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Plant Growth Hormones and Salt Stress-Mediated Changes in Acid and Alkaline Phosphatase Activities in the Pearl Millet Seeds

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ABSTRACT

The effect of ABA, GA_3, and NaCl on germination and phosphatases in pearl seeds was investigated. Germination decreased markedly under ABA (65%) and salt (nil) treatments; however, germination under GA_3 was 90%, respectively. With comparison to the control, a considerable increase in both acid and alkaline phosphatase activities was observed in embryos after ABA and GA_3 treatments. In the endosperm, a significant increase in acid phosphatase activity was observed after ABA, GA_3 and NaCl treatments. Alkaline phosphatase activity was apparently higher after GA_3 treatment. From above findings it is clear that with less germination under ABA and NaCl, a dramatic increase in phosphatase activities were observed than that of control. However, under GA_3 no significant decrease in germination was observed but higher phosphatase actives were recorded, suggesting that metabolism of phosphatases in germinating pearl millet seeds are regulated differentially by ABA, NaCl and GA_3. These findings further suggest that changes in the phosphatase enzymes might play important roles in acclimation of pearl millet seeds, to the changing environmental conditions. Based upon these results, a possible physiological role of phosphatases in the germinating pearl millet seeds is discussed.

Key Words: Acid phosphatase; Alkaline phosphatase; Growth; Pearl millet

INTRODUCTION

Water stress affects every aspect of plant growth and metabolism. Plant responses to water deficit depend upon various factors such as duration and degree of stress, the stage of growth, time of stress exposure and tissue type (Gupta & Sheoran, 1983). Due to their sedentary mode of life, plants involve many adaptive strategies in response to different abiotic stresses such as high salt, dehydration, cold and heat, which ultimately affect plant growth and productivity (Gill *et al*., 2003). Against these stresses, plants adapt themselves by different mechanisms including change in morphological and developmental pattern as well as physiological and biochemical responses (Bohnert *et al*., 1995). Adaptation to all these stresses is associated with metabolic adjustments including modulation of different enzymes (Shinozaki & Yamaguchi- Shinozaki, 1996; Yan *et al*., 2001; Ehsanpour & Amini, 2003). Phosphatases are one among them, which are believed to be important for the regulation of soluble phosphorous (Pi) (Yan *et al*., 2001) and traditionally classified as being acid and alkaline phosphatases ascending to their optimum pH for enzyme activity above and below pH 7.0 (Barrett-Lennard *et al*., 1982). Phosphorous (Pi) is an essential macronutrient for plant growth and development that plays key role in many processes, including energy metabolism and synthesis of nucleic acids and membranes (Fincher, 1989; Ehsanpour & Amini, 2003). Although, some abiotic stresses like salt, osmotic and water have been reported to increase acid phosphatase activity by maintaining a certain level of inorganic phosphate in the plant cells (Olmos & Hellin, 1997), however, the exact role of phosphatases at germination level is unknown, because, metabolism of these compounds can be affected by a number of environmental factors such as stress type, irridance, temperature, and type of ions present (Bohnert *et al*., 1995). Germination of grains is initiated by uptake of water and its successful completion is signaled by emergence of the developing root and shoots. Following uptake of water, hormone signals, probably released from the embryos are believed to result in the synthesis of hydrolytic and other enzymes in the endosperm (Fincher, 1989). Like mature plants, germinating seeds and seedlings can also be subjected to environmental stresses. Even when they imbibe water, seeds may be exposed to elements of a hostile environment, which include high temperature of soil, salinity and varying moisture content. Failure to cope with the adversity caused by these extremes results in poor germination, seedling development, and eventually, reduced crop yields. Thus, the variation that occurs in phosphatase activities during germination is poorly understood and information on physiological events involved in this process is scarce. Therefore, in this study, growth and status of phosphatase enzyme activities in the germinating sorghum seeds under salt stress and to the application of ABA and GA_3 was studied. Pearl millet is well adapted to semiarid and arid tropics, where salinity and drought are the major problem due to limited water supply. There is very little information available on these aspects in tropical crops. In view of this pearl millet has been selected for the present investigation.

MATERIALS AND METHODS

Seed germination and growth conditions. Washed grains of pearl millet (*Pennisetum glaucum* (L.) *R. Br.*) Were surface sterilized with 1% (w/v) mercuric chloride followed by 70% ethanol (Gill *et al*., 2003). Seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibation, seeds were placed in petriplates containing sterile filter sheets, moistened with 2 ml of distilled water (in case of control), ABA (0.1 mM), GA_3 (0.1 mM), and NaCl (410 mM) (Soderman *et al*., 1996; Gill *et al*., 2003). The plates were incubated at 37±1°C in a seed germinator. Germination percentages and biochemical analysis were determined after 14 h using radicle protrusion (appearance of radicle 2 mm in length) as a criterion (Gill *et al*., 2003). Each treatment was repeated three times independently of each other and each replicate included 100 seeds (i.e. 300 seeds per treatment). In order to determine the influence of different treatments on germination, mean of the three replicates were taken and seed germination per 100 seeds was calculated. For biochemical analysis, tissues from each replicate independently of other replicate were combined and used for further studies. Embryos and endosperm in water-irrigated control, GA_3 and ABA treatments were separated. After NaCl treatment (nil germination) it was difficult to separate the embryos from endosperm, therefore, whole seed was stored immediately in liquid nitrogen until further analysis. Parts of these tissues were weighed to obtain the fresh weight (FW). The dry weight (DW) was obtained after drying the different tissues at 75°C till constant weight. Tissue water content was obtained from the (FW-DW/FW) ratio.

Extraction and assay of acid and alkaline phosphatases. Both acid and alkaline phosphatases were extracted from the tissues essentially following the method of Sawhney and Singh (2000), by grinding the tissues with mortar and pestle at 0-4°C using 50 mM sodium acetate (pH 5.0) for acid phosphatase and 50 mM glycine NaOH buffer (pH 10.5) for alkaline phosphatase. The homogenate was centrifuged at 12,000 rpm for 15 min, and the supernatant collected. Phosphatase activities were assayed by measuring the amount of p-nitrophenol produced. One unit (U) of phosphatase (acid and alkaline) is equivalent to the amount of enzyme liberating 1 µmole of product per min under assay conditions.

Statistical analysis. The data obtained was subjected to ANOVA and student's *t*-test.

RESULTS

Seed germination under different treatments. Seed germination in distilled water reached the maximum (99%) after 14 h of plating. However, imposition of ABA and salt treatments resulted in a considerable delaying in germination. After 14 h of plating, nil germination was observed under NaCl and 65% germination was observed under ABA. No significant change in germination was observed after GA_3 treatment (90%) (Table I). Subsequently, the germination percentages increased to more than 90% after 72 h under ABA and 120 h in NaCl (data not shown).

ABA, GA_3 and NaCl-modulated physiological and biochemical changes. As compared with the control, no significant reduction in FW (Fresh weight) and DW (dry weight) of embryos was observed after GA_3 treatment (Table I), however, a significant decrease in both FW and DW was observed after ABA treatment. The tissue water content (FW-DW/FW) ratio, a measure of expansion growth of embryos in distilled water showed no substantial change with respect to GA_3 treated embryos, however, a dramatic decrease was observed after ABA treatment. As compared with control, a significant increase in acid phosphatase activity was observed after ABA and GA_3 treatments (Table I); however, the rate of enhancement was relatively same under ABA as well as GA_3 treatments. Similarly, a significant amount of alkaline phosphatase activity was observed after ABA and GA_3 treatments.

No significant change in endosperm FW, DW and tissue water content was observed after all treatments (Table II). The Acid phosphatase activity was significantly higher after ABA, GA_3 and NaCl treatments (Table II). The greatest activity was observed only after NaCl treatment. Alkaline phosphatase activity was apparently higher after ABA, GA_3 and NaCl treatments. Contrary to maximum acid phosphatase activity after NaCl treatment, the alkaline phosphatase activity was greater after GA_3 treatment. The rate of increase in alkaline phosphatase activity was also higher after GA_3 treatment.

DISCUSSION

The present investigation monitored changes caused plant growth hormones and salt stress treatments in the phosphatase enzymes of pearl millet seeds. Imposition of NaCl treatment resulted in nil germination; however, germination rate after GA_3, ABA, was 90%, 65%, respectively. Similar decreases in seed germination have been reported in the literature (Garciarrubio *et al*., 2003; Gill *et al*., 2003). The decrease in germination particularly under salt stress may be due to the fact that seeds seemingly develop an osmotically enforced "*dormancy*" under water stress conditions (Gill *et al*., 2003). This may be an acclimation strategy of seeds to prevent germination under stressful environmental conditions thus ensuring proper establishment of the seedlings. This assertion was supported by the fact that after 72 and 120 h, respectively, ABA- and NaCl-treated seeds recorded almost equal germination with respect to water-irrigated control (data not shown). However, the decrease in germination rate observed after ABA treatment may be attributed to metabolic alternations. The dormancy-inducing hormone (ABA) may also be involved in inhibiting seed germination by restricting the

Table I. ABA, GA_3 and NaCl-induced changes in germination along with effect of ABA and GA_3 on fresh weight (FW), dry weight (DW), tissue water content, acid and alkaline phosphatase activities in the embryos of pearl millet

Treatment	Germination (%)	FW (mg embryo^{-1})	DW (mg embryo^{-1})	Tissue water content	Phosphatase activity (Units.10^{-5} embryo^{-1})	
					Acid	Alkaline
Control	99±1	2.03±0.03	0.36 ±0.02	0.82± 0.03	5.10±0.85	4.21±0.77
ABA	*65±2	*0.62±0.02	*0.15 ±0.03	*0.75 ±0.02	*7.12±0.54	*8.80±0.12
GA_3	b90±6	b2.61±0.02	b0.33 ±0.05	b0.87 ±0.06	*7.51±0.32	*7.81±0.32
NaCl	nil	nd	nd	nd	nd	nd

*, represent significant difference vs. control at (P≤ 0.05). b, not significantly different, nd: not determined.

Table II. Effect of ABA, GA_3, and NaCl on endosperm fresh weight (FW), dry weight (DW,) tissue water content, acid and alkaline phosphatase activities of pearl millet seeds

Treatment	FW (mg endosperm^{-1})	DW (mg endosperm^{-1})	Tissue water content	Phosphatase activity (Units.10^{-5} embryo^{-1})	
				Acid	Alkaline
Control	6.01± 1.20	2.56± 0.09	0.57± 0.05	0.79±0.23	0.33±0.08
ABA	b5.23 ±1.54	b2.33 ±0.08	b0.55 ± 0.29	*6.96±0.45	*2.32±0.12
GA_3	b4.82 ±1.80	b1.63 ±0.99	b0.65 ± 0.11	*9.17±0.08	*205.8±6.80
NaCl	b6.00 ±1.30	b2.81 ±0.08	b0.53 ± 0.18	*17.1±2.30	*2.01±0.33

availability of energy and metabolites (Garciarrubio *et al*., 2003). Exogenous application of ABA has been shown to inhibit embryonic germination by modulating the endogenous level of ABA (Dewar *et al*., 1998). During germination, another plant hormone, gibberellin (GA), induces embryo growth and, therefore, stimulates the grain germination process. Therefore, with comparison to water-irrigated seeds, no substantial change in germination was observed under GA_3 treatment. As GA_3 is well-documented regulator of germination and associated enzymes with generally having promotive effects (Fincher, 1989). So, it may be clear that at onset of germination, ABA and GA_3 appear to act in a fully antagonistic manner. Embryo fresh and dry weights were significantly reduced under ABA, which is also known as stress hormone. They were smaller than in distilled water because of reduced fresh weight resulting from reduced water absorption (Prado *et al*., 1995). The FW increase in distilled water was mainly due to an increase in tissue water content and was reflected in (FW-DW/FW) ratios. Contrary to embryo, no significant changes in fresh and dry weights were observed under any treatment. These findings indicate the tissue dependent responses. The maintained dry weight of embryos after GA_3 treatment and of endosperm after NaCl as well as ABA treatments may be due to the osmotic adjustment provided by phosphatases inside the seeds.

In earlier research, Gill and Singh (1985) has reported that germination, growth, respiration and other related processes can be affected in seeds that are subjected to environmental stresses. Changes in any one of these processes can affect other metabolic activities, particularly the enzymes of phosphate metabolism that plays an important role in germination and seed development (Fincher, 1989). Therefore, the effect of phytohormones ABA and GA_3 on phosphatase activities was studied, which are well known regulators of germination, with GA_3 generally having promotive effects and ABA having inhibitory effects on germination and related enzymes through multiple regulatory mechanisms (Fincher, 1989). In the present study, with comparison to the untreated embryos, a significant increase in acid and alkaline phosphatase activities were observed after ABA and GA_3 treatments, which is in agreement with the earlier reports (Fincher, 1989). In endosperm, with comparison to the control, a significant increase in acid and alkaline phosphatase activities were observed after ABA as well as GA_3 treatments, however, the rate of increase in alkaline phosphatase activity was greater after GA_3 treatment than that of acid phosphatase after NaCl treatment. From all these data, it is clear that ABA and GA_3 mediated responses are tissue dependent and regulated through different pathways.

Normally, salt and water stresses affect physiology and biochemistry of plant cells under *in vitro* and *in vivo* conditions. These stresses have also been reported to enhance acid phosphatase activity in pea and wheat (Barrett-Lennard *et al*., 1982). It may be due to fact that under conditions of stress, growth is restricted and delivery of phosphate is impaired, thus resulting in the activation of the cellular phosphatases that release soluble phosphate from its insoluble compounds inside or outside of the cells thereby modulates osmotic adjustment by free phosphate uptake mechanism (Fincher, 1989). Here, we also demonstrated that the endosperm acid and alkaline phosphatase activities were significantly higher after salt treatment, than that of the control. Olmos and Hellin (1997) observed that acid

phosphatases are known to act under salt and water stress by maintaining a certain level of inorganic phosphate which can be co-transported with H^+ along a gradient of proton motive force. In contrast, Szabo-Nagy *et al.* (1992) and Barrett-Lennard *et al.* (1982), reported results, indicating that phosphatase activities are independent of phosphate levels. In future, we hope to find out more with regards to phosphate and correlating enzymes. Additionally, in this study we could hardly found any positive correlation between germination and phosphatases under plant growth regulators or salt stress. Although, ABA, NaCl decreased germination and GA_3 promoted, however, in all the treatments the levels of phosphatases were increased in embryos and endosperm. In future, we could find out more with context to germination and phosphatases. But from the above data it became apparent that under less germination higher levels of phosphatases might be involved in acclimation (keeping developing embryonic axis in resting state) of germinating seeds by modulating internal phosphate levels. Further, GA_3 modulated germination studies with phosphatases clearly reveled that other factors apart from phosphatases might also be involved in the germination process.

In conclusion, the present study indicates that changes observed under various abiotic conditions are associated with acclimation of pearl millet seeds to different environmental conditions that lead to an increase in synthetic activity and associated changes. Further, investigations are needed to enhance the understanding on the effect of different abiotic stresses and growth hormones during early seed development.

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