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Study of Some Aspects of Anaerobic Metabolism in Roots of Finger Millet and Rice Plants Subjected to Waterlogging Stress

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Abstract: Waterlogging stress is one of the major environmental constraints on agriculture in many parts of the world. Waterlogging leads to oxygen deficit in the soil which adversely affects root respiratory metabolism causing far reaching effects on plant growth and development. Crop species differ in waterlogging tolerance and crops such as rice possess excellent tolerance potential. An attempt has been made to study some facets of anaerobic respiratory metabolism in the roots of rice and finger millet. One month old plants raised in pot culture were subjected in 4, 8 and 12 days waterlogging. Activities of enzyme Alcohol Dehydrogenase (ADH) and Lactate Dehydrogenase (LDH), levels of ethanol and lactic acid and release of acetaldehyde from roots were estimated spectrophotometrically. The contents of alanine and γ -aminobutyric acid (GABA) were estimated with liquid chromatography with mass spectroscopy. Activity of ADH was increased in finger millet roots due to waterlogging while in case of rice roots opposite trend was noticed. Activity of LDH was lowered in roots of both the species in response to waterlogging. In finger case of millet roots an elevation in acetaldehyde level occurred due to 4 and 8 days of waterlogging while there was continuous decline in acetaldehyde level in rice roots with the increasing waterlogging period. In contrast to rice roots content of ethanol was increased during waterlogging in finger millet roots. Increase in waterlogging duration caused considerable decrease in lactic acid content in root tissue of rice and a slight lowering in the finger millet roots. Contents of alanine and GABA were reduced in finger millet roots while in case of rice roots short duration of waterlogging lead to increase in level of these metabolites. In number of instances, the pattern of these metabolic changes is not similar in these two species and this is related to difference in waterlogging tolerance in these two species.

Key words: Finger millet, rice, root metabolism, waterlogging

INTRODUCTION

Although waterlogging is becoming an important environmental constraints limiting crop production in many parts of the World, as compared to water deficit relatively less attention has been paid to physiological responses of different crops to waterlogging stress. The root system is the major target of oxygen deficiency resulting due to waterlogging stress and several metabolic disturbances occur in the root tissue which ultimately affect overall performance of the plant. Finger millet (*Eleusine coracana* L. Gaertn.) is cultivated in Southern and Eastern Africa and across most of South Asia (Hilu and De Wet, 1976) and the millet species is reputed for its hardy nature. In the present investigation, an attempt is made to perform comparative studies of effect of waterlogging on anaerobic metabolism in roots of finger millet and well known wetland crop rice.

MATERIALS AND METHODS

Cultivation of plants and waterlogging treatment:

Authentic seeds of finger millet variety GPU 28 and rice variety Indrayani were procured and sown in earthenware pots (30 cm dm) containing a mixture of garden soil+FY manure in the ratio 3:1 in the month of June in polyhouse. The holes in base of pots which were to be subjected to waterlogging conditions latter were sealed with cement so as to avoid leaching of water. After 20 days, twenty vigours healthy seedlings in each pot were maintained. The pots were supplied with equal amount of tap water and every care was taken to ensure proper growth of the plants and control of weeds. When the plants were one month old these were subjected to waterlogging treatments by adding excess of water and keeping water table close to soil surface. Control plants received regular water supply. Control plants received normal water supply. The waterlogging treatments were of 4, 8 and

12 days duration and these were arranged in such a way that at the end of treatments it was possible to obtain plants subjected to different treatments at the same time. These plants were carefully uprooted and roots were separated and washed thoroughly with distilled water.

Enzyme assays and analysis of anaerobic compounds:

Activity of enzyme alcohol dehydrogenase from the roots of finger millet and rice plants subjected to different treatments was studied by the method of Garmczarska (2002) with minor changes. Method of Garmczarska (2002) was followed with slight modifications for study of activity of enzyme lactate dehydrogenase. The soluble proteins in the enzyme extract were estimated following the method of Lowry *et al.* (1951). Estimation of acetaldehyde was done by the method of Sarkar (2001). Method of Bergmeyer *et al.* (1974) was followed for determining the amount of ethanol and lactic acid content in plant extract.

Analysis of amino acids by LC MS MS: The quantitative analysis of alanine and GABA contents was carried out from ethanol soluble fraction of roots with the help of Doctors' Analytical Laboratory, Mumbai following the method of LC MS MS. The experiment was performed in triplicate.

RESULTS AND DISCUSSION

Enzyme alcohol dehydrogenase and lactate dehydrogenase:

The influence of waterlogging treatments on the activities of enzymes and level of various metabolites is recorded in Fig. 1-7. From the Fig. 1 it is evident that activity of alcohol dehydrogenase is increased in finger millet roots during waterlogging while in case of rice opposite trend is seen. Activity of lactate dehydrogenase is decreased in roots of both the crop plants in response to waterlogging (Fig. 2).

Enzyme alcohol dehydrogenase catalyzes the terminal reaction of anaerobic glycolytic process, conversion of acetaldehyde to ethanol. Dennis *et al.* (2000) opined that increase in ADH may be helping to maintain ATP production in the absence of O_2 . In the roots of waterlogged finger millet plants increased alcohol dehydrogenase might be playing similar role, but this has elevated the level of ethyl alcohol. Umeda and Uchimiya (1994) found that transcription of alcohol dehydrogenase gene in 7 days old rice seedlings increased after 1 h of submergence and this increase was remained steady for

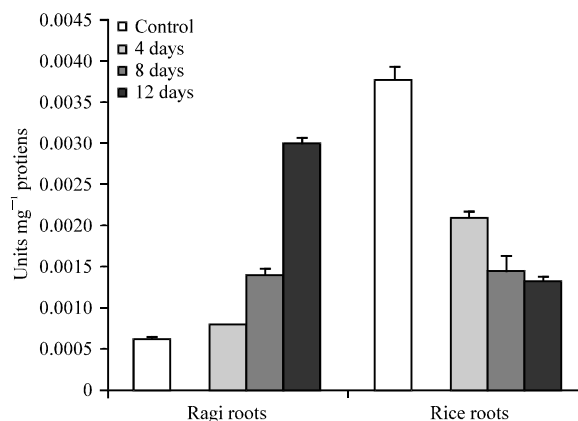


Fig. 1: Effect of waterlogging on activity of enzyme alcohol dehydrogenase in ragi and rice roots, average values Control ragi roots-0.0006 Units mg^{-1} proteins and Control rice roots-0.0037 Units mg^{-1} proteins

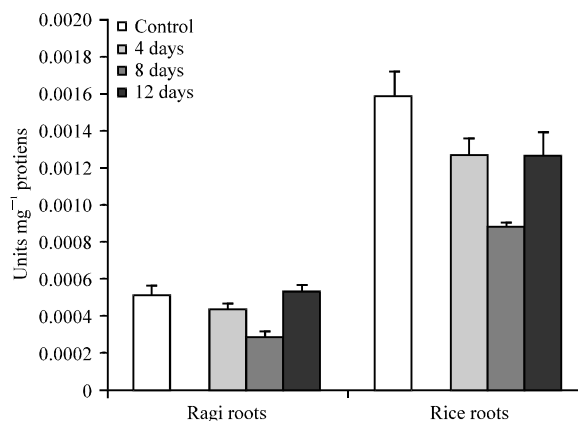


Fig. 2: Effect of waterlogging on activity of enzyme lactate dehydrogenase in ragi and rice root, average values Control ragi roots-0.00051 Units mg^{-1} proteins and Control rice roots-0.0016 Units mg^{-1} proteins

72 h and reduced afterwards. A decline in ADH activity indicates that in root system operation of normal glycolytic pathway may prevail or some metabolic modifications in the glycolysis avoiding alcohol formation may take place under the conditions of waterlogging.

Reduction of pyruvate catalyzed by Lactate Dehydrogenase (LDH). LDH activity is suggested to be involved in the short term hypoxic response by mediating the switch from ethanolic fermentation through acidification of the cytoplasm (Rivoal and Hanson, 1994). Chen and Quallis (2003) reported increase in LDH activity

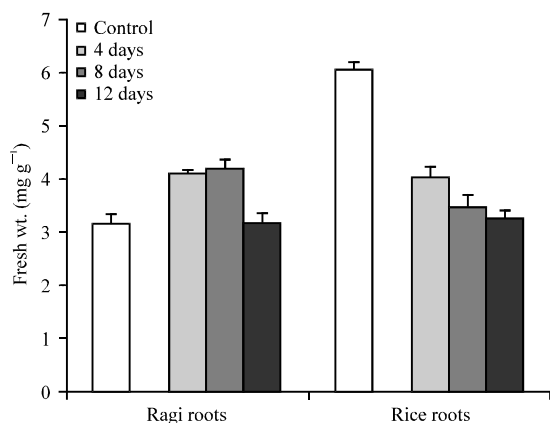


Fig. 3: Effect of waterlogging on acetaldehyde release from ragi and rice roots, average values Control ragi roots-3.50 mg g⁻¹ fresh wt. and Control rice roots-6.04 mg g⁻¹ fresh wt.

of seedlings of *Lepidium latifolium* when exposed to 7 days of anoxia. According to Davies-Roberts hypothesis, during preliminary stage of anoxia there is bursting of lactate formation with simultaneous drop of cytoplasmic pH (Davies, 1980; Roberts *et al.*, 1984). But cells of some plants, including rice this acidification is completed within 10-20 min of anaerobiosis (Felle, 1996). It is suggested by many workers lactic acid formation and activity of LDH is the first step of fermentation mechanism which is completed within early hours of stress. In present work, we noticed decline in lactate dehydrogenase activity as period of waterlogging increased from 4 days to 12 days. Thus, the long term cytoplasmic acidosis is prevented in roots of both these species which would be helpful in avoiding cellular injury.

Acetaldehyde release: Effect of waterlogging on acetaldehyde release in the root tissue of finger millet and rice is recorded in Fig. 3. In finger millet roots there is increase in acetaldehyde level due to 4 and 8 days of waterlogging. There is continuous decline in acetaldehyde level in rice roots with the increase in duration of waterlogging.

Kimmerer and Macdonald (1987) recorded more acetaldehyde production in flooded leaves of plants *Quercus palustris*, *Q. rubra*, *Betula rubra* and *B. lenta* than aerobic control. Sarkar (2001) studied a mechanism of acetaldehyde releasing capacity and waterlogging stress tolerance in different rice cultivars. Their study revealed that susceptible cultivars released greater quantities of acetaldehyde from root tissue than the tolerant cultivars. In present investigation, lowering of release of acetaldehyde in rice roots under waterlogging is noticed

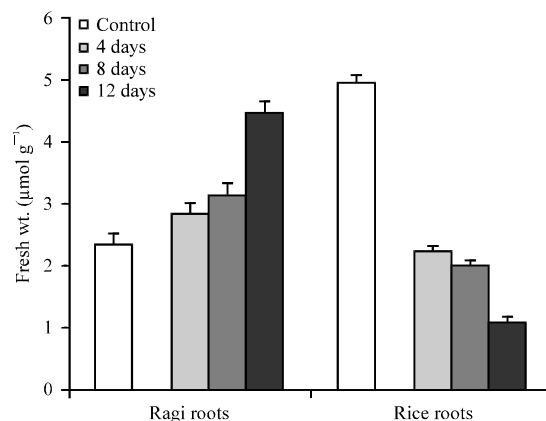


Fig. 4: Effect of waterlogging on ethanol content of ragi and rice roots, average values Control ragi roots-2.029 μmole g⁻¹ fresh wt. and control rice roots-5.15 μmole g⁻¹ fresh wt.

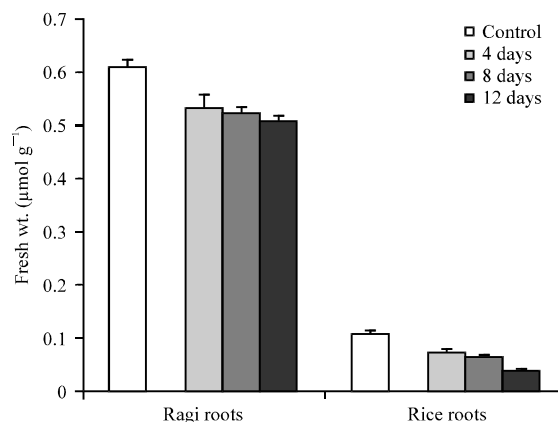


Fig. 5: Effect of waterlogging on lactic acid content of ragi and rice roots, average values Control ragi roots-0.638 μmole g⁻¹ fresh wt. control rice roots-0.122 μmole g⁻¹ fresh wt.

which indicate the presence of sufficient amount of O₂ in the root tissue due to possession of aerenchyma so that anaerobic fermentation does not predominate. On the contrary, in finger millet roots the release of acetaldehyde is promoted due to waterlogging indicating accelerated operation of anaerobic respiration.

Ethanol and lactic acid content: Effect of waterlogging on the level of ethanol and lactic acid content in the roots of finger millet and rice is shown in Fig. 4 and 5. In contrast to rice roots in finger millet roots, ethanol concentration is increased during waterlogging. Increase in waterlogging duration has led to considerable decrease in lactic acid content especially in root tissue of rice.

Hypoxic flooding of sunflower (*Helianthus annuus* L. cv. Delgren 131) roots caused rapid promotion of ethanol synthesis. Some of the root-synthesized ethanol was retained while a major portion was leaked into the flooding medium (Jayasekera *et al.*, 1990). In finger millet roots increase in ethanol level in the waterlogged roots is evident indicates continuation of glycolysis under anaerobic conditions through ethanolic fermentation pathway.

Menegus *et al.* (1989) indicated that flood tolerant species exhibit limited lactate production in comparison to flood sensitive species. Kato-Noguchi (2006) did not notice increase in lactate concentration and no initial burst of lactate production in rice coleoptile during anoxia. Wignarajah and Greenway (1976) reported very low content of lactate in *Zea mays* roots under anoxia. According to these workers this lowering was due to transport of O_2 in the root zone from shoot. In view of Rhodes (1980), lowering of tissue lactate content by secretion of lactic acid into medium can serve as a possible mechanism of avoidance of cytoplasmic acidosis. In both rice and finger millet plants increase in lactic acid level is not noticeable in waterlogged roots and on the contrary there is decline in lactic acid which is quite prominent in case of rice roots. Thus it appears that the cytoplasmic acidosis is avoided significantly in roots of both these species under waterlogged conditions.

Reduction in alanine and GABA content in finger millet:

Effect of waterlogging on the contents of alanine and Gamma (γ)-aminobutyric acid (GABA) in the roots of finger millet and rice is shown Fig. 6 and 7. It is evident from the figure that alanine level is decreased in roots of finger millet while there is increase in the alanine content in rice roots especially due to 4 days waterlogging. The content of γ -aminobutyric acid (GABA) is reduced due to waterlogging treatment in finger millet roots. On the other hand, in rice roots there is elevation due to 4 days waterlogging.

Alanine is one of the important building block of proteins in all living tissues. It is also a prominent component of free amino acid pool in the plant cells. It is indicated that alanine can also serve as alternate glycolytic product under waterlogged conditions. Accumulation of alanine was noticed by Reggiani *et al.* (2000) in response to waterlogging in 3-days old rice seedlings. In contrast to above report, Millar (2011) noticed that L-alanine did not significantly differ in abundance between control and anoxically-switched wheat coleoptiles. The experiment further revealed that pattern of amino acid profile was similar. In finger millet

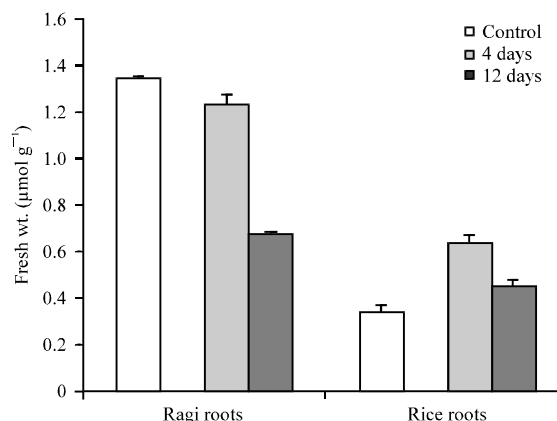


Fig. 6: Effect of waterlogging on alanine content of ragi and rice roots, average values Control ragi roots-1.352 $\mu\text{g g}^{-1}$ fresh wt. and Control rice roots-0.356 $\mu\text{g g}^{-1}$ fresh wt.

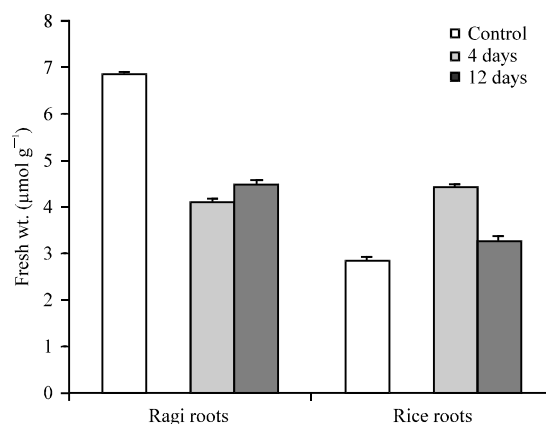


Fig. 7: Effect of waterlogging on GABA content of ragi and rice roots, Average values Control ragi roots-6.958 $\mu\text{g g}^{-1}$ fresh wt. and Control rice roots-2.964 $\mu\text{g g}^{-1}$ fresh wt.

roots free amino acid pool accumulation is not seen in response to waterlogging.

GABA represents a significant fraction of the free amino acid pool in plant cells. Sato *et al.* (2002) and Ricoult *et al.* (2005) stated that accumulation of GABA under stress condition is important as it store the C and N that would otherwise be lost under oxygen-deficient conditions. In rice coleoptiles, the anaerobic assimilation of inorganic nitrogen into amino acids, particularly alanine and γ -aminobutyrate (GABA)/glutamic acid may serve to supplement ethanolic fermentation in sustaining glycolytic energy production (Fan *et al.*, 1997). Millar (2011) noticed increase in concentration of GABA in

wheat and rice seedlings after 1 day of anoxia. In present investigation, we noticed slight increase in GABA content in waterlogged root tissue of rice. On the other hand, in finger millet roots there is reduction in GABA level under waterlogging stress. Since lactic acid production is not elevated in finger millet roots under waterlogged conditions a decrease in GABA level may not prove very crucial in relation to maintenance of cytoplasmic pH.

CONCLUSION

Waterlogging treatment was found to cause alternations in some facets of anaerobic metabolism in the roots of waterlogging tolerant crop rice and a hardy millet species finger millet. A decline in lactate dehydrogenase activity in roots of ragi and rice was noticed as period of waterlogging increased from 4 days to 12 days. Thus, the long term cytoplasmic acidosis is prevented in roots of both these species which would be helpful in avoiding cellular injury. But in case of waterlogged finger millet roots, activity of another anaerobic enzyme alcohol dehydrogenase was promoted along with increase in acetaldehyde and ethanol content. In this respect in rice roots opposite trend was noticed indicating avoidance of ethanol production. At the same time increase in level of some alternate protective compounds such as alanine and GABA was recorded in waterlogged rice roots in contrast to roots of finger millet.

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