International Journal of Plant & Soil Science

24(6): 1-9, 2018; Article no.IJPSS.44540
ISSN: 2320-7035

Biochemical Responses of Wolfbane (*Periploca angustifolia* Labill) to Water Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Author MMAEM put the paper idea, collecting plant samples, shared in reading growth parameters and wrote the manuscript. Author AEAAE collected the plant seeds, planting seeds, designed the experiment and shared in reading growth parameters. Author MFI carried out the chemical analyses and performed the statistical analysis. The three authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2018/44540
Editor(s):
(1) Dr. Abhishek Naik, Technology Development Department - Vegetable Crops, United Phosphorus Limited -Advanta, Kolkata, India.
Reviewers:
(1) Raúl Leonel Grijalva-Contreras, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, México.
(2) Köksal Aydinşakir, Batı Akderniz Agricultural Research Institute, Turkey.
(3) Fábio Henrique Portella Corrêa de Oliveira, Universidade Federal Rural de Pernambuco, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/26715

Original Research Article

Received 29 July 2018
Accepted 01 October 2018
Published 20 October 2018

ABSTRACT

Periploca angustifolia as an endangered plant species were grown at Balouza Research Station (North Sinai, Egypt) during the period from November, 2016 to April, 2018 including three months seedling stage, two months transplanting and establishment stage, and 12 months plant old after establishment, one meter between seedlings within each row as well as the drip irrigation system. Using three irrigation levels; 160, 120 and 80 mm/year distributed constantly every 10 days for one year along to investigate vegetative parameters, hydrogen peroxide (H_2O_2), polyphenol oxidase (PPO), peroxidase (POD), total free amino acids (FAA), total phenols and soluble sugars. All vegetative parameters (plant height, number of branches, number of pods, leaves fresh and dry weight, soft branches fresh and dry weight, hard branches fresh and dry weight, and branches height mean attained the highest reading at 160mm/year irrigation amount. The three irrigation amounts did not induce a significant change in H_2O_2 concentration in both leaves and roots of *P. angustifolia*. The irrigation with 80mm/year induced the highest PPO activity in leaves and roots and the highest POD activity in leaves of *P. angustifolia*. Also, the lowest used irrigation amount stimulated the highest accumulation of FAA, total phenols and soluble sugars in the leaves.

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Keywords: Periploca angustifolia; water stress; antioxidant enzymes and biochemical constituents.

1. INTRODUCTION

Drought is one of the most important factors of abiotic stress in plants. It controls growth, development, flowering and productivity of plant species. When plants are exposed to water stress, some reactive oxygen species (ROS) are produced [1]. These ROS may cause deleterious effects on plant cell such as lipid peroxidation, protein oxidation, photosynthesis and DNA damages [2,3]. These harmful influences of ROS on cell macromolecules may be retarded or inactivated by antioxidant systems which either enzymatic like PPO, POD, etc., or non-enzymatic like carotenoids, ascorbic acids, proline and polyphenolic compounds [4-6]. Many authors declared that the species exposed to mild and / or water stress conditions increased the activity of antioxidant enzymes [7] while some did not find water stress effects on the enzyme activities [8]. Phenolic compounds play important physiological and ecological roles, being involved in resistance to different types of stress [9]. A number of studies found the accumulation of phenolic compounds in plant tissues as a reaction to abiotic stress [10,11].

Besides antioxidant defense, plants accumulate metabolites such as soluble sugars, amino acids, total phenols and inorganic ions result in drought stress to regulate osmotic potential [12]. The highest elevation in soluble sugar content in Tibetan wild barley genotypes was observed in drought-tolerant XZ5 and XZ150 under 15 % SMC [13]. Soluble carbohydrates are considered as important metabolites in plants under drought stress [14]. Drought stress influenced a significant increase in soluble carbohydrate and a decrease in soluble protein in *Cicer arietinum* cultivars [15]. Also, it increased the accumulation of total free amino acids in maize seedlings [16] and *Brassica napus* [17]. Water stress reduced plant biomass but increased the concentrations of primary metabolites and hormones [18].

Periploca angustifolia (Labill) belonging to family Asclepiadaceae, is an erect glabrous shrubs, well adapted to arid climates and a high palatable for range animals, so the plants are often overgrazed by goats and camels. In Egypt, it distributes along the Western Mediterranean coastal region at calcareous ridges between Mersa Matruh and Sallum and the most common use is grazing [19]. In addition to its rich with secondary metabolites via flavonoids, flavonols and phenolic compounds that have pharmacologically useful antioxidant properties [20]. Moreover, it can be used in rehabilitation [21].

The present study aims to investigate the effect of water deficit on some biochemical parameters in leaves and roots of *Periploca angustifolia* (Labill). To achieve that we measured the activities of PPO and POD, H_2O_2 content, total free amino acids, total phenols and total soluble sugars.

2. MATERIALS AND METHODS

Plant seeds of *Periploca angustifolia* (Labill) were collected from natural population far from 5 km West Sidi Barrani city. Cleaning protocol was done after seed collection and dried immediately in order to minimise seed deterioration. The seeds were sterilised and soaked in tap water for 2 hours then were sown in polyethylene bags filled with sand and vermiculite (3:1) under controlled conditions in green house at Baloza Research Station (North Sinai, Egypt), one seed per each bag for three months with regular irrigation. After that the seedlings were transplanted in the permanent site. And one meter between seedlings within each row as well as the drip irrigation system using Elsalam Canal water ranged between 1800-2000 ppm. The experimental design used was complete block design in three replicates to control the amount of irrigation water. Before application of irrigation amounts, all transplants were irrigated according their needs for two months to establish their roots. The first block was irrigated with 80mm/ year, the second (120 mm/ year) and the third (160 mm/ year). The irrigation system was supported with a calculator to control the amount of water distributed constantly every 10 days for one year along. After one year from planting 3 guarded plants from each block were taken to determine the following growth characters i.e. plant height (cm) which was measured from ground surface to the end top of the main stem, number of branches/plant, number of pods/plant, leaves fresh and dry weight/plant, soft branches fresh and dry weight/plant, hard branches fresh and dry weight/plant, and branches height mean. The GPS reading where the experiment was done is 31°03' latitude, 32°36' longitude and 22 m altitude.

The colorimetric determination of hydrogen peroxide concentration at 390 nm in leaf tissues using potassium iodide was assayed as described by Loreto and Velikova [22]. A half gram of fresh weight was homogenised in 3 mL of 1% (w/v) tri-chloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm at 4°C for 10 min. Subsequently, 0.75 mL of the supernatant was added to 0.75 mL of 10 mM K-phosphate buffer (pH 7.0) and 1.5 mL of 1M KI. A Standard curve of H_2O_2 was done to calculate its concentration (µmol g^{-1} fresh weight) in plant tissues.

Total soluble sugars of 0.1 g fresh weight in leaves were extracted according to AOA [23] and estimated by phenol and sulfuric acid method as described by Chow and Landhäusser [24] by reading the yellow developed color at the wavelength 490 nm. A known weight of fresh leaves was extracted with MeOH 85% to determine the phenolic compounds as catechol according to the method of Folin-Denis as described by Shahidi and Naczk [25] by reading the developed blue colour at 725.

To prepare the extraction of free amino acids (FAA) and enzyme assays: Peroxidase (POD) and polyphenol oxidase (PPO), fresh leaves were homogenised in 5 mL phosphate buffer (0.1 mol/L, pH 7.8), centrifuged at 10,000 ×g for 20 min at 4°C and then the supernatant was used for assays. Free amino acids were determined according to Hamilton and Van [26]. One ml of each sample extract was treated with 1 ml of 10% pyridine and 1 ml of 2% ninhydrine solution. The optical densities of these colored solutions were then read at 570 nm. Glycine solution were prepared as standard. The activity of peroxidase, POD (EC 1.11.1.7) was assayed according to the method of Dias and Costa [27] whereas; polyphenol oxidase (PPO) (EC 1.14.18.1) activity was measured according to Oktay et al. [28]. The activity of both enzymes was expressed by calculation the changes in the absorbance per unit of time.

2.1 Statistical Analysis

Data were analysed using [29]. The standard error of the means was calculated and Tukey's test ($P \leq 0.05$) was used to determine significant differences between means at 5% level of probability.

3. RESULTS AND DISCUSSION

3.1 Changes in the Growth Parameters

The effect of three different irrigation amounts (160, 120 and 80 mm/year) on vegetative growth parameters of *Periploca angustifolia* are shown in Table 1. All the studied vegetative traits; plant height, number of branches/plant, number of pods/plant, leaves fresh weight/plant, fresh weight of soft branches/plant, fresh weight of hard branches/plant, pods weight/plant, leaves dry weight/plant, dry weight of soft branches/ plant, dry weight of hard branches/plant and branches height mean were decreased significantly by water stress, recording the lowest reading at 80 mm/year except number of branches at 120 mm/year.

As mentioned above, plant growth parameters increased with increased water application from 80 to 160mm/year. It is known to us that water plays important role in transport, nutrient uptake and photosynthesis. In this trend, [30] found that the vegetative growth parameters in *Coriandrum sativum* were improved by applying higher irrigation levels as compared to lower levels. Flower and pod numbers in *Lupinus albus* were reduced result in water stress [31]. Also, [32,33] observed that as water stress increased as the shoot length, diameter and plant total fresh and dry weights decreased. Moreover, water deficit can reduce directly plant growth through reducing turgor pressure of cells and inhibiting their mitosis and elongation [12].

Plants have developed different mechanisms to overcome the deleterious effects of abiotic stresses. Growth rate (including plant height, branching numbers and mass productivity) is the initial plant morphological response to water stress. Thus, the reduction in these morphological characters of *P. angustifolia* may be an adaptive response to water stress.

3.2 Changes in Reactive Oxygen Species

Data presented in Fig. 1 show that H_2O_2 content in *P. angustifolia*, did not affect significantly in the leaves and the roots by water stress. H_2O_2 is the most stable among reactive oxygen species which is produced in plant cells via photosynthesis and photorespiration. In addition to photosynthetic and respiratory metabolism, the extracellular matrix (ECM) plays a vital role in the generation of H_2O_2, which regulates plant growth, development, acclimatory and defence responses [34]. The role of H_2O_2 in stress-promoted damage has long been known [35], but it has also been reported to be involved in a wide range of hormone-dependent developmental signaling processes, as well as cell wall splitting and associated cell wall growth [36].

Table 1. Effect of irrigation rate on some growth parameters of *Periploca angustifolia* (Labill) grown at Baloza Research Station, North Sinai

Parameters	Irrigation rate (mm.year^{-1})			
	160	120	80	M.S.D≤0.05
Plant height (cm)	134 a	122 b	105.333 c	**11.14**
No. branches	35.67a	11.67b	12.67 b	**5.21**
No. pods	379.0 a	221.33 b	101.67 c	**12.99**
Leaves fresh weight (g)	863.33 a	643.00 b	328.33 c	**18.10**
Fresh weight of soft branches (g)	333.00 a	264.00 b	89.33 c	**19.95**
fresh weight of hard branches (g)	918.00 a	382,33 b	171.67 c	**11.08**
pods weight (g)	674.67 a	459.00 b	132.00 c	**11.14**
dry weight of leaves (g)	225.33 a	134.00 b	51.67 c	**10,29**
dry weight of soft branches (g)	105.33 a	66.00 b	42.00 c	**7.42**
dry weight of hard branches (g)	444.67 a	169.67 b	64.67 c	**4.79**
mean of branches height (cm)	129.17 a	114.10 b	88.87 c	**9.55**

Means followed by different letters are significantly different at P ≤0.05 according to Tukey's multiple range test

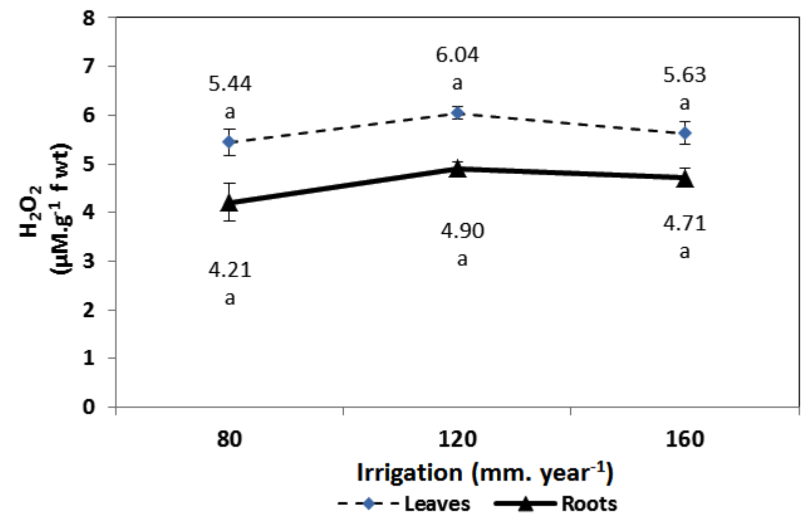

Fig. 1. Effect of Irrigation rate on the concentration of H_2O_2 in the leaf and root tissues of *Periploca angustifolia* (Labill) grown at Baloza Research Station, North Sinai

3.3 Changes in the Osmolytes

Data presented in Fig. 2 showed that soluble sugars in the leaves and the roots increased as water stress increased but the increase was only significant in the leaves. That increase in soluble sugar can be effective in keeping cell turgor, which is the protective reaction of *P. angustifolia* by improving water-holding capacity during water stress. Similar results were obtained for *P. angustifolia* [21], *Glycyrrhiza uralensis* [37] and *Lycium ruthenicum* [38]. Furthermore, [39] mentioned that water stress in the phyllode of *Acacia auriculiformis* induced inhibition of photosynthesis that decrease sucrose synthesis and the rate of sucrose export and transport in order to maintain higher pool of sucrose and total soluble sugars. The plant induces stress resistance by accumulating enormous amounts of soluble protein, free proline and soluble sugars to improve cell sap concentration, which can enhance cell turbidity and prevent excessive plasma dehydration [37].

Soluble sugars can act as signals regulating various processes associated with plant growth and development beside their function as metabolic resources and structural constituents of cells [40,41]. They are introduced in various metabolic events and act as molecule signals regulating different genes, especially those contributed in photosynthesis, sucrose metabolism and osmolyte synthesis [42].

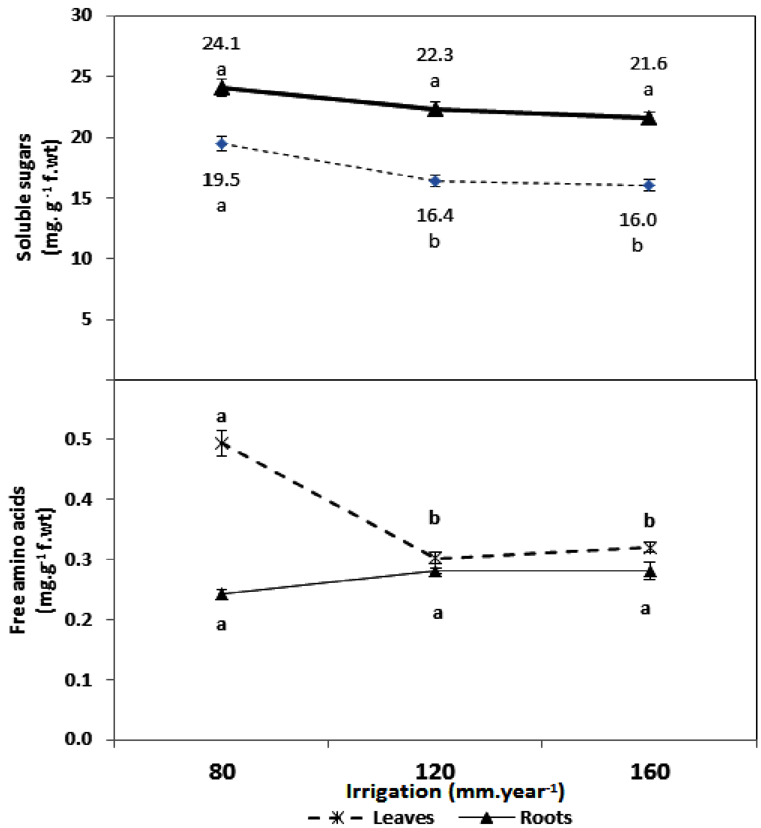

Fig. 2. Effect of Irrigation rate on the concentration of total soluble sugars and free amino acids in the leaf and root tissues of *Periploca angustifolia* (Labill) grown at Baloza Research Station, North Sinai

Like soluble sugars, F.A.A. extracted from the leaves increased significantly while the roots did not affect in F.A.A. content by water stress as observed from Fig. 2. In this trend, [43] declared that free amino acids in drought stressed leaves of *Lathyrus sylvestris* was increased by 11.9% as compared to the control. Also, Ranieri et al. [16] mentioned that total free amino acids were increased and a consistent rearrangement of the amino acid pool in maize seedling under water stress. Drought exposure increased pooled primary metabolites concentration in both leaves and roots, but this effect was significant only in leaves [18].

3.4 Changes in the Phenolic Compounds and Their Related Enzymes

Data presented in Fig. 3 showed that total soluble phenols in leaves and roots of *P. angustifolia* increased as water stress increased but the increase was only significant in the leaves. The increase in phenolic compounds in the leaves as affected by water stress due to their function as antioxidants [44]. In fact, that function may be less necessary in roots than in leaves, because roots lack the spikes in reactive oxygen species under stress that are combined with chloroplasts [45]. Large studies demonstrated that accumulation of total phenols rises under abiotic stress conditions [11,46-48]. In addition to, phenolic compounds take part in the defense against reactive oxygen species, which are inevitably produced when aerobic or photosynthetic metabolism is broken down by environmental stresses [49].

PPO and POD play a protective role in scavenging ROS [6,50]. In the present study, PPO increased significantly with water stress in leaves and roots attained the highest activity at irrigation range 80mm/year, while the change between S1 (160mm/year) and S2 (120 mm/year) was non-significant as represented in Fig. 4. Like PPO, POD increased significantly with water stress in leaves only, while in roots did not change significantly as shown in Fig. 4.

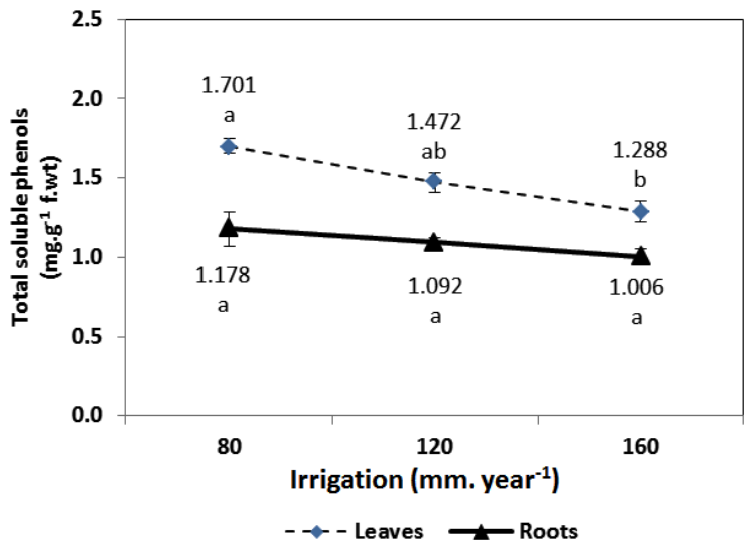

Fig. 3. Effect of Irrigation rate on the concentration of total soluble phenols in the leaf and root tissues of *Periploca angustifolia* (Labill) grown in at Baloza Research Station, North Sinai

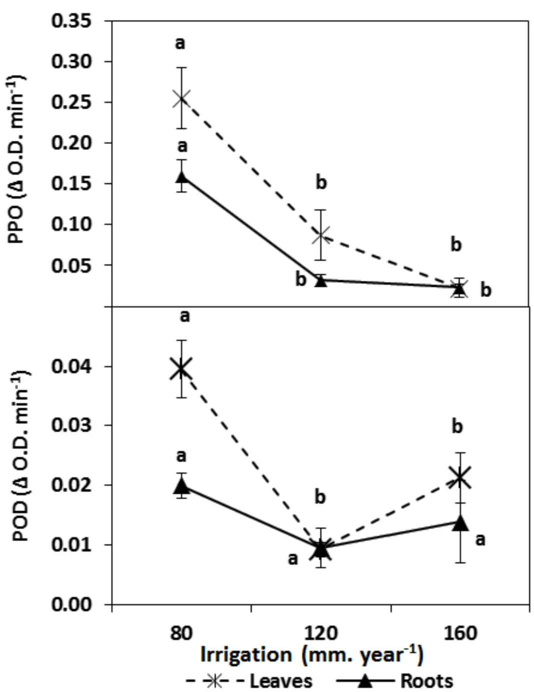

Fig. 4. Effect of Irrigation rate on the activity of polyphenol oxidase (PPO) and peroxidase (POD) in the leaf and root tissues of *Periploca angustifolia* (Labill) grown at Baloza Research Station, North Sinai

PPO motivates the oxidation of monophenols and/ or o-diphenols to o-quinones with the restriction of oxygen by reduction to water which results in protein complexing and the formation of melanin pigments. The formation of o- quinone-protein complexes may reduce the nutritional value of the tissue [51]. From our results it is obvious that PPO is an effective result in water stress tolerance in *P. angustifolia* leaves and roots. Consequently, the suggestion that an increase in PPO activity following a drought stress on *Trifolium repens* [52] and *Ramonda serbica* leaves which were exposed to near-complete water loss [53]. However, a conflicting result was given in tomato where suppression of PPO activity results in abiotic stress [54]. The increase of POD in the leaves results in water stress agreed with Salekjalati et al. [55], who found that POD activity significantly increase as compared with control will watered treatment in *Hordeum vulgare* L. Also, [56] observed the increased activity of POD in leaves and petioles of *Ctenanthe setosa* (Rosc.) Eichler under drought stress. Moreover, POD can enhance the degradation of phenols when coexisting with PPO [57]. In addition PODs play an important role in the fine regulation of reactive oxygen species in the cell through activation and deactivation of H_2O_2 [58].

4. CONCLUSION

Periploca angustifolia is a drought tolerant plant that can adapt morphologically to water stress by reducing its growth parameters to reduce transpiration process. Furthermore, plant keeps stability in the reactive oxygen species (H_2O_2

content). Biochemically, PPO plays a pivotal role in *P. angustifolia* to increase the tolerance to water stress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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