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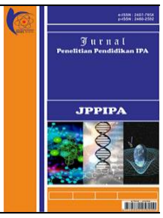

Analysis of Calcium Oxalate Content and Stomata Amaranth Leaves (*Amaranthus tricolor* var. Giti Red) as Response to Drought Stress

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Abstract: Amaranth is a plant that has calcium oxalate content in leaves. One of the roles calcium oxalate in plants is to increase drought tolerance. In addition, amaranth leaves also have anomocytic stomata. Stomata plant leaves are known to be formed genetically and not affected morphoanatomically under drought stress conditions, except density and conductance. Therefore, the research aimed to find out how the response of calcium oxalate levels and stomata of amaranth leaves (*Amaranthus tricolor* var. Red Giti) under drought stress. The research method used was a randomized block design (RBD) with two treatments, which are watering every day (WD) and watering at 50% wilting (SD) by observing stomatal density (stomata/mm^2) and calcium oxalate content (%). Data were analyzed statistically with independent T-test and chi-square. The results showed that stomatal density and calcium oxalate content were affected by drought stress with a significantly decreased response. This indicates that the amaranth plant may become a plant that is resistant to drought stress by decreasing stomatal density and calcium oxalate levels.

Keywords: Amaranth; Calcium Oxalate; Drought; Giti Red; Stomata

Introduction

Amaranth (*Amaranthus tricolor* L.) is one of the plant commodities cultivated and consumed by Indonesian people. Because amaranth contains nutrients, vitamins, and minerals that are beneficial for humans. This plant also grows in tropical and subtropical areas (Amalia et al., 2023; Kasmira et al., 2018). Amaranth has leaf micromorphology with anomocytic stomata (Jimoh, 2019). Amaranth leaves contain calcium oxalate and oxalic acid. The calcium oxalate in amaranth leaves has druce crystals (Capacio et al., 2018; Cerritos-Castro et al., 2022).

Drought stress research on plant calcium oxalate levels was carried out by Gouveia et al. (2020) and Tooulakou et al. (2019). Meanwhile, leaf stomata against drought stress by Liu et al. (2022), Zhu et al. (2021), and He et al. (2020) explained that stomata can be affected by drought stress by decreasing their density and conductivity. This is a form of plant response and mechanism to survive and prevent more water loss due to evapotranspiration. Research by Gouveia et al. (2020) and Tooulakou et al. (2019) explained that drought stress significantly affects calcium oxalate in plants with a response to decreasing its accumulation. Calcium oxalate is researched because it plays an important role in plant physiology, especially in increasing drought stress tolerance.

In plants, Ca^{2+} is usually stored as calcium oxalate crystals in plastids. The availability of Ca^{2+} is strongly linked to better plant growth, structural integrity of stems and the quality of fruit produced. It is also known that calcium acts as an activator of a number of enzymes, such as ATPase, phospholipases, amylase and

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succinidase. The metabolic pathway that uses ATP is mainly found in the cytoplasm and is kept separate from Ca^{2+} stores, which are mainly found in the apoplast, vacuole, endoplasmic reticulum ER and to a lesser extent in mitochondria, chloroplasts and nucleus (Robertson, 2013).

The strategies that plants use to survive water shortage include drought escape, drought avoidance, and drought tolerance. The first response of a plant's drought avoidance strategy is stomatal closure, which prevents excessive water loss (Osmolovskaya et al., 2018). In addition, plants respond to drought stress through various other morphological and physiological responses (Chen et al., 2016). To cope with current and future climate limitations and to ensure food security, the production of more climate-resilient crops is required.

This research uses amaranth (*Amaranthus tricolor* var. Red Giti) with the aim of finding out how calcium oxalate content and leaf stomata are resulted as response to drought stress. The Red Giti was chosen because it is one of the superior varieties produced in Indonesia and is stable in interacting with the environment (Tjapbukitmas, 2022). Amaranth leaves are an important source of phytochemicals including betalains and phenolic compounds (Tang et al., 2017). In addition, amaranth is capable of accumulating high levels of oxalate as calcium oxalate crystals, which are considered a major antinutrient (Vargas-Ortiz et al., 2021).

Method

This research used an experimental method with a Randomized Block Design (RBD) with two drought stress treatments using watering intensity, namely watering every day (WD) and watering at 50% wilting (SD). This treatment was applied to the Giti Red amaranth variety as a test plant. This design was carried out in 13 repetitions per treatment. Each replication consisted of 3 experimental units, for a total of 78 experimental units. In each experimental unit, 1 plant was observed to have the same height.

The research was carried out with 78 Giti Red amaranth seeds planted in each 1500 ml plastic bottle with a diameter of 8 cm as a pot containing red soil and cow manure in a ratio of 1:1 v/v in a Greenhouse. Seeds are grown until 10 HST and treated at 11-30 HST with treatment, namely watering every day (WD) and watering when they wilt 50% (SD). The daily watering (WD) treatment carried out to maintain water availability in media. The watering when wilted 50% (SD) treatment carried out when half of the population 78 plants wilted, that is, 39 new plants would be watered throughout the group. Criteria for wilted plants are observed in the wilting of leaves. Watering carried out on each plant per treatment group using 200 ml water (Mukhtar, 2016). Parameter measurements of calcium oxalate levels and leaf stomata carried out when the plants wilted.

Stomata density was measured using the imprint technique, namely printing leaf stomata using acetone. The imprint technique carried out by apply acetone from the edge of the leaf towards the midrib horizontally with a width of 2 cm. Preliminary research carried out on amaranth leaves to determine stable stomata density for use in actual research. Preliminary research carried out by apply nail polish liquid to the abaxial (bottom) part of amaranth leaves during the growth of plants. Take each leaf from the amaranth and clean it with a tissue. After that, the surface of the leaves is smeared with acetone. Then, wait until it dries and stick it with clear tape. The tape is pulled and attached to the slide. Leaf stomata were observed under a light microscope with a magnification of 400x with a field of view of 0.025 mm^2 (Humami et al., 2020). Then, leaf stomata density was calculated using the following formula and analyzed statistically using the chi-square test.

$$\text{Stomata Density} = \frac{Amount\ of\ Stomata}{Field\ of\ View} \quad (1)$$

Measurement of calcium oxalate content carried out in preliminary research to determine stable calcium oxalate levels in amaranth leaves for use in actual research. Standardization of permanganometric titration carried out using $KMnO_4$ 0.01 N, $H_2C_2O_4$ 0.01 N, and H_2SO_4 2 N. Standardization carried out by dissolving 10 ml of $H_2C_2O_4$ 0.01 N with 5 ml of H_2SO_4 2 N and heating at a temperature of 80°C for 10 minutes. The titration carried out in three repetitions until the color was completely purple. Amaranth leaves took as many as five leaf per repetition, pounded with a mortar, and filtered with Whatman filter paper into a beaker glass. The filtrate was boiled on a hot plate for 10 minutes at 100°C to obtain calcium oxalate extract. After that, the filtrate lefted for 5 minutes at room temperature 28°C. Analysis calcium oxalate content carried out by taking 1 ml of extract in each replication per treatment. The extract put into a 100 ml flask and distilled by water added until the mark reached. After that, shake it back and forth. The amaranth leaf extract diluted and transferred into an Erlenmeyer in 10 ml. Titration carried out by adding 5 ml of 2 N H_2SO_4 and heating at a temperature of 70-80°C for 10 minutes. Then, 0.01 N $KMnO_4$ solution added until it turned purple. Titration carried out in 3 repetitions for each repetition per treatment. Calcium oxalate levels were calculated using the following formula and analyzed statistically with the independent samples T-test.

$$\text{Calcium Oxalate (\%)} = \frac{V\ KMnO_4 \times N\ KMnO_4 \times BE\ CaOx}{V\ Filtrate} \times 100\% \qquad (2)$$

Where :
V $KMnO_4$: Volume potassium permanganate
N $KMnO_4$: Mol potassium permanganate
BE CaO_x : Ekiv weight calcium oxalate (128 g/mol)
V Filtrate: Volume leaf filtrate (mL)

Result and Discussion

The stomata density and calcium oxalate content of amaranth leaves of Red Giti which are watered every day (WD) and watered when they wilt 50% (SD) which can be seen in Table 1. The results chi-square test on the stomata density of Red Giti with a level of 5% (α <0.05) indicate a significant difference. It means that Red Giti watered every day (WD) and watered when it is 50% wilted (SD) significantly affects stomata density. The stomata density of Red Giti watered every day (WD) and watered when it wilts 50% (SD) illustrated in Figure 1. The Red Giti watered every day (WD) shows a greater stomata density than watered when it wilts 50% (SD). The Red Giti with smaller stomata density is hypothesized to be more tolerant under drought stress. This study is in accordance with Hasanuzzaman et al. (2023) explained that drought stress affects stomata density and will reduce significantly as a mechanism to increase water use efficiency and prevent increased leaf transpiration.

Reducing stomatal density refers to the number of stomata that close when drought stress occurs. Stomata closure prevents increased transpiration as a form of adaptation to conditions of lack of water availability in the environment. Stomata closure, apart from preventing transpiration, can also cause starvation. Because closed stomata also hinder gas exchange. Thus, it is difficult for CO_2 in the environment to enter and as a result plant photosynthesis is disrupted (Bertolino et al., 2019; Driesen et al., 2020; Pridgeon et al., 2021).

Agurla et al. (2018) explained that the mechanism of stomata closure due to drought stress occurs due to the mobilization of phytohormones as a defense signal against environmental stress. This phytohormone is known to be abscisic acid (ABA). Apart from that, it turns out that calcium ions also play a role in stomata closure. Because these component signals are known to inhibit potassium ion channels in the stomata when environmental stress occurs.

Bharath et al. (2021) stated that stomata closure due to induction from ABA is an important component of plant defense against abiotic stress. Because this signal can stimulate plants in a stressful environment. Apart from that, induction of ABA is also known to be related to other components, such as jasmonate metabolites and salicylic acid. These two components are also factors in increasing plant adaptation to environmental stress. Because this component increases secondary metabolite compounds in the plant.

In addition to the components ABA, jasmonate and salicylic acid. Eisenach et al. (2017) also explained that apart from ABA signals, it influences stomata closure. Potassium ions and malic acid also play an important role in the osmolyte concentration of guard cell vacuoles. Because mediation of malic acid release can stimulate the ABA signal response for stomata closure. Mediating the release of malic acid is also due to the activity of the phosphorylatable C-terminal serine channel.

Table 1. Stomata Density and Calcium Oxalate Content of Amaranth Leaves Red Giti

Treatment	Stomata density (stomata/mm^2)	Calcium oxalate content (%)	Chi-square test stomata density	T test calcium oxalate content
WD	11777.70 ±1812.60	0.19±0.01	0.000	0.037
SD	7554.90 ±691.90	0.18±0.01		

Numerical values are mean ± standard error tested by chi square and independent samples T-test (α<0.05)
Stomata density and calcium oxalate content of amaranth leaves Red Giti had a significant effect using chi-square test and independent samples T-test (α<0.05)
WD = watering every day
SD = watering when wilt 50%

Stomatal regulation is a known mechanism to avoid water shortages in response to drought stress (Zhang et al., 2018). Stomata density is closely related to stomata conductance and photosynthetic ability and smaller stomatal aperture size contributes to reducing water loss and increasing drought avoidance in plants (Bi et al., 2017; Mo et al., 2016; Zhang et al., 2018). Zhu et al. (2021) stated that drought stress on stomata significantly reduces their size and density. Adaptive changes in stomata occur especially in the early stages of drought stress conditions (Mo et al., 2016). This suggests that drought stress exerts a sensitive influence on stomatal adjustments that contribute to preventing water loss and increasing resistance to drought stress (Zhu et al., 2021). Gano et al. (2021) and Liu et al. (2022) explained that the appearance of stomata during drought stress decreased significantly as a form of plant response to tolerate conditions of water shortage and excessive transpiration. The appearance of stomata affects the number and density of stomata on plants. Himes et al. (2021) stated that abaxial leaf stomata density has a major impact on drought resistance parameters and is significantly influenced under drought stress.

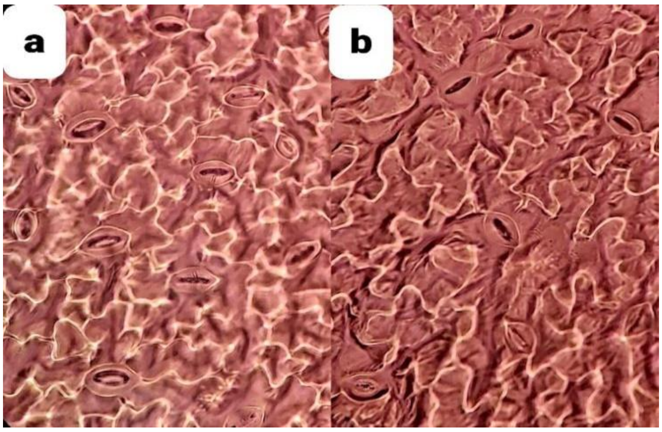

Figure 1. Densitas stomata bayam varietas Giti Red yang (a) disiram setiap hari (WD) dan (b) disiram ketika layu 50% (SD)

Calcium oxalate levels that were watered every day (WD) and watered when wilting was 50% (SD) showed that the results of independent samples T-test were significantly different which can be seen in Table 1. This states that the Giti Red amaranth variety that was watered every day (WD) and watered when wilted 50% (SD) significantly affected calcium oxalate content. The treatment watered every day (WD) showed that the calcium oxalate content of amaranth leaves of the Red Giti was greater than watered when wilted by 50% (SD). The results of these low calcium oxalate content hypothesized to be plants that are tolerant to drought stress.

The results of this study by Gouveia et al. (2020) explained that calcium oxalate levels in plants decrease under drought stress. Calcium oxalate can help regulate plants in drought stress conditions by adjusting oxalic acid which is synthesized by the oxidative process of photosynthesis. This explains that calcium oxalate in drought-stress conditions makes adjustments through a synthesis of oxalic acid from calcium oxalate with the enzyme oxalate oxidase to produce additional carbon, so that calcium oxalate levels in plants decrease under drought stress. Gouveia et al. (2020) explanation is by research results which showed low calcium oxalate levels in Red Giti under drought stress. It hypothesized that the Red Giti can tolerate drought stress.

Du et al. (2018) and Tooulakou et al. (2016) explained that the decrease in calcium oxalate levels occurred due to the activity of the oxalate oxidase enzyme which converts calcium oxalate into carbon dioxide and hydrogen peroxide. The carbon dioxide produced becomes additional carbon for plants experiencing drought stress to survive, so the lower the calcium oxalate levels, the more tolerant the plants are to drought stress. Gómez-Espinoza et al. (2020) also stated that conditions of CO_2 deficiency due to drought stress can reduce calcium oxalate levels due to decomposition. This was a form of plant response to provide carbon for photosynthesis when atmospheric CO_2 into the leaf mesophyll is limited by environmental factors, like drought stress. In addition, this lack of CO_2 occurs because it is influenced by the stomata closing during drought stress so that the more stomata are closed, the more CO_2 is blocked from entering the leaf mesophyll (Fan, 2019; Tooulakou et al., 2019).

Conclusion

Drought stress significantly affected calcium oxalate content and stomata of amaranth leaves of the Red Giti Red. This effect responds by decreasing calcium oxalate levels and stomatal density in plant leaves. This response also allows plants to be tolerant to drought stress.

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Author Contributions

Imam Safir Alwan Nurza: conceptualization, analysis, methodology, discussion, conclusion, validation, visualization, investigation, writing–original draft, review, editing, and proofreading; Chika Shafa Maura: funding acquisition and resources.

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Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

References

Agurla, S., Gahir, S., Munemasa, S., Murata, Y., & Raghavendra, A. S. (2018). Mechanism of Stomatal Closure in Plants Exposed to Drought and Cold Stress. In *Adv. Exp. Med. Biol* (Vol. 1081, pp. 215–232). https://doi.org/10.1007/978-981-13-1244-1_12

Amalia, D. R., & Rachmawati, D. (2023).

Morphophysiological responses of red amaranth (Amaranthus tricolor L.) to osmopriming treatment to overcoming salinity stress. *IOP Conference Series: Earth and Environmental Science*, *1165*(1), 012017. https://doi.org/10.1088/1755-1315/1165/1/012017

Bertolino, L. T., Caine, R. S., & Gray, J. E. (2019). Impact of Stomatal Density and Morphology on Water-Use Efficiency in a Changing World. *Frontiers in Plant Science*, *10*, 225. https://doi.org/10.3389/fpls.2019.00225

Bharath, P., Gahir, S., & Raghavendra, A. S. (2021). Abscisic Acid-Induced Stomatal Closure: An Important Component of Plant Defense Against Abiotic and Biotic Stress. *Frontiers in Plant Science*, *12*, 615114. https://doi.org/10.3389/fpls.2021.615114

Bi, H., Kovalchuk, N., Langridge, P., Tricker, P. J., Lopato, S., & Borisjuk, N. (2017). The impact of drought on wheat leaf cuticle properties. *BMC Plant Biology*, *17*(1), 85. https://doi.org/10.1186/s12870-017-1033-3

Capacio, A. F., & Belonias, B. (2018). Occurrence and variation of calcium oxalate crystals in selected medicinal plant species. *Annals of Tropical Research*, *40*(2), 45–60. https://doi.org/10.32945/atr4024.2018

Cerritos- Castro, I. T., Patrón- Soberano, A., Bojórquez-Velázquez, E., González- Escobar, J. L., Vargas-Ortiz, E., Muñoz- Sandoval, E., & Barba de la Rosa, A. P. (2022). Amaranth calcium oxalate crystals are associated with chloroplast structures and proteins. *Microscopy Research and Technique*, *85*(11), 3694–3706. https://doi.org/10.1002/jemt.24221

Chen, D., Wang, S., Cao, B., Cao, D., Leng, G., Li, H., Yin, L., Shan, L., & Deng, X. (2016). Genotypic Variation in Growth and Physiological Response to Drought Stress and Re-Watering Reveals the Critical Role of Recovery in Drought Adaptation in Maize Seedlings. *Frontiers in Plant Science*, *6*, 1241. https://doi.org/10.3389/fpls.2015.01241

Driesen, E., Van den Ende, W., De Proft, M., & Saeys, W. (2020). Influence of Environmental Factors Light, CO2, Temperature, and Relative Humidity on Stomatal Opening and Development: A Review. *Agronomy*, *10*(12), 1975. https://doi.org/10.3390/agronomy10121975

Du, X., Ren, X., Wang, L., Yang, K., Xin, G., Jia, G., Ni, X., & Liu, W. (2018). Calcium oxalate degradation is involved in aerenchyma formation in Typha angustifolia leaves. *Functional Plant Biology*, *45*(9), 922. https://doi.org/10.1071/FP17349

Eisenach, C., Baetz, U., Huck, N. V., Zhang, J., De Angeli, A., Beckers, G. J. M., & Martinoia, E. (2017). ABA-Induced Stomatal Closure Involves ALMT4, a Phosphorylation-Dependent Vacuolar Anion Channel of Arabidopsis. *The Plant Cell*, *29*(10), 2552–2569. https://doi.org/10.1105/tpc.17.00452

Fan, D. (2019). The Effect of Calcium to Maize Seedlings under Drought Stress. *American Journal of Plant Sciences*, *10*(08), 1391–1396. https://doi.org/10.4236/ajps.2019.108099

Gano, B., Dembele, J. S. B., Tovignan, T. K., Sine, B., Vadez, V., Diouf, D., & Audebert, A. (2021). Adaptation Responses to Early Drought Stress of West Africa Sorghum Varieties. *Agronomy*, *11*(3), 443. https://doi.org/10.3390/agronomy11030443

Gouveia, C. S. S., Lebot, V., & Pinheiro de Carvalho, M. (2020). NIRS Estimation of Drought Stress on Chemical Quality Constituents of Taro (Colocasia esculenta L.) and Sweet Potato (Ipomoea batatas L.) Flours. *Applied Sciences*, *10*(23), 8724. https://doi.org/10.3390/app10238724

Hasanuzzaman, M., Zhou, M., & Shabala, S. (2023). How Does Stomatal Density and Residual Transpiration Contribute to Osmotic Stress Tolerance? *Plants*, *12*(3), 494. https://doi.org/10.3390/plants12030494

He, X., Xu, L., Pan, C., Gong, C., Wang, Y., Liu, X., & Yu, Y. (2020). Drought resistance of Camellia oleifera under drought stress: Changes in physiology and growth characteristics. *PLOS ONE*, *15*(7), e0235795. https://doi.org/10.1371/journal.pone.0235795

Himes, A., Emerson, P., McClung, R., Renninger, H., Rosenstiel, T., & Stanton, B. (2021). Leaf traits indicative of drought resistance in hybrid poplar. *Agricultural Water Management*, *246*(1), 106676. https://doi.org/10.1016/j.agwat.2020.106676

Humami, D. W., Sujono, P. A. W., & Desmawati, I. (2020). Densitas dan Morfologi Stomata Daun Pterocarpus indicus di Jalan Arif Rahman Hakim dan Kampus ITS, Surabaya. *Rekayasa*, *13*(3), 240–245. https://doi.org/10.21107/rekayasa.v13i3.7869

Jimoh, M. O. (2019). Micromorphological assessment of leaves of amaranthus caudatus l. Cultivated on formulated soil types. *Applied Ecology and Environmental Research*, *17*(6), 13593–13605. https://doi.org/10.15666/aeer/1706_1359313605

Kasmira, K., Lahming, L., & Fadilah, R. (2018). Analisis Perubahan Komponen Kimia Keripik Bayam Hijau (Amaranthus tricolor.L) Akibat Proses Penggorengan. *Jurnal Pendidikan Teknologi Pertanian*, *1*, 49. https://doi.org/10.26858/jptp.v1i0.6232

Liu, X., Chen, A., Wang, Y., Jin, G., Zhang, Y., Gu, L., Li, C., Shao, X., & Wang, K. (2022). Physiological and transcriptomic insights into adaptive responses of Seriphidium transiliense seedlings to drought stress. *Environmental and Experimental Botany*, *194*, 104736. https://doi.org/10.1016/j.envexpbot.2021.104736

Mo, Y., Yang, R., Liu, L., Gu, X., Yang, X., Wang, Y., Zhang, X., & Li, H. (2016). Growth, photosynthesis and adaptive responses of wild and domesticated watermelon genotypes to drought stress and subsequent re-watering. *Plant Growth Regulation*, *79*(2), 229–241. https://doi.org/10.1007/s10725-015-0128-9

Osmolovskaya, N., Shumilina, J., Kim, A., Didio, A., Grishina, T., Bilova, T., Keltsieva, O. A., Zhukov, V., Tikhonovich, I., Tarakhovskaya, E., Frolov, A., & Wessjohann, L. A. (2018). Methodology of Drought Stress Research: Experimental Setup and Physiological Characterization. *International Journal of Molecular Sciences*, *19*(12), 4089. https://doi.org/10.3390/ijms19124089

Pridgeon, A. J., & Hetherington, A. M. (2021). ABA signalling and metabolism are not essential for dark-induced stomatal closure but affect response speed. *Scientific Reports*, *11*(1), 5751. https://doi.org/10.1038/s41598-021-84911-5

Robertson, D. (Niki). (2013). Modulating Plant Calcium for Better Nutrition and Stress Tolerance. *ISRN Botany*, *2013*, 1–22. https://doi.org/10.1155/2013/952043

Tang, Y., & Tsao, R. (2017). Phytochemicals in quinoa and amaranth grains and their antioxidant, anti-inflammatory, and potential health beneficial effects: a review. *Molecular Nutrition & Food Research*, *61*(7), 1–16. https://doi.org/10.1002/mnfr.201600767

Tjapbukitmas. (2022). *Amaranth Red Giti*. Retrieved from https://www.tjapbukitmas.co.id/product-details/12.

Tooulakou, G., Dimosthenis, N., Elissavet, D., Malvina, G. O., Christos, G. K., Georgios, L., Maria, I. K., & George, K. (2019). Changes in Size and Composition of Pigweed (Amaranthus hybridus L.) Calcium Oxalate Crystals Under CO2 Starvation Conditions. *Physiologia Plantarum*, *166*(3), 862–872. https://doi.org/10.1111/ppl.12843.

Tooulakou, G., Giannopoulos, A., Nikolopoulos, D., Bresta, P., Dotsika, E., Orkoula, M. G., Kontoyannis, C. G., Fasseas, C., Liakopoulos, G., Klapa, M. I., & Karabourniotis, G. (2016). Reevaluation of the plant "gemstones": Calcium oxalate crystals sustain photosynthesis under drought conditions. *Plant Signaling & Behavior*, *11*(9), e1215793. https://doi.org/10.1080/15592324.2016.1215793

Vargas-Ortiz, E., Ramírez-Tobias, H. M., González-Escobar, J. L., Gutiérrez-García, A. K., Bojórquez-Velázquez, E., Espitia-Rangel, E., & Barba de la Rosa, A. P. (2021). Biomass, chlorophyll fluorescence, and osmoregulation traits let differentiation of wild and cultivated Amaranthus under water stress. *Journal of Photochemistry and Photobiology B: Biology*, *220*, 112210. https://doi.org/10.1016/j.jphotobiol.2021.112210

Zhang, S., XU, X., SUN, Y., ZHANG, J., & LI, C. (2018). Influence of drought hardening on the resistance physiology of potato seedlings under drought stress. *Journal of Integrative Agriculture*, *17*(2), 336–347. https://doi.org/10.1016/S2095-3119(17)61758-1

Zhu, J., Cai, D., Wang, J., Cao, J., Wen, Y., He, J., Zhao, L., Wang, D., & Zhang, S. (2021). Physiological and anatomical changes in two rapeseed (Brassica napus L.) genotypes under drought stress conditions. *Oil Crop Science*, *6*(2), 97–104. https://doi.org/10.1016/j.ocsci.2021.04.003