

INVESTIGATION OF IMPROVING THE DROUGHT TOLERANCE IN PERSIAN PETUNIA (*Petunia* sp.) BY EXOGENOUS APPLICATION OF SALICYLIC ACID AND GIBBERELIC ACID

Morteza Goldani¹ ✉, Ali Dolatkahi², Mahdi Parsa¹, Navid Vahdati², Zahra Rasouli¹

¹ Department of Agrotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

² Department of Horticultural Science and Landscape, Ferdowsi University of Mashhad, Mashhad, Iran

ABSTRACT

Plants respond to water deficit through several mechanisms. Among which, plant hormones play an important role in induction to abiotic stress tolerance. To bring this issue closer, an experiment was conducted to examine whether salicylic acid (SA) and gibberellic acid (GA_3) exogenous applications through foliar spray could alleviate detrimental effects of water deficit on Persian petunia. Treatments consisted of water stress in three levels: 100% field capacity (F.C.) as control, 50, and 25% F.C linked with different concentrations of GA_3 and SA sprayed at four levels (0, 100, 200 and 300 mg L⁻¹). Results showed that the dry and fresh biomass, leaf area, photosynthetic pigments levels, relative water content (RWC) and the total number of flowers were significantly ($P \leq 0.01$) reduced in response to water deficit. However, free proline concentration and root-to-shoot ratio significantly increased in stressed plants. Plants subjected to severe water stress (25% F.C.), exhibit about 66% stomatal conductance rate lower than the plants grown at 100% F.C. The results showed that SA-treated plants exhibited reduced negative effect of water stress on most of the qualitative and quantitative attributes compared to control, while GA_3 application had little impact on studied traits. Importantly, stomatal conductance and RWC were improved in water-stressed plants treated with SA. Moreover, SA-treated plants produced more flowers at all water deficit levels compared to GA_3 sprayed plants. It can be concluded that Persian petunia plants respond to water deficit through adaptive changes at biochemical and morphological levels and that adverse effects of water deficit could be alleviated by exogenous application of SA.

Key words: ornamental flower, photosynthetic pigments, proline, relative water content, stomatal conductance, water deficit

INTRODUCTION

Persian petunia (*Petunia* sp.) is one of the most popular ornamental plants which is widely used in urban landscaping, because of its fragrant flowers [Shamshiri et al. 2011]. Water has long been considered as one of the main limiting factors in agricultural productions, especially in arid and semi-arid regions. Therefore, more efficient irrigation practices are important in landscape water management [Van Iersel et al. 2010]. Many researches have been done to un-

derstand alterations of morphological, biochemical, and physiological characteristics at leaf level under limited water conditions. In general, plants respond to water deficit through different types of strategies, including stomatal closure, leaf area reduction, product of compatible osmolytes, such as free proline, and changes in endogenous phytohormones status [Chaves et al. 2002, Athar and Ashraf 2005, Verbruggen and Hermans 2008]. Proline accumulation in plants is

✉ goldani@um.ac.ir

a mechanism by which plants resist water stress and also results in a decline in metabolic activity of plant cells, which is inevitably reflected in inhibition of their growth [Bezrukova et al. 2001, Umebese et al. 2009]. On the other hand, decreased GA_3 and increased ABA in plants exposed to osmotic stress were observed [Pirasteh-Anosheh et al. 2013]. Regarding this fact, exogenous application of phytohormones under stress conditions could be the possible means of alleviation of the effects of abiotic stress. It is well known that plant hormones play an important role in the regulation of signal transduction pathway involved in the induction of plant stress response [Waterland et al. 2010, Ali et al. 2012, Verma et al. 2016].

Salicylic acid (SA) among plant growth regulators (PGRs), is a common plant-produced signal molecule, which effectively participates in the regulation of physiological processes in plants like growth, photosynthesis, ethylene production and flowering [Hayat et al. 2010, Hesami and Dolatkhahi 2016] and also provides protection against biotic and abiotic stresses mainly drought through HR and SAR reactions [Kaya et al. 2002, Idrees et al. 2010]. The ameliorative effect of SA on growth of crop plants under abiotic stress conditions may have been due to its role in nutrient uptake, water relations, stomatal regulation, photosynthesis and production of reactive oxygen species (ROS) [Kawano and Muto 2000, Khan et al. 2003, Arfan et al. 2007]. ROS acts as signaling molecules that triggers the cascade of protective reactions in plants, including activation of antioxidant enzymes [Kar 2011]. Furthermore, SA induced abscisic acid (ABA)-mediated protective reactions in plants involves increased production of proline by the expression of the gene encoding D'-pyroline-5-carboxylate synthetase (P5CS) in proline biosynthesis [Delauney and Verma 1993].

In addition, GA_3 is another important endogenous growth regulator that has profound and various effects on regulating plant growth, development, and stress responses are known for it [Huerta et al. 2008, Sun et al. 2008]. It has been reported that GA_3 concentration rapidly decreased in corn leaves exposed to water deficit stress [Wang et al. 2008, Pirasteh-Anosheh et al. 2013]. In fact, reduction of GA_3 levels and signaling in plants subjected to water stress contributes to plant growth restriction [Colebrook et al. 2014]. Bano and

Yasmeen [2010] reported that water stress decreased GA_3 concentration in wheat leaves. Besides, exogenous application of GA_3 has been found to increase germination percentage and seedling growth under polyethylene glycol (PEG) induced water stress in chickpea [Kaur et al. 2000].

The aim of the present study was to evaluate the potential protective effect of SA and GA_3 on the development of Persian petunia (*Petunia* sp.) under water deficit stress during growth life cycle.

MATERIALS AND METHODS

Greenhouse and climate control. The experiment was performed in a venlo type greenhouse located at Ferdowsi University of Mashhad, Iran (Latitude 36°20'N, longitude 59°35'E, and 1065 m ASL) equipped with automated roof windows along with shading screens as well as evaporative cooling pads and exhaust fans during 2017. Growth conditions were as follows: max/min temperature 25/17°C (day/night), mean relative humidity 60% and photosynthetic photon flux density (PPFD) 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (photoperiod, 14h). Persian petunia (*Petunia* sp.) seeds were surface sterilized in 5% sodium hypochlorite solution for 5 min and then thoroughly rinsed 3 times with distilled water before sowing in 4-liter plastic containers. The soil used was sandy loam as follows: sand, 39.8%, silt, 51.4%, clay, 8.8%, pH, 7.3, electrical conductivity (EC), 2.3 dS m^{-1} , N, P, and K, 1420, 12.5, and 209 mg kg^{-1} , respectively. After emergence, the seedlings were thinned to 3 plants per pot. In this experiment, 3 irrigation regimes 100% as control, 50, and 25% of the field capacity (F.C.) combined with SA and GA_3 (Merck, Germany 2017) [Idrees et al. 2010] applications at concentration of 0, 100, 200 and 300 mg L^{-1} was planned in form of a factorial based on completely randomized design with 3 replicates. The growth regulators treatments have all been sprayed as foliage applied twice during the growing season by hand 10cc per plant watering can with a shelter between treatments to avoid the spray transfer to neighboring treatments. First spray was conducted in the first week after transplantation and first week before irrigation treatments, while the second was carried out before the flowering stage, as it was spraying in the evening times. The water stress applied was based on

the difference between soil dry and wet weight after watering and ceasing the runoff. Soil water contents were determined by weighing each container daily, then was added to maintain the predetermined water content in each pot. The amount of water applied for irrigation regimes of 100%, 50%, and 25% of the F.C. were 250, 125 and 62 ml, respectively.

Data collection. Shoots and roots were weighed immediately after harvest and then the shoots were dried at 80°C for 24 h (foliar dry weight, FDW). Plant leaf area (all leaves per plant) was measured by leaf area meter (Li-Cor, Li-1300, USA). Flower production is defined as full bloom stage and it was recorded for each plant individually. Days to flowering also is the interval between the presence of the florets and full bloom stage. These stages are all considered in this manner for the present experiment.

Relative water content. RWC from two young fully expanded leaves was calculated using the following equation [Yamasaki and Dillenburg 1999]:

$$\% \text{ RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100,$$

where FM is the leaf fresh mass (g), DW is the leaf dry mass (g) and TW is the leaf turgid weight (g). Turgid weight was determined by weighing the leaf after 12 h of immersion in distilled water at room temperature. Leaf DW was obtained after leaves were kept 48 h at 70°C in an oven.

Proline content. Proline content was determined by the method of Bates et al. [1973] with modifications using L-proline standard curve. Fresh leaves (0.1 g) from the outermost whorl were incubated with 5 ml 3% (w/v) sulphosalicylic acid at 100°C for 10 minutes and each 2 ml of extracts was mixed with 0.2% (w/v) ninhydrin reagent containing 70% (v/v) glacial acetic acid and incubated at 100°C for 60 min. The reaction was stopped under running tap water, the colored product was extracted with 4 mL toluene, and the absorbance of the toluene phase was measured at 520 nm.

Photosynthetic pigments measurements. Chlorophyll content was determined by spectrophotometric method [Lichtenthaler and Wellburn 1983]. Leaf samples were collected in early morning from fresh young leaves and were extracted in 80% acetone. Finally, the extraction's absorption was read at 663.2 and 646.8 nm, respectively. The results were expressed as mg g^{-1} FW.

Stomatal conductance. Leaf stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) was measured with a diffusion porometer (MK, Delta-T Devices, Cambridge, UK). Measurements were performed on the upper surface of fully expanded fifth to seventh leaves from the base of the plant.

Experimental design and analysis. The collected data were analyzed using SPSS software (*Version 16.0, SPSS Inc.*). The results were subjected to three-way analysis of variance (ANOVA) and the differences among the average of treatments were compared with each other using Tukey test at $P \leq 0.05$. Data normalization was done using Kolmogorov–Smirnov test.

RESULTS AND DISCUSSION

Plant growth parameters. Fresh and dry weights of root were significantly decreased in water-stressed plants in comparison with control plants (100% regime) – Table 1. The highest (3.49 g) and the lowest (3.23 g) fresh weight of root were found in 100% and 25% field capacity, respectively. Reductions in fresh weight of root due to water stress were more pronounced in GA₃ sprayed plants (2.93 g) than SA-treated plants (4.10 g) and the same results were obtained with dry weight of root. Dry weight of root was significantly ($P \leq 0.01$) affected by the interaction of stress \times PGRs type \times concentration (Tab. 1). Moreover, root-to-shoot ratio increased in stressed plants in comparison with well-irrigated plants (Fig. 1A). An increase in root-to-shoot ratio under water-stress is to facilitate water absorption and that it is related to the ABA content of roots and shoots [Shao et al. 2008].

In terms of foliar fresh (29.53 g) and dry weights (5 g), treatments were significantly ($P \leq 0.01$) affected by the interaction of stress \times PGRs type \times concentration (Tab. 1). The highest foliar fresh and dry weight was obtained in plants irrigated with 100% of water requirement along with application of SA (300 mg L^{-1}) – Table 2. As mentioned above, all the studied growth attributes decreased with an increase in drought stress intensity. This confirms the previous reports on various plant species such as *Helianthus annuus* L [Tahir and Mehid 2001], *Petunia hybrida* cv. 'Mix' [Shamshiri et al. 2011], and *Rosa hybrida* cv. 'Club Nika' [Dolatkahi et al. 2020].

Table 1. Analysis of variance to evaluate effects of water stress and hormone treatments on studied traits in Persian petunia (*Petunia* sp.)

Source of variation	df	Root FW	Root DW	Foliar FW	Foliar DW	Root/shoot ratio	Plant height	RWC	Leaf area	Day to flowering	Total number of flowers
Stress (A)	2	2.08*	1.99**	1186.07**	18.16**	0.478**	2188.1**	935.17**	14985.2**	8.51 ^{ns}	5436.16**
Hormone (B)	1	24.71**	0.876**	156.97**	4.63**	0.006 ^{ns}	1643.46**	597.19*	489.6 ^{ns}	34.72**	460**
Concentration (C)	3	3.03**	0.058 ^{ns}	6.86**	0.02 ^{ns}	0.006*	417.46**	193.27 ^{ns}	961.2 ^{ns}	22.33**	30.6**
A*B	2	3.35**	0.695**	1.59**	0.613**	0.018**	486.03**	153.12 ^{ns}	1447.9 ^{ns}	5.59 ^{ns}	89.38**
A*C	6	1.43*	0.05*	2.48**	0.310*	0.001 ^{ns}	201.36**	196.46*	1798.5*	11.73*	7.07**
B*C	3	3.05**	0.281**	107.08**	2.39**	0.029**	196.55**	134.51 ^{ns}	2436.1*	36.24**	290.9**
A*B*C	6	0.815 ^{ns}	0.18**	16.64**	1.50**	0.005*	284.55**	61.94 ^{ns}	1570.98 ^{ns}	7.11 ^{ns}	102.85**
Error	48	0.570	0.023	0.257	0.111	0.002	14.86	85.34	737.3	4.36	0.63

* significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; ns – not significant

In water-limitation conditions, morphological parameters such as fresh weight are greatly affected [Shao et al. 2008]. The reduction in growth attributes under water deficit may be due to the considerable decrease in plant growth, photosynthesis as well as suppression of cell expansion and cell growth in response to the low turgor pressure [Taiz and Zeiger 2006, Shao et al. 2008, Idrees et al. 2010]. Cell division, enlargement and differentiation, are the main processes that determine the quality and quantity of plant growth, affected by various internal and external factors, one of which is water stress [Patel and Golakiva 1988]. Reduction biomass production under water stress has been observed in many ornamental flowers species [Liu and Stützel 2004, Shamshiri et al. 2011, Zarghami et al. 2014]. In a research, SA-treated plants showed an increase in tolerance to water stress in measured growth parameters compared with plants faced water stress without SA application. Similar to our findings, Zarghami et al. [2014] reported a similar increase in the growth of shoots and roots of *Petunia* in response to SA treatment. The ability of SA in improve biomass under water stress is probably due to an increase in the activities of ribulose1,5-biphosphate carboxylase (RuBPC) and PEP carboxylase as reported by Idrees et al [2010] for lemongrass varieties.

Plant height and leaf area. Plant height was significantly ($P \leq 0.05$) affected by the interaction of stress ×

PGRs type × concentration (Tab. 1). The highest plant height was observed in well-irrigated plants with 200 mg L⁻¹ of GA₃ (Fig. 1B). The reduction in plant height under drought conditions might be associated with suppression of cell expansion and cell growth due to low turgor. Similar results were observed in *Petunia* [Shamshiri et al. 2011], *Rosa hybrid* [Dolatkhahi et al. 2020] and *Helianthus annuus* L. [Ahmad et al. 2009]. In general, plants sprayed with SA exhibit about 17% lower plant height than the plants sprayed with GA₃. Similarly, Taiz and Zeiger [2006] believed that stem growth may be dramatically enhanced by GA₃. It is well known that exogenous GA₃ application promotes internodal elongation in a wide range of species.

In the present study, plants developed under water stress conditions have a lower leaf area in comparison to control. SA and GA₃ foliar sprays applied at four concentrations were not effective on leaf area. Leaf area is the most important factor affecting crop productivity. Leaf area plasticity is important in maintaining the control of water use in crops [Shao et al. 2008]. Several studies have been devoted to plants under water deficit, where in general a decrease in leaf area was reported [Liu and Stützel 2004, Farooq et al. 2009], as compared to plants grown with adequate water supply. In these studies the fundamental physiological processes involved in the growth and development of leaves are cell division and cell expansion.

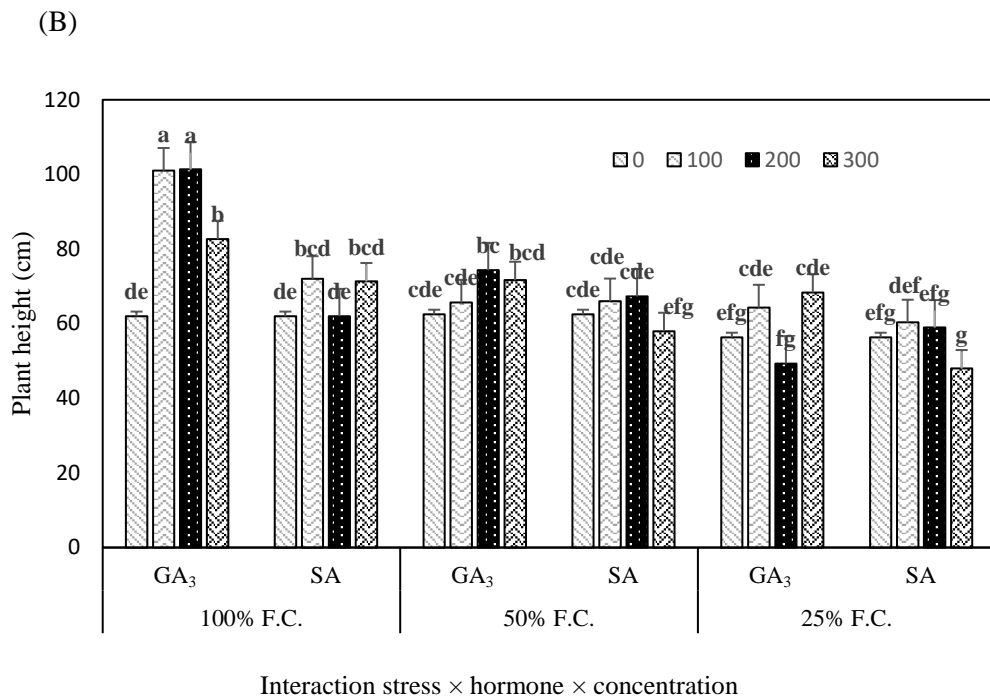
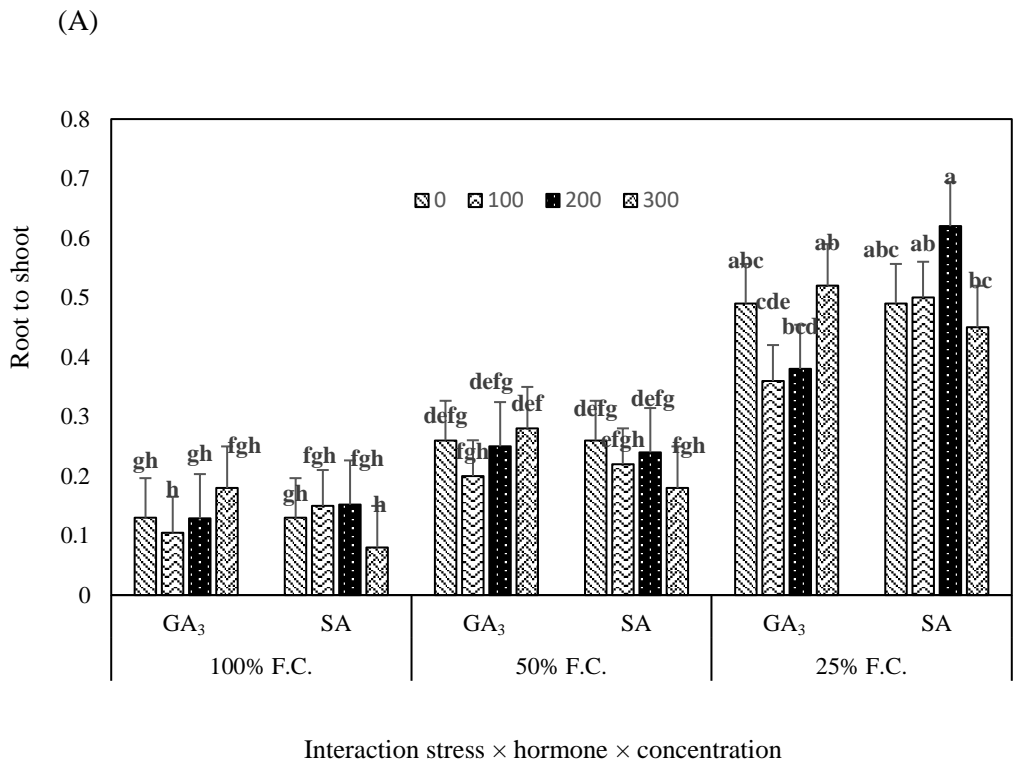


Fig. 1. Interaction between water stress × hormone type × concentration on: (A) root to shoot ratio and (B) plant height in Persian petunia (*Petunia* sp.). Each value represents the mean of four replicates with SE determined. Different letters within each column indicate significant differences according to Tukey test ($P \leq 0.05$)

Table 2. Analysis of variance to evaluate effects of water stress and hormone treatments on stomatal conductance and biochemical traits in Persian petunia (*Petunia* sp.)

Source of variation	df	Stomatal conductance	Proline	Chlorophyll a	Chlorophyll b	Chlorophyll total
Stress (A)	2	1797.88**	4.35*	3804.42**	5053.67**	17553.4**
Hormone (B)	1	436.1**	3.41 ^{ns}	685.85**	178.73**	1564.8**
Concentration (C)	3	257.09**	19.31**	4634.47**	35649.12**	32785.1**
A*B	2	358.8**	2.89 ^{ns}	2019.9**	35312.92**	53698.9**
A*C	6	141.1**	0.587 ^{ns}	12409.6**	3006.73**	9391.4**
B*C	3	311.4**	1.18 ^{ns}	2151.49**	15776.9**	19325.7**
A*B*C	6	74.91 ^{ns}	0.647 ^{ns}	331.36**	18102.4**	21520.03**
Error	48	34.33	1.02	3.76	12.83	19.63

* significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; ns: not significant

The reduction in plant height and leaf area observed in the present research under water stress can be related to a decline in both cell division and enlargement [Idrees et al. 2010].

Flower production and days to flowering. Water deficit treatment appeared to have no effect on the number of days to flowering (Tab. 1). However, PGRs showed significant effect on days to flowering, so that the earliest (60.41 d) and the latest (61.8 d) day to flowering were related to SA and GA₃ treatments, respectively. These results are in agreement with the findings of Martín-Mex et al. [2003] on *Gloxinia* and Martín-Mex et al. [2005] on *Saintpaulia* who reported that foliar spray of SA accelerated the flowering time compared to untreated control. The total number of flowers was significantly ($P \leq 0.01$) affected by the interaction of stress \times PGRs type \times concentration (Tab. 1). The highest total number of flowers (47.33) was obtained in plants irrigated with 100% F.C. along with SA 300 mg L⁻¹ (Fig. 2). Similarly, Cai et al. [2012] reported a decrease in flower production in garden roses when exposed to water deficit stress.

It was reported that SA can stimulate flowering, increase flower life [Singh and Usha 2003], and enhance photosynthetic and growth rates [Khan et al. 2003]. SA treatment also stimulates flowering in Lemna plants [Raskin 1992]. It seems that reduction in flower production under water deficit may be due to a decrease in stomatal conductance and the resulting rate of net photosynthesis under water stress.

Stomatal conductance and relative water content. In general, plants subjected to severe water stress exhibit about 66% stomatal conductance rate lower than control plants irrigated with 100% F.C. However, PGRs showed significant effect on stomatal conductance, as the plants sprayed with SA showed higher stomatal conductance than GA₃ treatments. Moreover, stomatal conductance was significantly ($P \leq 0.01$) affected by the interaction of stress \times PGRs type (Fig. 3). Under stress conditions, a reduction in stomatal conductance could have protective effects because it allows the plant to save water and to improve the water use efficiency [Chaves et al. 2009]. Stomatal control is the major physiological factor in the optimization of water use in drought conditions [Giorio et al. 1999]. This confirms previous reports on various plant species such as *Amaranthus* sp. [Liu and Stützel 2004], *Rosa hybrida* [Cai et al. 2012], and *Olea europaea* L. [Guerfel et al. 2009]. The ameliorative effect of SA on growth of crop plants in abiotic stress conditions may have been due to its role in nutrient uptake, water relations, stomatal regulation, photosynthesis and growth [Khan et al. 2003, Arfan et al. 2007]. Other researchers have confirmed that the decrease of stomatal conductance under drought stress was lower in SA-treated than untreated plants [Waseem et al. 2006, Saruhan et al. 2012]. However, it appears that the influence of SA application on stomatal conductivity may depend, among other things, on its concentration, the mode of application, duration of

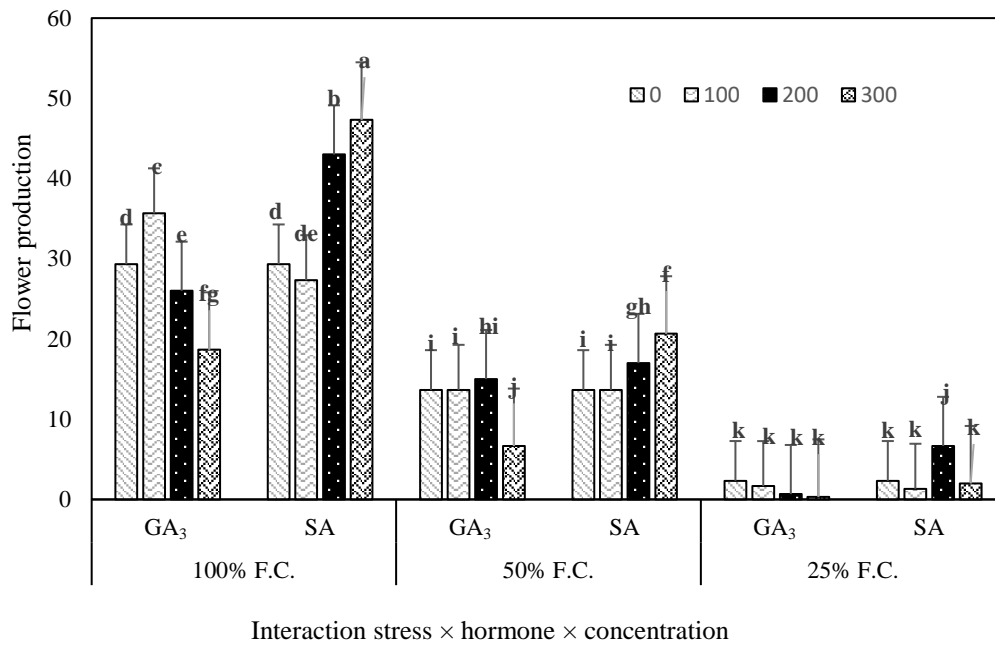


Fig. 2. Interaction between water stress × hormone type × concentrations on flower production in Persian petunia (*Petunia* sp.). Each value represents the mean of four replicates with SE determined. Different letters within each column indicate significant differences according to Tukey test ($P \leq 0.05$)

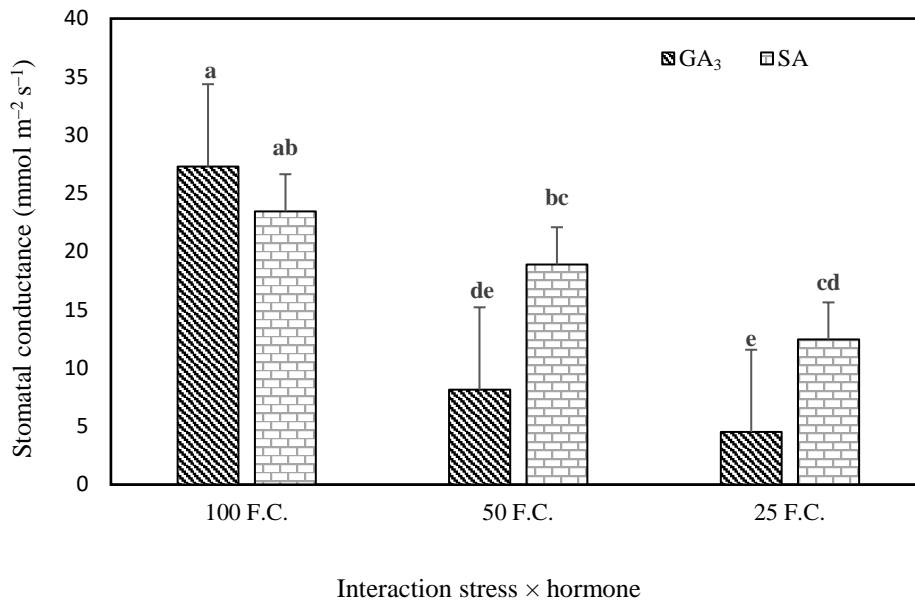
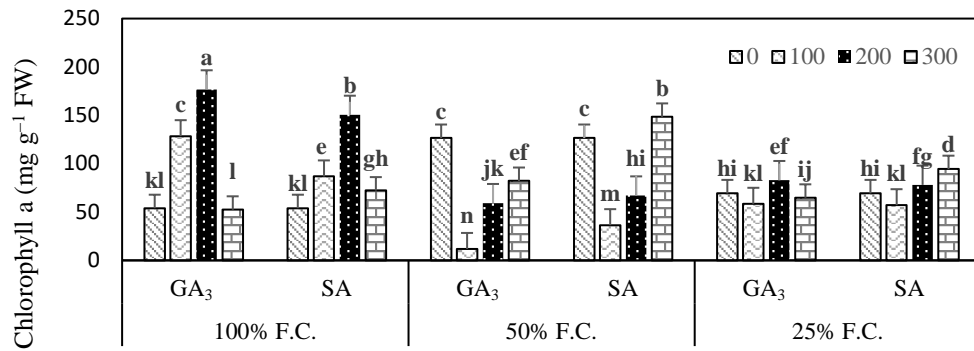
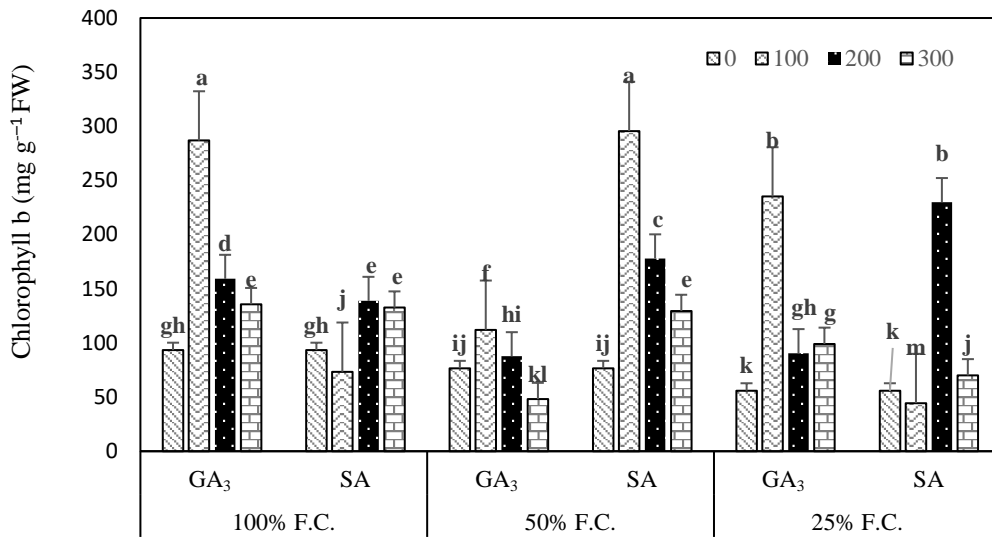


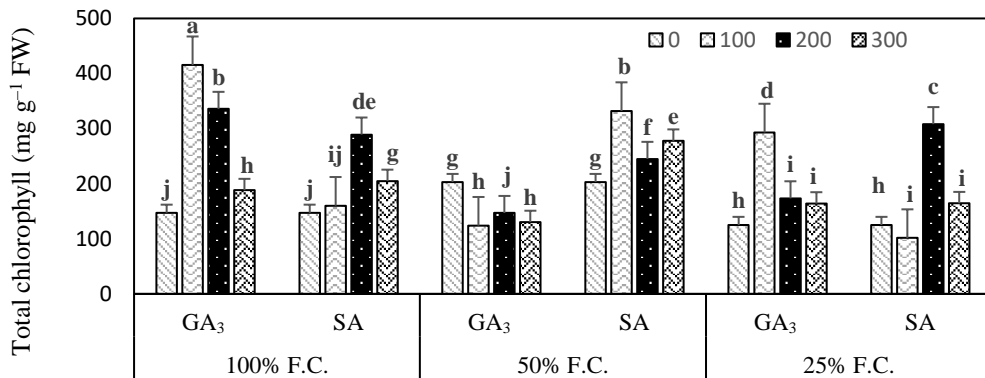
Fig. 3. Interaction between water stress × hormones on stomatal conductance in Persian petunia (*Petunia* sp.). Each value represents the mean of four replicates with SE determined. Different letters within each column indicate significant differences according to Tukey test ($P \leq 0.05$)



Interaction stress × hormone × concentration



Interaction stress × hormone × concentration



Interaction stress × hormone × concentration

Fig. 4. Interaction between water stress × hormone × concentration on: (A) chlorophyll a, (B) chlorophyll b and (C) total chlorophyll in Persian petunia (*Petunia* sp.). Each value represents the mean of four replicates with SE determined. Different letters within each column indicate significant differences according to Tukey test ($P \leq 0.05$)

treatment, species and physiological state of the plants [Hayat et al. 2013].

Statistical analysis of the leaf relative water content showed a significant difference between water treatments and the two PGR types. Water deficit treatment significantly ($P \leq 0.05$) decreased relative water content. The highest RWC (69.77%) was obtained in plants irrigated with 100% of water need, whereas the lowest one (57.55%) was observed in the plants exposed with 25% F.C. (Fig. 4). Furthermore, PGRs had significant effect on relative water content, so that plants sprayed with SA showed higher relative water content than GA_3 (Tab. 2). Similar results were obtained when sunflower plants were sprayed with SA, where RWC was significantly improved in stressed plants [Hussain et al. 2009]. Relative water content was significantly ($P \leq 0.05$) affected by interaction between water deficit \times concentration (Tab. 2). The decreased RWC under water deficit stress observed in this study is consistent with the previous studies on *Dianthus caryophyllus* [Álvarez et al. 2009], *Bougainvillea* [Cirillo et al. 2014], and *Rosa hybrida* [Dolatkahi et al. 2020]. The reduction in RWC under deficit irrigation could be the cause of the stomatal reductions and other physiological adaptations such as lower leaf area development, which both responses could contribute to reduce the total water consumption [Álvarez et al. 2009].

Photosynthetic pigments. Data presented in Table 2 show that chlorophyll content was significantly ($P \leq 0.01$) affected by interaction of stress \times PGRs type \times concentration. In our research petunia plants sprayed with SA showed higher chlorophyll content than non SA (Fig. 4). Photosynthetic pigments (chlorophyll a and b, and total chlorophyll) in the leaves diminished with water stress severity. The adverse effect of prolonged drought stress on photosynthetic pigments can be attributed to inhibition of pigment biosynthesis, instability of protein complexes and destruction of chlorophyll by increased activity of chlorophyll degrading enzyme chlorophyllase under stress condition [Flexas and Medrano 2002], as well as damage to the photosynthetic apparatus [Bolla et al. 2009]. A decrease in the chlorophyll concentration would be a typical symptom of oxidative stress and has been observed in water-stressed plants [Hazrati et al. 2016].

The enhancing effects of SA on photosynthetic pigment could be attributed to its stimulatory effects on Rubisco activity and photosynthesis. SA can induce the synthesis of protein kinases, which play an important role in regulating cell division, differentiation and morphogenesis [Zhang and Liu 2001, El-Tayeb 2005]. SA induced conservation of water in stressed plants also results in the protection of nitrate reductase activity (NRA) in SA treated and drought stressed plants.

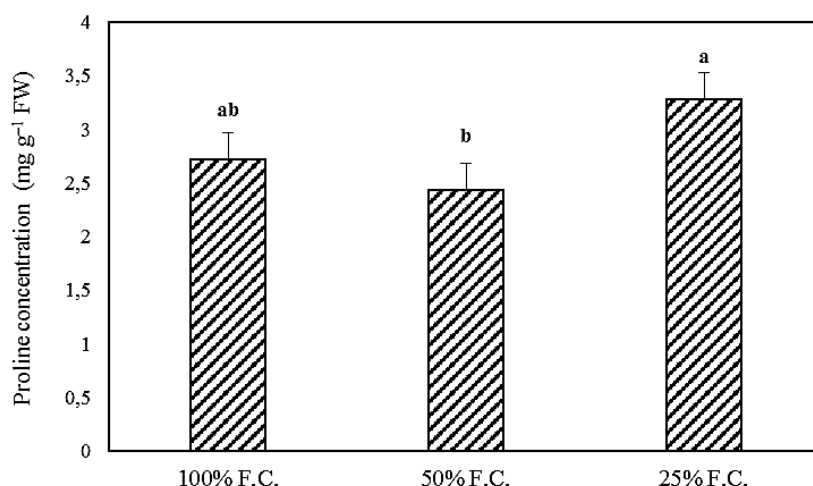


Fig. 5. Effect of water stress on proline accumulation in Persian petunia (*Petunia* sp.). Each value represents the mean of four replicates with SE determined. Different letters within each column indicate significant differences according to Tukey test ($P \leq 0.05$)

Proline accumulation. Proline accumulation significantly ($P \leq 0.05$) increased in the plants exposed to 25% F.C. (Tab. 2, Fig. 5). There was no desirable effect of GA₃ and SA on free proline concentration (Tab. 2). The accumulation of amino acids, such as proline, with decreasing soil matric potential might also have contributed to the process of osmoregulation inside the cell, as well as to other physiological and biochemical activities [Alhadi et al. 1999]. Plants exposed to water stress accumulate free proline, as a basic strategy for increasing the cell osmotic potential, facilitating water absorption [Ashraf and Foolad 2007] and reducing cell injury [Anjum et al. 2011]. Proline accumulation, considered a general marker of the drought tolerance [Ahmed et al. 2009, Liu et al. 2011], permits osmotic adjustment, which results in water retention and avoidance of cell dehydration [Blum 2005]. The results of the present study are in agreement with the findings of Idrees et al. [2010] for lemongrass and Yamada et al. [2005] for petunia, who reported increase in proline concentration in plants exposed to water stress. However, SA application under drought stress failed to alter proline content compared to the stressed plant grown without SA application.

CONCLUSION

In this study, fresh and dry weight of root and foliar, plant height, leaf area, the total number of flowers, stomatal conductance, relative water content and photosynthetic pigments were decreased, while free proline accumulation increased with decreasing water availability. Foliar application of SA at 200 mg L⁻¹ improved stomatal conductance and relative water content in water-stressed plants. Moreover, SA-treated plants produced higher flowers at all water deficit levels compared to GA₃ sprayed plants. This study confirms that exogenous application of SA not only can alleviate the detrimental effects of water stress, but exhibited a significant improvement in flower production of Persian petunia as compared to water stressed plants.

REFERENCES

- Ahmad, S., Ahmad, R., Ashraf, M.Y., Ashraf, M., Waraich, E.A. (2009). Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. *Pak. J. Bot.*, 41(2), 647–654.
- Ahmed, C.B., Rouina, B.B., Sensoy, S., Boukhris, M., Abdallah, F.B. (2009). Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. *Environ. Exp. Bot.*, 67, 345–352.
- Alhadi, F.A., Yasseen, B.T., Jabr, M. (1999). Water stress and gibberellic acid effects on growth of fenugreek plants. *Irrig. Sci.*, 18(4), 185–190.
- Ali, H.M., Siddiqui, M.H., Basalah, M.O., Al-Wahaibi, M.H., Sakran, A.M., Al-Amri, A. (2012). Effects of gibberellic acid on growth and photosynthetic pigments of *Hibiscus sabdariffa* L. under salt stress. *Afr. J. Biotechnol.*, 11(4), 800–804.
- Álvarez, S., Navarro, A., Bañón, S., Sánchez-Blanco, M.J. (2009). Regulated deficit irrigation in potted *Dianthus* plants: Effects of severe and moderate water stress on growth and physiological responses. *Sci. Hortic.*, 122(4), 579–585.
- Anjum, S.A., Xie, X.Y., Wang, L.C., Saleem, M.F., Man, C., Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *Afr. J. Agric. Res.*, 6, 2026–2032.
- Arfan, M., Athar, H.R., Ashraf, M. (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J. Plant Physiol.*, 164(6), 685–694.
- Ashraf, M.F., Foolad, M.R. (2007). Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.*, 59, 206–216.
- Athar, H., Ashraf, M. (2005). Photosynthesis under drought stress. In: *Handbook Photosynthesis*, Pessarakli, M. (Ed.), 2nd ed. CRC Press, New York, 795–810.
- Bano, A., Yasmeen, S. (2010). Role of phytohormones under induced drought stress in wheat. *Pak. J. Bot.*, 42(4), 2579–2587.
- Bates, L.S., Waldern, R.P., Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil.*, 39: 205–207.
- Bezrukova, M., Sakhabutdinowa, V., Fatkhutdinova, R. (2001). The role of hormonal changes in protective action of salicylic acid on growth of wheat seedlings under water deficits. *Agrochemiya (Russ.)*, 2, 51–54.
- Blum, A. (2005). Drought resistance, water-use efficiency, and yield potential are they compatible, dissonant, or mutually exclusive? *Aust. J. Agric. Res.*, 56, 1159–1168.
- Bolla, A., Koukourikou-Petridou, M., Voyiatzis, D., Chimonidou, D. (2009). Physiological responses associated

- to substrate water availability of *Rosa* ‘Eurored’ plants grown in soilless greenhouse conditions. *Sci. Hortic.*, 121(1), 80–83.
- Cai, X., Starman, T., Niu, G., Hall, C., Lombardini, L. (2012). Response of selected garden roses to drought stress. *HortScience*, 47(8), 1050–1055.
- Chaves, M.M., Flexas, J., Pinheiro, C. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.*, 103, 551–560.
- Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T., Pinheiro, C. (2002). How plants cope with water stress in the field? Photosynthesis and growth. *Ann. Bot.*, 89, 907–916.
- Cirillo, C., Rouphael, Y., Caputo, R., Raimondi, G., De Pascale, S. (2014). The influence of deficit irrigation on growth, ornamental quality, and water use efficiency of three potted *Bougainvillea* genotypes grown in two shapes. *HortScience*. 49(10), 1284–1291.
- Colebrook, E.H., Thomas, S.G., Phillips, A.L., Hedden, P. (2014). The role of gibberellin signalling in plant responses to abiotic stress. *J. Exp. Biol.*, 217(1), 67–75.
- Delauney, A.J., Verma, D.P.S. (1993). Proline biosynthesis and osmoregulation in plants. *Plant J.*, 4, 215–223.
- Dolatkhahi, A., Shoor, M., Bannayan, M., Tehranifar, A., Alizadeh, A. (2020). Water deficit decreases gas exchange parameters and marketable quality of *Rosa hybrida* ‘Club-Nika’ irrespective of training systems. *J. Agr. Sci. Tech.*, 22(3), 837–849.
- El-Tayeb, M.A. (2005). Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul.*, 45, 215–224.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. *Agron Sustain Dev.*, 29, 185–212.
- Flexas, J., Medrano, H. (2002). Energy dissipation in C3 plants under drought. *Funct. Plant Biol.* 29, 1209–1215.
- Giorio, P., Sorrentino, G., d’Andria, R. (1999). Stomatal behaviour leaf water status and photosynthetic response in field-grown olive trees under water deficit. *Environ. Exp. Bot.*, 42, 95–104.
- Guerfel, M., Baccouri, O., Boujnah, D., Chaïbi, W., Zarrouk, M. (2009). Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (*Olea europaea* L.) cultivars. *Sci. Hortic.*, 119(3), 257–263.
- Hayat, Q., Hayat, S., Irfan, M., Ahmad, A. (2010). Effect of exogenous salicylic acid under changing environment: a review. *Environ. Exp. Bot.*, 68(1), 14–25.
- Hayat, S., Ahmad, A., Alyemini, M.N. (2013). *Salicylic Acid: Plant Growth and Development*. Springer Science & Business Media, Dordrecht.
- Hazrati, S., Tahmasebi-Sarvestani, Z., Modarres-Sanavy, S.A.M., Mokhtassi-Bidgoli, A., Nicola, S. (2016). Effects of water stress and light intensity on chlorophyll fluorescence parameters and pigments of *Aloe vera* L. *Plant Physiol. Biochem.*, 106, 141–148.
- Hesami, A., Dolatkhahi, A. (2016). Plant Growth Regulators Impact on Vegetative and Reproductive Characteristics of *Gladiolus* Cut Flowers (*Gladiolus hybridus* Hort.). *JOP.*, 6(1), 33–38.
- Huerta, L., Forment, J., Gadea, J., Fagoaga, C., Pena, L., Pérez-Amador, M.A., García-Martínez, J.L. (2008). Gene expression analysis in citrus reveals the role of gibberellins on photosynthesis and stress. *Plant Cell Environ.*, 31, 1620–1633.
- Hussain, M., Malik, M.A., Farooq, M., Khan, M.B., Akram, M., Saleem, M.F. (2009). Exogenous glycine betaine and salicylic acid application improves water relations, allometry and quality of hybrid sunflower under water deficit conditions. *J. Agron. Crop Sci.*, 195, 98–109.
- Idrees, M., Khan, M.M.A., Aftab, T., Naeem, M., Hashmi, N. (2010). Salicylic acid-induced physiological and biochemical changes in lemongrass varieties under water stress. *J. Plant Interact.*, 5(4), 293–303.
- Kar, R.K. (2011). Plant responses to water stress: role of reactive oxygen species. *Plant. Signal. Behav.*, 6(11), 1741–1745.
- Kaur, S., Gupta, A.K., Kaur, N. (2000). Effect of GA₃, kintin and indole acetic acid on carbohydrate metabolism in chickpea seedlings germinating under water stress. *Plant Growth Regul.*, 30(1), 61–70.
- Kawano T., Muto, S. (2000). Mechanism of peroxidase actions for salicylic acid-induced generation of active oxygen species and an increase in cytosolic calcium in tobacco cell suspension culture. *J. Exp. Bot.*, 51, 685–693.
- Kaya, C., Kirnak, H., Higgs, D., Saltali, K. (2002). Supplementary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high salinity. *Sci. Hortic.*, 93, 65–74.
- Khan, W., Prithiviraj, B., Smith, D.L. (2003). Photosynthetic responses of corn and soybean to foliar application of salicylates. *J. Plant Physiol.*, 160(5), 485–492.
- Lichtenthaler, H.K., Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.*, 11, 591–592.
- Liu, C., Liu, Y., Guo, K., Fan, D., Li, G., Zheng, Y., Yu, L., Yang, R. (2011). Effect of drought on pigments, osmotic

- adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. *Environ. Exp. Bot.*, 71(2), 174–183.
- Liu, F., Stützel, H. (2004). Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Sci. Hortic.*, 102, 15–27.
- Martín-Mex, R., Villanueva-Couoh, E., Herrera-Campos, T., Larque-Saavedra, A. (2005). Positive effect of salicylates on the flowering of African violet. *Sci. Hortic.*, 103, 499–502.
- Martín-Mex, R., Villanueva-Couoh, E., Uicab-Quijano, V. (2003). Positive effect of salicylic acid on the flowering of gloxinia. In: *Proceedings 31st Annual Meeting. Plant Growth Regulatory Society America, Canada: Vancouver*, 149–151.
- Patel, M.S., Golakiya, B.A. (1988). Effect of Water-Stress on Yield Attributes and Yield of Groundnut (*Arachis Hypogaea* L.). *Indian J. Agric. Sci.*, 58(9), 701–703.
- Pirasteh-Anosheh, H., Emam, Y., Pessarakli, M. (2013). Changes in endogenous hormonal status in corn (*Zea mays*) hybrids under drought stress. *J. Plant Nutr.*, 36(11), 1695–1707.
- Raskin, I. (1992). Role of salicylic acid in plants. *Annu. Rev. Plant Physiol.*, 43, 439–463.
- Saruhan, N., Saglam, A., Kadioglu, A. (2012). Salicylic acid pretreatment induces drought tolerance and delays leaf rolling by inducing antioxidant systems in maize genotypes. *Acta Physiol. Plant.*, 34, 97–106.
- Shamshiri, M.H., Mozafari, V., Sedaghati, E., Bagheri, V. (2011). Response of petunia plants (*Petunia hybrida* cv. Mix) inoculated with *Glomus mosseae* and *Glomus intraradices* to phosphorous and drought stress. *J. Agr. Sci. Tech.*, 13, 929–942.
- Shao, H.B., Chu, L.Y., Jaleel, C.A., Zhao, C.X. (2008). Water-deficit stress-induced anatomical changes in higher plants. *C. R. Biol.*, 331, 215–225.
- Singh, B., Usha, K. (2003). Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul.*, 39(2), 137–141.
- Tahir, M.H.N., Mehid, S.S. (2001). Evaluation of open pollinated sunflower (*Helianthus annuus* L.) populations under water stress and normal conditions. *Int. J. Agric. Biol.*, 3, 236–238.
- Taiz, L., Zeiger, E. (2006). *Plant Physiology Fourth Edition* Sinauer Associates, Inc. Publishers. Sunderland, Massachusetts.
- Umebese, C.E., Olatimilehin, T.O., Ogunsusi, T.A. (2009). Salicylic acid protects nitrate reductase activity, growth and proline in amaranth and tomato plants during water deficit. *Am. J. Agric. Biol. Sci.*, 4(3), 224–229.
- Van Iersel, M.W., Dove, S., Kang, J.G., Burnett, S.E. (2010). Growth and water use of petunia as affected by substrate water content and daily light integral. *HortScience*, 45(2), 277–282.
- Verbruggen, N., Hermans, C. (2008). Proline accumulation in plants: a review. *Amino acids.*, 35(4), 753–759.
- Verma, V., Ravindran, P., Kumar, P.P. (2016). Plant hormone-mediated regulation of stress responses. *BMC Plant Biol.*, 16(1), pp. 86.
- Wang, C., Yang, A., Yin, H., Zhang, J. (2008). Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. *J. Integr. Plant Biol.*, 50(4), 427–434.
- Waseem, M., Athar, H.U.R., Asahrafi, M. (2006). Effect of salicylic acid applied through rooting medium on drought tolerance of wheat. *Pak. J. Bot.*, 38, 1127–1136.
- Waterland, N.L., Campbell, C.A., Finer, J.J., Jones, M.L. (2010). Abscisic acid application enhances drought stress tolerance in bedding plants. *HortScience*. 45(3), 409–413.
- Yamada, M., Morishita, H., Urano, K., Shiozaki, N., Yamaguchi-Shinozaki, K., Shinozaki, K., Yoshida, Y. (2005). Effects of free proline accumulation in petunias under drought stress. *J. Exp. Bot.*, 56(417), 1975–1981.
- Yamasaki, S., Dillenburg, L.R. (1999). Measurements of leaf relative water content in *Araucaria angustifolia*. *R. Bras. Fisiol. Veg.*, 11, 69–75.
- Zarghami, M., Shoor, M., Ganjeali, A., Moshtaghi, N., Tehranifar, A. (2014). Effect of salicylic acid on morphological and ornamental characteristics of *Petunia hybrida* at drought stress. *Indian. J. Fundam. Appl. Life. Sci.*, 4(3), 523–532.
- Zhang, S., Liu, Y. (2001). Activation of salicylic acid-induced protein kinase, a mitogen-activated protein kinase, induces multiple defense responses in tobacco. *Plant Cell.*, 13, 1877–1889.