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Full Length Research Paper

The Role of Salicylic acid Enhances Dry Matter Content, Water Relations, Respirations and Photosynthesis of Wheat (*Triticum aestivum* L.) under Water Stress conditions

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Abstract:
Exogenous application of salicylic acid (SA) could modify the physiological and morphological capacity wheat plants in water stress condition. A greenhouse experiment, at Makah Al Mukaramah City, Kingdom of Saudi Arabia. The wheat grains pure strain was obtained from Agricultural Research Station of King Saud University Kingdom of Saudi Arabia. Presoaked two contrasting wheat genotypes, a drought tolerant cv Pavon 76 and a drought sensitive cv Yecora Rojo were used in freshly prepared salicylic acid (0.5 mM SA) or distilled water (control) for 12 h at natural environmental conditions, to reduce the effect of water stress. Drought was induced by different water field capacity normal 80% control, 100% wet, 60% dry and 40% very dry treatments. The parameters investigated were succulence, dry matter content (DMC), relative water content (RWC), stomatal numbers and opening area (μ^2) and chloroplast pigment (chlorophyll a, b and carotenoids contents). Generally shoot and root succulence increased with increasing water treatments at all growth stages especially in +SA. Drought caused significant losses in relative water content (RWC %) especially in -SA. The shoot and root dry matter content (DMC %) decreased with increasing water field capacity stress, wet treatment (100 %). Salicylic acid seemed to alleviate the deleterious effect of water stress on stomatal number (No./mm^2). Water field capacity stress affected on the condition of stomatal apparatus for upper and lower epidermis, the rate of stomatal opening area (μ^2) increased. In this regard the most effective water stress was in upper epidermis because the number of stomata less than in lower epidermis. Salicylic acid (+ SA) treatment seemed to alleviate the deleterious effect of water stress on stomatal opening area (μ^2). Drought caused a significant decrease in chlorophyll pigments and net photosynthesis resulting in growth reduction of both wheat genotypes. However, this decrease was more severe in the genotype cv Yecora Rojo compared to cv Pavon 76. Salicylic acid (+ SA) was applied through grains soaking treatment in salicylic acid 0.5 mM.

Key words: *Wheat, drought, salicylic acid, succulence, dry matter content (DMC), relative water content (RWC), stomatal numbers, stomatal opening area (μ^2), chloroplast pigment.*

Introduction

Salicylic acid is (SA) an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants such as growth, photosynthesis, nitrate metabolism, ethylene production, heat production and flowering (Hayat *et al*. 2010). Salicylic acid is (SA) could be a very promising compound for the reduction of the abiotic stress sensitivity of crops by mitigates the damaging effects of various stress factors in plants (Kaya *et al*. 2002). Several methods of application (soaking the seeds prior to sowing, adding to the hydroponic solution, irrigating, or spraying with Salicylic acid (SA) is solution) have been shown to protect various plant species against abiotic stress factors by inducing a wide range of processes involved in stress tolerance mechanisms (Horvath *et al*. 2007).

Seed germination, stomata adjustment, absorption and transfer of ions are regulated by endogenous growth regulator salicylic acid. Salicylic acid (SA) is a conservative compound of some biotic and abiotic stresses. Salicylic acid (SA) acts as important molecular signal for plants adjustment under abiotic stress (Waseem *et al.*, 2006; Arfan *et al.*, 2007). In plants various physiological processes are regulated by salicylic acid (SA) such as growth, transpiration rates, photosynthetic processes and stomatal regulation, ion uptake and transport (Gunes *et al*., 2005). Moreover, salicylic acid also reduces negative effects of various abiotic stresses by increasing internal level of other plant growth regulators in plants (Sakhabutdinova *et al.,* 2003). Wheat is consumed as food by about 35% of the human population .Wheat is a staple food so, wheat plant growth and yield under different abiotic stress condition is compulsory (Zhu *et al*., 2000).

Crop plant production is affected by biotic and abiotic factors. Abiotic stress include water shortage, high salts, high and low temperature effect plant growth but water shortage is major limiting factor for crop plant production and it is increasing day by day (Gamze *et al*., 2005; Passioura, 2007). Water stress will be there when there is reduced water availability in the soil or high

salinity (Khaje Hosseini *et al*., 2003). Plant are severely affect by this and many factors involved in the response of plants to water stress, which are developmental stages, severity of the stress, duration of stress and cultivar genetics (Beltrano and Marta, 2008). Plants produce proteins as a reaction to biotic and abiotic stresses to reduce them which were induced by some phytohormones such as salicylic acid (Davis, 2005).

Reduced supply of water is known to hamper important physiological and biochemical mechanisms leading to reduction in plant growth. Shao et al. (2009) they found that a Limited availability of water is a serious constraint to agricultural production of major crops because water is vital factor in plant development. Substantial yield losses have been observed in different crops due to reduced supply of water even for a short period of time (Pinheiro et al., 2005). Drought stress causes reduced stomatal conductance resulting in decreased net photosynthetic rate. Chlorophyll degradation due to drought stress also inhibits photosynthetic rate in wheat (Moaveni, 2011).

The chloroplast pigments, chlorophylls "a" and "b" play an important role in photochemical reactions (Taiz and Zieger, 2006). The decrease of chlorophyll content under water limited conditions is reported to take place because of its photo-oxidation and degradation under drought (Anjum *et al.*, 2011). Under drought stress, degradation of chlorophyll takes place due to the increased activity of chlorophyllase enzyme (Mihailovic *et al.,* 1997). Non-stomatal decrease in photosynthesis due to drought stress in the present study may have been as a result of chlorophyll degradation (Guo and Li, 1996; Sairam *et al.*, 1998; Anjum *et al*., 2011). The present study was conducted to assess the role of salicylic acid (SA) in alleviating the adverse effects of drought stress on wheat plants.

Materials and Methods

Water Treatments and Plant Growth

Wheat Plant and Culture Techniques Plant Materials: The experimental plant used in this investigation was wheat (*Triticum aestivum*, L.) plants, two different types can be distinguished, (1) - cv. Pavon 76 resistance to drought, and (2) - cv. Yecora Rojo Sensitive to drought stress, pure strain of grains was obtained from Agricultural Research Station of King Saud University Kingdom of Saudi Arabia. Nutrient Solutions: The base nutrient solution used was similar to that applied by Hoagland and Arnon (1950).

Water Treatments (Irrigation System): After 12 a day, thinning was carried out 5 uniform plants per pot for experimentation. The 144 pots used were divided into 2 (72 Pots) groups (two cultivars) that were subjected to (4) different water treatments on the 10th days as indicated in the following scheme: (100%; 80% ©; 60%; 40%) in addition to Hoagland solution (nutrient solution) by using a hand spray, irrigated plants. The normal water holding capacity of the mixture of soil used was 80% © to maintain the field capacity characteristic of each of the 3 water treatment as indicted in the scheme as in Table (1), watering was carried out every day (100%), every two days 80% ©, every three days (60%) and every fourth day (40%). Watering was always made when the field capacities were lowered to 100%, 80% (C), 60%, 40% for wet, normal (control), dry and very dry respectively was found to differ with the progress in plant growth and with the climatic conditions during the duration of the experiment (El Sayed and Mujahed, 2016).

Growth measurements were carried out at 3 different growth stages thought the experimental period. These growth stages represented 30, 60 and 90 days old plants and referred vegetative stage, (growth stage I), flowering stage, (growth stage II) while fruiting stage (growth stage III) of plants, samples for all growth stages were always collected to the least field capacity (Before Irrigation) characteristic of each water treatment as shown in the above scheme. The data of different treatment were statistically analyzed using the least significant difference (*L.S.D*) at 1% and 5% levels

Table 1: The Time Table for Water Irrigation (W.F.C. %) 100%, 80% ©, 60%, 40% for wet, normal (control), dry and very dry respectively per Day.

Water Treatments (W.F.C. %)	Time for Irrigation (Day)	Conditions
100%	Every Day	Wet
80% ©	Every two Days	Normal (Control)
60%	Every Three Days	Dry
40%	**Every Four Days**	**Very Dry**

Plant water relationship parameters: Three plants of each treatment were washed with distilled water, blotted thoroughly and then divided into root and shoot. After weighing the shoot and root samples dried at 80°C reweighed, and the root/shoot ratio calculated for dry weight.

Succulence: The percentage of the succulence content and dry matter content (DMC) was determined after drying the shoot and root samples in air – circulation oven at 80°C after constant weight, and calculated as the following equation:
Succulence was determined according to the following equation:

$$\text{Succulence} = \text{Fresh Weight}/\text{Oven Dry Weight} \quad \text{.....(1)}$$

Measurement of Relative Water Content (RWC %); Relative Turgidity (RT %): Relative water content was estimated according to a modification of the method of Weatherly (1950); Weatherly and Barr (1962); Slatyer (1957); Fletcher *et al.* (1988) on the final day of the experiment and was calculated by the formula given by Kramer (1983). Detached leaf samples were weight

immediately and floated on distilled water in a darkened refrigerator (5˚C). Saturation of the leaves was attained after 24 h. and the leaves were rapidly and thorough blotted and weighed immediately. The leaves were then dried at 80˚C to constant weight in an air -circulation oven to constant weight. The relative water content of leaves was expressed according to the following equation:

$$\text{Relative Water Content (RWC)} = [(\text{Fresh weight} - \text{Oven Dry Weight})/(\text{Saturated Weight} - \text{Oven Dry Weight})]\text{x } 100 \quad (2)$$

Dry Matter Content (%): Dry Matter Content % was determined according to the following equation:

$$\%\ \text{Dry Matter Content} = (\text{Oven Dry Weight} / \text{Fresh Weight}) \text{ x } 100 \quad (3)$$

Determination Of The Stomatal Aperture and Frequency

Direct microscopic measurement were carried out on Lloyd's (1908) alcohol fixation method was used; strips were taken only from the underside of the young leaves, usually from just behind the tip, a region which was observed by Desai (1937) to have particularly sensitive stomata. Lloyd's Strips (1908) taken from the leaves under investigation and immediately immersed in absolute alcohol for fixation and preservation. Three leaves from a treated plant were selected for uniformity of appearance and status of growth. From each of these leaves, two epidermal strips were token and on each strip two areas of about 0.25 m^2 were selected for determining three stomatal counts in each upper and lower epidermis. For each determination three strips were taken from three different leaves of about the same age, ten stomata were chosen at random from each strip, and their widths were determined by means of a standardized ocular micrometer. The number of stomata per mm^2 (Stomatal Frequency) on upper and lower epidermis was determined using the square ocular micrometer.

Stomatal Opening Methods

The number of opened and closed stomata was determined using film of clear nail polish as follows: Obtain three leaves from different plants and paint a thick patch (at least one centimeter square cm^2) of clear nail polish on the underside of the leaf surface being studies. Then allow the nail polish to dry completely tape a piece of clear cellophane tape to the dried nail polish patch. Gently peel the nail polish patch from the leaf by pulling on a corner of the tape and "peeling" the fingernail polish off the leaf. This is the leaf impression will be examine. Tape the peeled impression to a very clean microscope slide. Use scissors to trim away excess tap, Label the slid with plant name. Examine the leaf impression under a light microscope at 400 x. Count all the stomata in one microscopic filed and record the number. Form the average number/400x microscopic field the stomata per mm^2 was calculated.

Determination of Photosynthetic Pigment

A known fresh weight (0.5 g) of five different leaves; leaf No. 3 from the down were homogenized immediately in a mortar with 5-10 ml cold aqueous acetone (85%) then centrifuged. The acetone extract was diluted with cold aqueous acetone (85%) to an appropriate volume. The pigment content of the extract obtained was measured Spectrophotometrically at wavelengths E 664; E 645; E 452 nm according to the method of Metzner *et al*. (1965). The following equations were used to determine the concentration of the pigments fractions as µg / ml.

$$\text{Chlorophyll a} = 10.3\ E664 - 0.918\ E645 \quad (4)$$

$$\text{Chlorophyll b} = 19.7\ E645 - 3.870\ E664 \quad (5)$$

$$\text{Carotenoids C} = 4.3\ E452 - (0.0264\ \text{Chl.a} + 0.426\ \text{Chl.b}) \quad (6)$$

The pigment fractions were then calculated as mg/g Leaf fresh weight for each treatment. The effect of various treatments on the interrelationships between these pigment fractions as well as their rate of biosynthesis could be represented as ratios of chlorophyll a / chlorophyll b (Chl. a / Chl. b); Chlorophyll a + Chlorophyll b / Carotenoids (Chl. a + Chl. b / Carot.); total chlorophyll (Chl. a + Chl. b) and Total Pigments Contents (Chl. a + Chl. b + Carotenoids) (El Khodary, 1973). The pigment fractions were calculated as: µg Chl. / mg Dry Weight.

Statistical Analysis

The data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Kotz, et. al., 2006).Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level (Kirkpatrick & Feeney, 2013). The used tests were as follows: (1). Student t-test: For normally quantitative variables, to compare between two studied groups (2). F-test (ANOVA): For normally quantitative variables, to compare between more than two studied groups, and Post Hoc test (LSD) for pair-wise comparisons 3. Two ways (ANOVA): Was assessed to showing the effect of each factor and the interaction between them (Kotz, et. al., 2006; Kirkpatrick and Feeney, 2013).

Results and Discussion

The Role of Salicylic acid (SA) On Succulence (Fresh weight/ Oven Dry Weight)

The data presented in Figure (1), Tables (1 & 2) found that the succulence (F. Wt. /Oven D. Wt.) increasing significantly ($P \leq 0.001$) with progressive in growth stages (I & II) then tended to decreased at growth stage III, in the present (+SA) or absent (-SA) of salicylic acid for all water field capacity treatments (wet, dry & very dry) (100%, 60% & 40%) in shoot and root of wheat plant

with both cultivars (cv. Pavon 76 & cv. Yecora Rojo), compared with control (80% ©). In the present of salicylic acid (+ SA), generally shoot succulence increased significantly ($P \leq 0.001$) with increasing water treatments with both cultivars (cv. Pavon 76 & cv. Yecora Rojo) at all growth stages compared with control. Whereas, in the absent of salicylic acid (- SA) the root succulence increased more than in the present of salicylic (+ SA). Overall the two ways analysis of variance (*ANOVA*) between different water treatments in both cultivars at all growth stages indicated that the *LSD* test highly significant at $P \leq 0.001$, for shoot. It is well-founded fact that salicylic acid (SA) potentially generates a wide array of metabolic responses in plants and also affects plant water relations (Hayat *et al*., 2010).

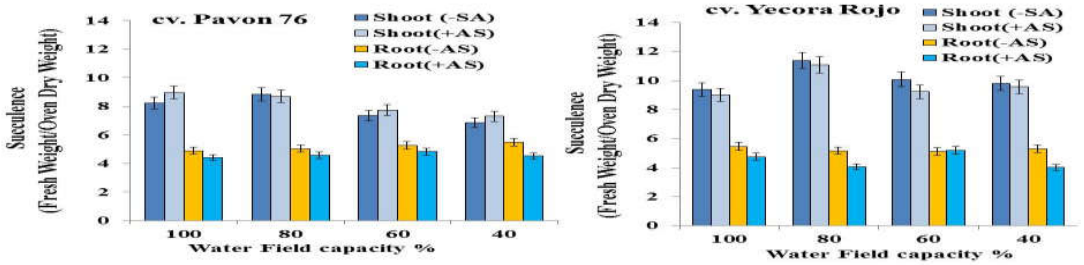

Figure (1): Interactive Effect of Salicylic acid (0.5 mM) and Water Stress On Root Succulence (Fresh Weight/Oven Dry Weight) of Wheat (*Triticum aestivum*, L. cv. Pavon 76 and cv. Yecora Rojo) Plant at Different Growth Stages (I, II & III) Grown Under Greenhouse Conditions, After Irrigation.

The Role of Salicylic acid (SA) On Relative Water Content (RWC); [Relative Turgidity (RT)]

Overall, the relative water content (RWC) [(Fresh Weight - Oven Dry Weight / Saturated Weight - Oven Dry Weight) X 100] increased with progressive in growth stages significantly ($P \leq 0.001$) before irrigation and non-significant after irrigation. The data presented Figure (2), Tables (1 & 2) found that the relations between both cultivars (cv. Pavon 76 & cv. Yecora Rojo), at all growth stages (I; II & III) and water field capacity treatments (wet, 100%; dry 60% & very dry 40%) in the present or absent of salicylic acid was highly significant ($p \leq 0.001$) for shoot RWC%. Whereas, RWC% in wheat shoot there is no differences in the present (+ SA) or in the absent (- SA) of salicylic acid at all growth stages (I, II & III) compared with control plants. Overall the two ways analysis of variance (*ANOVA*) between different water field capacity stress for both cultivars (cv. Pavon 76 & cv. Yecora Rojo), in the present (+ SA) and absent (- SA) of salicylic acid at all growth stages (I, II & III) indicated that the *LSD* test highly significant at $P \leq 0.001$. Drought caused significant losses in relative water content (RWC %), but in the present of salicylic acid (+ SA) application showed an increased RWC% compared to drought stress. Relative water content (RWC %) has been affected by the water deficit level as well as in the present of salicylic acid (+SA) treatment as compared to the control. It has been proved that salicylic acid (SA) triggers some metabolic processes in plants as well as affects plant water relations (Hayat *et al*., 2010). Singh and Usha (2003) observed that in wheat, while Kadioglu *et al*. (2011) found in *Ctenanthesetosa* plants grown under drought conditions, the results showed that wheat plants soaking with salicylic acid (SA) solution could maintain higher RWC compared with those of drought stressed plants without salicylic acid (SA) soaked before. These results show that application of salicylic acid (SA) is useful for drought tolerance improvement of wheat plants.

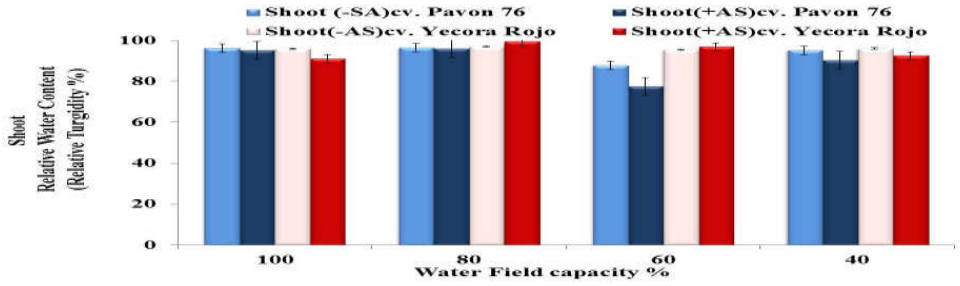

Figure (2): Interactive Effect of Salicylic acid (0.5 mM) and Water Stress On Shoot Relative Water Content (Relative Turgidity %) of Wheat (*Triticum aestivum*, L. cv. Pavon 76 and cv. Yecora Rojo) Plant at Different Growth Stages (I, II & III) Grown Under Greenhouse Conditions, Before Irrigation.

The Role of Salicylic acid (SA) On Dry Matter Content (DMC %)

The data presented shown in Figure (3), Tables (1 & 2) indicated that the dry matter content (DMC %) (Oven dry weight/Fresh weight x100) decreased significantly ($P \leq 0.001$) in wheat plant shoot and root for both cultivars with increasing water field capacity stress, wet treatment (100 %), the decreased occurred in the present of salicylic acid (+ SA) more in the absent (- SA) of salicylic acid with all water field capacity treatments (100, 60 & 40 %) at all growth stages compared with control (80% ©). Generally, the highest decrease in shoot and root dry matter content was registered by 0.5 mM SA with all water stress at all growth stages (I, II & III) compared to control (80% ©). So, the DMC %, increase significantly ($p \leq 0.001$) in roots more than in shoots for both cultivars. The shoot DMC% increased significantly ($P \leq 0.001$) at very dry (40%) in fruiting stage (stage III) especially in the absent (- SA) of salicylic acid. The dry matter content in shoot decreased after irrigation ($P \leq 0.001$), whereas, the shoot dry matter content increased significantly ($p \leq 0.001$) in cv. Yecora Rojo more than Pavon 76 especially at growth stage II (flowering stage).

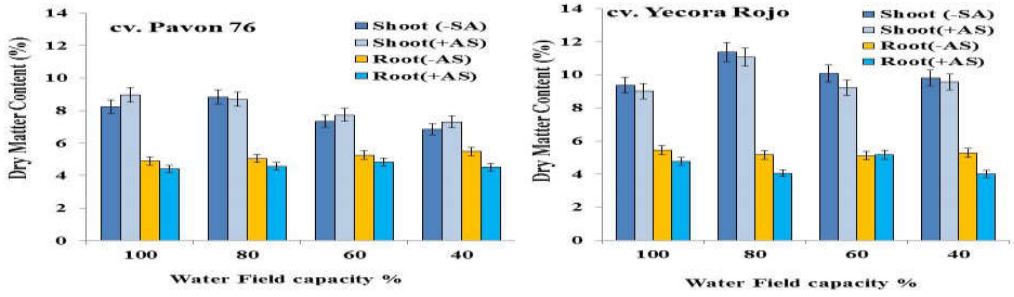

Figure (3): Interactive Effect of Salicylic acid (0.5 mM) and Water Stress On Shoot Dry Matter Content % [(Oven Dry Weight /Fresh Weight) x 100] of Wheat (*Triticum aestivum*, L. cv. Pavon 76 and cv. Yecora Rojo) Plant at Different Growth Stages (I, II & III) Grown Under Greenhouse Conditions, Before Irrigation.

The root dry matter content % increase significantly ($P \leq 0.001$) with dry and very dry water field capacity treatments (60% & 40%) respectively, more than wet water field capacity treatment (100%) compared with normal field capacity (control 80% ©) with both cultivars at all growth stages especially in the present (+ SA) of salicylic acid ($P \leq 0.001$). The results presented here found that in the present of salicylic acid (SA) tended to decrease the DMC % because increasing in antioxidant in cells, the antioxidant, able to remove the all free radicals (undesirable) and reducing the absorption of water. Overall, the two ways analysis of variance (*ANOVA*) between different water field capacity stress present (+ SA) and absent (- SA) of salicylic acid at all growth stages (I, II & III) at both cultivars (cv. Pavon 76 & cv. Yecora Rojo) indicated that the *LSD* test highly significant at $P \leq 0.001$, for both samples collected before and after irrigation.

The Role of Salicylic acid (SA) On Stomatal Apparatus [Number of Stomata (No./mm^2)] for Upper and Lower Epidermis

Data presented in Figure (4), Tables (3 & 4) showed that the clearly water field capacity stress affected on the condition of number of stomatal apparatus for upper and lower epidermis (No./mm^2) for both samples. The number of stomatal (No./mm^2) for upper and lower epidermis leaves increased significantly ($P \leq 0.001$) with growth stages (I, II & III) respectively. It is clear that drought stress dry and very dry (60% & 40%) respectively decreased significantly ($P \leq 0.001$) the number of stomatal (No./mm^2), whereas the wet treatment (100%) tended to increased significantly ($P \leq 0.001$) the number of stomatal (No./mm^2) for both cultivars compared with control (80%). In this regard the most effective of water stress was in lower epidermis because the number of stomata more than in upper epidermis. In the presence of salicylic acid (+ SA) treatment seemed to alleviate the deleterious effect of water stress on stomatal number (No./mm^2). Overall, the two ways analysis of variance (*ANOVA*) between different water field capacity stress in the present (+ SA) and in the absent (- SA) of salicylic acid at all growth stages (I, II & III) at both cultivars (cv. Pavon 76 & cv. Yecora Rojo) indicated that the *LSD* test highly significant at $P \leq 0.001$ for upper and lower epidermis..

Area of Stomatal Opening (μ^2) for Upper Epidermis

Data presented in Figure (4), Tables (3 & 4) showed that the clearly water field capacity stress affected on the condition of stomatal apparatus for upper epidermis, the rate of stomatal opening area (μ^2) increased significantly ($P \leq 0.001$) with growth stages respectively. It is clear that drought stress dry and very dry (60% & 40%) respectively decreased significantly ($P \leq 0.001$) the number of opened stomata and increased significantly ($P \leq 0.001$) the number of closed ones, whereas the wet (100%) tended to increased significantly ($P \leq 0.001$) the area of stomatal opening (μ^2) for both cultivars compared with control (80%). In case of after irrigation, the area of stomatal was more than before irrigation. The presence (+ SA) of salicylic acid treatment seemed to alleviate the deleterious effect of water stress on stomatal opening. Overall, the two ways analysis of variance (*ANOVA*) between different water field capacity stress in the present (+ SA) and in the absent (- SA) of salicylic acid at all growth stages (I, II & III) at both cultivars (cv. Pavon 76 & cv. Yecora Rojo) indicated that the *LSD* test highly significant at $P \leq 0.001$ upper epidermis.

Area of Stomatal Opening (μ^2) for Lower Epidermis

Data presented in Figure (4), Tables (3 & 4) showed that the clearly water field capacity stress affected on the condition of stomatal apparatus for lower epidermis, the rate of stomatal opening area (μ^2) increased significantly ($P \leq 0.001$) with growth stages respectively. It is clear that drought stress dry and very dry (60% & 40%) respectively decreased significantly ($P \leq 0.001$) opening area (μ^2) and increased significantly ($P \leq 0.001$) the number of closed ones, whereas the wet (100%) tended to increased significantly ($P \leq 0.001$) the opening area (μ^2) and decreased significantly ($P \leq 0.001$) the number of closed ones for both cultivars compared with control (80%). In this regard the most effective water stress was in upper epidermis because the number of stomata less than in lower epidermis. The 40% WFC at which the closed stomata for lower epidermis was out 80% and the opened one was 20% in the lower leaf surface. The presence of salicylic acid (+ SA) treatment seemed to alleviate the deleterious effect of water stress on stomatal opening area (μ^2). In this concern the number of closed stomata in the present of salicylic acid (+ SA) under water treated plant at 40% was about 80% in the upper leaf surface and 60% in the lower surface leaf. Overall, the two ways analysis of variance (*ANOVA*) between different water field capacity stress in the present (+ SA) and absent (- SA) of salicylic acid at all growth stages (I, II & III) for both cultivars (cv. Pavon 76 & cv. Yecora Rojo) indicated that the *LSD* test highly significant at $P \leq 0.001$ for both irrigation samples before and after for lower epidermis. The corresponding values in the lower surface were about 80% and about 20% respectively.

Table 2: Interactive Effect of Salicylic acid (0.5 mM) and Water Stress on Succulence, Dry Matter Content (%), and Relative Water Content of Wheat (*Triticum aestivum*, L. cv. Pavon 76) Plant at Fruiting Growth Stage (III) Grown under Greenhouse Conditions.

***Triticum aestivum*, L cv. Pavon 76**		**Succulence (F. Wt. /Oven D. Wt.)**		**Dry Matter Content (%) (Oven D. Wt. /F. Wt.)x100**		**Relative Water Content (Relative Turgidity %)**
W.F.C. (%) 0.5 mM SA		**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**
-SA	100%	$8.22^{abc}A \pm 0.29$	$4.88^{abc}A \pm 0.03$	$12.16^{cd}A \pm 0.20$	$20.48^{b}A \pm 0.54$	$96.25^{a}A \pm 1.15$
	80% ©	$8.83^{ab}A \pm 0.22$	$5.04^{abc}A \pm 0.20$	$11.32^{de}B \pm 0.01$	$19.82^{bc}A \pm 0.44$	$96.35^{a}A \pm 1.07$
	60%	$7.33^{cd}A \pm 0.54$	$5.26^{ab}A \pm 0.13$	$13.62^{b}A \pm 0.30$	$19.0^{cd}B \pm 0.18$	$87.67^{b}A \pm 2.27$
	40%	$6.84^{d}B \pm 0.36$	$5.47^{a}A \pm 0.16$	$14.60^{a}A \pm 0.39$	$18.27^{d}A \pm 0.14$	$95.03^{a}A \pm 0.78$
+SA	100%	$8.96^{a}A \pm 0.39$	$4.40^{c}A \pm 0.43$	$11.15^{e}A \pm 0.08$	$22.68^{a}A \pm 0.52$	$95.17^{a}A \pm 1.45$
	80% ©	$8.69^{ab}A \pm 0.50$	$4.56^{bc}A \pm 0.07$	$11.50^{de}B \pm 0.35$	$21.92^{a}B \pm 0.23$	$96.03^{a}A \pm 0.94$
	60%	$7.72^{bcd}A \pm 0.10$	$4.82^{abc}A \pm 0.41$	$12.94^{bc}A \pm 0.46$	$20.71^{b}A \pm 0.35$	$77.46^{c}B \pm 2.17$
	40%	$7.30^{cd}A \pm 0.47$	$4.51^{c}A \pm 0.14$	$13.68^{b}A \pm 0.19$	$22.16^{a}A \pm 0.04$	$90.23^{b}A \pm 1.07$
LSD (5%)		**1.150**	**0.717**	**0.860**	**1.052**	**4.386**

Table 3: Interactive Effect of Salicylic acid (0.5 mM) and Water Stress on Succulence, Dry Matter Content (%), and Relative Water Content of Wheat (*Triticum aestivum*, L. cv. Yecora Rojo) Plant at Fruiting Growth Stage (III) Grown Under Greenhouse Conditions.

***Triticum aestivum*, L cv. Yecora Rojo.**		**Succulence (F. Wt. /Oven D. Wt.)**		**Dry Matter Content (%) (Oven D. Wt. /F. Wt.)x100**		**Relative Water Content (Relative Turgidity %)**
W.F.C. (%) 0.5 mM SA		**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**
-SA	100%	$9.37^{c}A \pm 0.35$	$5.46^{a}A \pm 0.32$	$10.67^{abc}B^{*} \pm 0.46$	$17.60^{d}A^{*} \pm 0.46$	$95.89^{ab}A \pm 1.93$
	80% ©	$11.37^{a}A^{*} \pm 0.16$	$5.16^{a}A \pm 0.07$	$8.79^{d}B^{*} \pm 0.16$	$19.37^{c}A \pm 0.30$	$96.89^{a}B \pm 0.91$
	60%	$10.07^{bc}A^{*} \pm 0.40$	$5.11^{a}A \pm 0.39$	$9.92^{c}B^{*} \pm 0.21$	$19.54^{c}A \pm 0.23$	$95.4^{abc}A^{*} \pm 1.5$
	40%	$9.81^{c}A^{*} \pm 0.21$	$5.28^{a}A \pm 0.40$	$10.19^{bc}B^{*} \pm 0.19$	$18.93^{c}A \pm 0.40$	$95.99^{ab}A \pm 2.03$
+SA	100%	$9.01^{c}A^{*} \pm 0.32$	$4.75^{ab}A \pm 0.23$	$11.09^{a}A \pm 0.08$	$21.05^{b}A^{*} \pm 0.15$	$91.16^{c}A \pm 1.25$
	80% ©	$11.07^{ab}A \pm 0.53$	$4.06^{b}A^{*} \pm 0.07$	$9.02^{d}C^{*} \pm 0.34$	$24.62^{a}A^{*} \pm 0.18$	$99.38^{a}A \pm 1.27$
	60%	$9.22^{c}A^{*} \pm 0.43$	$5.17^{a}A \pm 0.19$	$10.83^{ab}B^{*} \pm 0.42$	$19.32^{c}A \pm 0.57$	$96.87^{a}A^{*} \pm 1.26$
	40%	$9.56^{c}A^{*} \pm 0.33$	$4.01^{b}A \pm 0.16$	$10.45^{abc}B^{*} \pm 0.23$	$24.92^{a}A^{*} \pm 0.07$	$92.46^{bc}A \pm 1.29$
LSD (5%)		**1.077**	**0.776**	**0.864**	**1.006**	**4.395**

Through their role in transpiration, stomata also help control leaf temperature, net stomatal conductance depend on both plant specific traits, such as stomata density leaf age and size, sub-stomata CO_2 concentration guard cells and epidermal cell turgor and on signals received from the environments (Reynolds-Henne *et al*., 2010). There is sample evidence for the effects of water status on stomata (Wikinson 2002; Lawson, 2009). Stomatal course in response to drought stress restricts CO_2 entry into leaves; thereby decreasing CO_2 assimilation as well as decreasing water loss fronts the leaves (Araus *et al*., 2002; Ober and Luterbacher, 2002).

Fischar *et al*. (1970) they reported that leaf water deficits of 30 to 40% had a marked after effect on tobacco stomata. The ability to open in light was depressed and complete recovery from this depression required 2 to 5 days after re-watering. This was probably related to a physiologically younger condition of leaves of stressed plants following turgor recovery. In beans similar stress caused after effects smaller in magnitude hat quantitatively similar to those in tobacco plant. In both tobacco and bean the magnitude of the after effect was approximately proportional the leaf water deficit attained immediately prior to re-watering.

Mohammadian *et al.* (2001) they found that water deficit caused stomatal closure, a reduced transpiration rate and elevated canopy foliage temperature. Moreover Dognatar *et al*. (2010) they found that the net photosynthesis transpiration rate and stomatal conductance were significantly affected by water stress. It seems that the reduction of water leads to the reduction of turgor and also reducing the turgor pressure would lead to the closeness of stomata (El Tayeb, 2005; Yazdanpanah *et al*., 2011).

Popava *et al*. (1977), they indicate that stomata behavior and regulation is a very important factor for photosynthetic ability. The established effects of salicylic acid (SA) on stomatal function, chlorophyll content, transpiration rate, and respiratory pathway lead to the assumption that SA might possess another physiological function most probably involved in regulation of some photosynthesis reaction.

Unless stated to the contrary, the chlorophyll a, chlorophyll b, carotenoids, chlorophyll content (Chl. a & b), and total pigment contents (Chl. a + Chl. b + Carotenoids) of wheat plant for both cultivars (cv. Pavon 76 and cv. Yecora Rojo) leaves increased progressively with growth stage I & II, then tended to decreased at growth stage III. The effect of water field capacity treatments increased with dry whereas decreased with wet and very dry treatments on chloroplast pigments at all growth stages (I, II & III), especially in the present of salicylic acid (+ SA).

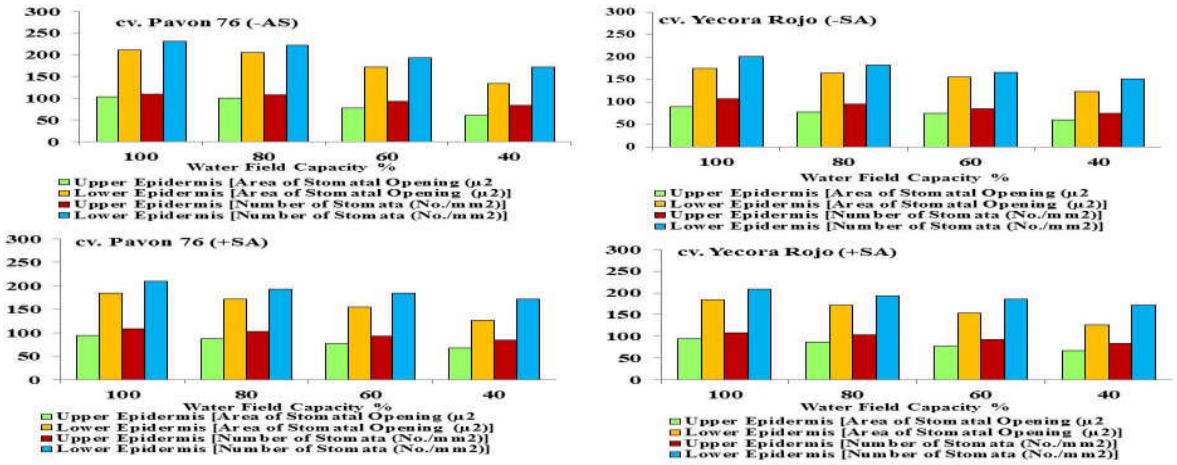

Fig 4: Interactive Effect of Salicylic acid (0.5 mM) and Water Stress On Upper and Lower Epidermis [Area of Stomatal Opening (μ^2)] and [Number of Stomata (No./mm^2)] of Wheat (*Triticum aestivum*, L. cv. Pavon 76 and cv. Yecora Rojo) Plant at Fruiting Growth Stages (III) Grown Under Greenhouse Conditions

Table 4: Interactive Effect of Salicylic acid (0.5 mM) and Water Stress On Upper and Lower Epidermis [Area of Stomatal Opening (μ^2)] and [Number of Stomata (No./mm^2)] of Wheat (*Triticum aestivum*, L. cv. Pavon 76) Plant at Fruiting Growth Stages (III) Grown Under Greenhouse Conditions.

***Triticum aestivum*, L cv. Pavon 76**		**[Area of Stomatal Opening (μ^2)]**		**[Number of Stomata (No./mm^2)]**	
W.F.C. (%) 0.5 mM SA		**Upper Epidermis**	**Lower Epidermis**	**Upper Epidermis**	**Lower Epidermis**
-SA	100%	$103.0^{a}A \pm 4.73$	$212.0^{b}A \pm 4.7$	$174.7^{ab}A^{*} \pm 4.33$	$106.7^{a}A \pm 2.85$
	80% ©	$99.67^{a}A \pm 6.69$	$205.7^{b}A \pm 3.8$	$164.3^{bc}A^{*} \pm 2.9$	$95.0^{b}A^{*} \pm 2.08$
	60%	$77.33^{b}A \pm 3.71$	$171.3^{c}A \pm 5.2$	$155.7^{c}A \pm 6.6$	$85.67^{c}A \pm 2.40$
	40%	$60.67^{c}A \pm 3.28$	$133.7^{d}A \pm 5.0$	$124.0^{d}A \pm 6.8$	$75.67^{d}A \pm 2.03$
+SA	100%	$107.7^{a}A \pm 3.0$	$232.7^{a}A \pm 3.5$	$184.7^{a}A^{*} \pm 3.0$	$108.7^{a}A^{*} \pm 2.0$
	80% ©	$101.7^{a}A \pm 4.3$	$212.7^{b}A \pm 4.9$	$171.3^{ab}A^{*} \pm 4.6$	$103.0^{a}A \pm 3.46$
	60%	$94.0A^{a} \pm 6.11$	$162.7^{c}A \pm 4.5$	$154.3^{c}A \pm 3.3$	$93.33^{b}A \pm 2.33$
	40%	$79.33A^{b} \pm 5.49$	$143.0^{d}A \pm 3.2$	$126.0^{d}A^{*} \pm 4.0$	$83.67^{c}A^{*} \pm 2.03$
LSD (5%)		**8.582**	**14.482**	**9.084**	**6.505**

Table 5: Interactive Effect of Salicylic acid (0.5 mM) and Water Stress On Upper and Lower Epidermis [Area of Stomatal Opening (μ^2)] and [Number of Stomata (No./mm^2)] of Wheat (*Triticum aestivum*, L. cv. Yecora Rojo) Plant at Fruiting Growth Stages (III) Grown Under Greenhouse Conditions.

***Triticum aestivum*, L cv. Yecora Rojo**		**[Area of Stomatal Opening (μ^2)]**		**[Number of Stomata (No./mm^2)]**	
W.F.C. (%) 0.5 mM SA		**Upper Epidermis**	**Lower Epidermis**	**Upper Epidermis**	**Lower Epidermis**
-SA	100%	$90.0^{ab}A \pm 4.16$	$174.7^{ab}A^{*} \pm 4.33$	$109.7^{bc}A \pm 2.33$	$231.0^{b}A \pm 1.7$
	80% ©	$78.67^{bcd}A \pm 4.33$	$164.3^{bc}A^{*} \pm 2.9$	$107.3^{bc}A \pm 3.3$	$221.7^{c}A \pm 2.4$
	60%	$75.33^{d}A \pm 4.26$	$155.7^{c}A \pm 6.6$	$92.33^{de}A \pm 4.37$	$193.7^{d}A \pm 2.6$
	40%	$61.0^{e}A \pm 4.73$	$124.0^{d}A \pm 6.8$	$84.33^{e}A \pm 2.91$	$171.7^{e}A \pm 2.4$
+SA	100%	$93.67^{a}A^{*} \pm 2.96$	$184.7^{a}A^{*} \pm 3.0$	$132.3^{a}A \pm 2.6$	$252.3^{a}A \pm 2.6$
	80% ©	$87.33^{abc}A \pm 4.33$	$171.3^{ab}A^{*} \pm 4.6$	$112.3^{b}A \pm 2.6$	$234.7^{b}A \pm 1.9$
	60%	$77.33^{cd}A \pm 3.28$	$154.3^{c}A \pm 3.3$	$103.0^{c}A \pm 3.2$	$218.3^{c}A \pm 1.8$
	40%	$67.33^{de}A \pm 3.48$	$126.0^{d}A^{*} \pm 4.0$	$93.67^{d}A \pm 2.40$	$189.0^{d}A \pm 1.7$
LSD (5%)		**7.172**	**11.945**	**7.344**	**8.748**

Chlorophyll 'a' Content (µg Chl. / mg Dry Weight)

Data recorded in Figures (5 & 6), Tables (5 & 6) showed that, generally chlorophyll 'a' content increased with progressive plant in age especially in vegetative and flowering stage (I & II) respectively, whereas, the chlorophyll 'a' decreased significantly ($P \leq 0.001$) at the fruiting stage (III). Moreover, drought stress especially at very dry (40%) water field capacity treatments decreased chlorophyll 'a' compared with control (80%) at both cultivars (cv. Pavon 76 & cv. Yecora Rojo), particularly at last stages of growth (III). It can be observed that the different levels of water field capacity induced to increase significantly ($P \leq 0.001$) the content of chlorophyll 'a' (pigment fractions) compared with control wheat plants. However, the chlorophyll a content increased significantly ($P \leq 0.001$) more in the present of salicylic acid (+ SA) at all growth stages. However, the highest increasing in chlorophyll a content was obtained with 0.5 mM SA concentration at all growth stages with dry and very dry compared to other water field capacity treatments and control plants. Overall the two ways analysis of variance (*ANOVA*) between different water field capacity in both cultivars (cv. Pavon 76 and cv. Yecora Rojo) leaves at growth stages (I, II & III) indicated that the *LSD* test highly significant at $P \leq 0.001$, for both samples collected before and after irrigation.

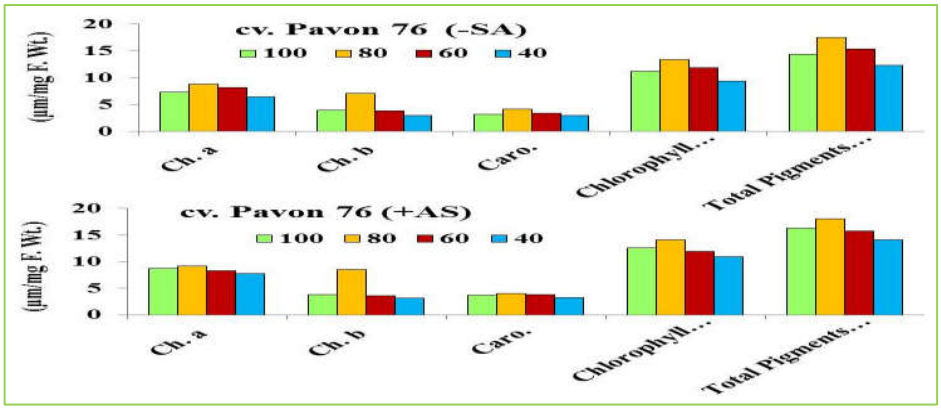

Fig 5: Interactive Effect of Salicylic acid (0.5 mM) and Water Stress On Chlorophyll a, Chlorophyll b, Carotenoids, Chlorophyll Content and Total Pigment Contents (mg/g Leaf D.Wt.) of Wheat (*Triticum aestivum*, L. cv. Pavon 76) Plant at Fruiting Growth Stages (III) Grown Under Greenhouse Conditions.

Chlorophyll 'b' Content (µg Chl. / mg Dry Weight)

Overall, the chlorophyll b content was affected by different water field capacity treatments on leaves of wheat plant as shown in Figures (5 & 6), Tables (5 & 6). The chlorophyll b content increased significantly ($p \leq 0.001$) in samples collected before and after irrigation with dry and very dry (60% & 40%) respectively more than wet (100%) at all growth stages (I, II & III) compared with control (80% ©). However, in the present of salicylic acid (+ SA) caused a significantly ($p \leq 0.001$) increased the chlorophyll b content significantly ($p \leq 0.001$) under water field capacity treatments (60% & 40%) compared with control (80 %©). So, the chlorophyll b content significantly increased ($p \leq 0.001$) in all samples collected before and after irrigation with different water field capacity treatments in the present of salicylic acid (+ SA) more than in absent of salicylic acid (- SA) at all growth stages (I, II & III). Generally the information show that use 0.5 mM of SA concentration gives higher chlorophyll b content in leaves of wheat plant compared to control at all growth stages. Overall the two ways analysis of variance (*ANOVA*) between different water field capacity in both cultivars at all growth stages (I, II & III) indicated that the *LSD* test highly significant at $P \leq 0.001$, for both samples collected before and after irrigation.

Table 6: Interactive Effect of Salicylic acid (0.5 mM) and Water Stress On Chlorophyll a, Chlorophyll b, Carotenoids, Chlorophyll Content and Total Pigment Contents (mg/g Leaf D.Wt.) of Wheat (*Triticum aestivum*, L. cv. Pavon 76) Plant at Fruiting Growth Stages (III) Grown Under Greenhouse Conditions.

W.F.C. (%) 0.5 mM SA		*Triticum aestivum*, L cv. Pavon 76 Chloroplast Pigments (µm/mg F. Wt.)				
		Chl. a	Chl. b	Carot.	Chlorophyll Content (Chl. a + Chl. b)	Total Pigments (Chl. a + Chl. b + Carot.)
-SA	100%	7.33^{bc}A±0.64	3.91^{ab}A±0.58	3.10^{a}A±0.58	11.24^{e}C ± 0.13	14.34^{f}C ± 0.06
	80% ©	8.82^{ab}A±0.52	7.01^{ab}A±0.58	4.15^{a}A±0.64	13.39^{b}B ± 0.11	17.54^{b}B ± 0.09
	60%	8.10^{abc}A±0.58	3.78^{ab}A±0.64	3.41^{a}A±0.64	11.88^{d}C ± 0.12	15.29^{e}C ± 0.09
	40%	6.37^{c}A±0.52	2.98^{b}A±0.58	2.91^{a}A±0.58	9.35^{f}C ± 0.05	12.26^{g}C ± 0.08
+SA	100%	8.71^{ab}A±0.64	3.87^{ab}A±0.52	3.71^{a}A±0.64	12.58^{c}C ± 0.07	16.29^{c}B ± 0.07
	80% ©	9.17^{a}A±0.64	8.53^{a}A±0.64	4.01^{a}A±0.58	14.08^{a}B ± 0.15	18.09^{a}B ± 0.08
	60%	8.33^{ab}A±0.52	3.61^{ab}A±0.64	3.78^{a}A±0.64	11.94^{d}C ± 0.16	15.72^{d}C ± 0.06
	40%	7.81^{abc}A±0.64	3.13^{b}A±0.58	3.24^{a}A±0.52	10.94^{e}C ± 0.10	14.18^{f}C ± 0.07
LSD (5%)		1.760	1.776	1.710	0.344	0.229

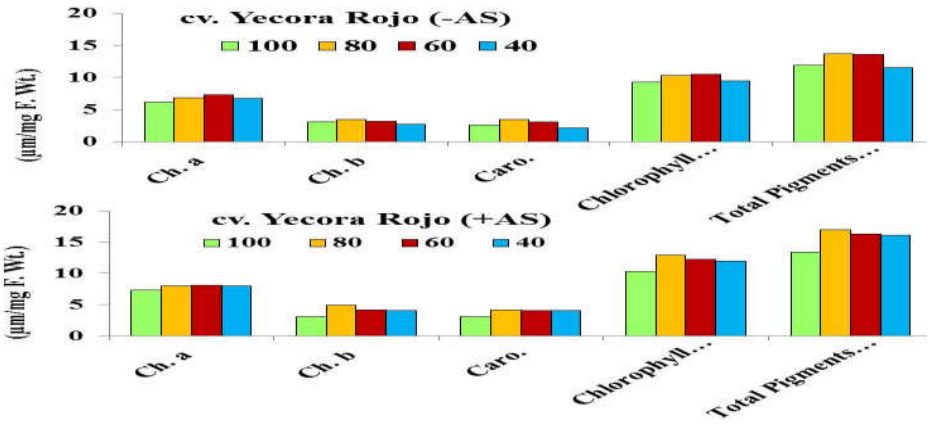

Fig 6: Interactive Effect of Salicylic acid **(**0.5 mM) and Water Stress On Chlorophyll a, Chlorophyll b, Carotenoids, Chlorophyll Content and Total Pigment Contents (mg/g Leaf D.Wt.) of Wheat (*Triticum aestivum*, L. cv. Yecora Rojo) Plant at Fruiting Growth Stages (III) Grown Under Greenhouse Conditions.

Chlorophyll Content (Chl. a & Chl. b)

Overall, the chlorophyll content (chlorophyll a+ chlorophyll b) of wheat leaves increase in the present (+ SA) than absent (- SA) of salicylic acid under water field capacity at all growth stages (I, II & III) compared with control Figures (5 & 6), Tables (5 & 6). However, in the present of salicylic acid (+ SA) the chlorophyll content (Chl. a + b) tended to increased significantly ($p \leq 0.001$) under water field capacity for dry and very dry water field capacity treatments (60% & 40%) respectively condition. Generally, the chlorophyll content of wheat leaves increased significantly ($p \leq 0.001$) with reducing water field capacity and present (+ SA) of salicylic acid (0.5 mM). Overall the two ways analysis of variance (*ANOVA*) between different water field capacity treatments in both cultivar at growth stages (I, II & III) in the present (+ SA) or absent (- SA) of salicylic acid indicated that the *LSD* test highly significant at $P \leq 0.001$, for both samples collected before and after irrigation.

Carotenoids Content (µg Chl. / mg Dry Weight)

Overall, the carotenoids content increased significantly ($p \leq 0.001$) in all samples collected before and after irrigation with growth stages (progressive with age) at all water field capacity with growth stages (I, II & III) in leaves of wheat plant compared to control as shown in Figures (5 & 6), Tables (5 & 6)**.** However, in the present of salicylic acid (+ SA) the carotenoids content increased significantly ($p \leq 0.001$) in vegetative and flowering stage (I & II) but decreased in fruiting stage (III) with all water field capacity treatment in cv. Pavon 76, whereas, in cv. Yecora Rojo the leaves before irrigated found that the carotenoids increased with progressive the plant stages (I, II & III) compared with the control. So, the interactions between water field capacity treatments and salicylic acid (SA) concentrations tended to increase ($P \leq 0.001$), the carotenoids content in wheat leaves compared to control plants. Generally, the both water field capacity, 60% and 40% (dry and very dry) respectively tended to increasing the carotenoids content in leaves of wheat plant with both cultivars at all growth stages in the present (+ SA) of salicylic acid compared with control. Overall the two ways analysis of variance (*ANOVA*) between different water field capacity in both cultivars at Growth Stages (I, II & III) in the present (+ SA) or absent (- SA) of salicylic acid indicated that the *LSD* test highly significant at $P \leq 0.001$, for both samples collected before and after irrigation.

Table 7: Interactive Effect of Salicylic acid **(**0.5 mM) and Water Stress On Chlorophyll a, Chlorophyll b, Carotenoids, Chlorophyll Content and Total Pigment Contents (mg/g Leaf D.Wt.) of Wheat (*Triticum aestivum*, L. cv. Yecora Rojo) Plant at Fruiting Growth Stages (III) Grown Under Greenhouse Conditions.

W.F.C. (%) 0.5 mM SA		*Triticum aestivum*, L cv. Yecora Rojo Chloroplast Pigments (µm/mg F. Wt.)				
		Chl. a	**Chl. b**	**Carot.**	**Chlorophyll Content (Chl. a + Chl. b)**	**Total Pigments (Chl. a + Chl. b + Carot.)**
-SA	100%	$6.18^{b}A\pm0.64$	$3.10^{ab}A\pm0.58$	$2.63^{ab}A\pm0.64$	$9.28^{d}B^{*}\pm0.11$	$11.91^{f}A^{*}\pm0.09$
	80% ©	$6.79^{ab}A\pm0.64$	$3.50^{ab}A\pm0.64$	$3.43^{ab}A\pm0.64$	$10.29^{c}C^{*}\pm0.16$	$13.72^{d}C^{*}\pm0.09$
	60%	$7.31^{ab}A\pm0.52$	$3.15^{ab}A\pm0.64$	$3.12^{ab}A\pm0.64$	$10.46^{c}B^{*}\pm0.21$	$13.58^{d}C^{*}\pm0.05$
	40%	$6.73^{ab}A\pm0.64$	$2.70^{b}A\pm0.64$	$2.13^{b}A\pm0.52$	$9.43^{d}C\pm0.10$	$11.56^{g}C^{*}\pm0.05$
+SA	100%	$7.38^{ab}A\pm0.47$	$3.13^{ab}A\pm0.64$	$3.10^{ab}A\pm0.58$	$10.30^{c}B^{*}\pm0.10$	$13.40^{e}B^{*}\pm0.05$
	80% ©	$8.01^{a}A\pm0.58$	$4.89^{a}A\pm0.64$	$4.13^{a}A\pm0.64$	$12.90^{a}B^{*}\pm0.12$	$17.03^{a}B^{*}\pm0.05$
	60%	$8.11^{a}A\pm0.52$	$4.17^{ab}A\pm0.52$	$4.07^{a}A\pm0.58$	$12.28^{b}B\pm0.18$	$16.35^{b}B^{*}\pm0.03$
	40%	$7.98^{a}B\pm0.58$	$4.01^{ab}A\pm0.58$	$4.01^{a}A\pm0.58$	$11.99^{b}B^{*}\pm0.24$	$16.12^{c}B^{*}\pm0.02$
LSD (5%)		**1.72**	**1.822**	**1.800**	**0.444**	**0.176**

Total Pigment Contents

Overall, the total pigment contents (Chl.'a' + Chl.'b'+ Carotenoids) of wheat leaves increase with both in the present (+ SA) or absent (- SA) of salicylic acid under water field capacity treatments at all growth stages especially at the second growth stages (flowering) compared with control Figures (5 & 6), Tables (5 & 6)**.** However, in the present of salicylic acid (+ SA) the total pigments content tended to significantly increasing ($p \leq 0.001$) under all water field capacity treatments. Generally, the total pigments content of wheat leaves increased significantly ($p \leq 0.001$) with decreased water field capacity treatments dry and very dry (60% & 40%) respectively especially in the present of salicylic acid (+ SA 0.5 mM). But maximum increased in total pigments content at 0.5 mM SA concentration with dry field capacity (60%) compared with control. The results observed that the Photosynthesis pigments (Chl. a & b and carotenoids) content of drought stressed wheat plants was significantly decreased below that of the control (100% WFC) however at mild water stress (80% WFC) the pigments content was improved as compared with control. This improvement might be attributed to the more concentration pigments in smaller leaf area under the mild water stress. Overall the two ways analysis of variance (*ANOVA*) between different water field capacity in both cultivar at all growth stages (I, II & III) in the present (+ SA) or absent (- SA) of salicylic acid indicated that the *LSD* test highly significant at $P \leq 0.001$, for both samples collected before and after irrigation.

The data presented by Zhu (2001); Munns (2002); Bartels and Sunkar (2005) they found that the osmotic stress (cell dehydration) and toxic (ion accumulation) effects on plants, impairing growth, ion homeostasis, photosynthesis and nitrogen fixation, among other key physiological processes. Chlorophylls contents (a&b) and carotenoids are main photosynthetic pigments and they play important role in photosynthesis.

Drought stress especially very dry (40%) resulted in a massive decrease ($P \leq 0.05$) in chlorophyll 'a' and 'b' contents at all growth stages in *Triticum aestivum* for leaves of both cultivars (cv. Pavon 76 and cv. Yecora Rojo). In the present of salicylic acid (+ SA) accumulation of chlorophyll 'a' and 'b' contents enhanced wet-watered as well as in drought stressed wheat plants. The increase in growth parameter of water stressed plants in response to salicylic acid (SA) may be related to the antioxidant responses that protect the plant from damage. Senartna *et al*. (2000); Daneshamand *et al*. (2010a& 2010b) suggested a similar mechanism to be responsible for salicylic acid (SA) in dead multiple stress tolerance in bean, tomato and potato plants, leaf chlorophyll, on important component of the photosynthetic system governing the dry matter accumulation, was increased significantly with salicylic acid (SA) application under water stress as compared to the control (- SA).

Daneshamand *et al*. (2010 a & b) found that the similar observations were recorded for photosynthetic rate. Salicylic acid (SA) treatment enable the plants to maintain the same photo synthetic rates under water stress as those of water sufficient plants. Drought stressed plant in absent of salicylic acid (- SA) showed significantly ($P \leq 0.001$) inhibited photosynthetic rate in this results which agreement with these Daneshamand *et al*. (2010 a& b) in potato plants. So, the ability of SA to increase plant mass and negate the adverse.

The present study showed that drought stress significantly reduced the leaf chlorophyll "a" and "b" content of drought sensitive as well as drought tolerant genotype. This is in line with what has been earlier reported in sunflower (Zhang and Kirkham, 1996; Manivannan *et al.*, 2007), rice (Pattanagul, 2011), barley (Havaux, 1998), maize (Dolatabadian *et al*., 2009a), okra (Amin *et al*. 2009) and wheat (Moaveni, 2011). Chlorophyll content increasing the efficiency of photosynthesis has long been a goal of plant research (Nar'tr and Lawlor, 2005). The site of the photosynthesis in plants is directly depends upon the chlorophyll bearing surface area, irradiance and its potential to utilize CO_2 (Hirose *et al*., 1997). Photosynthesis is a key metabolic pathway in plants. In fact, maintaining good photosynthetic rate leads to maintenance of growth under water stress (Dubey, 2005). The present results indicated that water stress caused marked decreases in the pigment contents in the leaves of the *Triticum aestivum* (wheat) plants. There is a common observation that leaf yellowing can occur when the leaves have had low water potentials for a considerable time. Chlorophyll is more sensitive to drought than carotenoids and consequently the ratio of total chlorophyll to carotenoids decreases with increasing drought severity (Barry *et al*., 1992). In accordance with these results, Manivannan *et al*. (2007b) found that the water deficit affected the early growth, biomass allocation and pigment of five varieties of sunflower (*Helianthus annuus L*.) plants.

Manivannan *et al*. (2007b) they found that there was a significant difference in early growth, dry matter accumulation and pigment among the studied varieties. The stimulating effect of salicylic acid (SA) may be due to the fact that SA led to increase leaf longevity on drought stressed plants by retaining their pigment content, therefore inhibit their senescence. In relation to these results, Chandra and Bhatt (1998) observed that an increasing or decreasing effect of SA on chlorophyll content of cowpea (*Vigna unguiculata*) depends on the genotype. In another study, treatment with SA increased pigment contents in soybean (Zhao *et al*., 1995), maize (Khodary, 2004), and wheat (Singh and Usha, 2003; Arfan *et al*., 2007) grown under normal or stress conditions. Furthermore, Arfan *et al*. (2007) found that the improvement in growth and grain yield of wheat salt-tolerance due to SA application was associated with improved photosynthetic capacity.

Drought induced reduction in growth and photosynthesis has been attributed to disturbance in the accumulation of nutrients, reduction in water potential and increase in osmotic potential, inhibition of photochemical processes (Sultana *et al*., 2000) and the increased production of ROS in the chloroplast (Gossett *et al*., 1994; Meneguzzo et al, 1999). The present study showed that SA treated plants exhibited higher values of pigment concentration in the present (+ SA) than in the absent (- SA) of salicylic acid compared with control or drought stressed samples (Figure and Table). Sinha *et al*., (1993) they found that the treatment with SA

the increase pigments content pointed out that chlorophyll and carotenoids content of maize and soybean plants leaves were increased upon treatment with SA .

Dela Rosa and Maiti (1995) they found that the inhibition in chlorophyll biosynthesis in sorghum plants because of abiotic stress, in this concern Bideshki and Rauin (2010) found that drought reduced chlorophyll a by 16% chlorophyll, 9% total chlorophyll 10%, total carotenoids and 10% in garlic plants as compare with the control (well watered plants) salicylic acid (SA) treatment, on the other hand had significant effects on most parameter recode red including the photosynthetic pigment

Wise and Naylor (1987) supposed in chlorophyll content of drought stressed plants is decrease of increasing the production of active oxygen radical (AOR) that cause peroxidation and degradation of these pigments. Similar reports exist for decreasing chlorophyll in wheat, cider and white mulberry row under drought conditions, in this report it has been reported that the accumulation of active oxygen types that produce during drought stress damage to many cell compound like fat protein and photosynthesis pigments (Sairam *et al*., 1998; Jianga and Nhunag, 2001)

There are contradictory reports about the role of SA on photosynthetic pigments; Lusia *et al*. (2005) reported that methyl salicylic did not have any effect on photosynthetic pigments but photosynthesis decreased under SA treatment. El Tayeb (2005) reported that the salicylic acid (SA) causes on increased in photosynthetic pigments in plant under biotic stress contradictory reports are from equivocally effects of SA which act as a phenol compound and probably with activation of O_2 cause damage of chloroplast protein and peroxidation of membrane lipids of thylakoid and finally decrease photosynthetic pigments under normal condition but at the time of stress, SA protect form photosynthetic apparatus through increasing the ability of cell anti apparatus through increasing the ability of cell anti oxidation and new proteins synthesis (Avancinid *et al*., 2003) increasing lipids peroxidation was regarded as an index of increasing oxidative stress, it was reported that in wheat SA cause decreasing damage effect of drought stress on photosynthetic pigments under stress condition (Hamada and Hamda, 2001).

Data recorded above indicated that drought stress was associated with change in sugar amount, and if the level of drought went up, the amount of sugar decreased much more. This reduction may be attributed to the negative effect of drought stress on the amount of chlorophyll and photosynthesis (Yazanpanah *et al*., 2011). In this regard, Dognalar *et al*. (2010) they found that net photosynthesis, transpiration rate and stomata conductance were significantly affected by stress due to changes in chlorophyll content and chlorophyll fluorescence, damage of photo synthetic apparatus and chloroplast structure some results were reported by Abd El Baki *et al*. (2000); Fidalgo *et al*. (20004); Kao *et al*. (2003); and Pinheior *et al*. (2008).

Moreover, Tang *et al*. (2002) showed that drought stress must also affect mesophyll 0.5 mMm. These mesophyll responses become as progressively more in important with increasing water deficiency (Gimenz *et al*., 1992; Tezare and lawlor, 1995). Parry *et al*. (2003) found that decreased photosynthetic capacity under water stress conditions resulted from impaired regeneration of ribulos, 1 S, bisphosphate (RUBP).

Miguel *et al*. (2006) they found that the treatment with salicylic acid (SA) causes improving resistance of plant on stress and as a result sugar approach to its normal conditions moreover SA appears to improve plant to clearance against water stress by activating the photosynthetic process.

Increasing parameters water stressed plants in response to SA may be retarder to the induction of antioxidant responses that protect the plant from damage, Sanaratna *et al*. (2000). The reduction of the sugar content could be as a result of photosynthesis reduction, because the reduction of water leads to the reduction the turgor pressure would lead to the closeness of the stomata and finally would affected the membrane of chloroplasts and decrease the photosynthesis (Redy *et al*., 2003; El Taybe, 2005; Yazdan panah, *et al*., 2011) in this respect, Bideskhi and Arvin (2010) they reported that drought stress significant inhibited photosynthetic rate in garlic plants. These results were in agreement with that of Daneshmand *et al*. (2010) on potato plants. The negative effect of drought on the photosynthesis was discussed by Parry *et al*. (2003) who stated that drought is a more limitation to the productivity of many corps. Stomata do sure in response to the drought stress restricts CO_2 entry into leaves thereby decreasing CO_2 assimilation as well as decreasing water loss from the leaves (Cornic 1994; Araus *et al*., 2002; Chaves 2002; Ober and luterbacher, 2002).

The cv. Pavon 76 maintained higher chlorophyll “a” and “b” content compared to that of genotype cv. Yecora Rojo under drought stress. Earlier studies have also reported that the chlorophyll content of drought resistant genotypes of barley, wheat and maize were higher compared to the sensitive genotypes in drought stress conditions (Pastori and Trippi, 1992; Rong-Hua *et al.*, 2006).
The present study revealed that exogenous application of salicylic acid via soaking the grains in 0.5 mM SA helped plants maintaining the chlorophyll pigments and hence mitigated the adverse effects of drought stress. These findings are in line with some earlier reports on *Cassia* (Singh *et al.*, 2001), okra (Amin *et al.*, 2009), wheat (Azzedine *et al.*, 2011), and maize (Dolatabadian *et al*., 2009a). Reactive oxygen species produced under stress conditions have been reported to cause pigment degradation (Sairam and Saxena, 2000; Anjum *et al*., 2011). However, ascorbic acid being an antioxidant actively scavenges these ROS, thereby reducing the chlorophyll degradation under stress (Ashraf, 2009).

Drought stress is also known to cause a significant reduction in the gas exchanges attributes; net photosynthesis, transpiration rate and stomatal conductance of plants (El-hafid *et al*., 1998; Shah and Paulsen, 2003; Flexas *et al*., 2004; Ali and Ashraf, 2011). In the present investigation, drought stress caused a marked reduction in net photosynthesis, transpiration rate and stomatal

conductance in both wheat genotypes. However, the drought tolerant cv Pavon 76 was superior to the drought sensitive genotype cv Yecora Rojo with respect to these gas exchange attributes. Drought-induced reduction in photosynthesis and transpiration rate has been reported earlier in a number of crops including wheat (El Hafid *et al*., 1998), maize (Ali and Ashraf, 2011), and sorghum (Kreig and Hutmacher, 1983). In the present study, exogenous application of salicylic acid mitigated the adverse effects of drought on photosynthesis in both wheat genotypes by increasing stomatal conductance. This could have also been due to the fact that salicylic acid as an antioxidant has the ability to mitigate the negative effects of stress on plants by neutralizing harmful oxidants which have been reported to damage plant membranes such as the thylakoid membranes of chloroplasts (Miguel *et al*, 2006; Dolatabadian *et al*., 2009b). In the present investigation, salicylic acid applied through soaking the grains before germinated was found to be more effective compared to control in alleviating the adverse effects of drought on different gas exchange attributes of both wheat cultivars.

In the present study, salicylic acid increased transpiration rate of wheat under drought stress compared to non-treated plants. Similar results were reported in okra plants under drought using ascorbic acid as a foliar spray (Amin *et al*., 2009). It has been reported that maintenance of water status is regulated by stomatal conductance and rate of transpiration (Ashraf, 2009). In the present study, there was a significant decline in stomatal conductance of drought tolerant as well as drought sensitive genotype under drought. Application of salicylic acid improved the stomatal conductance of plants compared to non-treated plants.

Conclusion

Overall, salicylic acid application was comparatively more effective in overcoming the adverse effects of drought stress in wheat compared to control in terms of respiration, photosynthetic rate and plant growth. Salicylic acid treated plants showed higher net photosynthetic rate, transpiration and stomatal conductance. shoot and root succulence increased with increasing water treatments at all growth stages especially in +SA. Drought caused significant losses in relative water content (RWC %) and dry matter content (DMC %) especially in –SA in shoot and root. Salicylic acid seemed to alleviate the deleterious effect of water stress on stomatal number (No./mm^2), the rate of stomatal opening area (μ^2) increased. Drought caused a significant decrease in chlorophyll pigments. However, this decrease was more severe in the genotype cv Yecora Rojo compared to cv Pavon 76. Salicylic acid (+ SA) was applied through grains soaking treatment in salicylic acid 0.05 mM. compared to control, (80% WFC). The chloroplast pigments of wheat plant for both cultivars (cv. Pavon 76 and cv. Yecora Rojo) leaves increased progressively with growth stage I & II, then tended to decreased at growth stage III. The effect of water field capacity treatments increased chloroplast pigments at all growth stages (I, II & III), especially in the present of salicylic acid (+ SA).Thus, it may be concluded that exogenously applied salicylic acid is effective in ameliorating the adverse effects of drought stress.

Ethics

All the authors read and approved the manuscript and no ethical issues involved.

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