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PLANT GROWTH REGULATORS INDUCED DROUGHT TOLERANCE IN SUNFLOWER (*Helianthus annuus* L.) HYBRIDS

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ABSTRACT

Present study was conducted to investigate whether plant growth regulators viz. salicylic acid and ascorbic acid can alleviate the adverse effects of drought stress on germination and seedling growth of two sunflower hybrids (Hysun-33 and Hysun-38). Two (-0.06 MPa and -0.12 MPa) levels of drought stress were created by adding different concentrations of (PEG8000) polyethylene glycol in growth medium. Results indicated that increase in drought stress significantly decreased germination percentage, plumule length, germination rate and seedling fresh biomass, except radical length, while, in contrast non significantly increased mean germination time with respect to their control. The adverse effects of drought stress (-0.12 MPa) level were more severe than (-0.06 MPa). Both hybrids differed significantly but Hysun-33 was less affected compared to Hysun-38. However, seed treatment of both hybrids with salicylic acid and ascorbic acid at different concentrations (0, 100mg $L^{-1}$and 200mg L^{-1}) proved significantly effective in alleviating the adverse effects of drought stress and increased the values of all the attributes, whereas, non significant decrease was noted for germination time. Moreover, drought stress alleviative effects due to salicylic acid were higher compared to ascorbic acid. Over all, most effective concentration was 200mg L^{-1} and maximum increase was recorded in Hysun-33.

Key words: *Helianthus annuus*; salicylic acid; ascorbic acid; drought stress

Abbreviations: SA=salicylic acid; AA=ascorbic acid.

INTRODUCTION

Drought is one of the major physical factors of environmental stresses which limits growth and distribution of natural vegetation more than that of any other factors viz. extreme temperature, cold, heavy metals, drought and salinity (Athar & Ashraf, 2005). Drought stress determines the success or failure of plant establishment. The adverse effects of drought on growth and development of crop plants are of multifarious nature and could affect at all the growth stages of plant growth. The susceptibility, severity and duration of plants exposition to drought stress varies in dependence of stress degree, different accompanying stress factors, plant species and their developmental stages but germination is regarded as most critical stage of plant life (Demirevska *et al*., 2009). Poor and delayed germination is considered serious setback in growth and yield (Willenborg *et al*., 2005). The basic aim and ultimate object of raising crop plants is to achieve maximum and uniform germination. If a seed starts germination under any type of stress, it may be hoped to enhance growth and development and may easily cope with environmental stresses. Decrease in moisture contents level in the germinating medium profoundly decreases moisture absorption, reduces the production of growth stimulators, increases the production of growth inhibitors that ultimately inhibit the germination of seeds (Corbineau *et al*., 1993). The seed treatment with plant growth regulators affects germination time leading to better growth and improved yield in stress conditions (Piri *et al.*, 2009).

Salicylic acid is a naturally occurring endogenous plant growth regulator having a role in regeneration of various physiological processes and biochemical functions of plants (Shakirova and Sakhabutdinova *et al.*, 2003). Salicylic acid acts as a signaling molecule influencing a wide range of diverse physiological processes including seed germination (Cutt & Klessig, 1992), enzymatic activity (Dolatabadian *et al.*, 2008), photosynthetic rate (Khan *et al.*, 2003), plant growth and yield (Hussein *et al.*, 2007), resistance against salinity (Shakirova *et al*., 2003) and drought stress (Bezrukova *et al.*, 2001). Moreover, salicylic acid also reduces negative effects of various abiotic stresses by increasing internal level of other plant growth regulators in plants (Sakhabutdinova *et al.*, 2003). Ascorbic acid commonly known as (vitamin C) is a non enzymatic compound that enables plants to resist stresses by reducing oxygenic free radicals constituted stress. Ascorbic acid is one of the abundantly occurring water-soluble antioxidant organic compounds and is mainly distributed in cytosol of the plant. It is required in trace amount to maintain normal plant growth in higher plants. Ascorbic acid is also required for the biosynthesis of different plant hormones, such as gibberellins and ethylene in addition to its role as an antioxidant and therefore could be important for germination (De Tullio and Arrigoni, 2003). Numerous studies have been conducted on the role of these plant growth regulators in different crops but very little work has been done with respect to drought stress alleviative role of these in sunflower. Thus, present study was conducted to further evaluate the role of these in alleviating the adverse effects of drought stress through seed treatment on germination and other growth related attributes of this crop.

MATERIALS AND METHODS

Seed material: The seeds of two sunflower hybrids viz. Hysun-33 and Hysun-38 were obtained from Oil Seed Department of Baluchistan Agriculture Research Institute, Quetta, Pakistan and experiment was also conducted there.

Drought stress treatment: An external osmotica (PEG8000) polyethylene glycol of Sigma Chemical Company, USA was used to create artificial drought stress. Polyethylene glycol (PEG8000) 17.5 and 34.5*g* was dissolved in 100mL of half strength Hoagland's nutrient solution separately to create two drought stress (-0.6 and -1.2 Mpa) levels respectively.

Seed treatment: Before the start of experiment seeds was surface sterilized with 1 % sodium hypochlorite solution for five minutes and then were given three washings with distal water to eliminate the residue. Then seeds were soaked for 1 hour in solutions of salicylic acid and ascorbic acid prepared at different (0, 100mg L^{-1} and 200mg L^{-1}) concentrations.

Germination test: Ten treated seeds were placed in each Petri plate having two moist layers of Whatman No 2 filter paper. The solution of 5mL containing different concentrations of PEG was added separately in each Petri plate daily after washing out previous one. Petri plates were kept in growth chamber for germination with alternate temperature of day and night (day 22 °C and night 18 °C), photoperiod of light/dark (14/10 hours) and photosynthetically active radiations 300 µmol. m^{-2}. s^{-1}. Seed was considered germinated when both plumule and radicle had grown about 10mm long or 5 mm in length respectively. The germinated seeds were counted and removed daily. Data were recorded for 14 days.

1- Germination percentage, germination rate and germination time were calculated according to (Jefferson and Penachchio, 2003) by using following formula:

Germination % = (n/N)*100

Where, n: number of seeds germinated, N: total number of seed in each Petri dish.

Germination rate = Σ (Ni /Di)

Ni: germinated seeds in each numeration, Di: day of each numeration.

Germination time = Σ (nx) / n

n is the number of newly germinated seeds on each day and x is the day of counting.

2-Shoot and root length was measured by millimetric ruler and fresh weight of seedling (shoot and root) was recorded by analytical balance (ALE-40 SM Shimatzu).

Statistical analysis: The data was analyzed using analysis of variance technique (ANOVA) under completely randomized design with four replications for each hybrid. Data was computed by using the MSTAT Computer Program (MSTAT Development Team, 1989). The Duncan's New Multiple Range test at 5% level of probability was used to compare means which were indicated by alphabets on data sets following Steel and Torrie (1986).

RESULTS

The mean square values received from statistical variance analysis of data present in (Table 1) revealed that maximum values of germination percentage, plumule and radical length were related to non stress condition, whereas increase in osmotic potential led to a significant ($P<0.001$) reduction in germination percentage, plumule length and non significant ($P>0.001$) reduction in radical length. The decrease noted due to drought stress (-1.2 MPa) level was higher than (-0.6 MPa). Both hybrids differed significantly. Hysun-33 showed less decrease compared to Hysun-38. However, seed treatment of both hybrids with salicylic acid and ascorbic acid at different concentrations (100 and 200 mg L^{-1}) significantly increased germination percentage and plumule length, while, non significant increase was observed for radical length. As for plant growth regulators influence, salicylic acid was found comparatively more effective in alleviating the adverse effects of drought stress than ascorbic acid. Moreover, among all the concentrations of salicylic acid, 200mg L^{-1} produced maximum values of these parameters. Hysun-33 responded significantly better than Hysun-38.

Any two letters not sharing a common letter in row or column differ significantly at 0.05% probability level: The results of statistical variance analysis of data regarding germination time, seedling fresh biomass and germination rate are present in (Table 2). Imposition of both levels of drought stress on both hybrids significantly ($P>0.001$) decreased seedling fresh biomass and germination rate, while, in contrast non significantly ($P<0.001$) increased mean germination time with respect to their control. Drought stress (0.6 MPa) level had a less sever effects than (-1.2 MPa). Both hybrids differed significantly. Adverse effects of drought stress were maximum on Hysun-38 than Hysun-33. However, pretreatment of seeds of both hybrids with salicylic acid and ascorbic acid at different (0, 100mg L^{-1} and 200mg L^{-1}) concentrations had a significant effects on seedling fresh biomass and germination rate and increased the values of these attributes, whereas, non significant effects of these were noted on decreasing mean germination time. Further, interaction of salicylic acid and ascorbic acid indicated that salicylic acid was more effective in reducing the harmful effects of drought stress. Overall, maximum increase for all the traits was observed in seeds treated with 200mg L^{-1} concentration of salicylic acid. Hysun-33 responded significantly better than Hysun-38.

Table. 1 Mean square values from variance analysis of data for germination %, plumule length and radical length of two sunflower hybrids subject to different levels of drought stress and role of salicylic acid and ascorbic acid.

Treatment	Germination %		Plumule length (cm)		Radical length (cm)	
	Hysun-33	Hysun-38	Hysun-33	Hysun-38	Hysun-33	Hysun-38
Control	99.02a	94.57b	13.25a	12.5ab	8.65a	8.12ab
SA 100mg L^{-1} (-0.6MPa)	79.2hi	73.39j	9.62ef	8.57gh	6.52ef	6fg
SA 200mg L^{-1} (-0.6MPa)	92.22bc	85ef	12.2ab	11.07c	7.85ab	7.45bc
AA 100mg L^{-1} (-0.6MPa)	73.25j	65.75k	8.7fg	7.8hi	6.2fg	5.45hi
AA 200mg L^{-1} (-0.6MPa)	88.45cd	81.95fg	11cd	9.82e	7.55bc	7.1cd
SA 100mg L^{-1} (-1.2MPa)	78.12i	72.02j	9.15ef	8hi	5.6gh	5.1ij
SA 200mg L^{-1} (-1.2MPa)	90.75cd	84.25fg	11.17c	10.05de	7.62bc	7.2cd
AA 100mg L^{-1} (-1.2MPa)	67.75k	61l	8.42gh	7.55ij	5.6hi	4.85ij
AA 200mg L^{-1} (-1.2MPa)	88.07de	80.55gh	9.7e	8.52gh	6.8de	6.4ef
Drought stress (-0.6MPa)	56.92m	50.35n	7.12jk	6.3k	4.5jk	4.1kl
Drought stress (-1.2MPa)	49.25n	44o	6.4k	5.27l	4.2kl	3.6l
LSD DXVXT	3.7887		0.9832		0.8314	

Table. 2 Mean square values from variance analysis of data for germination time, seedling fresh biomass and germination rate of two sunflower hybrids subject to different levels of drought stress and role of salicylic acid and ascorbic acid.

Treatment	Germination time (Day)		Seedling fresh biomass (g)		Germination rate	
	Hysun-33	Hysun-38	Hysun-33	Hysun-38	Hysun-33	Hysun-38
Control	3.25m	3.57lm	283.8a	275.5b	65.71a	57.4b
SA 100mg L^{-1} (-0.6MPa)	5hi	5.4gh	245.43f	238.3g	48.1ef	42.28ij
SA 200mg L^{-1} (-0.6MPa)	4kl	4.5jk	270.2c	262.72d	55.5bc	50.54de
AA 100mg L^{-1} (-0.6MPa)	5.6fg	5.9ef	232.65h	224.8j	45.9gh	39.5kl
AA 200mg L^{-1} (-0.6MPa)	4.8ij	5.2hi	264.7d	257.65e	52.75cd	46.78fg
SA 100mg L^{-1} (-1.2MPa)	6de	6.5cd	239.78g	232.27h	43.96hi	37.6lm
SA 200mg L^{-1} (-1.2MPa)	4.8ij	5.3gh	269.13c	261.57d	51.32cd	46.42fg
AA 100mg L^{-1} (-1.2MPa)	6.4cd	6.9c	226.87i	219.65j	41.76jk	35.42mn
AA 200mg L^{-1} (-1.2MPa)	6.3cd	6.8bc	262.1d	254.7e	48.64de	42.03jk
Drought stress (-0.6MPa)	7.22cd	7.76bccd	187.77k	180.8l	32.53n	25.44o
Drought stress (-1.2MPa)	8.77cd	9.55a	180.7l	173m	26.86o	20.52p
LSD DXVXT	1.3141		3.4636		4.3001	

Any two letters not sharing a common letter in row or column differ significantly at 0.055% probability level.

DISCUSSION

Drought is one of the most serious abiotic environmental stress factors that can reduce seed germination percentage by causing an increase in production of growth inhibitors and decrease in production of growth stimulators (Shakirova and Sahabutdinove, 2003), increase in mean germination time, decrease in radical and hypocotyl length, decrease in fresh weight of seedling (Gamze *et al*., 2005) and decrease in germination rate (Liusia *et al*., 2005). Application of different type of plant growth regulators via different modes and at different growth stages of crop plants especially at early stage has been reported very effective in alleviating the adverse effects of stress factors. Pre-sowing seed treatment has also shown considerable improvement in germination and other growth related attributes (Ashraf *et al*., 2008). From the results of present study, it is clear that drought stress caused a significant reduction in germination percentage, plumule length, fresh biomass of seedlings and germination rate, except radical length, while, in contrast increased mean germination time. However, seed treatment of both hybrids with salicylic acid and ascorbic acid alleviated the adverse effects of drought stress and showed a marked increase for all the attributes, while in contrast decreased mean time necessary for germination. Similar drought stress alleviative effects of salicylic acid and ascorbic acid on germination percentage, germination rate, radical and plumule length and fresh biomass of seedling (radical and plumule) has also been reported by (Smironoff, 1996). This increase in seedling length and fresh biomass after salicylic acid treatment in present study might be a result of increased shoot growth through increased cell division and cell extension by maintaining the hormonal balance (IAA and cytokinin levels) in the plant tissues, which enhanced the cell division by increasing internal level of other plant growth regulators (Sakhabutdinova *et al*., 2003). Baalbaki *et al*., (1999) explored that under deficit moisture conditions salicylic acid seed treatment stimulated germination of wheat seeds due to scavenging of reactive oxygen species, reduced oxidative damage and increased antioxidant activity. The increased root growth in terms of fresh biomass production due to salicylic acid treatments can be explained by the fact that salicylic acid enhanced the cell replication in root tips and thus resulted into increased root growth. Recently, Basra *et al*., (2006) reported that melon (*Cucumis melo* L.) seeds treated with 50 mg L^{-1} salicylic acid showed increased root and shoot length. In the same way, ascorbic acid plays multiple roles in developmental processes and has been acknowledged as an effective plant growth regulator and able to enhance cell division efficacy of competent cells, extend cell wall and alter cell metabolism (Pignocchi and Foyer, 2003). Thus increase in seedling length and fresh weight of radicle and pulmule of plants raised from ascorbic acid treated-seeds might be due to stimulated cell division and increase in cell number and elongation within these tissues (Citterio *et al.*, 1994). In very recent past days, Afzal *et al*., (2006) has reported significant increase in root and shoot length and fresh weight of seedlings due to exogenous application of ascorbic acid (50 mg L^{-1}) under saline stress conditions. The findings of Tavili *et al*., (2009) and our results suggested that hydropriming of *Agropyron elongatum* Host. seed with ascorbic acid and salicylic acid had a positive effects on germination percentage and germination rate under salinity stress conditions. Similar coinciding results has also been reported by Muhammad *et al*., (2011) that wheat seeds soaked for 48 hours in aerated solution of salicylic acid and ascorbic acid with 20ppm concentration of each increased final germination percentage, radical and plumule length. These results can also be best supported by the findings of Yasemin and Kutbay (2008) that different levels of salinity stress, temperature and their interactions significantly reduced germination percentage and germination rate of *spergularia marina* plants, whereas, seeds pretreated with 40 mM and 60 mM of salicylic acid and L-ascorbic acid alleviated the adverse effects of these stress factors and showed increase for all these attributes. From the results of this study, it could be envisaged that inhibition of germination under drought stress condition probably resulted from osmotic effect, while, seed treatment with plant growth regulators improved the performance. Hence, seed treatment is simple, cheap and an easy method that could be recommended to farmers for achieving higher germination and uniform emergence of seedlings under field conditions. However, detailed information on how salicylic acid and ascorbic acid can cause changes needs to be explored, so, further experimentation is necessary for comprehensive elucidation and conclusion .

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