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Response of Antioxidant Systems in Common Bean (*Phaseolus vulgaris)* Plants to Water Stress

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ABSTRACT

Common bean (*Phaseolus vulgaris*) is an important food crop that is often subjected to water stress conditions, which can negatively impact growth and yield. Antioxidant systems play a crucial role in plant responses to abiotic stresses, including water stress. In this study the effects of the response of antioxidant systems in broad bean plants to water deficit stresses were investigated. The plants were grown for three weeks in plastic pots containing 3 kg of soil and subjected to different soil moisture levels: 2-fold field capacity (waterlogging), 50% and 75% field capacity (water deficit), and one field capacity as a control. The data obtained revealed that the free phenolic compound in shoots and roots of the tested plants was significantly increased as a result of imposed to the levels of water deficit (75% F.C & 50% F.C) as compared to absolute controls. But in roots the free phenolic compound was significantly decreased especially under waterlogging stress. The hydrogen peroxide concentration of bean plants was significantly increased as a result imposed to decrease the soil moisture content WD and WL stresses. There is a marked and progressive increasing in the production of proline in plants shoots as the soil moisture level decreased, therefore the highest accumulation of proline was recorded in plants subjected to the lowest level of soil moisture content DW (50% FC) of the tested plants compared to absolute controls. Specific activity (Umg^{-1} protein) of catalase, guaical peroxidase and ascorbate peroxidase were significantly raised under high water deficit (50% FC) and waterlogging (2 FC), whereas SOD specific activity in common bean leaves and roots was unchanged under the LWD and HWD stresses while it was significantly increased under WL stress compared to absolute control. Antioxidant compounds and their enzyme activity are important mechanisms enabling plants to cope with drought.

Keywords: Antioxidant system; Drought resistance*; Phaseolus vulgaris*; Water stress.

INTRODUCTION

Water stress, including both drought and waterlogging, is a major abiotic factor that can significantly impact the growth, development, and productivity of crop plants. Common bean (*Phaseolus vulgaris* L.) is an important legume crop cultivated worldwide for its nutritious seeds and versatile culinary uses. However, this crop is often subjected to water stress conditions, which can negatively affect its growth and yield. Waterlogging of soil has major effects on natural vegetation and agricultural crops, limiting the growth of many plants mainly in humid regions Drew (1991). Waterlogging, as an important water-related stress, has been found to injure plants by rapidly reducing the rate of photosynthesis and stomatal conductance (Terazawa *et al.*, 1992). Excess moisture and drought are two important factors responsible for low yield (Zhou, 1994). Waterlogging also reduces yield in temperate to sub- tropical environments, especially when the water table remains near the soil surface for a period following transplanting and establishment (Cannell and Belford, 1980; Yin *et al.*, 1980; Zhou, 1994). Waterlogging is the major physiological constraint during the seedling stage, and there is a significant correlation between seed yield and growth during this stage (Macdonald and Gordon, 1978; Yin and Zhang, 1982; Hu, 1983; Zhou, 1994). Oxygen deficiency inhibits the root respiration of plants which results in substantial reduction in energy status of root cells. Since oxygen is a terminal electron acceptor in aerobic respiration, in its absence, Kreb's cycle and electron-transport system are blocked. Therefore, plants under waterlogged conditions use alternate pathways for energy extraction. This alternate pathway uses fermentative metabolism to produce Adenosine triphosphate (ATP), thereby, resulting in enhanced accumulation of ethanol. Moreover, the activity of alcohol dehydrogenase (ADH) has also increased (Davies, 1980; Vartapetian, 1991). Water stress has been found to activate the production of the reactive oxygen species (ROS) which is toxic for plant cells (Miyake, 2010). The common adverse effect of water stress on crops is the reduction in fresh and dry biomass production as well as crop yield (Lisar *et al*., 2012). Drought stress causes closing stoma and reducing leaf area (Kumudini, 2010); consequently, decreasing photosynthetic pigments and activity. Exogenously applied plant growth regulators are being used increasingly to enhance tolerance of crops to environmental stresses. Paclobutrazol, an active member of the triazole family, was developed for use as a plant growth retardant (Brian, 2015). Almost all types of abiotic stress, including waterlogging, are accompanied by an increased production of reactive oxygen species (ROS) such as

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superoxide radical (O2). Since ROS is highly reactive and toxic, overproduction of ROS will damage plant cells irreversibly by oxidation of cellular components (Mittler, 2002). Malondialdehyde (MDA) is the final product of membrane lipid peroxidation. Therefore, MDA content is often used to assess the extent of oxidative damage of cell plasma membrane (An *et al*. 2011).

To scavenge damaging ROS, plants have evolved antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and non-enzymatic antioxidants like ascorbate and glutathione (Mittler 2002). Enhancement of antioxidant capacity and reduction of oxidative damage have been reported as a critical mechanism behind ALA-induced resistance to multiple stresses such as salt (Zhen *et al*., 2012), drought (Li *et al*, 2011), and heat (Zhang *et al*.,2012). Therefore, we hypothesized that ALA might mitigate the damaging effect of waterlogging by stimulating the antioxidant defense system as well. Waterlogging blocks the oxygen supply to the roots, which inhibits respiration and hence greatly reduces the energy status of cells (Dennis *et al*, 200). Therefore, one of the best characterized responses for plants under waterlogging is the metabolic switch from oxidative phosphorylation to anaerobic fermentation in roots to maintain ATP production (Shabala *et al*. 2011 and Juntawong *et al*. 2014). The fermentation pathways are not used under aerobic conditions, but quickly activated by low oxygen conditions, suggesting a positive role in waterlogging adaptation mechanism. Kennedy *et al*. (1992) showed that plants which had more active fermentation pathways were more waterlogging tolerant. Ethanol and lactic acid are two main fermentation pathways in plants during waterlogging, where alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) are two key enzymes, respectively (Shabala *et al*. 2011). Yang *et al*. (2014) have reported that ALA mitigates salinity stress-induced suppression of plant respiratory activity and improves salt tolerance. Whether ALA can improve anaerobic fermentation pathways and consequently contribute to maintaining metabolic activities in roots under waterlogging is not known. This study was conducted to evaluate physiological responses, including changes in the activity of key antioxidant enzymes and levels of non-enzymatic antioxidants, in common bean plants subjected to water deficit or waterlogging treatments.

MATERIALS AND METHODS

Experimental design and growth: Ten seeds were sown at 0.5 g pot-1 at a depth of 1.5 cm. The plastic pots containing 3 Kg clay treated as follows (4 pots were used for each treatment). All pots were irrigated with tap water until the appearance of two true leaves (10 days). Some pots were irrigated with tap water until water logging (2x F.C.) carried out after 10 days from sowing with water. Some pots were irrigated with tap water until the water deficit (75% F.C. and 50 %F.C.) carried out after 10 days from sowing with water. At the end of the experiments plants were separated into shoots and roots and fresh weights (FW) of shoots and roots were recorded.

Determination of non-enzymatic antioxidants:

Free and cell wall-bound phenolics were determined according to Kofalvi and Nassuth (1995). Fresh leaves (0.5 g) were extracted in 50% methanol (12 v/v) for 90 min. at 80°C. The extract was centrifuged at 14000 rpm for 15 min. and the supernatant was taken for free phenolics determination using the Folin-Ciocalteu's phenol reagent. Phenolic concentration in the extract was determined from standard curve prepared with gallic acid. The level of lipid peroxidation in plant tissues was determined as 2-thiobarbituric acid (TBA) reactive metabolites, *i.e.* malondialdehyde (MDA). 0.45 g tissue sample was homogenized in 2.5 ml of 0.1 % trichloroacetic acid (TCA). The concentration of MDA was calculated by using an extinction coefficient (155 mM^{-1} cm^{-1}) and the results expressed as the level of lipid peroxidation in µmol MDA/g (Madhava Rao and Sresty, 2000). The H_2O_2 content of the shoots and roots samples, which is considered as a reactive oxygen species (ROS) and an oxidative stress marker, was calorimetrically measured as described by Mukherjee and Choudhuri (1983). The concentration of H_2O_2 was calculated from a standard curve plotted with known concentration of H_2O_2 and expressed as mg/g FW. The ascorbic acid was determined according to Jagota and Dani (1982). Leave tissues (0.2 g) were ground with liquid nitrogen and suspended in 2 ml of 5% TCA. After 10 min. the absorbance of the blue color developed was measured in Unico UV2100 spectrophotometer at 760 nm. A standard curve was prepared by different concentrations of ascorbic acid.

Assays of antioxidant enzymes activities

Collected leaf tissues (0.5 g) were ground to a fine powder in liquid nitrogen and then homogenized in 5 ml of 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM ethylenediamine tetraacetic acid (EDTA) and 0.1 g polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 18,000 rpm for 10 minutes at 4 °C. The supernatant was collected and used to assess the activity of antioxidative enzymes (Abdel-Aziz, 2019) specifically super-oxide dismutase (SOD; EC 1.15.1.1). The enzyme activity was assayed following the method of autoxidation of epinephrine (adrenochrome) as described by Misra and Fridovich (1972), with some modifications. Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the rate of H_2O_2 conversion to O_2 for 1 minute according to a modified method based on Aebi (1984).

Guaiacol peroxidase (POD; EC 1.11.1.7) activity was determined following the method of Tatiana *et al.* (1999) with some modifications. Meanwhile, Ascorbate peroxidase (APX; EC 1.11.1.11) enzyme activity was determined according to the modified method based on the described method of Nakano and Asada (1981). All colorimetric measurements, including enzyme activities, were conducted at 20°C using a Unico UV-2100 spectrophotometer (Mahdi, *et al*.,

2023). Protein concentration in the enzyme extract was determined using the method of Lowery *et al.* (1951).

RESULTS

Growth measurements

The impact of water logging and water deficit on fresh and dry weight of common bean plants were presented in Table (1). It is clear that dray matter accumulation of leaves, stem and roots significantly decreased in both stresses water deficit and water logging. Moreover, the most declines in fresh weight of leaves and stems were observed at the highest water deficit level (HWD). However, fresh, and dry matter accumulation of root and shoot of common bean plants were slightly decreased under water logging (WL) more than water deficit. On the other hand, HWD level exhibited the highest reduction in root fresh weight as compared to WL in relation to control. The total biomass of common bean (*Phaseolus vulgaris*) plants was significantly decreased under both stresses (water logging and water deficit).

In general, the different plant parts (leaves, stem, and roots) respond differently to the water-related stresses (waterlogging and drought) in terms of their fresh and dry weight. The leaves appear to be the most sensitive, showing the greatest reductions in both fresh and dry weights under the stress conditions.

Non-enzymatic antioxidant compounds

Phenolic compounds

Phenolic compounds are among the most influential and widely distributed secondary products in the plant species, being involved in resistance to different types of stresses. In the present study, the interactive effect of both stresses (water deficit and waterlogging) and phenolic compounds accumulation were studied.

The data in figure (1A) revealed that the free phenolic compound in shoots and roots of the tested plants was significantly increased as a result of imposed to the levels of water deficit (75% F.C and 50% F.C) or waterlogging compared to absolute con-trols. But the free phenolic compound of roots was significantly decreased especially under waterlogging stress. In common bean plants, the free phenolic comp-ound in shoots was significantly decreased underwater deficit while, it was unchanged under water logging stress. In roots, the free phenolic compound of com-mon bean plants was significantly increased under both stresses (WL and HWD) while it was unchanged under LWD.

The data in figure (1B) revealed that the bound phenolic compound in shoots significantly increased as a result of exposure to various levels of both stresses compared to control plants. In roots, the bound phenolic compound decreased under WL and increased under WD compared to absolute control plants.

Ascorbic acid content

According to the data in figure (2) the ascorbic acid concentration in shoots of bean plants was significantly unchanged as a result of imposed to level of drought (3/4FC) compared to absolute controls. The highest increase in the accumulation of ascorbic acids was recorded at higher water deficit content used (1/2FC) and waterlogging treatments while the ascorbic acid concentration in shoots was unchanged under LWD as compared to absolute control.

MDA content

Although determination of MDA as an estimation of oxidative damage to lipid membranes is a widely accepted methodology, it is known that carbohydrates and even some amino acids may undergo decomposition and produce MDA as an end-product (Hodges *et al*., 1999). Thus, MDA concentration does not always indicate the actual level of lipid peroxidation. In common bean plants, MDA concentration was significantly increased as a result of imposed to HWD, however, MDA concentration was unchanged under WL stress compared to absolute control.

Hydrogen peroxide

According to the data in Figure (3), the hydrogen peroxide concentration in shoots was significantly increased as a result imposed to decrease the soil moisture content WD and WL stress the percent of increase in the hydrogen peroxide concentration of common bean plants WD shoots was 133% and 172% respectively. The highest hydrogen peroxide concentration was recorded under WL stress the percent of increase in the hydrogen peroxide concentration of common bean plants was 238%. In roots, the hydrogen peroxide concentration of common bean plants was significantly increased as a result imposed to decrease the soil moisture content WD and WL stress the percent of increase in the hydrogen peroxide concentration roots of bean plants was 116% and 118% and 127% respectively.

Osmolyte and compatible solute

Proline which is considered as an osmoregulater and recorded to be accumulated in stressed plants; was measured in this investigation. In common bean plants,

Table 1. Effects of soil flooding and water deficit on fresh and dry weight of leaves, stem, and roots of common bean (*Phaseolus vulgaris)* plant.

Treatments	Fresh and dry weight of plant parts (g/plant)					
	leaves		Stem (g/ plant)		Root (g/ plant)	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
Control	6.12 ±0.721^{b}	0.79 ±0.102^{b}	2.304 ±0.142^{ab}	0.362 ±0.004^{b}	4.066 ±0.430^{c}	0.257 ±0.0326^{b}
Waterlogging	1.66 ±0.291^{a}	0.197 ±0.005^{a}	2.443 ±0.45^{4b}	0.122 ±0.003^{a}	2.443 ±0.034^{a}	0.001 ±0.0033^{a}
Low drought	5.46 ±.1.38^{b}	0.640 ±0.170^{b}	2.056 ±0.250^{ab}	0.334 ±5.015^{b}	3.230 ±0.638^{bc}	0.224 ±0.0409^{b}
High drought	2.55 ±1.074^{a}	0.311 ±0.128^{a}	1.491 ±0.153^{a}	0.179 ±0.638^{a}	2.464 ±0.543^{b}	0.165 ±0.040^{b}
F-value	14.097*	13.371*	2.841**	8.377*	11.56*	9.36*

Mean data, per column, with superscript letters are significantly different at $p \leq 0.05$.

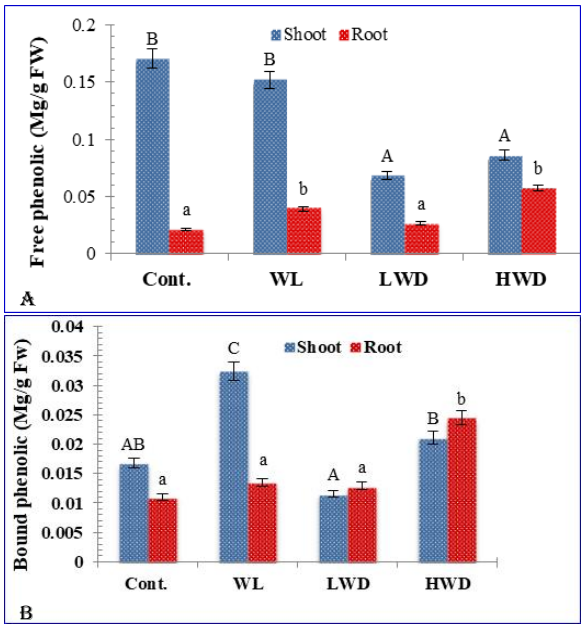

Figure (1): Effect of water deficit (WD) and waterlogging (WL: low and high) on non-enzymatic antioxidants of common bean plants. A, free phenolic and B, bound phenolic. Data are in mean ±SE, expressed as mg g^{-1} FW. Different letters, per column of shoot or root, indicate significant differences among treatments at p<0.01.

proline concentration in shoots was significantly increased under WD. There for the highest accumulation of proline was obtained in plants subjected to the lowest level of soil moisture content DW.In roots, a slight increase in proline at the LWD, then and suddenly a huge accumulation was recorded only at HWD in tested plants. WL stress imposed in the growth medium significantly stimulates proline biosynthesis in roots of the test plants compared to absolute controls, however, in shoots was not affected compared to absolute controls.

Enzymatic responses

Superoxide dismutase (SOD) Activity

The effect of WL and WD (LWD and HWD) stresses on activity of antioxidant enzyme superoxide dismutase activity (Δ abs. at 480 nm min-1 mg^{-1} FW) in leaves of faba bean plants is depicted in Figure (4) was superoxide dismutase activity (Δ abs. at 480 nm min^{-1} mg^{-1} FW) in leaves was significantly increased under the WL stress while unchanged under LWD and HWD compared to absolute control.

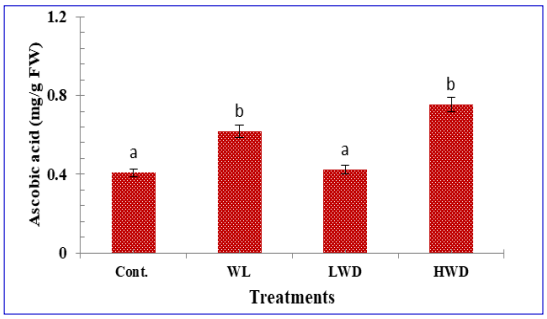

Figure (2): Effect of water deficit (WD) and waterlogging (WL: low and high) on ascorbic acid content of shoot of common bean plant expressed as mg g^{-1} FW. Data are the mean of three replica ±SE. Different letters, per column of shoot indicate significant differences among treatments at p<0.01.

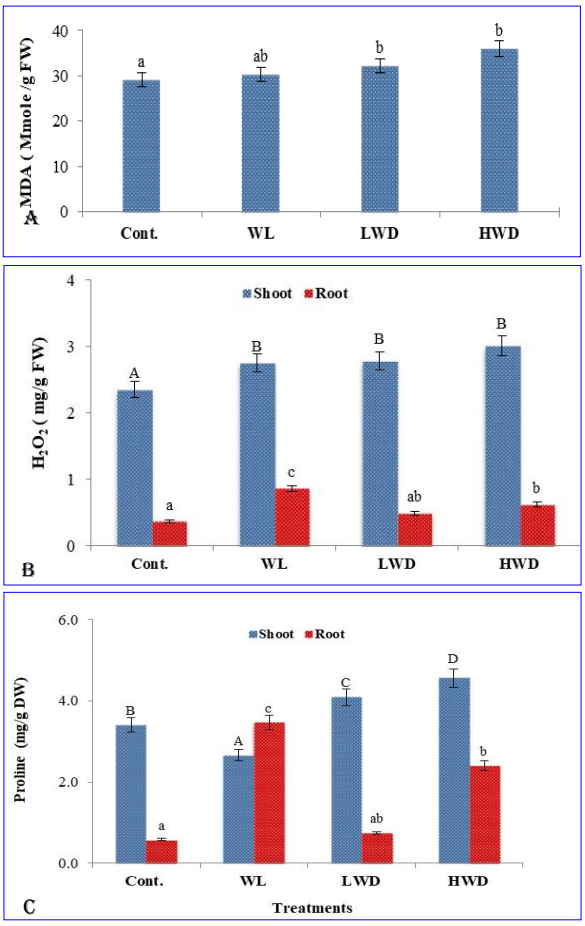

Figure (3): Effect of water deficit (WD) and waterlogging (WL: low and high) on measured parameters of common bean plant. A, malondialdehyde (MAD) expressed as mmol g^{-1} FW; B, hydrogen peroxide (H_2O_2) expressed as mgg^{-1} FW and C, Proline content expressed as mgg^{-1} DW. Data are the mean of three replica ±SE. Different capital/small letters, per column of shoot or root indicate significant differences among treatments at p<0.01.

SOD activity of roots of common bean plants was significantly increased up to both stresses (WL and HWD). As compared to common bean control plants no significant change in roots activity of SOD activity under LWD. According to the data in the figure (4A), SOD specific activity (U mg^{-1} protein) in common bean plants, SOD specific activity (U mg^{-1} protein) in leaves and roots was significantly increased under WL stress compared to absolute control. SOD specific activity in leaves and roots of common bean plants was unchanged under the LWD and HWD stresses; however, SOD specific activity up to HWD in roots was decreased compared to control.

Catalase enzyme

The specific activity of catalase (CAT) in common bean leaves under Low Water Deficit (LWD) and High Water Deficit (HWD) was significantly decreased, whereas it remained unchanged under Water Logging (WL) stress compared to the absolute control, as shown in Figure (4B). In roots, the specific activity of CAT of common bean leaves and roots was increased under WL stress while it was unchanged under LWD and HWD in common bean leaves compared to absolute control.

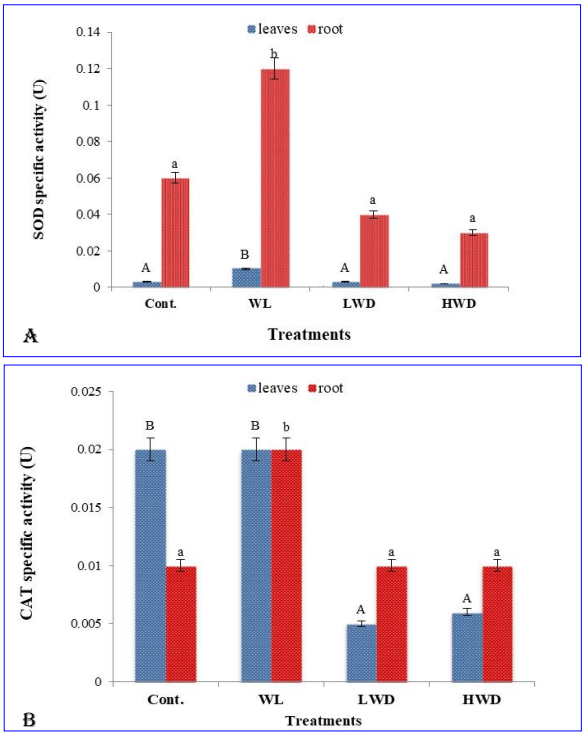

Figure (4): Effect of water deficit (WD) and waterlogging (WL: low and high) on: A, superoxide dismutase specific activity (SOD) and B, catalase enzyme activity (CAT) of leaves and roots of common bean plant expressed as unit per tissue. Data are the mean of three replica ±SE. Different letters, per column of leaves or roots indicate significant differences among treatments at $p<0.01$.

Ascorbate peroxidase and Glutathione peroxidase

The data in Figure (5A) revealed that the specific activity of ascorbate peroxidase (APX) in common bean leaves and roots have different responses where, under waterlogging stress, both the leaves and roots show a decrease in specific activity compared to the control. However, the reduction in specific activity is more pronounced in the leaves compared to the roots. At low water deficit (LWD) and High Water Deficit (HWD): As the water deficit stress increases from LWD to HWD, the specific activity in the leaves continues to decline further. In contrast, the roots are able to maintain a relatively higher specific activity, even under the more severe HWD condition.

For guaiacol peroxidase (GPx), different response pattern was recorded. Under waterlogging stress, both the leaves and roots show a decrease in glutathione peroxidase specific activity compared to the control condition (Fig. 5B) However, the reduction in guaiacol peroxidase activity is more pronounced in the leaves than the roots. Meanwhile, at low water deficit (LWD) and high water deficit (HWD), as the water deficit stress increases from LWD to HWD, the glutathione peroxidase specific activity in the leaves continues to decline further. In contrast, the roots are able to maintain a relatively higher guaiacol peroxidase specific activity, even under the more severe HWD condition. The leaves appear to be more sensitive, exhibiting a greater reduction in glutathione peroxidase activity, while the roots demonstrate more resilience in maintaining this important antioxidant enzyme's function under these stress conditions. The data indicates that the root system plays a crucial role in the plant's overall strategy to tolerate and adapt to water-related stresses, likely through mechanisms that preserve glutathione peroxidase activity and other stress-responsive enzymes.

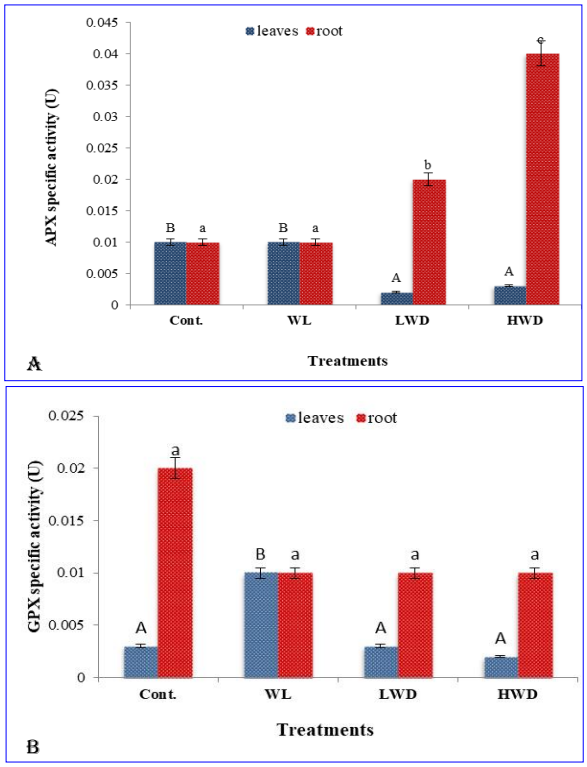

Figure (5): Effect of water deficit (WD) and waterlogging (WL: low and high) on: A, ascorbate peroxidase (APX) and B, guaiacol peroxidase (GPX) of leaves and roots of common bean plant expressed as unit per tissue. Data are the mean of three replica ±SE. Different capital/small letters, per column of leaves or roots indicate significant differences among treatments at $p<0.01$.

DISCUSSION

Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally death of plant (Jaleel *et al.*, 2008a). It reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and growth promoters (Jaleel *et al.*, 2008 b; Farooq *et al.*, 2008, Mohammed Farhard *et.al* 2014). It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion growth (Kusaka *et al.*, 2005; Shao *et al.*, 2008). The quantity and quality of plant growth depend on cell division, enlargement, and differentiation and all of these events are affected by water stress (Kusaka *et al.*, 2005). This might be the reason for the inhibition of plant growth under water deficit.

Hydrogen peroxide (H_2O_2), a form of reactive oxygen species, is regarded as a common cellular metabolite. H_2O_2 is continually synthesized through various sources including enzyme and non-enzyme pathways

in plants. To date, it has become accepted that H_2O_2 plays important roles in plant developmental and physiological processes including seed germination (Garcia-Becerra *et al*., 2023; Liu *et al*., 2013), programmed cell death (PCD; Cheng *et al*., 2015) senescence (Liao *et al*., 2012), flowering (Liu *et al*., 2013), root system development (Liao *et al*., 2009), stomatal aperture regulation (Ge *et al*., 2015) and many others. It is now understood that hydrogen peroxide (H_2O_2) functions as a signaling molecule in plant cells, responding to various stimuli. These findings suggest that H_2O_2 plays a role in cellular signaling pathways and modulates gene expression in plants. H_2O_2 is involved in mediating developmental and physiological processes in plants. This indicates that H_2O_2 may impact different areas of plants by increasing endogenous levels of H_2O_2, potentially affecting metabolic and antioxidant enzyme activity to promote plant growth and development (Liu *et al.,* 2013). However, further research is needed to fully understand the mechanisms underlying the diverse functions of H_2O_2 in plants. Among reactive oxygen species (ROS), H_2O_2 has a relatively long lifespan and small size, allowing it to pass through cellular membranes and enter different cellular compartments. García-Mata and Lamattina (2013) discovered that H_2O_2 may travel between cells through aquaporin channels for signaling purposes. Growing evidence suggests that H_2O_2 signaling plays a role in regulating various physiological processes in plants, such as participating in nitrosative stress-triggered cell death in kimchi cabbage (*Brassica rapa* var. glabra Regel) seedlings (Kim et al., 2015).

Proline is a compatible osmolyte, and performs multiple functions in stress adaptation, recovery and signalling, stabilization of proteins and protein complexes in the chloroplast and cytosol and protection of the photosynthetic apparatus in plants (Szabados, and Savoure, 2009). Ashraf and Foolad (2007) suggested that the application of proline successfully improved stress tolerance in plants. In plants under water or salt stress, proline content increases more than other amino acids, and this effect has been used as a biochemical marker to select varieties aiming to resist to such conditions (Bates, 1973). Moreover, there is additional evidence that these compatible solutes are accumulated in plants at high concentrations to alleviate enzyme inactivity or loss of membrane integrity due to water deficiency (Schwab and Gaff 1990).

The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Hare *et al*., 1999; Kavi Kishor *et al*., 2005; Ashraf & Foolad, 2007). The increase in proline content is the most remarkable parameter in rice grown under salt stress conditions (Roy *et al*., 1992). Proline's role as an osmolyte or osmoprotectant in leaves of drought-stressed plants has been debated (Seki *et al*., 2007; Szabados and Savoure, 2009; Gomes *et al*. 2010). Indeed, proline has been demonstrated to confer drought stress tolerance to wheat plants by increasing the antioxidant system rather than increasing osmotic adjustment (Vendruscolo *et al*., 2007; Szabados and Savoure, 2009).

CONCLUSION

In conclusion, the study on broad bean (*Phaseolus vulgare*) demonstrated that both water deficit and waterlogging conditions significantly inhibited the growth of common bean plants. The enzymatic and non-enzymatic antioxidant changes observed in response to these stresses pointed towards an amelioration of water deficit or waterlogging stresses tolerance in broad bean plants. The enhanced antioxidant defense system under water deficit stress, as shown by increased activities of key enzymes and elevated levels of non-enzymatic antioxidants, provided valuable insights into the strategies employed by common bean plants to adapt to water stress. Further research is necessary to unravel the underlying molecular mechanisms and explore potential interventions to enhance the water stress tolerance of this important legume crop.

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