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Influence of Drought Stress and its Interaction with Salicylic Acid on Medicinal Pumpkin (*Cucurbita pepo* L.) Seedling Growth

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Abstract: Salicylic acid increases the resistance to some environmental stresses such as drought salt and chilling stress. The experiment was conducted to evaluate the effects of SA (Salicylic Acid) on morphological and physiological characteristics of medicinal pumpkin seedlings under drought stress. A trial was conducted based on completely randomized block design with 5 levels of SA pre-treatment (0, 0.5, 1, 1.5 and 2 mM) and 5 levels of SA foliar spray (0, 0.5, 1, 1.5 and 2 mM) and 3 levels of drought stress, three water deficit levels (90% FC (I1), 70% FC (I2) and 50% FC (I3)) with 3 replications. Drought stress was started at 4 true leaf stage after SA spray. The results showed that SA increased leaf area, number of leaf, number of bud leaf and chlorophyll content, respectively. Stem diameter, plantlet height, shoot and root dry weight and biomass, increased by applying SA in both method application compared to control. Seedling height, shoot dry weight and leaf area, decreased with increasing of drought stress. However, higher salicylic acid concentrations inhibited above physiological characteristics. Results show that pre-treatment and spray of salicylic acid at (2 mM) are suitable to increase drought tolerance during germination and seedling growth on medicinal pumpkin plants under drought stress.

Key words: Salicylic acid, drought stress, medicinal pumpkin, chlorophyll content, relative water content

INTRODUCTION

One of the valuable plants in the pharmaceutical industry in the most developed countries is medicinal pumpkin (*Cucurbita pepo* var. *styriaca*) that belongs to Cucurbitaceae family (Wagner, 2000). Drought is one of the main a biotic stresses which limited planting in most farming land in Iran. Shortages of water limited produce agriculture products. Stress in plant is usually perceived as a decrease in photosynthesis and growth and is associated with alteration in carbon and nitrogen metabolism (Mwanamwenge *et al*., 1999; Hu and Schmidhalter, 2005). Arid conditions can lower the yield of many agriculture crops. When the available water in the soil is reduced drought stress occurs atmospheric conditions by transpiration or evaporation cause loss of water. Drought stress decreased seedling growth and vigor (Lusia *et al*., 2005).

Drought stress can affect plants in different ways in the frame of physiological window mild drought induces in plants regulation of water loss and uptake allowing maintenance of their leaf Relative Water Content (RWC) with the limits the photosynthetic capacity (Yordanov *et al*., 2003). Salicylic Acid (SA) is an endogenous growth regulator of phenolic nature which participates in the regulation of physiological processes in plants (Sakhabutdinova *et al*., 2003). Salicylic acid was a component of the plant resistance to pathogens and also plays an important role in mediating plant response to some abiotic stress (Gautam and Singh, 2009). Salicylic acid could induce resistance to pathogens as well as abiotic stress tolerance in plants and also growth and development such as seed germination flowering, heat production and fruit ripening (Jing *et al*., 2007). Plant grown under stress condition as drought, salt and heat increased the osmotic produce as amino acids. SA increased the defensive compounds as betaine, glycine and proline (Sakhabutdinova *et al*., 2003).

Hu and Schmidhalter (2005) studied the effect of drought stress on mineral nutrition of plants and indicated that drought disturb the mineral-nutrient relations in plants through their effects on nutrient availability, transport and partitioning in plants. Shekari *et al*. (2010) reported the pre-treated of borages seed with salicylate acid increased the speed and vigor of seedling. Hayat *et al*. (2005) reported wealth selling which treated by 10 mM SA had higher number of leaf and fresh and dry weight. Basra indicated the SA could improve the

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seeds germination and seedling growth in rice. Sakhabutdinova *et al.* (2003) reported that pre-sowing of wheat seeds with SA contributes the increase in the resistance of plants to stress factors of environment. Kumar *et al.* (2010) studied the effect of SA on in cucumber seedling growth and showed that salicylic acid increased contents of chlorophyll, total nonstructural carbohydrate and total nitrogen in isolated cucumber cotyledons. Amira reported that SA at 10 mM increased plant height and protein content in common bean. Khan *et al.* (2003) reported that Salicylic Acid (SA) spray enhanced the photosynthetic rates and growth of soybean and corn and showed SA and phenolic compounds can exert control over stomatal opening and chronic injection of SA increases the photosynthetic rate of corn. Baghizadeh and Hajmohammadrezaei (2011) reported seeds that were treated by salicylic acid, radical and plumule length, fresh and dry weight of radical and plumule were increased and showed that salicylic acid decrease effects and damages of drought stresses on okra germination and seedlings growth. Sakhabutdinova *et al.* (2003) said the protective SA action includes the development of anti-stress programs and acceleration of normalization of growth processes after removal stress factors. SA at 0.1 and 0.5 mM improved germination and seedling in wheat seedling under drought stress (Maghsoudia and Arvinb, 2011). The aim of this research was to investigate whether salicylic acid could be a protectant to ameliorate the influence of drought stress on medicinal pumpkin and thereby increasing its drought tolerance.

MATERIALS AND METHODS

The present investigation was carried out at the experimental greenhouse of Ferdowsi University of Mashhad, Iran. Medicinal pumpkin (*Cucurbita pepo* L.) cultivar kakai was used in the experiment. Seeds were surface sterilized with Mancozeb. The seeds were divided into three categories: one category was priming with different levels of SA solved into distilled water for 20 h then sown, other were priming with distilled water 20 h and sown then in four-leaf stage sprayed with different levels of SA solved into distilled water and the other were priming with distilled water 20 h before sowing (control). Seeds of pumpkin (two seed per pot) was sown in 2-3 cm depth in plastic pots (20×25 cm) and filled with soil-sand-litter mixture (1:1:1) Irrigation schedule according to water deficit treatments were performed by soil Field Capacity (FC).

Drought stress: Drought stress was imposed by three water deficit levels (90% FC (I1), 70% FC (I2) and 50% FC (I3)). Calculation of amount of the water in FC conditions performed by following equation:

$$\text{Water at the field capacity} = \frac{\text{Soil mass at field capacity} - \text{Over dry soil mass}}{\text{Over dry soil mass}} \times 100$$

Morphological measurements

Leaf area: The second developed leaf was recorded by leaf area meter (Li-Cor, Model Li-1300, USA) (Destructive was sampling).

Fresh and dry weight, leaf, stem and root: Fresh weight of leaf, stem and root was measured by scales 0.01. To measure the dry weight that samples were dried in oven at 60°C for 72 h. Then measured by the scales 0.01 mgr.

Length of shoot and root: Length of shoot measured from the point of emergence of the plant to the top of the vine on each tagged plant with meter scale and average length was calculated. Length of root measured from point of emergence of plant to top of main root on each tagged plant with meter scale and average length was calculated.

Physiological

Chlorophyll content measurement

To measure chlorophyll content a day before ending experiment, sections taken from the middle of leaf excluding main leaf veins was measured by portable chlorophyll meter (SPAD–502, Minolta Camera Co., Japan).

Relative Water Content (RWC): To determine the Relative leaf Water Content (RWC), leaves were collected, immediately weighted (fresh wt.), re-hydrated for 24 h at 4°C in darkness (turgid wt.) then oven-dried for 24 h at 80°C (dry wt.). The RWC was determined as:

$$RWC = \frac{FW\text{-}DW}{TW\text{-}DW}$$

Where:
FW = Fresh Weight
TW = Turgid Weight
DW = Dry Weight

Stomatal conductance: The stomatal conductance was measured by porometer (LI-1600, LI-COR Company).

Statistical analysis: The experiment was carried out in a factorial trial based on completely randomized design with three replicates. ANOVA was run for the variables by SAS 9.2 soft word and the Least Significant Difference (LSD) test was used to separate the means at <0.05.

RESULTS AND DISCUSSION

The results showed that drought stress decreased the growth and physiological characteristics in medicinal pumpkin seedlings and SA increased drought tolerance in pumpkin seedling growth under drought stress (Fig. 1-11).

No. of leaf/plant and leaf area: Drought stress cause reductions of number of leaf/plant and leaf area (Fig. 1 and 2). All treatment with SA pre-treatment and spray in combined drought stress enhanced the number of leaf and leaf area and had a significant different with control in $p<0.05$. Maximum of number of leaf were obtained by SA pretreatment at 2 mM with (83%) and leaf area up to 74.21 cm^2. Khan *et al.* (2003) reported that foliar application of SA enhanced the leaf area in corn and Soybean. SA improved the plant growth as leaf area and number of leaf/plant. Reduce in the leaf water potential

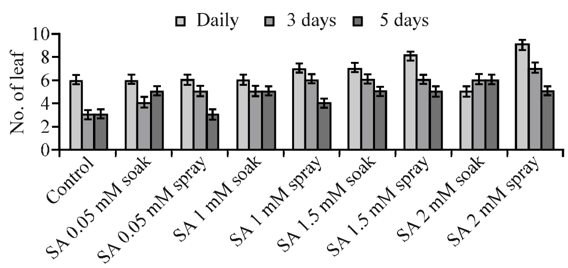

Fig. 1: Effects of exogenous SA application on mean number of leaf on medicinal pumpkin seedling

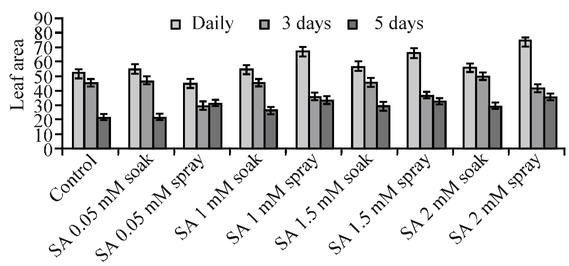

Fig. 2: Effects of exogenous SA application of leaf area on medicinal pumpkin seedling

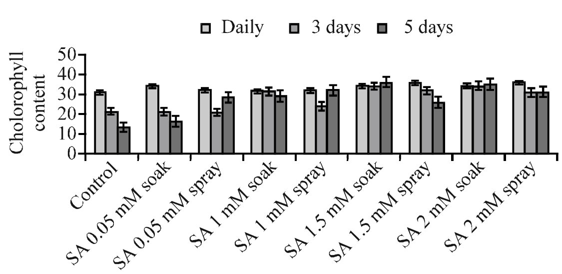

Fig. 3: Effects of exogenous SA application on chlorophyll content on medicinal pumpkin seedling

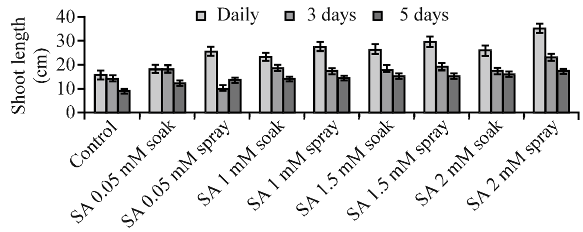

Fig. 4: Effects of exogenous SA application on mean shoot length (cm) on medicinal pumpkin seedling

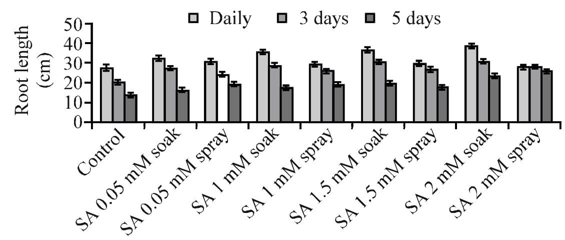

Fig. 5: Effects of exogenous SA application on mean root length (cm) on medicinal pumpkin seedling

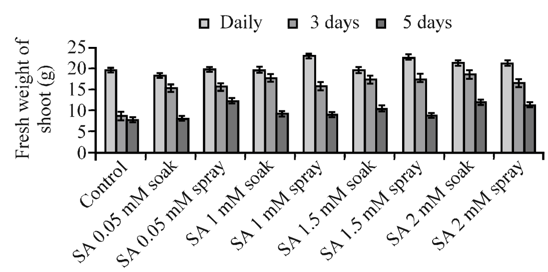

Fig. 6: Effects of exogenous SA application on mean fresh weight of shoot (g) on medicinal pumpkin seedling

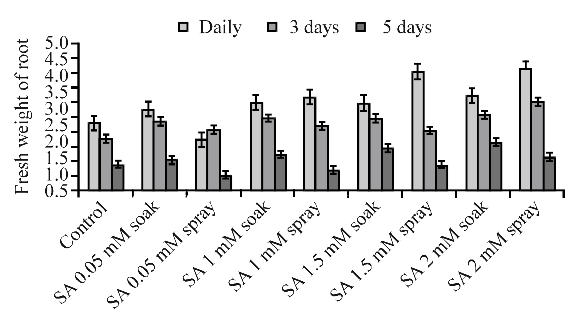

Fig. 7: Effects of exogenous SA application on mean fresh weight of root (g) on medicinal pumpkin seedling

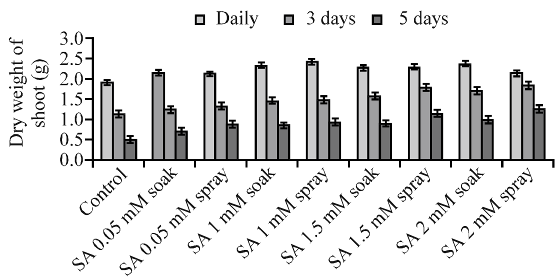

Fig. 8: Effects of exogenous SA application on mean dry weight of shoot (g) on medicinal pumpkin seedling

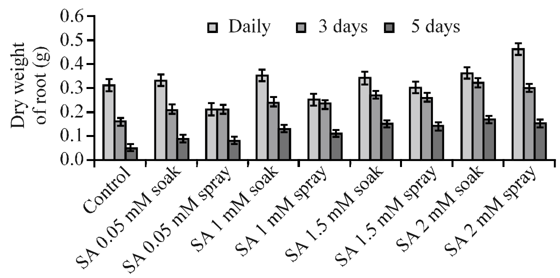

Fig. 9: Effects of exogenous SA application on mean fresh weight of shoot (g) on medicinal pumpkin seedling

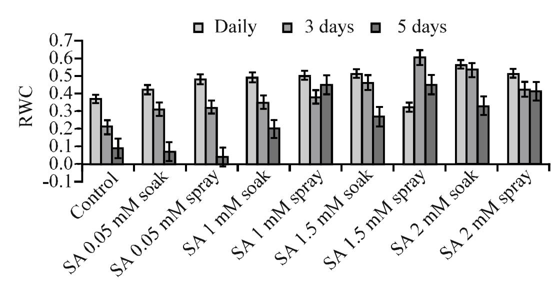

Fig. 10: Effects of exogenous SA application on relative water content on medicinal pumpkin seedling

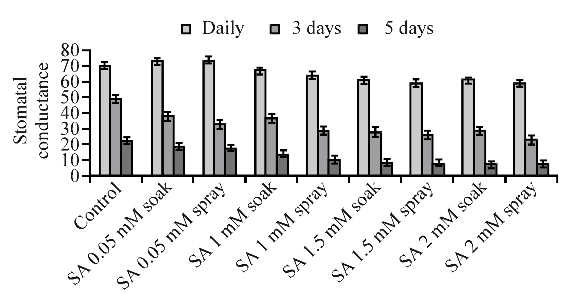

Fig. 11: Effects of exogenous SA application on stomatal conductance on medicinal pumpkin seedling

reduced turgor pressure of leaf cells and decreased the leaf growth. Decreased in leaf area was a response of plant to drought stress and limited the photosynthesis and plant growth.

Leaf area

Chlorophyll content: Chlorophyll content of second leaves in medicinal pumpkin seedlings is shown in Fig. 3 drought stress reduced the chlorophyll content in seedling. Chlorophyll content significantly increased by SA pre-treatment and sprays and the highest interaction between SA concentrations and water deficit levels was obtained under 2 Mm (44%) SA application. Exogenously application of SA in suitable concentrations may enhance the efficiency of antioxidant system in plants (Hayat *et al*., 2005). SA may be associated with Prevention of chlorophyll degradation. Foliar application of SA in the range of 1-3 mM chlorophyll content and the activity of Rubisco compared with non-SA-treated plants (Singh and Usha, 2003). These results are consistent to Khan *et al*. (2003).

Shoots and roots length: The results showed that foliar application of SA significant increased the length of shoot and root to 92 and 54%, respectively. Seedling biomass enhanced by SA treatments. Shah *et al*. (2002) reported that salicylic acid increased cell division on apical meristem of wheat seedling and increased of plant growth improve under water stress.

Fresh weight of shoots and roots: All SA treatment (pre- treat and spray) increased the shoots and roots fresh weight and the maximum obtained by SA per-treatment at 2 mM (61.33%) and (47%) (Fig. 6 and 7). Water stress caused the decline in metabolic activity of plant cells which should be inevitably reflected in inhibition of their growth. These results are consistent to Sadegi *et al*. (2010), Lu *et al*. (2002) and Gutierrez-Coronado *et al*. (1998).

Dry weight of shoots and roots: By increase of drought stress levels shoots and roots dry weights, reduced. The combined effects of drought stress and SA on shoot and root dry weights were statistically significant as compared to control $p<0.05$ (Fig. 8 and 9). By application SA sprays at 2 mM shoots and roots dry weights increased by 73% and 111% and priming with SA at 2 mM enhanced roots and shoots dry weights by 56% and 118%. SA could regulation the perspiration and water relative and that caused the increasing in dry plant mater and in finally enhanced the seedling growth. These results are consistent to Najafian *et al*. (2009) and Hamad and Hamada (2001).

Relative water content: The result indicated that all SA treatment significantly increased RWC with compared to control (Fig. 10). Maximum of RWC obtained by SA pretreatment at 2 mM with (51%). Decrease in Relative Water Content (RWC) by drought stress condition, photosynthesis reduced and therefore growth and yield decreased. SA may effected by regulators water in plant saved plant growth in front of drought stress. These results are consistent to Yordanov *et al*. (2003).

Stomatal conductance: With increasing drought stress level, the stomatal conductance was decreased in leaves of seedlings compared to the control. SA pre-treatment and spray at 2 Mm decreased the stomatal conductance by 42% and 46%. The stomatal closed in drought stress condition and available CO_2 decreased and nutrient absorption. drought stress caused stomatal closure and reduced transpiration rates, a decrease in the water potential of plant tissues, decrease in photosynthesis and growth inhibition SA by regulation on transpiration and photosynthesis can improve the efficiency of plant water and increased plant resistance to drought stress. The main factor on net photosynthesis is violated by stomata conduction. RuBPc is important enzyme in photosynthesis. Drought stress reduced the CO_2 absorption so the enzymes substrate is reduced and efficiency of RuBPc decreased and limited the photosynthesis and plant growth. SA caused severe damage to photosynthesis in wheat plants subjected to drought stress by decreasing stomatal conductance and transpiration (Nemeth *et al*., 2002). These result are consistent to Yordanov *et al*. (2003), Najafian *et al*. (2009), Molnar *et al*. (2005) and Popova *et al*. (2008).

CONCLUSION

Drought threatens crop productivity worldwide. Drought reduces nutrient uptake by the roots and transport from the roots to the shoots because of restricted transpiration rates and impaired active transport and membrane permeability (Hu and Schmidhalter, 2005). Photosynthesis rate can enhance by SA and it may be associated with chlorophyll increase and prevent of chlorophyll destruction. SA a natural endogenous growth regulator if used exogenously may improve plant growth and yield of medicinal pumpkin. This study was undertaken to identify the mechanisms and the influence of SA on the photosynthetic processes in drought stressed medicinal pumpkin plants. SA had protective role on the photochemical activity of chloroplast membranes and photosynthetic carboxylation in drought stressed medicinal pumpkin plants.

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