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Impact of Irrigation Water Deficit and Foliar Application with Salicylic Acid on the Productivity of Two Cowpea Cultivars

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THREE EXPERIMENTS were conducted at the Vegetable Experimental Farm, Faculty of Agriculture, Assiut University during two summer seasons 2015 and 2016 to study the effect of irrigation regime and salicylic acid foliar application on two cowpea cultivars. Each experiment subjected to one of three tested irrigation levels (70, 50 and 30 % of the available water which equal soil moisture tension of 0.35, 0.60 and 0.85 bar, respectively). Each experiment was laid out using strip plot arrangement in a randomized complete block design (RCBD) with three replications. Three salicylic levels (0, 150, 300 ppm) were randomized in horizontal plots while the two cowpea cultivars (Azmirly and Cream 7) were allocated in vertical plots. The results showed that the irrigation water deficit significantly decreased all the studied traits. Concerning salicylic acid (SA) application, obtained data revealed that increasing the SA concentrations from 0 (control) to 300 ppm, increased the plant ability to withstand drought. Also, Azmirly cultivar was better than Cream 7 for all studied traits. The highest mean values of seed yield per hectar were obtained from Azmirly cultivar which was irrigated at 70 % of the available water and sprayed with 300 ppm salicylic acid as foliar application. Protein patterns were analyzed in leaves of Azmirly and Cream 7 cultivars to study the changes in gene expression after one and two weeks of treatments with 0, 150 and 300 ppm of salicylic acid (SA). SA induced the expression of 11 new proteins as compared to the control treatment.

Keywords: Cowpea, Water deficit, Salicylic acid, RWC, Seed yield (kg/hectare), WUE, Protein patterns and Gene expression.

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is an essential legume mostly grown in arid and semi-arid regions (Muchero et al., 2009 and Afshari et al., 2013). It is usually better modified to water, heat and other biotic stresses compared with the other crops (Ehlers & Hall, 1997, Kuykendall et al., 2000 and Martins et al., 2003). In contrast, Turk et al. (1980) showed that it has a highly subtle to drought at the flowering and pod-filling stages. It is clear that the sensitivity of cowpea returns to water stress during different growth stages has not been sufficiently established.

Water deficit is one of the main factors limiting crop production (Mohamed, 1996, 1999). It decreases growth, interrupts water relations, and reduces water use efficiency in crops (Mohamed & Tawfik, 2007 and Farooq et al., 2012). Also, it reduces cell division rate and elongation, leaf size, stem expansion and root spread, and stomatal opening, as well as, water use efficiency (WUE), (Mohamed, 2003, Farooq et al., 2009 and Li et al., 2009).

Considerable differences among cowpea genotypes with regard to water stress tolerance were found. Also, cowpea genotypes differed significantly in number of pods and seed index which considered the most important traits for seed yield production under water deficit conditions (Faisal and Suliman, 2010). Also, many cowpea cultivars are affected by drought and high temperatures, especially at flowering stage (Dadson et al., 2005) and resulted in mainly reduction in productivity (Nielsen & Hall, 1985, Dow El-Madina & Hall, 1986 and Patel & Hall, 1990). Hence, there is a need for cowpea genotypes, that are more tolerant to drought and

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efficient in their water use (Anyia and Herzog, 2004).

Salicylic acid is one of the most important plant growth regulators which rises plant greenhouse or in open field (Larque-saavedra and Martin-mex, 2007). Also, it plays an important role in seed germination, transpiration, stomatal closure, glycolysis, flowering and fruit yield, (Raskin,1992, Klessig & Malamy, 1994 and Sadoun et al., 2016), and heat tolerance (Dat et al., 1998 a, b). Furthermore, SA could be used as a potential growth regulator to enhance plant growth, under water deficit conditions (Janda et al., 2007).

The effects of spraying salicylic acid on plants show many physiological and molecular processes including changes in gene expression, increasing the protein synthesis and activation of specific enzymes (Bekheta & Talaat, 2009 and Shakirova et al., 2003). However, Raskin (1992) reported that SA was categorized as endogenous growth regulator, a phytohormone which participates in regulation of plant growth and development. It can regulate ion transport and uptake (Kaydan et al., 2007), stomatal function (Raskin, 1992), increase stress tolerance and disease resistance (Klessig & Malamy 1994 and Ghorbani Javid et al., 2011) and finally enhance plant growth and yield. Electrophoretic protein profiles were used to detect changes in gene expression, in which presence or absence of protein bands as well as increasing or decreasing of band intensity reflect the change in gene expression (Khavkin & Zabrodina, 1994 and El-Aref, 2002).

The objective of this study was to evaluate the effects of irrigation water deficit and foliar application of salicylic acid on the productivity of two cowpea cultivars.

Materials and Methods

To study the effects of irrigation deficit and salicylic acid concentrations on two cowpea cultivars, three experiments were conducted at the Vegetable Experimental Farm, Faculty of Agriculture, Assiut University during two summer seasons 2015 and 2016. Each experiment was subjected to one irrigation level of the three levels 70, 50 and 30 % of the available water which equal to soil moisture tension of 0.35, 0.60 and 0.85 bar, respectively. Each experiment was laid out using strip plot arrangement in a randomized complete block design (RCBD) with three replications. Three salicylic acid levels (0, 150, 300 ppm) were arranged in the horizontal plots while the two cowpea cultivars (Azmirly and Cream 7) were allocated in the vertical one. Planting distance was 60 cm x 30 cm (Hasan, 2002, Agbogidi and Egho, 2012). The experimental unit area was 5.40 m^2 (3 rows 60 cm apart and 3-meter-long with 30 cm distance between hills producing about 52888 plant per hectare). The soil physical and chemical properties of experimental site are presented in Table 1 (Page et al., 1982).

TABLE1. Analysis of physical and chemical characteristics of the experimental soil.

Characteristic	Values	Characteristic	Values
pH(1:2.5)	8.01	Caly %	48.95
EC1:1 dSm^{-1}	1.20	Silt %	29.37
ECe dSm^{-1}	1.85	Sand %	21.68
Soluble Cations, (meq/kg soil)		Soil texture	Clay
Ca^{2+}	9.00	Bulk density, (g/cm^3)	1.70
Mg^{2+}	3.00	Field capacity, (F.C)%	42.40
Na^+	6.50	Wilting point (W.P)%	22.33
K^+	1.00	*Available soil moisture %	20.07
Soluble Anions,(meq/kg soil)		W.Saturation %	65.01
Cl^-	4.00	**Soil depth (cm) used in this study**	**Bulk density (kg/m^3)**
$HCO_3^-+CO_3^{2-}$	6.50		
SO_4^{2-}	9.00	0-15	1610.00
Total nitrogen (ppm)	8.00	15-30	1650.00
Available Phosphorus (ppm)	11.20	30-45	1720.00
Available Potassium (ppm)	300.00	45-60	1740.00

*Available soil moisture (ASM) calculated as the difference between the field capacity, (F.C) % and the wilting point (W.P) %.

The cowpea seeds were sown on 30th April and 5th May in the first and second seasons, respectively. The plants were sprayed to the drip point with salicylic acid as foliar application once after 30 days from sowing using plastic sheet one-meter height as a separator between treatments. Untreated plants were sprayed with tap water. All the other agricultural practices were done as recommended for cowpea by the Egyptian Ministry of Agriculture (CAAE, 2012).

In this study deficit irrigation strategies were applied to detect the difference between two cowpea cultivars to water shortage. Three deficit irrigation regimes were applied as follows:

- Irrigation water was applied after 30% of soil moisture depletion (SMD) or at 70% of the available soil moisture (70% ASM).
- Irrigation was applied after depletion of 50% (SMD) or at 50% of the available soil water (ASM).
- Irrigation was applied at 30% of the available soil moisture (ASM) (the stress treatment) or 70% of the soil moisture depletion (SMD).

The irrigation due time was detected by using soil tensiometers which was caliparated to achieve the above mentioned soil moister levels. The 70, 50,30 % ASM matched 0.35, 0.60 and 0.85 bar, respectively. Three tensiometers, (Irrometer Reg. U.S. PAT off moisture Indicator. Irrometer Company Riversid, CALIF., USA) were refilled with oxygen free water and closed tightly without any air puppies inside after each irrigation and inserted at depth of 30 cm in the root zone in each experiment. The surface irrigation system was used. The consumed water during each season was calculated by measuring the difference in the soil moisture before and after each irrigation in four soil depths (0-15, 15-30, 30-45, and 45-60 cm) according to the following equation:

Calculation of water consumptive use (CU)

Actual evapotranspiration was estimated by the sampling method and calculated according to the following formulas:

C.U = {D x Bd x (Q2-Q1)/100} (Israelsen and Hansen,1962)

Where:

C.U. = actual evapotranspiration (mm). (consumptive use)

D = soil depth (m) of each layer (0 – 0.15, 0.15-0.30, 0.30-0.45 and 0.45-0.60 m)

Bd = bulk density of soil (ton/m^3) for each layer.

Q2 = the percentage of soil moisture one day after irrigation (field capacity)

Q1 = the percentage of soil moisture before next irrigation.

The applied water in the three experiments were 6906.67, 5340.72 and 3772.30 m^3 ha^{-1} during the first season and 8898.82, 6309.38 and 3717.56 m^3 ha^{-1} during the second season for 70%, 50% and 30% ASM, respectively.

Also, reference evapotranspiration (ETo) was calculated using the meteorological data (Penman Monteith equation) as applied by CROPWAT model (Smith, 1991) as follows:

$$ET_0 = \frac{\left[o.408\times(Rn-G)+\gamma\left[\frac{900}{T+273}U2(ea-ed)\right]\right]}{\Delta+\gamma(1+0.34U2)}$$

Where:

ETo: reference evapotranspiration [mm day^{-1}],
Rn: net radiation at the crop surface [MJ m^{-2} day^{-1}],
G: soil heat flux density [MJ m^{-2} day^{-1}],
T: mean daily air temperature at 2 m height [°C],
U2: wind speed at 2 m height [ms^{-1}],
es ed: saturation vapour pressure [kPa],
ea: actual vapour pressure [kPa],
es ed – ea: saturation vapour pressure deficit [kPa],
Δ: slope vapour pressure curve [kPa $°C^{-1}$],
γ: psychrometric constant [kPa $°C^{-1}$].

The amount of applied water was matched the values calculated by Cropwat program as shown in Table 2 after using the cowpea crop factor (Kc) and the four growth stages periods and irrigation application efficiency.

The measurements were recorded on ten guarded plants in each experimental unit as follows:

- Relative water content (RWC%) were calculated as follows as described by Barr and Weatherly (1962). Ten leaf discs excised using a 1-centimetre-diameter cork borer were used for the measurements.

TABLE 2. Meteorological data used to calculate evapotranspiration according to penman- monteth, (Smith, 1991).

Year	Month	T max (°C)	T min (°C)	RH %	W.S (km/day)	ETo (mm/day)
2015	**January**	20.5	5.5	44.0	191.9	1.7
	February	22.7	7.6	38.8	196.8	2.3
	March	27.2	12.2	34.0	232.6	3.9
	April	29.3	14.6	25.6	238.3	5.1
	May	35.5	19.7	27.1	208.8	5.9
	June	36.6	21.3	37.4	156.3	7.0
	July	38.8	22.8	35.9	98.4	6.7
	August	40.3	24.8	38.6	100.8	6.8
	September	38.5	23.8	38.5	175.2	5.0
	October	33.0	19.5	51.3	195.6	4.0
	November	26.3	13.2	59.5	182.4	2.5
	December	20.4	7.2	63.2	201.6	1.8
2016	**January**	19.0	5.1	60.3	348.0	1.7
	February	24.5	8.3	50.7	408.0	2.6
	March	28.0	13.1	41.0	408.0	3.4
	April	35.1	17.1	31.5	487.2	4.3
	May	36.1	20.0	27.7	468.0	6.6
	June	40.7	24.6	28.0	468.0	7.4
	July	37.4	24.1	37.9	468.0	7.1
	August	37.5	24.1	36.8	520.8	6.7
	September	35.0	21.6	43.5	460.8	6.2
	October	32.8	17.7	49.5	362.4	4.6
	November	27.0	12.7	54.7	403.2	2.5
	December	19.9	6.3	59.7	348.0	1.7

T max = monthly average maximum temperature

T min = monthly average minimum temperature

RH% = Relative humidity

WS = Wind speed (km/day)

ET0=Stander evapo- transpiration (mm/day)

RWC (%) = (FW – DW) / (TW – DW) x 100

Where:
FW = fresh weight of leaves

TW = turgid weight of leaves after incubating leaves in distilled water for 24 h in refrigerator

DW = dry weight of leaves after oven drying at 70 °C for 48 h.

- Number of branches per plant at the end of the season
- One hundred seed weight (g) after drying
- Seed yield per plant (g)
- Seed yield (kg per hectare).
- Water use efficiency (WUE kg / m^3) as follows (Vites, 1965):

$$\text{Water use efficiency (kg/m}^3\text{)} = \frac{\textit{Seeds yield (kgha}^{-1}\textit{)}}{\text{Total water applied } (m^3ha^{-1})}$$

Each experiment was separately analyzed and bartlett test of error variances homogeneity was examined upon homogeneity combined analysis among the three studied irrigation treatments to detect the effect of irrigation factor and their interactions with the other tested factors. Collected data were analyzed using MSTAT-C Statistical Software Package (Michigan State University, Freed et al. 1991) and means were compared using the LSD at P≤ 0.05 according to Gomez and Gomes (1984). Simple correlation coefficients among yield and its attributes traits over both seasons (n=108) were also computed and studied.

Protein pattern analysis

Leaf samples of Azmirly and Cream 7 cultivars were collected from the SA treated (150 and 300 ppm) and un-treated (control) plants after one and two weeks of treatment. Soluble proteins were extracted by crushing 1.0 g of each sample in 1.0 ml extraction buffer (0.1 M Tris-HCl +2.0mM EDTA, pH 7.8). Electrophoresis of soluble proteins was applied as described by Laemmli (1970) using 12 % polyacrylamide and 1 % SDS (w/v) under denaturing conditions. The gel was stained with Commassie Blue R and the detected bands were scored and molecular weights were determined against protein marker consisted of 70, 60, 50, 40, 30, 20 and 10 KD using GS 365 electrophoresis data system program version 3.01 (Microsoft Windows @ version).

Results and Discussion

Relative water content (RWC) %

Collected data reveal that the irrigation, salicylic and cowpea cultivars had a significant effect on RWC % in both seasons (Table 3). But, the all interactions involved in this respect were not significant in both seasons. The highest mean values of RWC were obtained from irrigation at 70% of the available water (ASM) with no significant differences between this irrigation treatment and irrigation at 50% of available water (ASM) in both seasons. While, the significant lowest mean values of RWC (72.43 and 68.57% in the first and second seasons, respectively) were recorded when irrigation was done at 30% of available water. A high quantity of leaves relative water content was found in some of the cowpea genotypes due to stomata closure and a leaf area reduction (Anyia & Herzog 2004 and Afshari et al., 2013). Furthermore, the data reveal that salicylic acid significantly increased RWC trait in favor of high level (300 ppm) in both seasons. While, the lowest mean values of RWC were obtained from the control treatment with value of 73.12 and 69.85 in the first and second seasons, respectively. Larque-saavedra and Martin-mex, (2007) stated that SA enhanced rooting and root growth and development. Moreover, the data show that plants of Azmirly cultivar had significant contents of relative water as compared with Cream 7 cultivar in both seasons.

Number of branches per plant

Data illustrated in Table 4 show that the irrigation water deficit had a highly significant influence on the number of branches per plant in both seasons. Irrigation at 70% of available water (ASM) produced the highest mean values of 6.72 and 5.97 branch per plant in the first and second seasons, respectively. While, the lowest mean values in this respect were 5.00 and 3.87 branch per plant in the first and second seasons, respectively, were obtained when irrigation was applied at 30% of available water (ASM). This is explained by the shortage of irrigation water caused a reduction in nutrients uptake, metabolism translocation as well as cell devission and elongation which decreased number of branches per plant (Nonami,1998 and Farooq et al., 2009). Furthermore, data reveal that salicylic acid had a significant effect on number of branches per plant in both seasons. The 300 ppm SA concentration recorded the highest mean values of branches number per plant (6.42 and 5.61) in the first and second seasons, respectively. SA was reported to increase the shoot growth in many crop species (Larque-saavedra and Martin-mex, 2007). Similar trend was obtained in mung bean by Ali and Mohamed, (2013). Moreover, the obtained data show that the studied cowpea cultivars varied significantly in this trait in both seasons. Azmirly cultivar surpassed the Cream 7 in this respect by 40.62 and 30.09 % in the first and second season, respectively. This may be due to the genotypic, environmental interaction (Hanaa and Ali, 2011) .All the interactions involved in this respect were not significant in both seasons.

One hundred seed weight(g)

Data exhibited in Table 5 show that the irrigation water deficit had a highly significant influence on the 100 seed weight in both seasons. Thus, irrigation at 70% of available water (ASM) resulted in the highest mean values of the hundred seed weight (20.20 g and 16.36 g in the first and second seasons, respectively). While, the lowest mean values of 18.11 and 14.07 g for

100 seed weight in the first and second seasons, respectively, were obtained when irrigation was applied at 30% of available water (ASM). The shortage of irrigation water caused inhabitation of metabolism products translocation from the source to sink (seeds) which resulted in lower seed weight. Similar trend was obtained by Umebese and Bankole (2013). Also, data reveal that salicylic acid had a highly significant effect on the 100 seed weight in both seasons. The highest mean value of the 100 seed weight (20.28 and 16.53 in the first and second seasons, respectively) was in favor of 300 ppm treatment. Similar result was reported by Afshari et al. (2013). Moreover, the obtained data show that the studied cowpea cultivars varied significantly in the 100 seed weight in the two growing seasons. Azmirly cultivar surpassed the Cream 7 one in this respect by 17.37 and 27.84 % in the first and second seasons, respectively. This is logic since the Azmirly cultivar surpassed the other one in regards to relative water content and number of branches per plant. This may increase the sources of metabolism translocation into the sink (seeds) and consequently increased seed weight. Also, the first order interaction between SA x CV was significant in both seasons and the interaction between (I x CV) was highly significant in the second season only. However, the second order interaction (I x SA x CV) was not significant in both seasons.

Seed weight per plant (g)

Concerning the effect of irrigation water deficit, salicylic acid, cultivars and their interactions, data in Table 6 revealed a highly significant effect on the seed weight per plant in the two growing seasons except the interaction between (SA x CV) in the second season only which was not significant. Thus, decreasing the amount of water applied to cowpea plants from 70 to 50 or 30 % of available water (ASM) resulted in reduction in seed weight per plant by 21.08 and 37.89 % in the first season, being 21.00 and 38.23 % in the second season, respectively. The same trend was observed as it was for the 100 seed weight character. Also, the obtained data show that the superiority of spraying with 300 ppm SA over the other SA treatments in both seasons and produced the highest mean which were 38.57 and 36.90 g in the first and second seasons, respectively. This is to be expected since the same trend was true with regard to the 100 seed weight character. Furthermore, Azmirly cultivar surpassed Cream 7 in this respect by 14.10 % and 14.89 % in the first and second seasons, respectively. The same cultivar produced the highest mean value of 100 seed weight and consequently produced the highest seed weight per plant. Also, from the results, it is clear that Azmirly cultivar plants which were irrigated at 70% of available water (ASM) and sprayed with 300 ppm salicylic acid gave the highest seed weight per plant in both seasons (49.20 and 47.50 g in the first and second season, respectively). On the other hand, Cream 7 plants which were subjected to high water deficit (30% of available water) and sprayed with tap water (without salicylic) gave the lowest weight of seeds per plant in both seasons (23.00 and 22.5 g in the first and second season, respectively).

Seed yield (kg/ha)

Data in Table 7 denote that irrigation water deficit, salicylic acid, cultivars and their interactions had a significant effect on the seed yield (kg/ha) in both seasons except the interaction between Salicylic acid and cultivars (SA x CV) in the second season only. Irrigation at 70% of available water (ASM) surpassed the other two tested irrigation treatments in this respect. It produced 2447.9 and 2363.8 kg/ha in the first and second seasons, respectively. Ogbonnaya et al. (2003) found that cowpea seed yield was reduced by about 60–71% as a result of water deficit. Water deficit led to yield reduction which depends on the severity and duration of the stress period. A similar result was obtained by Faisal and suliman, (2010). This can be logic since the same trend was observed for seed yield per plant. Also, the obtained data show the superiority of spraying with 300 ppm SA over 150 and zero ppm SA treatments in both seasons and produced the highest mean values of 2142.5 and 2050.4 kg/ha in the first and second seasons, respectively. This is to be expected since the same trend was true with regard to seed yield per plant. Moreover, Azmirly cultivar surpassed Cream 7 one by 14.08 and 14.87 % in the first and second seasons, respectively. This is to be expected since the Azmirly cultivar produced the highest mean values with regard to seed yield per plant and consequently produced the highest seed yield per hectar. The same results were reported by Faisal and Suliman, (2010). Concerning the second order interaction, data obtained from these results clearly showed that that Azmirly cultivar plants which irrigated at 70% of available water (ASM) and sprayed with 300 ppm salicylic acid gave the highest seed yield kg/ha in both seasons of (2732.5 and 2640.0 kg/ha in the first and second seasons, respectively. On the other hand, Cream 7 plants which subjected to a water deficit as irrigated at 30% of available water (ASM) and sprayed with tap water (without salicylic) gave the lowest seed yield (kg/ha) in both seasons with values of 1277.5 and 1250.0 (kg/ha) in the first and second seasons, respectively. This is to be expected since the same interaction had a similar trend regarding the seed yield per plant in both seasons.

Water use efficiency (WUE kg / m^3)

The obtained data (Table 8) show that irrigation water deficit, salicylic acid, cultivars and their interactions had a highly significant effect on water use efficiency (WUE kg / m^3) in both seasons except the interaction between salicylic acid and cultivars (SA x CV) as well as the second order interaction (I x SA x CV) in the second season only. One cubic meter (m^3) of water produced the highest yield when 30% of available water (ASM) used in both seasons with values of 0.408 and 0.393 kg/m^3 in the first and second seasons, respectively. Abbate et al. (2004) and Subramanian et al. (2006) reported higher WUE in wheat and tomato under water stress than well irrigated due to transpiration reduction. Also, the highest mean values of water use efficiency (WUE kg / m^3) were obtained from plants treated with 300 ppm salicylic acid in both seasons of 0.408 and 0.345 kg / m^3 in the first and second seasons, respectively. This is logic since the same salicylic concentration produced the highest seed yield per hectare. That may be due to the effect of salicylic acid on stomata closure. Also, Azmirly cultivar surpassed cream 7 in water use efficiency (WUE) in both seasons with value of 0.400 and 0.341 kg/m^3 in the first and second seasons, respectively, this is also expected since Azmirly cultivar surpassed Cream 7 in seed yield per hectare and consequently increased WUE. Similar trend was found by Gwathmey & Hall (1992) and Hall (2004). From these data, it was found that Azmirly cultivar which treated with 300 ppm salicylic acid and irrigated at 30% of available water (ASM) produced the highest mean value of water use efficiency (WUE) with value of 0.464 and 0.455 kg/m^3 in first and second seasons, respectively.

The correlation coefficient among the studied characters over both seasons

Data presented in Table 9 show highly positive significant correlation between number of branches per plant, 100 seed weight and seed yield per plant with seed yield per hectare. That means the treatment which increased these traits led to increase the seed yield per hectare.

Electrophoretic changes of protein patterns

Electrophoretic Changes of protein patterns were analyzed in leaves of Azmirly and Cream 7 cultivars (Fig. 1 and 2), in order to follow the possible alterations in gene expression in leaves of cowpea plants after one and two weeks of spraying with 0, 150 and 300 ppm salicylic acid (SA). Protein profiles of these treatments are summarized in Tables 10 and 11 and illustrated in Fig. 1 and 2. The most noticeable change in protein patterns is that SA induced and enhanced protein synthesis, as compared to the control treatment.

After one week of spraying with SA, it was induced the appearance of four new polypeptides (21.1, 29.8, 46.1 and 50.2 KD) in Azmirly cultivar (Table 10). These proteins were induced with 300 ppm SA treatments, except the 50.2 KD protein which was induced under both levels of 150 and 300 ppm of SA. In case of Cream7 cultivar, four newly proteins at molecular weights 29.8, 46.1, 50.2 and 57.6 KD were induced in 150 and 300 ppm SA treated plants while one band at 11.8 KD was newly expressed under high level of SA (300 ppm). These results indicated that the three proteins 29.8, 46.1 and 50.2 KD were commonly induced in Azmirly and Cream 7 after one week of SA treatment, as compared to non-treated plants. Only one band at 42.3 KD was reduced under low level of SA treatment in Cream7.

After two weeks of spraying with SA (Table 11), five proteins were newly expressed in Azmirly, two of them (29.8 and 86.4 KD) were induced by both 150 and 300 ppm SA treatments while the other bands (46.1, 54.8 and 76.3 KD) were induced by only 300 ppm SA. In case of Cream7, the 29.8, 46.1 and 76.3 KD proteins were induced under both levels of SA while the three bands of 40.1, 54.8 and 65.4 KD were induced under the 300 ppm level of SA. These results revealed that four bands at 29.8, 46.1, 54.8 and 76.3 KD were commonly induced in both Azmirly and Cream7 cultivars after two weeks of SA treatment, as compared to non-treated plants. Only one band at 44.2 KD was reduced in Cream7 under both treatments of SA.

It appears clearly that spraying with SA induced the expression of 11 proteins, four of them (11.8, 21.1, 50.2 and 57.6 KD) induced only after one week of treatment and five proteins (46.1, 54.8, 65.4, 76.3 and 86.4 KD) induced only after two weeks of SA treatment while, two new proteins (29.8 and 46.1KD) were expressed along the two periods of SA treatments. The results revealed that most proteins, including the low molecular weight (MW) proteins (e.g. 11.8 and 21.1 KD), which induced in the first week of SA treatment were down-regulated after two weeks while newly high MW proteins 65.4, 76.3 and 86.4 KD were up-regulated after the second week of SA treatment.

The results of polyacrylamide gel electrophoresis indicated that the SA induced alternations in protein expression and 11 proteins were newly expressed as compared to the control treatment. Since SA increased plant growth and yield, these de novo syntheses of specific proteins may play an important role in the biochemical pathways to enhance plant growth and yield. These results reflect the potentiality of SA to enhance the expression of specific genes in the treated plants and enhanced a cascade biochemical reaction which might determine the ultimate appearance of growth patterns and yield (Jacobsen & Beach, 1985, Raskin, 1992 and Abdel-Hamid, 2002). Songhai et al. (1999) found that treatment of rice with exogenous SA induces the expression of the genes resulting in an increased number of proteins, increases in some band densities, enhanced specific enzyme activity and strengthened resistance. Bekheta and Talaat (2009) reported that treatment of cowpea with bioregulator SA increased total protein and caused positive changes in protein expression which induced some proteins and reduced other proteins. In addition, Jalal et al. (2012) found that water stress and SA treatments in shara (Plectranthus tenuiflorus) plants induced new high molecular weight protein bands and reduced other proteins.

Conclusion

Azmirly cultivar which was irrigated at 70 % of the available water and sprayed with 300 ppm salicylic acid as foliar application produced the highest mean values of seed yield per hectare (2732.5 and 2640 kg/hectare in the first and second seasons, respectively). Also, SA induced the expression of 11 new proteins as compared to the control treatment. Since SA increased plant growth and yield, these de novo syntheses of specific proteins may play an important role in the biochemical pathways to enhance plant growth and yield.

TABLE 3. Effect of irrigation deficit, salicylic acid application, cultivars and their interactions on relative water content (RWC).

Season		2015				2016			
Irrigation (I)	Salicylic acid (SA) / Cultivar (CV)	0 ppm	150 ppm	300 ppm	Mean	0 ppm	150 ppm	300 ppm	Mean
I_1 70 % of available water	CV_1 (Azmirly)	78.85	83.33	89.88	84.02	75.74	81.93	83.98	80.55
	CV_2 (Cream 7)	74.98	78.06	82.09	78.38	71.81	76.29	79.78	75.96
Mean		76.92	80.70	85.99	81.20	73.78	79.11	81.88	78.26
I_2 50 % of available water	CV_1 (Azmirly)	75.23	78.61	81.91	78.58	72.51	74.54	78.38	75.15
	CV_2 (Cream 7)	71.66	75.39	78.89	75.31	69.62	72.08	75.55	72.42
Mean		73.45	77.00	80.39	76.95	71.07	73.31	76.97	73.78
I_3 30 % of available water	CV_1 (Azmirly)	71.63	73.45	77.76	74.28	67.32	70.24	75.89	71.15
	CV_2 (Cream 7)	66.34	69.56	75.83	70.58	62.08	65.53	70.36	65.99
Mean		68.98	71.51	76.79	72.43	64.70	67.89	73.13	68.57
General Mean		73.12	76.40	81.06	76.86	69.85	73.44	77.32	73.54
CV x SA Interaction	CV_1	75.24	78.46	83.18	78.96	71.86	75.57	79.42	75.62
	CV_2	70.99	74.34	78.94	74.76	67.84	71.30	75.23	71.46
		F value		LSD 0.05		F value		LSD 0.05	
I		*		5.3001		*		5.8158	
SA		*		5.4306		*		5.1958	
CV		*		----		*		----	
I x SA		N.S		----		N.S		----	
I x CV		N.S		----		N.S		----	
SA x CV		N.S		----		N.S		----	
I x SA x CV		N.S		----		N.S		----	

NS, * non-significant and significant at 0.05 probability level, respectively.

TABLE 4. Effect of irrigation deficit, salicylic acid application, cultivars and their interactions on number of branches per plant.

Season		2015				2016			
Irrigation (I)	Salicylic acid (SA) / Cultivar (CV)	0 ppm	150 ppm	300 ppm	Mean	0 ppm	150 ppm	300 ppm	Mean
I_1 70 % of available water	CV_1 (Azmirly)	7.50	8.17	8.25	7.97	6.43	6.64	7.08	6.72
	CV_2 (Cream 7)	5.00	5.29	6.08	5.46	4.63	5.13	5.92	5.23
Mean		6.25	6.73	7.17	6.72	5.53	5.89	6.50	5.97
I_2 50 % of available water	CV_1 (Azmirly)	6.33	6.50	6.78	6.54	5.57	6.44	6.50	6.17
	CV_2 (Cream 7)	4.59	4.61	5.92	5.04	4.29	4.33	5.33	4.65
Mean		5.46	5.56	6.35	5.79	4.93	5.39	5.92	5.41
I_3 30 % of available water	CV_1 (Azmirly)	5.45	6.00	6.43	5.96	4.00	4.22	4.89	4.37
	CV_2 (Cream 7)	3.50	3.54	5.09	4.04	3.00	3.17	3.95	3.37
Mean		4.48	4.77	5.76	5.00	3.5	3.70	4.42	3.87
General Mean		5.40	5.69	6.42	5.84	4.65	4.99	5.61	5.08
CV x SA Interaction	CV_1	6.43	6.89	7.15	6.82	5.33	5.77	6.16	5.75
	CV_2	4.36	4.48	5.70	4.85	3.97	4.21	5.07	4.42
		F value		LSD 0.05		F value		LSD 0.05	
I		**		0.4478		**		0.6659	
SA		*		0.5006		**		0.3595	
CV		*		-----		*		-----	
I x SA		N.S		-----		N.S		-----	
I x CV		N.S		-----		N.S		-----	
SA x CV		N.S		-----		N.S		-----	
I x SA x CV		N.S		-----		N.S		-----	

NS, * and ** non-significant and significant at 0.05 and 0.01 probability level, respectively.

TABLE 5. Effect of irrigation deficit, salicylic acid application, cultivars and their interactions on 100 seed weight (g).

Season		2015				2016			
Irrigation (I)	Salicylic acid (SA) / Cultivar (CV)	0 ppm	150 ppm	300 ppm	Mean	0 ppm	150 ppm	300 ppm	Mean
I_1 75 % of available water	CV_1 (Azmirly)	20.82	21.51	22.76	21.70	17.37	18.25	19.85	18.49
	CV_2 (Cream 7)	17.82	18.95	19.32	18.70	13.04	14.18	15.48	14.23
Mean		19.32	20.23	21.04	20.20	15.21	16.22	17.67	16.36
I_2 50 % of available water	CV_1 (Azmirly)	19.71	20.16	21.80	20.56	15.57	16.87	18.47	16.97
	CV_2 (Cream 7)	16.30	17.90	18.95	17.72	12.89	13.83	14.55	13.76
Mean		18.01	19.03	20.38	19.14	14.23	15.35	16.51	15.36
I_3 30 % of available water	CV_1 (Azmirly)	18.67	19.73	20.92	19.77	14.43	15.80	17.57	15.93
	CV_2 (Cream 7)	15.13	16.31	17.91	16.45	11.15	12.21	13.26	12.21
Mean		16.90	18.02	19.42	18.11	12.79	14.01	15.42	14.07
General Mean		18.08	19.09	20.28	19.15	14.07	15.19	16.53	15.26
CV x SA Interaction	CV_1	19.73	20.47	21.83	20.68	15.79	16.97	18.63	17.13
	CV_2	16.42	17.72	18.73	17.62	12.36	13.41	14.43	13.40
		F value		LSD 0.05		F value		LSD 0.05	
I		**		0.6318		**		0.2293	
SA		**		0.3645		**		0.3548	
CV		**		-----		**		-----	
I x SA		N.S		-----		N.S		-----	
I x CV		N.S		-----		**		0.4586	
SA x CV		*		0.2079		*		0.9054	
I x SA x CV		N.S		-----		N.S.		-----	

NS, * and ** non-significant and significant at 0.05 and 0.01 probability level, respectively.

TABLE 6. Effect of irrigation deficit, salicylic acid application, cultivars and their interactions on seed yield per plant (g).

Season		2015				2016			
Irrigation (I)	Salicylic acid (SA) / Cultivar (CV)	0 ppm	150 ppm	300 ppm	Mean	0 ppm	150 ppm	300 ppm	Mean
I_1 70 % of available water	CV_1 (Azmirly)	44.00	45.70	49.20	46.30	42.00	44.60	47.50	44.70
	CV_2 (Cream 7)	37.50	42.80	45.20	41.83	36.90	41.20	43.00	40.37
Mean		40.75	44.25	47.20	44.07	39.45	42.90	45.25	42.53
I_2 50 % of available water	CV_1 (Azmirly)	34.50	38.00	41.00	37.83	33.10	37.00	39.70	36.60
	CV_2 (Cream 7)	28.00	32.20	35.00	31.73	26.90	31.20	33.70	30.60
Mean		31.25	35.10	38.00	34.78	30.00	34.10	36.70	33.60
I_3 30 % of available water	CV_1 (Azmirly)	27.50	29.40	31.50	29.47	26.10	28.00	30.50	28.20
	CV_2 (Cream 7)	23.00	25.50	29.50	26.00	22.50	23.50	27.00	24.33
Mean		25.25	27.45	30.50	27.73	24.30	25.75	28.75	26.27
General Mean		32.42	35.60	38.57	35.53	31.25	34.25	36.90	34.13
CV x SA Interaction	CV_1	35.33	37.70	40.57	37.87	33.73	36.53	39.23	36.50
	CV_2	29.50	33.50	36.57	33.19	28.77	31.97	34.57	31.77
		F value		LSD 0.05		F value		LSD 0.05	
I		**		0.6659		**		0.6257	
SA		**		0.6757		**		0.5376	
CV		**		-----		**		-----	
I x SA		**		1.9977		**		1.8771	
I x CV		**		1.3318		**		1.2514	
SA x CV		**		0.1698		N.S		-----	
I x SA x CV		**		0.5094		**		3.2166	

NS and ** non-significant and significant at 0.01 probability level, respectively.

TABLE 7. Effect of irrigation deficit, salicylic acid application, cultivars and their interactions on seed yield (kg / hectare).

Season		2015				2016			
Irrigation (I)	Salicylic acid (SA) / Cultivar (CV)	0 ppm	150 ppm	300 ppm	Mean	0 ppm	150 ppm	300 ppm	Mean
I_1 70 % of available water	CV_1 (Azmirly)	2445.0	2540.0	2732.5	2572.5	2332.5	2477.5	2640.0	2483.3
	CV_2 (Cream 7)	2082.5	2377.5	2510.0	2323.3	2050.0	2292.5	2390.0	2244.2
Mean		2263.8	2458.8	2621.3	2447.9	2191.3	2385.0	2515.0	2363.8
I_2 50 % of available water	CV_1 (Azmirly)	1917.5	2110.0	2277.5	2101.7	1840.0	2055.0	2205.0	2033.3
	CV_2 (Cream 7)	1555.0	1790.0	1945.0	1763.3	1495.0	1732.5	1872.5	1700.0
Mean		1736.3	1950.0	2111.3	1932.5	1667.5	1893.8	2038.8	1866.7
I_3 30 % of available water	CV_1 (Azmirly)	1527.5	1632.5	1750.0	1636.7	1450.0	1555.0	1695.0	1566.7
	CV_2 (Cream 7)	1277.5	1417.5	1640.0	1445.0	1250.0	1305.0	1500.0	1351.7
Mean		1402.5	1525.0	1695.0	1540.8	1350.0	1430.0	1597.5	1459.2
General Mean		1800.8	1977.9	2142.5	1973.7	1736.3	1902.9	2050.4	1896.5
CV x SA Interaction	CV_1	1963.3	2094.2	2253.3	2103.6	1874.2	2029.2	2180.0	2027.8
	CV_2	1638.3	1861.7	2031.7	1843.9	1598.3	1776.7	1920.8	1765.3
		F value		LSD 0.05		F value		LSD 0.05	
I		**		42.991		**		35.857	
SA		**		33.443		**		16.134	
CV		**		-----		**		-----	
I x SA		**		128.973		**		107.571	
I x CV		**		85.982		**		71.714	
SA x CV		**		75.063		N.S		-----	
I x SA x CV		**		225.189		*		221.526	

NS and ** non-significant and significant at 0.01 probability level, respectively.

TABLE 8. Effect of irrigation deficit, salicylic acid application, cultivars and their interactions on water use efficiency (WUE kg / m^3):

Season		2015				2016			
Irrigation (I)	Salicylic acid (SA) / Cultivar (CV)	0 ppm	150 ppm	300 ppm	Mean	0 ppm	150 ppm	300 ppm	Mean
I_1 70 % of available water	CV_1 (Azmirly)	0.354	0.368	0.396	0.372	0.262	0.278	0.296	0.279
	CV_2 (Cream 7)	0.302	0.344	0.363	0.336	0.230	0.258	0.269	0.252
Mean		0.328	0.356	0.380	0.354	0.246	0.268	0.283	0.266
I_2 50 % of available water	CV_1 (Azmirly)	0.359	0.395	0.426	0.394	0.292	0.326	0.349	0.322
	CV_2 (Cream 7)	0.291	0.335	0.364	0.330	0.237	0.275	0.296	0.269
Mean		0.325	0.365	0.395	0.362	0.264	0.300	0.323	0.296
I_3 30 % of available water	CV_1 (Azmirly)	0.405	0.433	0.464	0.434	0.390	0.418	0.455	0.421
	CV_2 (Cream 7)	0.339	0.376	0.435	0.383	0.336	0.351	0.403	0.364
Mean		0.372	0.404	0.449	0.408	0.363	0.385	0.429	0.393
General Mean		0.342	0.375	0.408	0.375	0.291	0.318	0.345	0.318
CV x SA Interaction	CV_1	0.373	0.399	0.429	0.400	0.315	0.341	0.367	0.341
	CV_2	0.310	0.352	0.387	0.350	0.268	0.294	0.323	0.295
		F value		LSD 0.05		F value		LSD 0.05	
I		**		0.0022		**		0.0065	
SA		**		0.0060		**		0.0029	
CV		**		-----		**		-----	
I x SA		**		0.0066		**		0.0195	
I x CV		**		0.0044		**		0.0130	
SA x CV		**		0.0180		N.S		-----	
I x SA x CV		**		0.0540		N.S		-----	

NS and ** non-significant and significant at 0.01 probability level, respectively.

TABLE 9. Correlation coefficients (Pearson Correlation) among different studied traits over both seasons (n=108).

Characters	number of branches per plant	100 seed weight (g)	seed yield per plant (g)	seed yield (kg per hectare)
number of branches per plant	--			
100 seed weight (g)	0.78838**	--		
seed yield per plant (g)	0.79063**	0.61615**	--	
seed yield (kg per hectare)	0.79161**	0.61564**	0.99856**	--

** significant at 0.01 probability.

TABLE 10. Molecular weights of protein bands detected in leaves of cowpea plants after one week of treatment with 0, 150 and 300 ppm salicylic acid (SA) (Data were obtained by GS 365 electrophoresis data system program version 3.01).

Band No.	KD	Azmirly			Cream 7		
		0 (ppm SA)	150(ppm SA)	300(ppm SA)	0 (ppm SA)	150(ppm SA)	300(ppm SA)
1	67.5	+	+	+	+	+	+
2	65.4	+	+	+	+	+	+
3	63.3	+	+	+	+	+	+
4	60.1	+	+	+	+	+	+
5	57.6	+	+	+	-	+	+
6	54.8	+	+	+	-	-	-
7	50.2	-	+	+	-	+	+
8	46.1	-	-	+	-	+	+
9	44.2	+	+	+	+	+	+
10	42.3	+	+	+	+	-	+
11	40.1	+	+	+	+	+	+
12	36.1	+	+	+	+	+	+
13	29.8	-	-	+	-	+	+
14	27.2	+	+	+	+	+	+
15	23.3	+	+	+	+	+	+
16	21.1	-	-	+	-	-	-
17	19.5	+	+	+	+	+	+
18	14.2	+	+	+	+	+	+
19	11.8	+	+	+	-	-	+
Number of bands		**15**	**16**	**19**	**12**	**15**	**17**

TABLE 11. Molecular weights of protein bands detected inleaves ofcowpea plants after two weeks of treatment with 0, 150 and 300 ppm salicylic acid (SA)(Data were obtained by GS 365 electrophoresis data system program version 3.01).

Band No.	KD	Azmirly			Cream 7		
		0 (ppm SA)	150(ppm SA)	300(ppm SA)	0(ppm SA)	150(ppm SA)	300(ppm SA)
1	88.7	+	+	+	+	+	+
2	86.4	-	+	+	-	-	-
3	80.2	+	+	+	+	+	+
4	76.3	-	-	+	-	+	+
5	67.5	+	+	+	+	+	+
6	65.4	-	-	-	-	-	+
7	63.3	+	+	+	+	+	+
8	57.6	+	+	+	+	+	+
9	54.8	-	-	+	-	-	+
10	46.1	-	-	+	-	+	+
11	44.2	+	+	+	+	-	-
12	40.1	-	-	-	-	-	+
13	36.1	+	+	+	+	+	+
14	29.8	-	+	+	-	+	+
15	27.2	+	+	+	+	+	+
16	23.3	+	+	+	+	+	+
17	19.5	+	+	+	+	+	+
18	14.2	+	+	+	+	+	+
19	11.8	+	+	+	+	+	+
Number of bands		**13**	**14**	**17**	**12**	**14**	**17**

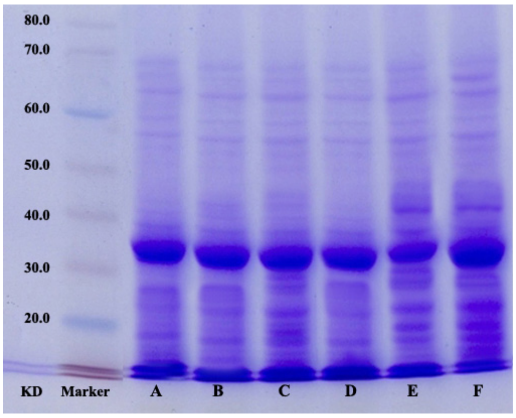

Fig. 1. SDS-polyacrylamide gel electrophoresis for protein profiles detected in leaves of cowpea plants after one week of treatment with 0, 150 and 300 ppm salicylic acid (SA). Where: protein marker in lane-1, Azmirly in lanes A (0 ppm SA), B (150 ppm SA) and C (300 ppm SA), and Cream 7 in lanes D (0 ppm SA), E (150 ppm SA) and F (300 ppm SA).

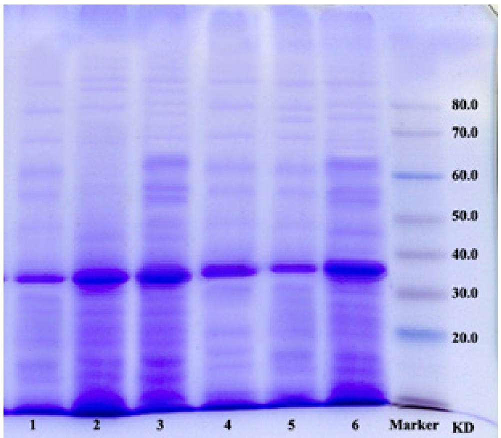

Fig. 2. SDS-polyacrylamide gel electrophoresis for protein profiles detected in leaves of cowpea plants after two weeks of treatment with 0 , 150 and 300 ppm salicylic acid (SA). Where: protein marker in lane-1, Azmirly in lanes 1 (0 ppm SA), 2 (150 ppm SA) and 3 (300 ppm SA), and Cream 7 in lanes 4 (0 ppm SA), 5 (150 ppm SA) and 6 (300 ppm SA).

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تأثير نقص مياه الري والرش بحمض السالسيليك على انتاجية صنفين من اللوبيا

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أجرى هذا البحث في مزرعة الخضر البحثية بكلية الزراعة – جامعة أسيوط خلال صيف موسمي ٢٠١٥ و٢٠١٦ لإختبار تأثير نقص مياه الري مع الرش بتركيزات مختلفة من حمض السالسليك (صفر و١٥٠ و٣٠٠ جزء في المليون) على انتاجية صنفين من اللوبيا (الازميرلى وكريم ٧). تم اجراء ثلاث تجارب منفصلة كل منها تحت مستوى مختلف من مستويات الري (٧٠ او ٥٠ او ٣٠ ٪ من الماء الميسر فى التربة). اشتملت كل تجربة على عاملين وهما حمض السالسليك والاصناف واستخدم تصميم القطاعات كاملة العشوائية بترتيب الشرائح بثلاث مكررات حيث وزع حامض السالسليك في الشرائح الافقية بينما الأصناف فى الشرائح الرأسية. أوضحت النتائج أن هناك تأثير معنوي واضح لمستويات الري لكل الصفات المدروسة فى الموسيمين، وأعطى الري عند ٧٠٪ من الماء الميسر للتربة أعلى القيم لكل الصفات المدروسة للموسيمين ماعدا صفة كفاءة استخدام الماء بينما أعطى الري عند ٣٠٪ من الماء الميسر للتربة أقل القيم لكل الصفات المدروسة للموسيمين ما عدا صفة كفاءة استخدام الماء. أيضا وجد ان الرش بحمض السالسليك بتركيز ٣٠٠ جزء فى المليون أعطى أعلى القيم لكل الصفات المدروسة للموسمين مقارنة بالكنترول . فيما يخص الاصناف وجد أن صنف الازميرلى تفوق على صنف كريم ٧ فى كل الصفات المدروسة فى الموسمين.

وتم تحليل أنماط البروتين في أوراق صنفى أزميرلي وكريم ٧ لدراسة التغيرات في التعبير الجيني بعد أسبوع واحد وأسبوعين من المعاملة بتركيزات صفر، ١٥٠، ٣٠٠ جزء في المليون من حمض السلسليك. ادت المعاملات بحمض السلسليك الى استحثاث تعبير ١١ من البروتينات الجديدة مقارنة بمعاملة الكنترول. وحيث ان المعاملة بحمض السلسليك ادت الى زيادة النمو والمحصول فإن هذه البروتينات الجديدة قد تلعب دورا هاما في المسارات البيوكيميائية لتحسين نمو النبات والمحصول.

للحصول على أعلى محصول (كجم للهكتار) يزرع الصنف أزميرلى ورشه بـ ٣٠٠ جزء في المليون بحمض السالسليك وريه عند ٧٠٪ من الماء الميسر. بينما تم الحصول على أعلى معدل كفاءة استخدام الماء من زراعة الصنف الازميرلى ورشه بـ ٣٠٠ جزء فى المليون من حمض السالسليك واجراء الري عند ٣٠٪ من الماء الميسر.