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Short Communication

Starch Phosphorylase Enzyme is Over-expressed in Submerged Rice (*Oryza sativa* L) Plant

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Activity of starch phosphorylase enzyme and its isozymes were studied in one-week and two-week-old seedlings of four cultivars of rice, *viz*. FR13A, Gangasiuli (submergence tolerant), Sarala and IR42 (submergence susceptible) under submerged as well as control conditions. In one-week-old seedlings there was no apparent increase in enzyme activity, whereas in the two-week-old seedlings the activity rose steadily and reached to a maximum after 144 h of submergence and then declined. The increase in activity was also reflected in the isozyme profiles of this enzyme wherein both qualitative as well as quantitative (suppression and over-expression of certain bands) differences were observed. It was concluded that, in addition to other enzymes of starch degradation, starch phosphorylase too may be involved in starch degradation during submergence stress in rice plants.

***Key words*:** *rice, Oryza sativa*, submergence, starch, starch phosphorylase, isozymes,

Complete submergence not only decreases O_2 supply to the rice plants but also drastically reduces rate of photosynthesis due to low penetration of light and slow diffusion of CO_2. As a result plants utilize reserve carbohydrates for their survival during submergence. However, before being used as energy source, reserve carbohydrates, particularly starch are first degraded to simpler fermentable form. Starch degradation can occur *via* hydrolytic and or probably phosphorolytic reactions. Rice seeds germinating under anoxia appeared to have complete set of enzymes namely α-amylase, β-amylase, debranching enzyme, α-glucosidase and maltase (1,2). The concerted action of these enzymes seemed to bring about complete conversion of starch to glucose. Phosphorolytic degradation of starch is carried out by starch phosphorylase enzyme. The reaction is potentially reversible under physiological conditions depending on the levels of glucose-1-phosphate and inorganic phosphate in the plastid (3). This enzyme has been reported from various tissues of rice plants (4,5). Although the role of other starch degrading enzymes (e.g. amylases) during submergence stress is well documented, the role of starch phosphorylase enzyme in starch degradation under submergence stress is still not clear. Here we report the activity of this enzyme and its isozyme profile in submerged rice plants of different growth stages.

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Seeds of four rice (*Oryza sativa* L) cultivars namely, FR13A, Gangasiuli (as submergence tolerant), Sarala and IR42 (as submergence susceptible) were surface sterilized for 10 min in a 4% solution of commercial sodium hypochlorite, followed by rinsing three times with glass-distilled water. Seeds were then placed on a petri plate lined with two layers of filter paper moistened with tap water for germination at 30±1 ^{o}C in dark under the ambient atmospheric conditions. For one-week-old seedlings, petri plates containing the seedlings were completely submerged in water in a plastic tray (15 cm deep). The tray was covered with another tray to cut off light. For two-week-old seedlings, seeds were sown in plastic pots containing farm soil and organic manure (3:1) and the two-week-old seedlings were completely submerged in a concrete tank (1 m deep) for 192 h. Only stems (culm and sheath) were taken for analysis.

Extraction and assay of starch phosphorylase enzyme was done according to Dhaliwal and Sharma (6). The amount of inorganic phosphorus released by the action of starch phosphorylase was estimated by the Fiske and Subba Row method (7). One unit of enzyme activity is defined as the amount of enzyme that liberates 1µmol of inorganic phosphate per h and specific activity as the number of enzyme units per mg of protein. Protein estimation was carried out by the method of Lowry *et al* (8) using BSA as standard.

For isozyme analysis crude enzyme extract was prepared from submerged and non-submerged plants as above. Polyacrylamide gel electrophoresis was performed using a vertical electrophoresis unit. Comparable amounts of protein (75 μg) from each cultivar under each treatment were loaded in lanes of a 7.5% polyacrylamide gel. Both the buffer and the gel contained 0.002 M EDTA. The gel also contained 0.1% starch as primer for phosphorylase reaction. All the samples were run under identical conditions (constant current of 20 mA). Staining for starch phosphorylase isozymes was done following the method of Vallejos (9).

Enzyme assay showed that in the one-week-old rice seedlings starch phosphorylase activity decreased after 48 h of submergence in all the cultivars irrespective of the level of tolerance to submergence stress. The enzyme activity then rose slowly and reached almost the control level after 96 h of submergence (Fig. 1). In the case of two-week-old seedlings the enzyme activity rose steadily up to 144 h of submergence, thereafter declined or did not change significantly (Fig. 2). However, there was no significant difference among the cultivars with respect to the activity of this enzyme.

Irrespective of the cultivars, one-week-old control seedlings exhibited five isozymes after 96 h of submergence, whereas the submerged seedlings had only two (Fig. 3a).

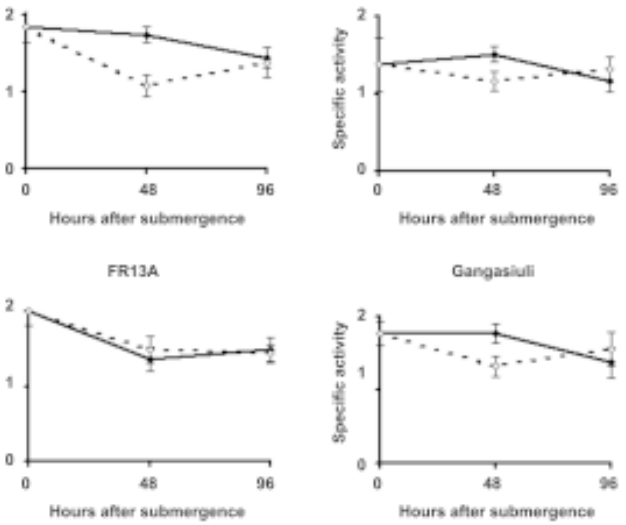

Fig. 1. Starch phosphorylase activity in one-week-old control (—) and submerged (-----) seedlings of different rice cultivars after different periods of submergence. Vertical bars represent ± SE of the mean (n = 4). Enzyme was extracted from the whole seedlings (except root).

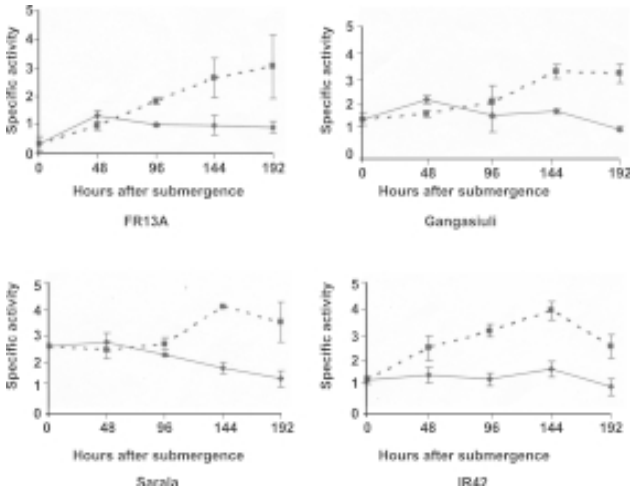

Fig. 2. Starch phosphorylase activity in two-week-old control (—) and submerged (-----) seedlings of different rice cultivars after different periods of submergence. Vertical bars represent ± SE of the mean (n = 4). Enzyme was extracted from the stem (culm and sheath).

Under both the conditions the 4th band appeared to have most of the activities than the rest. In the submerged seedlings while the 2nd, 3rd and 5th bands disappeared, activity of the 1st band slightly increased and that of the 4th band decreased. Starch phosphorylase zymogram from the two-week-old seedlings (both control and submerged) showed a total of three isozymes after 144 h of submergence and out of them activity of the fastest moving band appeared to have increased under submergence stress. While the activity of the first band remained unchanged, the second band seemed disappeared (Fig. 3b).

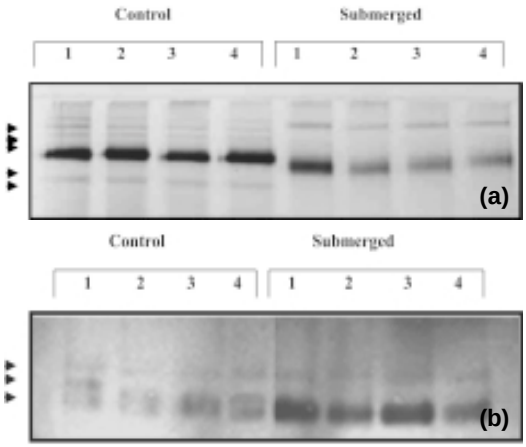

Fig. 3. Native PAGE of starch phosphorylase isoforms. Soluble proteins from the crude extracts of (**a**) one-week-old whole seedlings (96h after submergence), and (**b**) or stems of two-week-old seedlings(144h after submergence) were subjected to native PAGE in gels containing soluble starch. Lanes 1,2,3,4 reprsent FR13A, Gangasiuli, Sarala and IR42, respectively.

A number of enzymes have already been reported to be involved in starch degradation during submergence stress. Out of these amylases, especially α-amylase is considered to be the main enzyme of starch degradation during submergence (1,2). Starch phosphorylase, carrying out phosphorolytic degradation of starch, had already been reported from various tissues of growing rice plants. However, how this enzyme behaves during submergence stress is still not clear. In a preliminary study by Das *et al* (10) it was reported that this enzyme was greatly induced in the stem (culm and sheath) of three-week-old submerged rice seedlings. However, the observation was not substantiated by further evidence.

Although there was no apparent increase in enzyme activity in one-week-old seedlings, in the two-week-old seedlings starch phosphorylase activity increased by 1.61 - 7.20 fold as compared to pre-submergence level (Figs. 1 and 2). As there was no significant varietal difference (with respect to susceptibility to submergence stress) in the enzyme activity, the increased activity might be part of a general response to submergence stress without any regard to tolerance or susceptibility. However, late induction of this enzyme in the two-week-old seedlings might pose some questions on its exact role under submergence. Considering that the main enzyme for starch degradation during submergence is α-amylase which is greatly induced during the initial hours under submergence stress (1,2,11), starch phosphorylase may be activated or induced when submergence stress is prolonged and may bring about further degradation of reserve starch for plant's survival. Thus, starch phosphorylase induced by submergence stress is likely to perform a more general function of starch degradation during submergence stress, irrespective of the cultivar background.

Starch phosphorylase is a polymorphic enzyme whose expression is tissue specific and varies with the developmental stages (4,5). In one-week-old seedlings although a particular isozyme was induced under submergence stress (Fig. 3a) it was not reflected in the overall activity because activity of some other isozymes either decreased or disappeared. Among the three isozymes observed in the zymogram of two-week-old seedlings, activity of one of the isozymes, the faster moving band, increased while the band just following it disappeared under submergence stress (Fig. 3b). It is this faster moving band which might have been responsible for the observed rise in activity of the enzyme. Thus, submergence stress resulted in quantitative as well as well as qualitative differences in the pattern of starch phosphorylase expression in rice seedlings indicating that the genes involved were controlled by the surrounding environment (submergence or normal air). The degree of expression / suppression of individual genes may govern the growth of rice plants under submergence stress. It is, therefore, concluded that starch phosphorylase enzyme is over-expressed under submergence stress and, in addition to the primary starch degrading enzymes i.e. amylases, this enzyme may also be involved in starch degradation during submergence. However, further studies are needed to ascertain the role of individual isozyme during the stress.

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