Effect of salinity stress on growth, peroxidase and IAA oxidase activities in vigna seedlings

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Abstract

The present work was carried out with the aim of studying the effect of salinity stress on growth and IAA oxidizing system (i.e., peroxidase and IAA oxidase) in vigna (Vigna unguiculata L.) seedlings. The seedlings were treated with two concentrations of sodium chloride (NaCl) 0.1 M and 0.25 M. Length, fresh and dry weight were the parameters considered for growth. Salinity effect was distinct in fresh weight and dry weight of different organs. Peroxidase and IAA oxidase activities were measured at different time intervals for both cytoplasmic and wall bound fractions. Peroxidase activity was maximum at higher stress conditions bringing about the hypocotyl growth restriction. Thus there was a clear inverse correlation between elongation and peroxidase activity. IAA oxidase activity also showed a similar trend for both cytoplasmic and wall bound fractions. The role of IAA oxidizing system in defense mechanism in response to salinity stress is discussed.

Introduction

Salinity is one of the major widespread environmental stresses that can limit growth and development of plants. In fact this is a worldwide problem, particularly in arid and semi arid areas. Salinity generally affects the plant growth adversely and these adverse effects may be attributed to non-availability of water, disturbance in nutrients causing deficiency and ion toxicity to plant which lead to alteration in different physiological and biochemical processes in response to high internal salt concentration (Munns 1993). Levels of protein, nucleic acids and carbohydrate in plants growing under saline stress are affected by salt induced alteration in hydrolytic enzymes. In fact salinity affects plant processes by interfering with acquisition of nitrates and other essential nutrients as well as through direct interference with enzyme molecules.

Crop sensitivity varies with species, genotypes and growth stages (Gill and Dutt 1987). Injurious effects of salinity depend upon the stage of plant growth at which salinity is experienced. Available information indicates that early seedling stage is most salt sensitive for most crops (Gill and Sharma 1990). Salinity induced inhibition of growth and metabolic activities was reported in peanut (Silberbush and Lips 1988), cotton (Leidi et al. 1992), maize and wheat (Lewis et al. 1989), bean (Gouia et al. 1994), etc.

Retardation in plant growth and development under various stresses is attributed to inhibition of various physiological processes. Imbalance in phytohormone level in general, in particular seem to be one of the causes of growth retardation in stressed plants (Levitt 1980). Thus the response of plant to
excess NaCl involves changes in their morphology, physiology and metabolism. Peroxidases (EC 1.11.1.7, donor: hydrogen peroxide oxidoreductase) perform single electron oxidation on a wide variety of compounds in the presence of H$_2$O$_2$ (Dunford and Stillman 1976). Plant peroxidases are widely distributed in all higher plants and these enzymes are involved in various physiological processes of plants viz. lignification (Lagrimini et al. 1987), suberization (Espelie et al. 1986), cross linking of hydroxyproline rich wall proteins and feruloylated polysaccharide (Fry 1986), both oxidation and polymerization of soluble phenolics (Srivastava and van Huystee 1977), formation of H$_2$O$_2$ (Mader et al. 1980), chlorophyll degradation and senescence (Yamaguchi and Watada 1991) and auxin degradation (Jinnman and Lang 1965). One of the main functions is connected with its role as a part of defence enzyme complex in cells, ensuring detoxification of activated O$_2$ forms. This function is very important in the formation of metabolic response of plants to different stress factors (Bakardjieva et al. 1996).

Considering the aforesaid, in the present work effect of salinity (NaCl) stress was studied on growth, peroxidase and IAA oxidase activities in Vigna unguiculata during early seedling stages.

Material and Methods

Seeds of uniform size of vigna (Vigna unguiculata L.) were soaked in tap water for 3 h after which a thorough wash with distilled water was done. The seeds were then spread on a moist filter paper and kept in dark for 24 h for germination. Uniformly germinated seeds were then transferred to sieve culture dishes (190 x 50 cms mesh size 3 mm x 3 mm) containing nutrient media (Doddemma and Telkamp 1979) and either distilled water as control or salt incorporated in the form of NaCl with two concentrations: 0.1 M designated as S1 and 0.25 M as S2. All the sieve culture dishes were transferred to light room at 25±1 °C, with constant illumination with 6 fluroscent tubes at a height of 0.5 m height (ca 200 mmol m$^{-2}$s$^{-1}$). The solutions in the sieve culture dishes were renewed every 24 h. The time of transfer of the sieve culture dishes to the light room was taken as zero hour. Various salt concentrations were tried initially and 0.25 M concentration was such that maximum inhibition was obtained. Beyond this concentration the seedlings did not survive.

Growth analysis

Seedlings from each sieve culture dishes were selected and analyzed for growth and biochemical analysis at an interval of 12 or 24 h till 96 h. At the time of analysis the seedlings were excised into root, hypocotyl, leaf and cotyledon. The length of root and hypocotyl was measured to nearest mm and the fresh weight of each organ was taken separately; then they were oven dried at 70 °C for 3 - 4 days after which their dry weight was measured.

Extraction of cytoplasmic and wall bound enzyme

Required number of hypocotyls (about 500 mg) was ground in cold mortar in K-phosphate buffer (pH 6.4, 0.02 M) and centrifuged at 10,000 g for 10 min. The supernatant was used to estimate cytoplasmic peroxidase activity. The pellet after centrifugation was washed thoroughly with distilled water to make it free from cytoplasmic enzymes. About 12-15 washes were given, till the washings were free of peroxidase reaction with guaiacol. Then the pellet was kept with 10 ml of 1 M NaCl for 1h at room temperature with intermittent shaking to release ionically wall bound enzyme. After centrifugation at 10,000 g for 10 min., the supernatant served as wall bound enzyme.

Peroxidase activity

Peroxidase activity was measured by recording changes in absorbance at 470 nm (ΔA 470) using guaiacol as hydrogen donor in presence of H$_2$O$_2$. The assay mixture consisted of 12 mM K-phosphate buffer (pH 6.4), 4 mM guaiacol, enzyme and 1 mM H$_2$O$_2$. The activity is expressed as ΔA470 g$^{-1}$ of fresh weight-min$^{-1}$.

IAA oxidase assay

IAA oxidase activity was determined by a modified method of Gordon and Weber (1951). The assay mixture consisted of 0.16 mM 2,4-dichlorophenol, 0.2 mM MnCl$_2$, 8 mM K-phosphate buffer (pH 6.4), 0.2 mM IAA and 1 ml enzyme extract. The re-