

## Effects of foliar treatment of ascorbic acid on industrial hemp seedlings under drought stress

Qi LI<sup>a</sup>, Wenjing OUYANG<sup>b</sup>, Yinhong ZHAO, Yang YANG,  
Kailei TANG, Guanghui DU\*

Yunnan University, School of Agriculture, Kunming 650500, China; [liqi1@stu.ynu.edu.cn](mailto:liqi1@stu.ynu.edu.cn); [wenjing.ouyang@ynu.edu.cn](mailto:wenjing.ouyang@ynu.edu.cn); [zybzjb@mail.ynu.edu.cn](mailto:zybzjb@mail.ynu.edu.cn); [yjy@ynu.edu.cn](mailto:yjy@ynu.edu.cn); [kailei.tang@ynu.edu.cn](mailto:kailei.tang@ynu.edu.cn); [dgh2012@yun.edu.cn](mailto:dgh2012@yun.edu.cn) (\*corresponding author)

<sup>a,b</sup>These authors contributed equally to this work

### Abstract

Industrial hemp (*Cannabis sativa* L.) seedlings are sensitive to drought stress, which is a prevalent factor influencing its growth seriously. To explore the effect of ascorbic acid (AsA) on hemp seedlings under drought condition, a pot experiment was carried out to apply AsA on two cultivars, 'Yunma1' (YM) and 'Bamahuoma' (BM). Three drought treatments were imposed: control (normal water), drought (50% substrate moisture) and drought + AsA (50% substrate moisture + 200 mg/L AsA). The results showed that drought stress significantly suppressed plant height and reduced plant fresh weight, with reductions of 59% and 75% observed in YM, and 43% and 67% in BM, respectively. Although foliar treatment with AsA had little effect on increasing plant fresh weight under drought conditions, it significantly enhanced the concentrations of photosynthetic pigments and the activities of antioxidant enzyme (superoxide dismutase, SOD and peroxidase, POD). The results suggested that harmful effects of drought stress on hemp seedlings were mitigated by exogenous application of AsA, which decrease the breakdown of photosynthetic pigments, enhancing antioxidant enzyme activities and strengthening the antioxidative defense system. Among these, compared to drought treatment the carotene content and SOD activity exhibited the most significant increases after AsA spraying. Specifically, exogenous AsA treatment increased the carotene content by approximately 108.40% in YM and 88.53% in BM. Meanwhile, POD activity increased by 33.33% in YM and 60.94% in BM. Furthermore, the study found that hemp plant response and tolerance to drought were cultivar-dependent. Overall, these results provide a theoretical basis for understanding the mechanism by which AsA alleviates drought stress.

**Keywords:** *Cannabis sativa* L.; exogenous substances; plant growth; physiological index; water deficit

### Introduction

Hemp (*Cannabis sativa* L.) is an annual herbaceous belonging to the Cannabaceae family, and it is one of the oldest cultivated crops (Amaducci *et al.*, 2015). In China, industrial hemp is classified as having a tetrahydrocannabinol (THC) content of less than 0.3% (m/m) in its dried inflorescences and leaves. Because of its high commercial value, hemp has been involved into almost every aspect of people's lives, including food,

Received: 28 Nov 2024. Received in revised form: 05 Jan 2025. Accepted: 07 Mar 2025. Published online: 11 Mar 2025.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

paper making, textile industry, and the new energy field (Chandra *et al.*, 2017). In recent years, hemp has attracted considerable attention from both the public and the scientific community because of its wide-ranging applications and high therapeutic value.

Global climate change arouse a number of abiotic stresses (Raza *et al.*, 2023), and drought is one of the most severe factors impacting crop fields and quality (Alamri *et al.*, 2020). Many plants physiological processes, such as photosynthesis, membrane integrity, and phytohormones balance were interfered with drought. Moreover, this disturbance leads to a decrease in biomass accumulation (Raza *et al.*, 2023). Additionally, drought-induced oxidative stress disrupts plant metabolism (affecting protein, carbohydrate and lipid contents), and plants rely on their antioxidant systems for protection against such stress (Khan *et al.*, 2020). During the growth period of hemp, drought stress can increase the incidence of diseases and insect pests, slow growth, and delay seed maturation, ultimately affecting yield and quality (Jiang *et al.*, 2021). In addition to the general effects of drought on plants, drought conditions also exert a significant influence on the linear density, wax and fat content, as well as crystallinity of hemp fibers (Kwiatkowska *et al.*, 2024). However, the land competition contradiction between food crops and hemp crops has intensified due to the shortage of cultivated land resource (Gupta *et al.*, 2020). Consequently, the transfer of hemp cultivation to non-cultivated lands, which are often characterized by drought conditions, has become an inevitable trend of the future (Asma *et al.*, 2023). Wayne *et al.* (2024) found that during the growth period close to inflorescence maturity, light drought stress can increase THC levels in high-THC hemp cultivar, and intense drought treatments led to significantly decreased yield and THC and CBD concentrations. Therefore, it is necessary to study the tolerance of hemp to drought stress and develop cultivation methods to improve yield and quality of industrial hemp under drought.

Agricultural research is exploring various cultivation techniques to enhance plant tolerance under stress, including the development of stress-tolerant varieties and the use of chemical and physical treatment methods (Qin *et al.*, 2021; Raza *et al.*, 2023). In production practice, foliar spray or seeds pre-treatment by chemicals phytohormones are commonly used to mitigate deleterious effects of drought and enhance crop drought tolerance (Noman *et al.*, 2015). Ascorbic acid (AsA) can be used as an antioxidant that can effectively remove reactive oxygen species within a certain physiological range (Asada, 1992). It has been reported that exogenous AsA played a vital role in promoting seed germination, improving plant growth and reducing the losses of yield under stress conditions in maize, cauliflower, and sweet pepper (Noman *et al.*, 2015; Latif *et al.*, 2016; Khazaei *et al.*, 2020). AsA could protect plant cells from reactive oxygen species (ROS) generated during the plant's response to stress, and play a crucial role in several key physiological processes, including: photosynthesis, respiration, and carbohydrate metabolism by acting as a co-factor for metabolic enzymes or by regulating cellular redox-status (Mishra *et al.*, 2024). Thus, we also speculated that the application of exogenous AsA could improve the drought resistance of hemp plants by altering the contents of photosynthetic pigments, increasing the antioxidant enzymes activity, and adjusting the osmoregulation substances. Nevertheless, there is limited research on the relieving effects of exogenous AsA on hemp plants under drought condition. The present study aimed to: (i) assess the effects of drought on the growth of hemp seedlings, (ii) evaluate the effects of exogenous AsA in alleviating impairments caused by drought stress on hemp seedlings, and (iii) investigate the differences in response and tolerance to drought stress among hemp cultivars. These findings will provide a valuable reference and foundation for the cultivation practices and future studies of hemp.

## Materials and Methods

### *Plant materials*

Two industrial hemp cultivars, 'Yunma 1' (YM) for fiber production and 'Bamahuoma' (BM) for seed production, were used in this study. Seeds of YM were sourced from Yunnan Industrial Hemp Co., Ltd.,

Kunming, China and seeds of BM were obtained from Guangxi Academy of Agricultural Sciences, Nanning, China.

#### *Experimental treatments*

This experiment was conducted in the greenhouse of School of Agriculture, Yunnan University, China. Each pot (upper diameter: 18.5 cm, lower diameter: 14 cm, height: 14 cm) was filled with 500 g medium (peat soil), into which 30 seeds were evenly sown. Seedlings were thin out to 10 plants per pots when seedling reached a length of 5 cm. Throughout this period, hemp plants were watered regularly and weed were removed manually. Once most plants reach approximately 20 cm in height, three treatments were imposed: control, drought and drought with AsA application. Hemp plants in control group received normal watering (water 500 mL per pot every other day), while moisture content in the drought-stress pots was maintained at 50% substrate moisture by weighing the pots every day and add water to the target stress level. For the drought with AsA treatment, hemp leaves were sprayed with AsA solution (200 mg/L, confirmed by our preliminary experiments) once every other day for a total of 3 times, starting one week after the drought initiation. Each treatment was replicated three times, totaling 18 pots.

#### *Determination of morphological indexes*

Morphological attributes were assessed 15 days after the treatment, when its effects were most pronounced (it was confirmed by our preliminary experiments). Plant height of hemp was measured from the base of the plant main stem to the highest growing point using a flexible ruler, and its stem diameter was measured at 10 cm from the plant's base using a vernier caliper. The fresh weight of the above-ground plant was determined using a digital scale.

#### *Determination of physiological and biochemical indices*

In order to evaluate the physiological and biochemical indexes, a whole plant and the third pair of true leaves from 10 hemp plants in each treatment, which were sprayed with AsA after 15 days, were taken. Moreover, each treatment was repeated three times.

The concentrations of chlorophyll a, chlorophyll b, and carotenoid were determined using the method described by Arnon (1949). The spectrophotometer used in this experiment was Shanghai Metash Instruments UV8000. SOD (superoxide dismutase) and POD (peroxidase) activities, MDA (malondialdehyde) contents, and free proline amounts were measured using kits from Boxbio (Beijing Boxbio Science & Technology Co., Ltd.). The quantification of soluble protein and soluble sugar in plant leaves were performed by Bradford test (Bradford, 1976) and the thiobarbituric acid (TBA) test, respectively. To assess the cell membrane damage after drought stress, the relative conductivity of plant leachates was measured as described by Lutts *et al.* (1996).

#### *Data analyses*

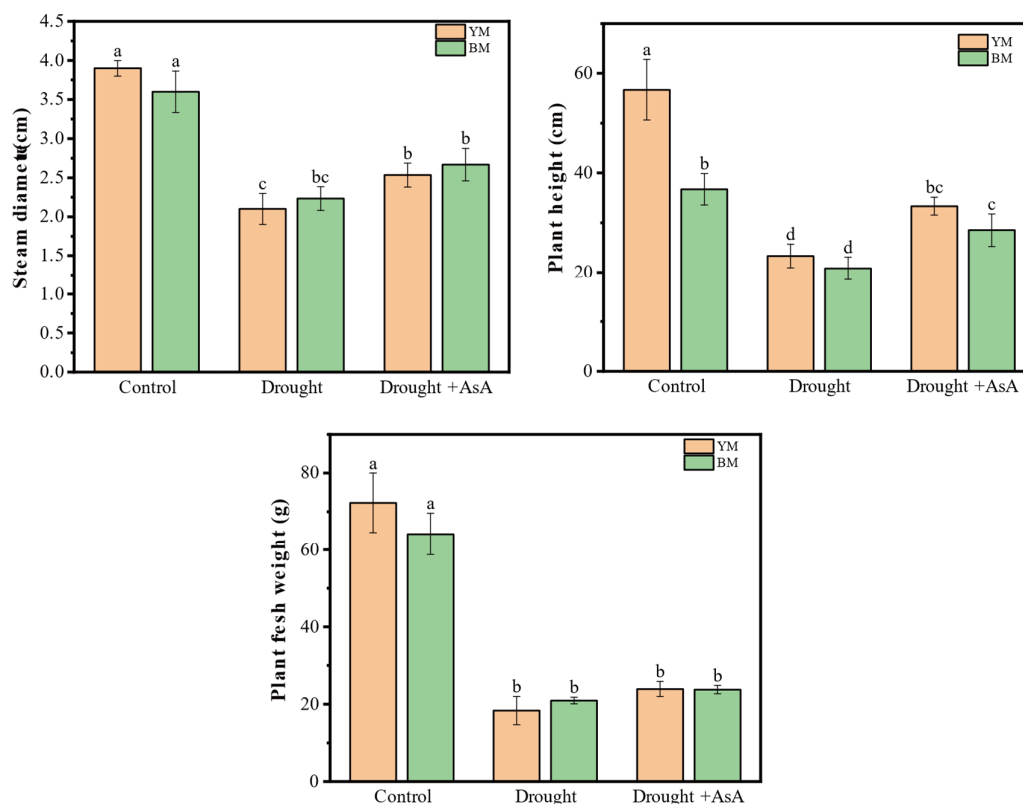
The data were tested for normality and homogeneity of variance before being subjected to analysis of variance (ANOVA) using SPSS 21.0. Means of treatments were compared using Duncan's Multiple Range Test at the 0.05 level of significance. Graphical representations were generated using OriginPro 2021 (9.8.0.200) software.

## **Results**

#### *Effect of drought and AsA on hemp growth*

Drought significantly reduced stem diameter, plant height, and plant fresh weight of both hemp cultivars (**Error! Reference source not found.**). Under drought stress, the plant fresh weight was decreased by 74.63% and 67.38%, the plant height was decreased by 58.91% and 43.38%, and the stem diameter was

decreased by 46.15% and 37.96% in YM and BM, respectively, compared to the control. Moreover, compared with drought treatment, the application of AsA under drought significantly increased plant height by 43.06% and 37.01% in YM, BM, respectively. Additionally, plant fresh weight was heavier with AsA application, but no significant increases were detected.

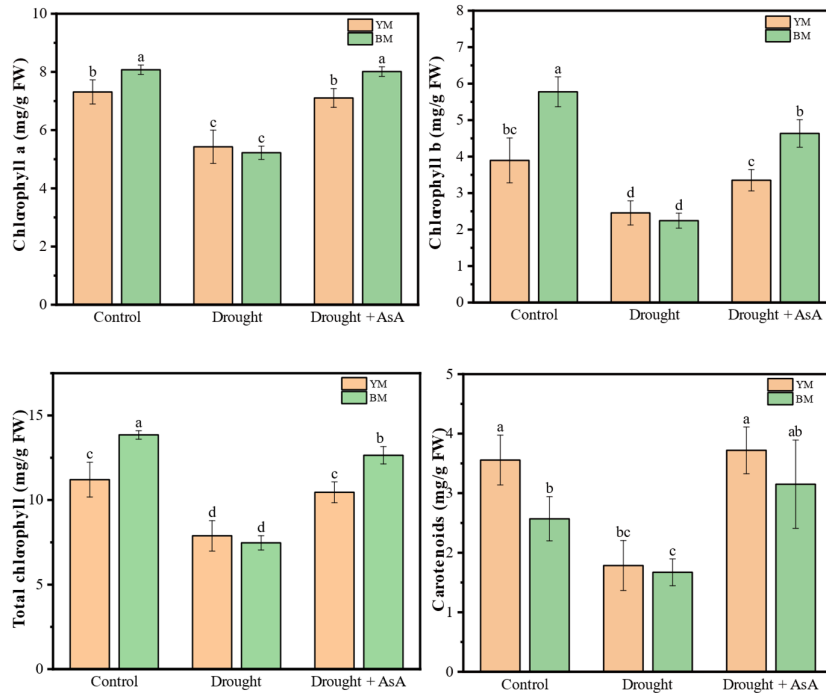


**Figure 1.** Effects of drought and AsA on hemp growth index

Different lowercases above the bars show significant difference between treatments ( $p < 0.05$ ). YM: industrial hemp cultivars ‘Yunma 1’; BM: industrial hemp cultivars ‘Bamahuoma’

#### *Effect of drought and AsA on photosynthetic pigments in hemp leaves*

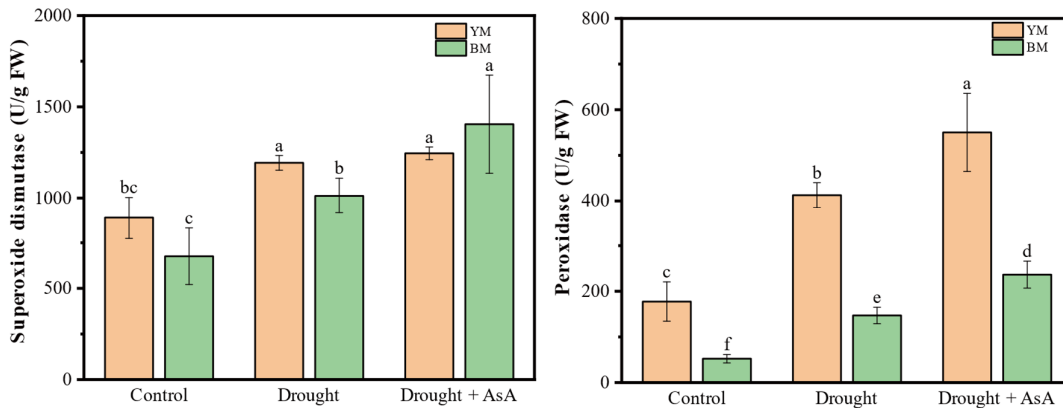
Drought has a significant impact upon all assessed photosynthetic pigments, including chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll (Figure 2). Compared with the control, drought induced decreases of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids contents by 25.77%, 36.95%, 29.65%, and 49.81% in YM, while the reduction were 35.31%, 61.14%, 46.09%, and 34.96% in BM, respectively. However, the content of photosynthetic pigment increased after AsA was applied compared to the drought treatments without AsA application. Specifically, chlorophyll a and carotenoids contents increased by approximately 30.88% and 108.40% in YM, and by 53.41% and 88.53% in BM, respectively. The application of AsA allowed the chlorophyll a and carotenoid contents to recover to the level that showed no significant difference from the control treatment.



**Figure 2.** Effects of drought and AsA on photosynthetic pigments in hemp seedling. Different lowercases above the bars show significant difference between treatments ( $p < 0.05$ ). YM: industrial hemp cultivars ‘Yunma 1’; BM: industrial hemp cultivars ‘Bamahuoma’

*Effects of drought and AsA antioxidant enzymes in hemp plant*

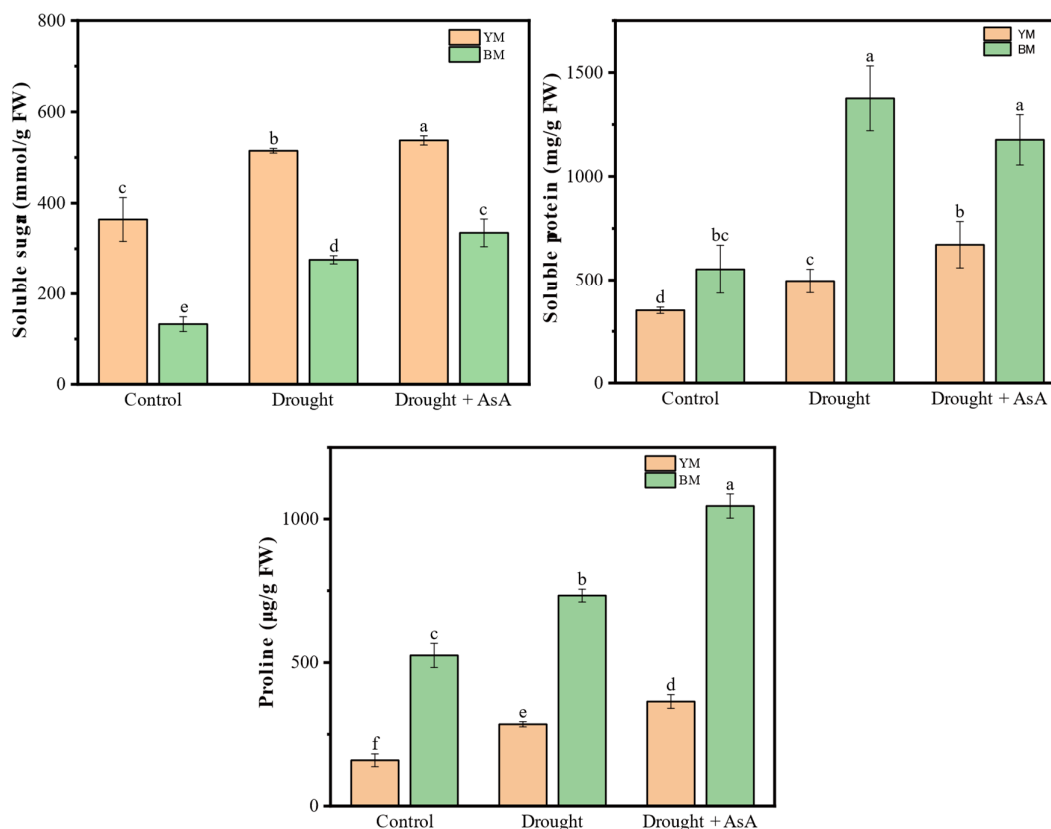
As shown in Figure 3, drought led to a significant increase in the activities of SOD and POD in hemp plants compared to the control treatment. Specifically, drought stress significantly increased SOD activities by 34.36% in YM and 49.70% in BM, while POD activities also showed a substantial increase, rising by 132.62% in YM and 181.55% in BM. The application of AsA under drought further enhanced activities of antioxidant enzymes in hemp plants, with SOD increasing by 4.37% in YM and 38.81% in BM, and POD increasing by 33.33% in YM and 60.94% in BM.



**Figure 3.** Effects of drought and AsA on antioxidant enzymes in hemp seedling. Different lowercases above the bars show significant difference between treatments ( $p < 0.05$ ). YM: industrial hemp cultivars ‘Yunma 1’; BM: industrial hemp cultivars ‘Bamahuoma’

*Effects of drought and AsA on osmotic regulation substance in hemp plant*

Compared to control group, the level of osmotic regulation substances (soluble sugar, soluble protein and proline contents) in hemp plants were increased significantly under drought stress (**Error! Reference source not found.**). For YM, the increase was about 41.31%, 40.66%, 78.87% in soluble sugar, soluble protein and proline, respectively. For BM, the increase was about 107.77%, 149.17%, 39.65% in soluble sugar, soluble protein and proline respectively. The highest soluble sugar and proline contents were obtained in drought + AsA treatment. Compared to drought treatment, there were about 4.44% and 21.50% increase in soluble sugar contents, and 27.83% and 42.62% increase in proline contents in YM and BM respectively. However, soluble protein contents of BM decreased (about 14.51%) at AsA treatment as compared to drought only treatment. Meanwhile, there were differences between the two varieties in the preference for producing osmotic potential regulating substances. In BM, drought stress is alleviated by producing numerous soluble protein and proline, in contrast to YM, which produces a large amount of soluble sugar to response drought.

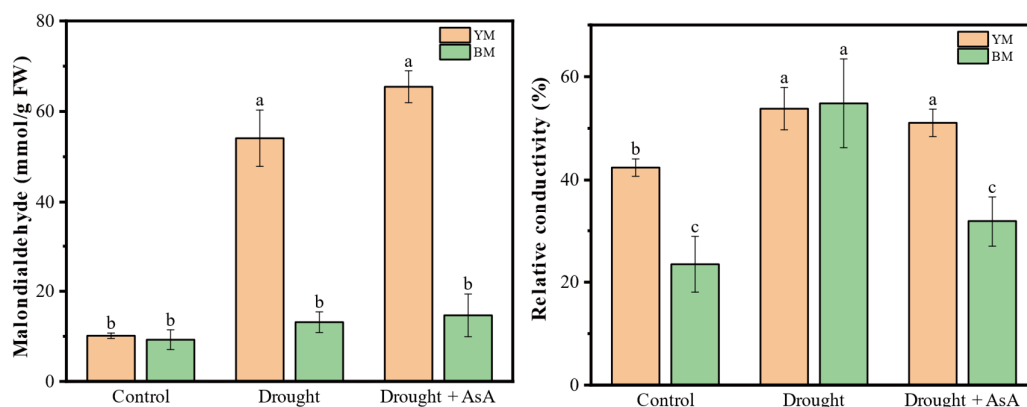


**Figure 4.** Effects of drought and AsA on osmotic regulation substance in hemp seedling. Different lowercases above the bars show significant difference between treatments ( $p < 0.05$ ). YM: industrial hemp cultivars ‘Yunma 1’; BM: industrial hemp cultivars ‘Bamahuoma’

*Effects of drought and AsA on cell membrane damage in hemp plant*

Drought disturbed the membrane stability in both cultivar (**Error! Reference source not found.**). There was a marked difference for MDA concentration between hemp cultivars when grown under drought and drought + AsA conditions. Compared to the control, MDA concentration increased in both cultivars under drought. Among them, drought caused an increase of about 433.85% in MDA concentration in YM, while it increased by 21.01% in BM. However, neither drought nor drought + AsA treatment had significant effects on MDA concentration of BM. Compared to the control, relative conductivity increased by

approximately 26.85% in YM and 133.59% in BM under drought stress. However, the application of AsA during drought conditions reduced the relative conductivity in BM by 41.89%, bringing it to a level that showed no significant difference from the control.



**Figure 5.** Effects of drought and AsA on cell membrane damage in hemp seedling. Different lowercases above the bars show significant difference between treatments ( $p < 0.05$ ). YM: industrial hemp cultivars 'Yunma 1'; BM: industrial hemp cultivars 'Bamahuoma'

## Discussion

### *Drought stress and AsA significantly affects the growth of hemp plants*

Drought stress disrupts plant growth, reducing plant height (Shemi *et al.*, 2021), reducing shoot and grain mass (Parveen *et al.*, 2021). In the present study, a marked disturbance in growth of hemp was also observed in plants under drought. According to these results, plants growth is susceptible to drought stress. Moreover, this research found that the AsA spraying treatment under drought stress promotes on stem diameter, plant fresh weight, and plant height (Figure 1). Those indicated that AsA spraying under drought conditions has a mitigation effect on hemp plants growth. Similar results have also been observed in other plant species. In maize, under 65% field water capacity, application of 100 mmol/L AsA slightly increased plant height as compared to without AsA treatment (Noman *et al.*, 2015). In cucumber, foliar-applied AsA (50 mg/L) was effective in enhancing AsA improved the fresh and dry weights under 60% field water capacity (Naz *et al.*, 2016). These results may be due to AsA's role in promoting cell elongation and increasing cell division in cotton and *Nicotiana tabacum* (de Pinto *et al.*, 1999; Song *et al.*, 2019).

### *AsA significantly increase the contents of photosynthetic pigments in hemp leaves*

As crucial components of the thylakoid membrane, photosynthetic pigments play a role in receiving light energy and directly participating in the photosynthetic process (Ansari *et al.*, 2019). In various plant species, such as cotton (Zahoor *et al.*, 2017) and strawberries (Li *et al.*, 2021), drought stress adversely impacted the levels of photosynthetic pigments, damaged the photosynthetic apparatus, and reduced the concentration of key enzymes involved in photosynthetic reactions. Consequently, substantial losses in plant growth and yield occur as a result of drought. In this study, under drought stress, the chlorophyll content of hemp showed a substantial decline, consistent with the results of in other species (Figure 2) (Khazaei *et al.*, 2020; Noman *et al.*, 2015). However, photosynthetic pigment content in drought treated plants significantly increased when exogenous AsA applied, compared with plant grown under drought without the application of AsA. It is worth mentioning that carotenoids were the most affected among the photosynthetic pigments. This may be because carotenoids play a role as photo protectant and antioxidant (Morelli and Rodriguez-Concepcion, 2023), and

thus, are usually preferentially degraded over chlorophyll under stress conditions (Seel *et al.*, 1992). Therefore, when exogenous AsA was applied, it effectively mitigated the degradation of carotenoids, and increasing the content of carotenoids. In Zheng's study, chlorophyll content was promoted due to the over-accumulation of AsA that scavenged reactive oxygen species (ROS) and inhibited the expression of senescence-related genes in *Arabidopsis thaliana* (Zheng *et al.*, 2020). In this sense maybe the AsA plays a crucial role in restoring plant photosynthetic capacity, helping to alleviate the damage caused by drought stress.

#### *Exogenous AsA could affect the physiological indexes of hemp under drought*

Membrane lipids peroxidation and impair of plant cell functions were due to the formation of superoxide radical and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) which triggered by drought stress (Farooq *et al.*, 2020). To alleviate such lasting effects and adapt to drought condition, plants usually accumulate antioxidants that enable them to maintain ROS homeostasis and withstand drought stress for a period of time (Dumanovic *et al.*, 2021). In *Arabidopsis thaliana*, contents of POD and SOD increased after 10 days of no watering (Liu *et al.*, 2020). Similarly, when subjected to 35-40% field water capacity, rapeseed increased SOD and POD activities to better scavenge ROS (Khan *et al.*, 2020). Consistent with studies in other plant species, drought affects the antioxidant enzymes activity of hemp plants in a significant way. In this study, we observed additionally enhancement of antioxidant enzymes with the application of AsA (Figure 3). This suggested AsA could serve as an antioxidant that reducing the generation of active oxygen, thus promoting the accumulation of biomass in plant (Loutfy *et al.*, 2020). Drought stress disturb osmotic regulation by three main reasons: affecting osmotic regulation substances contents, influencing cell membrane related substances, and damaging membrane integrity (Raza *et al.*, 2023). Sugar molecule act as an important nutrient as well as regulators of metabolism, stress response and development (Sami *et al.*, 2016). In herbaceous peony (*Paeonia lactiflora* Pall.), the content of soluble sugar increased to maintain osmotic homeostasis when seven days after watering was stopped, and the soluble sugar content continued to increase as the drought period prolonged (Li *et al.*, 2020). Proline plays a crucial role in osmoregulation, accumulating as a small molecule during drought stress and raising the osmotic pressure within cells to help them withstand drought conditions (Raza *et al.*, 2023). In the present study, proline and soluble sugar contents had increased significantly in both cultivars under drought and drought + AsA treatments compared with control (Figure 4). Especially, the greater impact of drought on proline contents of BM compared to YM cultivar indicates that BM is more responsive to osmoregulation. Moreover, Malondialdehyde (MDA) is one of the products of membrane lipid peroxidation, which can increase the permeability of cell membrane (Davey *et al.*, 2005). The change of MDA content can directly reflect the damage degree of cell membrane. Studies have shown that MDA content usually increases when plants receive stress (Ma *et al.*, 2015). As the result of the destruction of the cell membrane caused by drought stress, electrolyte leakage in the tissue increased, and the relative conductivity changed, consequently (Rolny *et al.*, 2011). In both cultivars, MDA contents and relative conductivity were improved under drought (Figure 5), this result is similar to that in pepper, where plants under 60% field water capacity had MDA contents 25% higher than those under full irrigation (Yildirim *et al.*, 2019), further proves that drought has a significant impact on the integrity of plant cell membranes. Meanwhile, enhancing of MDA content and electrolyte leakage indicates that drought stress caused oxidative damage to the membrane can be due to enhancing H<sub>2</sub>O<sub>2</sub> level (Hemmati *et al.*, 2018). Shafiq *et al.* (2014) reported that AsA reduces the generation of active oxygen species and mitigates their effects on the cell membrane, which aligns with the findings of this study, showing that relative conductivity decreases significantly in BM treated with AsA spraying under drought stress.

#### *Differences in responsive to exogenous AsA and drought stress between two cultivars*

Crop cultivars vary in their tolerance (or sensitivity) to stress conditions (Hu *et al.*, 2019). In this study, two industrial hemp varieties (BM and YM) were used. BM is mainly cultivated for seeds, while YM is grown

primarily for fiber. Moreover, the two varieties originate from different province of China (Guangxi and Yunnan provinces, respectively). The different origins and uses of the hemp cultivars may lead to varying response to drought. In this study, YM and BM plants treated with AsA showed improved drought tolerance, but with different physiological responses. Specifically, regarding osmotic regulation substance contents, the increase in soluble sugar was greater in YM compared to BM. Meanwhile, BM showed a more significant increase in soluble protein accumulation than YM. The differences in osmotic regulation substance contents under drought stress between two hemp cultivars suggest that drought stress and exogenously applied AsA are associated with the genetic nature of hemp. Similarly, Mohammad *et al.* (2024) reported changes in soluble protein and proline contents in several barley species under drought stress and AsA treatment. Their results showed a similar tendency to this study, the content of osmoregulatory substances increased under drought stress, but the extent of the increase varied among different cultivars. Additionally, there were notable differences in MDA contents and peroxidase activity between BM and YM. In the control treatment, the MDA content in leaf was similar in the tested two varieties was almost the same. However, YM accumulated significantly higher contents of MDA than BM under drought and drought + AsA. Moreover, compared to YM, BM showed a larger reduction in relative conductivity and a smaller accumulation of MDA contents, when sprayed with AsA under drought. In this study, regardless of the treatment, POD activity in YM was consistently significantly higher than that in BM. These differences indicated that effect of AsA is crop cultivar depended, which was also reflected in maize(Noman *et al.*, 2015).

## Conclusions

Our results indicated that AsA spraying could enhance the resistance of plants under stress, possibly due to its antioxidant function. There were differences in drought response between the tested hemp cultivars, BM and YM. In summary, this study had confirmed the alleviating effect of exogenous AsA on drought stress in hemp. However, we focused solely on examining the impact of AsA on the morphological traits and physiological parameters of hemp seedlings in a pot experiment. Thus, further study is still needed to address the molecular mechanism by which AsA alleviates drought stress in hemp. Moreover, the feasibility and effectiveness of applying AsA in field conditions should be assessed to support the cultivation of industrial hemp in arid and semi-arid regions.

## Authors' Contributions

Conceptualization, GD and QL; performed the experiments, WO and YZ; analyzed data and formal analysis, YY; writing original draft, QL and WO; revised the manuscript, KT, WO and GD.

All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

## Acknowledgements

This work was supported by the China Agriculture Research System of MOF and MARA (CARS-16-E15).

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

## References

- Alamri S, Hu Y, Mukherjee S, Aftab T, Fahad S, Raza A, ... Siddiqui MH (2020). Silicon-induced postponement of leaf senescence is accompanied by modulation of antioxidative defense and ion homeostasis in mustard (*Brassica juncea*) seedlings exposed to salinity and drought stress. *Plant Physiology and Biochemistry* 157:47-59. <https://doi.org/10.1016/j.plaphy.2020.09.038>
- Amaducci S, Scordia D, Liu FH, Zhang Q, Guo H, Testa G, Cosentino SL (2015). Key cultivation techniques for hemp in Europe and China. *Industrial Crops and Products* 68:2-16. <https://doi.org/10.1016/j.indcrop.2014.06.041>
- Ansari WA, Atri N, Pandey M, Singh AK, Singh B, Pandey S (2019). Influence of drought stress on morphological, physiological and biochemical attributes of plants: A review. *Biosciences Biotechnology Research Asia* 16:697-709. <http://dx.doi.org/10.13005/bbra/2785>
- Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24:1-15. <https://doi.org/10.1104/pp.24.1.1>
- Asada K (1992). Ascorbate peroxidase—a hydrogen peroxide-scavenging enzyme in plants. *Physiologia Plantarum* 85:235-241. <https://doi.org/10.1111/j.1399-3054.1992.tb04728.x>
- Hussain I, Ashraf MY, Saleem MH, Ashraf MA, Ali B, Shereen A, ... Yasin G (2023). Alleviating effects of salicylic acid spray on stage-based growth and antioxidative defense system in two drought-stressed rice (*Oryza sativa* L.) cultivars. *Turkish Journal of Agriculture and Forestry* 47:79-99. <https://doi.org/10.55730/1300-011X.3066>
- Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72:248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Chandra S, Lata H, ElSohly MA (2017). *Cannabis sativa* L. -botany and biotechnology. Springer. <https://doi.org/10.1007/978-3-319-54564-6>
- Davey MW, Stals B, Panis B, Keulemans J, Swennen RL (2005). High-throughput determination of malondialdehyde in plant tissues. *Analytical Biochemistry* 34:201-207. <https://doi.org/10.1016/j.ab.2005.09.041>
- de Pinto MC, Francis D, de Gara L (1999). The redox state of the ascorbate-dehydroascorbate pair as a specific sensor of cell division in tobacco BY-2 cells. *Protoplasma* 209:90-97. <https://doi.org/10.1007/BF01415704>
- Dumanović J, Nepovimova E, Natić M, Kuća K, Jačević V (2021). The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. *Frontiers in Plant Science* 11:552969. <https://doi.org/10.3389/fpls.2020.552969>
- Farooq A, Bukhari SA, Akram NA, Ashraf M, Wijaya L, Alyemeni MN, Ahmad P (2020). Exogenously applied ascorbic acid-mediated changes in osmoprotection and oxidative defense system enhanced water stress tolerance in different cultivars of safflower (*Carthamus tinctorious* L.). *Plants* 9:104. <https://doi.org/10.3390/plants9010104>
- Gupta A, Rico-Medina A, Caño-Delgado AI (2020). The physiology of plant responses to drought. *Science* 368:266-269. <https://doi.org/10.1126/science.aaz7614>
- Hu H, Liu H, Du G, Fei Y, Deng G, Yang Y, Liu F (2019). Fiber and seed type of hemp (*Cannabis sativa* L.) responded differently to salt-alkali stress in seedling growth and physiological indices. *Industrial Crops and Products* 129:624-630. <https://doi.org/10.1016/j.indcrop.2018.12.028>
- Hemmati K, Ebadi A, Khomari S, Sedghi M (2018). Influence of ascorbic acid and 24-epibrassinolide on physiological characteristics of pot marigold under water-stress condition. *Journal of Plant Interactions* 13(1):364-372. <https://doi.org/10.1080/17429145.2018.1483033>
- Jiang Y, Sun Y, Zheng D, Han C, Cao K, Xu L, ... Feng N (2021). Physiological and transcriptome analyses for assessing the effects of exogenous uniconazole on drought tolerance in hemp (*Cannabis sativa* L.). *Scientific Reports* 11(1):14476. <https://doi.org/10.1038/s41598-021-93820-6>

- Karataş İ, Öztürk L, Demir Y, Ünükara A, Kurunç A, Düzdemir O (2014). Alterations in antioxidant enzyme activities and proline content in pea leaves under long-term drought stress. *Toxicology and Industrial Health* 30:693-700. <https://doi.org/10.1177/0748233712462471>
- Khan MN, Khan Z, Luo T, Liu J, Rizwan M, Zhang J, ... Hu L (2020). Seed priming with gibberellic acid and melatonin in rapeseed: Consequences for improving yield and seed quality under drought and non-stress conditions. *Industrial Crops and Products* 156:112850. <https://doi.org/10.1016/j.indcrop.2020.112850>
- Khazaei Z, Esmailpour B, Estaji A (2020). Ameliorative effects of ascorbic acid on tolerance to drought stress on pepper (*Capsicum annuum* L.) plants. *Physiology and Molecular Biology of Plants* 26:1649-1662. <https://doi.org/10.1007/s12298-020-00846-7>
- Kwiatkowska E, Zimmiewska M, Róžańska W, Puchalski M, Przybylska P (2024). Assessing the impact of drought stress on hemp (*Cannabis sativa* L.) fibers. *Materials* 17(17):4198. <https://doi.org/10.3390/ma17174198>
- Latif M, Akram NA, Ashraf M (2016). Regulation of some biochemical attributes in drought-stressed cauliflower (*Brassica oleracea* L.) by seed pre-treatment with ascorbic acid. *The Journal of Horticultural Science and Biotechnology* 91:129-137. <https://doi.org/10.1080/14620316.2015.1117226>
- Li M, Yang Y, Raza A, Yin S, Wang H, Zhang Y, ... Jin W (2021). Heterologous expression of Arabidopsis thaliana rry gene in strawberry (*Fragaria × ananassa* Duch.) improves drought tolerance. *BMC Plant Biology* 21:1-20. <https://doi.org/10.1186/s12870-021-02839-4>
- Li T, Wang R, Zhao D, Tao J (2020). Effects of drought stress on physiological responses and gene expression changes in herbaceous peony (*Paeonia lactiflora* Pall.). *Plant Signaling & Behavior* 15(5):1746034. <https://doi.org/10.1080/15592324.2020.1746034>
- Liu G, Li B, Li X, Wei Y, He C, Shi H (2020). MaWRKY80 positively regulates plant drought stress resistance through modulation of abscisic acid and redox metabolism. *Plant Physiology and Biochemistry* 156:155-166. <https://doi.org/10.1016/j.plaphy.2020.09.015>
- Loutfy N, Azooz M, Abou Alhamed MF (2020). Exogenously-applied salicylic acid and ascorbic acid modulate some physiological traits and antioxidative defense system in *Zea mays* L. seedlings under drought stress. *Egyptian Journal of Botany* 60:313-324. <https://doi.org/10.21608/ejbo.2020.20077.1400>
- Lutts S, Kinet JM, Bouharmont J (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of Botany* 78:389-398. <https://doi.org/10.1006/anbo.1996.0134>
- Ma J, Du G, Li X, Zhang C, Guo J (2015). A major locus controlling malondialdehyde content under water stress is associated with Fusarium crown rot resistance in wheat. *Molecular Genetics and Genomics* 290:1955-1962. <https://doi.org/10.1007/s00438-015-1053-3>
- Mishra S, Sharma A, Srivastava AK (2024). Ascorbic acid: a metabolite switch for designing stress-smart crops. *Critical Reviews in Biotechnology* 44(7):1350-1366. <https://doi.org/10.1080/07388551.2023.2286428>
- Morelli L (2023). Open avenues for carotenoid biofortification of plant tissues. *Plant Communications* 4:100466. <https://doi.org/10.1016/j.xplc.2022.100466>
- Mohammad AA, Ammara S, Minahil T, Sheza AK, Zahoor AS, Koloko BL, ... Parvaiz A (2024). Modulation of the polyamines, osmolytes and antioxidant defense system to ameliorate drought stress tolerance in *Hordeum vulgare* L. using ascorbic acid. *South African Journal of Botany* 171:726-736. <https://doi.org/10.1016/j.sajb.2024.06.032>
- Naz H, Aisha N, Ashraf M (2016). Impact of ascorbic acid on growth and some physiological attributes of cucumber (*Cucumis sativus*) plants under water-deficit conditions. *Pakistan Journal of Botany* 48(3):877-883. [http://inis.iaea.org/search/search.aspx?orig\\_q=RN:47116071](http://inis.iaea.org/search/search.aspx?orig_q=RN:47116071)
- Noman A, Ali S, Naheed F, Ali Q, Farid M, Rizwan M, Irshad MK (2015). Foliar application of ascorbate enhances the physiological and biochemical attributes of maize (*Zea mays* L.) cultivars under drought stress. *Archives of Agronomy and Soil Science* 61:1659-1672. <https://doi.org/10.1080/03650340.2015.1028379>
- Parveen A, Arslan Ashraf M, Hussain I, Perveen S, Rasheed R, Mahmood Q, ... Abd Allah EF (2021). Promotion of growth and physiological characteristics in water-stressed *Triticum aestivum* in relation to foliar-application of salicylic acid. *Water* 13:1316. <https://doi.org/10.3390/w13091316>
- Qin X, Huang T, Lu C, Dang P, Zhang M, Guan XK, ... Siddique KH (2021). Benefits and limitations of straw mulching and incorporation on maize yield, water use efficiency, and nitrogen use efficiency. *Agricultural Water Management* 256:107128. <https://doi.org/10.1016/j.agwat.2021.107128>

- Raza A, Mubarik MS, Sharif R, Habib M, Jabeen W, Zhang C, ... Varshney RK (2023). Developing drought-smart, ready-to-grow future crops. *The Plant Genome* 16:e20279. <https://doi.org/10.1002/tpg2.20279>
- Rolny N, Costa L, Carrión C, Guimet JJ (2011). Is the electrolyte leakage assay an unequivocal test of membrane deterioration during leaf senescence? *Plant Physiology and Biochemistry* 49:1220-1227. <https://doi.org/10.1016/j.plaphy.2011.06.010>
- Sami F, Yusuf M, Faizan M, Faraz A, Hayat S (2016). Role of sugars under abiotic stress. *Plant Physiology and Biochemistry* 109:54-61. <https://doi.org/10.1016/j.plaphy.2016.09.005>
- Seel WE, Hendry GAF, Lee JA (1992). The combined effects of desiccation and irradiance on mosses from xeric and hydric habitats. *Journal of Experimental Botany* 43:1023-1030. <https://doi.org/10.1093/jxb/43.8.1023>
- Shemi R, Wang R, Gheith ESM, Hussain HA, Hussain S, Irfan M, ... 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>
- Song W, Wang F, Chen L, Ma R, Zuo X, Cao A, ... Li H (2019). *GhVTC1*, the key gene for ascorbate biosynthesis in *Gossypium hirsutum*, involves in cell elongation under control of ethylene. *Cells* 8:1039. <https://doi.org/10.3390/cells8091039>
- Wayne M, Jagdeep S, Katelyn K, Jeanine D, Alvaro S (2024). Severe drought significantly reduces floral hemp (*Cannabis sativa* L.) yield and cannabinoid content but moderate drought does not. *Environmental and Experimental Botany* 219:105649. <https://doi.org/10.1016/j.envexpbot.2024.105649>
- Yildirim E, Ekinici M, Kul R, Turan M, Gur A (2019). Mitigation of drought stress effects on pepper seedlings by exogenous methylamine application. *International Letters of Natural Sciences* 76:111-123. <https://doi.org/10.56431/p-8o3bh8>
- Zahoor R, Dong H, Abid M, Zhao W, Wang Y, Zhou Z (2017). Potassium fertilizer improves drought stress alleviation potential in cotton by enhancing photosynthesis and carbohydrate metabolism. *Environmental and Experimental Botany* 137:73-83. <https://doi.org/10.1016/j.envexpbot.2017.02.002>
- Zheng XT, Yu ZC, Chen XF, Cai ML, Wang YZ, Peng CL (2020). Endogenous ascorbic acid delays ethylene-induced leaf senescence in *Arabidopsis thaliana*. *Photosynthetica* 58:720-731. <http://dx.doi.org/10.32615/ps.2020.028>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



**License** - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.  
© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

**Notes:**

- **Material disclaimer:** The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- **Maps and affiliations:** The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- **Responsibilities:** The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.