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Full Length Research Paper

Effect of plant growth hormones and abiotic stresses on germination, growth and phosphatase activities in *Sorghum bicolor* (L.) Moench seeds

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Phosphatases are widely found in plants having intracellular and extracellular activities. Phosphatases are believed to be important for phosphorous scavenging and remobilization in plants, but its role in adaptation to abiotic stresses and growth hormones at germination level has not been critically evaluated. To address this issue, the effect of ABA, GA_3, NaCl and drought on germination, growth, acid and alkaline phosphatases in sorghum embryos and endosperm was investigated. Germination decreased markedly under ABA, NaCl and drought treatments. Subsequently, a remarkable decrease in fresh weight and dry weight was observed in embryos under ABA and NaCl treatments, whereas a significant decrease in endosperm fresh weight was observed only under drought stress. However, no significant change in endosperm dry weight was observed under other any treatment. Furthermore, a considerable increase in acid phosphatse activity was observed in embryos under GA_3 and NaCl treatments, however, alkaline phosphatase activity was substantially higher under all treatments. In endosperm, a significant increase in acid phosphatase activity was observed under ABA and NaCl treatments. Alkaline phosphatse activity was apparently higher under GA_3. However, no substantial changes in acid or alkaline phosphatase activities were observed after drought treatments. These findings suggest that changes in the phosphatase enzymes might play important roles in adaptation of germinating seeds, to changing environmental conditions. Based upon these results, a possible physiological role of phosphatases in germinating sorghum seeds is discussed.

Keywords: Growth, sorghum, acid phosphatase, alkaline phosphatase.

INTRODUCTION

Water stress affects practically every aspect of plant growth and metabolism. Plant responses to water deficit depend upon various factors such as duration and degree of stress, growth stage and time of stress exposure (Gupta and Sheoran, 1983). Due to their sedentary mode of life, plants resort to many adaptive strategies in response to different abiotic stresses such as high salt, dehydration, cold and heat, which ultimately affect the 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 that lead to the modulation of different enzymes (Shinozaki and Shinozaki, 1996; Yan et al., 2001; Ehsanpour and Amini, 2003). Among these enzymes are phosphatases, which are believed to be important for many physiological processes, including regulation of soluble phosphorous (Pi) (Yan et al., 2001). Phosphateases are traditionally classified as being acid and alkaline depending on their optimum pH for enzyme activity, above and below pH 7.0 (Barret-Lennard et al., 1982). Free soluble phosphate reserves plays vital role in energy transfer, metabolic regulation, important structural constituent of biomolecules like phytin bodies in the ungerminated seeds, protein and nucleotide phosphorylation (Fincher 1989; Ehsanpour and Amini, 2003).

Although, some abiotic stresses like salt, osmotic and water have been reported to increase phosphatase activities by maintaining a certain level of inorganic

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phosphate in plant cells (Olmos and Hellin, 1997), the exact role of phosphatases in the germinated seeds is still not clear, 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 water uptake and its successful completion is signaled by emergence of the developing root and shoot. Following uptake of water, hormone signals, probably released from the emergence, are believed to result in the synthesis of hydrolytic and other enzymes in the endosperm (Fincher, 1989). Moreover, like mature plants, germinating seeds and seedlings also can 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 cause by these extremes results in poor germination, seedling development, and eventually, reduced crop yields.

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, we present details on germination, growth and status of phosphatase enzyme activities in germinating sorghum seeds under salt, drought stresses and to the application of ABA and GA_3. Sorghum is a C_4 grass that is well adapted to semiarid and arid tropics (Quinby, 1974) where salinity is the major problem due to limited water supply. This grain crop is the fifth most important cereal grown worldwide, due in large parts to its unusual tolerance to adverse environmental conditions (Doggett, 1988). ABA and GA_3 are well-documented regulators of germination, with GA generally having promotive effects and ABA having inhibitory effects on germination and related changes through multiple regulatory mechanisms, including transcriptional control and synthesis of specific enzymes (for review see Fincher, 1989).

MATERIALS AND METHODS

Plant material and chemicals

The seeds of *Sorghum bicolor* (L.) Moench cv. CSH-6 was purchased from National Seed Corporation, Pusa, and New Delhi, India. The fine chemicals and reagents used in this study were purchased from Sigma chemicals, corporation, St. Louis, USA. All the other chemicals were of analytical grade.

Seed germination and growth conditions

Washed grains of *S. bicolor* were surface sterilized with 1% (w/v) mercuric chloride followed by 70% ethanol. Seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibation, the seeds were placed in petriplates containing sterile filter sheets, and different treatments viz: ABA, GA_3, NaCl and drought were imposed as described in Gill et al. (2003) and Soderman et al. (1996). The plates were incubated at 37±1°C in a seed germinator. Germination percentages and biochemical analysis were estimated 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 were combined and used for further studies. Embryos and endosperm in water-irrigated control, ABA, GA_3 and NaCl treatments were separated whereas whole seeds after drought treatment (nil germination), were 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)/DW 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 in 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

A statview ANOVA program was used for statistical analysis of the data. Values for different treatments with in each tissue were compared using one-way analysis of variance with repeated measures and student's *t*-test for differences between pairs of data if the ANOVA ($LSD_{0.05}$) revealed significance. Means were tested by LSD at P=0.05 level ($LSD_{0.05}$).

RESULTS

Seed germination under different treatments

Seed germination in distilled water reached the maximum (99%) in 14 h. Imposition of ABA and NaCl treatments resulted in a considerable decrease in germination as compared to GA_3 (98%). However, nil germination was observed after drought treatment (Table 1).

Table 1. Effect of ABA, GA3, NaCl and drought on germination of sorghum seeds. Data represent mean of three independent experiments.

Treatment	Germination (%)
Control	99±2
ABA	$65±4^{s}$
GA_3	$98±2^{ns}$
NaCl	$35±4^{s}$
Drought	nil

^{s}, significant difference vs. control at ($P \leq 0.05$).
^{ns}, not significantly different.

Table 2. Effect of ABA, GA_3, and NaCl on fresh weight(FW), dry weight(DW) and tissue water content of embryos of sorghum. Data represent mean of three independent experiments.

Treatment	FW (mg embryo^{-1})	DW (mg embryo^{-1})	Tissue water content
Control	7.01±1.10	3.50±0.20	1.00±0.14
ABA	0.53±0.12^{s}	0.22±0.08^{s}	1.40±0.35^{ns}
GA_3	6.90±1.20^{ns}	3.20±0.25^{ns}	1.15±0.17^{ns}
NaCl	2.10±0.11^{s}	1.60±0.13^{s}	0.31±0.05^{s}

^{s}, significant difference vs. control at (P≤0.05).
^{ns}, not significantly different.

Table 3. Effect of ABA, GA_3, NaCl and drought on endosperm fresh weight (FW), dry weight (DW) and tissue water content of sorghum. Data represent mean of three independent experiments.

Treatment	FW (mg endosperm $^{-1}$)	DW (mg endosperm $^{-1}$)	Tissue water content
Control	47.01±2.35	41.10±2.70	0.14±0.05
ABA	45.03±3.10^{ns}	39.00±2.35^{ns}	0.15±0.07^{ns}
GA_3	46.20±2.50^{ns}	40.00±2.11^{ns}	0.15±0.06^{ns}
NaCl	46.21±2.81^{ns}	41.54±2.13^{ns}	0.11±0.08^{ns}
Drought	40.01±2.01^{s}	38.12±3.14^{ns}	0.04±0.008^{s}

^{s}, significant difference vs. control at (P≤0.05).
^{ns}, not significantly different.

Changes in embryo

No significant reduction in embryo FW and DW was observed after GA_3 treatment (Table 2). However, a significant decrease in both FW and DW was observed after ABA as well as NaCl treatments. The tissue water content (FW-DW)/DW ratio, a measure of expansion growth of embryo in distilled water showed no substantial change with respect to ABA and GA_3 treated embryos, however a dramatic decrease was observed under NaCl treatment.

A significant increase in acid phosphatase activity was observed after GA_3 and NaCl treatment, however, a considerable decrease was observed in ABA-irrigated embryos (Table 4). On the contrary, a significant amount of alkaline phosphatase activity was observed after all treatments.

Changes observed in endosperm

No significant difference in FW was observed under all treatments, except a substantial decrease was observed in the endosperm under drought (Table 3). Parallel to this, no significant change in DW was observed under all treatments. A significant decrease in TWC was observed only under drought treatment.

Acid phosphatase activity was significantly higher under ABA and NaCl treatments. A substantial decrease in acid phosphatase activity was observed after GA_3 treatment (Table 5). On the contrary, alkaline phosphatase activity was apparently higher under all treatments. No significant change was observed after drought treatment.

Table 4. ABA-, GA_3-, NaCl-induced changes in acid and alkaline phosphatase activities in the embryos of sorghum. Data represent mean of three independent experiments.

Treatment	Phosphatase activity (Units.10^{-5} mg^{-1} DW)	
	Acid	Alkaline
Control	4.00±0.85	nd
ABA	2.12±0.54^{s}	7.80±0.12^{s}
GA_3	7.51±0.32^{s}	4.81±0.32^{s}
NaCl	163.2±5.50^{s}	41.3±3.40^{s}

^{s}, significant differences vs. control at (P≤ 0.05).
nd; not determined.

DISCUSSION

The present investigation monitored changes caused ABA, GA_3, NaCl and drought treatments in germination rate, growth, and phosphatase enzymes of *S. bicolor*. Imposition of drought treatment resulted in a very significant decrease in germination rate (nil), while

Table 5. Effect of ABA, GA_3, NaCl and drought on endosperm acid and alkaline phosphatase activities in the seeds of sorghum. Data represent mean of three independent experiments.

Treatment	Phosphatase activity (Units.10^{-5} mg^{-1} DW)	
	Acid	Alkaline
Control	1.61±0.23	0.33±0.08
ABA	6.96±0.45^{s}	2.32±0.12^{s}
GA_3	0.17±0.08^{s}	205.8±6.80^{s}
NaCl	17.1±2.30^{s}	2.01±0.33^{s}
Drought	1.51±0.35^{ns}	0.32±0.09^{ns}

^{s}, significant difference vs. control at (P≤0.05).
^{ns}, not significantly different.

germination rate under GA_3, ABA, and NaCl were 98%, 65%, and 35%, respectively. Similar declines in seed germination have been reported in the literature (Gill et al., 2003, Garciarrubio et al., 2003). The decrease in germination rate particularly under drought and salt stress conditions may be due to the fact that seeds seemingly develop an osmotically enforced "*dormancy*" under water stress conditions. This may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedlings (Gill et al., 2003). However, decrease in germination rate observed under ABA treatment may be attributed to metabolic alternations. ABA may also be involved in inhibiting the seed germination by restricting the 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), and when ABA synthesis is inhibited by fluridone, precocious germination occurs. During germination, another plant hormone, gibberellin (GA), induces embryo growth and stimulates the germination process. Therefore, in comparison to water-irrigated seeds, no substantial change in germination was observed under GA_3 treatment. GA_3 is well-documented regulator of germination and associated enzymes with generally having promotive effects (Fincher, 1989). Thus at onset of germination, ABA and GA_3 appear to act in a fully antagonistic manner (see Jacobsen and Beach, 1985; Fincher, 1989). Further, a significant reduction in tissue water content was observed in the embryos under NaCl treatment and under drought stress in the endosperm, indicating that respective tissues were under water stress. Similar observations on decrease in water level under stress conditions were made by Siddique et al. (2000) in wheat and Pennypacker et al. (1990) in alfalfa.

In relation to growth, embryos were suppressed by ABA and NaCl treatments. Endosperm FW was significantly reduced under drought stresses conditions. The reduced FW results from reduced water absorption (Prado et al., 1995). Experimental evidence have shown that osmotic adjustment can reduce growth sensitivity to water stress (Cutler et al., 1980) or allow growth to proceed at a slow rate under water stress by maintaining turgor. It can then be concluded that contribution of growth at lower water potential is a result of turgor maintenance, whereas the inhibition of growth is not entirely dependent on turgor (BassiriRad and Coldwell, 1992). The FW increase in distilled water was mainly due to an increase in tissue water content and is reflected in the (FW-DW)/DW) ratios. Our results showed that imposition ABA and NaCl treatments resulted in a very significant reduction in dry matter of embryos. Chartzoulakis et al. (1993), Finkelstein and Lynch, (2000) and Kirnak et al. (2001) reported similar declines in dry matter. It is well known that as soil water availability becomes limited, plant growth usually decreases. This was previously considered to be due to turgor loss in expanded cells. However, some studies have shown that growth may be inhibited at low water potential despite complete maintenance of turgor in the growing regions as a result of osmotic adjustment. This suggests that the growth inhibition may be metabolically regulated possibly serving an adaptive role by restricting the development of transpiring leaf area in the water-stressed plants (Sharp, 1996).

In order to gain further insight into the physiological changes occurring during hormonal and stress conditions, we studied acid and alkaline phosphatases activities in sorghum embryos and endosperm. 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 anyone 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). Normally, salt and water stresses affect the physiology and biochemistry of plant cells under *in vitro* and *in vivo* conditions. These stresses have 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, resulting in the activation of the cellular phosphatases that release soluble phosphate from its insoluble compounds inside or outside of the cells thereby modulating free phosphate uptake mechanism. A water deficit has also been shown to affect acid phosphatase in wheat (Thakur and Thakur, 1993). Here, we also demonstrated that the embryo acid and alkaline phosphatase activities were significantly higher under salt stress. Similarly in endosperm, the acid and alkaline phosphates activities were significantly higher. Interestingly, in contrary to the earlier report in wheat (Barrett-Lennard et al., 1982), we could hardly find any increase in acid or alkaline phosphatase activities under drought stress, indicating the cultivar and stress

dependent nature of phosphatases. 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-Negy et al. (1992) and Barrettt-Lennard et al. (1982) reported results, indicating that phosphatse activities are independent of phosphate levels. In future, we hope to expand our investigation with regards to phosphate and correlating enzymes.

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