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Antioxidant response of *Impatiens walleriana* L. to drought

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Abstract: Stress caused by drought induces plant morphology, biochemistry, and physiology changes, leading to considerable reductions in plant growth and productivity. This study aimed to evaluate the antioxidant defence system of impatiens seedlings (*Impatiens walleriana* L.) under drought. The antioxidant response of impatiens to drought was evaluated using following parameters: the activity of catalase, guaiacol peroxidase, pyrogallol peroxidase and ascorbate peroxidase, total phenolic and flavonoids contents and total antioxidant capacity. The experiment was conducted during 2020 in a greenhouse under controlled conditions. Half of the impatiens seedlings (20 plants), after the acclimation period in the greenhouse, were exposed to drought for a period of five days, while the second half was not (20 plants were regularly watered). The results of the study showed that the exposure of impatiens seedlings to drought increased the activity of enzymatic components, total phenolics and flavonoids contents and total antioxidant capacity of leaves. Greater exposure of impatiens to drought (in the observed period) implied a higher plant enzymatic and non-enzymatic antioxidant defence system activity. These results confirm that impatiens has evolved both enzymatic and non-enzymatic antioxidant defence mechanisms to adapt and survive the short-term drought exposure.

Key words: defence system; free radicals; leaves; plant growth; stress

Antioksidacijski odziv vodenke (*Impatiens walleriana* L.) na sušo

Izvleček: Stres, ki ga povzroča suša sproži v rastlinah spremembe v morfologiji, biokemični zgradbi in fiziologiji, kar vodi k znatnemu zmanjšanju rasti in produktivnosti rastlin. Namen raziskave je bil ovrednotiti antioksidacijsko obrambo sejank vodenke (*Impatiens walleriana* L.) v sušnem stresu. Antioksidacijski odziv vodenke na sušo je bil ovrednoten z naslednjimi parametri: aktivnostjo katalaze, guajakol peroksidaze, pirogalol peroksidaze in askorbat peroksidaze, vsebnostjo celokupnih fenolov in flavonoidov in celokupne antioksidacijske kapacitete. Poskus je bil izveden v rastni sezoni 2020 v rastlinjaku v nadzorovanih razmerah. Polovica sejank vodenke (20 rastlin), je bila po aklimatizaciji razmeram rastlinjaka izpostavljena sušnemu stresu za pet dni, medtem ko je bila druga polovica (20 rastlin) redno zalivana. Rezultati raziskave so pokazali, da je izpostavitev sejank vodenke sušnemu stresu povečala aktivnosti analiziranih encimov, vsebnosti celokupnih fenolov in flavonoidov ter celokupno antioksidacijsko sposobnost listov. Večja izpostavitev vodenk suši je v opazovanem obdobju povzročila večji encimski in neencimski antioksidacijski obrambni odziv. Rezultati potrjujejo, da ima vodenka sposobnost razvoja encimskega in neencimskega antioksidacijskega obrambnega sistema in lahko preživi krajša obdobja izpostavitve suši.

Ključne besede: obrambni sistem; prosti radikali; listi; rast rastlin; stres

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1 INTRODUCTION

Drought is the most important abiotic factor limiting crop productivity. The lack of water in soil reduces the soil water potential and the ability of plants to take up water, resulting in growth inhibition and reproductive failure (Fahad et al., 2017). In addition, the inevitable consequence of drought is an increase in the production of reactive oxygen species (ROS) in plant cells. ROS include free radicals such as superoxide radical, hydroxyl radical as well as non-radical molecules like hydrogen peroxide (H_2O_2). Increased levels of ROS can cause cellular damage and even cell death (Tola et al., 2021).

Plants, however, have evolved numerous mechanisms to contend with oxidative stress, including the enzymatic and non-enzymatic antioxidant systems. Non-enzymatic defences include compounds with antioxidant properties such as phenolic compounds, vitamin C and carotenoids, while the enzymatic defences include antioxidant enzymes associated with ROS scavenging in plants such as superoxide dismutase (SOD), guaiacol peroxidase (GPX), pyrogallol peroxidase (PPX), ascorbate peroxidase (APX) and catalase (CAT) (Mehla et al., 2017).

SOD protects cells against ROS by catalysing the dismutation of highly toxic superoxide anions to less toxic hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). After dismutation of the superoxide anions by SOD into O_2 and H_2O_2, the CAT decomposes the released H_2O_2 into H_2O and O_2 (Berwal & Ram, 2018). GPX and PPX also protect cells against the damaging effect of H_2O_2 by catalysing their decomposition through oxidation of phenolic substrates (Gill & Tuteja, 2010). APX is also a H_2O_2-scavenging enzyme. APX utilizes ascorbic acid as specific electron donor to reduce H_2O_2 to H_2O (Hasanuzzaman et al., 2019).

The aim of this study was to evaluate the enzymatic and non-enzymatic antioxidant defence system of impatiens seedlings (*Impatiens walleriana* L.) under drought stress. Impatiens was selected as subject of this study primarily because the global production of this flowering plant species is consistently increasing. Therefore any new knowledge about the behaviour of these plants, especially under stress conditions, is of great interest to both producers and scientists.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL CONDITIONS

The experiment was conducted in May 2020 under controlled conditions in the greenhouse of public communal company 'Park' in Sarajevo. The temperature in the greenhouse during the experiment was maintained at 24 °C/21 °C during day/night, while the relative humidity (RH) was maintained between 60 % and 70 %, with combined venting to reduce RH and fogging systems to increase RH.

In the beginning of the experiment, the impatiens seedlings were in the initial stage of flowering. The first part of the study involved transplanting impatiens seedlings into individual pots (20 cm diameter × 13 cm height), containing substrate Florahum-SP. Impatiens seedlings used in the experiment were produced in the nursery near the greenhouse and showed no significant difference in size and appearance.

Ten days after transplanting, half of the impatiens (20 plants) were exposed to drought for next five days (non-watering). However, the second half was not exposed to drought, they served as controls (20 plants were regularly watered). Leaves of impatiens were sampled at the beginning and at the end of experiment (2nd and 5th day after drought treatment). Each leaf sample consisted of three fully expanded and healthy impatiens leaves. Fresh leaves were cut and immediately frozen with liquid nitrogen and then stored in ultra-freezer at -20 °C until further use.

2.2 PROTEIN AND ENZYME ACTIVITY MEASUREMENTS

To obtain the extracts that were used to determine the protein content and activities of catalase and peroxidases, 0.5 g of fresh leaf sample was macerated using a mortar and pestle with liquid nitrogen and 0.015 g polyvinylpyrrolidone (PVP). The powder thus obtained was homogenized in 1.5 ml 50 mM potassium phosphate buffer (pH 7) containing 1 mM dithiothreitol (DTT) and 1mM ethylenediaminetetraacetic acid (EDTA). The homogenized material was centrifuged at 10,000 g for 10 min at 4 °C, and the supernatant was used for protein and enzyme activity measurements.

The extracted proteins were quantified using the Bradford method with bovine serum albumin (BSA) as the standard (Bradford, 1976). The pyrogallol peroxidase (PPX) activity was determined by the oxidation of pyrogallol according to the method of Chance & Maehly (1955) and the results were expressed as μmol purpurogallin per min per mg protein. The guaiacol peroxidase (GPX) activity was determined by the oxidation of guaiacol according to the method of Chance & Maehly (1955) and the results were expressed as μmol tetraguaiacol per min per mg protein. The ascorbate peroxidase (APX) activity was determined by the oxidation of ascorbic acid

according to the method of Nakano & Asada (1981) and the results were expressed as µmol ascorbic acid oxidized per min per mg protein. The catalase (CAT) activity was determined by monitoring the decrease in absorbance at 240 nm at an interval of 5 to 120 sec as a result of H_2O_2 consumption (Aebi, 1984). Results were expressed as µmol of H_2O_2 consumed per min per mg protein.

2.3 EXTRACTION OF PHENOLIC COMPOUNDS FROM LEAVES

The collected fresh leaves of impatiens were oven-dried at 40 °C (3 days) to avoid degradation of their phenolic compounds. After that, dried leaf samples were ground to a fine powder using an electric blender and stored at 4 °C until extraction and analysis. Extraction of phenolic compounds from dried leaf sample was done as follows: 1 g of sample was extracted with 30 ml of 60 % ethanol aqueous solution at room temperature for 24 h. Thereafter, the extract was filtered through Whatman filter paper (11 µm pore size) into 50 ml volumetric flask and diluted to the mark with 60 % ethanol aqueous solution. The extract thus obtained was used to estimate total phenolic content, total flavonoid content and total antioxidant capacity.

2.4 TOTAL PHENOLICS CONTENT

The colorimetric reaction with Folin-Ciocâlteu reagent was performed to determine the content of phenolic compounds in leaf samples of impatiens (Ough & Amerine, 1988). The reaction mixtures consisted of 0.1 ml of extract, 6 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent (before use diluted in distilled water 1:2, v/v) and 1.5 ml of 20 % Na_2CO_3 were mixed thoroughly. Thereafter, the mixture was heated in a water bath at 40 °C for 30 min. After cooling to room temperature, the absorbance of the mixture was read at 765 nm. The results were calculated on the basis of the calibration curve for gallic acid (0-500 mg l^{-1}) and were expressed as mg of gallic acid equivalents per g of dry mass (mg GAE g^{-1} DM).

2.5 TOTAL FLAVONOIDS CONTENT

The aluminum chloride colorimetric assay was performed to determine the total flavonoid contents (Zhishen et al., 1999). The reaction mixtures consisted of 1 ml of extract, 4 ml of distilled water, 0.3 ml of 5 % $NaNO_2$, 0.3 ml of 10 % $AlCl_3$ and 2 ml of 1 M NaOH were mixed thoroughly. The mixture was made up to 10 ml with distilled water and incubated at room temperature for 1 h, and then the absorbance of the mixture was read at 510 nm. The results were calculated on the basis of the calibration curve for catechin (0-100 mg l^{-1}) and were expressed as mg of catechin equivalents per g of dry mass (mg C g^{-1} DM).

2.6 FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

Ferric reducing antioxidant power (FRAP) assay was performed to estimate the total antioxidant capacity (Benzie & Strain, 1996). The reaction mixture consisted of 80 µl of extract, 240 µl of distilled and 2080 µl of fresh FRAP reagent were mixed thoroughly. The FRAP reagent was prepared immediately before use by mixing acetate buffer (300 mM, pH = 3.6), 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM HCl and 20 mM $FeCl_3$ in a volume ratio of 10:1:1. Thereafter, the mixture was heated at 37 °C for 15 min in a water bath. After cooling to room temperature the absorbance was read at 595 nm. The results were calculated on the basis of the calibration curve for $FeSO_4 \times 7\ H_2O$ (0-2000 µM) and were expressed as µmol of Fe^{2+} per g of dry mass (µmol Fe^{2+} g^{-1} DM). Amersham ultrospec 2100 spectrophotometer (Biochrom, USA) was used for all spectrophotometric measures.

2.7 STATISTICAL ANALYSIS

All experimental measurements were done in triplicates and the results were presented as mean ± standard deviation. Pearson correlation coefficient was used to reflect relationship total phenolic, total flavonoids and total antioxidant activities. One-way analysis of variance (ANOVA) and least-significant-difference test (LSD) at 0.05 level of probability ($p < 0.05$) were performed to evaluate statistical significance between the means using Microsoft Excel 2010 software (Office 2010, Redmond, WA, USA).

3 RESULTS

The activity of all tested enzymes (GPX, PPX, APX and CAT) in the observed period were higher in leaves of impatiens exposed to drought compared to impatiens grown under standard growth conditions (without stress), as shown in Table 1. The results of this study also showed that the activities of all enzymes were increased with the progress of stress.

Table 1: Effect of short-term exposure to drought on antioxidant enzymes of impatiens leaves

	Enzyme activity (μmol min^{-1} mg^{-1} protein)			
Treatment	GPX	PPX	APX	CAT
2nd day of exposure to drought	0.25 ± 0.11[b]	0.41 ± 0.13[b]	0.27 ± 0.26[b]	0.009 ± 0.001[b]
2nd day without stress	0.16 ± 0.07[c]	0.33 ± 0.21[b]	0.13 ± 0.12[c]	0.008 ± 0.002[b]
5th day of exposure to drought	0.31 ± 0.04[a]	1.03 ± 0.13[a]	0.51 ± 0.16[a]	0.033 ± 0.014[a]
5th day without stress	0.18 ± 0.04[c]	0.48 ± 0.07[b]	0.22 ± 0.08[bc]	0.009 ± 0.004[b]
$LSD_{0.05}$	0.054	0.159	0.114	0.006

The activity of GPX and APX were significantly higher in the leaves of impatiens exposed to drought than in the control, regardless of the duration of plant exposure to stress. However, the activity of PPX and CAT in leaves of all stressed impatiens seedlings was significantly higher only at the end of the experiment i.e. on the fifth day of plant exposure to drought. In controls i.e. in variants where impatiens seedlings were not exposed to drought, the activity of all enzymes tested did not change significantly during the experiment.

In this study, the non-enzymatic antioxidant defence system (total phenolic contents (TPC), total flavonoids (TFC) and total antioxidant capacity (TAC) were also affected by growth conditions (Table 2).

As shown in Table 2, TPC, TFC and TAC were higher in the leaves of impatiens exposed to drought than in control. The increases were statistically significant for both the second and the fifth day of plant exposure to drought.

In this study, there was a positive and strong significant relationship between the total phenolic/flavonoids and the total antioxidant capacity of impatiens leaves regardless of growth conditions, indicating that phenolic compounds are mainly responsible for total antioxidant capacity of plants (Table 3).

4 DISCUSSION

A key sign of drought stress at the cellular level is the overproduction of reactive oxygen species (ROS), which is being considered as the most common cause of cellular damage. However, plants have evolved an efficient enzymatic and non-enzymatic antioxidant system to protect themselves against ROS. Within a cell, the SOD constitutes the first line of plant antioxidant defence against ROS. However, H_2O_2, which results from the action of SOD, is toxic to cells. Therefore, the efficient scavenging of H_2O_2 is regarded as a key feature in the cellular antioxidant defence system. Fortunately, plant cells are endowed with H_2O_2-metabolizing enzymes such as peroxidases and catalase. Peroxidases are group of enzymes that catalyse the conversion H_2O_2 into H_2O using a wide variety of substrates as an electron donor (Abedi & Paknyat, 2010).

In this study, generally, stress caused by drought increased the CAT and peroxidase enzymatic activity, and the increase was in line with stress duration; greater exposure of impatiens to drought (in the observed period) implied a higher activity of antioxidant enzymes. However, there was a differential level of activity among enzymes. The activities of GPX and APX enzymes at the early stage of drought stress (2nd day after drought treatment) were significantly higher as compared to CAT, although both peroxidases and CAT act on the same substrate (H_2O_2). Lower CAT activities in plants at the early stage of stress have been reported in many studies (Chugh et al, 2013; Antonić et al., 2016; Wang et al., 2019). Smirnof & Araound (2019) noted that CAT does not have a high affinity for H_2O_2 and this is probably one of the main reasons for its low activity. However, CAT has

Table 2: Effect of short-term exposure to drought on non-enzymatic antioxidants of impatiens leaves

Treatment	TPC (mg GAE g^{-1} DM)	TFC (mg C g^{-1} DM)	TAC (μmol Fe^{2+} g^{-1} DM)
2nd day of exposure to drought	6.92 ± 0.18[b]	2.08 ± 0.24[b]	92.91 ± 5.82[b]
2nd day without stress	5.65 ± 0.22[c]	1.50 ± 0.18[c]	65.23 ± 3.65[c]
5th day of exposure to drought	7.98 ± 0.70[a]	2.68 ± 0.34[a]	103.95 ± 4.17[a]
5th day without stress	6.37 ± 0.75[bc]	2.20 ± 0.20[b]	89.42 ± 12.38[b]
$LSD_{0.05}$	0.83	0.26	10.58

Table 3: Pearson's correlation between total phenolic (TPC), total flavonoids (TFC) and total antioxidant capacity (TAC)

Treatment		TPC	TFC	TAC
2nd day of exposure to drought	TPC	1	0.95	0.93
	TFC		1	0.94
	TAC			1
2nd day without stress	TPC	1	0.92	0.93
	TFC		1	0.91
	TAC			1
5th day of exposure to drought	TPC	1	0.94	0.95
	TFC		1	0.96
	TAC			1
5th day without stress	TPC	1	0.93	0.94
	TFC		1	0.92
	TAC			1

a very high reaction rate (Smejkal & Kakumanu, 2019). The Braunschweig Enzyme Database (BRENDA) reports that one molecule of catalase can convert over 2.8 million molecules of hydrogen peroxide to water and oxygen per second (Schomburg et al., 2017). Therefore, CAT is a sink for H_2O_2 and is indispensable for plant defence system against oxidative stress (Willekens et al., 1997).

Besides enzymatic antioxidants, plants synthesize a wide range of non-enzymatic antioxidants capable of decreasing ROS-induced oxidative damage (Kasote et al., 2015). Non-enzymatic antioxidants include vitamin C, vitamin E, phenolic compounds, carotenoids, etc. Among all non-enzymatic antioxidants, phenolic compounds appear to be the most important since they have a great potential to clear ROS. The antioxidant properties of phenolic compounds are mainly due to their high redox potential, allowing them to act as reducing agents, hydrogen donors or singlet oxygen quenchers (Liang et al., 2010).

In the present study, the accumulation of phenolic compounds was significantly higher in leaves of impatiens exposed to drought than in controls (without stress). Moreover, an increase in phenolics contents was more significant in impatiens exposed to drought for longer duration. These results suggest that plant initiates the intensive synthesis of phenolic compounds as a response to drought, and this hypothesis has been confirmed by many other scientists (Basu et al., 2010; Cramer et al., 2011; Šamec et al., 2021).

Sharma et al. (2019) reported that the considerable accumulation of phenolic compounds in plants is usually a consistent feature of non-enzymatic antioxidant defence mechanisms under stress. However, the capacity of antioxidant defence mechanisms depends on each phenolic compound's chemical structure. Among the phenolic compounds with known antioxidant activity, flavonoids are highlighted (Dibacto et al., 2021). In this study, TFC in leaves of impatiens were progressively influenced by drought. An increase in TFC in leaves of impatiens was already recorded in the 2nd days after drought treatment, and with the progress of stress (5th days after drought treatment), TFC was gradually increased. In this study, an increase of TFC was in line with increase of TPC in impatiens leaves regardless of growth conditions. This was expected since the flavonoids are the biggest group of phenolic compounds.

In the present study, the total antioxidant capacity level estimated with FRAP assay was also significantly higher in leaves of impatiens exposed to drought than in controls. Furthermore, the present study indicates a very strong relationship between the TPC/TFC and TAC in leaves of impatiens, regardless of growth conditions. In short, the antioxidant activity in leaves of impatiens increased by increasing the total phenolic and flavonoid contents. These results were also expected since it is known that phenolic compounds are among the most potent antioxidants from plants.

The levels of enzymatic and non-enzymatic antioxidants in impatiens leaves were very high in the fifth day after drought treatment. Accumulation of these antioxidants suggests a high level of stress convened to the impatiens during this period (Sharma et al., 2012). It can also be assumed that the impatiens during this period continues to defend itself against ROS by producing a high amount of enzymatic and non-enzymatic antioxidants (Kim et al., 2014). However, numerous studies reported a decline in the activity of antioxidant enzymes in various plants in the final stage of stress (three days or more after exposed plant to stress), indicating that antioxidant capacity and thus drought tolerance can vary among plants (Almeselmani et al., 2006; Sabzmeydani et al., 2021). It is evident that plant response to drought depends not only on the extremity and time duration of the stress but also on the plant genetic background.

5 CONCLUSIONS

Exposure of impatiens seedlings to drought increased the activity of enzymatic antioxidants, total phenolic and flavonoid contents and total antioxidant capacity of leaves. Greater exposure of impatiens to drought (in the observed period) implied a higher activity of plant enzymatic and non-enzymatic antioxidant defence systems. These results confirm that impatiens have evolved both enzymatic and non-enzymatic antioxidant

defence mechanisms to adapt and survive the short-term drought exposure.

6 REFERENCES

Abedi, T., & Pakniyat, H. (2010). Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). *Czech Journal of Genetics and Plant Breeding, 46*, 27–34. https://doi.org/10.17221/67/2009-CJGPB

Aebi, M. (1984). Catalase in vitro. *Methods in Enzymology, 105*, 121–126. https://doi.org/10.1016/s0076-6879(84)05016-3

Almeselmani, M., Deshmukh, P.S., Sairam, R.K., Kushwaha, S.R., Singh, T.P. (2006). Protective role of antioxidant enzymes under high temperature stress. *Plant Science, 171*(3), 382–388. https://doi.org/10.1016/j.plantsci.2006.04.009

Antonić, D., Milošević, S., Cingel, A., Lojić, M., Trifunović-Momčilov, M., Petrić, M., Subotić, A., Simonović, A. (2016). Effects of exogenous salicylic acid on *Impatiens walleriana* L. grown in vitro under polyethylene glycol-imposed drought. *South African Journal of Botany, 105*, 226–233. https://doi.org/10.1016/j.sajb.2016.04.002

Basu, S., Roychoudhury, A., Saha, P.P, Sengupta D.N. (2010). Differential antioxidative responses of indica rice cultivars to drought stress. *Plant Growth Regulation, 60*(1), 51–59. https://doi.org/10.1007/s10725-009-9418-4

Benzie, I.F., & Strain, J.J. (1996). Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Berwal, M.K. & Ram, C. (2018). Superoxide Dismutase: A Stable Biochemical Marker for Abiotic Stress Tolerance in Higher Plants. In A. B. De Oliveira (Ed), *Abiotic and Biotic Stress in Plants*. London, UK: IntechOpen. https://doi.org/10.5772/intechopen.82079

Bradford, M.M. (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/10.1006/abio.1976.9999

Chance, B., & Maehly A.C. (1955). Assay of catalases and peroxidases. *Methods in Enzymology, 2*, 764–775. https://doi.org/10.1016/S0076-6879(55)02300-8

Chugh, V, Kaur, N, Grewal, M.S., Gupta, A.K. (2013). Differential antioxidative response of tolerant and sensitive maize (*Zea mays* L.) genotypes to drought stress at reproductive stage. *Indian Journal of Biochemistry and Biophysics, 50*(2), 150–158.

Cramer, G.R., Urano, K., Delrot, S., Pezzotti, M., Shinozaki, K. (2011). Effects of abiotic stress on plants: a systems biology perspective. *BMC Plant Biology, 11*, 163. https://doi.org/10.1186/1471-2229-11-163

Dibacto, R.E.K., Tchuente,B.R.T., Nguedjo, M.W., Tientcheu, Y.M.T., Nyobe, E. C., Edoun, F.L.E., Kamini, M.F.G., Dibanda, R.F., Medoua, G.N. (2021): Total polyphenol and flavonoid content and antioxidant capacity of some varieties of *Persea americana* peels consumed in Cameroon. *Scientific World Journal, 2021*, e8882594. https://doi.org/10.1155/2021/8882594

Fahad, S., Bajwa. A.A., Nazir, U., Anjum, S.A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M.Z., Alharby, H., Wu, C., Wang, D., Huang, J. (2017). Crop production under drought and heat stress: Plant responses and management options. *Frontiers in Plant Science, 8*, e1147. https://doi.org/10.3389/fpls.2017.01147

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

Hasanuzzaman, M., Bhuyan, M., Anee, T.I., Parvin, K., Nahar, K., Mahmud, J.A., Fujita, M. (2019). Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. *Antioxidants, 8*(9), 384. https://doi.org/10.3390/antiox8090384

Kasote, D.M., Katyare, S.S., Hegde, M.V., Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences, 11*(8), 982–991. https://doi.org/10.7150/ijbs.12096

Kim, Y.H., Khan, A.L., Kim, D.H., Lee, S.Y., Kim, K.M., Waqas, M., Jung, H.Y., Shin, J.H., Kim, J.G., Lee, I.J. (2014). Silicon mitigates heavy metal stress by regulating P-type heavy metal ATPases, Oryza sativa low silicon genes, and endogenous phytohormones. *BMC Plant Biology, 14*, 13. https://doi.org/10.1186/1471-2229-14-13

Liang, T., Yue, W., Li, Q. (2010): Comparison of the phenolic content and antioxidant activities of *Apocynum venetum* L. (Luo-Bu-Ma) and two of its alternative species. *International Journal of Molecular Sciences, 11*(11):4452–4464. https://doi.org/10.3390/ijms11114452

Mehla, N., Sindhi, V., Josula, D., Bisht, P., Wani, S.H. (2017). An introduction to antioxidants and their roles in plant stress tolerance. In M. I. R. Khan & N. A. Khan (Eds.), *Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress* (pp. 1–23). Singapure, SG: Springer. https://doi.org/10.1007/978-981-10-5254-5_1

Nakano, Y., & Asada K. (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiology, 22*(5), 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Ough, C.S., & Amerine, M.A. (1988). *Methods for Analysis of Musts and Wines* (pp. 196–221). New York, NY: John Wiley & Sons.

Sabzmeydani, E., Sedaghathoor, S., Hashemabadi, D. (2021). Effect of salicylic acid and progesterone on physiological characteristics of Kentucky bluegrass under salinity stress. *Revista de Ciencias Agrícolas, 38*(1), 111–124. https://doi.org/10.22267/rcia.213801.151

Šamec, D., Karalija, E., Šola, I., Vujčić Bok, V., Salopek-Sondi, B. (2021). The role of polyphenols in abiotic stress response: The influence of molecular structure. *Plants 10*(1), 18. https://doi.org/10.3390/plants10010118

Schomburg, I, Jeske, L, Ulbrich, M, Placzek, S., Chang, A., Schomburg, D. (2017). The BRENDA enzyme informa-

tion system–from a database to an expert system. *Journal of Biotechnology 261*, 194–206. https://doi.org/10.1016/j.jbiotec.2017.04.020

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., Zheng, B. (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules (Basel, Switzerland), 24*(13), 2452. https://doi.org/10.3390/molecules24132452

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany, 2012*, e217037. https://doi.org/10.1155/2012/217037

Smejkal G.B., & Kakumanu S. (2019). Enzymes and their turnover numbers. *Expert Review of Proteomics, 16*(7), 543–544. https://doi.org/10.1080/14789450.2019.1630275

Smirnoff, N., & Arnaud, D. (2019). Hydrogen peroxide metabolism and functions in plants. *New Phytologist, 221*(3), 1197–1214. https://doi.org/10.1111/nph.15488

Tola, A.J., Jaballi, A., Missihoun, T.D. (2021). Protein carbonylation: Emerging roles in plant redox biology and future prospects. *Plants, 10*(7), e1451. https://doi.org/10.3390/plants10071451

Wang, X, Liu, H, Yu, F, Hu, B, Jia, Y, Sha, H, Zhao, H. (2019). Differential activity of the antioxidant defence system and alterations in the accumulation of osmolyte and reactive oxygen species under drought stress and recovery in rice (*Oryza sativa* L.) tillering. *Scientific Reports 9*(1), 8543. https://doi.org/10.1038/s41598-019-44958-x

Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., Inzé, D., Van Camp, W. (1997). Catalase is a sink for H_2O_2 and is indispensable for stress defence in C3 plants. *The EMBO journal, 16*(16), 4806–4816. https://doi.org/10.1093/emboj/16.16.4806

Zhishen, J., Mengcheng, T., Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*(4), 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2