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Research article

INFLUENCE OF LACTIC ACID ON SEED GERMINATION OF FINGER MILLET (*ELEUSINE CORACANA* GAERTN.)

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ABSTRACT: Lactic acid is regarded as a harmful product of anaerobic respiration occurring under waterlogged conditions in plant tissues. Effect of exogenous application of lactic acid on seed germination and activities of some important enzyme systems in seed of finger millet cultivar GPU28 was investigated. High concentration (0.5%) of lactic acid caused a marked reduction in germination percentage during initial phase (first 24 h) of seed germination. Lactic acid treatment caused suppression of seedling growth; radical growth being more sensitive than the coleoptiles growth. The analysis of enzyme activities at 24h germination stage revealed that lactic acid treatment caused decrease in the activities of enzyme amylase, acid phosphatase, ATPase, peroxidase, and catalase while there was elevation of alkaline phosphatase activity.

Key words: Anaerobiosis, Lactic acid, Ragi

INTRODUCTION

Environmental stresses such as waterlogging, drought, salinity etc. can cause severe effects on plant cells, sufficient to cause cell death. Among these stresses, waterlogging or flooding stress is found to pose severe threat to growth and yield of various crops. Gas diffusion from the atmosphere to the soil is drastically limited during flooding. As consequence, the respiratory activity of roots and micro- organisms rapidly depletes the bulk soil solution of oxygen and anoxic conditions are created in the soil environment (Drew and Lynch, 1980). In such conditions fermentation pathway actively generates ethyl alcohol and lactic acid as end products of respiration. Lactic acid has been regarded as the major factor causing cellular damage in waterlogged tissue through the process of cytoplasmic acidosis (Hochachaka and Mommensen, 1983). However no information is available regarding effect of lactic acid on different facets of plant cell metabolism. Hence in the present investigation an attempt has been made to study effect of exogenous application of lactic acid on some aspects of germination physiology of finger millet cultivar GPU28.

MATERIAL AND METHODS

Seeds of finger millet variety GPU-28 (red seeds) were collected from Agricultural Research Station of Agriculture College, Kolhapur (MS).

For study of effect of lactic acid on seed germination of ragi, different concentrations of lactic acid (0.1, 0.2, 0.4 and 0.5%) were prepared in distilled water. For germination study 10 seeds in each petriplate placed on the filter paper moistened with 10 ml of lactic acid solution while, seeds placed on filter paper moistened with distilled water served as control. Seeds were allowed to germinate at room temperature. The germination percentage was recorded after 24h. After 96h the seedling growth with respect to average root length and shoot length was recorded for seedlings of each treatment. For further metabolic studies two concentrations of lactic acid (0.1 and 0.5%) were chosen and the enzyme assays were performed from seeds germinated from 24h.

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The study of α-amylase was done by following the method of Katsumi and Fukuhara (1969). The activity of acid phosphatase was assayed according to the method of McLachlan (1980). The method of Weimberg (1970) was adopted for determination of activity of enzyme alkaline phosphatase. Enzyme ATPase from ragi seedlings was extracted following the method of Weimberg (1970) and its assay was performed according to the method described by Todd and Yoo (1964). The liberated inorganic phosphorus was estimated by the method of Fiske and Subba Rao (1925).Peroxidase activity was studied by the method of Horiguchi, (1988). Catalase activity was assayed following the method of Luck (1974) as described by Sadasivam and Manikam (1992). The soluble proteins in the enzyme extract were determined according to the method described by Lowry *et al*.,(1951). The values depicted in the figure represent average of three determinations.

RESULT AND DISCUSSION

Germination and seedling growth

Effect of different concentrations of lactic acid on the seed germination and seedling growth is depicted in the fig.1,2 and plate no.1. It is evident from the figure that germination percentage is markedly reduced due to high concentration of lactic acid (0.5%) especially during initial phase (24h). But at the same time, the germination percentage is very slightly affected by lower doses of lactic acid.

Lactic acid is found to exert marked negative effect on seedling growth. This effect is becomes more noticeable in case of 0.2% and higher concentrations of lactic acid. Due to 0.5% lactic acid the seedling growth is almost totally hampered. Among the coleoptiles and radicle, the growth of radicle is more sensitive to lactic acid treatment than that of coleoptiles and this is clearly evident in the seeds treated with 0.4% lactic acid. Lynch (1980) studied effect of different aliphatic, aromatic and amino acids on barley seed root elongation at 5.5 and 6.5 pH. They reported that at a concentration of 5 mol m^{-3} at pH 6.5, acetic acid, citric acid, lactic acid and glycine hardly affected the extension of roots while, at pH-5.5 aliphatic acids such as acetic acid, butyric, citric, formic, propionoic, succinic and aromatic acids such as benzoic, p-coumaric, p-hydroxybenzoic, 3-phenylpropionic, salicylic, syringic and vanillic acid inhibited root elongation. In contrast to barley, root growth in ragi seedlings appears to be quite sensitive to higher doses of lactic acid. They further observed that benzoic acid and salicylic acid decreased seed germination to 60% in barley.

Thus, cytoplasmic acidosis caused by lactic acid probably results in death of existing cells and also challenges the possibility of further cell division.

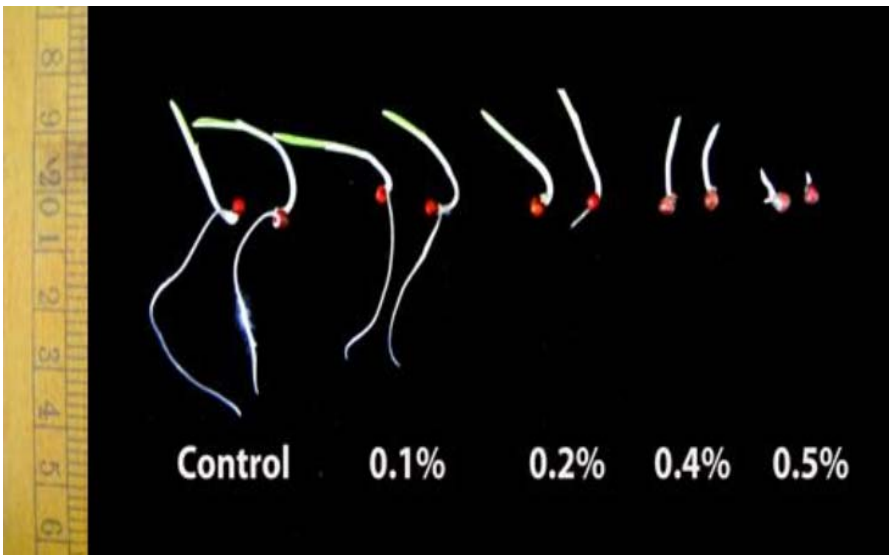

Plate No. 1 Effect of lactic acid treatment on seedling growth of finger millet cultivar (GPU-28)

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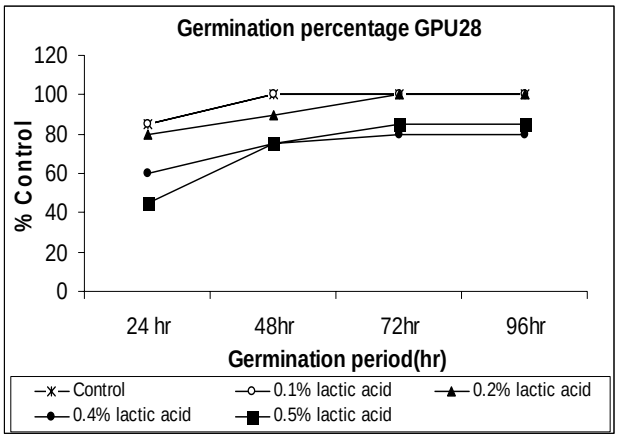
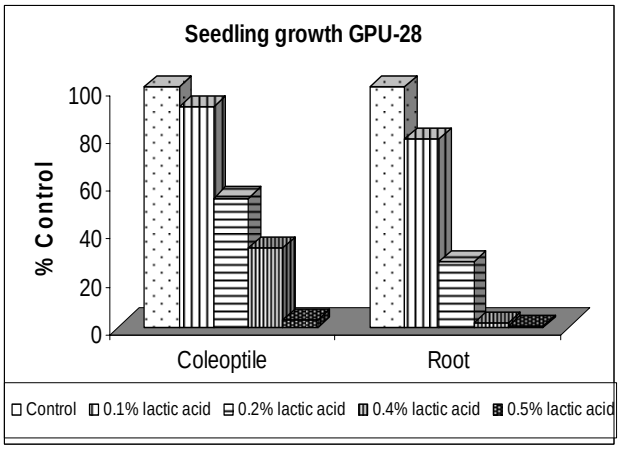


Control (24h)- 85 % Control (48h)-100% Control (72h)-100% Control (96h)-100%

Fig.1. Effect of lactic acid treatment on seed germination of finger millet cultivar GPU 28.



Control coleoptile-1.68 cm.Control radicle-1.95cm.

Fig.2. Effect of lactic acid treatment on seeding growth of finger millet cultivar GPU 28

Enzymatic studies

Effect of two different concentrations of lactic acid (0.1 and 0.5%) on the activity of α- amylase in germinating (24h) seeds of finger millet is shown in fig 3. It is clear from figure that the activity of α-amylase is significantly reduced due to lactic acid treatments.

α- amylase represents one of the most important enzymes of reserve carbohydrate catabolism during seed germination. The activity of this enzyme is considerably increased during seed germination in cereals. The interaction of ethyl alcohol and gibberellins in regulation of α- amylase activity has been also studied by some workers. In 1967, Jones and Varner reported that the aleurone tissue of barley was sensitive to the presence of fermentative product, ethyl alcohol which partly inhibited the induction of α- amylase triggered by gibberellic acid at concentrations more than 37mM. Perata *et al*., (1996) also made similar observations. Loreti *et al*., (2002) noticed that ethanol is produced by both barley and rice embryoless half seeds under anaerobic conditions. The amount of ethanol produced in barley (upto13mM) was significantly higher than that in rice (about 1mM). These workers fed exogenous ethanol under aerobic conditions to barley and rice grains treated with GA_3.The α- amylase produced was assayed.

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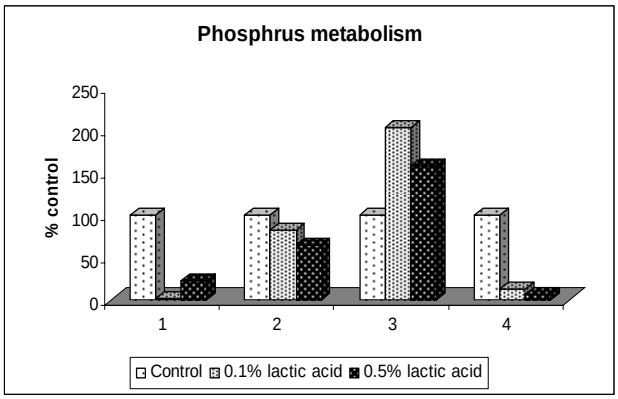
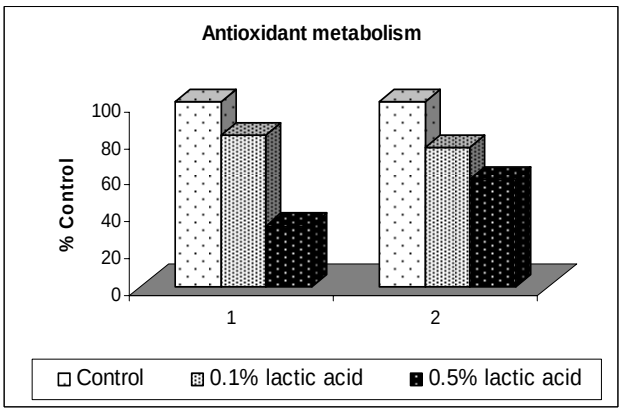
The results showed that upto 20mM ethanol concentration α- amylase production was not repressed in either barley or rice. Hence, these workers concluded that repression of α- amylase under anoxia is not brought about by ethanol. It is surprising that although, some attention has been paid to the effect of ethanol on α- amylase production, practically no attention has been given to the influence of another product of anaerobic metabolism lactic acid on this aspect. Our observations reveal that lactic acid is a potent inhibitor of α- amylase production in germinating ragi seeds. It would be interesting to see whether lactic acid treatment is able to prevent GA-mediated induction of α-amylase activity.



Acid phosphatase control- 98.55 µmoles p-nitrophenol $h^{-1}mg^{-1}$ protein Catalase control- 6.91 h^{-1} mg^{-1}protein

Fig.3. Effect of lactic acid treatment on phosphorus metabolism of finger millet cultivar GPU 28



Alkaline phosphatase control-0.00996 µmoles p-nitrophenol $h^{-1}mg^{-1}$ protein ATPase control- 0.0329 µg ip h^{-1} mg^{-1}protein

Fig.4. Effect of lactic acid treatment on antioxidant metabolism of finger millet cultivar GPU 28

Figure 3 shows effect of lactic acid treatment on the activity of enzyme acid phosphatase (ACP) in germinating finger millet seeds. It is evident from figure that higher concentration of lactic acid has also caused inhibition of the enzyme activity.

Acid phosphatase is a broad spectrum hydrolytic enzyme of phosphorus metabolism and it plays role in breakdown of phosphate reserves during seed germination. The influence of various phytohormones on the activity of acid phosphatase in germinating seeds has been studied by several workers. In the presence of GA_3, enzyme production is stimulated and the enzyme was released into the medium (Ashford and Jacobson, 1974).

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It is stated by Loreti *et al*., (2002) that overarching disturbance to general metabolism by ethanol or other causes such as cytoplasmic acidification is a probable reason of germination failure under waterlogging conditions. Our observations indicate that eventhough cytoplasmic acidification may take place under the influence of lactic acid, this does not lead to intensification of acid phosphatase in germinating seeds. Effect of treatment of lactic acid on the activity of alkaline phosphatase in germinating seeds (24h) is depicted in fig.3. It is evident from the figure that treatment of lactic acid has caused increase in activity of alkaline phosphates. The alkaline phosphatase is regarded as a hydrolytic enzyme involved in catabolic activity in case of animals (Pickering and Pickering, 1978; Patil, 2008).But in case of plants the exact role of this enzyme is not fairly understood. It is noticed in the present investigation that lactic acid affect the seedling growth of ragi. This effect might be mediated through its action on enzymes such as alkaline phosphatase. Effect of treatment of lactic acid on the activity of enzyme ATPase in the germinating finger millet seeds (24h) is shown in the fig. 3. It is evident from figure that activity of enzyme ATPase is considerably inhibited due to lactic acid treatment.

Seed germination has been defined as the transformation from the state of low metabolic activity to the state of very high metabolic activity (Bewley and Black 1978). It is obvious that during state of such high metabolic turnover the enzymes involved in synthesis and degradation of ATP would play a key role. Through inhibition of ATP ase activity, lactic acid treatment may pose limitations on availability of energy for various synthetic and growth processes in the seedling. The above findings do not support the idea of Sachs *et al*., (1980) that ATPase interssification due to cytoplasmic acidosis by lactic acid can cause decline in ATP level which may become a limiting factor for cell growth. Effect of lactic acid concentrations on the activity of enzyme peroxidase in the germinating seeds (24h) of ragi is shown in fig. 4. It is clear from figure that activity of enzyme peroxidase is inhibited due to the lactic acid treatment. Peroxidase is a multifunctional enzyme involved in various cellular growth processes, cell wall formation and antioxidant metabolism.

Among various plant enzymes, enzyme peroxidase is perhaps the most intensively studied enzyme with respect to influence of various factors on this enzyme (Gaspar, 1986). But at the same time not much information is available about influence of lactic acid on this enzyme. The work of Waffenschmidt *et al*., (1993), has demonstrated that in *Chlamydomonas* ethylene reduced activity of peroxidase as well as peroxidase induced lignification and protein assembly of cell wall.

Effect of lactic acid concentration on activity of enzyme catalase in the germinating ragi seeds (24h) shown in fig.4. The activity of enzyme catalase is decreased due to both concentrations of lactic acid.

Catalase is one of the oxidative enzyme in the plants and the activity of this enzyme is found to have correlation with respiration rate in germinating seeds (Vora *et al*., 1976) .Several compounds have been found to cause inhibition of catalase activity. Various plant acids such as α-Ketoglutaric acid , malic acid, citric acid, fumaric acid and oxalic acid were found to inhibit catalase activity in cotton leaf catalase (Lane and King 1968). Inhibition of catalase activity was observed when seeds of black gram were treated with 4mM of phenolic compounds such gallic, p-coumaric and p-hydroxybenzoic acids (Djanaguiraman *et al.,* 2005). According to Monk *et al.,* (1987), when anoxic intolerant rhizomes of *Glyceria maxima* exposed to ethanol and acetaldehyde vapors there was increase in catalase activity followed by rapid death of rhizomes. In contrast to ethanol treatment, in the present investigation lactic acid treatment is found to be inhibitory for catalase activity in ragi seeds. The inhibition of catalase by lactic acid can affect the free radical scavenging mechanism and there by disturb the overall cellular metabolism.

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