Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

POTENTIAL ROLE OF PRE-SOWING SEED TREATMENT WITH GLYCINEBETAINE IN ANTIOXIDANTS MACHINERY, GROWTH AND BIOCHEMICAL ATTRIBUTES OF FLAX (*Linum usitatissimum* L.) UNDER WATER DEFICIT CONDITIONS

ZYMA BASHARAT *
Department of Botany, University of Agriculture, Faisalabad-Pakistan.
*Corresponding Author Email: zymabasharat93@gmail.com

MUHAMMAD SHAHBAZ
Department of Botany, University of Agriculture, Faisalabad-Pakistan.

EJAZ AHMAD WARAICH
Department of Agronomy, University of Agriculture, Faisalabad-Pakistan.

Abstract

Water scarcity has a profound impact on crop yield and productivity, affecting numerous crop plants globally. Flax, a versatile crop with high nutritional value, is an essential oil seed and fiber crop. To combat drought stress, plants employ various mechanisms, and glycinebetaine (GB) plays a vital role as a compatible solute, mitigating stress through osmotic adjustment and membrane protection. A pot experiment was conducted at the Old Botanical Garden, University of Agriculture, Faisalabad, to investigate GB's role in flax under water stress. Two flax varieties, Roshini and Chandini, were subjected to two drought stress levels (controlled and 50% FC) and four GB levels (non-soaked, water-soaked, 10, and 20 mM) via pre-soaking seed treatment for 8 hours. Twenty seeds of each variety were sown in plastic pots, and a CRD design with four replicates was used to arrange the experiment. The results showed that drought stress significantly hampered morphological attributes, including shoot fresh weight, shoot length, and reduced chlorophyll pigments (chlorophyll *a*, *b*, total chlorophyll, and carotenoids). However, drought stress increased reactive oxygen species (ROS) like MDA and H_2O_2, triggering a response in enzymatic antioxidants (CAT, SOD, and POD). Exogenously applied GB as osmolytes enhanced shoot fresh weight, shoot length, and chlorophyll pigments (except chl *a*/*b* ratio), increased enzymatic antioxidants, and decreased ROS. Yield attributes were significantly improved, the Chandini variety performed better under stress conditions and 20 mM GB showing the best results in pre-soaking seed treatment.

Keywords: Drought Stress, Flax, Glycinebetaine, ROS, Photosynthetic Pigments, Antioxidants, Yield.

1. INTRODUCTION

Crop production is being threatened by escalating the risk of drought worldwide, a consequence of climate change. Plant physiology is being altered by drought stress, resulting in changes to mineral content, chloroplast pigments and production of specialized compounds [1]. Plants have evolved remarkable resilience to environmental stresses, including both physical and biological factors. Plants can tailor their physiology and development to meet these challenges. This adaptive capacity empowers plants to flourish in diverse environments and respond dynamically to changing conditions [2].

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

The phytosphere offers a boundless source of medicinal herbs, employed in both modern medicine and traditional healing practices to cure countless diseases. In the last three decades, researchers have devised groundbreaking approaches to evaluate the safety and therapeutic potential of these plants [3].

Therapeutic plants are great source of diverse bioactive molecules and ingredients, which contribute to their curative abilities [4]. The Flax plant is globally most prominent and one of the oldest cultivated crops with a rich history of widespread, harvesting and spanning thousands of years, from the advent of civilization to the recent days.

Flax seeds superb for intestine and colon, improved the immunity, best for respiratory, skin, heart and brain health because flax is the great source of alpha linolenic acid. Flax seeds also used for slimming body purpose because it has great abilities for fat loss and fat burning. Grounded flax also used for diabetic patients to lower down their blood glucose level [5]

Glycinebetaine (GB) is a versatile, quaternary ammonium compound that accumulates in various plants thriving in water-scarce or high-salinity environments. By behaving as a non-toxic, cell-friendly solute, GB effectively alleviates osmotic and solute potential within stressed cells, safeguarding them against the deleterious impacts of drought stress [6]. GB stands apart for its exceptional ability to safeguard membrane integrity, enzymes, and critical complexes like PSII and Rubisco, unmatched in efficacy by other compatible solutes [7].

Cells counter stress by maintaining homeostasis through precise osmotic regulation. In plants, this entails synthesizing electrically neutral, low-molecular-weight [8]. Osmolytes that are soluble and effective at physiological pH, thereby accumulating to raise cytoplasmic osmotic pressure and safeguard critical organelle membrane stability [9]. Importantly, compatible solutes accumulated in response to abiotic stress do not perturb the plant's central metabolic pathways, thereby ensuring the continuity of core functions, even at exceptionally higher concentrations [10].

This investigation seeks to elucidate the potential role of glycinebetaine to augment flax production in environments characterized by water scarcity. Despite its well-established benefits to crop production, the physiological and biochemical responses of flax to glycinebetaine under water deficit conditions remain poorly understood. This study aims to investigate the effects of glycinebetaine application on flax morphology, physiology, and biochemistry during water stress, with the objective of optimizing its use to enhance production and yield in both drought and well-watered conditions. Furthermore, the research will examine the impact of glycinebetaine on various flax attributes, determine the optimal concentrations required to mitigate drought stress, and assess the efficacy of foliar glycinebetaine application in alleviating drought stress in flax. The findings of this study will inform evidence-based strategies for improving flax production in water-limited environments.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

2. MATERIALS AND METHODS

A pot experiment was conducted in the Old Botanical Garden at the University of Agriculture in Faisalabad, Pakistan, to investigate the effects of pre-sowing seed treatment with glycinebetaine (GB) on two flax varieties under water-scarce conditions.

The experiment followed a completely randomized design and was carried out under specific climatic conditions: relative humidity of 72.62%, temperature of 17.38°C, photoperiod of 6.8 hours, and rainfall of 29.53 mm. Each pot contained 7 kg of clay-loam soil with a 2:1 soil to humus ratio, comprising 60% clay, 25% sand, and 15% silt, with a pH of 6.3.

The two flax varieties used in the experiment were Roshini and Chandini, which were obtained from the Ayub Agricultural Research Institute, Faisalabad. Seeds of these varieties were soaked in GB solution for 8 hours at four levels: non-soaked, water-soaked, 10mM, and 20mM. The GB levels were optimized based on prior research showing positive impacts on various crops. Twenty seeds were sown in each pot. Thinning was performed to maintain six plants per pot. Two drought stress levels were maintained: well-watered and 50% field capacity after 60 days of sowing. Morphological data were collected, and harvesting was done at crop maturity.

2.1 Morphological attributes

Two plants from each pot were carefully removed and rinsed with tap water. Shoot length was measured using a meter rod, and fresh weight was recorded using a weighing machine.

2.2 Chlorophyll estimation

Chlorophyll pigments in flax leaves were estimated by extracting 100 mg fresh leaves of flax in 5 ml of 80% acetone, incubating overnight, and measuring absorption at 645, 663, and 480 nm using a spectrophotometer [11].

2.3 Quantification and calculation of enzymatic antioxidants

For enzymatic antioxidant evaluation, 0.25 g of fresh flax leaves were extracted in 5 ml of phosphate buffer (50mM, pH 7.8). The mixture was centrifuged at 12,000 rpm for 15 minutes at 4°C. The resulting supernatant was collected for enzyme quantification, while the remaining material was discarded.

2.3.1 Superoxide dismutase (SOD)

Superoxide dismutase activity was measured using a reaction mixture containing 400 µl water, 100 µl methionine, 250 µl phosphate buffer, 100 µl triton, 50 µl NBT, 50 µl enzyme extract, and 50 µl riboflavin. After 15 minutes incubation under a lamp, absorbance was measured at 560nm using a spectrophotometer. SOD activity was determined by the enzyme concentration that inhibited 50% NBT photo reduction, with a control sample lacking enzyme extract [12].

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

2.3.2 Catalase (CAT)

The reaction mixture consisted of 1.9 ml potassium phosphate buffer, 0.1 ml sample extract, and 1 ml distilled H_2O_2. The sample was analyzed at 240 nm using a spectrophotometer, with readings taken every 20 seconds for 2 minutes. The decrease in optical density per minute was used to calculate catalase enzyme activity [13].

2.3.3 Peroxidase (POD)

The reaction mixture consisted of 100 µl H_2O_2, 0.1 ml guaiacol, 50 µl sample extract, and 750 µl phosphate buffer. Absorbance was measured at 470 nm using a spectrophotometer, with readings taken with an interval of 20 seconds for 2 minutes [13].

2.4 Melondialdehyde (MDA) concentration

A 0.25 g fresh leaf sample was extracted in 3ml TCA solution and centrifuged at 12,000 rpm for 15 minutes. Then, 3 ml 0.5% TBA in 20% TCA was added, and the samples were heated at 95°C for 50 minutes. After cooling, the samples were centrifuged again at 10,000 rpm for 10 minutes. Finally, absorbance was measured at 532 nm and 600 nm [14]

2.5 Hydrogen peroxide (H_2O_2)

A 0.5 g fresh leaf sample was crushed in 5 ml of 0.1% tri-chloroacetic acid (TCA) and then centrifuged at 12,000 rpm for 15 minutes. The resulting extract (0.5 ml) was mixed with 0.5 ml potassium phosphate buffer (pH 7.0) and 1 ml KI in a test tube. After vortexing, the optical density was measured at 390 nm at spectrophotometer [15].

2.6 Yield related attributes

At plant maturity, various yield-related parameters of flax were evaluated, including the number of seed pods per plant and the weight of seeds per plant, to assess the crop's productivity.

2.7 Statistical analysis

Three factor factorial completely randomized design (CRD) was used to arrange the both experiments. CO- STAT computer software by [16] method was used to analyze the data with 3-way ANOVA.

3. RESULTS

Moisture shortage significantly reduced shoot fresh weight (SFW) in both Chandini and Roshini flax varieties ($P \leq 0.001$). However, pre-sowing seed treatment with glycinebetaine (GB) significantly mitigated this effect, increasing SFW under water stress conditions in both varieties ($P \leq 0.001$). Interestingly, 10mM GB was more effective in Roshini, while in Chandini, both 10 and 20mM GB levels performed equally well under both stressed and non-stressed conditions. The interaction between variety, drought, and GB was found to be non-significant (Table 1; Fig. 1).

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

Drought stress, imposed by 50% field capacity (FC), significantly reduced shoot length (P ≤ 0.001) in flax. However, varietal differences were observed, with Chandini exhibiting longer shoots compared to Roshini (P ≤ 0.001). The application of glycinebetaine (GB) significantly increased shoot length in both varieties (P ≤ 0.001). Specifically, 10mM GB was more effective in Roshini, while 20mM GB performed better in Chandini, under both controlled and stress conditions (Table 2; Fig. 1).

Water scarcitic condition markedly ($P \leq 0.001$) lessened the chlorophyll *a* (chl *a*) content in both flax varieties (Roshini and Chandini). Varietal difference was observed non-significant. Pre-sowing seed treatment with GB showed uniform behavior in both flax varieties. No overall interaction was observed between V×D×GB (Table 1; Fig. 1)

Hydrological drought substantially ($P \leq 0.001$) diminished the chlorophyll *b* ratio in both flax varieties (Roshini and Chandini). Varietal difference was non-significant. Glycinebetaine as seed priming marginally ($P \leq 0.05$) increased the chlorophyll *b* ratio in both flax varieties under water shortage circumstances (Table 1; Fig. 1)

Water scarce situation noticeably ($P \leq 0.001$) augmented the chl *a*/*b* ratio in both flax varieties. Varietal variation was not noticeable. GB seeds priming prior to planting slightly ($P \leq 0.05$) increased the chl *a*/*b* ratio in Chandini under controlled condition but chl *a*/*b* ratio decreased when GB was applied in Roshini under non-stress situation. Overall, 20mM GB level considerably ($P \leq 0.01$) performed better in Chandini under both conditions (Table 1; Fig. 1)

Imposition of 50% field capacity (FC) profoundly ($P \leq 0.001$) experienced reduction in total chlorophyll in both flax varieties (Roshini and Chandini). Non-significant varietal difference was observed. Under drought stress situation glycinebetaine as pre-sowing seed treatment slightly ($P \leq 0.05$) enhanced the total chlorophyll content in both flax varieties. Overall, 20mM in Roshini and 10mM GB performed better in Chandini under stress situations (Table 1; Fig. 2)

Water stress induction abridged the carotenoids contents significantly ($P \leq 0.001$) in both varieties. A significant ($P \leq 0.001$) varietal difference was observed as Chandini showed higher value for carotenoids as compared to Roshini. Under water scarce condition 20mM GB treated seed performed better in both flax varieties. Maximum values for carotenoids were observed in Chandini with 20mM glycinebetaine application under non-stress situation (Table 1; Fig. 2)

Drought stress considerably ($P \leq 0.001$) increased the action of superoxide dismutase (SOD) in both flax varieties. Varietal difference ($P \leq 0.001$) was also significant as SOD activity was observed maximum in Chandini as compared to Roshini. GB application as pre sowing seed treatment for 8 hours noticeably ($P \leq 0.05$) enhanced the SOD functioning in both varieties under water scarce situation. Overall, 20mM GB performed better in Chandini and 10mM level performed better in Roshini under controlled and stress conditions (Table 1; Fig. 2)

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

Water limitation (50% FC) profoundly ($P \leq 0.001$) increased the catalase activity (CAT) in both varieties of flax. Significant ($P \leq 0.01$) varietal difference was noticed as maximum catalase activity was recorded in Roshini as compared to Chandini. Priming seeds with GB before sowing for 8 hours amplified the activity of CAT profoundly ($P \leq 0.001$). Of various level of glycinebetaine 20mM level performed best in both flax varieties under drought conditions. The interaction between V×D×GB was noticed not significant (Table 1; Fig. 2)

Water shortage expressively ($P \leq 0.001$) increased the peroxidase (POD) activity in both flax varieties. Glycinebetaine as seed priming treatment profoundly ($P \leq 0.001$) amplified POD activity under stress and controlled conditions. Varietal difference was also significant ($P \leq 0.001$) as Chandini showed more SOD activity as compared to Roshini. Overall, 20mM GB level performed better in both flax traits under all situations (Table 1; Fig. 2)

Trend in malondialdehyde (MDA) was not significantly differing in both flax varieties. Drought stress significantly ($P \leq 0.001$) escalated the MDA content in both flax varieties. Exogenously applied GB profoundly ($P \leq 0.001$) reduced the MDA content in both flax varieties. Overall, 20mM GB treated seeds showed less concentration of MDA content in Chandini while in Roshini 10mM GB level lower down the maximum MDA content. The combined interaction between V×D×GB was also highly significant ($P \leq 0.001$) as Chandini performed better in drought situation with exogenous application of GB (Table 1; Fig. 3)

Hydrogen peroxide (H_2O_2) concentration increased slightly ($P \leq 0.05$) in Chandini under water shortage conditions. A slight ($P \leq 0.05$) varietal variation was noted as Roshini showed reduced H_2O_2 concentrations as compared to Chandini. Exogenously applied glycinebetaine slightly ($P \leq 0.05$) reduced the hydrogen per oxide content. Roshini showed lower H_2O_2 concentration in moisture stress situations. The interaction between V×D×GB was also observed non-significant. (Table 1; Fig. 3)

Drought stress situations remarkably ($P \leq 0.001$) reduced the number of pods/plant on both flax varieties. Varietal difference was slightly ($P \leq 0.05$) variable as Roshini showed more number of pods. Seed priming with different levels of glycinebetaine considerably ($P \leq 0.001$) increased the pods number on both varieties. Of various level of GB 20mM level performed better in Chandini in controlled and stress conditions but in Roshini 10mM performed slightly better in stress and 20mM showed better result in control situations (Table 1; Fig. 3)

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

Table 1: Mean squares from analysis of variance of data for growth attributes, chlorophyll pigments, SOD, CAT, POD, Total soluble protein, MDA, H_2O_2 and Number of pods/ plants of flax (*Linum usitatissimum* L.) when seed were treated as pre-sowing treatment with glycinebetaine for 8 hours under drought stress conditions

SOV	df	SFW	SL	Chl. *a*	Chl. *b*	Total chlorophyll	Chl *a*/ Chl *b*	Carotenoi-ds
Varieties (V)	1	0.107 ns	66.626***	0.098 ns	0.098 ns	0.627 ns	0.238 ns	0.015***
Drought (D)	1	71.396***	1795.640***	2.118***	3.973***	13.053***	3.677***	0.016***
Glycinebetaine (GB)	3	3.935***	159.114***	0.030 ns	0.292 ns	0.707 ns	0.797*	0.004***
V × D	1	0.200 ns	72.675***	0.164 ns	0.168 ns	0.029 ns	0.744 ns	3.0603e-4**
V × GB	3	0.069 ns	25.761***	0.069 ns	0.440*	0.378 ns	0.998**	6.3895e-4**
D × GB	3	0.132 ns	7.125*	0.110 ns	0.225 ns	0.793*	0.709*	4.3985e-4*
V × D × GB	3	0.040 ns	24.731***	0.130 ns	0.257 ns	0.618 ns	0.277 ns	0.001***
Error	48	0.068	2.278	0.078	0.105	0.266	0.213	1.399
SOV	**df**	**SOD**	**CAT**	**POD**	**Malondial-dehyde**	**H_2O_2**	**Number of pods/ plants**	**Total seed weight/ plant**
Varieties (V)	1	660.142***	3.1876e-5**	0.004***	0.867 ns	3.0096e-5*	5.640*	0.271**
Drought (D)	1	666.284***	3.7609e-4***	0.0012***	17.228***	3.098e-5*	289***	10.280***
Glycinebetaine (GB)	3	100.821*	8.5365e5***	3.421e-4***	5.903***	2.0256e-5*	20.890***	0.681***
V × D	1	270.738**	9.0817e-6 ns	7.3489e-6 ns	0.506 ns	2.9791e-6 ns	1.233e-32 ns	0.121*
V × GB	3	62.962 ns	1.93322e-6 ns	3.1252e-5 ns	0.773*	2.0214e-5*	0.432 ns	0.0178 ns
D × GB	3	6.435 ns	3.4898e-5***	8.7766e-5*	2.952***	1.8282e-5*	17.125***	0.368***
V × D × GB	3	3.726 ns	4.7789e-6 ns	6.2923e-5 ns	1.691***	1.6286e-5 ns	1.625 ns	0.022 ns
Error	48	31.763	2.953	2.4492e-5	0.261	5.696e-4	1.361	0.024

, * and ******* significant at 0.05, 0.01 and 0.001 levels, respectively ns = non-significant

SFW= Shoot fresh weight, SDW= Shoot dry weight; SL= Shoot length Chl= chlorophyll; SOD = Superoxide dismutase; CAT = Catalase; POD = Peroxidase; H_2O_2 = Hydrogen per oxide

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

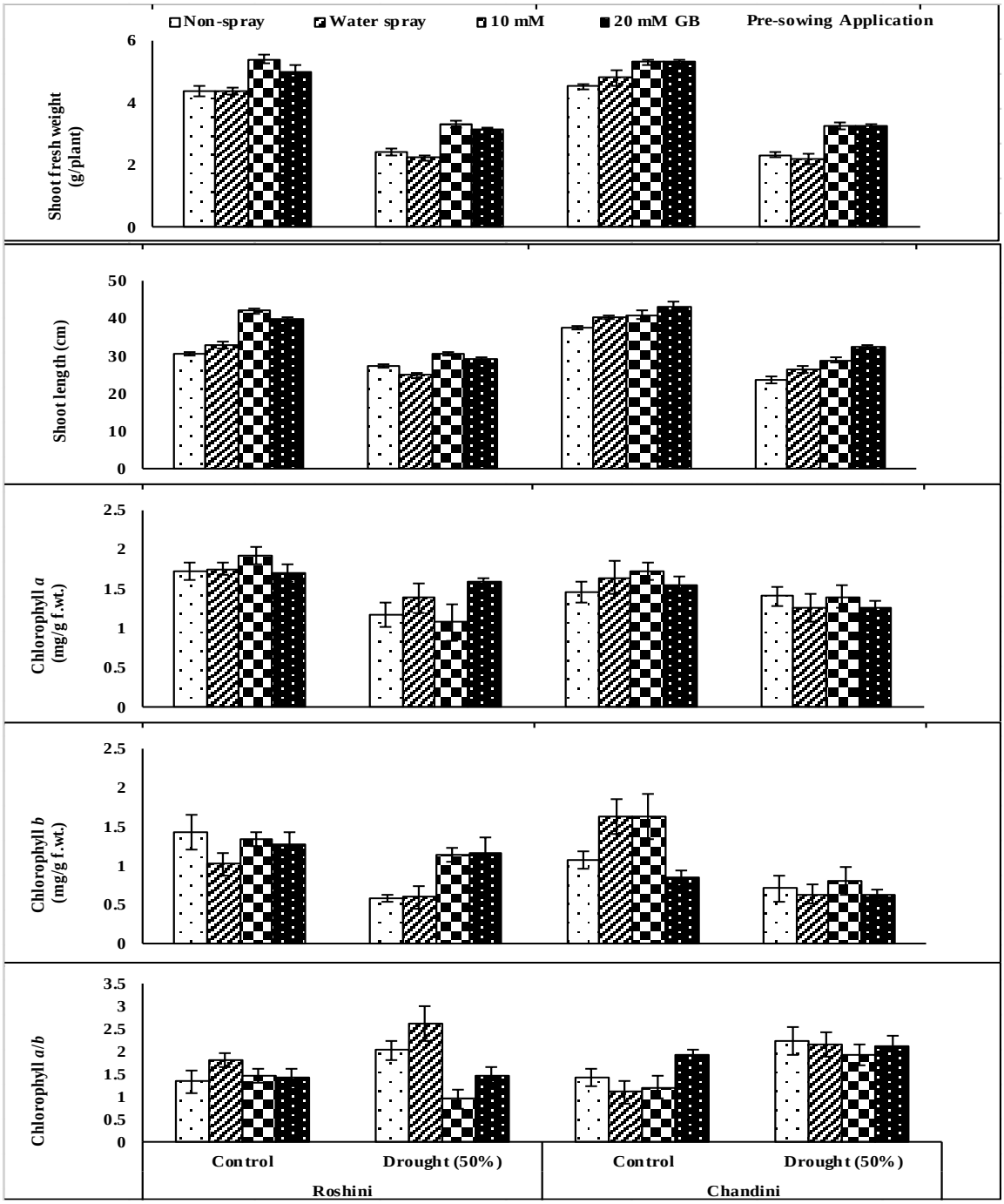

Fig 1: Growth attributes and chlorophyll pigments of flax plant (*Linum usitatissimum* L.) varieties (Roshini and Chandini) when pretreated seeds were sown under control and drought stress conditions

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

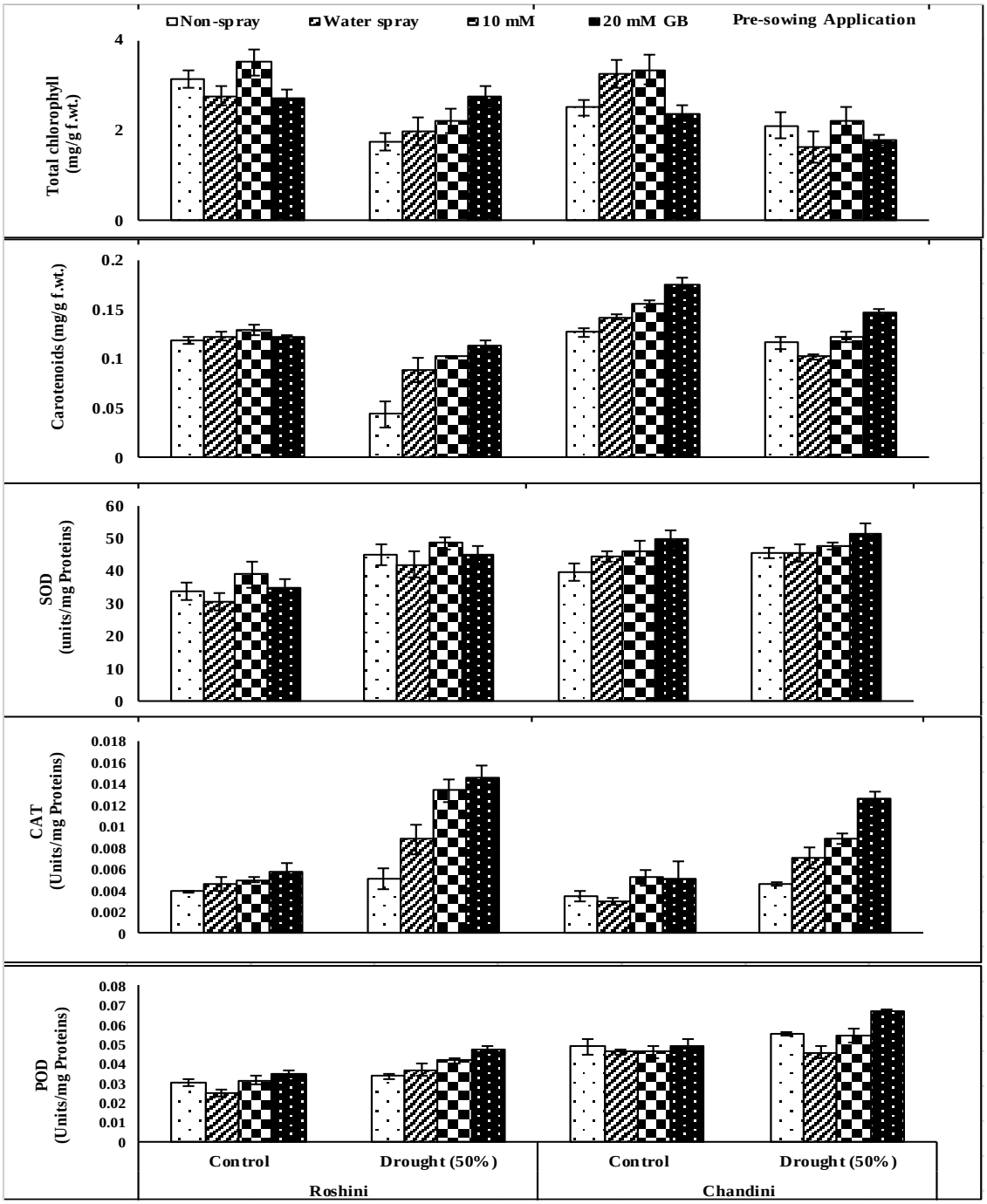

Fig 2: Chlorophyll pigments and enzymatic antioxidants of flax plant (*Linum usitatissimum* L.) varieties (Roshini and Chandini) when pretreated seeds were sown under control and drought stress conditions

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

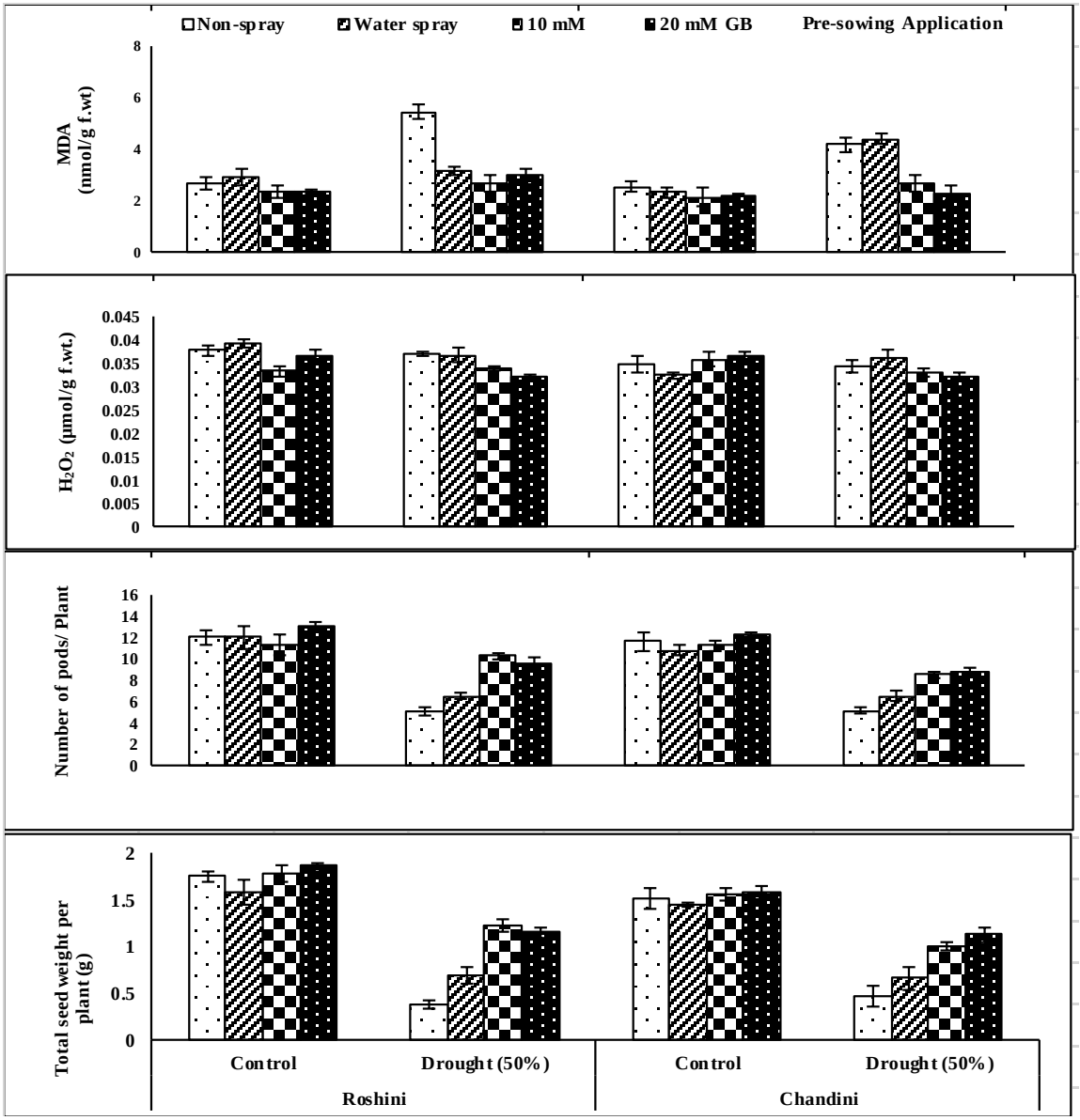

Fig 3: Malondialdehyde, hydrogen per oxide and yield attributes of flax plant (*Linum usitatissimum* L.) varieties (Roshini and Chandini) when pretreated seeds were sown under control and drought stress conditions

Moisture stress significantly ($P \leq 0.001$) dropped the total seed weight/plant in both flax varieties. Varietal difference was significantly ($P \leq 0.01$) as Roshini showed higher seed weight as compared to Chandini. Pre-soaking seed treatment with different level of GB profoundly ($P \leq 0.001$) increased the total seed weight in both flax varieties. Of various level of GB 20 mM level performed better in Chandini in controlled and stress situation but in Roshini 20 mM level performed better in controlled and 10 mM level performed best in stress condition (Table 1; Fig. 3).

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

4. DISCUSSION

Drought or limited water supply had a profound influence on the morphology and productivity of flax plants in our recent findings, leading to substantial drops in shoot fresh weight, shoot dry weight, and flax shoot length. The water shortage severely impaired cellular processes, resulting in decreased cell growth and enlargement [17] reduced turgidity, and diminished water holding capacity. These effects ultimately compromised the plants' ability to maintain optimal performance and vigor [18].

Furthermore, the water stress triggered a significant increase in the production of reactive oxygen species (ROS), which can lead to oxidative stress and further exacerbate the negative impacts of water scarcity on flax plants [19] and perturbed the metabolic and enzymatic pathways as a result developmental characteristics severely disturbed similar observations was noted in flax traits [20]. Glycinebetaine seeds priming exerts antioxidant impacts by optimizing enzyme expression, scavenging ROS, and modulating redox balance, hence, mitigating cellular oxidation [21].

Pre- soaking seed treatment with glycinebetaine enhanced the plant height and fresh weight in both flax traits 10mM boosted up the all morphological attributes in Roshini. This act may be due to the GB's contribution to cellular homeostasis and water balance is likely the underlying cause of this increase, which may have a cascading effect that ultimately boosts photosynthetic efficiency [22]. Consistent effects were found in quinoa [23] and duck weed [24].

Recent observations showed a downturn in photosynthetic pigments due to drought stress. The reduction in photosynthetic pigments during drought stress may result from disrupted biosynthetic pathways or enhanced catabolic processes affecting chlorophyll pigments and associated molecules [25]. GB's protective effects on the photosynthetic apparatus, including Rubisco and membrane stabilization may enhance chlorophyll pigment concentrations by mitigating water deficit impacts. Moreover, GB's optimization of photosynthetic machinery efficiency likely plays a key role in this enhancement as in rice [24]. Similar results were noted in bread wheat [25]. In recent observations SOD, POD and CAT activity increased in stressed and controlled flax traits similar results were noted in rice [26] and cotton [27] and in as superoxide dismutase and catalase activity increased in stressed plants as SOD act as the first line of defense, and shows a vital role in safeguarding cells against the harmful effects of reactive oxygen species. Through its metalloenzyme activity, SOD efficiently converts superoxide anions (O_2.-) into harmless oxygen (O_2) and hydrogen peroxide (H_2O_2), maintaining cellular homeostasis and preventing oxidative damage [28].

Reactive Oxygen Species (ROS)-induced disruptions to cell equilibrium are counteracted by the synergistic behavior of catalase (CAT), Superoxide Dismutase (SOD), and Peroxidase (POD) enzymes [29]. However, the ability of plants to withstand abiotic stresses is intricately linked to their capacity to modulate ROS levels through a balance of ROS generation and anti-oxidative scavenging mechanisms.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

Glycinebetaine application as seed priming increased the functioning of antioxidants enzymes activity in recent investigations which are related with previous observations as in soybean [30], oats [31] and rice [32]. Seeds priming with glycinebetaine boosts SOD, POD, and CAT activity by increasing endogenous GB levels and decreasing $O_2 \cdot -$ concentrations, H_2O_2 which enhances moisture stress tolerance by neutralizing harmful oxidative radicals [33]. Water stress provokes an accumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in flax plants, characteristic of oxidative injury.

This echoes earlier research by Mohamed and [34], who documented analogous augmentations in H_2O_2 and MDA in drought-stressed soybean plants, pointing to a conserved oxidative stress response in plants facing water deficit situations. Pre-soaking seeds treatment with glycinebetaine declined the MDA and H_2O_2 content in both flax traits. GB treatment substantially mitigated drought-induced stress, leading to enhanced membrane stability, as evidenced by reduced ion leakage and decreased concentrations of H_2O_2 and MDA in flax leaves. Water scarcity declined the yield and its components in both flax traits.

Reduced yield content depends on the total duration of drought its intensity and plant developmental stage. Rhizosphere water scarcity induces a decline in leaf turgor pressure [35] triggering stomatal closure to alleviate transpirational water losses. Reduced leaf area and damaged chlorophyll pigments may synergistically contribute to yield loss under severe drought conditions, disrupting photosynthetic processes [36]. Similar findings were noted in maize [37].

Glycinebetaine as pre-soaking seed treatment increased yield attributes in our findings which correlates with previous observation in wheat [38] and maize [32]. Applying exogenous GB may improve assimilate transport and water relations, leading to enhanced grain yield in flax. This yield increase is attributed to the upregulation of endogenous GB, chlorophyll pigments and essential amino acid production, which collectively drove grain yield improvement [39]. Overall, the variety Chandini exhibited superior drought tolerance, and the application of 20 mM glycinebetaine through pre-soaking seed treatment emerged as the most effective strategy to enhance flax productivity under water stress conditions.

5. CONCLUSION

In conclusion, the application of glycinebetaine at various levels significantly enhanced the morphological, oxidative defense machinery, biochemical and yield parameters of two flax varieties, particularly by increasing the number of pods per plant under moisture stress conditions. Glycinebetaine also augmented the activity of enzymatic antioxidants, thereby mitigating the adverse effects of reactive oxygen species induced by moister stress. While glycinebetaine effectively alleviated the detrimental impacts of water scarcity on flax plants, further research is warranted to elucidate its precise role at the rooting and molecular levels.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

References

1) W.S. Wang, Y.J. Pan, X.Q. Zhao, D. Dwivedi, L.H. Zhu, J. Ali and F. By, "Drought-induced site-specific DNA methylation and its association with drought tolerance in rice (*Oryza sativa* L.)" J. Exp. Bot. 6: 1951-60. 2011

2) K.B. Bhavana, A.N. Babu, J. Lakshmi, B. Deepthi and G. Kavya, "A review on pharmacological properties and laboratory outcomes of flaxseed diet (*Linum usitatissimum*)" Int. J. Pharma Sci. Rev Res.70:100. 2021.

3) D. Grujicic, A. Markovic, J.T. Vukajlovic, M. Stankovic, M.R. Jakovljevic, A. Ciric, K. Djordjevic, N. Planojevic, M. Milutinovic and O. Milosevic-Djordjevic, "Genotoxic and cytotoxic properties of two medical plants Teucrium arduini L. and Teucrium flavum L. in relation to their polyphenolic contents" Mutat. Res. Genet. Toxicol. Environ. Mutagen. 852: e503168. 2020.

4) D. Douketis and James, "Pharmacologic properties of the new oral anticoagulants: a clinician-oriented review with a focus on perioperative management" Current Pharmaceutical Design. 16: 3436-3441. 2010

5) S.I. Allakhverdiev, H. Hayashi, Y. Nishiyama, A.G. Ivanov, J.A. Aliev, V.V. Klimov, N. Murata, R. Carpentier, "Glycinebetaine protects the D1/D2/Cytb559 complex of photosystem II against photo-induced and heat-induced inactivation" J Plant Physiol. 160: 41-9. 2003

6) X.L. Ma, Y.J. Wang, S.L. Xie, C. Wang and W. Wang, "Glycinebetaine application ameliorates negative effects of drought stress in tobacco". Russian J Plant Physiol. 54: 472-9. 2007

7) Z. Basharat and M. Shahbaz, "Morpho-physiological and biochemical responses of Flax (*Linum usitatissimum* L.) to exogenously applied glycinebetaine under water deficit conditions". Journal of Xi'an Shiyou University, Natural Science Edition. 20: 664-700. 2024

8) H.A. Alhaithloul, M.H. Soliman, K.L. Ameta, M.A. El-Esawi and A. Elkelish, "Changes in ecophysiology, osmolytes, and secondary metabolites of the medicinal plants of *Mentha piperita* and *Catharanthus roseus* subjected to drought and heat stress" Biomolecules. 10: 43. 2019

9) M.S. Raza, M.F. Saleem, M. Jamil and I.H. Khan, "Impact of foliar applied glycinebetaine on growth and physiology of wheat (*Triticum aestivum* L.) under drought conditions". Pak. J. Agric. Sci. 51: 327-34. 2014

10) D.I. Arnon, "Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*" Plant Physiol. 24: 1-1. 1949

11) C.N. Giannopolitis and S.K. Ries, "Superoxide dismutases: I. Occurrence in higher plants" Plant Physiol. 59: 309-314. 1977

12) B. Chance and A.C. Maehly. 1955. "Assay of catalases and peroxidases" Methods Enzymol. 2: 764-775. 1955

13) I. Carmak and W.J. Horst," Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*)". Physiol. Plant. 83: 463-468. 1991

14) V. Velikova, I. Yordanov and A. Edreva, "Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines" Plant Sci. 151: 59-66. 2000

15) G.W. Snedecor and W.G. Cochran, "Statistical Methods (7th ed.)". Iowa State University Press. 1980

16) S.M. Abass and H.I. Mohamed, "Alleviation of adverse effects of drought stress on common bean (*Phaseolus vulgaris* L.) by exogenous application of hydrogen peroxide". Bangladesh J. Bot. 41: 75-83. 2011

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

17) F. Zulfiqar, J. Chen, P.M. Finnegan, A. Younis, M. Nafees, W. Zorrig and K.B. Hamed, “Application of trehalose and salicylic acid mitigates drought stress in sweet basil and improves plant growth” Plants 10:1-14. 2021

18) A. Abid and M. Shahbaz, “Influence of exogenously applied trehalose on morphological and physiological traits of flax (*Linum usitatissimum*) plant under water shortage conditions” Pak. J. Agric. Sci.1: 59. 2022

19) N. Ahmed, Y. Zhang, K. Li, Y. Zhou, M. Zhang and Z. Li, “Exogenous application of glycinebetaine improved water use efficiency in winter wheat (*Triticum aestivum* L.) via modulating photosynthetic efficiency and antioxidative capacity under conventional and limited irrigation conditions” Crop J. 7: 635-650. 2019

20) M. Ashraf and M.R. Foolad, “Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59: 206-16. 2007

21) M.F. Maqsood, M. Shahbaz, M. Arfan, S.M. Basra and M. Ahmed, “Presowing seed treatment with glycine betaine confers NaCl tolerance in quinoa bymodulating some physiological processes and antioxidant machinery” Turk. J. Bot. 45: 1-4. 2021

22) F. Duman, A. Aksoy, Z. Aydin and R. Temizgul, “Effects of exogenous glycinebetaine and trehalose on cadmium accumulation and biological responses of an aquatic plant (*Lemna gibba* L.)” Water Air Soil Pollut. 217: 545-56. 2011

23) T.F. Bhuiyan, K.U. Ahamed, K. Nahar, J. Al Mahmud, M.B. Bhuyan, T.I. Anee, M. Fujita and M. Hasanuzzaman, “Mitigation of PEG-induced drought stress in rapeseed (*Brassica rapa* L.) by exogenous application of osmolytes”. Biocatal. Agric. Biotechnol. 20: e101197. 2019

24) S.U. Khan, A. Khan and S. Naveed, “Effect of exogenously applied kinetin and glycinebetaine on metabolic and yield attributes of rice (*Oryza sativa* L.) under drought stress” Emirates J. Food Agric. 27:75-82. 2015

25) N. Gupta and S. Thind, “Improving photosynthetic performance of bread wheat under field drought stress by foliar applied glycine betaine” J. Agric. Sci. Technol. 17:75-86. 2015

26) M. Farooq, S.M. Basra, A. Wahid, Z.A. Cheema, M.A. Cheema and A. Khaliq, “Physiological role of exogenously applied glycinebetaine to improve drought tolerance in fine grain aromatic rice (*Oryza sativa* L.)”. J. Agron. Crop Sci. 194: 325-33. 2008

27) P. Prajapat, D. Singh, S. Tripathi, K. Patel, H. Abbas and A. Patel, “Effect of water stress on antioxidative enzymes and glycinebetaine content in drought tolerant and drought susceptible cotton (*Gossypium hirsutum* L.) genotypes” Indian J. Biochem. Biophy. 55:198-204. 2018

28) B. Hisyam, M.A. Alam, N. Naimah and M.S. Jahan, “Roles of glycinebetaine on antioxidants and gene function in rice plants under water stress” Asian J. Plant Sci. 16:132-40. 2017

29) M.H. Siddiqui, M.Y. Al-Khaishany, M.A. Al-Qutami, M.H. Al-Whaibi, A. Grover, H. M. Ali, M.S. Al-Wahibi and N.A. Bukhari, “Response of different genotypes of faba bean plant to drought stress. Int. J. Mol. Sci. 16: 10214-27. 2015

30) H.I. Mohamed and S.A. Akladious, “Influence of garlic extract on enzymatic and non enzymatic antioxidants in soybean plants (*Glycine max*) grown under drought stress. Life Sci. J.11: 46-58. 2014

31) A. Shehzadi, N.A. Akram, A. Ali and M. Ashraf, “Exogenously applied glycinebetaine induced alteration in some key physio-biochemical attributes and plant anatomical features in water stressed oat (*Avena sativa* L.) plants”. J. Arid Land. 11: 292–305. 2019

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13756913

32) S. Shafiq, N.A. Akram, M. Ashraf, P. García-Caparrós, O. M. Ali and A.A. Latef, “Influence of glycine betaine (natural and synthetic) on growth, metabolism and yield production of drought-stressed maize (*Zea mays* L.) plants” Plants 10: 2540. 2021

33) S. Donnini, M. Dell'Orto and G. Zocchi, “Oxidative stress responses and root lignification induced by Fe deficiency conditions in pear and quince genotypes” Tree Physiol. 31:102-13. 2011

34) H.A. Alhaithloul, M.H. Soliman, K.L. Ameta, M.A. El-Esawi and A. Elkelish, “Changes in ecophysiology, osmolytes, and secondary metabolites of the medicinal plants of *Mentha piperita* and *Catharanthus roseus* subjected to drought and heat stress” Biomolecules. 10: 43. 2019

35) L. Borras and L.N. Vitantonio-Mazzini, “Maize reproductive development and kernel set under limited plant growth environments. J. Exp. Bot. 69: 3235-3243. 2018

36) R.N. Mutava, P.V.V. Prasad, M.R. Tuinstra, K.D. Kofoid and J. Yu, “Characterization of sorghum genotypes for traits related to drought tolerance” Field Crop Res. 123: 10-18. 2011

37) M. Luqman, M. Shahbaz and E.J. Waraich, “Effect of different concentrations of GR24 as seed priming treatment on physio-chemical and yield related attributes of maize (*Zea mays*) hybrids under drought stress. Pak. J. Bot.. 55: 1257-66. 2023

38) N. Ahmed, Y. Zhang, H. Yu, A. Gabar, Y. Zhou, Z. Li and M. Zhang, “Seed priming with Glycine betaine improve seed germination characteristics and antioxidant capacity of wheat (Triticum aestivum L.) seedlings under water-stress conditions”. Appl. Ecol. Environ. Res. 1: 4. 2019

39) H.R. Miri and M. Armin, “The interaction effect of drought and exogenous application of glycine betaine on corn (*Zea mays* L.). Euro. J. Exp. Biol. 3: 197-206. 2013