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Growth of chick pea (*Cicer arietinum*) in response to salicylic acid under drought stress

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Abstract

Chick pea (*Cicer arietinum* L.) is an important legume crop and consumed all over the world, especially in the developing country because it is a good source of carbohydrates and protein, and its protein quality is considered to be better than other legumes. The objective of the present work was to determine the effect of salicylic acid (SA, 0.00, 0.5 and 1mm) and different irrigation regimes (I1, I2, I3 and I4 were irrigation treatments after 60, 100, 140 and 180mm evaporation from class A pan, respectively) on plant performance of check pea. Exposure of plants to drought stress leads to serious physiological and biochemical dysfunctions, and ultimately results in a significant reduction in plant performance. The lowest grain yield of chick pea was observed under severe drought stress (I_4), whereas in the absence of drought stress the chick pea had the highest grain yield. SA as natural signalling molecule is able to reduce the adverse effect of environmental stresses on plants and increase crop productivity through morphological, physiological and biochemical mechanisms. The biological yield was increased by foliar application of salicylic acids as compared with control plants significantly. The results showed that exogenous application of SA resulted in an increase of yield components (pods per plant, grains per pod, and grains per plant and 100 grains weight traits).

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Introduction

Chick pea (*Cicer arietinum* L.) is one of the most important pulse crop grown in the world. Chickpea is cultivated in many countries around the world, particularly in Asian countries (Ganjeali and others, 2011). Legumes, especially chickpea, are used as dry grains in appreciable amounts for human nutrition (de Almeida Costa and others, 2006). Chickpea is one of the most popular vegetarian foods in populations of developing country because it is an important source of proteins, carbohydrates, B vitamins, and certain dietary minerals such as iron and phosphorus (Chavan and others, 1987).

Drought as an abiotic stress leads to reduction of agricultural crop production in worldwide. Drought stress during the flowering and grain-filling period have negative effects on morphological, physiological and biochemical activities and ultimately leads to low seed yield (Coste and others, 2001). Many plants have improved their resistance to tolerate drought stress through different physiological, morphological, biochemical and molecular changes (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). Plants respond to stresses by the synthesis of signalling molecules, which activate a range of signal transduction pathways. Several signalling molecules have been identified in plants such as calcium, abscisic acid (ABA), jasmonic acid, ethylene, salicylic acid (SA), glycinebetaine (GB) and etc. (Gunes and others, 2005a). Phytohormones play central roles in the ability of plants to self-adaptation to abiotic stresses by mediating a wide range of adaptive responses (Santner and Estelle, 2009).

Different techniques could be used to enhance crop yield, particularly under adverse environmental conditions. Application of plant growth regulators is an important tool for improvement of drought tolerance in plants. Salicylic acid (SA), a stress-related phenolic phytohormone, acts as a signal for induction of specific plant responses to biotic and abiotic stresses (Lu, 2009). Several studies indicate that SA plays an important role in the plant response to different abiotic stresses including drought, salinity, temperature, heavy metal, UV radiation (Hayat and others, 2010; Horváth and others, 2007). It has been reported that exogenous application of SA could help to reduce adverse effects of drought in *Ctenanthe setosa* (Kadioglu and others, 2011). Exogenous treatment of *Zea mays* with different levels of SA (0.5 and 1.0mm) under drought stress Led to a decrease in adverse effect of drought and induced plant resistance to drought stress (Elgamaal and Maswada, 2013). There are some reports about the positive effects of salicylic acid on yield improvement in plants like wheat (Sharafizad and others, 2013) and tomato (Yıldırım and Dursun, 2008). This research was carried out to investigate the effects of salicylic acid on yield improving of *C. arietinum* under different irrigation regimes.

Material and methods

Plant material

Seeds of *C. arietinum* L. cv. Azad were obtained from Agricultural-Jihad Organization of East Azarbaijan, Iran. The seeds were thoroughly washed in running tap water and were surface sterilized with 5% sodium hypochlorite for 10min followed by repeated washing with distilled water. The surface sterilized seeds were sown by hand in 4cm depth of a sandy loam soil.

Experimental condition

The study was conducted during the growing season of 2014 at Hashtrood, East Azerbaijan, Iran (Latitude 37°28′N, Longitude 46°52′ E, Altitude 1643m above sea level), under field conditions. The experiment was carried out in a randomized complete block design with split-plot arrangement and was replicated thrice. Each plot included 6 rows 25cm apart, 3 meter long, 1 and 2 meter distances were taken between test plots and replicates, respectively.

SA application

SA was initially dissolved in absolute ethanol and then added drop wise to water (ethanol/water, 1/1000, v/v) (Stevens and others, 2006). Foliar spray treatment of SA was applied at the vegetative (VSA) and the flowering (FSA) stages with concentrations of 0.00 (control), 0.5mm and 1mm (as SA0, SA1 and SA2) using a hand held sprayer.

Irrigation treatments

In this experiment, irrigation treatments (I1, I2, I3 and I4 were irrigation treatments after 60, 100, 140 and 180mm evaporation from class A pan, respectively) were designated to main plots. It was measured evaporation every day and when reaching interest level, the irrigation was applied in the next morning.

Sampling method

The total score for ground cover was assessed from summation of all scores in 100 parts of each plot and given to percent. At maturity time, 10 plants were harvested from each plot and plant height, pods per plant, grains per pod and grains per plant were recorded. Finally, plants of $1m^2$ in the middle part of each plot were harvested and plant biomass and grain yield per unit area as well as harvest index were determine.

Statistical analysis

Each treatment was analyzed with at least three replicates and standard deviation (S.D.) was calculated. Statistical analysis was performed using Duncan test; $p < 0.05$ and $p < 0.001$ were considered statistically significant and highly significant, respectively.

Results and discussion

Irrigation treatment

Analysis of variance indicated that irrigation treatments significantly influenced yield components (pods per plant, grains per pod, and grains per plant and 100 grains weight traits) (Table 1) as well as biological yield, grain yield, harvest index and ground cover (Table 2). The yield response to drought stress of chickpea is given in Table 3. Drought stress had a significant effect on performance of chick pea. The stress resulted in declined pods per plant, grains per pod, grains per plant and 100 grain weight compared with well-irrigated conditions. The plant showed a higher performance at the well-irrigated treatment (no drought stress) than the other irrigated treatments (Table 3). Giunta *et al.* (1993) reported that yield and yield components of wheat are differently affected by drought stress in relation to its timing and intensity. An intense drought stress mainly affects the number of grains per unit area through a general decrease in fertility, whereas a mild drought stress causes only a decrease in the mean grain weight (Giunta and others, 1993).

Table 1. Analysis of variance for the effects of salicylic acid foliar spraying on yield components of chickpea under different irrigation treatments.

Source of Variation	df	Mean Square			
		Pods per plant	Grains per pod	Grains per plant	100 Grains weight
Replication	2	1.75	0.0808	223.5	7.67
Irrigation (I)	3	595.88^{**}	1.731^{**}	7995.0^{**}	73.88^{*}
Error	6	54.38	0.1149	101.3	10.58
Salicylic acid (SA)	2	963.58^{**}	0.986^{**}	8537.8^{**}	43.59^{*}
I × SA	6	30.77^{ns}	0.105^{ns}	139.2^{ns}	6.03^{ns}
Error	16	81.35	0.1539	119.8	11.73
CV (%)	-	21.3	22.9	14.1	12.1

Ns, * and **: Not significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 2. Analysis of variance for the effects of salicylic acid foliar spraying on biological yield, grain yield, harvest index and ground cover under different irrigation and salicylic acid treatments.

Source of Variation	df	Mean Square			
		Biological yield	Grain yield	Harvest index	Ground cover
Replication	2	550	1568	38.42	49.33
Irrigation (I)	3	261669.1^{**}	92456.7^{**}	162.99^{**}	909.33^{**}
Error	6	4838	509	21.53	40.44
Salicylic acid (SA)	2	262231.0^{**}	88326.1^{**}	105.64^{**}	379.08^{*}
I × SA	6	4522.6^{ns}	859.3^{ns}	10.63^{ns}	40.33^{ns}
Error	16	7571	2100	4.13	64.67
CV (%)	-	20.1	19.9	3.9	11.3

Ns, * and **: No significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

The results indicated that percentage of ground cover significantly varied depending on irrigation rate. The lowest ground cover of chick pea was observed under severe drought stress (I_4), whereas in the absence of drought stress it had the highest ground cover (Fig 1).

Compare with control, the losses in ground cover in response to stress treatment were: 10% at I_2, 20% at I_3, and 37.5% at I_4 (Fig 1). Reduction in percentage of ground cover due to drought stress can be attributed to competition of plants to absorb water and nutrients (Ghassemi-Golezani and others, 2016).

Effects of drought stress on biological yield efficiency were statistically significant ($p \leq 0.01$). The highest biological yield was recorded in control treatment (I_1) and mild stress (I_2).

Compare with control, the losses in ground cover in response to stress treatment were: 16.9% at I2, 57.3% at I3, and 85.8% at I4 (Fig 2). A major effect of Water deficit is reduction in leaf size and expansion which cause of reduction in the amount of light absorption for plant photosynthesis and finally leads to reduced biological yield (Wahid and Close, 2007).

It has been reported that the first and foremost effect of drought is impaired germination and poor stand establishment which leads to a decrease in biological yield (Harris and others, 2002).

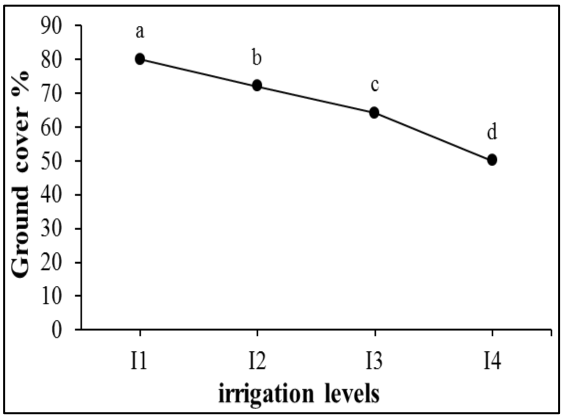

Fig. 1. The mean values of ground cover in chick pea under different irrigation regimes. I_1, I_2, I_3 and I_4 (60, 100, 140 and 180mm evaporation from evaporation pan A class.

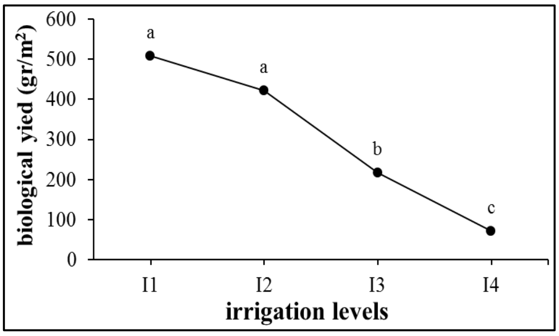

Fig. 2. The mean values of biological yield in chick pea under different irrigation regimes. I_1, I_2, I_3 and I_4 (60, 100, 140 and 180mm evaporation from evaporation pan A class).

Effects of drought stress on grain yield were statistically significant ($p \leq 0.01$). The highest grain yield was obtained by the control (fully watered) treatment. The percentage of grain yield under water deficit decreased 89.5% as compared with control plants (Fig. 3).

The 1000-grain weight is a decisive factor donating towards the concluding grain yield. It was determined that the drought stress had noteworthy effect on 1000-grain weight (Shah and others, 2017). Gholinezhad *et al.* (2009) reported that drought stress caused significant decrease in grain weight in sunflower so that, in their study, the maximum and minimum 1000-grain weight were obtained in well-irrigation and severe drought stress, respectively (Gholinezhad and others, 2009).

Analysis of variance showed that the harvest index was affected by drought stress significantly ($p \leq 0.01$). The highest amount of that was recorded in control (well-irrigated) treatment (Table 2). However, there was significant difference between I_1, and I_4 as well as between I_2, and I_4 (Fig. 4).

One of the limiting factors of plant growth and development is water deficit that not only reduces production of dry matter but also causes a disorder to the partitioning of carbohydrates to grain thus reducing the harvest index (Setter, 1990).The factors that affect plant growth and development can be classified as genetic or environmental cues. Division, enlargement and differentiation of plant cells depend on environmental and endogenous factors as well as interaction of them. Water is one of the environmental factors that influence biochemical, physiological, ecological and morphological reaction and regulates plant developmental plasticity. Drought stress is the most important limiting factor for crop production, adversely affect growth and crop production. The quality and quantity of plant growth are affected by water deficit (Farooq and others, 2009; Fathi and Tari, 2016). It has been reported that plants exposed in water stress exhibited a significant decline in photosynthetic parameters, membrane stability index, leaf water potential, activity of nitrate reductase, carbonic anhydrase, chlorophyll and relative water content (Hayat and others, 2008). Ghaseme-Golezani *et al.* (2015) reported that drought stress cause to decrease in grain yield of lentil through negative effects on morphological, physiological and biochemical activities. In barley (*Hordeum vulgare*), drought stress leads to decrease in grain yield, that was associated with reductions in number of tillers, spikes and grains per plant and individual grain weight. In conclusion, drought stress was detrimental to grain yield (Samarah, 2005).

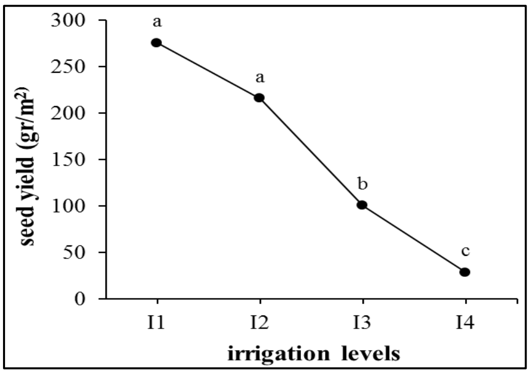

Fig. 3. The mean of grain yield in chick pea under different irrigation regimes. I1, I2, I3 and I4: 60, 100, 140 and 180mm evaporation from evaporation pan A class.

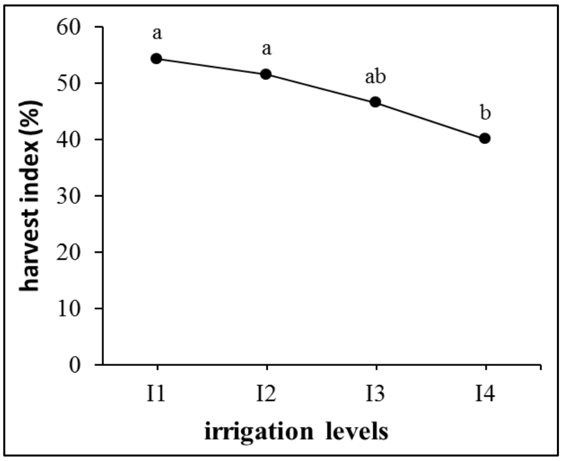

Fig. 4. The mean of harvest index in chick pea under different irrigation regimes. I1, I2, I3 and I4: 60, 100, 140 and 180mm evaporation from evaporation pan A clas.

Salicylic acid treatment

Analysis of variance showed that foliar application of salicylic acid significantly affected all growth characteristics (Tables 1 and 2). The exogenous application of SA resulted in an increase of yield components (pods per plant, grains per pod, and grains per plant and 100 grains weight traits) (Table 3). However, there was significant difference among SA_0, SA_1 and SA_2 (Table 4). The results presented in Table 4 and Fig 7 show that, seed yield and 100 grains weight significantly enhanced in chick pea under SA treatment compared with controls. The highest rate of 100 grains weight was obtained in SA_2 treatment (30.14g). Grown (2012) reported that salicylic acid significantly enhanced sunflower seed yield and yield components compared with the untreated plants (Grown, 2012).

Table 3. Means of yield components (pods per plant, grains per pod, grains per plan And 100 grain weight) of chickpea influenced by irrigation.

Treatments	Pods per plant	Grains per pod	Grains per plant	100 Grains weight (g)
I_1	50.78^{a}	2.111^{a}	108.78^{a}	31.12^{a}
I_2	46.22^{ab}	1.989^{ab}	93.14^{b}	29.83^{a}
I_3	40.00^{bc}	1.633^{b}	68.61^{c}	27.83^{ab}
I_4	32.00^{c}	1.133^{c}	40.58^{d}	24.54^{b}

Different letters in each column indicate significant difference at $p \leq 0.05$. I_1, I_2, I_3, I_4: irrigation after 60, 100, 140 and 180 mm evaporation from class A pan, respectively.

Table 4. Means of pods per plant, grains per pod, grains per plan and 100 grain weight of chickpea influenced by d salicylic acid foliar spraying.

Treatments	Pods per plant	Grains per pod	Grains per plant	100 Grains weight (g)
SA_0	33.42^c	1.475^b	54.55^c	26.34^b
SA_1	42.00^b	1.642^b	71.87^b	28.52^{ab}
SA_2	51.33^a	2.033^a	106.91^a	30.14^a

Different letters in each column indicate significant difference at $p \leq 0.05$. SA_0, SA_1 and SA_2: 0, 0.5 and 1 mm of salicylic acid, respectively.

The application of SA increased ground cover significantly compared to control treatment. The percentage of ground cover at 1mm SA increased at the ratio of 18% as compared with control plants (Fig 5). Exogenous application of salicylic acid enhances the photosynthetic rate, maintains the stability of membranes, improves nutrition absorption, and thereby additional ground cover of plants under stress condition (Joseph and others, 2010).

The effects of salicylic acid on biological yield were significant ($p \leq 0.01$). The application of that resulted in a considerable increase in plant biological yield compared to control plants. Foliar spraying of salicylic acid in concentrations 0.5 and 1 mM, enhanced biological yield in ratio 14.5 and 48.4 percent, respectively (Fig. 6).

The improvement of this trait may be due to effect of SA application on improve and increase of photosynthetic capacity (Arfan and others, 2007).

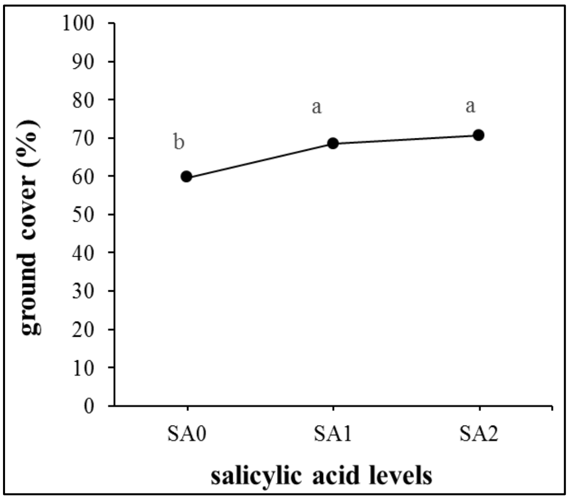

Fig. 5. The mean of ground cover in chick pea under different salicylic acid levels. SA1, SA2 and SA3: 0, 0.5 and 1 mmol salicylic acid.

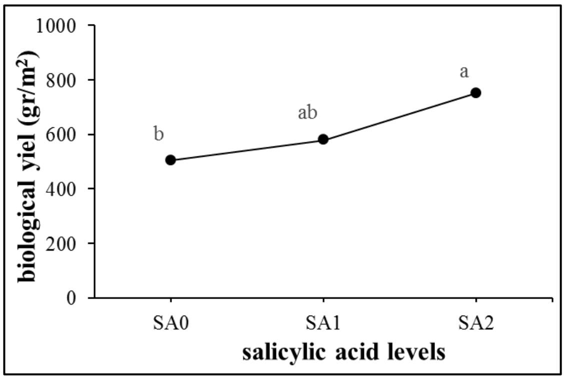

Fig. 6. The mean of biological yield in chick pea under different salicylic acid levels. SA1, SA2 and SA3: 0, 0.5 and 1 mmol salicylic acid.

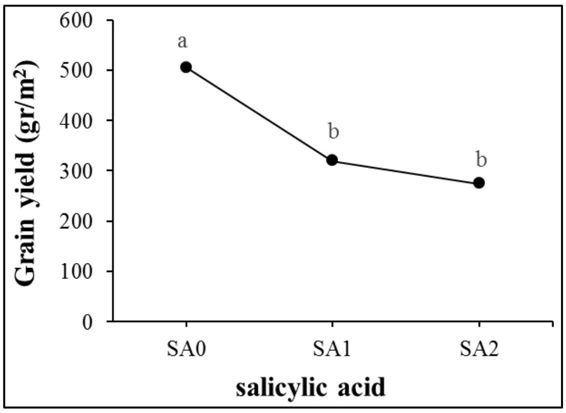

Fig. 7. The mean of seed yield in chick pea under different salicylic acid levels. SA1, SA2 and SA3: 0, 0.5 and 1 mmol salicylic acid.

Signalling compounds are able to reduce the deleterious effect of stresses on plants and increase crop productivity through morphological, physiological and biochemical mechanisms. SA is known as natural endogenous signalling molecule that plays a key role in governing and mediating the responses of plants to diverse environmental stresses such as drought (Hayat and others, 2010; Raskin, 1992).

It has been reported that SA affects the plant growth under stress through improving nutrient absorption, membrane stabilization, water relations, stomatal regulation and photosynthesis (Gunes and others, 2005b; Hayat and others, 2008; Stevens and others, 2006). SA can modulate important enzymatic (such as monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase, GSH peroxidase, glutathione peroxidase) and non-enzymatic (such as GSH) components of AsA–GSH pathway as well as glyoxalase enzymes (Gly I and Gly II) and assisted the plants to become more tolerant to drought stress-induced oxidative damage by enhancing their antioxidant defense and glyoxalase systems (Alam and others, 2013). Exogenously applied SA resulted in increased net CO2 assimilation rate due to increased stomatal conductance and eventually in increased plant dry mass (Habibi, 2012; Pancheva and others, 1996). It has been cleared that application of SA causes expression of plant genes which involved in signaling pathway in plants under drought stress condition. This include genes encoding chaperone, heat shock proteins (HSPs), sinapyl alcohol dehydrogenase (SAD), cinnamyl alcohol dehydrogenase (CAD) and Cytochrome P450 (CYP 450). These genes play essential role in major physiological functions such as photosynthesis, carbohydrate metabolism, protein metabolism, stress and defense, energy production, signal transduction, and toxin metabolism, ultimately increase plant performance under drought stress (Jumali and others, 2011; Khan and others, 2015).

Positive effects of exogenous application of SA on plant growth and final crop yield under drought stress have been demonstrated in many plants, including sunflower (Hussain and others, 2008), peach (Mohamadi and Pakkish, 2014), chickpea (Rokhzadi, 2014), milk thistle (Ghassemi-Golezani and others, 2017), wheat (Aldesuquy and others, 2012), coriander (Hesami and others, 2012).

Conclusion

Water deficit is one of the most acute abiotic stresses that affects growth and development of plants. Drought stress can significantly reduce plant performance.

The data obtained from the present study suggest that foliar SA applications can ameliorative the deleterious effects of drought stress and increase the yield components (pods per plant, grains per pod, and grains per plant and 100 grains weight traits) of *C. arietinum* L. These results indicate that SA application is useful for improving the plant growth and performance under drought stress.

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