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Effects of Foliar Application of Salicylic Acid on Growth and Physiological Attributes of Cowpea under Water Stress Conditions

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Abstract-The possible alleviating effects of salicylic acid on protection of cowpea (*Vigna unguiculata*) cv. Parastu under water stress conditions were investigated in the University of Zanjan Agriculture Research Station during 2008. Water stress was imposed by withholding irrigation at 50% flowering (flowering water stress, FWS) and when 50% of the pods grew 2-3cm (pod-formation water stress, PFWS). Salicylic acid (SA) was sprayed when plants had approximately ten fully expanded leaves with different concentrations of 0, 150, 300, 450 and 600 µM. Water stress was found to affect net photosynthesis rate, relative water content and Chlorophyll index. Proline content and leaf temperature increased in response to water stress. In both water stress treatments, plants treated with 300µM SA showed the highest values for net photosynthesis rate, transpiration rate and proline concentration. In FWS, the lowest leaf temperature was recorded in the control plants. However, in PFWS, leaf temperature was not affected by SA treatment. In FWS condition, the application of 300 µM SA showed the highest leaf area. It seems that the application of SA in 300 µM concentration may improve plant functions in both normal and stress conditions.

Keywords: Correlation, Flowering-stage, Pod-formation stage, Salicylic acid, Water Stress

INTRODUCTION

Water availability is an important factor affecting plant growth and yield, mainly in arid and semi-arid regions where plants are frequently subjected to periods of drought

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(Panozzo and Eagles 1999). Genotype susceptibility to drought is often measured by reduction in yield under drought stress (Blum, 2011). However, drought stress induces an array of morphological, physiological, biochemical, and molecular responses in order to help the plants to cope with water stress conditions (Wang and Huang, 2004). Many physiological factors could be involved in the drought stress injury (Jiang and Huang, 2001).

In order to activate defense mechanisms, plants must perceive the stress condition and signal (Park *et al.*, 2007; Qiao and Fan, 2008). Many molecules have been suggested as signal transducers or messengers including salicylic acid (Misra and Saxena, 2009). Salicylic acid is a phytohormone that plays an important role in the regulation of plant growth and development (Horvath *et al.*, 2007). The exogenous application of SA results in the enhancement of plant resistance against different biotic and abiotic stress through different mechanisms (Stevens *et al.*, 2006; Horvath *et al.* 2007; Shi and Zhu 2008; Hayat *et al.* 2010; Bai *et al.*, 2009; Kadioglu *et al.*, 2011). However, the effectiveness of SA in stress tolerance depends on the type of species and experiment conditions such as the concentration of the SA applied, the type of stress and the level and duration of stress (Hayat *et al.* 2010; Németh *et al.*, 2002; Waseem *et al.,* 2006; Arfan *et al.*, 2007).

Cowpea is an important legume mostly grown in arid and sub-arid zones of the world (Muchero *et al.*, 2009). The inherent capacity of cowpea to survive under moderate to severe drought stress conditions has been shown previously (Ewansiha and Singh, 2006; Dadson *et al.*, 2005). Cowpea exhibits broad adaptation mechanisms to drought, such as drought escape, avoidance of drought by decreasing leaf area, avoidance of dehydration, and vegetative stage drought tolerance by delayed leaf senescence (Lopez *et al.*, 1987; Hall, 2004). It is also able to maintain high leaf water potential or high leaf relative water content during water stress (Küppers et al., 1988; de Carvalho *et al.*, 1998). This strategy, however, may lead to decrease in CO_2 assimilation by involving stomatal closure (Chaves, 1991). In cowpea, stomatal conductance is the major limitation under drought conditions; nevertheless, a pronounced non-stomatal limitation can occur leading to the impairment of photosynthetic activity. Significant genotypic variations in leaf gas exchange parameters have been found among cowpea genotypes under drought stress (Herzog and Anyia, 2001; Hall, 2004; Singh and Reddy, 2011). Umebese and Bankole (2013) reported that stress caused reductions in all parameters studied in cowpea plants. 3 and 5 mM SA foliar treatment caused an increase of 27% in leaf ψw, 94% in chlorophyll content, 75% in plant biomass, 7% in nitrate reductase activity and 38% in proline content. However, variation in the vegetative stage was much lower compared to the reproductive stage.

Biochemical studies have shown that plants under water stress accumulate a number of metabolites that do not interfere with plant metabolism. In addition, the accumulation of these solutes was found to contribute to turgor maintenance. Among these solutes, the accumulation of low molecular weight solutes such as proline and glycine betaine have been shown to act as osmo protectants (Serraj and Sinclair, 2002; Ahmed *et al.*, 2009). Proline has multiple functions, such as regulation of osmotic pressure, protection of membrane integrity and stabilization of enzymes/proteins (Hare and Cress, 1997). Proline accumulation is one of the most frequently reported modifications induced by osmotic stress in plants. Proline also protects plants against free radical induced damage by quenching singlet oxygen (Matysik *et al.*, 2002).

Although different mechanisms responsible for drought resistance in cowpea have been studied in detail, the role of exogenous SA under salt stress in morphological and physiological traits are not much studied. The main aim of this experiment was to study the effects of exogenous SA on parameters of plant growth and proline accumulation in cowpea under water stress conditions. In fact, this experiment was conducted to assess whether exogenously applied SA could alleviate the adverse effects of drought on plant growth and its functions.

MATERIALS AND METHODS

Field experiment

The experiment was conducted during 2008 at the agricultural research station of Zanjan University, North West of Iran (1620 m above sea level., latitude: 36°41' N, longitude: 48°27' E). The site is in a semi-arid zone with a mean annual temperature of 11°C and mean annual precipitation of 298 mm. Soil characteristics of the field are presented in Table 1.

Table 1. Characteristics of the upper soil layer (0- 60cm) of the experimental site

	Soil Depth (cm)	
Soil Characteristics	**0 -30**	**30-60**
Soil Texture	**Loam**	**Loam**
Electrical Conductivity (dSm^{-1})	**1.51**	**2.42**
pH (in Water Solution)	**7.4**	**7.2**
Bulk Density (g cm^{-3})	**1.57**	**1.61**
Organic Matter (%)	**1.11**	**0.85**

Plant material and experimental conditions

Seeds of cowpea *Vigna unguiculata* cv. Parastu (supplied by the Seed and Plant Improvement Institute, Karaj, Iran) were sown in April 2008. Each plot consisted of five rows, each 5 m long, 0.5 m apart. Seeds were planted every 15cm. Stressed and non stressed plots were planted adjacent to each other, separated by a 2 m space to reduce lateral infiltration of water from NS to WS plots.

A number of meteorological variables were recorded from the Agrimet Station (1000 m away from the plots) of Zanjan University throughout the crop growing season (Table 2). Class A pan evaporation was located approximately 50 m from the experimental field. Average reference evapotranspiration during the experiment was 2 mm higher than the long-term average for the same period (Table 2). Total rainfall was quite negligible (<20 mm), therefore, soil water availability was almost totally dependent on irrigation.

Table 2. Main monthly climate parameters in the year of the field experiment during growth season and with a long term average

Year	Months	Rainfall (mm)	T_{min} (°C)	T_{max} (°C)
2008	April	46.8	-2	20
	May	52.8	0	25
	June	16.2	8	23
	July	6.3	10	36
	August	4.7	14.9	33

The experiment was conducted as a strip split plot experiment with two factors: (i) water stress (WS) and (ii) salicylic acid (SA). Water stress treatments were Well-Watered (WW), flowering-stage drought stress (induced by withholding irrigation at 50% flowering) and pod-formation stage drought stress (induced by withholding irrigation when 50% of pods grew to be 2-3cm long). Different concentrations of salicylic acid (MW = 138.1 g.mol^{-1}) (*Fluka*, USA) were 0, 150, 300, 450 and 600 µM, sprayed onto foliar parts of plants when they had approximately ten fully expanded leaves before flowering. Data for different traits were recorded based on 8 m^2 of each plot. In control treatments, plants were irrigated when 80 mm water evaporated from the Class A pan. Irrigation in stress plots was performed when 120 mm water evaporated.

Measured Parameters

Proline Concentration:

Proline accumulation was determined as described by Bates *et al.* (1973). Proline concentrations (µM.mol^{-1}) were calculated using proline standards (0- 8- 16- 24- 32 and 40 µM.mol^{-1}) in identical manner. Finally, proline concentrations (µM) were calculated per 1 g of leaf dry weight.

Leaf relative water content:

Leaf relative water content (RWC) was determined using 10 to 15 fully expanded leaves from each plot. The leaf before the upper fully expanded leaf was detached from the plants and immediately weighed and recorded as fresh weight (FW). Samples were placed in covered Petri dishes filled with distilled water for leaves to reach full hydration. After approximately 12 h at room temperature, leaf samples were blotted dry with paper towels and weighed immediately to determine turgid weight (TW). The leaf tissue was then dried in an oven at 80°C for 48 h to determine dry weight (DW). Leaf RWC was calculated as: RWC = (FW − DW)/ (TW − DW) × 100. (Barrs and Weatherley, 1962)

ΔT: ΔT was calculated by the equation

$\Delta T = TL - TA$,

where TL is leaf temperature and TA is air temperature.

Leaf temperatures were measured using a hand-held laser thermometer (MiniTemp, RayTeck, China) at flowering and pod formation stages. Air temperature was measured by a digital thermometer (model). In each plot, five identical leaves from different plants were measured by the laser thermometer.

Photosynthetic parameters:

Photosynthetic parameters of leaves were determined using a portable photosynthesis analysis system LCA4 (ADC Co., UK). Net photosynthesis rate (Pn) and transpiration rate (E) of plant leaves were measured 3 days after water stress. Measurements were done on three plants in each plot at 9–11 am on a sunny day.

Chlorophyll (Chl) Index:

Leaf Chlorophyll index in special leaves (the 2nd fully expanded leaf) was determined using Chlorophyll Meter CMM-200 (Opti Science, UK) after pod formation stage stress. Measurements were done on six plants in each plot.

Chlorophyll a and b Content:

Chlorophyll *a* and *b* were extracted from leaves using the protocol described by Meidner (1981). After chlorophyll extraction, the amount of these two pigments was measured using a spectrophotometer V-530 (JASCO, Japan).

Leaf Area (LA):

At the initiation of the pod formation stage, 5 plants in each plot were sampled to determine leaf area (LA) using a Leaf Area Meter (LA-200-ADC-Co-UK).

Statistical Analysis:

A two-way analysis of variance (ANOVA) was run. Differences among means of treatments were compared by Duncan's multiple range tests (Sokal and Rohlf, 1988). All data were analyzed by the MSTATC statistical software. Simple correlation coefficients among different traits were also determined using SPSS 15 software.

RESULTS AND DISCUSSION

Proline Concentration:

Water stress significantly increased proline concentration. In both water stress conditions, foliar spray of 300μM SA resulted in the greatest proline concentration (Tables 3 and 4). In both water stress conditions, proline concentration was highly correlated with leaf temperature. In contrast, negative correlations were found between these parameters, chlorophyll content, Chl *a*, and Chl*b* (Tables 5 and 6). It is assumed that the accumulation of this amino acid is the general form of adaptation to osmotic stress. A direct consequence of higher osmolyte concentration is the maintenance of comparatively higher RWC (Misra and Dwivedi, 2004). Different studies have demonstrated correlations between proline accumulation and drought stress (Gunes*et al.*, 2008; Wu *et al.*, 2012; Souza *et al.*, 2004). However, the absence of this positive correlation in some other studies did not exclude the important role of proline from stress adaptation. Souza *et al.* (2004) observed late and small increases in proline levels in cowpea during water stress and recovery. Other studies suggest that this amino acid has no role in osmotic adjustment, and might thus be related with some stress-imposed injury (Campos *et al.*, 1999; Singh and Reddy 2011); still, no evidence of osmotic adjustment has been found in cowpea (Souza *et al.*, 2004; Singh and Reddy, 2011).

Table 3. Effects of exogenous salicylic acid on different physiological traits of cowpea (cv. Parastu) under flowering stage and pod formation stage water stress. Each value represents the mean of three replications

Flowering stage water stress

		Proline (mg/g)	RWC (%)	Δ T(°C)	Pn (µmol/m^{2}s)	E (mmol/m^{2}s)	Chlorophyll Index
Stress	Well watered plants	2.72b	83.01a	-7.75b	7.05a	1.48a	95.03a
	Water stress	4.25a	73.23b	-4.81a	5.41b	1.31a	84.74b
	±SE	0.03	0.49	0.24	0.09	0.05	1.78
Salicylic acid (µm)	Control	3.08c	75.15b	-7.43 c	4.63c	1.13c	73.12c
	150	3.39b	79.3ab	-6.7bc	6.22b	1.46ab	88.07b
	300	3.97a	81.23a	-5 a	7.1a	1.62a	105.27a
	450	3.56b	78.3 ab	-5.73ab	6.64ab	1.39b	93.53b
	600	3.43b	76.98ab	-6.53bc	6.57ab	1.41b	89.45b
	±SE	0.15	1.43	0.31	0.22	0.06	2.05

Pod formation stage water stress

		Proline (mg/g)	RWC (%)	Δ T(°C)	Pn (µmol/m^{2}s)	E (mmol/m^{2}s)	Chlorophyll Index
Stress	Well watered plants	2.6b	75.55a	-3.87a	2.85a	0.95a	71.97a
	Water stress	5.68a	66.42b	-2.24a	1.78a	0.65b	60.59a
	±SE	0.071	1.71	0.44	0.08	0.023	2.52
Salicylic acid (µm)	Control	3.43c	68.74a	-4.06a	1.57b	0.65c	61.87b
	150	4.04b	72.13a	-2.83a	2.29ab	0.7 b	63.74ab
	300	4.45a	71.68a	-2.68a	2.66a	0.98a	71.13a
	450	4.36a	71.56a	-2.48a	2.51a	0.83ab	68.31ab
	600	4.41a	70.8a	-3.23a	2.54a	0.79b	66.36ab
	±SE	0.09	1.6	1.37	0.24	0.05	2.3

Means within a column followed by the same letter are not significantly different. The data shown are mean of five replicates. RWC: Relative Water content, LT: Leaf temperature, Pn: Net Photosynthesis rate, E: Transp. Means followed by the same letter are not significantly different by Duncan's multiple range (P= 0.05)

Thus the observed metabolic alterations may have been the consequence of stress rather than being an adaptive response. Exogenous SA-induced increase in proline level is also observed in other species of plants under abiotic stress (Yusuf *et al.* 2008).

Table 4. Interaction between SA concentrations and water stress and different physiological and morphological traits of cowpea (cv. Parastu) during flowering stage and pod formation stage water stress. Each value represents the mean of three replications. Means followed by the same letter are not significantly different by Duncan's multiple range (P= 0.05)

Flowering stage water stress

water stress	Salicylic acid (µm)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a + b (mg/g)	Chlorophyll a/b (mg/g)	LA (cm^2)
Control	Control	0.1885 d	0.0753 b	0.2638 d	2.5098 a	1152 bcd
	150	0.1940 c	0.0840 a	0.278 c	2.3102 b	1414 a
	300	0.2167 a	0.0852 a	0.3019 a	2.5425 a	1391a
	450	0.2112 b	0.0835 a	0.2947 b	2.5284 a	1376 a
	600	0.2121 b	0.0822 a	0.2943b	2.5801 a	998 de
Flowering stage water stress	Control	0.1685 g	0.0691 c	0.2376 g	2.4395 ab	1049 cde
	150	0.1747 f	0.0699 c	0.2446 f	2.4985 a	1324 ab
	300	0.1804 e	0.0720 c	0.2524 e	2.5056 a	1462 a
	450	0.1779 ef	0.0696 c	0.2475 ef	2.5611 a	1169 bcd
	600	0.1786 ef	0.0719 c	0.2505 e	2.4847 a	988 de
	±SE	0.0012	0.0011	0.0015	0.0462	6.3

Pod formation stage water stress

water stress	Salicylic acid (µm)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a + b (mg/g)	Chlorophyll a/b (mg/g)	LA (cm^2)
Control	Control	0.1703de	0.0423b	0.2126d	4.0407ab	1152 bcd
	150	0.1759cd	0.0527a	0.2286c	3.336b	1414a
	300	0.1914a	0.0580a	0.2495a	3.2988b	1391a
	450	0.1814bc	0.0570a	0.2384b	3.1840b	1376a
	600	0.1857ab	0.0554a	0.2411b	3.3707b	998de
Pod formation stage ater stress	Control	0.1557f	0.0335c	0.1893f	4.6794a	1291ab
	150	0.1613ef	0.0368bc	0.1981e	4.4686a	1198bc
	300	0.1682de	0.0355bc	0.2037e	4.7342a	1015de
	450	0.1644ef	0.0345c	0.1989e	4.7659a	934ef
	600	0.1647ef	0.0343c	0.1990e	4.8628a	798f
	±SE	0.0026	0.0022	0.0018	0.0293	6.54

Relative Water Content (RWC):

In general, water stress considerably reduced RWC. A high amount of RWC in leaves was maintained in some of the cowpea genotypes as a result of stomata closure and a reduction in leaf area (Anyia and Herzog, 2004). Studies have demonstrated that cowpea is able to maintain high leaf water potential during water stress (Küppers et al., 1988; de Carvalho et al., 1998). However, by involving stomata closure, this strategy may lead to decreases in CO_2 assimilation (Chaves, 1991) and hence in growth and yield. Exogenous applied 300 μM SA increased RWC in FWS condition, while different SA treatments during PFDS had no significant effect on this parameter (Tables 3). Similarly, Kadioglu *et al*. (2011) reported that, in comparison with control plants, SA treated plants exhibited a slower decrease in RWC during drought stress. RWC was positively correlated with proline concentration. Therefore, the improvement in RWC by the exogenous application of SA may be the result of osmotic adjustment because of the accumulation of compatible solutes like proline. This result is similar to the findings of Misra and Saxena (2009) and Kadioglu*et al*. (2011).

Leaf temperature (LT):

Application of water stress at flowering stage caused LT to increase. It has been reported that LT increases in response to drought stress because of stomata closure. Sedigh *et al*. (2006) reported that leaf and canopy temperature increased in wheat under drought stress. In drought tolerant genotypes, lower LT is the result of mechanisms that help the plant to keep its stomata open for a longer time. Increasing plant water deficit leads to stomata closure, decreases transpiration cooling and consequently increases LT (O'Neill *et al*. 2006). LT was negatively correlated with RWC in both drought stress conditions (Tables 5 and 6). This finding is in agreement with the results of Khan *et al*., (2007). They reported that RWC was lower, whereas LT was higher in stressed faba bean plants, probably due to restricted transpiration cooling induced by stomata closure. The lowest leaf temperature was recorded in 300 μM SA treated plants. In the pod formation-stage, drought stress leaf temperature was affected by neither drought stress nor the SA treatment (Table 3).

Net photosynthesis rate (Pn*)* and Transpiration rate (E):

In general, water stress significantly reduced Pn. Spraying SA improved Pn in cowpea in water stress conditions (Table 3). This result is in agreement with some earlier studies on different crops (Kumar *et al*., 2002; Khodary, 2004; Stevens *et al*., 2006). The application of SA affects the regulation of photosynthesis in plants grown under normal or stressful conditions; however, these effects are inconclusive. Nemeth *et al*. (2002) reported that SA caused severe damage to photosynthesis in wheat plants subjected to drought stress by decreasing stomata conductance and transpiration. Hamada and Al-Hakimi (2001) have shown that the treatment of wheat plants with 100 ppm SA could stimulate growth by enhancing Pn. The decrease of Pn induced by water deficit can be associated with different physiological parameters including stomata conductance. FWS did not affect E significantly but water stress at pod formation stage reduced this parameter. In this water stress condition, plants treated with 300μM SA showed the highest values for these parameters (Tables 3). Studies have shown that drought stress decreases Pn, chl content and E (Wu *et al*., 2012, Cheruth*et al*., 2009). The exogenous application of SA was found to enhance the Pn and E in *B. juncea* (Fariduddin *et al*., 2003), corn and soybean (Khan *et al*. 2003).

Table 5. Correlation coefficients (Pearson Correlation) among different morphological and physiological traits evaluated in cowpea plant under flowering stage water stress

	Chlorophyll a/b	Chlorophyll a+b	Chlorophyll b	Chlorophyll a	Chlorophyll Content	E (mmol/m^{2}s)	Pn (µmol/m^2 s)	LT(^{0}C)	RWC	Proline
Proline	0.103	-0.668**	-0.696**	-0.634**	-0.114	-0.163	-0.369(*)	0.913**	-0.644**	1
RWC	0.112	0.847**	0.797**	0.838**	0.647**	0.513**	0.703**	-0.568**	1	
LT (^{0}C)	0.113	-0.524**	-0.560**	-0.491**	0.073	0.033	-0.200	1		
Pn (µmol/m^{2}s)	0.127	0.830**	0.769**	0.825**	0.766**	0.706**	1			
E (mmol/m^{2}s)	-0.080	0.617**	0.635**	0.589**	0.797**	1				
Chlorophyll Content	0.194	0.705**	0.621**	0.713**	1					
Chlorophyll a	0.248	0.990**	0.880**	1						
Chlorophyll b	-0.240	0.937**	1							
Chlorophyll a+b	0.113	1								
Chlorophyll a/b	1									

RWC: Relative Water content, LT: Leaf temperature, Pn: Net Photosynthesis rate, E: Transpiration rate

*** Indicates significance at *P* =0.05. ** Indicates significance at *P* = 0.01.**

Reports show that photosynthesis in water-stressed cowpea plants is limited by stomata and non-stomata factors (de Carvalho*et al*., 1998; Souza *et al*., 2004). However, water-stressed cowpea plants presented reductions in stomata conductance paralleled by transpiration rates (Souza *et al*., 2004). Anya and Herzog (2004) reported the reduction of transpiration surfaces as a drought avoiding strategy in cowpea plants. Pn was negatively correlated with proline concentration. A positive correlation was found between Pn and RWC (Tables 5 ad 6).

Table 6. Correlation coefficients (Pearson Correlation) among different morphological and physiological traits evaluated in cowpea plant under Pod formation stage water stress

	Chlorophyll a/b	Chlorophyll a+b	Chlorophyll b	Chlorophyll a	Chlorophyll Content	E (mmol/m^{2}s)	Pn (µmol/m^{2}s)	LT(^{0}C)	RWC	Proline
Proline	0.744**	-0.738**	-0.764**	-0.655**	-0.546 **	-0.548**	-0.535**	0.426*	-0.742**	1
RWC	-0.703**	0.780**	0.768**	0.726**	0.393(*)	0.619**	0.592**	-0.148	1	
LT (^{0}C)	0.300	-0.217	-0.273	-0.148**	-0.184	-0.072	-0.050	1		
Pn (µmol/m^{2}s)	-0.721**	0.834**	0.810**	0.787**	0.605**	0.755**	1			
E (mmol/m^{2}s)	-0.638**	0.759**	0.711**	0.740**	0.673**	1				
Chlorophyll Content	-0.451*	0.630**	0.577**	0.626**	1					
Chlorophyll a	-0.652**	0.961**	0.831**	1						
Chlorophyll b	-0.955**	0.953**	1							
Chlorophyll a+b	-0.832**	1								
Chlorophyll a/b	1									

RWC: Relative Water content, LT: Leaf temperature, Pn: Net Photosynthesis rate, E: Transpiration rate
*** Indicates significance at *P* =0.05.**
**** Indicates significance at *P* = 0.01.**

Chlorophyll:

Water stress reduced Chl index significantly (Table 3). Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*) and Chlorophyll *a+b* (Chl *a+b*) decreased in response to FWS as well as PFWS (Tables 4). The reduction in Chl concentration is identified as a drought response mechanism to minimize light absorption by chloroplasts (Pastenes *et al.*, 2005). However, the application of 300 µM SA increased these variables in both water stress conditions (Tables 1 and Tables 4**)**. There was strong interaction between the Chl *a/b* ratio, SA application and water stress (Tables 4). In FWS, the maximum index was observed for 300 µM application. On the other hand, SA application caused this index to increase dramatically. It is generally accepted that photosynthetic efficiency depends on photosynthetic pigments like chl *a* and *b* (Taize and Zieger, 2002). Singh and Usha (2003) reported that the foliar application of SA increased chl content and stomata conductance possibly causing higher fresh weight. Moharekar *et al.* (2003) reported that salicylic acid activated the synthesis of carotenoids and xanthophylls and also enhanced the rate of deep oxidation with a concomitant decrease in chlorophyll pigments and chl a/b ratio in wheat and moong. In the present study, a positive relationship was found between chl contents. Also, there was a negative and significant correlation between chlorophyll a, b and total with proline content both in flowering and pod formation stages (Tables 5, 6). It was stated that reduction of chlorophyll content under stress conditions was because of changes in nitrogen metabolism due tonthesis of compounds such as proline which has a role in osmotic adjustment. On the other hand, our finding show that there was a positive and significant relationship between chlorophyll (a, b, total and content) and net photosynthesis and transpiration rate. It seems that chlorophylls has a key role in the photosynthesis process as an intercepting antenna for light energy (Taize and Zieger, 2002). Chl *a*, also chl *b* and Chl *a+b*. This suggests that the increase in Pn, as a result of SA treatment, is largely due to Chl content.

Leaf Area (LA):

Water stress and SA treatment were found to affect LA significantly. LA decreased in response to drought stress, especially PFWS. Under different water stress treatments, the highest values were achieved in different SA treatments. Under regular irrigation there were no differences between SA treatments from 150 to 450 µM. On other hand, under water stress conditions, different reactions were found. In FWS, the highest LA was found in 300 µM, but the PFWS application of SA lead to a downward trend in LA (fig.1). Cowpea exhibited broad adaptation mechanisms to drought, such as drought avoidance, by decreasing LA, dehydration avoidance, and vegetative stage drought tolerance by delayed leaf senescence (Bates and Hall, 1981; Lopez *et al*., 1987; Hall, 2004). Decreased stomata conductance and LA reduction were the main drought avoidance strategies in some genotypes of cowpea (Anyia and Herzog, 2004). In *Jatropha curcas*, drought significantly reduced LA and relative growth rate (Maes *et al*., 2009). Reduction in LA by water stress was an important cause of decrease in crop yield through photosynthesis reduction (Correia *et al*., 2001; Li and Wang, 2003). Studies have shown that the exogenous application of SA counteracts the drought stress inhibiting plant growth in different crop species (Haider and Saifullah. 2001; Wang *et al*., 2010; Kadioglu *et al*., 2011). In contrast, Nemeth *et al*. (2002) reported that exogenously applied SA through rooting mediums caused an increase in growth inhibition in maize. It can thus be concluded that increasing LA together with improved RWC resulting from SA treatments could alleviate drought stress. In conclusion ,conclusion, the exogenous treatment of SA might have alleviated the deleterious effect of water stress on the physiological traits of cowpea. This role of SA may be attributed to its ability to improve photosynthetic parameters and plant water status. Increased transpiration rate and proline accumulation as a result of exogenous SA might also be effective mechanisms that protect the cowpea plant against the injuring effects of water deficit.

Conclusion:

Our results showed that water stress reduced characteristics such as leaf area, chlorophyll content (a, b total and index) net photosynthesis, transpiration rate and relative water content, and increased proline content and leaf temperature. The application of SA improved all the measured traits and induced drought tolerance in the treated plants. Among SA concentrations, 300 µM SA had better effects on plants as compared to others.

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تاثیر محلول‌پاشی سالیسیلیک اسید بر صفات رشدی و فیزیولوژیک لوبیا چشم بلبلی تحت تنش آبی

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چکیده- اثر محلول‌پاشی با سالیسیلیک اسید در غلظت‌های مختلف و نقش آن به عنوان یک محافظ در برابر تنش خشکی بر روی کارکردهای فیزیولوژیک گیاه لوبیا چشم بلبلی، رقم پرستو به صورت یک آزمایش مزرعه‌ای در سال ۲۰۰۸ در مزرعه تحقیقاتی دانشگاه زنجان انجام شد. آزمایش. تنش آبی در دو مرحله ۵۰٪ گلدهی و ۵۰٪ غلافدهی اعمال گردید. محلول پاشی با سالیسیلیک اسید درسطوح ۰ ، ۱۵۰ ، ۳۰۰ و ۴۵۰ میکرو مولار در مرحله ۱۰ برگی گیاه صورت گرفت. تنش خشکی اثر معنی‌داری بر روی صفات سرعت فتوسنتز خالص، محتوای نسبی آب و محتوای کلروفیل داشت. در پاسخ به تنش خشکی میزان پرولین و دمای برگ افزایش یافت. در هر دو سطح تنش آبی در سطح غلظت۳۰۰ میکرو مولار سالیسیلیک اسید سرعت فتوسنتز خالص، سرعت تعرق و میزان پرولین افزایش معنی‌داری را داشت. در تیمارهای تنش گلدهی کمترین دمای برگ در تیمارهای شاهد مشاهده گردید، اگر چه در تیمار های تنش گلدهی سالیسیلیک اسید اثر معنی داری در دمای کانوپی نداشت. در تیمارهای اعمال تنش آبی در زمان گلدهی بیشترین میزان سطح برگ در سطح ۳۰۰ میکرو مولارسالیسیلیک اسید دیده شد. بطور کلی، در این آزمایش تیمار محلول‌پاشی با ۳۰۰ میکرو مولار سالیسیلیک اسید موجب بهبود اکثر صفات مورد ارزیابی گردید.

واژه های کلیدی : تنش آبی، سالیسیلیک اسید، مرحله گلدهی، مرحله غلافدهی، همبستگی

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** مکاتبه کننده