DROUGHT STRESS IMPARED PHYSIOLOGY OF SUNFLOWER (*Helianthus annuus* L.) HYBRIDS REPARED BY PLANT GROWTH REGULATORS

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ABSTRACT: The present study was under taken to examine the modic effects of salicylic acid and ascorbic acid on mineral elements and leaf proline contents of two drought stressed sunflower hybrids (Hysun-33 and Hysun-38). Drought stress was imposed at vegetative and reproductive growth stages. Two concentrations of salicylic acid and ascorbic acid (100 and 200 mg L^{-1}) were applied as a foliar spray at vegetative and reproductive stages and root treatment. Results revealed that imposition of drought stress at both the growth stages of both hybrids caused significant reduction for shoot nitrogen, potassium and calcium contents, whereas non significant reduction for phosphorous contents was noted. However, in contrast significant increase was noted for proline contents. However, exogenous application of all the (0, 100 mgL^{-1} and 200 mgL^{-1}) concentrations of salicylic acid and ascorbic acid applied by either mode had a significant drought stress alleviative effects and increased the values of all the attributes, whereas, non significant increase was noted for phosphorus contents. Effect of foliar spray application was significantly higher for calcium and leaf proline than root treatment. Salicylic acid proved more effective than ascorbic acid, particularly, when applied at vegetative stage. Most effective concentration was 200 mg L^{-1} and maximum increase was recorded in Hysun-33.

Key words: Sunflower; drought; salicylic acid and ascorbic acid; mode of application

Abbreviations: SA=salicylic acid; AA= ascorbic acid; Irri= irrigation; V= vegetative; R= reproductive; S= stage; DS=drought stress.

(Received 08-11-2013 Accepted 09-09-2015)

INTRODUCTION

Plants thrive best within the ranges of certain ideally available climatic factors but in contrast harsh weather conditions badly check their growth, development, reproduction, physiological and biochemical processes, however, deficient availability of water expressed in terms of drought stress is regarded prime limiting factor for crop production but unfortunately water is increasingly becoming a scarce entity and expected to pose a severe threat to herbage cultivation and growth in many regions of the universe (Passioura, 2007). The possibility, occurrence and frequency of climatic stress factors including drought stress is expected to worsen in future and may prove to be a critical constraint to the business of crop growing, productivity, internal physio-chemical processes and their products at global scale (Fischer *et al*., 2001). Plant community being sessile and non-mobile remains fixed to a single place over the full episode of their life, periodic or full scale droughts, soil salinity, soil toxicity, environmental pollution are regular problematic features that are most generally supposed notorious for their adverse effects on all type of founa (Sakamoto and Murata, 2002). Right from germination to crop maturity assured availability of moisture contents in growing medium guarantees the successful crop production but plant growth stages are very critically in this regard (Athar and Ashraf, 2005). Menace of drought stress typically influences the physico-biochemical processes including rate of respiration, process of photosynthesis and translocation of its product to different parts, absorption of the elements of soil fertility and their movement across the cell wall, biosynthesis of carbohydrates, nutrient metabolism and finally decrease the production of internal growth promoters (Farooq *et al*., 2008). The response of each plant specie to different biotic and abiotic stress factors is different, moreover, difference is further prominent when judged even at different levels of plant growth stages, cell structure and organizational level, however, rate of resistance is best measured depending upon exposure of plants to severity, intensity and longativity of stress (Jaleel *et al*., 2007). The success of the business of crop plants raising depends upon the sharp knowhow of plant responses to all the stress factors (Zhao *et al.*, 2008).

A new and possible approach to minimize losses due to drought stress is the exogenous application of some chemical desiccants on plants (Gaballah and Mandour, 2000). Salicylic acid and ascorbic acid are organic compounds that could be synthesized but generally occur naturally in plants in a very small amount. Salicylic acid and ascorbic acid are known for their influence on the maintenance, regulation and expedition of several physio-biochemical processes occurring within the cell viz-a-viz absorption and transportation of the elements of fertility (N^+, P^+, K^+ and Ca^+), production of the contents of chlorophyll, stimulation of the protein biosynthesis pathway, retardation of ethylene biosynthesis, channelization and effectuation of the photosynthetic apparatus and machinery, induction of stomatal closer or opening and finally adjustment of transpiration rate in bread wheat varieties (Shakirova *et al.,* 2003). Plant growth regulators, their analogies and other derived products having the similarity of morph and origin can positively stimulate plant growth, development and finally contribute a lot towards resistance to all the stress factors in cereal crops (Rajala and P-Sainio, 2000). Generally, the used plant growth regulators on one hand have got ability to simultaneously trigger some physiological processes, while on the other hand inhibit the others but it is in consonance with the plant species, their phonological growth stages and suited environmental conditions (Mateo *et al*., 2006). Thus, present investigation was hypothesized to evaluate the efficacy of chemical used for the experimentation and compare and contrast the effectivity of their mode of application on physiological attributes of this crop.

MATERIALS AND METHODS

The present research work was designed to evaluate the efficacy of two organic hormonal properties containing products i.e. salicylic acid and ascorbic acid and their mode through which these prove most effective in drought stress mitigation. The venue of experimentation was Agriculture Research Institute, Quetta, Pakistan (latitude = 31°- 30' N, longitude = 73°-10' E and altitude = 5500ft). Two most recently introduced sunflower hybrids (Hysun-33 and Hysun-38) were chosen. Seeds of both the hybrids were obtained from oil seed directorate of the above stated institute. Before the onset of experimentation, the physioc-chemical properties of experimental site were determined and soil was found medium textured (sandy loam) having 8.03 pH. The main plot was divided into three plots and further subdivided into 16 subplots each measuring 15x15meters. Between rows 75 cm and plants 20 cm distance was maintained. A Randomized Complete Block Design (RCBD) with four replications was followed. In each replication there were two rows and each row had six plants. Filed was sown with hand drawn rabbi drill using the seed rate 10kg/ha. First irrigation was maintained to all the three plots after the two weeks of emergence. After that drought stress was imposed to the plants grown in second and third subplots by skipping first irrigation at vegetative stage (after 6 weeks) and other at reproductive stage after another 4 weeks or 10 weeks soon after the emergence of seedlings respectively, however, the seedling grown in first subplot were maintained usual number of irrigations. The suspension of salicylic acid and ascorbic acid priorly prepared at different (0, 100mg L^{-1} and 200 mg L^{-1}) concentrations was foliarly applied at vegetative and reproductive growth stages which at the irrigation was missed. While, in case of root treatment part of experiment, seedlings were raised in polystyrene foam trays containing peat-moss-sand as a growing medium. Two weeks after the emergence of seedlings, irrigation was applied to the trays in order to soften the soil and plants were uprooted. Soon after the removal from trays the roots of seedlings were rinsed thoroughly in distill water and all the dust and debris was removed. After that roots of uprooted seedlings were dipped in the solution of both the growth regulators prepared at different (0, 100 and 200 mg L^{-1}) concentrations for 6 hours. Then seedlings were transplanted the field and irrigation was applied. Drought stress treatment was maintained at vegetative and reproductive growth stages, respectively.

Determination of mineral elements: The shoot samples after washing with distal water and air drying were chopped into small pieces with sterilized scalpels. The representative samples were oven dried and grinded. Then 0.5 *g* of ground material was digested with 2 mL of sulfuric acid-hydrogen peroxide mixture following the method introduced by (Wolf, 1982). The plant samples were digested with HNO_3-$HCLO_4$ mixture (2:1, V/V). The digested extract was filtered and used for the determination of N^+, P^+, K^+ and Ca^{2+}. Mineral nutrient elements were determined simultaneously by ICP-AES (inductively coupled plasma atomic emission spectroscopy. SPS 1200VR, Seiko, Japan).

Determination of proline contents: The contents of this variable were determined by the method introduced by (Bates *et al*., 1973). The fresh leaves sample of 0.5 gram dry weight was homogenized in 10 cm^3 of 3% aqueous sulfosalicylic acid and after 72 hours proline was released; the homogenate was centrifuged at 22000g for 5 min. Then 2 cm^3 of the supernatant of acid ninhydrin was added. Further, 2 cm^3 of glacial acetic acid was added and the contents were boiled for 1 hour at 100 °C in a water bath. The mixture was further extracted with 10 cm^3 of toluene by mixing the two thoroughly in a test tube with vigorous stirring. The absorption of choromophore was read at 515 nm in an *Ecloli 24* spectrophotometer (Hyderabad, India), 2 mL proline (*Sigma*) was used for the preparation of the standard curve. The amount of proline in samples was calculated in mg (proline) g^{-1} (d.m.).

Statistical Analysis: There were four factors viz-a-viz varieties, stages, plant growth regulators and mode of applications. The analysis of variance (ANOVA) of the data for all the attributes was computed by using the MSTAT Computer Program (MSTAT Development Team, 1989). To judge differences among the mean values the Duncan's New Multiple Range test introduced by (Steel and Torrie, 1986) was followed by using 5% level of probability.

RESULTS AND DISCUSSION

Statistical variance analysis of data recorded in (Table 1) clearly indicated that the imposition of drought stress at both the (vegetative and reproductive) growth stages of both hybrids induced significant ($P<0.001$) reduction in shoot nitrogen and potassium, while, non significant ($P>0.001$) reduction in phosphorous contents accumulation but reduction observed at vegetative stage was significantly higher. Both hybrids differed significantly. Hysun-33 showed less reduction compared to Hysun-38. However, exogenous application of salicylic acid and ascorbic acid through foliar spray and root treatment led to significant increase in shoot nitrogen and potassium contents accumulation and non significant increase in phosphorous contents. Interaction of foliar spray and root treatment mode of application of both the growth regulators revealed a non significant difference for the parameters under study. However, drought stress alleviative effects of foliar spray application of salicylic were higher than ascorbic acid particularly, when was applied at vegetative stage. Among all concentrations 200 mg L^{-1} produced highest values of all the parameters. Overall, Hysun-33 responded significantly better than Hysun-38.

Shared lettered values in rows and columns do not differ significantly: Statistical variance analysis of data for shoot calcium and leaf proline contents accumulation contained in (Table 2) illustrated that imposition of drought stress at both the growth stages of both hybrids significantly ($P<0.001$) reduced shoot calcium contents, while, in contrast significantly ($P<0.001$) increased leaf proline accumulation. Reduction observed for shoot calcium, while, increase of proline was significantly higher at vegetative stage. Both hybrids also differed significantly from each other. Hysun-33 was less affected compared to Hysun-38. However, exogenous application of salicylic acid and ascorbic acid by both the modes proved significant effective to alleviate the harmful effects of drought stress and increased the values of all the parameters. Further, foliar spray mode of application also proved significantly more effective than root treatment. As for influence of both the plant growth regulators, salicylic acid applied as a foliar spray at vegetative stage had a significant increase in values of all the attributes than ascorbic acid. Finally taken, 200 mg L^{-1} concentration produced maximum increase, particularly, in Hysun-33.

Table. 1 Shoot nitrogen, phosphorous and potassium contents accumulation of two drought stressed sunflower hybrids in response to plant growth regulators applied through different modes at different phonological growth stages.

Treatments	Shoot nitrogen contents (µmol kg^{-1} d.wt.)			
	Foliar spray		Root treatment	
	Hysun-33	Hysun-38	Hysun-33	Hysun-38
Control	39.5a	37.8ab	39.5a	37.8ab
DS at V/S	24.4lm	22.5m	24.4jk	22.5kl
DS at R/S	26.3kl	24.4lm	26.3ij	24.4jk
DS at VS +SA 100 mg L^{-1}	31.3ef	29.3ij	30.5ef	28.7fg
DS at VS +SA 200 mg L^{-1}	35bc	33.3cd	34.3cd	32.4cd
DS at VS +AA100 mg L^{-1}	29.5fg	27.6jk	29.2cd	27.2hi
DS at VS +AA 200 mg L^{-}	33.7cd	31.8de	33.1cd	31.2de
DS at RS +SA 100 mg L^{-1}	32.7cd	30.8fg	31.5de	29.6ef
DS at RS +SA 200 mg L^{-1}	36.2bc	34.4bc	35.4bc	33.4cd
DS at RS +AA100 mg L^{-1}	30.5egh	28.5ij	29.8ef	27.9gh
DS at RS +AA 200 mg L^{-1}	34.5cd	32.6cd	34cd	32.1cd
LSD treatment x variety 1.1662				
Treatments	**Shoot phosphorous contents (µmol kg^{-1} d.wt.)**			
Control	18.1a	17ab	18.1a	17ab
DS at V/S	10.8hi	9.9i	10.8gh	9.9hi
DS at R/S	12.2fg	11.1gh	12.2ef	11.1fg
DS at VS +SA 100 mg L^{-1}	14.3bc	13.4de	13.5cd	12.4ef

DS at VS +SA 200 mg L^{-1}	16.2ab	15ab	15.7ab	14.6bc
DS at VS +AA100 mg L^{-1}	14bc	13ef	13.3de	12.3ef
DS at VS +AA 200 mg L^{-}	15.6ab	14.6bc	15.2bc	14cd
DS at RS +SA 100 mg L^{-1}	15.8ab	14.5bc	14.2bc	13de
DS at RS +SA 200 mg L^{-1}	16.8ab	15.9ab	16.3ab	14.9ab
DS at RS +AA100 mg L^{-1}	14.7bc	13.6cd	13.8cd	12.7ef
DS at RS +AA 200 mg L^{-1}	16.4ab	15.5ab	15.8ab	14.7bc
LSD treatment x variety 1.5592				
Treatments	**Shoot potassium contents (µmol kg^{-1} d.wt.)**			
Control	43.5a	41.4ab	43.5a	41.4ab
DS at V/S	24kl	21.9l	24lm	21.9m
DS at R/S	26.5ij	24.4jk	26.5jk	24.4kl
DS at VS +SA 100 mg L^{-1}	31.5ef	29.6gh	30.6ef	28.7hi
DS at VS +SA 200 mg L^{-1}	36.8bc	34.7cd	35.6cd	33.8cd
DS at VS +AA100 mg L^{-1}	31fg	29gh	30.1ef	28.1hi
DS at VS +AA 200 mg L^{-}	33.5de	31.4ef	32.6de	30.5ef
DS at RS +SA 100 mg L^{-1}	32.5de	30.4fg	31.6de	29.5ef
DS at RS +SA 200 mg L^{-1}	38.5ab	36.5bc	37.6bc	35cd
DS at RS +AA100 mg L^{-1}	29.5gh	27.4hi	28.9gh	26.6i
DS at RS +AA 200 mg L^{-1}	34.5cd	32.4de	33.6cd	31.5e
LSD treatment x variety 1.5622				

Table. 2 Shoot calcium and leaf proline contents accumulation of two drought stressed sunflower hybrids in response to plant growth regulators applied through different modes at different phonological growth stages.

Treatments	**Shoot calcium contents (µmol kg^{-1} d.wt.)**			
	Foliar spray		Root treatment	
	Hysun-33	Hysun-38	Hysun-33	Hysun-38
Control	38.6a	36.8ab	38.6a	36.8ab
DS at V/S	18.7no	16.6o	18.7op	16.6p
DS at R/S	21.4lm	19.6mn	21.4mn	19.6no
DS at VS +SA 100mg L^{-1}	28.5gh	26.6ij	26.5fg	24.6ij
DS at VS +SA 200mg L^{-1}	33.7cd	32.2de	32.5bc	30.8cd
DS at VS +AA100mg L^{-1}	24.9jk	22.7kl	24jk	22.1lm
DS at VS +AA 200mg L^{-}	30.5ef	28.7gh	29.6de	27.5ef
DS at RS +SA 100mg L^{-1}	29.7ef	27.5hi	27.5fg	25.6hi
DS at RS +SA 200mg L^{-1}	34.5bc	32.5cd	33.5b	31.8bc
DS at RS +AA100mg L^{-1}	26.5ij	24.8jk	25.5hi	23.6kl
DS at RS +AA 200mg L^{-1}	31.7de	29.6fg	30.5cd	28.7de
LSD treatment x variety 1.0191				
Treatments	**Leaf proline contents (µmol g^{-1}f.wt.)**			
Control	191.1j	182.6jk	191.1j	182.6jk
DS at V/S	221.9gh	208.1hi	221.9fg	208.lhi
DS at R/S	210.4hi	195.3ij	210.4gh	195.3ij
DS at VS +SA 100mg L^{-1}	259.2bc	245.6de	253.2bc	239.6de
DS at VS +SA 200mg L^{-1}	278.3a	267.1ab	272.2a	265.9ab
DS at VS +AA100mg L^{-1}	250cd	236.4ef	244bc	230.4de
DS at VS +AA 200mg L^{-}	264.4ab	251.6cd	258.4ab	245.6cd
DS at RS +SA 100mg L^{-1}	248de	236.1ef	242de	230.1ef
DS at RS +SA 200mg L^{-1}	272.6ab	261.9ab	266.6ab	256.1ab
DS at RS +AA100mg L^{-1}	242.4ef	230fg	236.4ef	224fg
DS at RS +AA 200mg L^{-1}	260.8bc	247.9de	254.7bc	241.9de
LSD treatment x variety 6.5930				

Shared lettered values in rows and columns do not differ significantly

DISCUSSION

Macro nutrients are known to have significant role in abiotic stress tolerance and their increased accumulation on one hand possibly guaranteed the triggering of defense mechanism and on the other hand enhanced the activity of enzymes responsible for the resistance (Cherki *et al*., 2002). All the macro nutrient (N^+, P^+, K^+, and Ca_2^+) are regarded important constituents for the activities of all kinds of enzymes for example these may act as a building block of cell wall, can regulate the synthesis of protein and integrate protein components, similarly may serve as essential factors for cell wall, plasma membrane, photosynthetic protein complexes and constitute bases for the synthesis of genetic material (RNA and DNA) (Taiz and Zeiger, 2002). On the basis of previous studies conducted by Babu *et al*., (2012) on tomato cultivars it was apparent that observed increase in the level of salinity stress was reciprocal to the decreased accumulation of N^+, P^+, K^+ contents and K^+/Na^+ ratio. It is also evident from the earlier research reports published by Nafees *et al*., (2010) that exogenous application of salicylic acid alleviated the salinity-inhibitory effects in mung bean. Same are the findings of Rao *et al*., (2012) that salicylic acid proved substantially effective in minimizing the adverse effects of drought stress in maize and caused a dramatic increase in nitrogen, phosphorous, calcium and potassium contents accumulation. Moreover, Sarangthem and Singh (2003) while working with *Phaseolus vulgaris* have also expressed similar view that exogenous application of salicylic acid under water stress conditions caused a considerable increase in root nitrogen, phosphorous and potassium contents. These findings can also be supported by the research work of Raafat and Radwan (2011) that salicylic acid and ascorbic acid being the main constituent organic acids have a definite role in increasing the contents of nitrogen, phosphorous and potassium in wheat plants. In another study Gadallah (2004) observed that safflower shoots sprayed with solutions of distilled water having 2.0 pH that served as a control and then sprayed with 0 and 100 mg L^{-1} ascorbic acid under salinity stress conditions produced dominant increase for shoot nitrogen, potassium and calcium contents. Bartles and Sunkae, (2005) hold the view that biochemical and physiological changes deemed to occur in plants when these are exposed to deficit moisture conditions, however, abiotic stresses namely drought, salinity and high temperature were often supposed to reduce water potential in the growing media and led to increased accumulation of proline contents in plants. From the results of present study it was eminent that imposition of drought stress at vegetative stage induced the hybrids to produce more proline contents compared to reproductive stage but exogenous application of salicylic acid and ascorbic acid caused further increase in the accumulation of this variable. These results could also be best interpreted in the light of the findings of Hussain *et al*., (2007) that foliar spray application of salicylic acid increased free proline contents accumulation in maiz plants. Similarly, Shakirova *et al*., (2003) while working with wheat and El-Tayeb, (2005) working with barley found that salicylic acid induced more proline contents accumulation in salinity and drought stressed seedlings, respectively. Our results could also be best supported by the findings of Gadallah (2004) who while working with safflower found similar results. In the same way research reports published by Cengiz *et al*., (2010) and Dolatabadian *et al*., (2008) are also in consonance with the results of our study that foliar application of ascorbic on salinity stressed maize (*Zea mays* L. cv., DK 647 F1) and canola (*Brassica napus* L. cv. Okapi) seedlings significantly increased proline contents synthesis.

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