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Ameliorating effects of exogenous paclobutrazol and putrescine on mung bean [*Vigna radiata* (L.) Wilczek] under water deficit stress

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Abstract: Plant growth regulators play crucial roles in modulating plant response to environmental stresses. In this experiment, the effect of different doses of paclobutrazol (PBZ) and putrescine (Put), i.e., 0, 50, 100 and 150 mg/L on mung bean in two conditions of water deficit (WD) and well-watered (WW) was investigated. The seed yield decreased due to water deficit stress, while the PBZ and Put application alleviated the damage of drought stress through increasing proline and leaf chlorophyll content and improving membrane stability, and thus increased plant yield compared to untreated control plants. According to regression equations, the high PBZ levels (150 mg/L or more) and moderate levels of Put (about 90 mg/L) were determined as the optimal concentrations to maximise mung bean yield in WD conditions. In WW conditions, the mung bean responses to PBZ were inconsistent, whereas Put application positively affected some physiological traits and seed yield. In conclusion, the physiological attributes and, subsequently, the seed yield of drought-stressed mung bean plants could be improved by foliar application of PBZ and Put.

Keywords: dehydration stress; green manure; irrigation; plant tolerance; polyamines; triazoles

Water deficit stress is a key factor in reducing crop yield and hindering sustainable production (Bodner et al. 2015). One of the strategies to increase plant tolerance against environmental stresses, including drought stress, is to use plant growth regulators (Tesfahun 2018).

Paclobutrazol (PBZ), a plant growth regulator of the triazole group, with a wide variety of physiological, growth and developmental effects on plants, has been extensively used in horticulture and crop production. PBZ has been shown to modify the pattern of photoassimilates distribution among plant organs, which can affect the crop productivity (Senoo and Isoda 2003, Kumar et al. 2012) and improve plant tolerance to drought stress (Cohen et al. 2019).

Polyamines (PAs) are a group of low molecular weight aliphatic organic compounds having at least two amino groups and are found in cells of all living organisms (Singh et al. 2018). The main PAs in plants are putrescine (Put), spermidine (Spd) and spermine (Spm), which are involved in a wide variety of plant functions, including development, growth, phenology and response to environmental stresses (Chen et al. 2019). Nowadays, it has been clearly shown that polyamines play a role in increasing plant tolerance to environmental stresses (Singh et al. 2018).

Mung bean [*Vigna radiata* (L.) Wilczek] is a short-duration pulse crop cultivated in many regions of the world as food, fodder, green manure and cover crop, which is considered as a suitable rotational plant in cropping systems (Raina et al. 2016, Chand et al. 2018, Zhu et al. 2018).

Although there are many reports on different plants' response to growth regulators under environmental stresses, little information is available on the response of mung bean to foliar-applied PBZ and Put under both water deficit and normal irrigation conditions. Therefore, this study aimed to: (*i*) compare the effects of different doses of PBZ and Put on yield and physiological traits of mung bean under well-watered and water-deficit stress conditions, and (*ii*) evaluate the possibility of compensating for dehydration stress in mung bean using PBZ and/or Put in field conditions.

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MATERIAL AND METHODS

This study was performed as a two-year experiment, in 2016 and 2017, at the experimental farm of Sanandaj Azad University, Iran (35°10'N, 46°59'E; 1 390 m a.s.l.). The long-term annual precipitation and the average temperature of the study area are 471 mm and 13.4 °C, respectively. The meteorological status of the region and the farm soil traits are shown in Tables 1 and 2, respectively.

The experiment was laid out as a split-plot based on a randomised complete block design with three replications. Two irrigation regimes of water deficit (WD) and well-watered (WW) were allocated to the main plots, and seven treatments of growth regulators (GRs) including: control (distilled water); 50, 100 and 150 mg/L of Put and 50, 100 and 150 mg/L of PBZ were assigned as the sub-plots. In each subplot, there were four planting rows of 6 m in length, 0.5 m between the rows, and 7 cm between the plants. Seeds of mung bean cultivar Gowhar were manually sown in the first year, on July 5, and in the second year, on June 24. All plots were equally well irrigated during the three weeks after planting to ensure plant establishment. Then the irrigation treatments were implemented according to the soil moisture depletion and the effective rooting depth of mung bean. In the WW treatment, the plots were irrigated at intervals of 5 to 7 days to bring the soil water content to field capacity, and in the WD treatment, the same amount of water was supplied by irrigating every 10–14 days. Foliar application of Put and PBZ treatments was done four times, started at the 6-leaf stage and repeated every 14 days.

The mean value of SPAD (soil-plant analysis development) readings from ten randomly selected leaves in each plot was recorded using a SPAD chlorophyll meter (Minolta SPAD-502 meter, Tokyo, Japan). The contents of photosynthetic pigments were determined based on the method of Lichthenthaler (1987). The electrolyte leakage (EL) and proline content of leaves were determined according to the procedures described by Lutts et al. (1996) and Bates et al. (1973), respectively. The seed yield was determined after harvesting the plants of a 2 m^2 area from the central rows of each plot.

The collected data of the two growing seasons were subjected to a combined analysis of variance, and the least significant difference (*LSD*) test was used to compare the means. The trend of variation *versus* Put and PBZ levels were plotted by regression analysis for some of the traits. The statistical operations were done using SAS (SAS Institute Inc., Cary, USA).

Table 1. Mean temperature and rainfall during the months of two growing seasons (2016 and 2017)

Month	Mean air temperature (°C)		Rainfall (mm)	
	2016	2017	2016	2017
June	24.8	24.9	0.25	0
July	28.7	29.2	0	0
August	28.9	28.4	0	0
September	21.4	23.3	0	0
October	15.2	15.1	5.08	0.25

Table 2. Soil properties of the experimental farm

Depth	2016		2017	
	0–30	30–60	0–30	30–60
	(cm)			
Clay (%)	32.3	32	13.6	9.5
Silt (%)	34	28.3	37.4	35.4
Sand (%)	33.7	39.7	49	55.1
OC (%)	1.13	0.83	1.1	0.94
TNV (%)	4.5	7.25	14.25	10.57
pH	7.69	8.01	7.41	7.52
EC (ds/m)	0.489	0.535	0.516	0.27
N (%)	0.11	0.08	0.11	0.09
P (ppm)	14.03	10.86	14.2	13.2
K (ppm)	234.4	205	306	235

OC – organic carbon; TNV – total neutralising value; EC – electrical conductivity

RESULTS AND DISCUSSION

Electrolyte leakage and proline content. The electrolyte leakage and proline content were significantly increased in WD condition compared with WW treatment over the two years (Table 3). Environmental stresses, such as drought, disrupt the cell membrane stability, leading to increasing ions leakage from cells (Bodner et al. 2015). Proline can protect the plant from various stresses through contribution to osmotic adjustment, detoxification of reactive oxygen species, maintaining membrane integrity and stabilising proteins and enzymes (Ashraf and Foolad 2007). The elevated content of leaf proline in this study is in line with the findings of Bangar et al. (2019).

The EL rate decreased with increasing Put level in both WD and WW conditions (Figures 1A, B), suggesting the protective effect of Put on cell membranes. Polyamines prevent lipid peroxidation and macro-

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Table 3. Effects of irrigation regime and growth regulators application on various traits of mung bean in 2016 and 2017

Year	Irrigation regime	Growth regulator	EL (%)	Proline (μmol/g FW)	SPAD value	Chl *a* (mg/g FW)	Chl *b* (mg/g FW)	Seed yield (kg/ha)
2016	WD	control	52.6	7.227	34.0	0.928	0.176	622
		Put 50	50.6	6.823	38.3	0.963	0.234	770
		Put 100	50.9	7.505	38.5	1.500	0.418	1 111
		Put 150	50.7	6.988	38.3	1.304	0.230	726
		PBZ 50	52.8	8.682	36.4	1.195	0.339	1 541
		PBZ 100	52.5	7.790	39.9	1.427	0.386	1 555
		PBZ 150	47.1	7.874	37.7	1.314	0.291	1 921
	WW	control	43.1	4.119	42.7	1.802	0.291	1 702
		Put 50	40.6	2.639	52.0	1.421	0.453	1 696
		Put 100	42.1	5.742	48.5	1.650	0.481	2 175
		Put 150	36.3	4.988	47.6	1.359	0.376	1 299
		PBZ 50	35.8	2.705	39.6	1.343	0.302	1 239
		PBZ 100	44.6	4.183	41.2	1.528	0.347	1 278
		PBZ 150	40.6	4.950	44.8	1.819	0.294	1 347
		$LSD_{0.05}$	3.9	2.076	5.5	0.340	0.110	240
2017	WD	control	49.8	5.221	31.8	0.888	0.207	908
		Put 50	51.0	7.064	36.1	1.013	0.255	1 265
		Put 100	48.1	7.905	37.0	1.256	0.262	1 475
		Put 150	47.2	6.892	34.6	1.132	0.227	1 215
		PBZ 50	51.4	6.347	35.6	1.080	0.423	1 114
		PBZ 100	51.1	8.189	38.0	1.509	0.351	1 217
		PBZ 150	48.5	7.968	37.2	1.703	0.286	1 361
	WW	control	40.0	3.931	41.2	1.438	0.334	1 674
		Put 50	39.6	2.572	45.3	1.617	0.379	1 569
		Put 100	37.9	5.185	47.5	1.829	0.483	2 045
		Put 150	34.7	3.792	43.2	1.619	0.299	1 688
		PBZ 50	34.4	4.680	39.9	1.516	0.333	1 348
		PBZ 100	39.4	3.616	42.0	1.490	0.366	1 537
		PBZ 150	36.3	4.044	43.0	1.120	0.268	1 558
		$LSD_{0.05}$	6.6	1.258	4.4	0.286	0.089	320

EL – electrolyte leakage; SPAD – soil-plant analysis development value; WD – water deficit; WW – well-watered; *LSD* – least significant difference; FW – fresh weight; putrescine (Put) – 50, 100 and 150 mg/L; paclobutrazol (PBZ) – 50, 100 and 150 mg/L

molecules destruction in stress conditions (Nahar et al. 2016). Application of the highest dose of PBZ (150 mg/L) reduced the EL compared with control in WD conditions (Figure 1A), indicating the protective impact of PBZ on cell membranes. Triazoles have been reported to play a role in preventing oxidative damage (Soumya et al. 2017).

Regression analysis of proline in WD conditions showed a second-order elevation in response to PBZ and Put application so that the proline content in WD status can be maximised by applying about 116 mg/L of PBZ and 95 mg/L of Put (Figure 1C). The trend of proline content in WW conditions was inconsistent (Figure 1D). In line with these results, Yooyongwech et al. (2017) found that different doses of PBZ caused a remarkable increase in leaf proline of sweet potato under severe water deficit stress but had no effect in non-stress conditions.

SPAD and photosynthetic pigments. WD stress significantly decreased chlorophyll content and SPAD over both years (Table 3, Figure 2). PBZ levels of 100 and 150 mg/L in WD significantly increased SPAD

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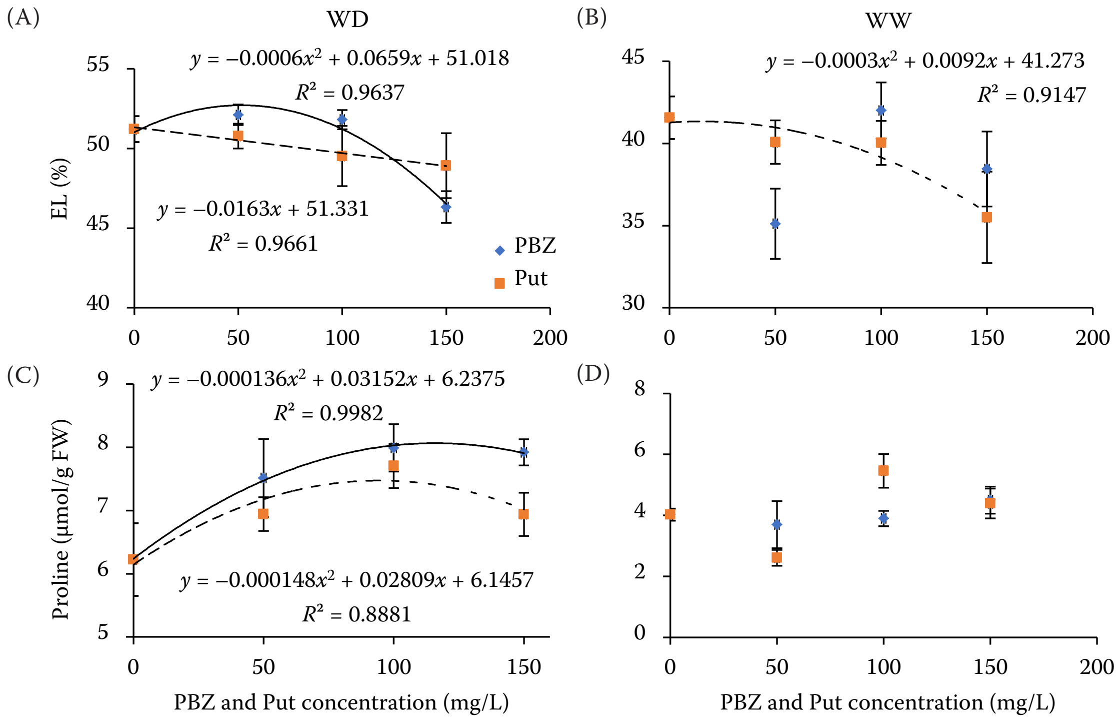

Figure 1. Changing trends of electrolyte leakage (EL) (A, B) and proline content of leaves (C, D) *versus* paclobutrazol (PBZ) and putrescine (Put) levels in water deficit (WD) and well-watered (WW) conditions. Symbols indicate data averaged over the two years of the experiment. Vertical bars represent ± standard error of the mean (n = 6); FW – fresh weight

compared to the control, while in WW conditions, PBZ had no effect on SPAD (Figures 2A, B). All levels of the Put in both WD and WW conditions significantly increased SPAD (Figures 2A, B). In WD conditions, the chlorophyll *a* and *b* contents increased significantly using all levels of PBZ as well Put rates of 100 and/or 150 mg/L had a significant effect on photosynthetic pigments (Figures 2C, E). In WW conditions, PBZ had no effect on chlorophyll pigments, whereas the chlorophyll *b* content was significantly increased by Put application at 50 and 100 mg/L rates compared with control (Figures 2D, F). PBZ has been reported to retard chlorophyll degradation or accelerate its biosynthesis (Berova and Zlatev 2000), and it has been reported that polyamines are involved in preventing the breakdown of chlorophyll-protein complexes (Besford et al. 1993). In a similar study, Kumar et al. (2012) reported that all PBZ treatments ranging from 0–120 mg/L increased chlorophyll contents compared with control, and the PBZ level of 100 mg/L was the most effective treatment. Another report by Mahdavian et al. (2020) showed an increase in photosynthetic pigments and SPAD of leaves following the Put application in different soil water conditions.

Seed yield. The seed yield means over the two years in WD condition decreased by 24% compared to WW condition (Table 3). In WD conditions, the seed yield was significantly increased by the application of all doses of PBZ and Put compared with the control (Figure 2G). Regression modeling showed that the seed yield response to PBZ and Put levels was quadratic so that the optimal levels of PBZ and Put to produce maximum seed yield in WD conditions were estimated at about 162 and 90 mg/L, respectively, for which the seed yields of 1 610 and 1 205 kg/ha can be obtained respectively (Figure 2G). In WW conditions, PBZ had no positive effect on seed yield, while a significant increase was shown by applying 100 mg/L of Put compared with the control (Figure 2H).

The improving effect of PBZ on mung bean seed yield under stress conditions can be attributed to the positive effects of this growth regulator on the production of pods and seeds as well as promoting effect on the accumulation of proline and the biosynthesis of leaf chlorophyll. In addition, it has been reported that PBZ may play a role in altering the distribution of assimilates to the reproductive parts of the plant (Senoo and Isoda 2003). Since the changing trend of seed yield against PBZ levels in stress condition was similar to that of leaf chlorophyll and proline content, the way in which the plant

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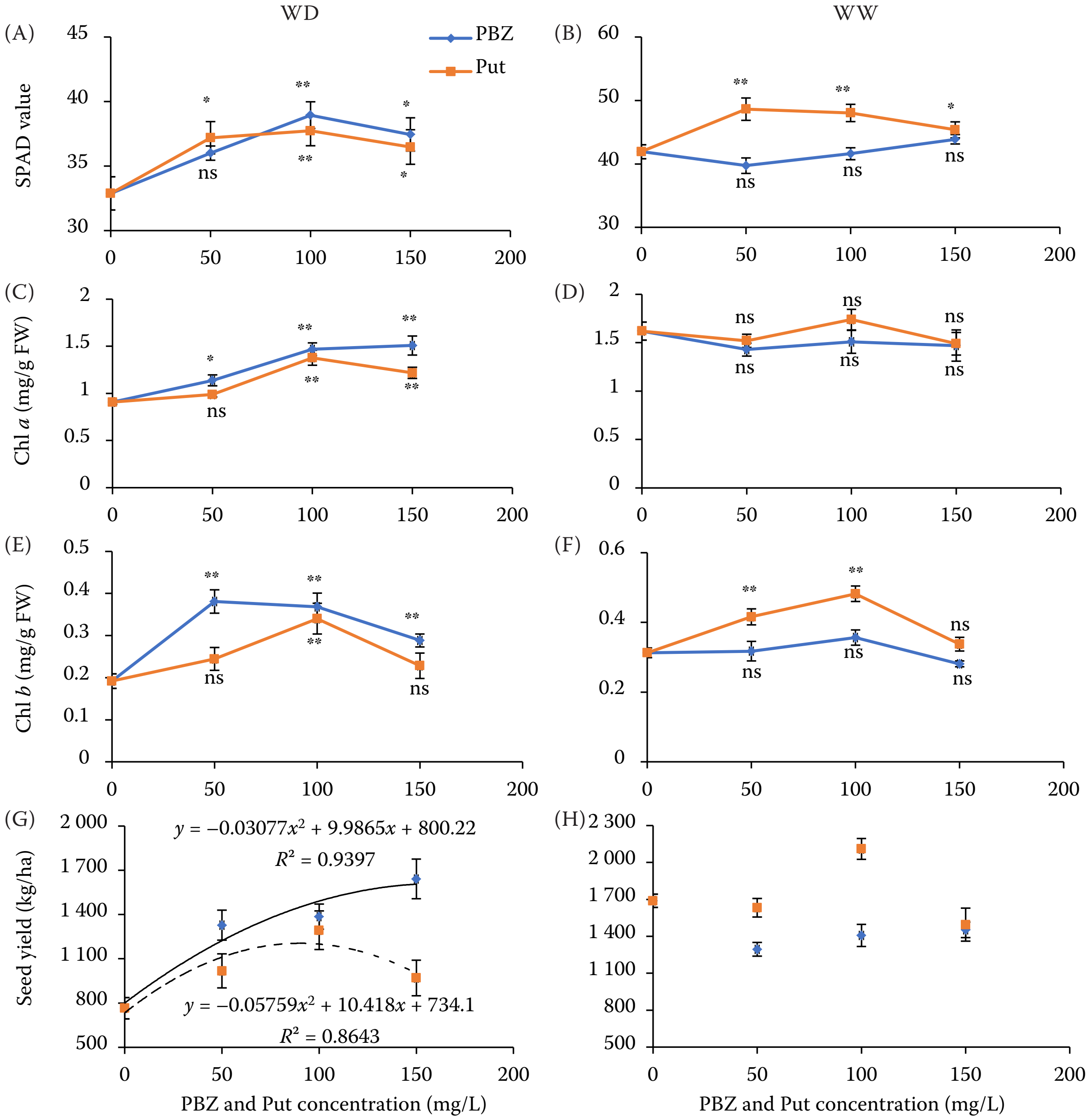

Figure 2. Changing trends of SPAD (soil-plant analysis development) (A, B), chl *a* (C, D), chl *b* (E, F) and seed yield (G, H) *versus* paclobutrazol (PBZ) and putrescine (Put) levels in water deficit (WD) and well-watered (WW) conditions. Symbols indicate data averaged over the two years of the experiment. Vertical bars represent ± standard error of the mean (n = 6). ns – not significant; $^*P < 0.05$; $^{**}P < 0.01$

responded to PBZ in terms of seed yield can be linked to chlorophyll content and the consequently improved photosynthesis as well as the protective effect of proline. In accordance with our results, Plaza-Wüthrich et al. (2016) demonstrated that in drought stress conditions, the yield of the tef plant significantly increased through PBZ application, and they declared that the increase in photosynthetic pigments due to PBZ, which is involved in photosynthesis improvement, had a positive effect on the plant production.

The seed yield increase by Put application can be ascribed to pod formation enhancement, the elevation of proline accumulation, improving membrane stability and increasing chlorophyll biosynthesis. In line with these results, Ebeed et al. (2017) declared that exogenously applied polyamines improved drought tolerance of wheat through increasing endogenous polyamines and osmolytes such as proline, which ultimately increased the seed yield under drought stress.

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In conclusion, water deficit stress resulted in increased damage to the cell membrane, reducing chlorophyll content of leaves and a consequent decrease in seed yield of mung bean. Application of PBZ and Put in stress conditions was able to alleviate the detrimental effects of drought stress by increasing proline and leaf chlorophyll content and improving cell membrane stability, thereby increasing the crop yield. Regression analysis in stress conditions revealed that the optimal rates of exogenous PBZ and Put to maximise seed yield were found to be at 150 mg/L (or higher) and 90 mg/L, respectively. In non-stressed condition, the PBZ impact on crop traits was not consistent, while supplying the plant with Put had promoting effects. In general, results indicated that the crop response to growth regulators and their concentrations depends on the status of soil water content.

REFERENCES

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

Bangar P., Chaudhury A., Tiwari B., Kumar S., Kumari R., Bhat K.V. (2019): Morphophysiological and biochemical response of mungbean [*Vigna radiata* (L.) Wilczek] varieties at different developmental stages under drought stress. Turkish Journal of Biology, 43: 58–69.

Bates L.S., Waldren R.P., Teare I.D. (1973): Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205–207.

Berova M., Zlatev Z. (2000): Physiological response and yield of paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.). Plant Growth Regulation, 30: 117–123.

Besford R.T., Richardson C.M., Campos J.L., Tiburcio A.F. (1993): Effect of polyamines on stabilization of molecular complexes in thylakoid membranes of osmotically stressed oat leaves. Planta, 189: 201–206.

Bodner G., Nakhforoosh A., Kaul H.-P. (2015): Management of crop water under drought: a review. Agronomy for Sustainable Development, 35: 401–442.

Chand G., Nandwal A.S., Kumar N., Devi S., Khajuria S. (2018): Yield and physiological responses of mungbean *Vigna radita* (L.) Wilczek genotypes to high temperature at reproductive stage. Legume Research, 41: 557–562.

Chen D.D., Shao Q.S., Yin L.H., Younis A., Zheng B.S. (2019): Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. Frontiers in Plant Science, 9: 1945.

Cohen I., Netzer Y., Sthein I., Gilichinsky M., Tel-Or E. (2019): Plant growth regulators improve drought tolerance, reduce growth and evapotranspiration in deficit irrigated *Zoysia japonica* under field conditions. Plant Growth Regulation, 88: 9–17.

Ebeed H.T., Hassan N.M., Aljarani A.M. (2017): Exogenous applications of Polyamines modulate drought responses in wheat through osmolytes accumulation, increasing free polyamine levels and regulation of polyamine biosynthetic genes. Plant Physiology and Biochemistry, 118: 438–448.

Kumar S., Ghatty S., Satyanarayana J., Guha A., Chaitanya B.S.K., Reddy A.R. (2012): Paclobutrazol treatment as a potential strategy for higher seed and oil yield in field-grown *Camelina sativa* L. Crantz. BMC Research Notes, 5: 137.

Lichthenthaler H.K. (1987): Chlorophylls and carotenoids – pigments of photosynthetic biomembranes. Methods in Enzymology, 148: 350–382.

Lutts S., Kinet J.M., Bouharmont J. (1996): NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Annals of Botany, 78: 389–398.

Mahdavian M., Sarikhani H., Hadadinejad M., Dehestani A. (2020): Putrescine effect on physiological, morphological, and biochemical traits of carrizo citrange and volkameriana rootstocks under flooding stress. International Journal of Fruit Science, 20: 164–177.

Nahar K., Hasanuzzaman M., Rahman A., Alam Md.M., Mahmud J.-A., Suzuki T., Fujita M. (2016): Polyamines confer salt tolerance in mung bean (*Vigna radiata* L.) by reducing sodium uptake, improving nutrient homeostasis, antioxidant defense, and methylglyoxal detoxification systems. Frontiers in Plant Science, 7: 1104.

Plaza-Wüthrich S., Blösch R., Rindisbacher A., Cannarozzi G., Tadele Z. (2016): Gibberellin deficiency confers both lodging and drought tolerance in small cereals. Frontiers in Plant Science, 7: 643.

Raina S.K., Govindasamy V., Kumar M., Singh A.K., Rane J., Minhas P.S. (2016): Genetic variation in physiological responses of mungbeans (*Vigna radiata* (L.) Wilczek) to drought. Acta Physiologiae Plantarum, 38: 263.

Senoo S., Isoda A. (2003): Effects of paclobutrazol on dry matter distribution and yield in peanut. Plant Production Science, 6: 90–94.

Singh P., Basu S., Kumar G. (2018): Polyamines metabolism: a way ahead for abiotic stress tolerance in crop plants. In: Wani S.H. (ed.): Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants. San Diego, Academic Press, 39–55. ISBN: 978-0-12-813066-7

Soumya P.R., Kumar P., Pal M. (2017): Paclobutrazol: a novel plant growth regulator and multi-stress ameliorant. Indian Journal of Plant Physiology, 22: 267–278.

Tesfahun W. (2018): A review on: response of crops to paclobutrazol application. Cogent Food and Agriculture, 4: 1525169.

Yooyongwech S., Samphumphuang T., Tisarum R., Theerawitaya C., Cha-Um S. (2017): Water-deficit tolerance in sweet potato [*Ipomoea batatas* (L.) Lam.] by foliar application of paclobutrazol: role of soluble sugar and free proline. Frontiers in Plant Science, 8: 1400.

Zhu Y.S., Sun S., FitzGerald R. (2018): Mung bean proteins and peptides: nutritional, functional and bioactive properties. Food and Nutrition Research, 62: 1290.

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