1) moistened with 8.0 ml of distilled water. The germinated seeds were allowed to grow at 27°C in dark for different time interval (0, 24, 48, 72 and 92 h). Germination was recorded on daily basis for seven days. First count was taken on the 3rd day and the last count on the 7th day. Germination percentage (Krishnasamy and Seshu, 1990) and vigor index (Copeland, 1976) was calculated using the following equation:

$$\text{Germination percentage (\%)} = \frac{\text{No. of seed that germinated at 48 h}}{\text{No. of seed that germinated at 96 h}} \times 100$$

$$\text{Germination vigor Index} = A_1/T_1 + A_2/T_2 + \ldots\ldots/+ A_n/T_n$$

Where, A = Number of seeds that germinated; T = time (days) corresponding to A; n = no. of days to final count

α-Amylase extraction

0.5 g dry, soaked and germinated seeds after 0, 24, 48, 72 and 96 h were crushed using a pre-chilled mortar and pestle in 5.0 ml phosphate buffer (pH 7.0, 50 mM) and centrifuged at 15000 rpm for 15 min at 4°C. The supernatant was separated and stored at -20°C for further analysis.

Enzyme assay

The enzyme assay was carried out by incubating 0.1 ml enzyme with 1.0 ml soluble starch (1%) (50 mM phosphate buffer, pH 7.0) at 50°C for 15 min, the reaction was stopped and the reducing sugars released were estimated by the addition of dinitrosalicylic acid (DNS) reagent (Miller, 1959).

One unit of amylase activity was defined as “the amount of enzyme which liberates 1 µmol of reducing sugar as maltose per minute under standard assay conditions”.

Estimation of total carbohydrate and reducing sugar

Total carbohydrate estimation was carried out by anthrone method (Yemm and Willis, 1954) and reducing sugars were determined by the DNS method (Miller, 1959).

RESULTS AND DISCUSSION

Varietal differences were observed regarding germination studies of five rice cultivars (Figure 1). Germination started after 24 h in all cultivars and gradually increased up to 72 h. Overall, all the varieties exhibited same mode of pattern for germination percentage, but the maximum germination percentage was observed in SADA HAYAT after 72 h of incubation, followed by other varieties. IR-8 seeds showed a little bit low germination percentage from the rest of the cultivars. Germination vigor indices showed significant varietal differences among all varieties and maximum vigor index was observed for SADA HAYAT and DR-82 (Figure 2) while, IR-8 showed minimum vigor index. Similarly, α-amylase activity was low at initial stages of germination and increased as germination proceeded up to 72 h and then decrease was observed after 92 h. α-Amylase activity for dry seeds and imbibed seeds exhibited very negligible detection but maximum enzyme activity was found in germinating seeds of SADA HAYAT after 72 h of incubation followed by DR-82 (Figure 3). This shows that at 72 h, maximum conversion of reserved starch into reducing sugars occurs through α-amylase which is then utilized for seed germination.

Data showed that SADA HAYAT and DR-82 exhibited maximum germination percentage and high α-amylase activity among all rice cultivars. The results are in agreement with the findings of Karrer et al. (1993) and Jazayeria et al. (2007) that amylase activity was lowest at initial stages of germination and steeply increased at 72 h and decreased at the end of germination. It showed that germination percentage positively correlated with activity of α-amylase activity. Reducing sugars accumulation showed variable pattern among varieties and with time periods (Figure 4).

SADA HAYAT showed gradual accumulation of sugar contents followed by IR-6 and SAHAKAR as germination proceeded. The sugar contents were significantly increased up to 92 h after sowing during the period of germination, while total carbohydrates progressively decreased with time, showing the lowest at 92 h of incubation. Varietal differences were observed in carbohydrate consumption pattern in the whole germination process (Figure 5). Reduction in total carbohydrates was reported in germinating seeds of SADA HAYAT, SAHAKAR and IR-6 at 92 h indicating faster ability of starch degradation and its mobilization to seed germination for faster seedling establishment.

Results also revealed that α-amylase triggers the fast rate of carbohydrate breakdown in rice seeds after three days of germination. This initial decrease in carbohydrates in germinating seeds coupled with gradual increase in sugars in SADA HAYAT may be attributed to rapid utilization of carbohydrates in endosperm during the

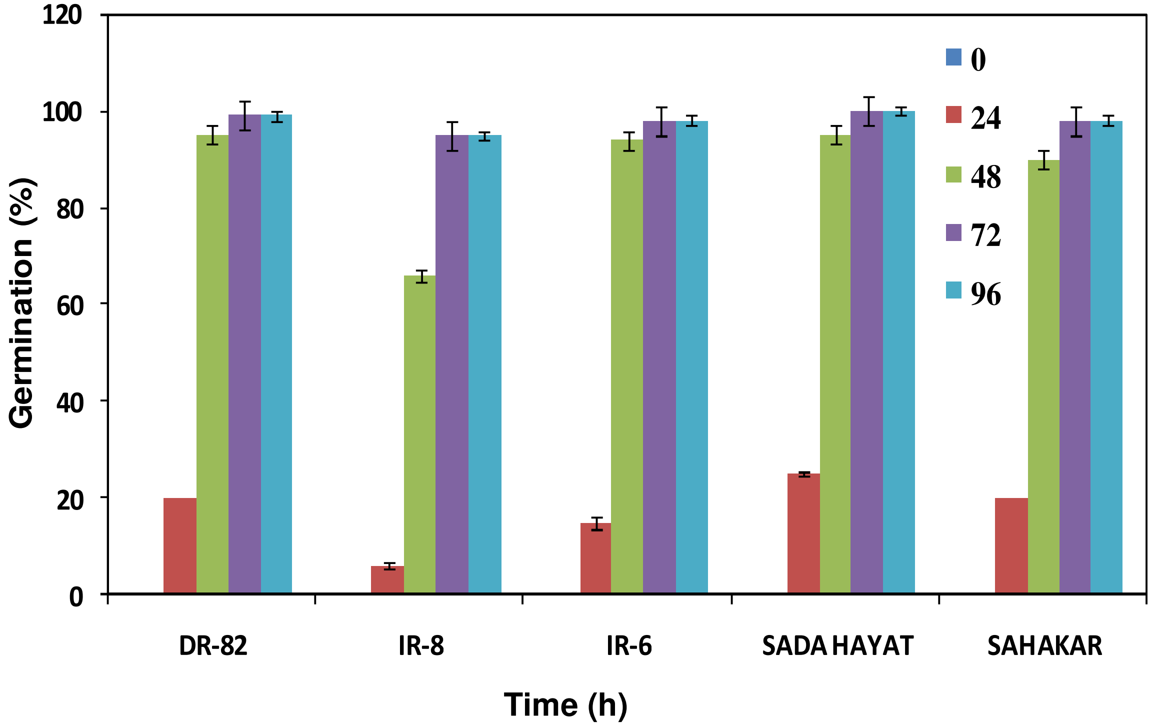

Figure 1. Germination percentage of different rice cultivars with varying time incubations.

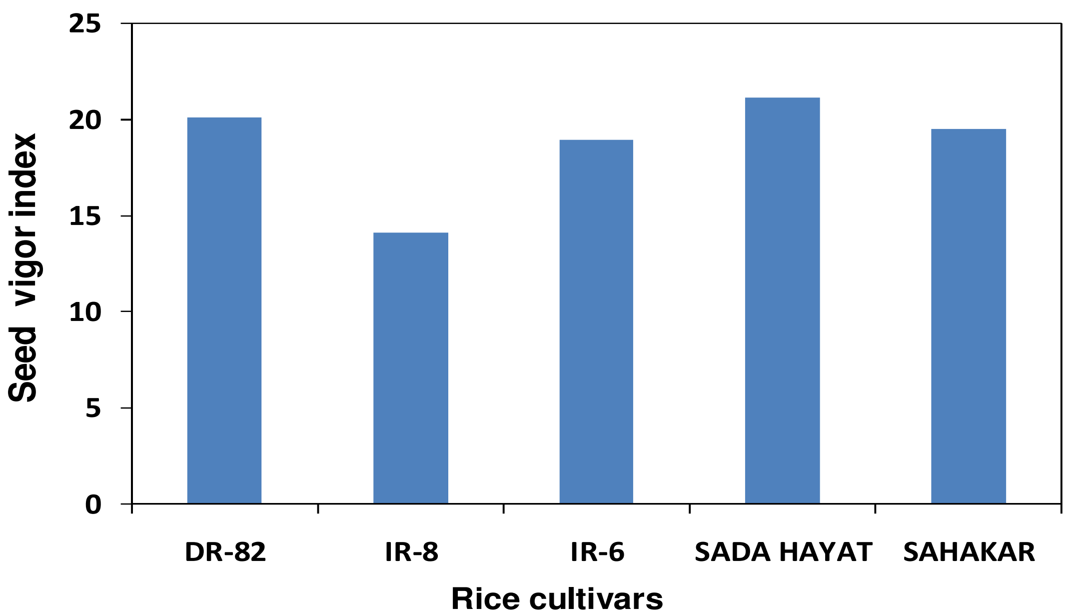

Figure 2. Seed vigor index of different rice cultivars.

initial few days of germination (Kashem et al., 1995; Lee and Kim, 2000; Basra et al., 2006; Ismail et al., 2008; Elabri et al., 2009) which presumably provided the substrates for energy generation for fast and uniform seedling emergence.

Conclusions

It was concluded that varietal differences exist with respect to germination potential and level of hydrolytic enzymes within rice varieties. α-Amylase actually represents

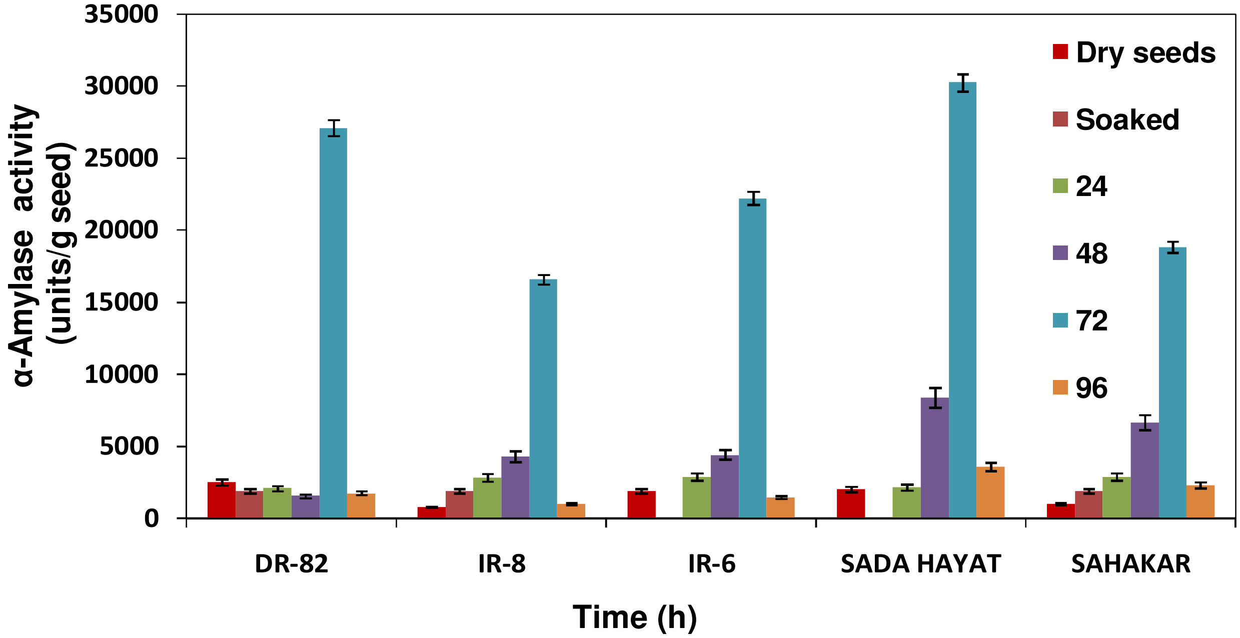

Figure 3. Alpha amylase activities of different rice cultivars at various time intervals of germination.

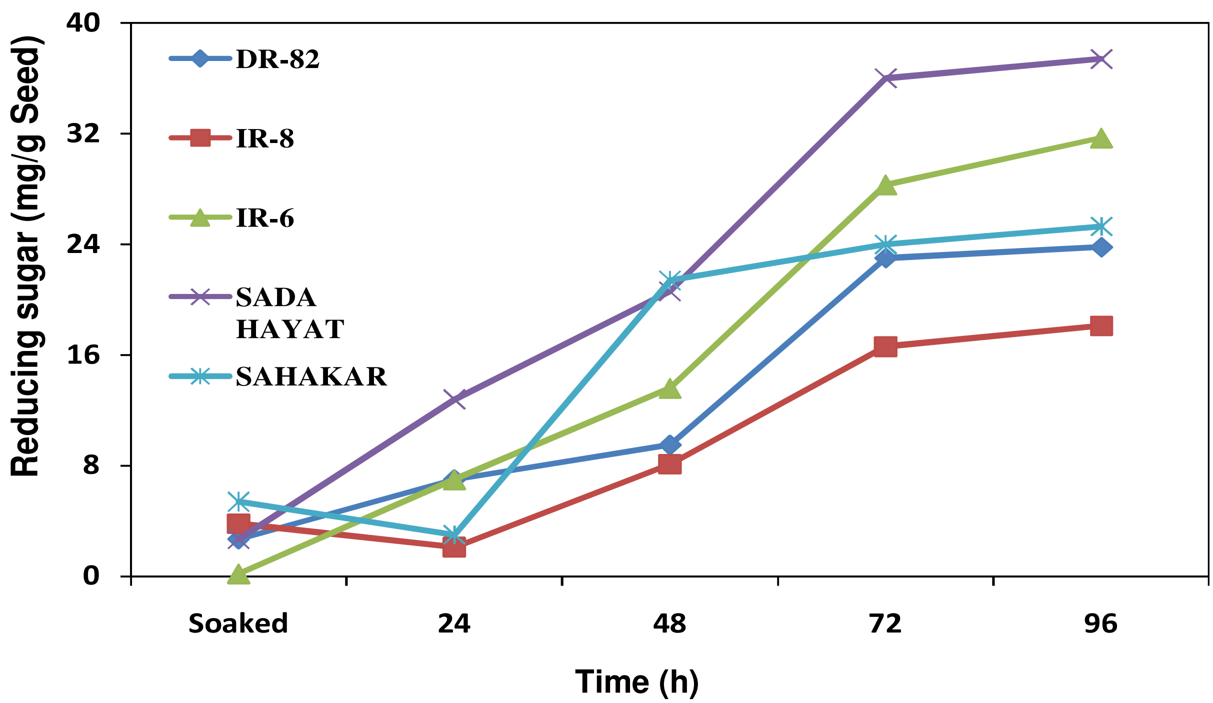

Figure 4. Reducing sugars of different rice cultivars at various time intervals of germination.

the predominant contribution of the carbohydrate metabolism in endosperm of rice seeds and represents the variable spectrum of germination potential (seed vigor) of Sindh rice cultivars.

Dry and imbibed seeds showed reduced α-amylase activity, while germinating seeds of SADA HAYAT and DR-82 showed differential response because of activetion of residual enzyme synthesis and *de novo* synthesis of α-amylase during germination, and consequently depicting maximum germination potential. Such information will enable breeders to select potential varieties that possess high seed and seedling vigor traits in attaining

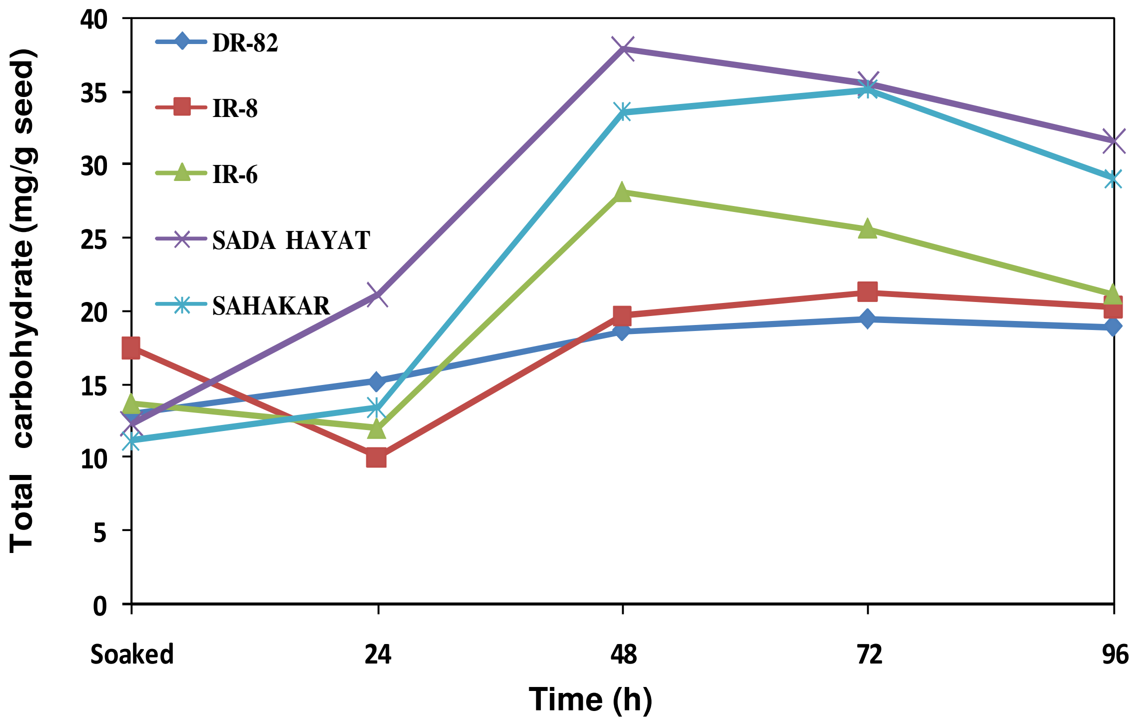

Figure 5. Total carbohydrates of different rice cultivars at various time intervals of germination.

maximum production.

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