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Foliar application of Salicylic Acid reduced the harsh influences of water deficit and biochemical contents in varying degree in sunflower

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Abstract: Water deficit is considered the most important restricted factor for plant products, in that several chemicals have been used to reduce the harmful effects of water stress. It is known that the role of salicylic acid (SA) is quite important in this mechanism. To study the effect of water deficit and four levels of salicylic acid (0, 0.375, 0.75 and 1.5 mM) on some biochemical criteria of three sunflower hybrids including tolerant (NX-19012, NX-00989) and sensitive (FH-352) at vegetative and flowering growth stages. Two sets of pots were arranged in green house, water stress given to one set at vegetative stage and to second at flowering stage, and sprayed with SA. Proline and Sugar enhanced significantly under drought conditions and further increased by the application of SA 0.75 mM. The decrease of protein indicated that water stress affected protein synthesis, but it was increased with the spray of SA. Tolerant hybrids produced better results than the sensitive. It seemed that SA had the ability to increase the tolerant ability of the plant to drought stress.

Keywords: Sunflower, exogenous application of SA, biochemical parameters, drought stress.

1. INTRODUCTION

Plants are always confronted with environmental constraints of both biotic and abiotic nature and its response to both stresses can be additive, synergistic or antagonistic and are affirmed by quantifying different quantitative and qualitative traits (Ehdaie *et al*., 2008). Water stress is characterized by water deficiency, followed by unpredictable and low rainfall, high water requirements or merging of all these environmental constraints which twist productive land into barren terrain annually (Ramakrishna *et al.*, 2000). Almost all plants show water stress tolerance, but its degree varies from species to species (Chaitanya *et al*., 2003).

Sunflower (*Helianthus annuus* L.) has been accepted as a high potential crop that can successfully convene future oil requirements. In varying climates of Pakistan sunflower is being grown productively. It can endure a wide range of temperatures from 8 to 34°C, the optimum temperature for better crop production falls between 20 and 25°C (Shah *et al*., 2005). Even though sunflower is considered as moderately tolerant crop to moisture stress yet drought greatly affects its area and production. The yield of sunflower is destabilized by both biotic and abiotic stresses. Drought stress causes severe adverse effect on growth, biomass and yield of the crop.

Plant growth hormone Salicylic Acid (SA is known to produce protective effects under the activation of stress factors of different abiotic nature on plants (Sakhabutdinova *et al*., 2003). SA, an ubiquitous plant phenolic was recognized as an endogenous growth regulator in plants after the finding that it is involved in many plant physiological processes, and regulator of plant metabolism, mainly involved in biotic and abiotic stress Aydin and Nalbantoglu (2011).

Plant cells encounter the unfavorable environmental conditions by accumulating a variety of small organic metabolites referred collectively as compatible solutes such as proline, soluble sugars and proteins. Properties of compatible solutes allow the maintenance of turgor pressure during water stress Sakamoto and Murata (2002). This phenomenon of accumulation of compatible solutes is called osmotic adjustment. Osmotic adjustment accepted as an effectual way of drought resistance in many crops (Zhang *et al*., 2004).

Keeping in view the above facts, because of the insufficient information available on the SA effects on sunflower, an experiment was performed to investigate the optimized concentration of SA effective on biochemical attributes of sunflower at different stages of growth under drought stress.

2. MATERIALS and METHODS

For this greenhouse experiment, initially soil was sun dried and sieved to avoid any plant residues. 12 kg of this soil was filled in each pot. The experiment was laid out in a completely randomized design in factorial arrangement with three replications. Five seeds were

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sown and then watered with tap water. In the beginning all pots were kept at field capacity level for obtaining good germination and emergence. Later on the water was applied according to the water stress level specified for the experiment. Before imposing water stress the plants were thinned out and three healthy plants were kept in each pot.

This greenhouse experiment was performed to evaluate the genotypic response of three sunflower hybrids towards four concentrations (0, 0.375, 0.75 and 1.5mM) of salicylic acid at vegetative and flowering stages under water stress conditions. Before experimentation, achenes of each sunflower hybrid were sterilized for 5 minutes in 5% solution of sodium hypochlorite. Water stress was given to one set at vegetative stage and to second set at flowering stage by withholding water till wilting and sprayed with varying concentrations of SA (0, 0.375, 0.75 and 1.5mM in 0.1% solution of Tween-20) to plants. The control plants were irrigated normally and sprayed with 0.1% Tween-20. The pH of the solution should be 6.5. Data regarding all the plant parameters were performed using standard procedures, and were statistically analyzed by using Statistix 8.1 software through analysis of variance technique. The LSD test at 5% probability was used to compare the differences among treatments' means (Steel *et al*., 1997).

For Proline 1.0 g of fresh leaf was homogenized in 10 ml of 3% sulfosalicylic acid. The mixture was then filtered and one ml of filtrate was added in 1 ml of glacial acetic acid + 1 ml of ninhydrin solution. Incubate the mixture at 100^o C for 1 h and cooled it. Add Toluene 4 ml to the mixture and vortex for 5 min. Place it at room temperature and the absorbance was read at 520 nm as estimated by Bates *et al.* (1973).

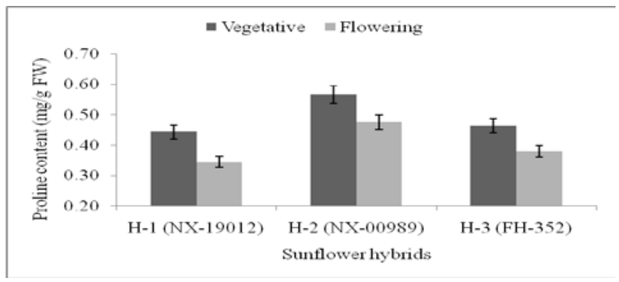
Soluble sugars were estimated following Dubois, 1951. Take 0.5g fresh leaf in test tubes having 10 ml of 80% ethanol and heat them at 80^{o}C for one hour in water bath. In another set of test tubes put 0.5 ml of this extract then 1ml of 18% phenol and left at room temperature for one hour then add 2.5ml of sulphuric acid, shake and absorbance was read at 490nm.

Add fresh leaves 0.2g in 4 mL of 50 mM cooled PO_4 buffer. Centrifuge the homogenate at 6000 × *g* at 4°C for 10 min. Measure the protein content of the extract as described by Bradford (1976).

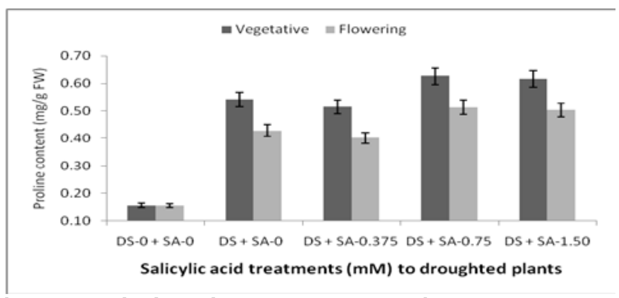
3. RESULTS

Leaf Proline Contents

Difference between two growth stages of sunflower plants for SA application was significant with respect to proline content. Generally, all the genotypes had lower proline content if drought stressed / sprayed with salicylic acid at flowering stage. All the sunflower hybrids responded equally to SA application at two growth stages. However, differential response of drought stressed sunflower hybrids to foliar application of SA at two growth stages was significant **(Fig 1a).** The SA application at concentrations higher than 0.375 mM caused a significant increase of proline content sprayed at both stages of drought stress, whereas, control treatment (DS-0+SA-0) rendered the lowest value for proline content **(Fig 1b).** Within all the treatments, interaction of DS with various SA concentrations showed better response of sunflower hybrids to SA foliar application at vegetative growth stage. The shielding effect of salicylic acid on drought-stressed sunflower plants improved with increasing SA concentration, being best at 0.75 mM.



a. Hybrids× Growth stages



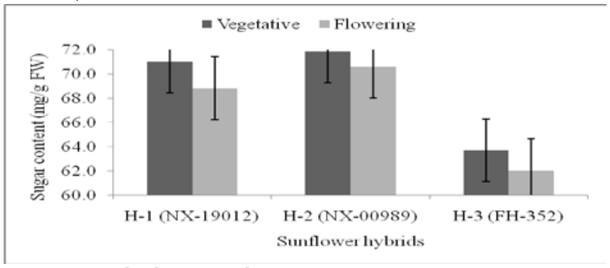
b. Salicylic acid concentrations × Growth stages

Fig: 1 Leaf proline content of drought stressed sunflower hybrids under foliar applied various concentrations of salicylic acid at two growth stages.

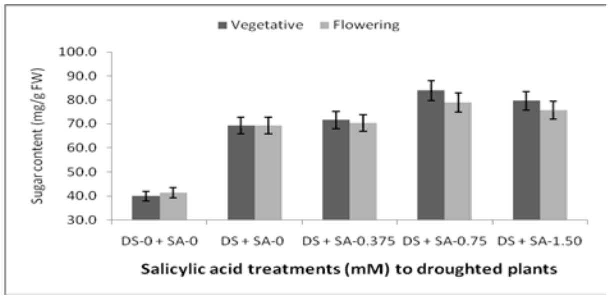
Soluble Sugar Contents

Total content sugars differed significantly for salicylic acid application at two growth stages of sunflower plants. Foliar application of salicylic acid at vegetative growth stage produced significantly greater sugar content as compared to that with SA applied at flowering stage. The H-3 (FH-352) contained the lowest sugars, while H-1 (NX-19012) and H-2 (NX-00989) had statistically higher contents although both differed non significantly **(Fig 2a).** Response of drought stressed sunflower hybrids to foliar application of SA at two growth stages was significant **(Fig 2b).** The SA application in 0.75 mM concentration at both stages produced significantly higher sugar content of drought stressed plants as compared to those receiving no SA. Whereas, control treatment (DS-0+SA-0) rendered the lowest value for sugar content, and it was significantly inferior to all the treatments in drought stressed plants.

Positive effect of salicylic acid on sugar content of sunflower plants under drought stress, enhanced by increasing SA concentration being the highest at the 0.75 mM, but lesser at further higher concentration.



a. Hybrids × Growth stages



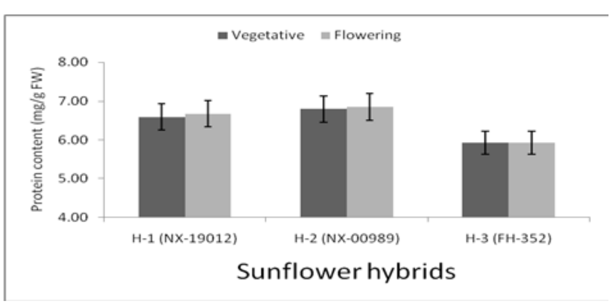
b. Salicylic acid concentrations × Growth stages

Fig: 2 Soluble sugar contents of drought stressed sunflower hybrids under foliar applied various concentrations of salicylic acid at two growth stages.

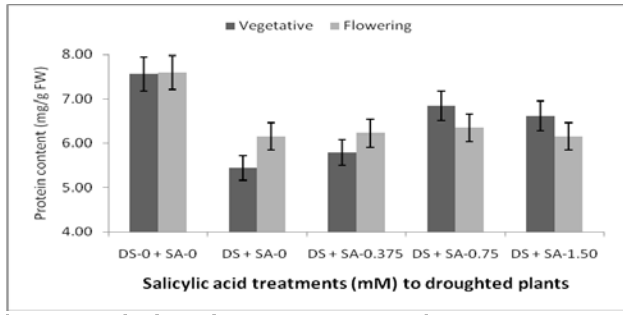
Leaf Protein Contents

Difference between foliar application of salicylic acid at two growth stages of sunflower plants was non significant for protein content. However, three sunflower genotypes differed significantly with each other for protein content under foliar application of salicylic acid. H-2 (NX-00989) showed the highest protein content, while H-3 (FH-352) had the lowest one **(Fig 3a).** Similarly, there was statistically significant difference among different concentrations of salicylic acid application at two growth stages.

Sunflower hybrids showed no observable difference when SA was applied at vegetative and flowering growth stages. SA application in 0.75 mM concentration at both stages produced significantly higher protein content of drought stressed plants as compared to those receiving no SA **(Fig 3b).**



a. Hybrids× Growth stages



b. Salicylic acid concentrations × Growth stages

Fig: 3 Protein content of drought stressed sunflower hybrids under foliar applied various concentrations of salicylic acid at two growth stages.

4. DISCUSSIONS

Proline accumulation increased under the water stress in all sunflower hybrids. Osmotic adjustment is the main part of physiological processes through which plants react to the drought stress (Zhu, 2003) and proline plays important role in this adjustment.

The present investigation revealed that proline accumulation enhanced by the foliar application of SA concentrations at both stages significantly in all hybrids. The accumulation of proline, mainly in the cytosol, often occurs in plants under stress with a correlation between stress tolerance and proline accumulation (Desnigh and Kanagaraj, 2007), but the relationship is not universal and may be species dependent (Ashraf and Foolad, 2007). The SA ameliorates the harmful effects of osmotic stress through increased proline and carbohydrate content (Marcińska *et al.,* 2013). Sayyari *et al.* (2013) studied the effect of SA on lettuce (*Lactuca sativa* L.) in drought conditions; proline increased significantly by SA application.

The present research indicates that accumulation of soluble sugar increased under water deficit conditions in all hybrids, significantly in tolerant ones. The enhancement of soluble sugars is strongly correlated to the attainment of stress resistance in plants (Hoekstra and Buitink, 2000). Soluble sugars act as osmoprotectants as they stabilize the cellular membranes and maintain the turgor potential.

Exogenous application of SA to water stressed sunflower plants presented high levels of sugar in tolerant hybrids. Results agreed with the findings of El-Tayeb (2005) who found an additional increase in Na, soluble proteins and soluble sugars in salt-stressed barley grains with application of SA. Kabiri *et al.* (2014) reported that salicylic acid protected the *Nigella* plant against drought stress through increase of photosynthetic pigments and soluble sugar contents.

The present investigation showed that leaf protein decreased under drought stress. Increased /decreased levels of amino acids and protein caused by water stress have been mentioned in many reports which stated that it depends on the stress level and type of plant. A stress event which inhibits cell division and expansion, and thus leaf expansion, will also arrest protein synthesis.

Results also indicated that SA foliar application augmented the leaf protein and reduced the effect of water stress. Degree of decrease in protein contents under drought was higher in the sensitive genotype as compared to the tolerant ones. Exogenous application of SA protects the plants against drought stress by increasing the protein content (Kabiri *et al.,* 2014). Application of 100 ppm SA enhanced the contents of total soluble proteins and grain proteins (Sivakumar *et al*., 2002).

5. CONCLUSION

The results of this experiment revealed that water stress significantly influence the plant biochemical composition. Drought tolerant hybrids better performed in arid regions and application of 0.75mM Salicylic acid improved the harmful effects of water deficit especially at vegetative stage in sunflower. Considering the results it is suggested that SA 0.75mM can alleviate oxidative damage caused by the drought stress conditions.

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