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Effect of salt and drought stress on acid phosphatase activities in alfalfa (*Medicago sativa* L.) explants under *in vitro* culture

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Acid phosphatase is wildly found in plants. This enzyme has intra and extra cellular activity. For instance, it dephosphorylase organic phosphate and change it to inorganic phosphate. However, acid phosphatase activity is increased by salt and osmotic stress. In this experiments, calluses were produced from *invitro* grown explants of *Medicago* sativa cv. Yazdi and cv. Hamedani under aseptic condition on MS medium containing NAA, 2,4-D. Then calluses and seedling were transferred to the same medium containing 0,0.2, 0.4, 0.6, 0.8, 1% NaCl and 0, 2, 4, 6, 8, 10% Mannitol as osmotic stress. After 2 weeks acid phosphatse activities were measured and data statistically analyzed. Clearly acid phosphatase activities was increased by salt and drought stress in both cultivars, and the difference between two genotype indicating that the acid phosphatase activity is highly genotype dependent.

Key words: Acid phosphatase, *Medicago sativa*, osmotic, salt, drought, stress.

INTRODUCTION

Phosphatase have been traditionally classified as being alkaline and acid phosphatase (Apase) acceding to their optimum pH for catalytic activity above or below pH 7.0 (Barret-Lannard et al., 1982). Acid phophatase is widely distributed in plants and variations in different plant species that have been studied. Many electrophoretically distinguishable isoforms are often present in a tissue or cell type. This enzyme is localized as intracellular in cytoplasm and vacuoles and extracellular in cell wall (Lee, 1998). Acid phophatase isosyme (Apase-1-1) has been identified as a marker for nematode resistance gene in *Lycopersicon peruvianum* (Ehsanpour and Jones, 1996). Orthophosphate anion "inorganic phosphate" (Pi) plays a vital functional role in energy transfer and metabolic regulation and is also an important structural constituent of many biomulacules. Consequently, Pi assimilation, storage and metabolism are of critical importance to plant growth and development (Vincent et al., 1992). Direct uptake of organic phosphate compounds by plants is considered unlikely. Rather phosphate is acquired by plant roots as inorganic phosphate from the soil solution. Thus to contribute to plant phosphate nutrition, soil organic phosphate must first be dephosphorylated by phosphatases (Julie et al., 2000).

It has been reported that acid phosphatase activities of roots *in Medicago polymorpha* is increased under phosphate deficiency (Julie et al., 1999). Barrett-Lannared et al. 1982) also demonstrated that salt and water stress increase acid phosphatase activity in *Pisum sativum*. Acid phosphatase is known to act under stress by maintaining a certain level of inorganic phosphate in plant cells (Lefebvre et al., 1990, Chiung-Yueh et al., 1998, Olmos and Hellin, 1997, Deleo and Sacher 1970). The aim of this study is to assess the effect of salt (NaCl) and osmotic stress (water stress) on acid phosphatase activities of *Medicago sativa* callus, leaf, stem and roots under *in vitro* condition.

MATERIAL AND METHODS

Seeds of *Medicago sativa* cv. Yazdi and cv. Hamedani were obtained from Natural Resource Center of Esfahan, Iran. Seeds then were surface sterilized in 20% (v/v) sodium hypochlorite for 20 min, followed by 3 washes with sterile distilled water under aseptic condition. Calli were produced from stem segments of *in vitro* grown plants on MS (Murashige and Skooge, 1962) medium containing kinetin, 2,4-D (2,4 Dichlorophenoxyacetic acid and NAA (Naphthalene acetic acid), each 2 mg/l. Propagated calli, after 3 subcultures, were then transferred to the same medium, 1) containing 0, 0.2, 0.4, 0.6, 0.8, 1.0 % NaCl and 2) medium containing 0, 2, 4, 6, 8, 10 % mannitol as osmotic stress. *Invitro* grown seedlings were also transferred into MS medium containing the above mentioned salt and mannitol concentrations. At the end of week 1 and week 2, callus, leaf+stem, and root (1gr, each) were ground with a mortar and pestle in 1 ml extraction buffer as recommended by Szabo-Zagy et al. (1992) and enzyme assays were conducted using three replicates according to Julie et al. (1999) method.

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Table 1. Statistical analysis of acid phosphatase activities in different concentration of salt and mannitol. Uncommon letters are significant (p<0.5).

Cultivar	Explant	Mannotol (%)	NaCl (%)
Yazdi	Callus	0a,2b,4b,6b,8b,10b	0a,.0.2b,0.4b,0.6b,0.8b,1b
	Leaf and stem	0a,2b,4b,6c	0a,.0.2b,0.4b,0.6b,0.8c,1b
	Root	0a,2c,4b,6b,	0a,.0.2b,0.4b,0.6b,0.8b,1b
Hamedani	Callus	0b,2b,4b,6b,8b,10a	0a,.0.2a,0.4a,0.6a,0.8a,1a
	Leaf and stem	0a,2b,4b,6b	0a,.0.2a,0.4a,0.6b,0.8b,1b

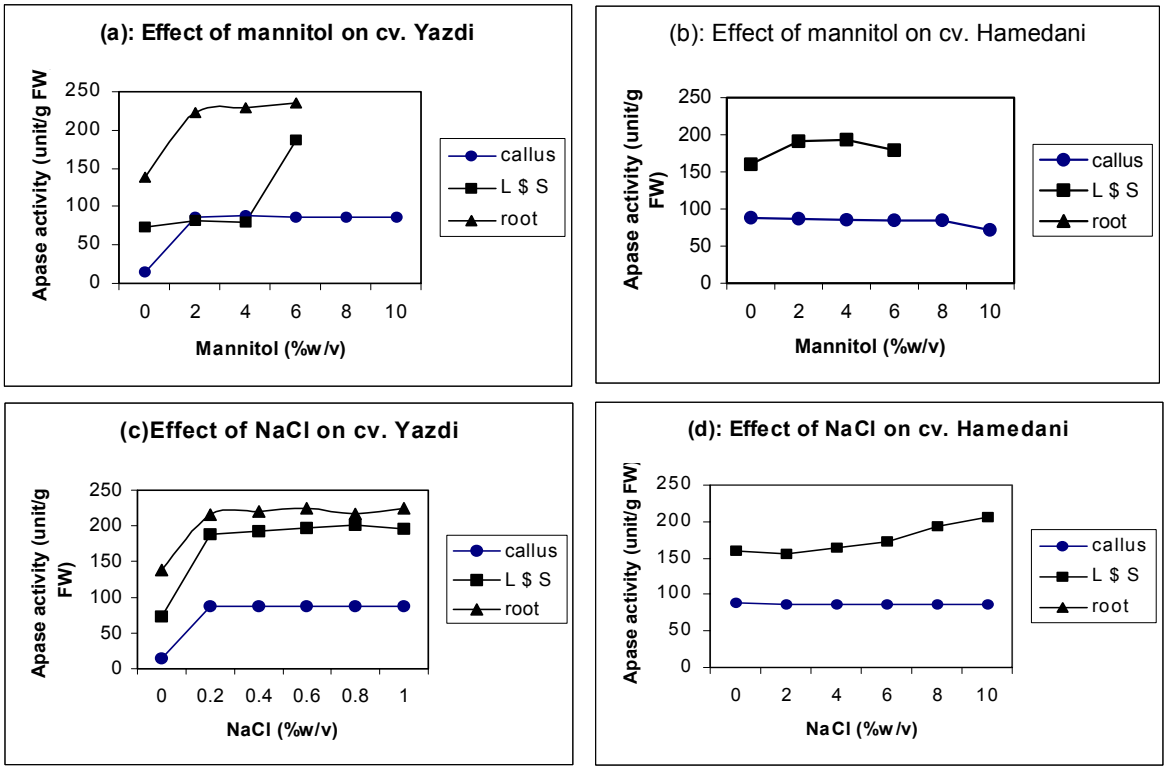

Figure 1. Effect of different concentrations of mannitol (a and b) and NaCl (c and d) on acid phosphatase (Apase) activities of callus, leaf (L), stem (S) and roots of *M. sativa* cv. Yazdi and Hamedani.

RESULTS

The effects of salt and non-ionic osmotic stress (drought stress) on acid phosphatase activities of calluses from cultivar Yazdi and Hamedani are shown in Figure 1 and Table 1. The *M. sativa* Yazdi cultivar has significantly increased acid phosphatase activities in callus, leaf, stem and roots after treatment with salt and osmotic stress. However, acid phosphatase activities of leaf and stem was only began increasing at 4% mannitol (Figure 1a). In cv. Hamedani (Figure 1b), the acid phosphatase activities of leaf and stem, after initial increase, started decreasing at 6% mannitol. Four important patterns were observed (1). As a result of salt and osmotic stress the acid phosphatase activities in all explants were increased. (2). The acid phosphatase activities of cultivar Hamedani in medium without salt and mannitol was higher than that of cv. Yazdi. (3): Cultivar Hamedani did not produce any roots under salt and mannitol stress. (4): The level of acid phosphatase activities was highest in roots (for Yazdi at least), followed by leaf and stem, while callus expressed the lowest activities.

DISCUSSION

Normally, salt and water stress affect the physiology and biochemistry of plant cells under *in vitro* and *in vivo* conditions. These stresses have been reported to increase acid phosphatase activity (Barrett-Laennard et al., 1982). It has been demonstrated that the induction of acid phosphatase activity under osmotic and salt stresses is not accompanied by a decrease in inorganic phosphate level. Pervious reports have showed that acid phosphatase activity in *P. sativum* is increased under salt stress (87mM) (Olmos and Hellin, 1997; Stephen et al., 1994). Here we also demonstrated that the acid phosphatase activities in *Medicago sativa* cv. Yazdi and Hamedani are increased under stress. However, the acid phosphatase activities in cv. Hamedani are not similar to cv. Yazdi.

One of the mechanisms for salt and drought stress tolerance in plants is the increase of the root length or root branches. In this study, the roots of cv. Yazdi were increased, while those of cv. Hamedani were not. Consequently, although cv. Hamedani had higher level of acid phosphatase activities in the medium without salt or mannitol, it was more sensitive to salt and osmotic stress. Nevertheless, more investigations are needed to determine whether or not having higher level of acid phosphatase activities in plant increases its tolerance to stress? According to our experiments (not published), when a plant increases its acid phosphatase activities under stress condition it becomes more resistant to stress conditions.

Olmos and Hellin (1997) observed that acid phosphatase 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), reported results indicating that phosphatase activities are independent of phosphate levels. In the future, we hope to determine interacellular and exteracellular phosphate level under salt and osmotic stress. However, whether or not levels of inorganic phosphate can serve as a signal for induction of acid phosphatase activities in *Medicago* explants need to be further studied. Since extracellular walls of cv. Yazdi and Hamedani are not identical (Szabo-Nagy et al., 1992), the different acid phosphatase responses of these 2 cultivars to salt and water stress may be genotype dependent.

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