LRF-750
[1-6]

RESEARCH ARTICLE   Legume Research- An International Journal

Effects of Drought Stress on SOD Activity and Pro Content in Different Parts of Soybean Leaves

Yumei Tian[1], Xiaomei Li[2], Xinyu Zhou[1], Zhipeng Qu[1], Xiyue Wang[1], Shoukun Dong[1]

10.18805/LRF-750

ABSTRACT

Background: Drought is the main restricted agent for growth and development of soybean and the physiological characteristics of different parts of soybean leaves changed significantly.

Methods: To explore the impacts of drought stress on the physiological characteristics of leaves in different parts of soybean, in this study, Williams 82 was used as the experimental material and drought stress was simulated by a watering nutrient solution containing 10% PEG-6000. Under different treatment time conditions, the superoxide dismutase (SOD) activity and proline (Pro) content in different parts of soybean leaves were determined.

Result: The results indicate that the SOD activity and Pro content in the upper and bottom leaves of soybean increased significantly under drought stress conditions and with the increase in drought stress time, the SOD activity in the upper and bottom leaves of soybean increased first and then declined and the content of Pro increased gradually. In general, soybean relief drought damage by increasing SOD activity and Pro content under drought stress. This study offered the theoretical foundation for exploring the mechanism of soybean drought resistance.

Key words: Drought, Proline, Soybean, Superoxide dismutase.

INTRODUCTION

Soybean is a significant grain and oilseed crop in the world, it is rich in nutrients, including isoflavones, oligosaccharides, saponins, phospholipids, vitamins and so on (Song *et al.*, 2023). At present, some countries use soybean ink in prints. Soybean plays an important role in the world. Drought is common in many parts of the world (Islam *et al.*, 2022), it has the characteristics of high frequency, wide influence range, large follow-up influence, long duration and shows a normal development trend (Hussain *et al.*, 2016). Therefore, plants are inevitably exposed to drought during their growth, they will generate a large number of hazardous substances, such as reactive oxygen species (ROS) and reactive nitrogen (RNS) under drought conditions (Chen *et al.*, 2022). These harmful substances will destroy the structure of cell membranes in plants, cause damage to cell physiological function and produce lipid peroxidation and eventually, caused cells death (Kunert *et al.*, 2016). Heilongjiang is one of the main soybean-producing areas in China, the precipitation in the growing season is between 350-480mm and more than 85% of the annual precipitation is concentrated from July to September. In recent years, due to lack of rain during May to June, is posing a great challenge to the growth of soybean. Drought in seedling stage resulted in a significant decrease in the quantity of branches of soybean, the increase of reactive oxygen species in the body damages the oxidized body of soybeans, which leads to a decrease in soybean yield (Chun *et al.*, 2021, Buezo *et al.*, 2019). Plants regulate the accumulation and breakdown of metabolites and there are response mechanisms for protection in the face of drought stress and among them, superoxide dismutase (SOD) is a key antioxidant enzyme (Shao *et al.*, 2005) and proline (Pro) is a key osmotic regulator (Gill *et al.*, 2010).

SOD is an antioxidant enzyme capable of converting superoxide free radicals into less active substances (Wang *et al.*, 2012). Under sufficient moisture conditions, the antioxidant enzyme system of plants is usually in equilibrium. However, under water stress, the water pressure inside the plant water potential will drop, resulting in a large number of superoxide anion free radicals (Wang *et al.*, 2016). If unattended, the loss of soil moisture in an arid environment leads to the decrease of water absorbed by plant roots, which breaks the balance of water and air pressure inside plants and this environment increases, the activity of SOD activity to deal with the oxidative stress response caused by drought stress (Wei *et al.*, 2020). Studies have shown that 10% PEG simulated drought stress and soybean seedling leaf SOD activity increased (Wang *et al.*, 2019).

[1]College of Agriculture, Northeast Agricultural University, Harbin, Heilongjiang Province, 150030, China.
[2]Heilongjiang Agricultural Engineering Vocational College, Harbin, Heilongjiang Province, 150025, China.

Corresponding Author: Shoukun Dong, College of Agriculture, Northeast Agricultural University, Harbin, Heilongjiang Province, 150030, China. Email: shoukundong@163.com

How to cite this article: Tian, Y., Li, X., Zhou, X., Qu, Z., Wang, X. and Dong, S. (2023). Effects of Drought Stress on SOD Activity and Pro Content in Different Parts of Soybean Leaves. Legume Research. doi:10.18805/LRF-750.

Submitted: 21-04-2023 **Accepted:** 11-07-2023 **Online**: 31-07-2023

Proline, as a multifunctional amino acid, has many functions such as stabilizing protein, cell membranes and subcellular structures under stress and protect cell function by scavenging ROS. Proline has strong water solubility, which form polymers with some compounds in cells and has the function of water retention and scavenging hydroxyl radical, slowing down the process of cell membrane peroxidation to resist drought stress (Filippou *et al*., 2014). Studies have shown that proline is the most effective substance to regulate osmosis in plants, therefore, the change in free proline content can be used as an important indicator to measure the abiotic stress of plants. Under high osmotic stress conditions, the synthesis of proline in plants has increased (Hayashi *et al*., 2000). Studies have shown that under the treatment of high concentrations of sucrose and glucose, the accumulation of proline in leaves increased (Larher *et al*., 1993).

Due to the difference in growth times of plant leaves, the bottom leaves usually senescence earlier than the upper leaves. In the process of aging, the changes in SOD activity and Pro are worth exploring. Especially under drought stress, the changes in SOD activity and Pro content in the upper and bottom leaves of soybean are unknown. Therefore, it was focused on Williams 82, to explore the changes of superoxide dismutase and proline in the upper and bottom leaves of soybean under drought stress and sufficient irrigation conditions. Analysis will be done for their differences when soybean is at five leaves stage under aridity stress. This study will provide a scientific and effective theoretical basis and reference for understanding the antioxidant mechanism of plants under drought stress especially in soybean.

MATERIALS AND METHODS

Trial design

The experiment was conducted from June to September 2022 within the rooftop glass rain shelter at the production internship base of Northeast Agricultural University (45°44′ 43.87 N, 126°430′50.42 E). The test variety was Williams 82, which is native to the United States and was the first soybean variety selected for soybean genome sequencing. The test was carried out by sand culture method, a circular plastic bucket with a height of 35 cm and an inner diameter of 30 cm was selected. A gauze was put on the bottom of the barrel and each barrel is filled with 18 kg of washed sand. Six soybean seeds with full grains and uniform size were selected for sowing, three seedlings with consistent growth in each barrel were left after seedling. After the true leaves grew out, 500 mL of Hoagland's nutrient solution was irrigated every day to meet the growing needs of plants, Hoagland's nutrient solution is a nutrient solution dedicated to plant cultivation. It contains different kinds of nutrient elements, which can effectively promote plant growth, but also make plants healthier and stronger and improve plant resistance (Waheed *et al*., 2019). Two processing methods were emplaced in the test. At the five-leaf stage of soybean growth, 500 mL of nutrient solution mixed with PEG-6000 (10%) was irrigated every day to simulate drought stress and the nutrient admixture without PEG-6000 was used as the control group (CK), each treatment was repeated three times. Samples were collected and stored on the 1 d, 3 d, 5 d, 7 d and 9 d after irrigation. The sampling time was 8:00-9:00 a.m. every day and the fourth and fifth compound leaves (upper leaves) and the first and second compound leaves (bottom leaves) were taken. The upper and bottom leaves of normally watered soybean were recorded as CK-up and CK-down, respectively, the upper and bottom leaves of PEG-irrigated soybean were recorded as drought-up and drought-down, respectively. After sampling, the samples were quickly frozen in liquid nitrogen and then transferred to -80°C ultra-low temperature refrigerator for preservation.

Index determination

SOD activity was determined by the colorimetric method (Hayat *et al*., 2012) and Pro content was determined by the ninhydrin hydrate colorimetric method (Gao, 2006).

Contribution analysis

$$\text{SOD relative magnitude of change (\%)} = \frac{\frac{\Delta SOD}{SOD}}{\frac{\Delta SOD}{SOD} + \frac{\Delta POD}{POD}}$$

Note: ΔSOD: SOD activity (different leaf parts and different days-blank group). The relative variation (%) of Pro was calculated by the same method.

Data statistics

Excel 2019 was used for collation and charting; data analysis was performed using SPSS25.0 multivariate analysis of variance.

RESULTS AND DISCUSSION

SOD is involved in the formation of the number one line of defense against active oxygen radicals, under drought stress conditions, changes in SOD activity and Pro content have been used as indicators of changes in plant redox status (Moran *et al*., 1994, Schwanz *et al*., 2001). In an aridity environments, SOD activity and Pro content in the upper and bottom leaves of soybean were significantly increased. Under water stress of plants, calcium signaling, ABA signaling, ROS and other signaling pathways will be activated, thereby activating the production of SOD. The production of proline is also considered to be related to the drought signaling pathway, such as water deficiency leads to increased cytoplasmic osmotic pressure, which cause proline accumulation.

Effects of drought stress on SOD activity in soybean leaves

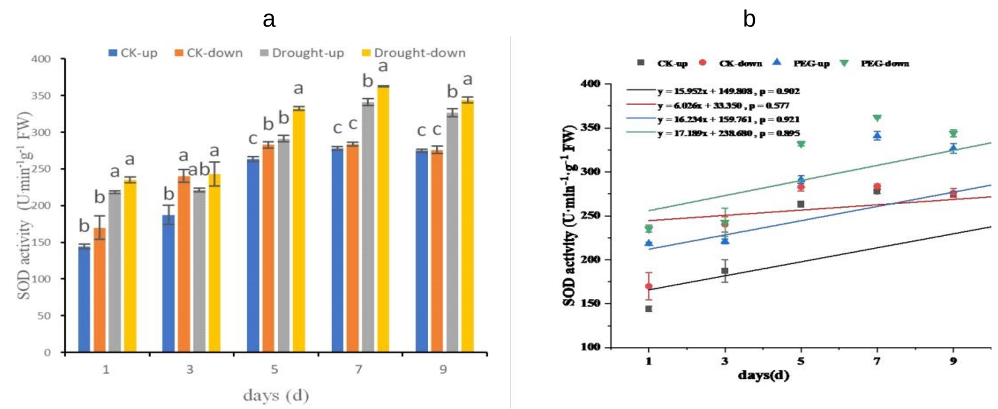
The SOD activity of soybean leaves is shown in Fig 1. With the prolongation of treatment time, the SOD activity of leaves in both parts increased first and then tapered off, reaching peak on the seventh day of the treatment. On days 1-3 of treatment, the SOD activity of soybean leaves had shown that the control group increased rapidly and the drought treatment group increased slowly; in the treatment of 5-7 days, the drought treatment group increased faster than the control group. The SOD activity of the two parts of soybean leaves increased the fastest in 3-5 days.

The SOD activity of soybean leaves in two parts (upper and lower) was higher in the drought group than in the control group under drought stress. On the 1, 3, 5, 7 and 9th days of treatment, the SOD activity of the upper leaves in the drought group (Drought-up) increased by 51.65%, 18.01%, 10.57%, 22.75% and 18.90%, respectively, compared with the upper leaves in the control group (CK-up); the SOD activity of the lower leaves in the drought group (Drought-down) increased by 38.44%, 1.07%, 17.49%, 29.71% and 24.65%, respectively, compared with the lower leaves in the control group (CK-down). Except for the third day, the differences at other treatment times were significant, indicating that drought stress could induce the expression of SOD activity. In both treatment groups, the SOD activity of soybean leaves showed that the lower leaves were greater than the upper leaves. Compared with CK-up, the SOD activity of CK-down increased by 18.02%, 28.27%, 7.35%, 0.54% and 1.87% on days 1, 3, 5, 7 and 9th, respectively. Compared with Drought-up, the SOD activity of Drought-down increased by 7.75%, 9.85%, 14.07%, 6.23% and 5.27% on days 1, 3, 5, 7 and 9th, respectively. The difference was significant on days 3 and 5 in the control group and on days 5, 7 and 9th in the drought group.

In this study, the SOD activity in the upper leaves of soybean was lower than that in the bottom leaves in CK, the difference caused by leaf senescence. Studies have shown that the bottom leaves senescence earlier than the upper leaves and SOD activity in senescent leaves was significantly higher than that in tender leaves (Wen and Zhang, 2022). The upper leaves of CK and the bottom leaves of CK gradually approached after 5 days, because the fifth leaf gradually transformed into old leaves with the occurrence of new leaves. The SOD activity in upper leaves of soybean in drought treatment was always lower than that in the bottom leaves. The antioxidant system like SOD eliminates the accumulation of ROS in leaves and ensure the function of chlorophyll and the greenness of leaves (Del Río, 2015). SOD activity in the bottom leaves of soybean was higher than that in upper leaves under drought stress, it ensures the function of leaves and delays the senescence of leaves. Studies have shown that SOD enzyme activity in the bottom leaves of spring maize after silking is higher under different cultivation modes (Menezes-Benavente *et al*., 2004). At the seedling stage of soybean drought treatment, SOD activity increased, but after 10 days of drought treatment, SOD activity was decreased (Dong *et al*., 2019). The SOD activity in drought-treated tomato leaves increased by 74.3% (Wang *et al*., 2022) and reached a significant level. The reason for the above situation may be that short-range drought stress can induce the expression of SOD enzyme, so that plants have certain drought resistance, however, with the prolongation of stress time, the active oxygen in leaves continued to increase, ROS cause damage to plant tissues, thereby affecting the SOD activity, result in the drought resistance of plants was inhibited. Our results are similar to previous studies.

Effects of drought stress on pro content in soybean leaves

As shown in Fig 2, the proline (Pro) content in soybean leaves gradually increased with the extension of treatment time. The trend of Pro content changes in the two parts of soybean leaves tended to be consistent. Moreover, the Pro content in both parts of the leaves showed that the drought treatment group was higher than the control group. On the 1, 3, 5, 7 and 9th days of treatment, compared with CK-up, the Pro content of Drought-up increased by 46.54%, 113.56%, 70.91%, 43.84% and 56.70%, respectively.



Note: 'a' is a bar graph and 'b' is a fitting graph. The X axis is the number of days of treatment, CK-up, CK-down is the upper and bottom leaves of normal watered soybean, Drought-up, Drought-down is the upper and bottom leaves of PEG-6000 watered soybean.

Fig 1: Effects of drought stress on SOD activity in soybean leaves.

Compared with CK-down, the Pro content of Drought-down increased by 115.07%, 57.50%, 78.97%, 23.14% and 47.75%, respectively. This indicates that soybeans will accumulate a large amount of Pro under drought stress conditions to improve their adaptability to drought stress. In both treatment groups, the Pro content of soybean leaves showed that the upper leaves were higher than the lower leaves. The difference in the control group was not significant. Compared with CK-down, CK-up increased by 77.12%, 36.96%, 24.94%, 15.90% and 11.03% on days 1, 3, 5, 7 and 9th, respectively. Compared with Drought-down, Drought-up increased by 20.95%, 46.16%, 19.38%, 35.34% and 17.57% on days 1,3,5,7 and 9th respectively. In both treatment groups, the Pro content of soybean leaves showed that the upper leaves were higher than the lower leaves. Except for the first day of drought treatment, the differences were significant on other days.

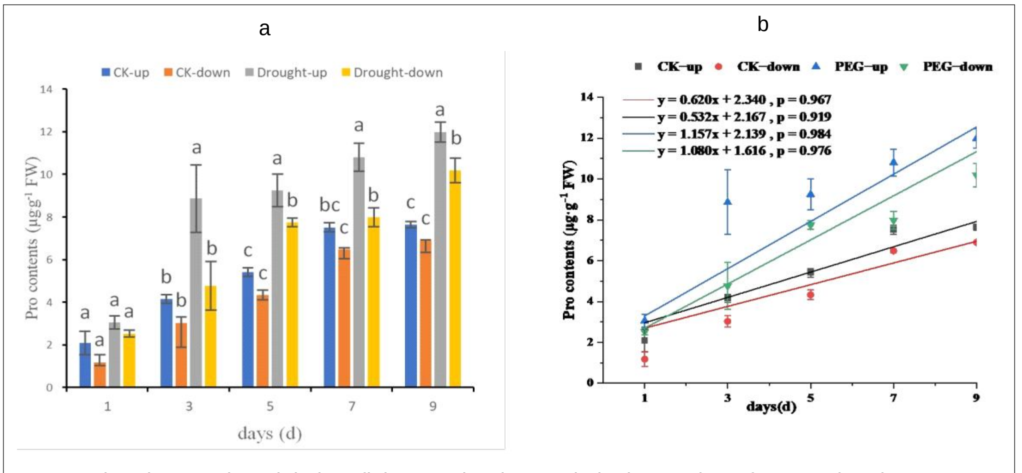
Osmotic regulation is an important coping mechanism of plants to water and salt stress. It can reduce the osmotic potential of plant tissues by accumulating low molecular weight organic solvents and inorganic ions, minimizing water loss and maintaining water absorption (Parida and Das, 2005). Under stress conditions, the increase of Pro is an adaptability of plants, which can control their water balance and ensure that plants are not or less damaged by stress, Pro accumulation is a protective response of plants to drought stress (Ashraf and Follad, 2007). In this study, it can be found that the impact of drought treatment on the proline metabolism of soybean is different. Early stages of drought treatment (such as the third day of treatment), proline content in upper leaves increased significantly after drought treatment, indicating that drought stress was more sensitive to the synthesis and accumulation of proline in this part. With the passage of drought treatment time, the accumulation of proline in the bottom leaves gradually increased and reached the summit on the 9th day, this shows that with the raise of drought treatment time, Pro synthesis may have different responses in different parts and this helps to understand the response mechanism of plant vulnerability and also helps to carry out plant regulation and cultivation. This is similar to the results of other scholars on barley (Singh *et al*., 1972).

Analysis of variance

In order to further explore the independent effects of drought, days and leaf parts on the measured indicators, we used multivariate analysis of variance to analyze different influencing factors. It can be seen from Table 1 that the sig values corresponding to drought, days and F values of leaf parts are less than 0.05, which indicates that there are significant effects on drought, days and leaf parts. The interaction between leaf parts and drought, the interaction between leaf parts and days and the interaction between drought days had significant effects on SOD activity. The interaction of leaf position, drought and days had no significant effect on SOD activity and proline content.

Relative change amplitude analysis

The relative changes of SOD and Pro in different parts of leaves under drought stress in different days are shown in Table 2. With the extension of drought treatment time, in the early stage of drought treatment (1-3 d), the relative variation of SOD in upper leaves was higher than that in bottom leaves. In the late stage of drought treatment (5-9 d), the relative change of SOD in bottom leaves was higher than that in upper leaves, which infers that in the process of resisting drought stress, the effect on leaves was small in the early stage of stress, the upper leaves of SOD played a major role, due to the extension of stress time and the role of upper leaves gradually decreased. The increase of SOD in bottom leaves was greater than that in upper leaves, which played a major role in resisting drought stress.



Note: 'a' is a bar graph and 'b' is a fitting graph. The X axis is the number of processing days. CK-up, CK-down were upper and bottom leaves of normal watered soybean, Drought-up, Drought-down were upper and bottom leaves of PEG-6000 watered soybean.

Fig 2: Effects of drought stress on pro content in soybean leaves.

Table 1: Multifactor analysis of variance.

	SOD		Pro	
	F	Sig.	F	Sig.
Leaf position	52.185	0.001	37.955	0.001
Drought	277.529	0.001	125.454	0.001
Days	253.745	0.001	92.793	0.001
Leaf parts * drought	0.175	0.678	5.3	0.027
Leaf parts * days	2.851	0.038	1.612	0.19
Drought * days	10.792	0.001	3.666	0.012
Leaf position * drought * days	2.595	0.051	1.248	0.292

Note: Analysis of variance, using the F test method, the F value in the result represents a specific value obtained by the F test formula and the corresponding p value is obtained according to the numerical table, which is sig. The sig value <0.05 indicates that it affects the result, otherwise it has no effect. '*' representative 'and'.

Table 2: The relative changes of SOD activity and Pro content in different parts of leaves.

Treatment	1	3	5	7	9
SOD-up	23.15%	14.42%	8.68%	20.70%	15.26%
SOD-down	18.93%	1.04%	13.54%	24.23%	19.01%
Pro-up	21.56%	50.14%	37.71%	34.07%	34.64%
Pro-down	36.36%	34.40%	40.07%	21.00%	31.09%

Note: Relative magnitude of change (%): It indicates the regulation ability of SOD and Pro in drought resistance regulation. SOD-up is the SOD of upper leaves, SOD-down is the SOD of bottom leaves and Pro is expressed in the same way. Table 2 shows the difference in the relative changes (%) of SOD and Pro in the leaves of two parts of soybean under drought stress.

With the extension of drought treatment time, under drought treatment for 1 d, the relative variation of Pro in bottom leaves was higher than that of upper leaves. In the middle and late stages of drought treatment (3-9 d), the relative variation of Pro in upper leaves was higher than that of bottom leaves. It indicated that the bottom leaves of Pro played a major role in the process of resisting drought stress, the role of upper leaves gradually decreased in the later stage of stress. The increase of Pro in upper leaves was greater than that in bottom leaves, which played a major role in resisting drought stress.

CONCLUSION

In this experiment, Williams 82 was used as the material to determine the SOD activity and Pro content in the upper and bottom leaves of enough water and drought stress at different periods. The results proved that SOD activity of soybean leaves increased at first then reduced, with the prolongation of drought stress time and the bottom leaves were higher than the upper leaves. The content of Pro increment with the elongation of drought stress time and the upper leaves were higher than the bottom leaves, leaf position, drought, days and SOD activity, Pro content showed a significant correlation. This finding indicates that in actual production, it is necessary to delay the senescence of the bottom leaves, thereby increasing the yield of soybean. In the experiment, a comparative method was used, soybean was subjected to drought stress and leaves at different positions were studied. There is a lack of other antioxidant enzymes and amino acid metabolism-related indicators in the experiment. In the next experiment, the determination of nitrogen metabolism and antioxidant enzymes should be the center.

Funding

The research was funded by Natural Science Foundation of Heilongjiang Province of China, grant number LH2021C023.

Conflict of interest: None.

REFERENCES

Ashraf, M.F.M.R. and Foolad, M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany. 59(2): 206-216.

Buezo, J., Sanz Saez, Á., Moran, J.F., Soba, D., Aranjuelo, I. and Esteban, R. (2019). Drought tolerance response of high yielding soybean varieties to mild drought: Physiological and photochemical adjustments. Physiologia Plantarum. 166(1): 88-104.

Chen, Q., Cao, X., Nie, X., Li, Y., Liang, T. and Ci, L. (2022). Alleviation role of functional carbon nanodots for tomato growth and soil environment under drought stress. Journal of Hazardous Materials. 423: 127260. doi: 10.1016/j.jhazmat.2021. 127260.

Chun, H.C., Sanghun, L.E.E., Choi, Y.D., Gong, D.H. and Jung, K.Y. (2021). Effects of drought stress on root morphology and spatial distribution of soybean and adzuki bean. Journal of Integrative Agriculture. 20(10): 2639-2651.

Del Río, L.A. (2015). ROS and RNS in plant physiology: An overview. Journal of Experimental Botany. 66(10): 2827-2837.

Dong, S., Jiang, Y., Dong, Y., Wang, L., Wang, W., Ma, Z., Yan, C., Chen, M. and Liu, L. (2019). A study on soybean responses to drought stress and rehydration. Saudi Journal of Biological Sciences. 26(8): 2006-2017.

Filippou, P., Bouchagier, P., Skotti, E. and Fotopoulos, V. (2014). Proline and reactive oxygen/nitrogen species metabolism is involved in the tolerant response of the invasive plant species *Ailanthus altissima* to drought and salinity. Environmental and Experimental Botany. 97: 1-10.

Gao, J. (2006). Instruction for Plant Physiology Experiments. Higher Education Press, Beijing. pp. 211-214.

Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 48(12): 909-930.

Hayashi, F., Ichino, T., Osanai, M. and Wada, K. (2000). Oscillation and regulation of proline content by P5CS and ProDH gene expressions in the light/dark cycles in *Arabidopsis thaliana* L. Plant and Cell Physiology. 41(10): 1096-1101.

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J. and Ahmad, A. (2012). Role of proline under changing environments: A review. Plant Signaling and Behavior. 7(11): 1456-1466.

Hussain, M.I., Lyra, D.A., Farooq, M., Nikoloudakis, N. and Khalid, N. (2016). Salt and drought stresses in safflower: A review. Agronomy for Sustainable Development. 36: 1-31.

Islam, W., Idrees, A., Waheed, A. and Zeng, F. (2022). Plant responses to drought stress: MicroRNAs in action. Environmental Research. 215: 114282. doi: 10.1016/j.envres.2022.114282.

Kunert, K.J., Vorster, B.J., Fenta, B.A., Kibido, T., Dionisio, G. and Foyer, C.H. (2016). Drought stress responses in soybean roots and nodules. Frontiers in Plant Science. 7: 1015. https://doi.org/10.3389/fpls.2016.01015.

Larher, F., Leport, L., Petrivalsky, M. and Chappart, M. (1993). Effectors for the osmoinduced proline response in higher plants. Plant Physiology and Biochemistry (Paris). 31(6): 911-922.

Menezes-Benavente, L., Kernodle, S.P., Margis-Pinheiro, M. and Scandalios, J.G. (2004). Salt-induced antioxidant metabolism defenses in maize (*Zea mays* L.) seedlings. Redox Report. 9(1): 29-36.

Moran, J.F., Becana, M., Iturbe-Ormaetxe, I., Frechilla, S., Klucas, R.V. and Aparicio-Tejo, P. (1994). Drought induces oxidative stress in pea plants. Planta. 194: 346-352.

Parida, A.K. and Das, A.B. (2005). Salt tolerance and salinity effects on plants: A review. Ecotoxicology and Environmental Safety. 60(3): 324-349.

Schwanz, P. and Polle, A. (2001). Differential stress responses of antioxidative systems to drought in penduculate oak (*Quercus robur*) and maritime pine (*Pinus pinaster*) grown under high CO_2 concentrations. Journal of Experimental Botany. 52(354): 133-143.

Shao, H.B., Liang, Z.S., Shao, M.A. and Sun, Q. (2005). Dynamic changes of anti-oxidative enzymes of 10 wheat genotypes at soil water deficits. Colloids and Surfaces B: Biointerfaces. 42(3-4): 187-195.

Singh, T.N., Aspinall, D. and Paleg, L.G. (1972). Proline accumulation and varietal adaptability to drought in barley: A potential metabolic measure of drought resistance. Nature New Biology. 236: 188-190.

Song, W., Sun, S., Wu, T., Yang, R., Tian, S., Xu, C. and Han, T. (2023). Geographic distributions and the regionalization of soybean seed compositions across China. Food Research International. 164: 112364. doi: 10.1016/j.foodres.2022.112364.

Waheed, H., Javaid, M.M., Shahid, A., Ali, H.H., Nargis, J. and Mehmood, A. (2019). Impact of foliar-applied Hoagland's nutrient solution on growth and yield of mash bean (*Vigna mungo* L.) under different growth stages. Journal of Plant Nutrition. 42(10): 1133-1141.

Wang, H., Y ang, L., Li, Y., Hou, J., Huang, J. and Liang, W. (2016). Involvement of ABA- and H_2O_2-dependent cytosolic glucose -6-phosphate dehydrogenase in maintaining redox homeostasis in soybean roots under drought stress. Plant Physiology and Biochemistry. 107: 126-136.

Wang, S., Shao, B., Liu, S., Ye, X. and Rao, P. (2012). Purification and characterization of Cu, Zn-superoxide dismutase from black soybean. Food Research International. 47(2): 374-379.

Wang, W., Zheng, W., Lv, H., Liang, B., Jin, S., Li, J. and Zhou, W. (2022). Animal-derived plant biostimulant alleviates drought stress by regulating photosynthesis, osmotic adjustment and antioxidant systems in tomato plants. Scientia Horticulturae. 305: 111365. https://doi.org/10.3390/plants12132535.

Wang, W.J., Li, S., Ma, Z.Z., Ma, Y.L., Liu, L.J. and Dong, S.K. (2019). Effects of water stress on protective enzyme activity and rel-ative conductivity of spring soybean leaves. Chinese Agronomy Bul-letin. 35(11): 14-18.

Wei, J., Xu, C., Li, K.X., He, H.J. and Xu, Q.J. (2020). Research progress of superoxide dismutase and plant stress resistance. Journal of Plant Physiology. 56(12): 2571-2584.

Wen, Y. and Zhang, D. (2022). Alternative oxidase is involved in leaf senescence *via* regulation of salicylic acid accumulation in tomato. Biochemical and Biophysical Research Communications. 625: 81-86.