https://doi.org/10.1590/1678-4499.20230041

Spermine decreases ethylene and increases sugars and phenolic compounds in nasturtium flowers grown under drought and salt stress

Toshik Iarley da Silva[1], Marlon Gomes Dias[2], Lucas Bretas Barbosa[2], Nícolas Oliveira de Araújo[2], Felipe Douglas Ferreira[2], José Antônio Saraiva Grossi[2], Franciscleudo Bezerra da Costa[1], Cláudia Araújo Marco[3], Dimas Mendes Ribeiro[4]

1. Universidade Federal de Campina Grande – Centro de Ciências e Tecnologia Agroalimentar – Pombal (PB), Brazil.
2. Universidade Federal de Viçosa – Departamento de Agronomia – Viçosa (MG), Brazil.
3. Universidade Federal do Cariri – Centro de Ciências Agrárias e da Biodiversidade – Crato (CE), Brazil.
4. Universidade Federal de Viçosa – Departamento de Biologia Vegetal – Viçosa (MG), Brazil.

Received: Mar. 3, 2023 | **Accepted:** May 8, 2023

Section Editor: Juliana Sanches

***Corresponding author:** iarley.toshik@gmail.com

How to cite: Silva, T. I., Dias, M. G., Barbosa, L. B., Araújo, N. O., Ferreira, F. D., Grossi, J. A. S., Costa, F. B., Marco, C. A. and Ribeiro, D. M. (2023). Spermine decreases ethylene and increases sugars and phenolic compounds in nasturtium flowers grown under drought and salt stress. Bragantia, 82, e20230041. https://doi.org/10.1590/1678-4499.20230041

ABSTRACT: Nasturtium (*Tropaeolum majus*) is an ornamental and medicinal plant that has edible flowers. Drought and salt stress decrease flower production and quality, including affecting sugar metabolism and ethylene production. Ethylene can accelerate the nasturtium senescence process, decreasing its postharvest quality. The use of polyamines, mainly spermine, may be a strategy for reducing the harmful effects of these stresses on the metabolism of sugars and phenolic compounds and for decreasing the production of ethylene, which accelerates senescence, in nasturtium flowers. Therefore, the objective here was to evaluate the role of spermine application on sugar and phenolic compounds and on ethylene production in nasturtium flowers grown under drought and salt stress. Two experiments were performed in isolation and at the same time in order to achieve this. Spermine down-regulated ethylene production and up-regulated the content of sugars and phenolic compounds on nasturtium flowers grown under drought and saline stress. Sugars and phenolic compounds down-regulated ethylene production in nasturtium flowers. Spermine can be used to mitigate the harmful effects of drought and salt stress on nasturtium flowers by increasing sugar and phenolic compounds and decreasing ethylene production.

Key words: *Tropaeolum majus*, abiotic stresses, polyamine, sugar and phenolic metabolism.

INTRODUCTION

Nasturtium (*Tropaeolum majus* L., Tropaeolaceae) is a plant with edible flowers grown in several regions of the world. Nasturtium flowers are one of the main commercialized edible flowers. The spicy flavor of its flowers gives this plant a peculiar characteristic. Fresh leaves and flowers are mainly consumed in salads and sandwiches (Xu et al. 2021), but they are also excellent for the decoration of dishes and an alternative cultivation for small and medium producers. In addition, this plant has glucosinolates, flavonoids, fatty acids and thiocyanates, a set of nutraceutical substances that are very important for human health (Valsalam et al. 2019).

Nasturtium is a plant cultivated in many parts of the world. However, many regions have several abiotic stresses that can decrease plant growth and production. Plants, under natural and/or agricultural conditions, are exposed to various environmental stresses (Seleiman et al. 2021). Salt stress and drought are two of the main abiotic stresses that can affect plant metabolism (Ma et al. 2020). About 10% of arable land worldwide suffers from drought stress and salinity, especially in arid or semi-arid regions (Liu et al. 2022). Soil salinization increases annually around the world due to climate change, high evaporation, low precipitation, poor water management, fertilization in crop areas and irrigation with saline water (Nachshon 2018, Ors et al. 2021). Drought is also a concern for current agricultural production, given the scarcity of water and irregular rainfall in several regions (Shemi et al. 2021). Plant responses to both salt stress and drought are similar, such as initial osmotic stress and decreased water potential, which lead to the production of reactive oxygen species (ROS), stomatal closure, decreased water absorption, and reduced growth and production (Yolcu et al. 2021).

Drought and saline stresses make it difficult to grow edible flowers in several places around the world, especially in arid and semi-arid regions. These are the two most common abiotic stresses, which cause disturbances in the growth and productivity of crops at all stages of development (Ors et al. 2021). These stresses can cause various types of damage to plant metabolism, such as osmotic stress, damage to the photosynthetic apparatus, decreased water use efficiency and even death in more extreme situations (Abd El-Mageed and Semida 2015). The search for alternatives to minimize the damage caused by abiotic stresses is constant. The use of phytohormones, such as polyamines, is a promising alternative to allow the acclimation of plants to drought and salt stress.

Polyamines participate in the regulation of plant growth and development. However, their metabolism undergoes deep changes during abiotic stress (Gonzalez et al. 2021). Polyamines are a group of aliphatic, polycationic and low-molecular-weight molecules with two or more amine groups synthesized from amino acids and are found in all living things (Michael 2016, Navakoudis and Kotzabasis 2022). Spermidine and putrescine are present in all organisms that synthesize polyamines, whereas spermine, thermospermine and cadaverine are not present in all organisms (Gerlin et al. 2021). Polyamines play a key role in redox homeostasis, as the increase in polyamines, especially spermine, acts to scavenge hydroxyl radicals and participates in increasing H_2O_2 levels through their catabolism by amine oxidases, thereby controlling ROS levels (Pottosin et al. 2014) and reducing the damage caused by them.

Polyamines, particularly spermine, interact with oxidative balance and sugar and nitrogen metabolism (amino acid transport/biosynthesis) (Sequera-Mutiozabal et al. 2016). Furthermore, polyamines influence the production of ethylene by impeding the transcription and activity of the enzyme 1-carboxylic acid-1-aminocyclopropane synthase (ACS), as well as the action of ethylene (Dias et al. 2010, Champa et al. 2015). This combined action of these factors can delay senescence by decreasing oxidative damage. Sugars can interact with ethylene biosynthesis and signaling to regulate flower senescence of ethylene-sensitive species (Yuan et al. 2012). Ethylene increases senescence, while polyamines inhibit it (Del Duca et al. 2014). The anti-senescence effect of polyamines is due to the inhibition of biosynthesis and polyamine-mediated ethylene action (Dias et al. 2010, Hasan et al. 2021).

The role of spermine in influencing the levels of sugars and phenolic compounds and in producing ethylene in nasturtium flowers has not yet been elucidated. Neither has this action on nasturtium flowers grown under drought and salt stress been adequately studied. Therefore, the objective here was to evaluate the role of spermine application on sugar and phenolic compounds and ethylene production in nasturtium flowers grown under drought and salt stress.

MATERIAL AND METHODS

Experiment location

Two experiments were performed in isolation at the same time to evaluate the role of spermine application on sugar and phenolic compounds and ethylene production in nasturtium flowers grown under drought and salt stress. The experiments were carried out in a greenhouse at the Department of Agronomy, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

Plant material and experimental design

Nasturtium seeds (var. Semi-dobrada sortida, Feltrin®) were sowing in a 128-cell polystyrene tray with commercial substrate (Topstrato). Seedlings were tranplanted in 1.2-L pots with commercial substrate (Topstrato), and transplanted at 12 days after planting. Spermine diluted in deionized water with 0.05% Tween 20 (v:v) was used as a surfactant to increase plant uptake. The control treatment was deionized water and 0.05% Tween 20. Plants were sprayed with about 10 mL of each solution or until completely wet. Spermine applications were made every seven days for four weeks.

The experiment to evaluate drought stress was distributed in a completely randomized design, in a 3 × 2 factorial scheme, with three irrigation depths (30, 50 and 80% of the pot's holding capacity) and two spermine doses (0 and 1 mM of spermine), with five replications. A pre-test was performed to determine the dose of spermine applied. The beginning of the treatments was 20 days after planting (eight days after transplanting). The determination of pot holding capacity (PHC) was performed according to the methodology described by Kämpf et al. (2006). The formula used was Eq. 1:

$$PHC = PW - (PWwhc - PWdry) \times WRD + PWdry \quad (1)$$

in which: PW: pot weight; PWwhc: water holding capacity (weight); PWdry: pot weight filled with completely dry substrate; WRD: water replacement depth (Girardi et al. 2016).

PHC maintenance was carried out daily in all vessels, weighing them and replacing the volume of water lost by evapotranspiration, using a scale with a capacity of 10 kg. The plants were fertigated with 4 $g \cdot L^{-1}$ of 20-20-20 fertilizer + micronutrients (Peters), once a week.

The experiment to evaluate salt stress was also distributed in a 3 × 2 factorial scheme, with three saline stress intensities–0, 40 (moderate salt stress) and 80 (severe salt stress) mM of NaCl, two spermine doses (0 and 1 mM of spermine – Silva et al. 2022a) and five repetitions. Salt stress was started at 20 days after planting.

Relative water content

Ten discs of flowers (1 cm in diameter) were used to determine the relative water content (RWC). After weighing and obtaining the fresh mass (FM), the flower discs were immersed in 10 mL of deionized water for 3 hours until reaching the turgid mass (TM). Then, the discs were placed in an oven at 65 °C for 48 hours to obtain the dry mass (DM). The RWC was calculated using Eq. 2:

$$RWC (\%) = [(FM-DM)/(TM-DM)] \times 100 \quad (2)$$

Analysis of reducing and non-reducing sugars

Soluble sugars were extracted from approximately 2 g of fresh leaf and homogenized in 80% ethanol heated to 85 °C. The extract was centrifuged at 12,000 g for 8 min. The supernatant was collected, and the precipitate was extracted once more with 80% ethanol. The content of total soluble sugars (TSS) was estimated using the sulfuric phenol method (Dubois et al. 1956). The assay containing 0.25 mL of supernatant, 0.25 mL of 5% phenol and 1.25 mL of concentrated H_2SO_4 was incubated at 30 °C for 20 minutes. After cooling, absorbance was measured at 490 nm. Sucrose was used as the standard, the TSS content being expressed as a percentage of TSS per fresh leaf mass.

The reducing sugar (RS) content was quantified using the 3,5-dinitrosalicylic acid (DNS) method proposed by Gonçalves et al. (2010), with modifications. An aliquot of 0.5 mL of the supernatant was added to 0.5 mL of the DNS reagent, and the tubes were heated in a water bath for 5 minutes. After cooling in an ice bath, 4 mL of water was added, and absorbance was read at 540 nm. Fructose was used as the standard, and the RS content was determined in %RS per fresh leaf mass. The non-reducing sugar content (NRS) was estimated by the difference between TSS and RS, with results expressed as %NRS per fresh leaf mass.

Analysis of total phenolic compounds

Total phenolic compounds were extracted from the same extract used for sugar analysis. The phenolic content was determined according to Fu et al. (2010), using gallic acid as a standard. The absorbance was determined by a spectrophotometer at 760 nm, and the content was expressed in $mg \cdot g^{-1}$ fresh mass.

Ethylene measurement

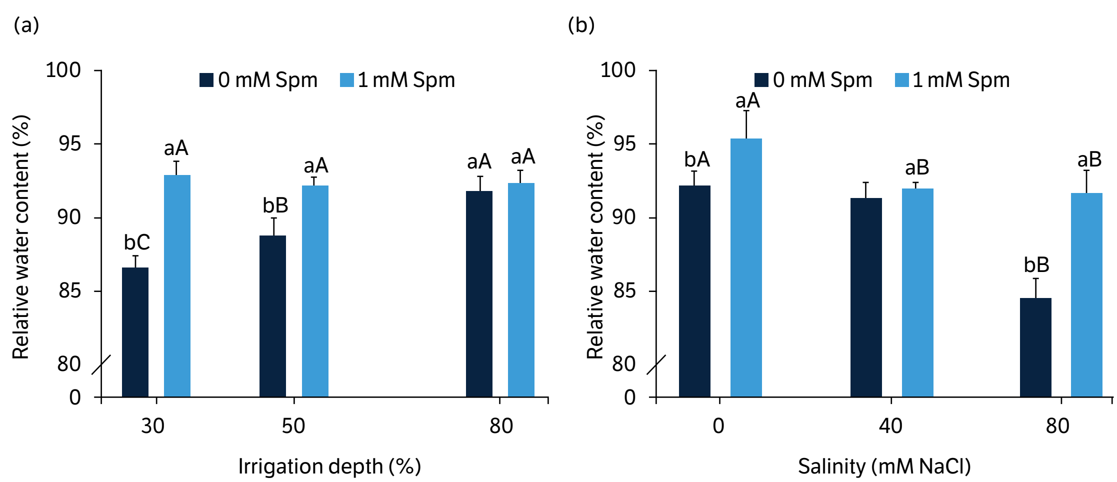
Ethylene production was determined using a sample of one flower placed in a 38-mL hermetically sealed container. After 17 hours, 1 mL of the internal atmosphere of the container was extracted with a syringe, and ethylene was quantified using a gas chromatograph (Hewlett-Packard 5890, series II). Column, inlet, and flame ionization detector temperatures were maintained at 60, 110 and 150 °C, respectively. Ethylene production was expressed as pmol ethylene g^{-1} FM h^{-1}.

Statistical analysis

Data was subjected to analysis of variance (ANOVA), and, when significant ($p \leq 0.05$), a comparison of means (Tukey test) was performed using the ExpDes statistical package (Ferreira et al. 2018). An analysis of canonical correspondence and confidence ellipses ($p \leq 0.01$) was performed to study the interrelationship between variables and factors using the candisc package (Friendly and Fox 2017). Pearson's correlation analysis was performed using the corrplot (Wei and Simko 2017) and PerformanceAnalytics (Peterson and Carl 2020) packages. The R statistical program (R Core Team 2021) was used to perform the statistical analyses.

RESULTS

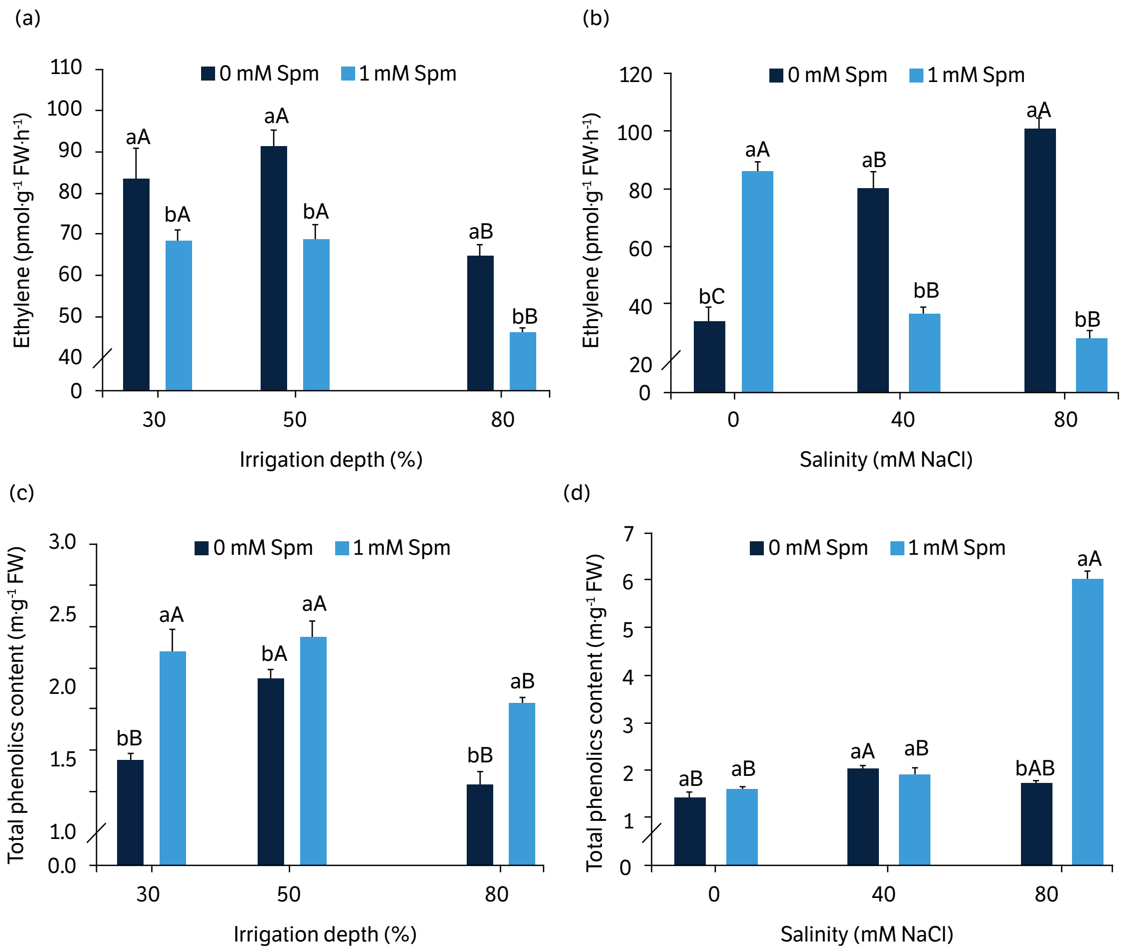
Spermine application increased the relative water content in nasturtium flowers grown under severe (6.83%) and moderate (3.66%) drought stress, as well as increased in flowers grown under severe salt stress (10.07%) (Fig. 1).



*Means followed by the same lowercase and uppercase letters do not differ by Tukey's test at 5% probability for spermine and drought/salt stresses, respectively. Values are mean ± standard deviation (n = 5).

Figure 1. Relative water content of *Tropaeolum majus* flowers grown under (a) drought stress and (b) salt stress*.

Spermine application decreased ethylene production in nasturtium flowers grown under moderate (24.35%) and severe (17.95%) drought stress and moderate (55.48%) and severe (72.31%) salt stress. Ethylene production was the same in flowers grown under moderate and severe drought stress, and the same behavior occurred in flowers under moderate and severe salt stress. Spermine application increased the content of total phenolic compounds in flowers grown under both moderate drought stress (48.91%) and severe drought stress (13.99%) and in flowers grown under severe salt stress (252.11%) (Fig. 2).

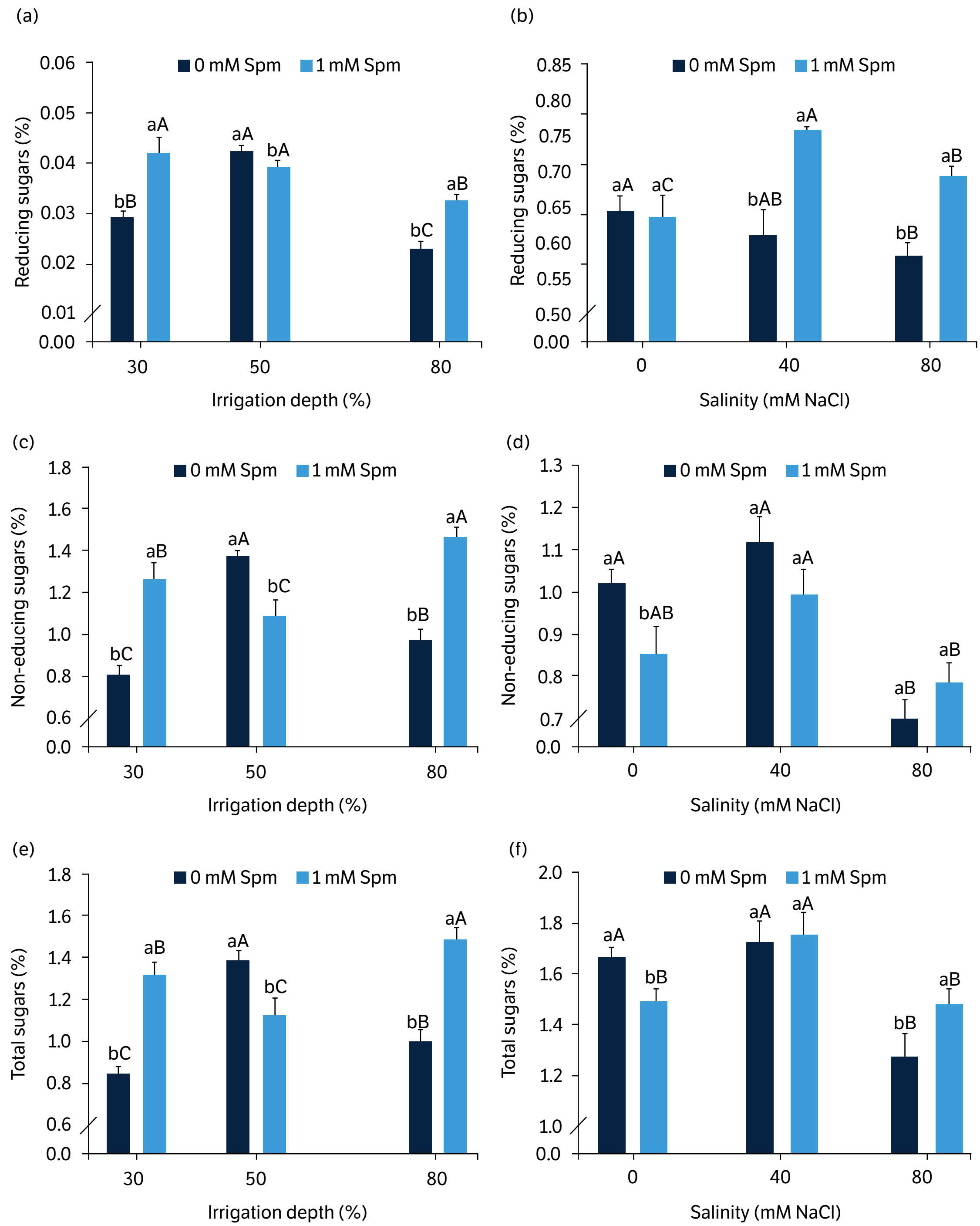


*Means followed by the same lowercase and uppercase letters do not differ by Tukey's test at 5% probability for spermine and drought/salt stresses, respectively. Values are mean ± standard deviation (n = 5).

Figure 2. Ethylene production and total phenolic compounds content of *Tropaeolum majus* flowers grown under (a and c) drought stress and (b and d) salt stress*.

Spermine application increased the levels of reducing sugars (44.75%), non-reducing sugars (56.51%) and total sugars (56.10%) in flowers grown under severe drought stress, but all of these parameters decreased in flowers under moderate drought stress (6.68, 18.49 and 18.13%, respectively). Flowers grown under spermine application and severe and moderate drought stress had a higher content of reducing sugars compared to plants without stress and with spermine (29.63 and 20.62%, respectively). Spermine increased reducing sugar levels in flowers grown under moderate (24.20%) and severe (19.06%) salt stress, as well as increasing total sugar levels in flowers under severe salt stress (16.30%) (Fig. 3).

A canonical variables analysis and confidence ellipses were performed to understand the interrelation between factors and variables. Ethylene production was more related to flowers grown without spermine and under moderate (50P0) and severe (30P0) drought stress. The content of total phenolic compounds (Phenolic) and reducing sugars (RS) had a greater relation with flowers grown under spermine application and under moderate (50P1) and severe (30P1) drought stress, while the content of NRS and TSS had a higher relation with flowers under spermine application and under severe drought stress and without this stress (80P1). The RWC had a greater relationship with plants under spermine application (Fig. 4a). For saline stress, ethylene production was more related to flowers grown under severe salt stress and without spermine application (80P0). The sugar contents and relative water content were more related with flowers grown under spermine application and moderate salt stress (40P1) and in plants without stress and without spermine application (0P0); there was no difference between these two treatments. The content of phenolic compounds was more related to flowers under severe salt stress and spermine application (80P1) (Fig. 4b).



*Means followed by the same lowercase and uppercase letters do not differ by Tukey's test at 5% probability for spermine and drought/salt stresses, respectively. Values are mean ± standard deviation (n = 5).

Figure 3. Reducing sugars, non-reducing sugars, and total sugars of *Tropaeolum majus* flowers grown under (a, c and e) drought stress and (b, d and f) salt stress*.

The content of NRS and TSS and RWC downregulated the ethylene production of nasturtium flowers grown under drought stress. The sugar contents upregulated the content of phenolic compounds (Fig. 5a). The contents of total phenolic compounds and sugars and relative water content downregulated the ethylene production of nasturtium flowers grown under salt stress. The contents of reducing and non-reducing sugars upregulated the content of phenolic compounds. The relative water content upregulated the sugar contents in nasturtium flowers (Fig. 5b).

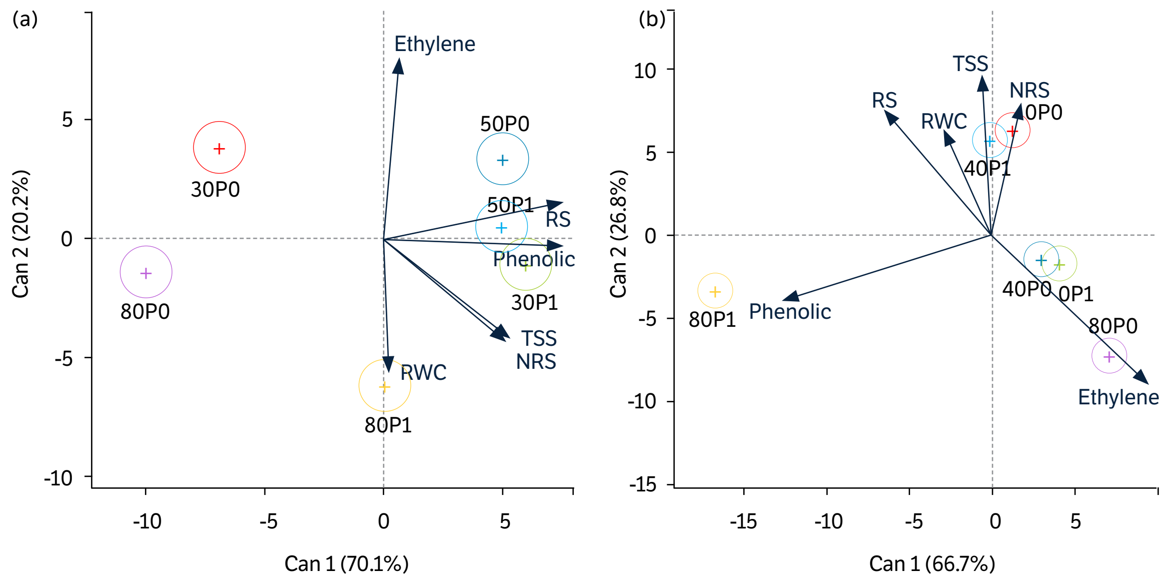
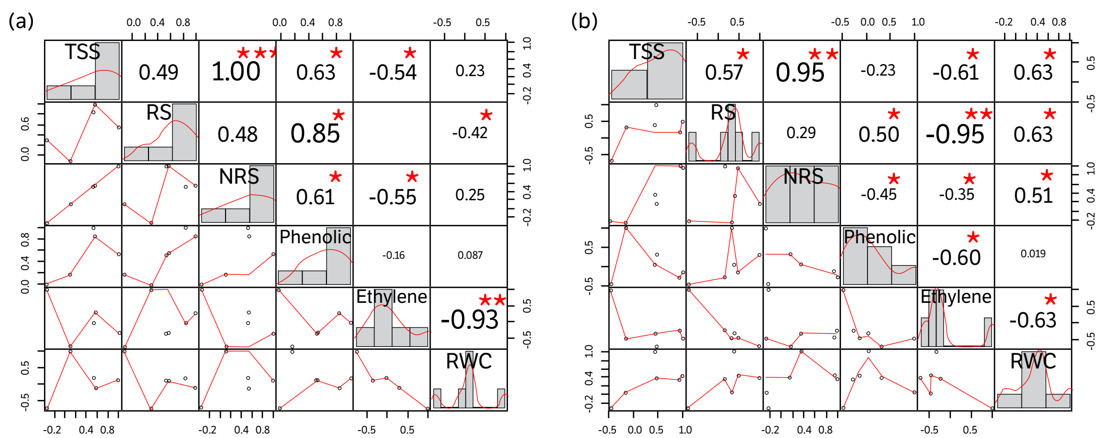


Figure 4. Canonical variables analysis and confidence ellipses of flowers of *Tropaeolum majus* grown under (a) drought stress and (b) salt stress.



TSS: total soluble sugars; RS: reducing sugar; NRS: non-reducing sugar content; RWC: relative water content; *significant at 5%; **significant at 1%.
Figure 5. Pearson's correlation between the analyzed variables of flowers of *Tropaeolum majus* grown under (a) drought stress and (b) salt stress.

DISCUSSION

Spermine application increased the relative water content of nasturtium flowers grown under drought and salt stress because this phytohormone improves the water status of cells (Ebeed et al. 2017), through osmoprotection and in the osmotic regulation that helps maintain turgor and cellular water status (Slama et al. 2015). Similar results were observed in nasturtium leaves grown under drought (Silva et al. 2022b) and salt stress (Silva et al. 2022a). In addition, the application of polyamines can induce the production of nitric oxide in plants (Tun et al. 2006, Wimalasekera et al. 2011, Silveira et al. 2021), which has been reported as signaling in plants under stress, which leads to the maintenance of water content, greater antioxidant capacity, and better stability of cell membranes (Gan et al. 2015).

Spermine application decreased ethylene production in nasturtium flowers grown under drought stress and salt stress due to antagonistic competition of polyamines with ethylene for the common precursor, S-adenosylmethionine (SAM) (Hasan et al. 2021). This precursor is converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by the action of the ACC synthase enzyme; in the final step of the hormone biosynthesis, ACC is oxidized to ethylene by the ACC oxidase enzyme (Pan et al. 2019).

Spermine decreased ethylene production in maize (*Zea mays* L.) under drought stress (Talaat and Shawky 2016). Spermine application increased the content of total phenolic compounds in flowers grown under the aforementioned stresses because this phytohormone facilitates the accumulation of phenolic compounds that act as scavengers of ROS or essential antioxidants to protect plants against oxidative damage (Ghabel and Karamian 2020). The increase in phenolic compounds in *Glycyrrhiza glabra* L. under cold stress has been reported to be due to the capacity of this antioxidant and ROS neutralization in response to stress (Ghabel and Karamian 2020). Thus, the increase in phenolic compounds may be an induced response to deal with oxidative stress (Bashandy et al. 2020). Spermine has been described as producing an antioxidant effect in flowers of *Nicotiana plumbaginifolia* L. (Nisar et al. 2015).

Spermine application increased the content of sugars in flowers grown under drought and salt stress, as this phytohormone plays an important role in carbohydrate synthesis and as a growth regulator in some biological processes associated with carbohydrate synthesis (Ghabel and Karamian 2020). Sugars are active osmolytes in decreasing abiotic stress in plants. Spermine has a key role in the regulation of active osmolytes in soybean (*Glycine max* L.) genotypes susceptible to drought stress due to maintenance of water status under adverse conditions (Dawood and Abeed 2020). In addition, spermine is associated with the modification of carbohydrate metabolism enzymes and promotes the maintenance of a higher content of sugars, especially sucrose, in plants (Song et al. 2015).

Spermine application increased the content of water-soluble carbohydrates (fructose and sucrose) in white clover (*Trifolium repens* L.) cultivars grown under drought stress (Li et al. 2015). It relieved the carbohydrate metabolism damage caused by cold in spinach (*Spinacia oleracea* L.) (He et al. 2002). The harmful effects of salt stress on sugar content are related to increased ROS production, which leads to carbohydrate oxidation. Furthermore, polyamines play an important role in scavenging ROS and maintaining membrane stability (Yi et al. 2018).

The accumulation of sugars produces an osmolytic effect which plays a large role in osmoprotection, regulation of osmotic adjustment, scavenging of free radicals and in providing membrane protection to alleviate the damaging effects of both salt stress and drought stress (Krasensky and Jonak 2012, Singh et al. 2015). Soluble sugars are associated with both ROS anabolism and catabolism, such as that which occurs in the pentose phosphate oxidative pathway entailed in NADPH production, which involves ROS elimination (Hu et al. 2012). Accumulation of sugars prevents cell membrane oxidation (Arabzadeh 2012), reduces the photosynthetic rate and stomatal closure (Osakabe et al. 2014) and maintains leaf water content (Xu et al. 2007) of plants under drought stress.

Ethylene production was more closely related to flowers grown under drought stress and salt stress without spermine because polyamines, including spermine, inhibit ACS (Takahashi et al. 2010), a key enzyme in ethylene synthesis, and ethylene is a direct inhibitor of arginine decarboxylase (ADC) and S-adenosylmethionine decarboxylase (SAMDC) (Pál et al. 2015). Spermine application decreased ethylene production in carnation (*Dianthus caryophyllus* L.) flowers (Lee et al. 1997). Moreover, the content of total phenolic compounds and sugars had a greater relationship with flowers grown under drought and salt stress with spermine application. This behavior is related to the fact that spermine is a phytohormone which acts on the metabolic defense mechanism against oxidative stress (Sequera-Mutiozabal et al. 2016). Phenolic compounds and sugars are non-enzymatic pathways for maintaining cellular homeostasis and eliminating ROS in plants under abiotic stresses. The increase in phenolic compounds and consequent decrease in ROS production in plants under stress was observed in *Anthurium andraeanum* Linden ex André (Simões et al. 2018), *Brassica napus* L. (Bashandy et al. 2020), *G. max* (Dawood and Abeed 2020) and *Oryza sativa* (Farooq et al. 2009).

The contents of total phenolic compounds and sugars downregulated the ethylene production of nasturtium flowers grown under drought stress and salt stress due to ethylene inducing respiratory activity and, consequently, a depletion of the carbohydrate content in flowers (Costa et al. 2020). Furthermore, sugars can downregulate ethylene synthesis, as observed in *Paeonia suffruticosa* Andrews flowers under glucose application, through decreased ethylene production due to delay and inhibition of ACC and ACS activity and suppression of ACC oxidase (Wang et al. 2014).

Additionally, it is worth noting that the crosstalk between ethylene and sugars, mainly glucose, occurs partially through the transcriptional regulation of genes involved in the biosynthesis of this phytohormone (Andriunas et al. 2011). The upregulation of sugars in proportion to the content of phenolic compounds may have been due to the activation of the flower system under stress to eliminate ROS (Torras-Claveria et al. 2012).

CONCLUSION

Spermine application downregulated ethylene production and upregulated the content of sugars and phenolic compounds in nasturtium flowers grown under drought stress and salt stress. Sugars and phenolic compounds downregulate ethylene production in these nasturtium flowers. Spermine can be used to mitigate the harmful effects of drought stress and salt stress in nasturtium flowers by maintaining sugar and phenolic compounds and decreasing ethylene production.

AUTHORS' CONTRIBUTION

Conceptualization: Silva, T. I., Dias, M. G., Barbosa, L. B., Araújo, N. O. and Ferreira, F. D.; **Methodology:** Silva, T. I.; **Investigation:** Silva, T. I., Dias, M. G., Barbosa, L. B., Araújo, N. O., Ferreira, F. D., Grossi, J. A. S., Costa, F. B., Marco, C. A. and Ribeiro, D. M.; **Writing – Original Draft:** Silva, T. I. and Dias, M. G.; **Writing – Review and Editing:** Silva, T. I., Dias, M. G., Barbosa, L. B., Araújo, N. O. and Ferreira, F. D.; **Supervision:** Grossi, J. A. S., Costa, F. B., Marco, C. A. and Ribeiro, D. M.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

FUNDING

Conselho Nacional de Desenvolvimento Científico e Tecnológico
[https://doi.org/10.13039/501100003593]

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
[https://doi.org/10.13039/501100002322]
Financial Code 001

ACKNOWLEDGMENTS

Not applicable.

REFERENCES

Abd El-Mageed, T. A. and Semida, W. M. (2015). Effect of deficit irrigation and growing seasons on plant water status, fruit yield and water use efficiency of squash under saline soil. Scientia Horticulturae, 186, 89-100. https://doi.org/10.1016/j.scienta.2015.02.013

Andriunas, F. A., Zhang, H. M., Weber, H., McCurdy, D. W., Offler, C. E. and Patrick, J. W. (2011). Glucose and ethylene signalling pathways converge to regulate trans-differentiation of epidermal transfer cells in *Vicia narbonensis* cotyledons. The Plant Journal, 68, 987-998. https://doi.org/10.1111/j.1365-313X.2011.04749.x

Arabzadeh, N. (2012). The effect of drought stress on soluble carbohydrates (sugars) in two species of *Haloxylon persicum* and *Haloxylon aphyllum*. Asian Journal of Plant Sciences, 11, 28-35. https://doi.org/10.3923/ajps.2012.28.35

Bashandy, S. R., Abd-Alla, M. H. and Dawood, M. F. (2020). Alleviation of the toxicity of oily wastewater to canola plants by the N2-fixing, aromatic hydrocarbon biodegrading bacterium *Stenotrophomonas* maltophilia-SR1. Applied Soil Ecology, 154, 103654. https://doi.org/10.1016/j.apsoil.2020.103654

Champa, W. H., Gill, M. I. S., Mahajan, B. V. C. and Bedi, S. (2015). Exogenous treatment of spermine to maintain quality and extend postharvest life of table grapes (Vitis vinifera L.) cv. Flame seedless under low temperature storage. LWT – Food Science and Technology, 60, 412-419. https://doi.org/10.1016/j.lwt.2014.08.044

Costa, L. C., Luz, L. M., Nascimento, V. L., Araujo, F. F., Santos, M. N., Franca, C. D. F., Silva, T. P., Fugate, K. K. and Finger, F. L. (2020). Selenium-ethylene interplay in postharvest life of cut flowers. Frontiers in Plant Science, 11, 2055. https://doi.org/10.3389/fpls.2020.584698

Dawood, M. F. and Abeed, A. H. (2020). Spermine-priming restrained water relations and biochemical deteriorations prompted by water deficit on two soybean cultivars. Heliyon, 6, e04038. https://doi.org/10.1016/j.heliyon.2020.e04038

Del Duca, S., Serafini-Fracassini, D. and Cai, G. (2014). Senescence and programmed cell death in plants: polyamine action mediated by transglutaminase. Frontiers in Plant Science, 5, 120. https://doi.org/10.3389/fpls.2014.00120

Dias, L. L. C., Ribeiro, D. M., Catarina, C. S., Barros, R. S., Floh, E. I. S. and Otoni, W. C. (2010). Ethylene and polyamine interactions in morphogenesis of *Passiflora cincinnata*: effects of ethylene biosynthesis and action modulators, as well as ethylene scavengers. Plant Growth Regulation, 62, 9-19. https://doi.org/10.1007/s10725-010-9478-5

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Biochemistry, 28, 350-356. https://doi.org/10.1021/ac60111a017

Ebeed, H. T., Hassan, N. M. and Aljarani, A. M. (2017). Exogenous applications of polyamines modulate drought responses in wheat through osmolytes accumulation, increasing free polyamine levels and regulation of polyamine biosynthetic genes. Plant Physiology and Biochemistry, 118, 438-448. https://doi.org/10.1016/j.plaphy.2017.07.014

Farooq, M., Wahid, A. and Lee, D. J. (2009). Exogenously applied polyamines increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. Acta Physiologiae Plantarum, 31, 937-945. https://doi.org/10.1007/s11738-009-0307-2

Ferreira, E. B., Cavalcanti, P. P. and Nogueira, D. A. (2018). ExpDes: Experimental Designs. R package version 1.2.0. Available at: https://CRAN.R-project.org/package=ExpDes. Accessed on: Jul. 12, 2022.

Friendly, M. and Fox, J. (2017). candisc: visualizing generalized canonical discriminant and canonical correlation analysis. R package version 0.8-0. Available at: https://CRAN.R-project.org/package=heplots. Accessed on: Jul. 12, 2022.

Fu, L., Xu, B.-T., Xu, X.-R., Qin, X.-S., Gan, R. Y. and Li, H. B. (2010). Antioxidant capacities and total phenolic contents of 56 wild fruits from South China. Molecules, 15, 8602-8617. https://doi.org/10.3390/molecules15128602

Gan, L., Wu, X. and Zhong, Y. (2015). Exogenously applied nitric oxide enhances the drought tolerance in hulless barley. Plant Production Science, 18, 52-56. https://doi.org/10.1626/pps.18.52

Gerlin, L., Baroukh, C and Genin, S. (2021). Polyamines: double agents in disease and plant immunity. Trends in Plant Science, 26, 1061-1071. https://doi.org/10.1016/j.tplants.2021.05.007

Ghabel, V. K. and Karamian, R. (2020). Effects of TiO2 nanoparticles and spermine on antioxidant responses of *Glycyrrhiza glabra* L. to cold stress. Acta Botanica Croatica, 79, 137-147. https://doi.org/10.37427/botcro-2020-025

Girardi, L. B., Peiter, M. X., Bellé, R. A., Robaina, A. D., Torres, R. R., Kirchner, J. H. and Ben, L. H. B. (2016). Evapotranspiration and crop coefficients of potted *Alstroemeria* × *Hybrida* grown in greenhouse. Irriga, 21, 817-829. https://doi.org/10.15809/irriga.2016v21n4p817-829

Gonçalves, M., Rodrigues-Jasso, M. R., Gomes, N., Teixeira, J. A. and Belo, I. (2010). Adaptation of dinitrosalicylic acid method to microliter plates. Analytical Methods, 2, 2046-2048. https://doi.org/10.1039/c0ay00525h

Gonzalez, M. E., Jasso-Robles, F. I., Flores-Hernández, E., Rodríguez-Kessler, M. and Pieckenstain, F. L. (2021). Current status and perspectives on the role of polyamines in plant immunity. Annals of Applied Biology, 178, 244-255. https://doi.org/10.1111/aab.12670

Hasan, M., Skalicky, M., Jahan, M. S., Hossain, M., Anwar, Z., Nie, Z. F., Alabdallah, N. M., Brestic, M., Hejnak, V. and Fang, X. W. (2021). Spermine: its emerging role in regulating drought stress responses in plants. Cells, 10, 261. https://doi.org/10.3390/cells10020261

He, L., Nada, K., Kasukabe, Y. and Tachibana, S. (2002). Enhanced susceptibility of photosynthesis to low-temperature photoinhibition due to interruption of chill-induced increase of S-adenosylmethionine decarboxylase activity in leaves of spinach (*Spinacia oleracea* L.). Plant and Cell Physiology, 43, 196-206. https://doi.org/10.1093/pcp/pcf021

Hu, M., Shi, Z., Zhang, Z., Zhang, Y. and Li, H. (2012). Effects of exogenous glucose on seed germination and antioxidant capacity in wheat seedlings under salt stress. Plant Growth Regulation, 68, 177-188. https://doi.org/10.1007/s10725-012-9705-3

Kämpf, A. N., Takane, R. J. and Siqueira, P. T. V. (2006). Floricultura: técnicas de preparo de substratos. Brasília: LK.

Krasensky, J. and Jonak, C. (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. Journal of Experimental Botany, 63, 1593-1608. https://doi.org/10.1093/jxb/err460

Lee, M. M., Lee, S. H. and Park, K. Y. (1997). Effects of spermine on ethylene biosynthesis in cut carnation (*Dianthus caryophyllus* L) flowers during senescence. Journal of Plant Physiology, 151, 68-73. https://doi.org/10.1016/S0176-1617(97)80038-7

Li, Z., Jing, W., Peng, Y., Zhang, X. Q., Ma, X., Huang, L. K. and Yan, Y. H. (2015). Spermine alleviates drought stress in white clover with different resistance by influencing carbohydrate metabolism and dehydrins synthesis. PLoS One, 10, e0120708. https://doi.org/10.1371/journal.pone.0120708

Liu, M., Liu, X., Zhao, Y., Korpelainen, H. and Li, C. (2022). Sex-specific nitrogen allocation tradeoffs in the leaves of *Populus cathayana* cuttings under salt and drought stress. Plant Physiology and Biochemistry, 172, 101-110. https://doi.org/10.1016/j.plaphy.2022.01.009

Ma, Y., Dias, M. C. and Freitas, H. (2020). Drought and salinity stress responses and microbe-induced tolerance in plants. Frontiers in Plant Science, 11, 591911. https://doi.org/10.3389/fpls.2020.591911

Michael, A. J. (2016). Polyamines in eukaryotes, bacteria, and archaea. Journal of Biological Chemistry, 291, 14896-14903. https://doi.org/10.1074/jbc.R116.734780

Nachshon, U. (2018). Cropland soil salinization and associated hydrology: Trends, processes and examples. Water, 10, 1030. https://doi.org/10.3390/w10081030

Navakoudis, E. and Kotzabasis, K. (2022). Polyamines: -bioenergetic smart switch for plant protection and development. Journal of Plant Physiology, 270, 153618. https://doi.org/10.1016/j.jplph.2022.153618

Nisar, S., Tahir, I. and Ahmad, S. S. (2015). Modulation of flower senescence in *Nicotiana plumbaginifolia* L. by polyamines. Indian Journal of Plant Physiology, 20, 186-190. https://doi.org/10.1007/s40502-015-0154-7

Ors, S., Ekinci, M., Yildirim, E., Sahin, U., Turan, M. and Dursun, A. (2021). Interactive effects of salinity and drought stress on photosynthetic characteristics and physiology of tomato (*Lycopersicon esculentum* L.) seedlings. South African Journal of Botany, 137, 335-339. https://doi.org/10.1016/j.sajb.2020.10.031

Osakabe, Y., Yamaguchi-Shinozaki, K., Shinozaki, K. and Tran, L. S. P. (2014). ABA control of plant macroelement membrane transport systems in response to water deficit and high salinity. New Phytologist, 202, 35-49. https://doi.org/10.1111/nph.12613

Pál, M., Szalai, G., and Janda, T. (2015). Speculation: polyamines are important in abiotic stress signaling. Plant Science, 237, 16-23. https://doi.org/10.1016/j.plantsci.2015.05.003

Pan, C., Zhang, H., Ma, Q., Fan, F., Fu, R., Ahammed, G. J., Yu, J. and Shi, K. (2019). Role of ethylene biosynthesis and signaling in elevated CO_2-induced heat stress response in tomato. Planta, 250, 563-572. https://doi.org/10.1007/s00425-019-03192-5

Peterson, B. G. and Carl, P. (2020). PerformanceAnalytics: econometric tools for performance and risk analysis. R package version 2.0.4. Available at: https://CRAN.R-project.org/package=PerformanceAnalytics. Accessed on: Jul. 12, 2022.

Pottosin, I., Velarde-Buendía, A. M., Bose, J., Zepeda-Jazo, I., Shabala, S., and Dobrovinskaya, O. (2014). Cross-talk between reactive oxygen species and polyamines in regulation of ion transport across the plasma membrane: implications for plant adaptive responses. Journal of Experimental Botany, 65, 1271-1283. https://doi.org/10.1093/jxb/ert423

R Core Team (2021). R: A language and environment for statistical computing. Vienna, Austria. Available at: http://www.r-project.org/index.html. Accessed on: Jul. 12, 2022.

Seleiman, M. F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Abdul-Wajid, H. H. and Battaglia, M. L. (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants, 10, 259. https://doi.org/10.3390/plants10020259

Sequera-Mutiozabal, M. I., Erban, A., Kopka, J., Atanasov, K. E., Bastida, J., Fotopoulos, V., Alcázar, R. and Tiburcio, A. F. (2016). Global metabolic profiling of *Arabidopsis* polyamine oxidase 4 (AtPAO4) loss-of-function mutants exhibiting delayed dark-induced senescence. Frontiers in Plant Science, 7, 173. https://doi.org/10.3389/fpls.2016.00173

Shemi, R., Wang, R., Gheith, E. S., Hussain, H. A., Hussain, S., Irfan, M., Cholidah, L., Zhang, K., Zhang, S. and Wang, L. (2021). Effects of salicylic acid, zinc and glycine betaine on morpho-physiological growth and yield of maize under drought stress. Scientific Reports, 11, 3195. https://doi.org/10.1038/s41598-021-82264-7

Silva, T. I., Dias, M. G., Araújo, N. O., Santos, M. N. S., Cruz, R. R. P., Dias, T. J., Ribeiro, W. S., Grossi, J. A. S. and Barbosa, J. G. (2022a). Spermine reduces the harmful effects of salt stress in *Tropaeolum majus*. Physiology and Molecular Biology of Plants, 28, 687-696. https://doi.org/10.1007/s12298-022-01165-9

Silva, T. I., Dias, M. G., Araújo, N. O., Santos, M. N. S., Ribeiro, W. S., Santos Filho, F. B., Dias, T. J., Barbosa, J. G. and Grossi, J. A. S. (2022b). Spermine reduces the harmful effects of drought stress in *Tropaeolum majus*. Scientia Horticulturae, 304, 111339. https://doi.org/10.1016/j.scienta.2022.111339

Silveira, N. M., Ribeiro, R. V., de Morais, S. F., de Souza, S. C., da Silva, S. F., Seabra, A. B., Hancock, J. T. and Machado, E. C. (2021). Leaf arginine spraying improves leaf gas exchange under water deficit and root antioxidant responses during the recovery period. Plant Physiology and Biochemistry, 162, 315-326. https://doi.org/10.1016/j.plaphy.2021.02.036

Singh, M., Kumar, J., Singh, S., Singh, V. P. and Prasad, S. M. (2015). Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. Reviews in Environmental Science and Bio/Technology, 14, 407-426. https://doi.org/10.1007/s11157-015-9372-8

Slama, I., Abdelly, C., Bouchereau, A., Flowers, T. and Savouré, A. (2015). Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. Annals of Botany, 115, 433-447. https://doi.org/10.1093/aob/mcu239

Simões, A. N., Diniz, N. B., Vieira, M. R. S., Ferreira-Silva, S. L., da Silva, M. B., Minatel, I. O. and Lima, G. P. P. (2018). Impact of GA3 and spermine on postharvest quality of anthurium cut flowers (*Anthurium andraeanum*) cv. Arizona. Scientia Horticulturae, 241, 178-186. https://doi.org/10.1016/j.scienta.2018.06.095

Song, J., Wang, Y., Liu, C. and Li, D. (2015). Effect of exogenous spermine on quality and sucrose metabolism of vegetable soya bean (*Glycine max* L.) during cold storage. International Journal of Food Science and Technology, 50, 1697-1703. https://doi.org/10.1111/ijfs.12828

Takahashi, Y., Cong, R., Sagor, G. H. M., Niitsu, M., Berberich, T. and Kusano, T. (2010). Characterization of five polyamine oxidase isoforms in *Arabidopsis thaliana*. Plant Cell Reports, 29, 955-965. https://doi.org/10.1007/s00299-010-0881-1

Talaat, N. B. and Shawky, B. T. (2016). Dual application of 24-epibrassinolide and spermine confers drought stress tolerance in maize (*Zea mays* L.) by modulating polyamine and protein metabolism. Journal of Plant Growth Regulation, 35, 518-533. https://doi.org/10.1007/s00344-015-9557-y

Torras-Claveria, L., Jáuregui, O., Codina, C., Tiburcio, A. F., Bastida, J. and Viladomat, F. (2012). Analysis of phenolic compounds by high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry in senescent and water-stressed tobacco. Plant Science, 182, 71-78. https://doi.org/10.1016/j.plantsci.2011.02.009

Tun, N. N., Santa-Catarina, C., Begum, T., Silveira, V., Handro, W., Floh, E. I. S., and Scherer, G. F. (2006). Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. Plant and Cell Physiology, 47, 346-354. https://doi.org/10.1093/pcp/pci252

Valsalam, S., Agastian, P., Arasu, M. V., Al-Dhabi, N. A., Ghilan, A. K. M., Kaviyarasu, K., Ravindran, B., Chang, S. W. and Arokiyaraj, S. (2019). Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of *Tropaeolum majus* L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. Journal of Photochemistry and Photobiology B: Biology, 191, 65-74. https://doi.org/10.1016/j.jphotobiol.2018.12.010

Wang, Y., Zhang, C., Wang, X., Wang, W. and Dong, L. (2014). Involvement of glucose in the regulation of ethylene biosynthesis and sensitivity in cut *Paeonia suffruticosa* flowers. Scientia Horticulturae, 169, 44-50. https://doi.org/10.1016/j.scienta.2014.02.017

Wei, T. and Simko, V. (2017). R package “corrplot”: visualization of a correlation matrix. (Version 0.84). Available at: https://github.com/taiyun/corrplot. Accessed on: Jul. 12, 2022.

Wimalasekera, R., Tebartz, F. and Scherer, G. F. (2011). Polyamines, polyamine oxidases and nitric oxide in development, abiotic and biotic stresses. Plant Science, 181, 593-603. https://doi.org/10.1016/j.plantsci.2011.04.002

Xu, S. M., Liu, L. X., Woo, K. C. and Wang, D. L. (2007). Changes in photosynthesis, xanthophyll cycle, and sugar accumulation in two North Australia tropical species differing in leaf angles. Photosynthetica, 45, 348-354. https://doi.org/10.1007/s11099-007-0059-4

Xu, W., Lu, N., Kikuchi, M. and Takagaki, M. (2021). Continuous lighting and high daily light integral enhance yield and quality of mass-produced nasturtium (*Tropaeolum majus* L.) in Plant Factories. Plants, 10, 1203. https://doi.org/10.3390/plants10061203

Yi, Z., Li, S., Liang, Y., Zhao, H., Hou, L., Yu, S. and Ahammed, G. J. (2018). Effects of exogenous spermidine and elevated CO_2 on physiological and biochemical changes in tomato plants under iso-osmotic salt stress. Journal of Plant Growth Regulation, 37, 1222-1234. https://doi.org/10.1007/s00344-018-9856-1

Yolcu, S., Alavilli, H., Ganesh, P., Panigrahy, M. and Song, K. (2021). Salt and drought stress responses in cultivated beets (*Beta vulgaris* L.) and wild beet (*Beta maritima* L.). Plants, 10, 1843. https://doi.org/10.3390/plants10091843

Yuan, Y., Qian, H., Wang, Y., Shi, Y., and Tang, D. (2012). Hormonal regulation of Freesia cutflowers and FhACS1. Scientia Horticulturae, 143, 75-81. https://doi.org/10.1016/j.scienta.2012.06.012