INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
23–0290/2023/30–6–415–421
DOI: 10.17957/IJAB/15.2103
http://www.fspublishers.org

Full Length Article

Exogenous Supplementation of PGPs Improves Secondary Metabolites and Essential Nutrients of Stevia (*Stevia rebaudiana*) under Drought Stress

Fatima Ahmadzai[1], Kanval Shaukat[1*], Hafiz Mohammad Akram[2], Abdul Wahid[3] and Nazimah Maqbool[3]
[1]*Department of Botany, University of Balochistan, Quetta, 08770, Pakistan*
[2]*Directorate of Agronomy, Ayub Agricultural Research Institute, Faisalabad, 38000, Pakistan*
[3]*Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan*
[*]For correspondence: kanval_shaukat777@yahoo.com
Received 03 August 2023; Accepted 14 October 2023; Published 30 November 2023

Abstract

Drought is recognized as one of the major abiotic stresses for plant growth and development throughout the world. The objective of this study was to assess the effect of different Plant Growth Promoters (PGPs) on Stevia plants and how they modulate some secondary metabolites and nutrients profile to cope with drought. A pot experiment was conducted at Botanical garden, University of Balochistan, Quetta. The design of experiment was completely randomized design (CRD) with three replicates each treatment. Seeds were sown at 1.27 cm depth. After fifteen days of germination, the seedlings were subjected to drought stress (50% field capacity). Moreover, after seven days of drought stress foliar supplementation of different PGPs *i.e.*, salicylic acid (SA; 250 μM), ascorbic acid (AsA; 250 m*M*), moringa leaf extract (MLE; 10%), thiourea (TU;10 m*M*) and proline (PRO;1 μM) was applied to plants and after 15 days of foliar supplementation plants were harvested *i.e.*, approximately 37 days' seedlings. Results portrayed that ascorbic acid, salicylic acid and proline significantly enhanced soluble sugars and anthocyanins in roots and shoot of drought affected stevia plants. All the PGPs helped stevia plants to absorb more potassium, calcium and magnesium under water deficit conditions as compared to control. The accumulation of soluble sugars and Anthocyanins protects the cell membrane from reactive oxygen species produced under drought. Further, these soluble sugars high up the osmotic potential that induces the inwards movements of water and essential nutrients in shoot and root. Hence, foliar treatments of PGPs are suggested so that it may help Stevia plants to mitigate the stress pressure under drought. Thus, further studies are needed to unveil the omics and mechanisms involved in drought tolerance of stevia seedlings.

Keywords: Drought; Nutrients; Plant Growth Promoters; Stevia

Introduction

Drought is a key limiting factor in reducing the overall yield of field crops (Hussain *et al.* 2019; Ozturk *et al.* 2021). The loss of ecological balance and global climatic changes have water crisis for overall humanity. Drought stress has emerged as a major barrier to the expansion of agricultural output (Ahluwalia *et al.* 2021; Yang *et al.* 2021). Drought primarily influenced aquaporins and ion transporters of the plasma membrane that shift the absorption and transportation of water and nutrient away from roots. The efficiency of water and nutrient transport across cell layers is a key component of drought tolerance mechanisms (Barzana and Carvajal 2020). Drought affected plants exhibits buildup of reactive oxygen species (ROS) buildup, cell shrinkage, loss of cell turgor, limited water uptake, imbalanced nutrient distribution (Hussain *et al.* 2018; Ahanger and Ahmed 2019; Yang *et al.* 2019).

Drought stress causes significant changes in plant secondary metabolites, which are of great economic value to a variety of fields, including the pharmaceutical, aromatherapy and food industries. These changes are due to the obvious changes in plant growth characteristics under water stress conditions (Rezaei-Chiyaneh *et al.* 2021). The ability of plant cells to assimilate and absorb minerals including nitrogen, phosphorus, silicon, calcium and magnesium is negatively impacted under drought stress conditions causing diminished growth and development of plants (Abdelaal 2015; Batool *et al.* 2022). The mineral absorption and transport are reduced under water stress owing to a decrease in the nutrient diffusion rate; thus, different minerals, such as potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}), play critical role in plant physio-biochemical processes (Ali *et al.*

To cite this paper: Ahmadzai F, K Shaukat, HM Akram, A Wahid, N Maqbool (2023). Exogenous supplementation of PGPs improves secondary metabolites and essential nutrients of stevia (*Stevia rebaudiana*) under drought stress. *Intl J Agric Biol* 30:415–421

2018; Khan *et al.* 2020). Similarly, ascorbic acid concentrations and sugar content both noticeably decline during drought stress (Abdelaal *et al.* 2021).

The mechanisms by which plants respond to drought stress must be identified to reduce the impacts of drought stress on plants (Ilyas *et al.* 2021). Understanding how drought affects plants can help to manage the yield of Stevia (Volaire 2018; Marchin *et al.* 2020). Salicylic acid among PGRs is one of the most essential phenolics compound produced by plants which has a very positive role in overall biochemical and physiological attributes of plants under stressed conditions (Hussain *et al.* 2008; Nazar *et al.* 2015; Khalvandi *et al.* 2021). Foliar spray of salicylic acid improved the enhanced physiological characteristics, nutrients and tissue water status through an increase in membrane stability index and relative water content, decrease in electrolyte leakage of cotton plants to withstand drought stress (Mahdi *et al.* 2020; Iqbal *et al.* 2022). Foliar application of salicylic acid on sesame modulates osmoprotectants, antioxidants, mineral nutrients that led to overcome the unfavorable effects of drought stress (Pourghasemian *et al.* 2020; Shaukat *et al.* 2022). The ascorbate in sweet pepper was markedly enhanced by foliar application of ascorbic acid (Khazaei and Estaji 2020). Foliar application of proline had a substantial impact on grain production, chlorophyll a, b and total, carotenoids, and anthocyanin which enhanced osmotic compounds like proline and soluble sugars in chickpea under drought stress (Khalesi *et al.* 2023). Thiourea treatment of maize improved enzymatic and non-enzymatic antioxidants responsible for antioxidation under salinity (Granaz *et al.* 2022). The moringa leaf extract is enriched in antioxidants, proline, amino acids, soluble sugars, thocopherol and glutathione that boost the yield and growth of plants (Yuniati *et al.* 2022).

The perennial shrub Stevia (*Stevia rebaudiana* Bertoni), belonging to the Asteraceae family, is grown in many parts of the world. It is known for its sweetness because it contains steviol glycosides, which have 100–300 times the sweetness of sucrose and has been used as a sugar substitute in the food and pharmaceutical sectors as a sweetener (Ahmad *et al.* 2020). Powdered leaves of the antidiabetic herbaceous perennial shrub, Stevia are used in tea, coffee, juices and other drinks, best for diabetic and weight conscious people (Ghaheri *et al.* 2017). Different experiments suggested that use of PGPs is an effective strategy helping plants to combat the impacts of environmental stresses, but literature references are absent about its impact on Stevia under drought stress. PGPs increase plant growth and yields while being inexpensive and safe to use because they increase stress tolerance by triggering metabolic defense systems. Therefore, this study was conducted to assess the effect of different PGPs on stevia plants and how they modulate some secondary metabolites and nutrients profile to enhance drought tolerance of stevia plants grown in climatic regimes of Quetta, Balochistan, Pakistan.

Materials and Methods

Experimental site and design

A pot experiment was carried out at Botanical Garden, University of Balochistan Quetta to assess the potential impact of plant growth promoters, including Salicylic acid (SA), Ascorbic acid (AsA), Moringa leaf extract (MLE), Thiourea (TU) and Proline (PRO), on the physiological and biochemical attributes of Stevia under drought stress. The experimental design was Complete Randomized Design (CRD) with three replicates. Seeds of Stevia were obtained from Directorate of Agronomy, Ayub Agriculture Research Institute Faisalabad. Five seeds of Stevia were sown at a depth of 1.27 cm in each plastic pots filled with sandy loam soil (8 kg) and organic matter (2 kg). After fifteen days of germination, half of 42 pots were kept as control and other half were subjected to drought (50% field capacity). Foliar application of salicylic acid (SA; 250 μM), ascorbic acid (AsA; 250 m*M*), moringa leaf extract (MLE; 10%), thiourea (TU;10 m*M*) and proline (PRO;1 μM) was done after a week of drought treatment in comparison to water spray (H_2O Spray) and no foliar spray (NFS) to both control (CON) and drought stressed (DS) plant [Each concentration of used PGP was selected using preliminary trails; furthermore, each PGP was produced about 2 Liters for foliar supplementation]. The samples of root and shoot were collected for each treatment after fifteen days of foliar application. The fresh samples were washed and preserved at -80°C for anthocyanins and soluble sugar analysis. For Potassium, Calcium and Magnesium analysis, root and shoot samples were washed and dried in an oven at 65°C for 24 h.

Secondary metabolites analysis

Determination of anthocyanins: For the quantification of anthocyanins, 0.1 g of root and shoot samples from the plant were extracted in 1 to 2 mL of acidified methanol. The samples were then immersed in water bath at 50°C for 60 min and the absorbance was then measured at 535 nm using acidified methanol as a blank (Stark and Wray 1989).

Determination of soluble sugars

Plant sample 0.1 g of root and shoot were boiled in 5 mL of distilled water for 1 h and 1 mL from the extract was diluted with 9 mL of distilled water. Then 0.5 mL from this diluted extract was taken and 5 mL of Anthrone Reagent was added to it. Then the sample mixture was placed in water at 90°C for 20 min. The absorbance was measured at 620 nm. Distilled water was used as blank (Yoshida *et al.* 1976).

Mineral nutrients analysis

Harvested plants were oven dried at 65ºC for 24 h 0.1 g of both shoot and root of each treatment was digested in 5 mL

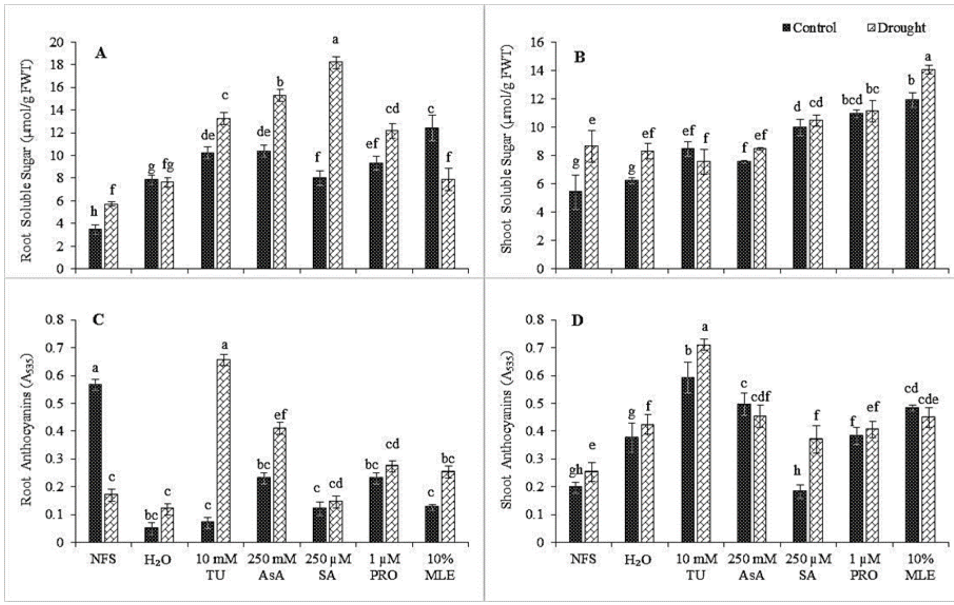

Fig. 1: Changes in secondary metabolites. (**A**) root soluble sugar, (**B**) shoot soluble sugar, (**C**) root anthocyanins, (**D**) shoot anthocyanins of Stevia as affected under drought stress and foliar treatments with various PGPs. Different alphabets/letters on bars represents statistically significant interactions (*P < 0.05*) according to LSD test

*NFS: no foliar spray; H_2O: water spray; SA: salicylic acid foliar spray; AsA: ascorbic acid foliar spray; MLE: moringa leaf extract foliar spray; TU: thiourea foliar spray; PRO: proline foliar spray; PGPs: plant growth promotors

nitric acid placing it on hot plate and diluting the samples to 30 mL using distilled water. Digested samples were then used for the nutrient quantification Potassium, Calcium and Magnesium content was determined by using Atomic Absorption Spectrophotometer (Yoshida *et al.* 1976).

Statistical analysis

This experiment was conducted following completely randomized design with factorial arrangement and replicated three times. The data collected for root and shoot of all attributes were analyzed with analysis of variance (ANOVA) using "STATISTICX 8.1" (Analytical software, Tallahassee, FL, USA). The statistical significance was tested with LSD (*P < 0.05*) and the two factors were compared pairwise represented as labels or alphabets above graph bars. The graphs were generated using MS EXCEL where bars represent the mean values, error bars represent standard deviation among means.

Results

Determination of secondary metabolites

Data for root soluble sugar showed highly significant (*P < 0.05*) variations among treatments and stress (Fig. 1A). The concentration of soluble sugar in roots was higher in drought conditions for all PGPs as compared to control and foliar water spray. The comparison among different PGPs showed that 250 *µM* SA and 250 m*M* AsA significantly produced high soluble sugar contents thus helped Stevia to strive under water deficit conditions. TU (10 m*M*) and 01 µM PRO showed less significant increase in root soluble sugars in Stevia under drought as compared to SA and AsA respectively (Fig. 1A).

Statistically significant differences were found among treatment and stress for shoot soluble sugar in Stevia (Fig. 1B). An increase in shoot soluble sugars was recorded under drought as compared to control for all the treatments. Among PGPs, 10% MLE significantly boosted the contents of soluble sugars in shoots of Stevia under drought. PRO (1 *µM*), 250 m*M* AsA and 250 *µM* SA slightly enhanced the shoot soluble sugars in Stevia under water stress as compared to MLE and control (Fig. 1B).

Significant differences among treatments and stress were analyzed for root and shoot anthocyanins in Stevia (Fig. 1C and D). Results obtained showed that 10 m*M* TU enhanced the root and shoot anthocyanin of Stevia under drought as compared to control as well as other treatments of PGPs respectively. Among other PGPs, 250 m*M* AsA, 01 *µM* PRO and 10% MLE slightly increased the root and shoot anthocyanins under drought as compared to 250 *µM* SA as well as their controls (Fig. 1C and D).

Determination of mineral nutrients

Significant differences (*P < 0.05*) among treatments and stress were analyzed for root and shoot Potasium in Stevia (Fig. 2A and B). Results obtained showed that 10% MLE and 10 m*M* TU enhanced the root and shoot Potassium of

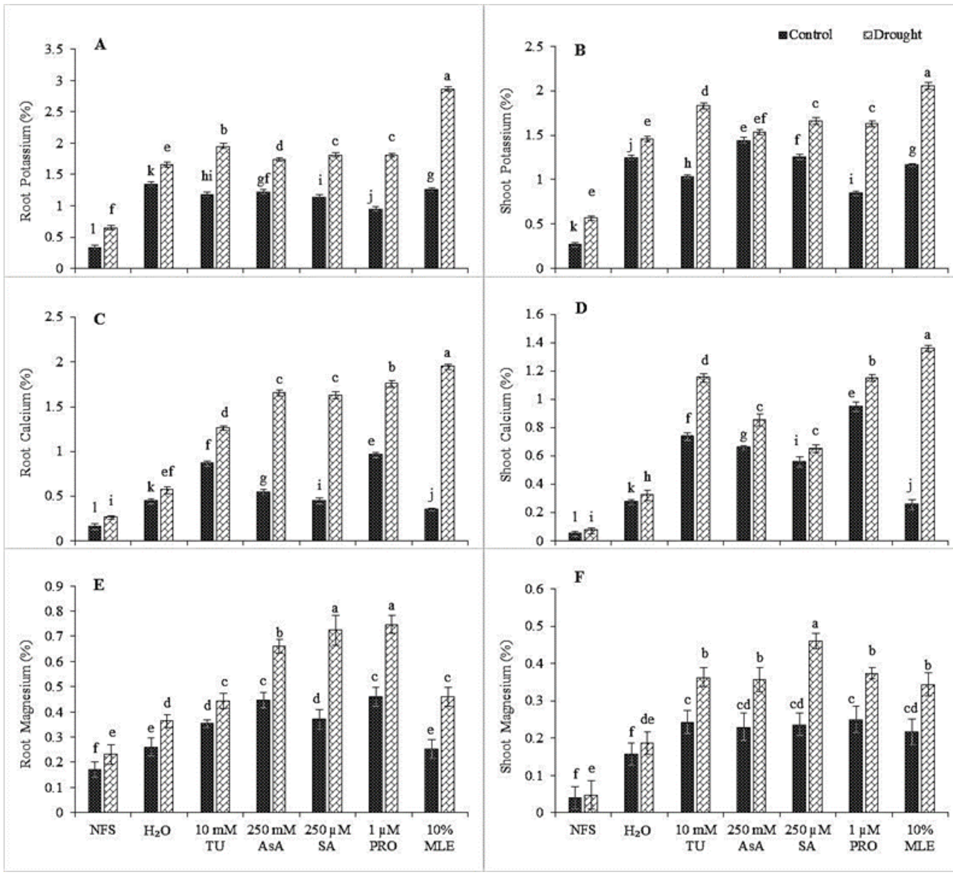

Fig. 2: Changes in nutrients accumulation. (**A**) root potassium, (**B**) shoot potassium, (**C**) root calcium, (**D**) shoot calcium, (**E**) root magnesium, (**F**) shoot magnesium content of Stevia as affected under drought stress and foliar treatments with various PGPs. Different alphabets/letters on bars represents statistically significant interactions ($P < 0.05$) according to LSD test

*NFS: no foliar spray; H_2O: water spray; SA: salicylic acid foliar spray; AsA: ascorbic acid foliar spray; MLE: moringa leaf extract foliar spray; TU: thiourea foliar spray; PRO: proline foliar spray; PGPs: plant growth promotors

Stevia under drought as compared to control as well as other treatments of PGPs respectively. Among other PGPs, 250 m*M* AsA, 01 *µM* PRO and 250 *µM* SA slightly increased the root and shoot Potassium under drought as compared to their controls (Fig. 2A and B).

Data for root and shoot Calcium showed highly significant variations among treatments and stress (Fig. 2C and D). The concentration of Calcium in roots and shoot was higher in drought conditions for all PGPs as compared to control and foliar water spray. The comparison among different PGPs showed that 1 *µM* PRO and 10% MLE significantly produced high Calcium contents in Stevia under water deficit conditions. SA (250 *µM*) and 250 m*M* AsA showed less significant increase in root and shoot Calcium under drought as compared to their respective controls (Fig. 2C and D).

Statistically significant differences were found among treatment and stress for root and shoot Magnesium contents in Stevia (Fig. 2E and F). An increase in root and shoot Magnesium was recorded under drought as compared to control for all the treatments. Among PGPs, 1 *µM* PRO, 250 *µM* SA and 250 m*M* AsA significantly boosted the contents of Magnesium in root and shoots of Stevia under drought as compared to 10% MLE and 10 m*M* TU respectively (Fig. 2E and F).

Discussion

Climate change is the primary cause of biotic and abiotic stresses and has significant impacts on vegetation of a region. As a result, agriculture and climate change are inextricably intertwined in several. Abiotic factors can affect crop chemical profiles and their quantity (Hinojosa-Gómez *et al.* 2020). Drought stress has numerous detrimental consequences on production due to aberrant physiological processes as loss of turgidity, rate of carbon absorption, gaseous exchange and oxidative damage (Hussain *et al.* 2018). Enormous loss in agricultural output was caused by drought stress during the past few decades but now there is a global interest in enhancing yield and plant drought resistance (Ilyas *et al.* 2021). The present study aims to investigate the impacts of PGPs supplementation *i.e.*, TU, AsA, SA, PRO and MLE on the physiological and nutrient attributes of stevia under drought stress (Fig. 3).

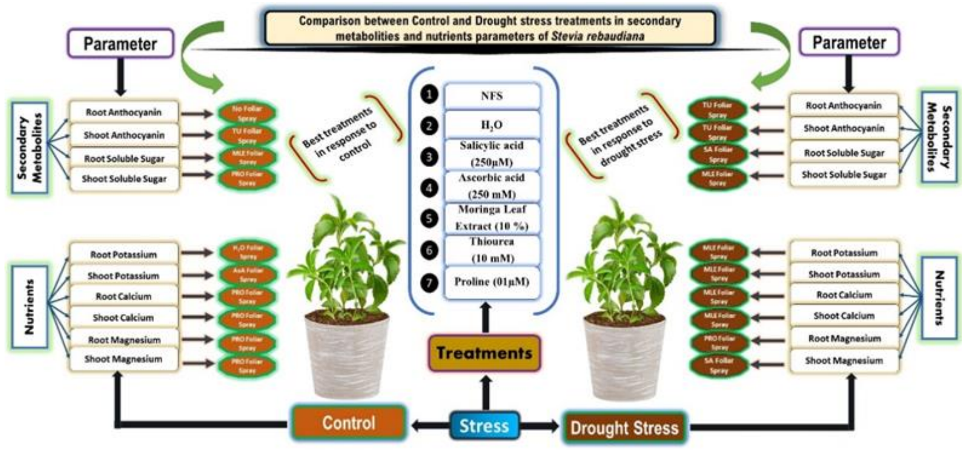

Fig. 3: Comparison of overall secondary metabolites and nutrients parameters in response to various treatments under control and drought stress of *S. rebaudiana*

Different studies have shown that various plant species have effective coping strategies to show tolerance in stressed conditions, such as drought stress (Ekinci *et al.* 2020). In order to protect cellular components and restore the osmotic balance, plants accumulate high intracellular levels of osmoprotectant compounds as stress severity increases. The main building blocks of energetic and biosynthetic metabolism, soluble sugars also function as protective macromolecules or ROS scavengers as well as suitable osmolytes, restoring the osmotic equilibrium (Singh *et al.* 2015; Pommerrenig *et al.* 2018; Nizar *et al.* 2022). Sperdouli and Moustakas (2012) reported a statistically significant accumulation of soluble sugars along with proline and anthocyanin content under moderate drought stressed Arabidopsis plants as compared to control. Correspondingly, present study findings also endorse that under drought stress and particularly with foliar supplementation of PGPs especially as SA highly enhanced the soluble sugar content in the both root and shoot of Stevia plants which is one of the potential solutions for overcoming osmotic stress caused by drought. SA played a significant role in increasing soluble sugar content in Stevia under drought thus being more effective than other PGPs (Fig 1A and B).

An important category of secondary plant metabolites known as anthocyanins protects plants against different biotic and abiotic challenges *i.e.*, drought and salinity (Naing and Kim 2021; Samad *et al.* 2023). Anthocyanins, a flavonoid family increase in plants under drought stress (Sanjarimijani *et al.* 2016). The findings of Lalay *et al.* (2022) reported that potential role of PGRs enhance anthocyanin content in plants subjected to drought stress. The present results are statistically similar with the above findings that drought stress had an adverse effect on the anthocyanin content of Stevia roots and shoots. According to Cirillo *et al.* (2021) anthocyanins are basic key regulators of drought stress tolerance, as they found substantial increase in anthocyanin content in transgenic tobacco plants under drought stress compared to control plants, thus validating that secondary metabolite particularly anthocyanins improves tolerance potential of plants. A similar tendency was observed in the current study that anthocyanin content of plants under drought stress was significantly higher specifically under PGPs treatments. Nevertheless, foliar applications of PGPs, notably TU and AsA, under both control and drought stress, significantly enhanced the anthocyanin content in Stevia (Fig. 1C and D).

The capacity of plants to absorb nutrients is influenced by a variety of variables, including environmental parameters like soil moisture as well as plant aspects like root features like the rate of nutrient uptake by roots, length of the complete root system and area of uptake by roots (Liang *et al.* 2018). Plant active transport decreases as the severity of the drought increases, and less nutrients are transferred from the roots to the shoots (Li *et al.* 2021). Potassium is the most prevalent cation in plants, accounting for 2% to 10% of the dry weight which is not only required for plant physiological and biochemical processes but it also plays a role in plants' adaptive response to abiotic or biotic stresses such as drought (Oosterhuis *et al.* 2014; Asif *et al.* 2017; Nizar *et al.* 2023). K^+ is essential for plants' adaptive response to drought stress. It has been established that greater K^+ uptake confers greater drought tolerance in many plant species (Osakabe *et al.* 2013). The present outcomes of the study suggested that K^+ content increased under MLE spray treatment in both root and shoot of Stevia followed by TU spray under drought stress as compared to control. This maximum accumulation of K^+ and other nutrients is the signifying role of plant growth promoters *i.e.*, TU, AsA, SA, MLE and PRO in impeding the negative impacts of drought stress in Stevia (Fig. 2A and B). In the same manner, Ca^{2+} is also a key signaling molecule in drought tolerance and affects a variety of physiological and cellular processes in plants. In plants that use a calcium-dependent signaling network, it is crucial for signal detection and transduction. (Tuteja and Mahajan 2007). Ca^{2+} in root and shoot of Stevia showed highly significant variations by accumulating minimum Ca^{2+}

content. The data further revealed that in shoot and root of Stevia, MLE and PRO spray proved to ameliorate drought stress effectively thus enhancing Ca^{2+} in shoot and root as the effective treatments. Additionally, drought stress affected Ca^{2+} in the shoots and roots of stevia; however, foliar application of PGPs, notably MLE and PRO, reduced the negative impacts of drought stress and improved Ca^{2+} in the shoot and root (Fig. 2C and D). Likewise, Mg^{2+} is loaded into the xylem after uptake from the soil for long-distance transfer to the shoots. The mobility of phloem encompasses the constant recycling of Mg^{2+} between shoots and roots (Tang and Luan 2017). High levels of cellular Mg^{2+} serve as the central element of chlorophyll molecules in green tissues and serve as cofactors for a variety of enzymes (Sun *et al.* 2017). Mg^{2+} is a highly mobile nutrient due to its connections to potassium and nitrogen, which gives it a lot of potential in the dry matter that moves from the sink to the source (Senbayram *et al.* 2015). The results of the current study showed substantial variations in shoot and root Mg^{2+} accumulation in Stevia, with SA and PRO foliar spray, both shoot and root under drought stress had maximum Mg^{2+} content, mitigating the detrimental effects of drought (Fig. 2E and F).

Conclusion

In this study, foliar application of different PGPs assisted the plants in accumulating anthocyanins and soluble sugars that increased the osmotic potential of stevia root and shoots. This higher osmotic potential increases the movement of water along with nutrients into the plants. Therefore, higher potassium, calcium and magnesium contents were recorded in root and shoot of stevia under drought and foliar applications of PGPs. Conclusively, this study highly recommends the supplementation of PGPs not only to mitigate the negative impacts of drought stress but help plants to flourish well by as per stevia's increasing demand to be used as a healthy alternative to sugar.

Acknowledgements

The authors are thankful to Department of Botany, University of Balochistan, Quetta, Pakistan, for providing research environment and support to conduct this study. Also thankful to Ayub Agricultural Research Institute, Faisalabad for providing assistance in various chemical analysis.

Author Contributions

KS, HMA and AW planned the experiments, FA performed the experiment. KS, AW, HMA and NM interpreted the results. FA and KS made the write up. FA, KS and AW read and revised the writeup. KS and NM statistically analyzed the data and made illustrations.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

Funding Source

No specific funding is provided for this research project

References

Abdelaal KAA (2015). Effect of salicylic acid and abscisic acid on morpho-physiological and anatomical characters of faba bean plants (*Vicia faba* L.) under drought stress. *J Plant Prod* 6:1771–1788

Abdelaal K, M AlKahtani, K Attia, Y Hafez, L Király, A Künstler (2021). The role of plant growth-promoting bacteria in alleviating the adverse effects of drought on plants. *Biology* 10:520-543

Ahanger MA, P Ahmad (2019). Role of mineral nutrients in abiotic stress tolerance: Revisiting the associated signaling mechanisms. *In*: *Plant Signaling and Behavior: Role and Regulation Under Stressful Environments*, pp:269–285. Ahanger MA, P Ahmad (Eds). Elsevier, Amesterdam, The Netherlands

Ahluwalia O, PC Singh, R Bhatia (2021). A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. *Resour Environ Sustain* 5:100032

Ahmad J, I Khan, R Blundell, J Azzopardi, MF Mahomoodally (2020). *Stevia rebaudiana* Bertoni.: An updated review of its health benefits, industrial applications and safety. *Trends Food Sci Technol* 100:177–189

Ali Q, MT Javed, A Noman, MZ Haider, M Waseem, N Iqbal, R Perveen (2018). Assessment of drought tolerance in mung bean cultivars/lines as depicted by the activities of germination enzymes, seedling's antioxidative potential and nutrient acquisition. *Arch Agron Soil Sci* 64:84–102

Asif M, O Yilmaz, L Ozturk (2017). Potassium deficiency impedes elevated carbon dioxide-induced biomass enhancement in well-watered or drought-stressed bread wheat. *J Plant Nutr Soil Sci* 180:474–481

Barzana G, M Carvajal (2020). Genetic regulation of water and nutrient transport in water stress tolerance in roots. *J Biotechnol* 324:134–142

Batool M, AM El-Badri, MU Hassan, Y Haiyun, W Chunyun, Y Zhenkun, G Zhou (2022). Drought stress in *Brassica napus*: Effects, tolerance mechanisms, and management strategies. *J Plant Growth Regul* 42:21–45

Cirillo V, V D'Amelia, M Esposito, C Amitrano, P Carillo, D Carputo, A Maggio (2021). Anthocyanins are key regulators of drought stress tolerance in tobacco. *Biology* 10:139-153

Ekinci M, S Ors, E Yildirim, METIN Turan, U Sahin, A Dursun, R Kul (2020). Determination of physiological indices and some antioxidant enzymes of chard exposed to nitric oxide under drought stress. *Russ J Plant Physiol* 67:740–749

Ghaheri M, D Kahrizi, G Bahrami (2017). Effect of mannitol on some morphological characteristics of *in vitro Stevia rebaudiana* Bertoni. *Bihar Biol* 11:94–97

Granaz, K Shaukat, G Baksh, N Zahra, MB Hafeez, A Raza, A Samad, M Nizar, A Wahid (2022). Foliar application of thiourea, salicylic acid, and kinetin alleviate salinity stress in maize grown under etiolated and de-etiolated conditions. *Discover Food* 2:27-40

Hinojosa-Gómez J, SC Martín-Hernández, JB Heredia, J León-Félix, T Osuna-Enciso, MD Muy-Rangel (2020). Anthocyanin induction by drought stress in the calyx of roselle cultivars. *Molecules* 25:1555-1565

Hussain M, S Farooq, W Hasan, S Ul-Allah, M Tanveer, M Farooq, A Nawaz (2018). Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agric Water Manage* 201:152–166

Hussain M, MA Malik, M Farooq, MY Ashraf, MA Cheema (2008). Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J Agron Crop Sci* 194:193–199

Hussain S, S Hussain, T Qadir, A Khaliq, U Ashraf, A Parveen, M Rafiq (2019). Drought stress in plants: An overview on implications, tolerance mechanisms and agronomic mitigation strategies. *Plant Sci Today* 6:389–402

Ilyas M, M Nisar, N Khan, A Hazrat, AH Khan, K Hayat, A Ullah (2021). Drought tolerance strategies in plants: A mechanistic approach. *J Plant Growth Regul* 40:926–944

Iqbal S, X Wang, I Mubeen, M Kamran, I Kanwal, GA Díaz, A Abbas, A Parveen, MN Atiq, H Alshaya, TKZ El-Abedin, S Fahad (2022). Phytohormones trigger drought tolerance in crop plants: Outlook and future perspectives. *Front Plant Sci* 12:3378-3391

Khalesi A, MSA Mirkalaeia, SAMM Sanavy, A Eftekhari, MN Moghadam (2023). Effect of foliar application of amino acids on grain yield and physiological traits of chickpea under drought stress. *Gesun Pflan* 75:1705–1718

Khalvandi M, A Siosemardeh, E Roohi, S Keramati (2021). Salicylic acid alleviated the effect of drought stress on photosynthetic characteristics and leaf protein pattern in winter wheat. *Heliyon* 7:1-11

Khan N, A Bano, S Ali, MA Babar (2020). Crosstalk amongst phytohormones from plants and PGPR under biotic and abiotic stresses. *J Plant Growth Regul* 90:189–203

Khazaei Z, A Estaji (2020). Effect of foliar application of ascorbic acid on sweet pepper (*Capsicum annuum*) plants under drought stress. *Acta Physiol Plant* 42:118-129

Lalay G, S Ullah, I Ahmed (2022). Physiological and biochemical responses of *Brassica napus* L. to drought-induced stress by the application of biochar and Plant Growth Promoting Rhizobacteria. *Microsc Res Tech* 85:1267–1281

Li Z, X Su, Y Chen, X Fan, L He, J Guo, Q Yang (2021). Melatonin improves drought resistance in maize seedlings by enhancing the antioxidant system and regulating abscisic acid metabolism to maintain stomatal opening under PEG-induced drought. *J Plant Biol* 64:299–312

Liang B, T Gao, Q Zhao, C Ma, Q Chen, Z Wei, C Li, C Li, F Ma (2018). Effects of exogenous dopamine on the uptake, transport, and resorption of apple ionome under moderate drought. *Front Plant Sci* 9:755-768

Mahdi HA, RS Taha, SM Emam (2020). Foliar applied salicylic acid improves water deficit-tolerance in Egyptian cotton. *J Plant Prod* 11:383–389

Marchin RM, A Ossola, MR Leishman, DS Ellsworth (2020). A simple method for simulating drought effects on plants. *Front Plant Sci* 10:1715-1728

Naing AH, CK Kim (2021). Abiotic stress-induced anthocyanins in plants: Their role in tolerance to abiotic stresses. *Physiol Plant* 172:1711–1723

Nazar R, S Umar, NA Khan, O Sareer (2015). Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. *S Afr J Bot* 98:84–94

Nizar M, K Shaukat, A Samad, N Zahra, I Saleem, A Raza (2023). Impact of exogenous salicylic acid and hydrogen peroxide on vitamins, essential nutrients and silymarin content of milk thistle grown at two different altitudes of Balochistan under cadmium stress. *J Agric Food Res* 13:100643

Nizar M, K Shaukat, N Zahra, MB Hafeez, A Raza, A Samad, Q Ali, MH Siddique, HM Ali (2022). Exogenous application of salicylic acid and hydrogen peroxide ameliorate cadmium stress in milk thistle by enhancing morpho-physiological attributes grown at two different altitudes. *Front Plant Sci* 12:1-18

Oosterhuis DM, DA Loka, EM Kawakami, WT Pettigrew (2014). The physiology of potassium in crop production. *Adv Agron* 126:203–233

Osakabe Y, N Arinaga, T Umezawa, S Katsura, K Nagamachi, H Tanaka, K Yamaguchi-Shinozaki (2013). Osmotic stress responses and plant growth controlled by potassium transporters in *Arabidopsis*. *Plant Cell* 25:609–624

Ozturk M, BUT Unal, P García-Caparrós, A Khursheed, A Gul, M Hasanuzzaman (2021). Osmoregulation and its actions during the drought stress in plants. *Physiol Plant* 172:1321–1335

Pommerrenig B, F Ludewig, J Cvetkovic, O Trentmann, PA Klemens, HE Neuhaus (2018). In concert: Orchestrated changes in carbohydrate homeostasis are critical for plant abiotic stress tolerance. *Plant Cell Physiol* 59:1290–1299

Pourghasemian N, R Moradi, M Naghizadeh, T Landberg (2020). Mitigating drought stress in sesame by foliar application of salicylic acid, beeswax waste and licorice extract. *Agric Water Manage* 231:105997

Rezaei-Chiyaneh E, MA Machiani, A Javanmard, H Mahdavikia, F Maggi, MR Morshedloo (2021). Vermicompost application in different intercropping patterns improves the mineral nutrient uptake and essential oil compositions of sweet basil (*Ocimum basilicum* L.). *J Soil Sci Plant Nutr* 21:450–466

Samad A, K Shaukat, MUR Ansari, M Nizar, N Zahra, A Raza, I Djalović (2023). Role of foliar spray of plant growth regulators in improving photosynthetic pigments and metabolites in *Plantago ovata* (Psyllium) under salt stress–A field appraisal. *Biocell* 47:523–532

Sanjarimijani M, AR Sirousmehr, B Fakheri (2016). The effects of drought stress and humic acid on morphological traits, yield and anthocyanin of roselle (*Hibiscus sabdariffa* L.). *Agroecology* 8:346–358

Senbayram M, A Gransee, V Wahle, H Thiel (2015). Role of magnesium fertilizers in agriculture: Plant–soil continuum. *Crop Past Sci* 66:1219–1229

Shaukat K, N Zahra, MB Hafeez, R Naseer, A Batool, A Raza, A Wahid (2022). Role of salicylic acid–induced abiotic stress tolerance and underlying mechanisms in plants. *In*: *Emerging Plant Growth Regulators in Agriculture*, pp:73–98. Naeem M, T Aftab (Eds). Elsevier Inc., Amsterdam, The Netherlands

Singh M, J Kumar, S Singh, VP Singh, SM Prasad (2015). Roles of osmoprotectants in improving salinity and drought tolerance in plants: A review. *Rev Environ Sci Biotechnol* 14:407–426

Sperdouli I, M Moustakas (2012). Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of Arabidopsis thaliana to drought stress. *J Plant Physiol* 169:577–585

Stark D, V Wray (1989). Anthocyanins. *In: Methods in Plant Biology, Plant Phenolics*, Vol. I, pp:32–356. Harborne JB (Ed). Academic Press/Harcourt Brace Jovanovich, London

Sun Y, R Yang, L Li, J Huang (2017). The magnesium transporter MGT10 is essential for chloroplast development and photosynthesis in *Arabidopsis thaliana*. *Mol Plant* 10:1584–1587

Tang RJ, S Luan (2017). Regulation of calcium and magnesium homeostasis in plants: From transporters to signaling network. *Curr Opin Plant Biol* 39:97–105

Tuteja N, S Mahajan (2007). Calcium signaling network in plants: An overview. *Plant Signal Behav* 2:79–85

Volaire F (2018). A unified framework of plant adaptive strategies to drought: Crossing scales and disciplines. *Glob Chang Biol* 24:2929–2938

Yang X, M Lu, Y Wang, Y Wang, Z Liu, S Chen (2021). Response mechanism of plants to drought stress. *Horticulturae* 7:50-85

Yang Z, J Liu, F Poree, R Schaeufele, H Helmke, J Frackenpohl, S Lehr, P von Koskull-Döring, A Christmann, H Schnyder, U Schmidhalter (2019). Abscisic acid receptors and coreceptors modulate plant water use efficiency and water productivity. *Plant Physiol* 180:1066–1080

Yoshida SD, A Forno, JH Cock, KA Gomez (1976*). Laboratory Manual for Physiological Studies of Rice*. International Rice Research Institute (IRRI), Los Banos, The Philippines

Yuniati N, K Kusumiyati, S Mubarok, B Nurhadi (2022). The Role of moringa leaf extract as a plant biostimulant in improving the quality of agricultural products. *Plants* 11:2186-2198