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ORIGINAL ARTICLE

Response of *Brassica napus* L grains to the interactive effect of salinity and salicylic acid

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Soil salinity is a serious environmental problem that has negative effect on plant growth, production and photosynthesis. Fresh and dry plant weights decreases with salinity treatments. The very important role of salicylic acid (SA) in response to different stress and modification and decline damages due to stresses has established in different studies. In this research, effect of grain soaking presowing in (0, 1, 1.5, 2 and 5 mM) of salicylic acid (SA) and NaCl (0, 4, 8 and 12 dsm^{-1}) on canola (*Brassica napus L*) was studied. Increasing of NaCl level reduced the germination percentage(GP), Average velocity of germination (AVG) and growth parameters of 15-day old seedlings in compared to control plants. pretreated of SA in content 1mM significantly increased the germination percentage, and in contents more than of 1mM reduced the germination percentage in seeds under salinity stress. SA in content 1mM increased RWC, root and shoot of fresh weight in the stressed seedlings. Increasing of NaCl level increased Electrolyte leakage and MDA content in the stress seedling. electrolyte leakage and MDA content were markedly reduced under salt stress with SA 1mM than without. It was concluded that SA could be used as a potential growth regulator to improve salt tolerance in canola. Our observations indicate that, although SA is not essential for germination under normal growth conditions, it plays a promotive role in seed germination under high salinity by reducing oxidative damage.

Key words: Salicylic acid;(SA), germination percentage;(GP), Average velocity of germination(AVG), relative water contents;(RWC) , Malondialdehyde;(MDA), Salinity

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Many species of higher plants, including most crops, are subjected to growth inhibition under high NaCl conditions. The salt-induced inhibition of plant growth is caused not only by osmotic effects on water uptake but also by variable effects on plant cell metabolism. While the first component can bring about water deficit, the excess of a specific ion can cause toxicity and can induce nutritional disorders (Khatoon et al. 2010). Several reports appearing in the literature revealed that salinity causes many adverse effects on the morphology, anatomy and physiology of pearl millet (Hussain et al., 2010). For instance, percent germination, height, grain and straw yield of pearl millet decreased with increasing concentration of salinity (Hussain et al., 2008). When plants are exposed to salt stress, they adapt their metabolism in order to cope with the changed environment. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly (Hasegawa et al., 2000). SA is a plant phenol and is used for rising plant resistance to undesirable effects of biotic and abiotic stresses and participates in regulation of plant physiological stages. SA has significant effect on different aspects of plant life like plant growth and development, photosynthesis, evaporation, ion transmission and absorption; also causes to special changes in leaf anatomy and chloroplast structure (Sakhabutdinova et al., 2003). Salicylic acid (SA) is a critical signaling molecule that modulates plant responses to pathogen infection. Upon infection, SA biosynthesis is stimulated, and a group of pathogenesis-related (PR) genes is induced. These molecular events underlie the hypersensitive response (HR) and systemic acquired resistance (SAR) to prevent propagation of pathogens in infected plants (Dempsey et al., 1999; Durrant & Dong, 2004). During the HR, necrotic damage occurs at the site of pathogen entry, which is usually accompanied by production of reactive oxygen species (ROS). Exogenous application of SA may influence a range of diverse processes in plants, including seed germination (Cutt and Klessig 1992), stomatal closure (Larquer-Saaveda1979), ion uptake and transport (Harper and Balke 1981), membrane permeability (Barkosky and Einhellig 1993), photosynthetic and growth rate (Khan et al. 2003). Khodary (2004) found that SA treatment increased the chlorophyll and carotenoids content in maize plants. SA is also known as an important signal molecule for modulating plant responses to environmental stress. (Senaratna et al. 2000). It is now clear that SA provide protection against a number of abiotic stress as heat stress in mustard seedlings (Dat et al. 1998), chilling damage in different plants (Tasgı´n et al.2003), heavy metal stress in barley (Metwally et al. 2003) and drought stress on wheat plants (Singh and Usha, 2003). The present investigation was carried out to study the effect of grain soaking presowing with salicylic acid on the salt stress responses of *Brassica napus*.

MATERIALS AND METHODS

Grains were sterilized with sodium hypochlorite solution (5%) for 5 min, washed 3 times with sterilized distilled water. Salicylic acid was dissolved in distilled water and the pH was adjusted at 6.5 with KOH (1 N). Preliminary screening the grains were soaked for 12 h in the dark at 22^0C, either in concentrations (0.5, 1, 1.5, 2 and 5 mM SA) or in distilled water as control. Then many pretreated grains were putted in Petri dishes (9 cm diameter) provided with two filter paper saturated with 20 ml Nacl in concentrations (0, 4, 8 and 12 dsm^{-1}). The final GP and Average velocity of germination (AVG) was recorded after a period of 5 days in grains. In this stage the optimum response of germination in concentration of 1 mM SA was selected. The next stage many pretreated grains in 1mM SA content were putted in Petri dishes saturated with 7 ml Nacl in concentrations (0, 4, 8 and 12 dsm^{-1}) and 20 ml of 50% strength Hoagland's nutrient solution (Hoagland and Arnon 1950). The pH of the nutrient solution was maintained at pH 6.5 three replicates were prepared for each treatment. The final fresh weights of shoot and root, RWC, Electrolyte leakage and MDA content was recorded after a period of 15 days in seedling. The comparison of mean was done with

LSD test to SPSS 14.o software in probability level of 1٪ for drawing graph, we use Excel 2003 software.

Leaf relative water contents (%):

The leaf relative water contents (RWC) were calculated at the time harvest according to Beadle et al. (1993) using the equation: RWC (%) = [(FW - DW)/ (TW - DW)] 100 Where FW is fresh weight, DW is dry weight, and TW is turgid weight.

Electrolyte leakage measurements:

Approximately 0.2 g of fresh leaves was cut into about 1-cm segments and placed in 5 ml of deionized water at room temperature. After 45 min, the conductivity (C_1) was measured and then the samples were incubated in a boiling water bath for 15 min to achieve 100% electrolyte leakage (C_2). The results were calculated according to the formula: (%)=(C_1C_2)100 (Dua at el; 2006).

Lipid peroxidation measurements:

Lipid peroxidation in leaves was evaluated by the malondialdehyde (MDA) content. Approximately 0.5 g of fresh leaves was homogenized in 5 ml of 5% (w/v) TCA in an ice bath. Then the homogenates were transferred into a tube and centrifuged at 1000g for 10 min at 4°C . Aliquots of the supernatant and 0.5% (w/v) thiobarbituric acid in 20% (w/v) TCA were added into a new tube. This mixture was incubated at 98°C for 40 min, then cooled to room temperature and centrifuged at 8000g for 5 min. The supernatant was subjected to spectrophotometric analysis. MDA content was calculated from the absorbance (A535–A600)using the extinction coefficient of 155/(mM cm). Karabal at el;(2003).

RESULTS AND DISCUSSION

The results of germination percentage(GP) and Average velocity of germination (AVG)*Brassica napus* grains under SA and salinity treatments has brought at figure (1). In the study, Increasing of NaCl levels reduced germination percent in *brassica napus*. These results are consistent with those of Steppuhn et al;(2001) who showed reduced germination percent in brassica and wheat plants. The seeds pretreated with (0.5, 1, 1.5, 2 and 5 mM) SA solution exhibited lower germination percentage than those of untreated(control) seeds. These results are consistent with those of (Zea mays) (Guan & Scandalios, 1995), Arabidopsis (Nishimura et al., 2005), and barley (Hordeum vulgare) (Xie et al., 2007) who reported SA inhibits seed germination in a dosage-dependent. In addition, the germination percentage of seeds under salinity stress was markedly higher with SA application than without. These results are consistent with those of Rajasekaran et al. (2002) and Shakirova et al.,(2003) who showed a promotion in seed germination with SA application. SA inhibit Synthesis of Etilen with affect on ACC Synthesis activity Raskin, I., (1992). SA increased GP in barley, wheat and carotte under low temperature in compared control plants. The results of fresh weights of roots and shoots *Brassica napus* grains under SA and salinity treatments has brought at figure (2). Fresh weights of roots and shoots decreased progressively with the rise of Nacl levels in compared with control plants. These results are in agreement with those of Ghoulam et al. (2001), who showed that salinity caused a marked reduction in growth parameters (fresh and dry weight) of shoots and roots of sugar beet plants. This may indicate that, salicylic acid pretreatment of barley grains exhibited an increase in salt tolerance. Salicylic acid plays an important role in the defense response to stresses (salts, water, etc) in many plant species (Yalpani et al., 1994; Senaratna et al., 2000). Exogenously applications of salicylic acid helped to increase plant growth significantly in saline conditions (Setevens et al., 2007). Gutierrrez-Coronado et al. (1998) also reported a similar increase in the growth of shoots and roots of soybean plants under normal conditions in response to salicylic acid treatment. salt stress with oxin and cytokinin reduced growth in wheat seedling. Shakirova, F.rn; et al (2003). SA treatment increased oxin and cytokinin contents in wheat under salt stress. Sakabutdinova at el; (2003). (Singh et al., 2003)found that SA application increased the dry mass of wheat seedlings under water stress. Khodary (2004) has reported that SA increased the fresh and dry weight of shoots and roots of stressed maize plants, which is consistent with our results in brassica plants. The results of RWC, MDA content and electrolyte leakage *Brassica napus* seedlings under SA and salinity treatments has brought at figure (3).

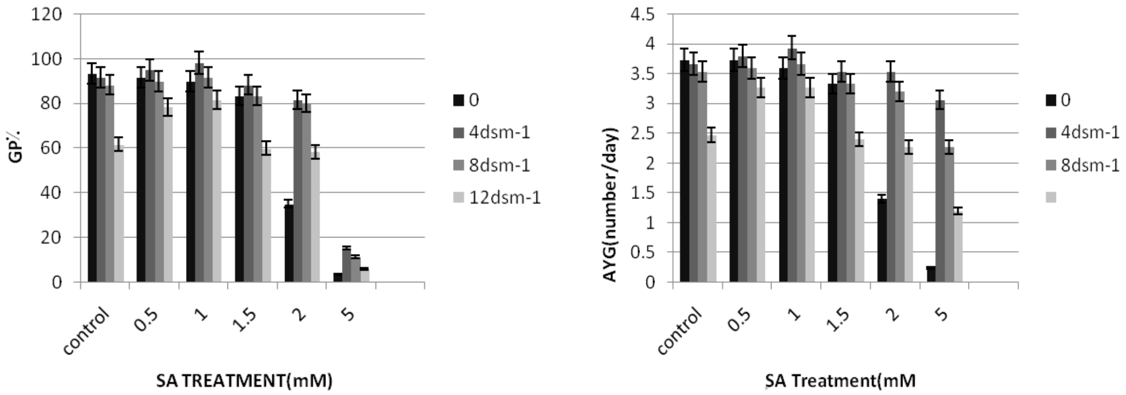

Figure 1 Effect of SA on germination percentage and germination speed mean.

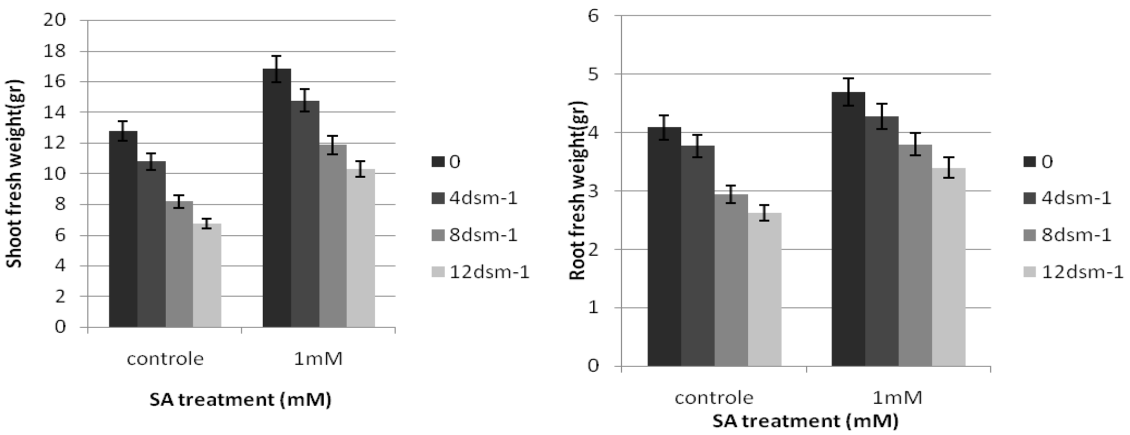

Figure 2 Effect of SA on shoot and root weight in Brassica.

In general, the RWC of leaves declined under salt stress. SA treatments induced an increase in RWC of the stressed seedlings, These observations are consistent with those of Barskosky and Einhellig (1993) who showed a consistently higher leaf diffusive resistance and lower transpiration and water potential in soybean plants with SA application. Because salinity stress enhanced free radicals levels in plants. peroxidation and electrolyte leakage in the stressed seedlings. The data showed that electrolyte leakage and lipid peroxidation increased as the stress level raised. These results agree with those of Bor et al. (2003) who found salt stress increases the lipid peroxidation in the leaves of two beet species. Grains soaking presowing with SA led to a significant decrease in the level of lipid peroxidation.

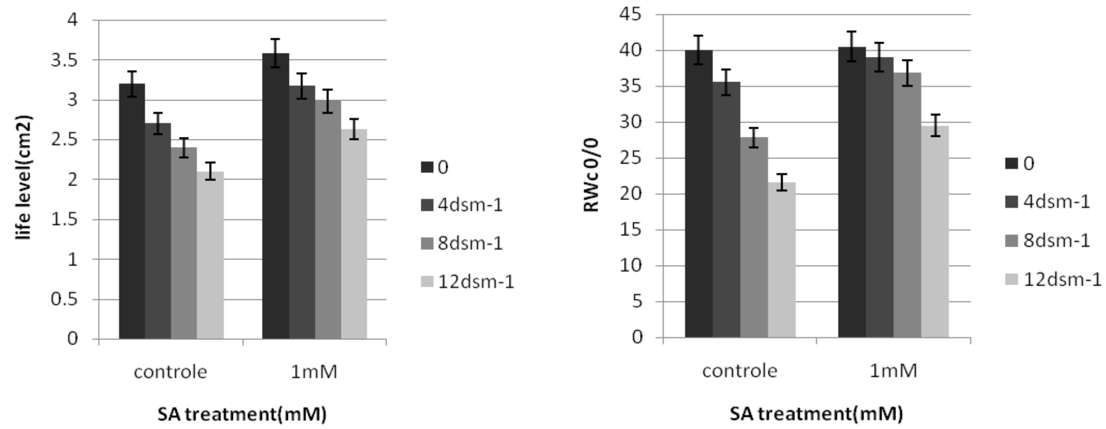

Figure 3. Effect of SA on relative water content (%) and level leaf Brassic.

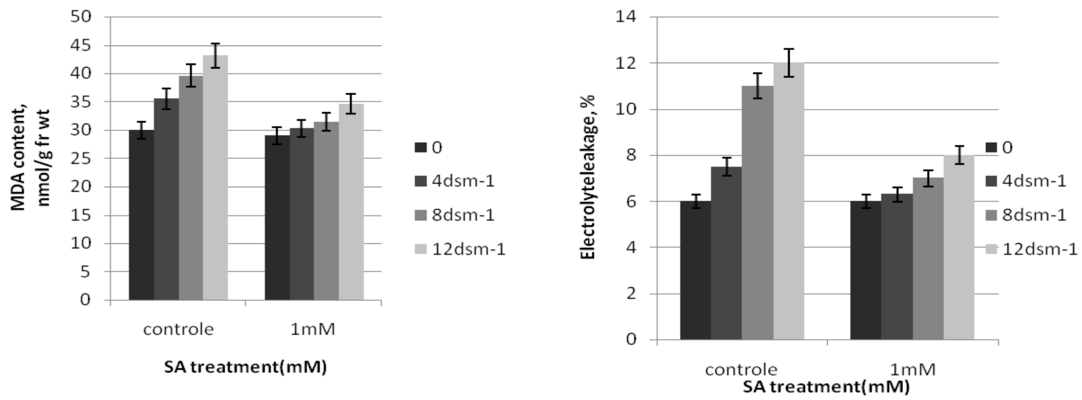

Figure 4. Effect of SA on relative Electrolyte leakage (%) and MDA content Brassica.

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