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ORIGINAL ARTICLE

Influence of Waterlogging on Carbohydrate Metabolism in Ragi and Rice Roots

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Effect of different durations of waterlogging (4, 8 and 12 days) stress on carbohydrate status and activities of some related enzymes in ragi and rice roots was studied. In both ragi and rice roots there was decrease in starch and total sugar content in response to waterlogging conditions. Activity of α amylase was decrease in ragi roots while opposite trend was noticed in case of rice roots. The activity of pyruvate kinase was markedly increased due to 4, 8 and 12 days waterlogging in ragi roots while such increase was noticed in rice roots due to 12 days stress. Treatment of waterlogging caused enhancement in the activity of alkaline inorganic pyrophosphatase in the roots of both ragi and rice.

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Root is a main plant part which is directly exposed to waterlogging condition. Various physiological, biochemical and anatomical changes are induced in root system during hypoxia and anoxia (Vartapetain, 1990). Meeting the energy demands of the root system under such situation is a serious problem. Since carbohydrates are the major energy source, alterations in the carbohydrate metabolism are probably of crucial importance in meeting energy demand under waterlogged conditions. In present study effect of waterlogging on changes in carbohydrate status and activities of related enzymes in ragi and rice (*Oryza sativa* L.) roots are investigated.

MATERIALS AND METHODS

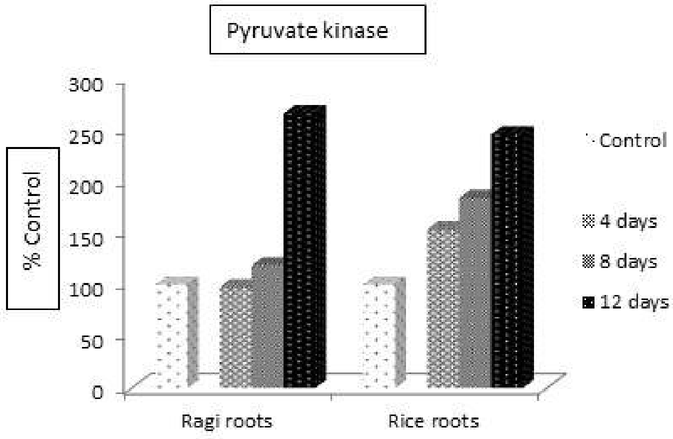
Seeds of ragi variety *GPU 28* and rice variety *Indrayani* were procured and after positive confirmation about viability were sown in earthen pots filled with equal amount of mixture of garden soil + FY manure in the ratio 3:1. These pots were kept in polyhouse in the month of June. Healthy and vigorous seedlings were maintained in the pots which were given equal water supply. After one month, plants of ragi and rice were subjected to waterlogging treatment of different duration (4, 8 and 12 days) by maintaining the water table above the soil surface through excessive water supply and preventing drain of water through the basal pore in the pots. The experiment was so arranged that at the end of treatments, it was possible to obtain plants receiving different waterlogging treatments (4, 8 and 12 days) at the same time for further investigation. Normal water supply was provided for control plants. The experiment was performed in triplicate.

The plants subjected to different treatments were carefully uprooted and roots were harvested. Soluble sugars in the root tissue were estimated by the method of Dey (1990). The reducing sugars liberated after hydrolysis of after extraction of root tissue with ethanol were insoluble starch residue collected estimated according to the method of Nelson (1944). Method of Katsumi and Fukuhara (1969) was employed to determine the activity of enzyme α-amylase from the root tissue. Extraction of the pyruvate kinase enzyme from the root tissue was done by using method of Kachmer and Boyer (1953) with slight modifications and enzyme assay was carried out following the method of Miller and Evans (1957). Activity of enzyme inorganic pyrophosphatase was estimated by the method of Rauser (1971). The soluble proteins were recorded from enzyme extract were determined by the method of Lowry *et al*., (1951).

RESULTS AND DISCUSSION

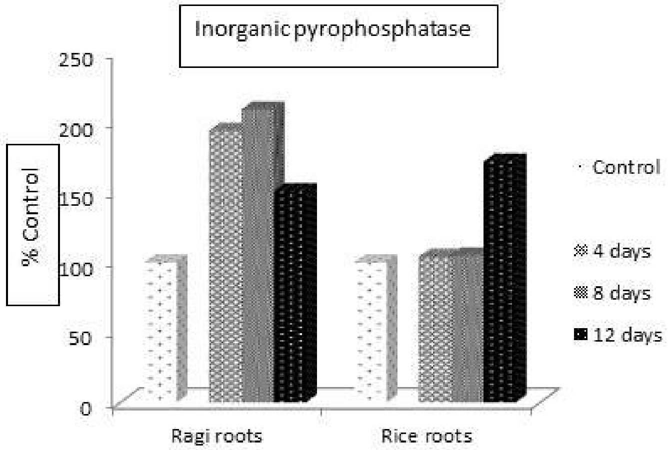
It is evident from the Figure 1 that in both ragi and rice roots there is decrease in starch and total sugar content under waterlogging conditions. The level of carbohydrates in the plant tissue gives an indirect idea of the metabolic status of the plant tissue as well as the energy content of the plant tissue. Rai *et al*., (2004) observed enhancement in sugar content in 20 days old maize (*Zea mays* L.) plants (during initial period of waterlogging) but after 96 hours of stress the level decreased. In the roots of both ragi and rice the total sugar level is reduced under the condition of waterlogging and this decrease is more prominent in rice roots. Thus in roots of both species there is rapid utilization of these compounds for respiratory process to fulfill the energy demands of waterlogging roots. Reduction in starch in roots of various plant species such as barley and wheat, in response to hypoxia has been reported (Geigenberger *et al*., 2000; Gibon *et al*., 2002). These changes were best explained as part of the adaptive response of plants to hypoxia: the increased demand for carbon to drive glycolysis during hypoxia can lead to the decrease of the starch pool. In case of both ragi and rice roots, the fresh synthesis of starch under such conditions might be also suspended due to diversion of precursor sucrose for respiratory process.

Effect of waterlogging on the activity of α-amylase is shown in the Figure 2. In case of ragi roots activity of this enzyme is decreased while opposite trend is noticed in case of rice roots. α-amylase are major amylolytic enzymes in germinating seeds for hydrolysis of stored starch. α-amylase hydrolyses α (1-4) bonds in amylose and amylopectin which results in production of glucan fragments that are further broken down by α-glucosidase and de- branching enzymes. Sewa (2000) studied behavior of different hydrolyzing enzymes in *Brachiaria mutica* (tolerant) and *B. brizantha* (intolerant) during submergence. Sucrose synthase activity in leaves decreased in both the species while α-amylase activity increased two fold in *B. mutica* and 40% in *B. brizantha* under flooding. Smith *et al*., (1987) noticed that under submerged conditions rapid intermodal growth of rice took place with degradation of starch. Seven α amylase genes, out of a total of 10 genes in the rice genome were characterized on the rice Gene Chip out of which only *RAMY3D* was significantly modulated by anoxia (Huang *et al*., 1990). Under waterlogging stress, a grater supply of sugars can be achieved through either increased synthesis or accelerated breakdown of reserve starch in roots and leaves. In case of ragi roots there is decline in α-amylase activity due to waterlogging and the breakdown of starch may be possible through β-amylase. On the other hand in rice roots there is marked increase in α-amylase which plays a definite role in waterlogging tolerance by hydrolyzing starch and supplying sugars for ATP production under potentially anaerobic conditions.



Average values a) Control ragi roots- 0.408 Δ OD h^{-1} mg $^{-1}$ protein
b) Control rice roots- 0.807 Δ OD $h^{-1}mg^{-1}$ protein

Fig.3. Effect of waterlogging on activity of pyruvate kinase in ragi and rice roots.



Average values a) Control ragi roots- 0.166 mg P liberated $h^{-1}mg^{-1}$ protein
b) Control rice roots- 0.0.603 mg P liberated $h^{-1}mg^{-1}$ protein

Fig.4. Effect of waterlogging on activity of inorganic pyrophosphatase in ragi and rice roots.

Activity of pyruvate kinase is found to be increased considerably in ragi roots over entire range of waterlogging treatment while in rice roots such increase is noticed in roots subjected to 12 days stress (Figure 3). Pyruvate kinase is one of the most important enzymes of glycolysis as it catalyzes the synthesis of pyruvic acid; the reaction coupled with ATP production. Barker *et al*., (1966) have stressed that the pyruvate kinase is the first glycolytic enzyme which respond to anaerobic conditions. Mohanty *et al*., (1993) found that anoxia tolerant cells of rice contained higher PK activity. PKc stimulated during anaerobiosis in order to partially offset the reduced ATP levels accompanying anoxia (Kobr and Beevers, 1971; Podesta and Plaxton, 1991). Pyruvate is therefore used as catabolic electrons sink (Ward *et al*., 2000). Although enhancement in pyruvate kinase would be helpful in maintainance of ATP supply it would also lead to increased availability of pyruvic acid for synthesis of anaerobic products ethyl alcohol and lactic acid.

It is evident from Figure 4 that activity of alkaline inorganic pyrophosphatase is increased due to waterlogging in the roots of both rice and ragi the increase being more conspicuous in rice. Pyrophosphate contains a high energy phosphate bond and its hydrolysis is exothermic under normal physiological conditions, releasing energy. Carystinos *et al*., (1995) noticed over expression of tonoplast bound PPiase in rice seedlings in response to anoxia and chilling stress. These workers observed strong induction (75 fold) of tonoplastic H+-PPiase in rice seedlings after 6 days of anoxia. Treatment of anoxia also caused enhancement of this enzyme in hypocotyl of mung bean (Darley *et al*., 1995). Compared to rice roots, ragi roots show low activity of the enzyme. The increased hydrolysis of pyrophosphate would be certainly advantageous for root system of both the crop species when ATP supply becomes limited under anaerobic conditions.

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