

Early warning signals of drought induced oxidative stress and genotype tolerance. A review

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Abstract

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Drought was shown to cause harm to plants – reducing growth, physiological activity, reproduction, and productivity. In an agricultural aspect, “drought” means not having sufficient soil moisture required by plants to maintain their normal growth and development throughout the life cycle. Drought is known as the most common environmental stress affecting plants through induced oxidative stress. Being sessile organisms, plants have developed various strategies to cope with water scarcity – escape, avoidance, and tolerance. In this review, we discuss the drought as an environmental factor, oxidative stress induced by drought, and some commonly used endpoints such as MDA and H₂O₂ for the evaluation of the magnitude of drought stress as well as the contribution of both Proline and HSP70/chaperone involved in the formation of plants tolerance to drought-induced stress. Based on the available data in the literature and our own results, it was shown that both biochemical endpoints MDA and H₂O₂ could play a dual role – as indicators of the magnitude of drought-induced stress, as well as markers of tolerance to drought. The great contribution of Pro and HSP70 for the formation of increased drought tolerance in plants was analyzed. The dynamics of cytoplasmic HSP70 chaperone levels in bean lines with different resistance to water deficiency revealed that HSP70 family proteins are one of the main components of plant stress tolerance. It could be said that these simple and not expensive endpoints could be successfully applied as early warning signals for revealing drought-induced stress and plants tolerance.

Keywords: drought; H₂O₂; MDA; Proline; plants; HSP70

Introduction

Drought as an environmental factor: The limited or below-average rainfall periods of time, as a result of some climate changes that can reduce soil water content were defined as “drought”. In an agricultural aspect, based on definitions available, it could be summarized that „drought“ means not having sufficient soil moisture required by plants in order to maintain their normal growth and development throughout their life cycle. Drought has been well documented to harm many processes in plants – photosynthesis, water relations,

nutrient uptake, oxidative status, osmotic balance, hormonal balance, plant growth, biomass and can (Ferioun et al., 2023; Urmi et al., 2023) reduce yields by more than 50% (Hossain et al., 2024).

Oxidative stress induced by drought: The biosynthesis of reactive oxygen species (ROS), is recognized as the most detrimental impact of drought stress on plants (Khodabin et al., 2020; Lee et al., 2022), inducing various free radicals, such as superoxide (O^{•−2}), hydroxyl radicals (.OH), perhydroxy radical (HO₂.), alkoxy radicals (RO.) and non-radical

(molecular) forms – singlet oxygen ($^1\text{O}_2$), and hydrogen peroxide (H_2O_2). Cell organelles that take part in active electron transport – chloroplasts, mitochondria, peroxisomes, apoplast, and their membranes are considered as generators of ROS (Impa et al., 2012) due to over-reduction in the electron transport chain under water stress (Melandri et al., 2020). The overproduction of ROS during drought stress can damage very important macro-molecules – nucleic acids, proteins, and lipids, resulting in some cases to cells/plants' dead (Dumanović et al., 2021; Lin et al., 2024).

How can plants cope with unfavorable environmental conditions including drought? Three main strategies against drought have been elaborated by plants – drought escape, avoidance, and drought tolerance by changing their morphology, physiological, and molecular mechanisms (Khatun et al., 2021; Subramani et al., 2024).

The drought escape strategy can be evolutionary developed or artificially created by altering plants' lifespan – shortening the life cycle or the period of growth and development. For agricultural plants, the planting period can also be changed. Mild and severe drought is avoided by reducing plant water loss using different approaches.

The term “drought tolerance” defines plants' ability to carry out their physiological processes, albeit to a certain extent, in severe and prolonged drought. In this case, the oxidative stress induced by drought can trigger stress tolerance mechanisms activating and regulating many genes and metabolic processes in order to:

- maintain cell membrane integrity by producing protective compounds;
- reduce toxic substances accumulation by activating ROS scavenging systems.

At present, unique anatomical structures, physiological and molecular mechanisms were found in plants, that could be associated with their drought tolerance strategy – cell wall-specific proteins or enzymes, membrane-linked proteins, transporters, transcription factors, R-proteins, signaling molecules, etc. (Ferioun et al., 2023; Lin et al., 2024; Subramani et al., 2024).

The magnitude of plants' tolerance to drought stress has been found to depend on the species, varieties within the same species, severity and duration of drought, stage of plant development, soil texture, and other climatic conditions (Khatun et al., 2021).

Three factors – climate change, expected further global warming (Mondal et al., 2023), as well as longer periods of drought, require the identification and selection of simple, not expensive early warning signals for the revealing of drought-induced oxidative stress, before seriously affecting

plants and plants' tolerance to drought. It is believed that this may help for better understanding of plant stress responses to drought and further selection of better drought-adapted cultivars (Aina et al., 2024).

The main task of this review was to clarify whether some very commonly used, simple, and not expensive endpoints, such as hydrogen peroxide (H_2O_2), lipid peroxidation (MDA, proline (Pro) and heat shock protein 70 (HSP70) could be used as early warning signals for oxidative stress and drought tolerance in plants.

Materials and Methods

Analyzing basic papers and up to date literature (more 56% from latest 5 years) it was clear that during the years two main approaches have been applied by scientists to provide some clarity on this issue: i) investigation of the stress response of tolerant/susceptible genotypes (Svetleva et al., 2012; Khodabin et al., 2020; Rao et al., 2020; Zlatev et al., 2020; Kirova et al., 2021); ii) investigation of the stress response of genetically closely related varieties or mutant lines (Angelova et al., 2017).

Here, some data, obtained as a result of experiments performed using genetically closely related genotypes of *Phaseolus vulgaris* L. Dobrudzhanski 2 (D2) and Dobrudzhanski 7 (D7) cultivars and mutant lines are presented. The three mutant lines – 1, 8 and 9 have been obtained by the methods of experimental mutagenesis by (Svetleva et al., 2012).

H_2O_2 , MDA and Pro were measured as described by us earlier (Svetleva et al., 2012; Zlatev et al., 2020). The level of heat shock proteins (HSPs) was determined by gel-electrophoresis and Western blotting (Chankova et al., 2014).

Results and Discussion

Dual role of H_2O_2 and MDA

Under physiological conditions, H_2O_2 is produced in plant cells during photosynthesis and photo-respiration. Under adverse environmental conditions, such as drought, its levels increase depending on the strength and duration of the drought. Hydrogen peroxide (H_2O_2) is a highly toxic radical that can react with cellular proteins, lipids, and deoxyribonucleic acids, can enhance lipid per-oxidation and thus damaging the membranes ultimately leading to oxidative stress-induced cell death (Gill and Tuteja, 2010). On the other hand, it is the most stable radical among ROS and, under mild or moderate stress, it can play the role of a signaling molecule in various physiological processes, interacting with thiols-containing proteins. Earlier, it was documented that H_2O_2 can

activate both different signaling pathways and transcription factors regulating gene expression and cell cycle processes (Mittler, 2002; Vranová et al., 2002; Slesak et al., 2007).

Two very important findings have been registered over the years: the first one revealed that the drought has resulted in increased H_2O_2 production and membrane lipid peroxidation (MDA) (Khodabin et al., 2020; Rao et al., 2020; Muhammad et al., 2021; Lee et al., 2022; Urmi et al., 2023), and the second – that H_2O_2 and MDA levels correlate well with both – stress magnitude (Rahim et al., 2020) and genotype characteristics. Overproduction of H_2O_2 and MDA has been documented to increase with increasing oxidative stress and is higher in cultivars or mutant lines more sensitive to drought (Muhammad et al., 2021; Kirova et al., 2021; Ferioun et al., 2023). Our experimental data presented in Figure 1, are good illustration of the written above. Based on induced levels of H_2O_2 and MDA, the correlation between PEG concentrations, stress response magnitude of *Phaseolus vulgaris* L. and genotype was confirmed. Approximately similar and not very high levels of induced H_2O_2 and MDA were measured for both cultivars D2 and D7, and mutant line 9. In contrast, both mutant lines 1 and 8 responded with about 2-fold higher levels of induced H_2O_2 and MDA. The higher levels of induced H_2O_2 and MDA levels after PEG treatment are a good reason to characterize mutant lines 1 and 8 as more susceptible to drought. Another important conclusion that could be drawn from the data presented in Fig. 1, is that these two endpoints are good enough to distinguish the difference in stress response even among genetically closely related genotypes. Increased levels of H_2O_2 and MDA could be considered as early warning signal for drought induced stress. Low levels of MDA are informative for membranes

stability and probably could be considered as an early signal for plants tolerance to drought.

Results from recent years found that under drought stress, ROS, H_2O_2 and MDA accumulations were higher in a sensitive/susceptible genotype compared to a more drought-tolerant genotype (Rao et al., 2020; Kirova et al., 2021; Hossain et al., 2024; Lin et al., 2024) regardless of species.

It should be mentioned that increased H_2O_2 amounts could be considered as an early warning signal for increased oxidation and a shifted redox balance, whereas MDA levels could be considered as an early warning signal for the degree of membrane lipid per-oxidation as a result of oxidative damage. Like H_2O_2 , MDA plays a dual role in plant drought stress response. As one of the final products of polyunsaturated fatty acids per-oxidation in the cells, MDA levels induced by drought are informative for the degree of plasma membrane damage and the plant's drought stress tolerance. Cell membrane stability could be considered as an indicator of drought tolerance (Lin et al., 2024).

In drought-stressed plants, overproduction of H_2O_2 and MDA is induced, which negatively affects the integrity of plant cell membranes and leads to increased electrolyte leakage and metabolic toxicity. The role of osmolytes to maintain turgor pressure and increase water uptake capacity by increasing cytoplasmic osmotic pressure was well described by (Urmi et al., 2023).

Basic role of Pro as one of the most common osmolytes in water-stressed plants was documented well more than 20 years ago. Pro was shown to act like a chaperone, redox buffer, and ROS scavenger, protecting membranes and proteins during drought stress (Verbruggen and Hermans,

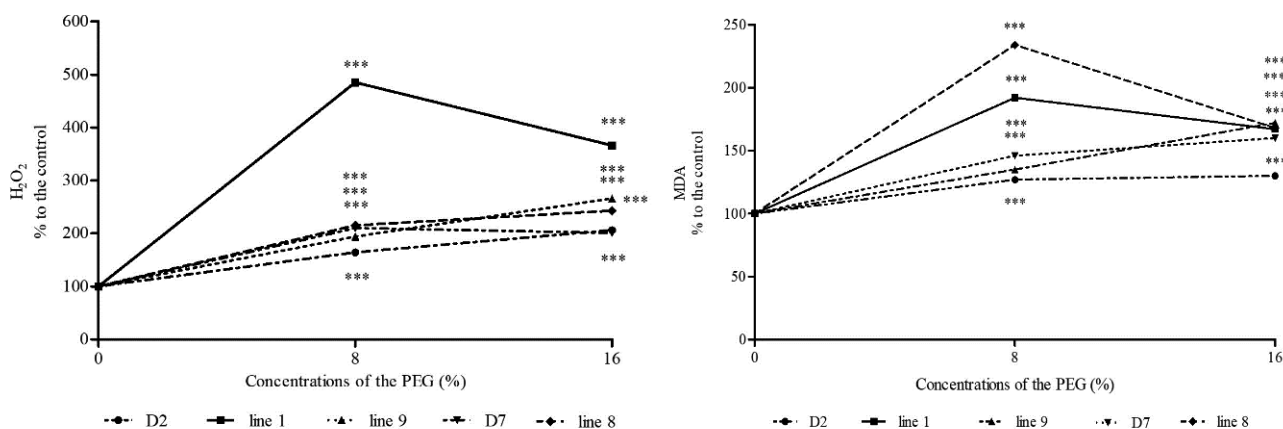


Fig. 1. The relationship between the magnitude of drought induced stress measured as H_2O_2 and MDA levels and the genotype. Data are from three independent experiments and statistically significant

2008). Its accumulation was also described to result from both activation of Pro biosynthesis and inactivation of Pro degradation (Yoshiba et al., 1997). Recently, the expression of Pro biosynthesis genes (P5CS1, P5CS2a, P5CS2b, P5CR) was found to increase in tolerant and sensitive cultivars of peanut plants upon exposure to polyethylene glycol. Other interesting finding documented that the relative expression of Pro catabolism genes (ProDH1, ProDH2) was increased only in the sensitive cultivar during drought stress (Furlan et al., 2020).

Several years ago an interaction between Pro and soluble sugars was defined by (Gurrieri et al., 2020). They proposed further investigation of this finding for complete understanding of this relationship.

Huge amount of data has been gathered that drought stress increases the accumulation of Pro and some relationship exists between the magnitude of the drought stress and Pro accumulation (Mafakheri et al., 2010; Khodabin et al., 2020; Rahim et al., 2020; Zlatev et al. 2020; Lee et al., 2022; Li et al., 2024). However, the relationship between Pro accumulation under drought conditions and genotype tolerance/susceptibility is not so clear. Quite contradictory data could be found in the literature (Arteaga et al., 2020; Zlatev et al., 2020; Kirova et al., 2021; Lin et al., 2024).

To provide some clarity on this issue, two main approaches could be applied. The first one includes an analysis of drought stress response of tolerant/susceptible genotype (Svetleva et al., 2012; Khodabin et al., 2020; Rao et al., 2020; Zlatev et al., 2020; Kirova et al., 2021) and the second one as it was proposed by us earlier- by analyzing stress response of genetically closely related varieties or mutant lines (Angelova et al., 2017).

Our own experiments with two cultivars *Phaseolus vulgaris* L. and mutant lines derived by them have provided an information that Pro accumulation could be considered as a good tool to distinguish differences in a cells' stress response even in genetically closely related cultivars or mutant lines (Figure 2). Comparing the curves representing Pro accumulation of the genotypes analyzed by us well expressed differences were obtained. The cultivar D2 and both mutant lines derived from this cultivar – mutant line 1 and mutant line 9 were shown to differ strongly in their response. Cultivar D2 and mutant line 9 were characterized with very well overproduction of Pro. Mutant line 1 was characterized by very low Pro accumulation and if we came back to data presented at Fig. 1, it could be seen that very high levels of H_2O_2 and MDA were induced after PEG treatment. Such difference was not found comparing D7 stress response and derived from this genotype mutant line 8. Our finding demonstrate that stress response of genetically closely related genotypes

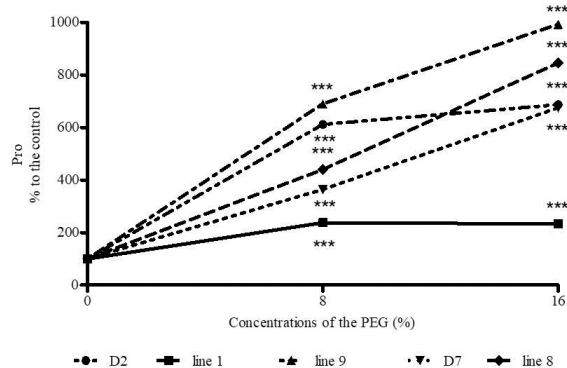


Fig. 2. Pro as a stress signal for drought stress in genetically closely related genotypes. Data are from three independent experiments and statistically significant

could be successfully distinguished on the basis of H_2O_2 , MDA and Pro accumulation and they could be used as early warning signals for oxidative stress, as well as both of them – low level of MDA and high level of Pro accumulation – for drought tolerance.

High levels of Pro accumulation have been considered a typical feature of stress-tolerant plants (Svetleva et al., 2012; Rao et al., 2020; Zlatev et al., 2020; Hossain et al., 2024). In a contrary, there are also contrasting reports where Pro content in the drought-sensitive cultivar was found higher, or equivalent to that in the drought-tolerant cultivar (Kirova et al., 2021; Lin et al., 2024). In other experiments with common bean a relationship between over-accumulation of Pro and stronger inhibition of growth was described by (Arteaga et al., 2020). Based on their finding these authors proposed to use Pro “as a suitable biochemical marker for simple, rapid, large-scale screenings of bean genotypes”.

Recently, Lee et al. (2022) discussed in a very precise way in their report based on experiments with rapeseed leaves that in the future more evidences are necessary to define the threshold at which Pro level switches from inducing cellular protection to hypersensitivity to over-produced ROS, especially H_2O_2 .

Giving all this information, currently, the question on the relationship between Pro overproduction and genotype drought tolerance remains open.

Chaperone system in protection against oxidative stress

Due to their chaperone activity, heat shock proteins are universal cytoprotectors. Among the HSP families, the HSP70 family of proteins has been shown to play a vital role in responses to both abiotic and biotic stresses. With their diverse functions, HSP70s chaperones play a protective role under various stresses, which is a key factor for plant growth

and development. Therefore, this gene family is an excellent candidate for enhancing resistance to multiple stresses (Masand and Yadav, 2016). Recently, higher levels of HSP70 expression have been reported in stress- and disease-resistant plants compared to susceptible plants (Cho and Hong, 2006; Song et al., 2014; Augustine et al., 2015; Pulido et al., 2017; Devarajan et al., 2021).

Previously, we obtained results indicating that HSP70 protein levels are good indicators of oxidative stress levels in green algae cells (Chankova et al., 2013; 2014). According to the literature, plant dehydration activates genes encoding proteins such as dehydrins, osmoregulatory proteins, antioxidant defense enzymes, etc. Of particular interest is the study of heat shock proteins as indicators of drought-induced oxidative stress (Masand and Yadav, 2016; Davoudi et al., 2022).

Our results showed that dehydration of bean cultivars and mutant lines induces the synthesis of heat shock proteins. The study of the effect of water deficit on the levels of cytoplasmic HSP70 chaperones in cultivars and mutant lines of bean *P. vulgaris* L., differing in drought tolerance, are presented in Figure 3. It was found that, compared to normal conditions, under drought, the level of cytoplasmic proteins HSP70 in cultivars and mutant lines can increase

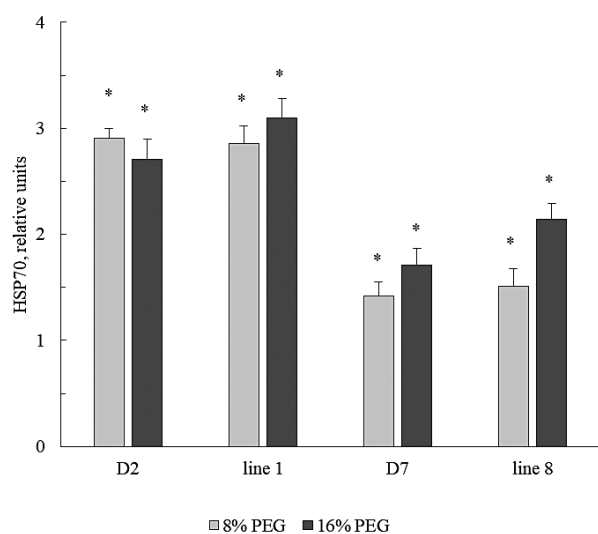


Fig. 3. Changes in cytoplasmic HSP70 protein levels in both bean cultivars D2 and D7 as well as in both mutant lines 1 and line 8 after PEG – polyethylene glycol-induced drought stress. HSP70 levels were calculated relative to HSP70 levels of the corresponding cultivars and lines in non-dehydrated controls. Data are from three independent experiments and statistically significant

several folds. A significant increase in the level of HSP70 chaperones relative to the control plants is observed at a PEG concentration of 8%: for D2 and line 1, an increase of 2.9 and 2.8 times, respectively, is shown, and for D7 and line 8, an increase of 1.4 and 1.5 times. A higher increase in the HSP70 level (an increase from 1.7 to 3.1 times) is detected during dehydration caused by higher PEG concentrations (16%). The increased level of HSP70 in bean varieties indicates its ability to actively tolerate drought and survive under stressful conditions.

Comparing the HSP70 response of both cultivars – D2 and D7, it should be said that the accumulation of HSPs strongly depends on the genotype (Figure 3). Thus, HSP70 levels were 1.3–1.5 times higher in D2 compared to D7 at 8% PEG concentration. As stated above, higher levels of heat shock proteins in D2 suggest higher resistance to dehydration compared to D7. On the other hand, no statistically significant differences were calculated between HSPs levels of genetically closely related genotypes – D2 and line 1, as well as D7 and mutant line 8. Although well expressed relationship between unicellular green algae strains resistance to environmental stimuli and overproduction of HSP70B was obtained by us previously, in our experiments with common bean we did not definitively find such a relationship. Perhaps further research in this direction will provide more clarity. A study of cytoplasmic HSP70 chaperone protein levels in bean cultivars and mutant lines revealed that this protein can serve as an early marker of drought-induced oxidative stress and its accumulation strongly depend on genotype. This is consistent with the hypothesis that heat shock proteins of the HSPs70 family are among the main components of stress tolerance.

Bioinformatics analysis of heat shock protein gene families of completely sequenced plant genomes revealed an excess number of HSP70 genes (18–114) in higher plants, in contrast to algae and other organisms (Swindell et al., 2007; Hu et al., 2009; Zhang et al., 2015; Singh et al., 2016; Kim et al., 2021). These genes are believed to have formed as a result of duplication. The functions of these excess HSP70 genes remain to be studied. Apparently, plants, as organisms that are unable to move and avoid stress, have developed defense mechanisms for survival or adaptation to stressful conditions. Thus, the HSP genes underlying genotype resistance are more numerous, diverse, and variable than previously thought, especially with respect to the diversity of their regulation.

According to genetic and genomics studies, heat shock protein genes and antioxidant genes closely interact in the plant response to drought stress (Rahman et al., 2022). Thus, in response to dehydration, a wide range of proteins

involved in defense mechanisms are activated in plant cells, and among them, heat shock proteins of the HSP70 family are cellular defense proteins, but can serve as early indicators of oxidative stress and drought resistance in plants.

Conclusion

In short, we can say that increased levels of H₂O₂, MDA, Pro, and HSP70 could be considered as reliable early warning signals of drought stress. The magnitude of stress response has been shown to be highly genotype dependent. The response of genetically closely related genotype can be successfully used as an early warning signal for drought stress using H₂O₂, MDA, Pro as endpoints. Low levels of MDA as a signal of membrane stability may provide preliminary information on genotype tolerance. Pro accumulation could be considered as early signal of plants tolerance when plants do not undergo very strong oxidative stress. Unfortunately, Pro's contribution to the formation of drought tolerance still remains open. HSP70 chaperones are integral components of adaptive strategies used by plants to overcome the adverse effects of drought. HSP70 functions as an early warning signal, allowing stress to be detected before it causes serious plant damage. HSP70 expression increases significantly under drought, which is important for plant adaptation to this type of stress.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References:

- Aina, O., Bakare, O. O., Fadaka, A. O., Keyster, M. & Klein, A. (2024). Plant biomarkers as early detection tools in stress management in food crops: a review. *Plants*, 259(3), 60. <https://doi.org/10.1007/s00425-024-04333-1>
- Angelova, T., Parvanova, P., Miteva, D. & Chankova, S. (2017). Set of Reliable Markers for the Evaluation of Drought Induced Stress in *Phaseolus vulgaris* L. Genotypes. *Comptes rendus de l'Académie bulgare des Sciences*, 70(10), 1411-1420 (C. R. Acad. Bulg. Sci.).
- Arteaga, S., Yabor, L., Díez, M. J., Prohens, J., Boscaiu, M. & Vicente, O. (2020). The use of proline in screening for tolerance to drought and salinity in common bean (*Phaseolus vulgaris* L.) genotypes. *Agronomy*, 10(6), 817. [doi:10.3390/agronomy10060817](https://doi.org/10.3390/agronomy10060817).
- Augustine, S. M., Cherian, A. V., Syamaladevi, D. P. & Subramonian, N. (2015). *Erianthus arundinaceus* HSP70 (EaHSP70) acts as a key regulator in the formation of anisotropic interdigitation in sugarcane (*Saccharum* spp. hybrid) in response to drought stress. *Plant and Cell Physiology*, 56(12), 2368-2380. <https://doi.org/10.1093/pcp/pcv142>.
- Chankova, S., Mitrovska, Z., Miteva, D., Oleskina, Y. P. & Yurina, N. P. (2013). Heat shock protein HSP70B as a marker for genotype resistance to environmental stress in *Chlorella* species from contrasting habitats. *Gene*, 516(1), 184-189. <http://dx.doi.org/10.1016/j.gene.2012.11.052>.
- Chankova, S. G., Dimova, E. G., Mitrovska, Z., Miteva, D., Mokerova, D. V., Yonova, P. A. & Yurina, N. P. (2014). Antioxidant and HSP70B responses in *Chlamydomonas reinhardtii* genotypes with different resistance to oxidative stress. *Ecotoxicology and environmental safety*, 101, 131-137. <https://doi.org/10.1016/j.ecoenv.2013.11.015>.
- Cho, E. K. & Hong, C. B. (2006). Over-expression of tobacco NtHSP70-1 contributes to drought-stress tolerance in plants. *Plant cell reports*, 25, 349-358. doi.org/10.1007/s00299-005-0093-2.
- Davoudi, M., Chen, J. & Lou, Q. (2022). Genome-wide identification and expression analysis of heat shock protein 70 (HSP70) gene family in pumpkin (*Cucurbita moschata*) root-stock under drought stress suggested the potential role of these chaperones in stress tolerance. *International Journal of Molecular Sciences*, 23(3), 1918.
- Devarajan, A. K., Muthukrishnan, G., Truu, J., Truu, M., Ostonen, I., Kizhaeral, S. S., Panneerselvam, P. & Kuttalینگam, G. S. (2021). The foliar application of rice phyllosphere bacteria induces drought-stress tolerance in *Oryza sativa* (L.). *Plants*, 10(2), 387. [Doi: 10.3390/plants10020387](https://doi.org/10.3390/plants10020387).
- Dumanović, J., Nepovimova, E., Natić, M., Kuča, 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>.
- Ferroun, M., Srhiouar, N., Bouhraoua, S., El Ghachtouli, N. & Louahlia, S. (2023). Physiological and biochemical changes in Moroccan barley (*Hordeum vulgare* L.) cultivars submitted to drought stress. *Heliyon*, 9(2).
- Furlan, A. L., Bianucci, E., Giordano, W., Castro, S. & Becker, D. F. (2020). Proline metabolic dynamics and implications in drought tolerance of peanut plants. *Plant Physiology and Biochemistry*, 151, 566-578.
- Gill, S. S. & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, 48(12), 909-930. <https://doi.org/10.1016/j.plaphy.2010.08.016>.
- Guirrieri, L., Merico, M., Trost, P., Forlani, G. & Sparla, F. (2020). Impact of drought on soluble sugars and free proline

- content in selected Arabidopsis mutants. *Biology*, 9(11), 367. doi:10.3390/biology9110367.
- Hossain, M. S., Li, J., Wang, C., Monshi, F. I., Tabassum, R., Islam, M. A., Faruquee, M., Mukhtadir, M. A., Mia, M. S., Islam, A. K. M. M., Hasan, A. K., Sikdar, A. & Feng, B.** (2024). Enhanced Antioxidant Activity and Secondary Metabolite Production in Tartary Buckwheat under Polyethylene Glycol (PEG)-Induced Drought Stress during Germination. *Agronomy*, 14(3), 619. <https://doi.org/10.3390/agronomy14030619>.
- Hu, W., Hu, G. & Han, B.** (2009). Genome-wide survey and expression profiling of heat shock proteins and heat shock factors revealed overlapped and stress specific response under abiotic stresses in rice. *Plant Science*, 176(4), 583-590. <https://doi.org/10.1016/j.plantsci.2009.01.016>.
- Impa, S. M., Nadaradjan, S. & Jagadish, S. V. K.** (2012). Drought stress induced reactive oxygen species and anti-oxidants in plants. In: *Abiotic stress responses in plants: metabolism, productivity and sustainability*, (Ahmad P., Prasad M. eds). Springer, New York, NY, 131-147.
- Khatun, M., Sarkar, S., Era, F. M., Islam, A. M., Anwar, M. P., Fahad, S., Datta, R. & Islam, A. A.** (2021). Drought stress in grain legumes: Effects, tolerance mechanisms and management. *Agronomy*, 11(12), 2374. <http://doi.org/10.3390/agronomy11122374>.
- Khodabin, G., Tahmasebi-Sarvestani, Z., Rad, A. H. S. & Modarres-Sanavy, S. A. M.** (2020). Effect of drought stress on certain morphological and physiological characteristics of a resistant and a sensitive canola cultivar. *Chemistry & biodiversity*, 17(2), e1900399. <https://doi.org/10.1002/cbdv.201900399>.
- Kim, T., Samraj, S., Jiménez, J., Gómez, C., Liu, T. & Begcy, K.** (2021). Genome-wide identification of heat shock factors and heat shock proteins in response to UV and high intensity light stress in lettuce. *BMC Plant Biology*, 21, 1-20. doi: 10.1186/s12870-021-02959-x.
- Kirova, E., Pecheva, D. & Simova-Stoilova, L.** (2021). Drought response in winter wheat: Protection from oxidative stress and mutagenesis effect. *Acta Physiologiae Plantarum*, 43(1), 8. <https://doi.org/10.1007/s11738-020-03182-1>.
- Lee, B. R., La, V. H., Park, S. H., Mamun, M. A., Bae, D. W. & Kim, T. H.** (2022). H₂O₂-responsive hormonal status involves oxidative burst signaling and proline metabolism in Rapeseed leaves. *Antioxidants*, 11(3), 566. <https://doi.org/10.3390/antiox11030566>.
- Li, G., Wei, J., Li, C., Fu, K., Li, C. & Li, C.** (2024). Amino acid metabolism response to post-anthesis drought stress during critical periods of elite wheat (*Triticum aestivum* L.) endosperm development. *Environmental and Experimental Botany*, 218, 105577.
- Lin, S., Zhang, W., Wang, G., Hu, Y., Zhong, X. & Tang, G.** (2024). Physiological Regulation of Photosynthetic-Related Indices, Antioxidant Defense, and Proline Anabolism on Drought Tolerance of Wild Soybean (*Glycine soja* L.). *Plants*, 13(6), 880. <https://doi.org/10.3390/plants13060880>.
- Mafakheri, A., Siosemardeh, A. F., Bahramnejad, B., Struik, P. C. & Sohrabi, Y.** (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Australian Journal of crop science*, 4(8), 580-585. <https://search.informit.org/doi/10.3316/informit.857341254680658>.
- Masand, S. & Yadav, S. K.** (2016). Over-expression of MuHSP70 gene from *Macrotyloma uniflorum* confers multiple abiotic stress tolerance in transgenic *Arabidopsis thaliana*. *Molecular biology reports*, 43, 53-64. doi 10.1007/s11033-015-3938-y.
- Melandri, G., AbdElgawad, H., Riewe, D., Hageman, J. A., Asard, H., Beemster, G. T., Kadam, N., Jagadish, K., Altmann, T., Ruyter-Spira, C. & Bouwmeester, H.** (2020). Biomarkers for grain yield stability in rice under drought stress. *Journal of Experimental Botany*, 71(2), 669-683. <https://doi.org/10.1093/jxb/erz221>.
- Mittler, R.** (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, 7(9), 405-410.
- Mondal, S., Karmakar, S., Panda, D., Pramanik, K., Bose, B. & Singhal, R. K.** (2023). Crucial plant processes under heat stress and tolerance through heat shock proteins. *Plant Stress*, 10, 100227. <https://doi.org/10.1016/j.stress.2023.100227>.
- Muhammad, A. A., Jiang, H. K., Shui, Z. W., Cao, X. Y., Huang, X. Y., Imran, S., Ahmad, B., Zhang, H., Yang, Y. N., Shang, J., Yang, H., Yu, L., Liu, C. Y., Yang, W. Y., Sun, X. & Du, J. B.** (2021). Interactive effect of shade and PEG-induced osmotic stress on physiological responses of soybean seedlings. *Journal of Integrative Agriculture*, 20(9), 2382-2394. [https://doi.org/10.1016/S2095-3119\(20\)63383-4](https://doi.org/10.1016/S2095-3119(20)63383-4).
- Pulido, P., Llamas, E. & Rodriguez-Concepcion, M.** (2017). Both Hsp70 chaperone and Clp protease plastidial systems are required for protection against oxidative stress. *Plant signaling & behavior*, 12(3), e1290039. doi 10.1080/15592324.2017.1290039.
- Rahim, D., Kalousek, P., Tahir, N., Vyhnanek, T., Tarkowski, P., Trojan, V., Abdulkhaleq, D., Ameen, A. H. & Havel, L.** (2020). *In vitro* assessment of kurkish rice genotypes in response to PEG-induced drought stress. *Applied Sciences*, 10(13), 4471. doi:10.3390/app10134471.
- Rahman, M. A., Woo, J. H., Song, Y., Lee, S. H., Hasan, M. M., Azad, M. A. K. & Lee, K. W.** (2022). Heat shock proteins and antioxidant genes involved in heat combined with drought stress responses in perennial rye grass. *Life*, 12(9), 1426. <https://doi.org/10.3390/life12091426>.
- Rao, D. S., Raghavendra, M., Gill, P., Madan, S. & Munjal, R.** (2020). Studies on role of proline, hydrogen peroxide and total antioxidant activity in wheat (*Triticum aestivum* L.) under drought stress after anthesis. *International Journal of Chemical Studies*, 8(6), 738-742. <https://doi.org/10.22271/chemi.2020.v8.i6k.10856>.
- Singh, R. K., Jaishankar, J., Muthamilarasan, M., Shweta, S., Dangi, A. & Prasad, M.** (2016). Genome-wide analysis of heat shock proteins in C4 model, foxtail millet identifies potential candidates for crop improvement under abiotic stress. *Scientific reports*, 6(1), 32641. <https://doi.org/10.1038/srep32641>.
- Slesak, I., Libik, M., Karpinska, B., Karpinski, S. & Miszalski, Z.** (2007). The role of hydrogen peroxide in regulation of plant metabolism and cellular signalling in response to environmental stresses. *Acta biochimica polonica*, 54(1), 39-50. <https://doi.org/10.3390/life12091426>.
- Song, A., Zhu, X., Chen, F., Gao, H., Jiang, J. & Chen, S.** (2014). A chrysanthemum heat shock protein confers tolerance to abi-

- otic stress. *International journal of molecular sciences*, 15(3), 5063-5078. doi.org/10.3390/ijms15035063.
- Subramani, M., Urrea, C. A., Tamatamu, S. R., Sripathi, V. R., Williams, K., Chintapenta, L. K., Todd, A. & Ozbay, G.** (2024). Comprehensive proteomic analysis of common bean (*Phaseolus vulgaris* L.) seeds reveal shared and unique proteins involved in terminal drought stress response in tolerant and sensitive genotypes. *Biomolecules*, 14(1), 109. https://doi.org/10.3390/biom14010109.
- Svetleva, D., Krastev, V., Dimova, D., Mitrovska, Z., Miteva, D., Parvanova, P. & Chankova, S.** (2012). Drought tolerance of Bulgarian common bean genotypes, characterized by some biochemical markers for oxidative stress. *Journal of Central European Agriculture*, 13(2), 349-361. DOI: 10.5513/JCEA01/13.2.1059.
- Swindell, W. R., Huebner, M. & Weber, A. P.** (2007). Transcriptional profiling of Arabidopsis heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. *BMC genomics*, 8, 125. doi:10.1186/1471-2164-8-125.
- Urmi, T. A., Islam, M. M., Zumur, K. N., Abedin, M. A., Haque, M. M., Siddiqui, M. H., Murata, Y. & Hoque, M. A.** (2023). Combined effect of salicylic acid and proline mitigates drought stress in rice (*Oryza sativa* L.) through the modulation of physiological attributes and antioxidant enzymes. *Antioxidants*, 12(7), 1438. https://doi.org/10.3390/antiox12071438.
- Verbruggen, N. & Hermans, C.** (2008). Proline accumulation in plants: a review. *Amino acids*, 35, 753-759. https://doi.org/10.1007/s00726-008-0061-6.
- Vranová, E., Inzé, D. & Van Breusegem, F.** (2002). Signal transduction during oxidative stress. *Journal of experimental botany*, 53(372), 1227-1236. https://doi.org/10.1093/jexbot/53.372.1227.
- Yoshida, Y., Kiyosue, T., Nakashima, K., Yamaguchi-Shinozaki, K. & Shinozaki, K.** (1997). Regulation of levels of proline as an osmolyte in plants under water stress. *Plant and cell physiology*, 38(10), 1095-1102. https://doi.org/10.1093/oxfordjournals.pcp.a029093.
- Zhang, L., Zhao, H. K., Dong, Q. L., Zhang, Y. Y., Wang, Y. M., Li, H. Y., Xing, G. J., Li, Q. Y. & Dong, Y. S.** (2015). Genome-wide analysis and expression profiling under heat and drought treatments of *HSP70* gene family in soybean (*Glycine max* L.). *Frontiers in plant science*, 6, 773. https://doi.org/10.3389/fpls.2015.00773.
- Zlatev, Z., Berova, M., Krastev, V., Svetleva, D., Parvanova, P., Miteva, D., Mitrovska, Z. & Chankova, S.** (2020). Drought tolerance of Bulgarian common bean genotypes, characterized by some physiological and biochemical parameters. *Agricultural Sciences/Agrarni Nauki*, 12(27). DOI: 10.22620/agris-ci.2020.27.003.

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