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EXOGENOUS APPLICATION OF SALICYLIC ACID AT DIFFERENT PLANT GROWTH STAGES IMPROVES PHYSIOLOGICAL PROCESSES IN MARIGOLD (*Tagetes erecta* L.)

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Marigold (*Tagetes erecta* L.) is a famous flowering plant in the world which has medicinal and industrial values. In Pakistan, it is an important floricultural crop and used widely as bedding plant. Pakistan is facing water shortage, which is affecting crops growth and productivity including marigold badly. Drought stress reduces plant growth, physiological and biochemical processes and flower yield in marigold. Salicylic acid (SA) is a phytohormone and its exogenous application is an effective approach to support plant during drought stress and mitigate its harmful impacts. Present study was planned to evaluate the effect of exogenous salicylic acid application on growth response and drought tolerance potential of marigold. For this purpose, a pot experiment was conducted in rain out shelter with four treatments of SA i.e., no SA application, SA foliar @ 100 mg L^{-1} application at vegetative growth, reproductive growth and at vegetative plus reproductive growth stages, under normal irrigation as well as under drought stress conditions were imposed. Drought stress was applied by skipping every alternate irrigation. The results showed that SA application at reproductive growth stage of marigold improved plant growth by improving its physiological processes during drought stress. It was concluded that exogenous application of SA, not only increased bioproductivity of marigold plants but also ameliorated the negative impacts of drought stress by enhancing drought tolerance potential in plants.
Keywords: Marigold, drought, foliar spray, salicylic acid, physiological processes

INTRODUCTION

Marigold (*Tagetes erecta* L.) belongs to genus *Tagetes* which is famous for its ornamental beauty and medicinal values. Marigold is an herbaceous annual plant with bright flowers of varying colors and sizes. China, India and Peru are leading producers of marigold in the world. In Pakistan, it is a major floricultural crop that is available in the market throughout the year. Due to its availability in various seasons, it has become a favorite bedding and container plant among the gardening community in Pakistan. Marigold is cultivated as crop to fulfill the demand of loose flowers and garlands for decoration at various ceremonies and as raw material in different industries in the country. Marigold is being used in folk medicine since ancient times. It is evident from history that this herb has been used in religious events as well as in different therapies (Khulbe, 2015). Its aromatic nature and essential oil contents make marigold more valuable for cosmetic and perfumery industry (Devika and Koilpillai, 2014). Petals of marigold contain lutein di-esters (xanthophyll) that is used for food value addition, manufacturing drugs, as natural colorant in textile dying and for manufacturing of poultry feed to get yellow egg skin and yolk naturally (Deineka *et al*., 2014). Its flower extract has mosquitocidal properties and can potentially be used as insecticide (Mavi, 2014). Extracts of leaves and roots of marigold have been reported to contain triterpene, thiophene, and steroids (Munhoza *et al*., 2014). For biological control of nematodes, marigold leaf extract is applied to nematode affected crops (Balbaa *et al*., 2008).

Among all abiotic stresses, drought stress affects plant growth the most and it is considered the major reason of yield loss in important crops all over the world. Shortage of irrigation water, puts stress on plant growth and reduces crop yield (Ashraf, 2010). Drought causes disturbances in photosynthetic rate as stomata are closed to avoid water loss in plants (Moussa, 2006; Mastalerczuk *et al*., 2017) and activities of many enzymes involved in Calvin cycle (Monakhova and Chernyadev, 2002). Water deficit is major cause of reduction in plants water contents and growth which leads to crop failure (Zhang *et al*., 2011). As a result of these changes some allied changes, like production of various unstable molecules of oxygen produce in mitochondria and chloroplast which are known as active/reactive oxygen species (ROS) (Arora *et al.,* 2002). When ROS are overproduced, they cause lipid peroxidation (Zhang *et al.,* 2011) and damage chlorophyll, protein, deoxyribonucleic acid (DNA), lipids and various important organic molecules in plant cells (Jiang and Zhang, 2001).

Drought is serious problem for marigold as it damages its growth and leads to the plant death. Drought effects seed germination, height of seedlings, water potential and growth by reducing photosynthesis and respiration (Zhang *et al.*, 2011). Poor flower quality, disturbance in flowering time, decrease in plant height and lower water potential in marigold are observed during drought (Burnett *et al*., 2006). Leaf ammonium concentration is increased, leaf chlorophyll contents are decreased and proteolysis is noticed in leaves of marigold as result of drought stress (Chyliński and Łukaszewska, 2008; Jamil *et al*., 2018). Under drought stress, leaves are wilted as a results of dehydration which is very common in marigold and its main reason is reduction in photosynthesis and increase in the production of ROS. Cellular membrane and leaf chlorophyll are damaged due to ROS in marigold.

In plant body, salicylic acid is produced naturally which is important for better growth and enhancing natural defense system of plants. During biotic and abiotic stresses salicylic acid exogenous application is found effective in major crop because it can repair the damage caused by these environmental hazards (Miura and Tada, 2014). Use of salicylic acid is suggested to increase drought tolerance in plants (Miura and Tada, 2014). Keeping in view the climatic prediction of upcoming drought and global warming and importance of marigold plant in ornamental and pharmacology industry, a study was planned to explore the effect of salicylic acid exogenous application during drought stress on different physiological processes and plant growth of marigold.

MATERIALS AND METHODS

***Pot experiment*:** This experiment was performed at rain-out shelter of the department of Agronomy, University of Agriculture Faisalabad, Pakistan using a local marigold variety of marigold (Gutta/ Desigainda). Seeds were collected from a local nursery and sterilization was done by using 5% sodium hypochlorite solution for three minutes and rinsed thoroughly with distilled water. Seeds of marigold were sown in germination trays having peat moss as growing media. After 24 days, the seedlings (5 seedlings /pot which were later thinned to 3 seedlings /plant) were transplanted into 240 mm diameter pots already filled with silt, sand and leaf manure mixture with 1:1:1 ratio. To meet nutrition requirement of plants, Hoagland solution ($1/5^{th}$ strength) was applied (Hoagland and Arnon, 1950). Before starting drought stress treatment, seedlings were given time to acclimatize and establish for 20 days.

***Drought stress and treatments*:** Drought stress was applied by skipping every alternate irrigation after 20 days of seedling establishment. After that, one treatment was without salicylic acid application and in other treatments salicylic acid was foliar sprayed with 100mgL^{-1} concentration, at vegetative (30 days after transplanting), reproductive (when first flower bud was opened) and at both vegetative plus reproductive growth stages of marigold. The experiment was arranged in complete randomized design (CRD) with three replications. There were total 24 pots which were divided into 4 sets having salicylic acid application at different crop growth/developmental stages of marigold after imposing moisture stress. Each set had six pots (three pots with normal irrigation and three pots with drought stress). After salicylic acid application at different growth stages of marigold, data for physiological indices including fresh and dry biomass, flowering yield were collected from randomly selected plants from each pot.

***Plant water relations*:** To evaluate leaf water potential (-MPa), a fully expanded third leaf from top of plants was harvested at mature stage. Samples were collected in the morning (06:00 – 09:00 a.m.) for measurement of water potential (Ψw) by using pressure chamber ("Scholander" type) (Model-1000, PMS instruments company, OR-USA). Osmotic potential (–MPa) was measured from the leaf samples by which water potential was determined. For seven days, these leaves were frozen at -20°C and then these leaves were thawed and crushed with a glass rod in a append of tube to extract cell sap. This cell sap was used to estimate the value of leaf osmotic potential (Ψs) using an osmometer (Osmomat-030, Germany). By subtracting the above value of osmotic potential (Ψs) from water potential (Ψw), turgor potential (MPa) (Ψp) of leaves was determined using following formula:

$$\Psi p = \Psi w - \Psi s$$

To determine the value of relative water content (RWC) (%), from the top of plant the third maximum expanded leaf was taken as sample. For collection of leaf samples, plastic bags were used and immediately taken to the laboratory. Digital electrical balance was used to know leaf fresh weight and then soaked these leaves in distilled water for 24 h in small cups made up of plastic. After that leaves were removed to obtain turgid weight with the help of weighing balance. To obtain the oven dry weight, these leaves were placed in oven at 65°C temperature for 74 hours. To calculate the amount of relative water contents, the method described by Cornic (1994) was followed.

$$RWC = [(FW-DW) / (TW-DW)] \times 100$$

Where, FW =Sample fresh weight, DW =Sample dry weight, TW = Sample turgid weight

***Gas exchange parameters*:** Gas exchange parameters were determined by portable infrared gas analyzer (LCA-4 ACD) (Analytical Development, Hoddesdon, UK). For this purpose fully expanded leaf from middle of plant was selected to take the readings of transpiration rate (E, mmol H_2O m^{-2} s^{-1}), photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}) and Sub-stomatal CO_2 rate (internal CO_2concentration) (Ci) during 09:00 a.m. to 11:00 a.m. At the end of each experiment, three plant per pot were harvested and dried in oven at 70°C till a constant weight.

***Dry matter*:** Dry weight was recorded using electric weighing balance. To calculate the average dry weight, added amount of dry weights of all samples and divided them by the total number of samples.

***Statistical data analysis*:** The data collected from the experimentation was statistically analyzed by following the method of analysis of variance and Statistix-8.1(software). For calculation of differences among the treatment means, applied least significant difference (LSD) test at probability P ≤ 0.05 (Steel *et al.,* 1997).

RESULTS

The results of leaf water potential (Ψw) of marigold are presented in Figure 1. The data showed a significant decline in leaf Ψw due to drought (Table 1). In normal moisture supply, 100 mg L^{-1} salicylic acid spray at vegetative plus reproductive growth stages, produced the maximum value of Ψw. However, during moisture deficit environment, the greater value of Ψw was observed when 100 mg L^{-1} salicylic acid was sprayed at reproductive growth stage. Whereas, plants grown without salicylic acid spray (No SA), exhibited the minimum value of leaf water potential in control and drought stress conditions (Fig. 1). As shown in the data that leaf osmotic potential (Ψs) was greatly affected by unavailability of water (Table 1). The foliar applied salicylic acid at reproductive growth stage produced the maximum value of leaf Ψs, in normal irrigation and during stressful conditions. However, the minimum Ψs was noted in plants without salicylic acid spray (No SA) and it was statistically at par with salicylic acid sprayed at vegetative plus reproductive growth stages of marigold plants. On imposing moisture stress, the leaf Ψs was minimum in plants without SA application. The results showed that both in regular irrigation and drought stress, SA@100 mg L^{-1} application at reproductive stage of marigold plant had the greatest value of leaf turgor potential (Ψp). However, plants where salicylic acid was applied at vegetative plus reproductive stage, produced the minimum leaf Ψp when water supply was normal. After drought stress application to plants, the minimum value of Ψp was found in those plant which received salicylic acid foliar spray at vegetative plus reproductive stage in moisture stress conditions (Fig. 3).

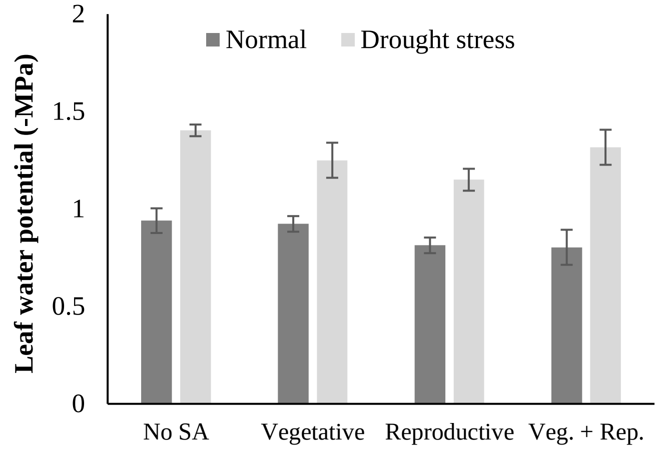

Figure 1. Effect of salicylic acid application on leaf water potential (Ψw) of marigold plants under normal and drought stress. Values are means ± standard error.

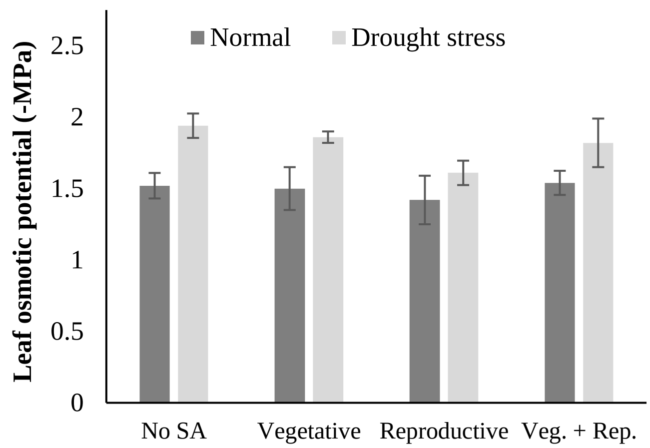

Figure 2. Effect of salicylic acid application (100 mg L^{-1}) on leaf osmotic potential (Ψs) of marigold (*Tagetes erecta* L.) plants under normal (no moisture stress) and drought stress (moisture stress) conditions. Values are means ± standard error.

Table 1. Mean sum of squares of the data for leaf water potential (-MPa), leaf osmotic potential (-MPa), leaf turgor potential (MPa) and relative water contents (%) of marigold (*Tagetes erecta* L.) under two drought stress levels in pots.

SOV[a]	DF[b]	Leaf water potential (-MPa)	Leaf osmotic potential (-MPa)	Leaf turgor potential (MPa)	Relative water contents (%)
Stress	1	1.04584**	0.55510**	0.07707**	515.227**
SA	3	0.03787**	0.05494**	0.01057**	207.283**
Stress*SA	3	0.01076**	0.01540**	0.01897**	15.072 ^{ns}
Error	14	0.00058	0.00163	0.00145	19.019
Total	23				

* = LSD p≤0.05, ** = LSD p≤0.01, ^{ns} = Non significant, [a]SOV = Source of variation, [b]DF = Degree of freedom, SA = Salicylic acid

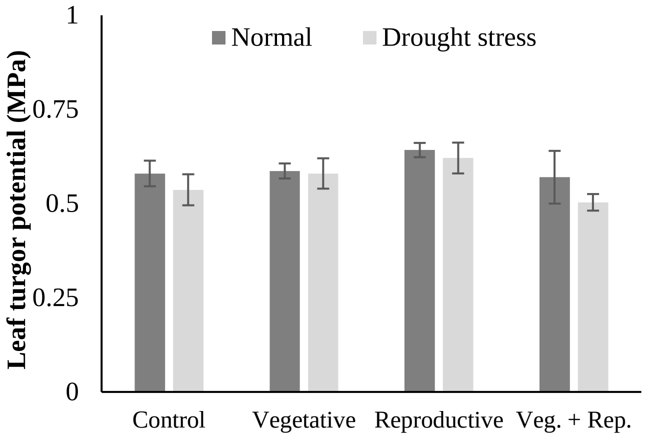

Figure 3. Effect of salicylic acid application (100mg L^{-1}) on leaf turgor potential (Ψp) of marigold (*Tagetes erecta* L.) plants under normal (no moisture stress) and drought stress (moisture stress) conditions. Values are means ± standard error.

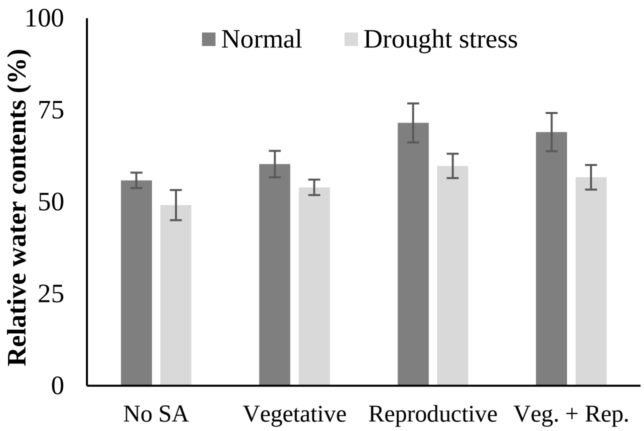

Figure 4. Effect of salicylic acid application (@ 100mg L^{-1}) on relative water contents (%) of marigold (*Tagetes erecta* L.) plants under normal (no moisture stress) and drought stress (moisture stress) conditions. Values are means ± standard error.

Relative water contents (RWC) were significantly decreased when moisture stress was imposed to plants (Table 1; Fig. 4). In plants grown under normal irrigation, RWC increased when foliar application of SA @100 mg L^{-1} was done at reproductive growth stage of marigold. While, the value of RWC was found minimum when no SA spray was applied in normal water supply. Whereas, the application of drought stress resulted in increase in RWC when SA was sprayed at reproductive growth stage of marigold. Absence of SA foliar application (No SA) caused reduction in RWC of marigold plants maintaining the least value for this variable under water stress environment.

The photosynthetic rate (*Pn*) of marigold plants decreased on reducing water supply. There were a significant variations in *Pn* of plants grown in normal moisture and in drought stress (Table 2). Photosynthetic rate was found the highest on applying SA @ 100 mg L^{-1} to marigold plants at reproductive growth stage under normal water supply as well as during drought stress conditions. Similarly, the plant grown without SA spray, exhibited the lowest values for photosynthetic rate, when grown with or without drought stress (Fig. 5). The transpiration rate (*E*) of marigold plants was significantly $p < 0.05$ decreased due to drought stress imposition (Table 2; Fig. 6). Higher transpiration rate was maintained by marigold plants when SA@ 100 mg L^{-1} was applied at reproductive growth stage. However, the lowest transpiration rate was recorded in plant grown without SA treatment in normal and water stress conditions (Fig. 6). The stomatal conductance (*gs*) was significantly influenced by drought stress (Table 2). The highest stomatal conductance was recorded when plants were sprayed with 100 mg L^{-1} SA at reproductive growth stage of marigold under normal or drought conditions. While, the minimum values for stomatal conductance were noted in treatment without salicylic acid application during drought stress (Fig. 7). The results of sub-stomatal conductance CO_2 rate was significantly reduced due to moisture stress (Table 2; Fig. 8). Marigold plants maintained better sub-stomatal CO_2 rate on applying SA @100 mg L^{-1} at reproductive stage when they received normal watering as well as during water deficit.

Table 2. Mean sum of squares of data for photosynthetic rate (A) (µmol CO_2 m-1 s-1), transpiration rate (E) (mmol H_2O m^{-1} s^{-1}), stomatal conductance (gs) (mmol H_2O m^{-1} s^{-1}), sub-stomatal CO_2 rate (Ci) (µmol CO_2 mol^{-1}) and dry weight of marigold (*Tagetes erecta* L.) plants under two drought stress levels in pots.

[a]SOV	[b]DF	Photosynthetic rate (*A*) (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (*E*) (mmol H_2O m^{-2} s^{-1})	Stomatal conductance (g_s) (mmol H_2O m^{-1} s^{-1})	Sub-stomatal CO_2 rate (*Ci*) (µmol mol^{-1})	Dry weight plant^{-1}(g)
Stress	1	522.014**	632.427**	0.66667**	1338.62**	2181.23**
SA	3	47.135**	93.914**	0.07326*	97.12^{ns}	288.15**
Stress*SA	3	6.615**	6.039**	0.00421 ^{ns}	4.14^{ns}	52.93**
Error	14	0.680	0.353	0.02003	30.18	5.74
Total	23					

* = LSD p≤0.05, ** = LSD p≤0.01, ^{ns} = Non significant, [a]SOV = Source of variation, [b]DF = Degree of freedom, SA = Salicylic acid

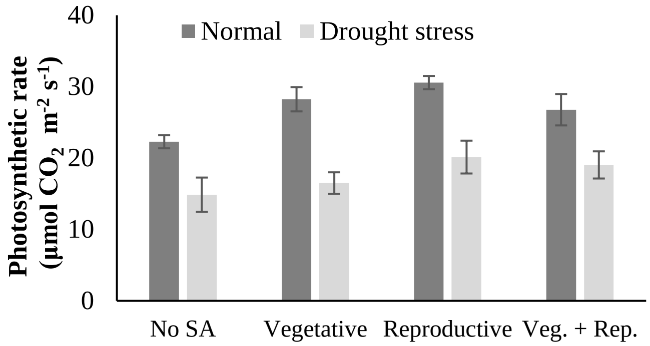

Figure 5. **Effect of salicylic acid application (@100 mg L^{-1}) on photosynthetic rate (*A*) (µmol CO_2 m^{-1} s^{-1}) of marigold (*Tagetes erecta* L.) plants under normal (no moisture stress) and drought stress (moisture stress) conditions. Values are means ± standard error.**

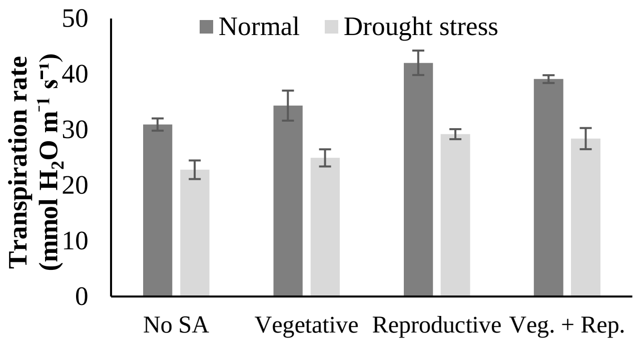

Figure 6. **Effect of salicylic acid application (@ 100 mg L^{-1}) on transpiration rate (*E*) (mmolH_2O m^{-1} s^{-1}) of marigold (*Tagetes erecta* L.) plants under normal (no moisture stress) and drought stress (moisture stress) conditions. Values are means ± standard error.**

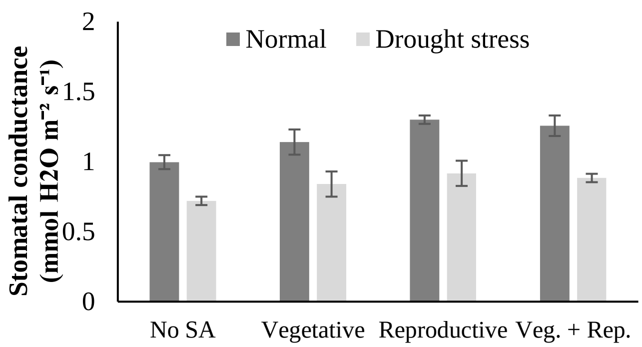

Figure 7. **Effect of salicylic acid application (@100 mg L^{-1}) on stomatal conductance (g_s) (mmol H_2O m^{-1} s^{-1}) of marigold (*Tagetes erecta* L.) plants under normal (no moisture stress) and drought stress (moisture stress) conditions. Values are means ± standard error.**

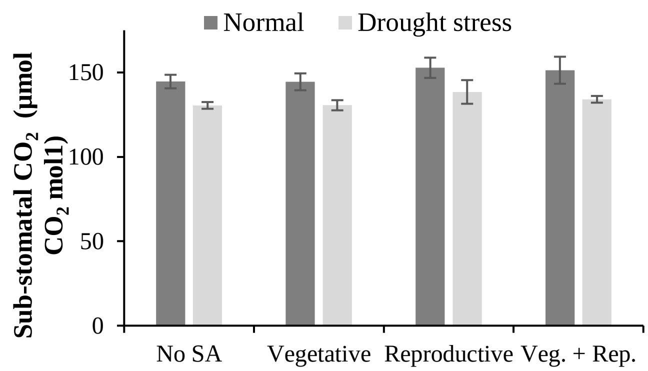

Figure 8. **Effect of salicylic acid application (100 mg L^{-1}) on sub-stomatal CO_2 rate (*Ci*) (µmol CO_2mol^{-1}) of marigold (*Tagetes erecta* L.) plants under normal (no moisture stress) and drought stress (moisture stress) conditions. Values are means ± standard error.**

The lowest values of sub-stomatal CO_2 rate were at par when SA was not applied (No SA) and when it sprayed at vegetative growth stage of marigold in normal and limited moisture supply (Fig. 8). The results of dry weight plant^{-1} of marigold are presented in Fig. 9. It is obvious from the data that the dry weight plant^{-1} of marigold was influenced significantly by drought stress (Table 2). During both normal moisture supply and drought stress, the maximum dry weight per plant was obtained by SA @100 mg L^{-1} foliar spray at reproductive growth stage of marigold plants. Minimum dry weight was recorded in marigold plants which were not treated with SA (No SA). The amount of plant dry weight was lowest with no salicylic acid application in water deficit condition and it was statistically increased with salicylic application at vegetative growth stage (Fig. 9).

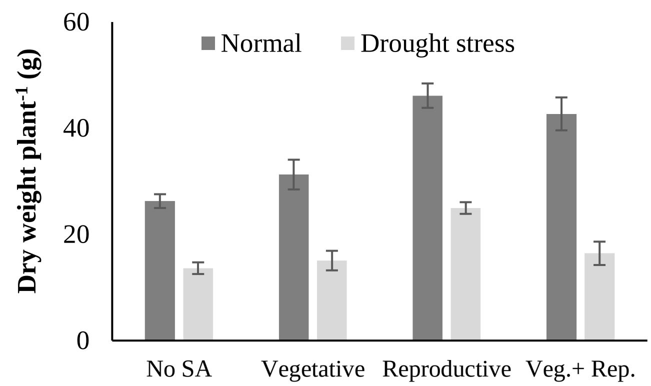

Figure 9. **Effect of salicylic acid application (100 mg L-1) on dry weight plant^{-1}(g) of marigold (Tagetes erecta L.) under normal (no moisture stress) and drought stress (moisture stress) conditions. Values are means ± standard error.**

DISCUSSION

Plant water relation were improved by 100 mg L^{-1} salicylic acid (SA) application at reproductive growth stage of marigold with or without imposing drought stress which is obvious from Figures 1 to 4. Similar observations were recorded by other scientists (Hayat *et al*., 2008; Hashmi *et al*., 2012) who found an improvement in the leaf water potential and relative water contents by SA foliar application to drought affected tomato. Water potential in leaves of wheat also increased by salicylic acid spray under drought stress (Singh and Usha, 2003; Dalmia and Sawhney, 2004). Application of SA@100 mgL^{-1}in rice plants, grown under drought stress environment, significantly decreased leaf osmotic potential (Ψs), turgor potential (Ψp), leaf water potential (Ψw) and photosynthetic rate (*Pn*). There was a correlation between RWC, leaf proline and SA found under limited water supply (Farooq *et al*., 2009). Similarly, on applying foliar spray of SA under drought stress increased RWC and other water relations were also pointed out by Hayat *et al*. (2010).Same trend was witnessed in wheat by Singh and Usha (2003) and in *Ctenanthe setosa* by Kadioglu *et al*. (2011). Figs. 1 to 4 show that by SA treatment caused a manipulation of Ψs and Ψp of plants which might be due to the process of osmotic adjustment, enhanced by SA application during deficit moisture environment. This phenomenon was also seen by others scientists (Machado and Paulsen, 2001; Hura *et al*., 2007). Transpiration causes more loss of water than the amount of water absorbed through roots. It was observed in earlier studies that SA treatment increased moisture absorption by plant roots and the amount of water loss by stomata was manipulated to decrease adverse effects of drought and it helped to regulate the availability of moisture in plants body (Makoto *et al*., 1990; Tas and Tas, 2007; Keyvan, 2010). The possible reasoning of our result are better explained by Sakhabutdinova *et al*. (2003) and He *et al*. (2005) who recorded that more production of photosynthates and sap in leaves caused an increase in RWC of plants when SA was foliar applied. Agarwal *et al*. (2005) described another reason of this process and explained that increase in amount of water contents of plants by SA foliar spray, in limited moisture availability, might be the result of less electrolyte leakage due to stabilization of membranes in plants. The data presented in Figures 5 to 8 show results of gas exchange parameters. Figure 5 depicts that photosynthetic rate was decreased on imposing drought stress to marigold plants. The possible reasons of decreased photosynthetic rate could be closure of stomata which reduces the supply and internal concentration of CO_2 (Tiwari *et al*., 2005) inhibited rubisco activity (Yardanov *et al*., 2003) as well as other enzymes involved in photosynthesis (Lawlor, 2002; Reddy *et al*., 2005). Less stomatal conductance is the reason of reduction in photosynthesis (Farooq *et al*., 2008) and chlorophyll contents (Loggini *et al*., 1999). Whereas, SA improved photosynthetic activity which was also found in tomato grown under moisture stress where photosynthetic rate along with chlorophyll contents were increased by salicylic acid application (Fariddudin *et al*., 2003; Hayat *et al*., 2008). By application of SA, transpiration rate and stomatal conductance were enhanced which might be another reason for increased photosynthetic rate in plants (Khan *et al*., 2003). Salicylic acid treatment improved stomatal conductance, transpiration and sub-stomatal CO_2 in marigold plants as shown in Figures 6 to 8. Khan *et al*. (2003) and Nazar *et al*. (2015) explained that SA spray improved stomatal conductance, rate of transpiration and sub-stomatal CO_2 rate which might be one reason of more photosynthetic rate. The results obtained by salicylic acid spray in okra plants during drought stress, showed more germination as a result of increased rate of stomatal conductance (Baghizadeh and Hajmohammadrezae, 2011). Noreen and Ashraf (2008) had similar observations regarding this phenomenon. Nazar *et al*. (2015) found better photosynthesis and plant growth by salicylic acid application. They explained its important role to regulate plant responses during moisture stress and suggested its use as potential growth regulator to improve plant drought tolerance. Under drought stress, there was a harmful impact on dry and fresh plant weights of marigold. However, salicylic acid foliar treatment at reproductive growth stage of marigold regained more plant dry weight (Fig. 9). Same results were also reported by different researchers in other crops (Fitter and Hay, 2002; Hamayun *et al*., 2010). Farooq *et al*. (2009) observed a decline in dry and fresh plant weight in rice during drought and SA application at reproductive growth stage with 100 mg L^{-1} repaired this loss. They further explain that decrease in plant fresh and dry weights during drought stress might be an indication of damage to photosynthesis process caused by unavailability of adequate moisture during drought stress conditions. Salicylic acid foliar application increase in dry matter of wheat in limited water availability (Singh and Usha, 2003).

***Conclusions*:** It is concluded that marigold plant is badly affected by drought stress if it is not managed timely. Foliar application of 100 mg L^{-1} salicylic acid at reproductive growth stage was the best dose and time to improve various physiological parameters resulting in increased plant growth and productivity.

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