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Changes in electrolyte leakage and lipid peroxidation in rosemary winter and summer cuts and concomitant antioxidants and oxidative enzymes, in response to soil type and irrigation water supply

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Abstract : The percentage of electrolyte leakage (EL) as a measure to cell membrane permeability and the concentration of malondialdhyde (MDA) as a product of lipid peroxidation damage of cell membranes by free radicals were determined in roots and shoots of rosemary (*Rosmarinus officinalis* L.) plants grown in sandy clay (SC) and sandy loam (SL) soils and irrigated once (I_1) or twice (I_2) per week, with each soil. The mentioned criteria were taken with two cuts at February and August (3, 9 months from transplanting, respectively). Higher values were recorded in leaves than in roots and at the second than the first cut. Planting in the SC soil combined with I_1 irrigation system induced higher EL and MDA values and H_2O_2 produced in leaves at the two cuts, as compared to the corresponding plants supplied with I_2 irrigation system at the 1st cut. Among the different treatments, a negative correlation was generally shown between the contents of total phenols (TPH), total flavonoides (TF), ascorbic acid (ASA) and consequently total antioxidant (TAC) capacity in addition to the antioxidant enzymes peroxidase (POX) and catalase (CAT) in leaves and soil moisture that was indirectly affected by the soil type (SC and SL). On the other hand, enhanced activities of the oxidative enzymes polyphenol oxidase (PPO), ascorbic acid oxidase (ASAO), and indoleacetic acid oxidase (IAAO) were recorded in leaves of the plants grown in the SC soil concomitant with I_1 and I_2 irrigation, at the two cuts, as compared to the plants grown in the SL soil. Thus, it might be assumed that rosemary plants as being affected by different soil types and irrigation levels, during the two cuts, could enhance their antioxidant metabolites and antioxidant enzyme activities in leaves in order to minimize EL and MDA rates in roots and leaves. Considering that soil water content within the different applied treatments was not stressful, our results might emphasize that the network of antioxidant system is also tuned to certain extents under variable favorable conditions with similar trends as those taking place under stress conditions.
Keywords: Rosemary (*Rosmarinus officinalis*), Soil type, Irrigation systems, Lipid peroxidation, Antioxidants, Oxidizing enzymes.

Introduction

Rosemary (*Rosmarinus officinalis* L.) is an aromatic, evergreen, perennial herb, grown under a wide range of climates, endogenous to Europe, Asia and Africa, mainly in areas surrounding the Mediterranean Sea[1]. It is cultivated in Egypt throughout the year. This plant is one of the most effective spices widely used in food

processing among the herbs of family Lamiaceae (Labiatae). Rosemary extract may be a good candidate for functional foods as well as for pharmaceutical plant-based products[2,3,4,5]. Rosemary extracts exhibit potent antioxidant activity that could be primarily attributed to its phenol constituents[6]. Phenol and flavonoid compounds contribute to about 90% of rosemary antioxidant activity[7]. A positive correlation was observed between total antioxidant activity and total phenol content of rosemary extract[7,8]. A greater amount of phenol compounds led to more powerful radical-scavenging effect[9,10]. Accumulation of phenolics has been also reported as a result of inhibiting their oxidation [11]. The authors also found that transgenic tomato (*Lycopersicon esculentum*) plants with suppressed polyphenol oxidase (PPO) exhibited more favorable water relations and delayed photo inhibition and photo oxidative damage during plant water stress, while PPO over expression increased photo-oxidative damage during water stress.

However, other antioxidants as ascorbic acid have been also considered to protect the plants against oxidative damage [12,13]. Suppressed expression of the ascorbate oxidase (AAO) gene in transgenic tobacco and Arabidopsis mutant also increased the tolerance for salt stress due to the accumulation of ascorbate[14]. Reactive oxygen species (ROS) could disrupt normal metabolisms of plants through lipid peroxidation of cellular membranes, as well as oxidation of proteins, nucleic acids, and photosynthetic pigments and enzymes[15,16]. Efficient scavenging of ROS produced during various environmental conditions requires the action of non enzymatic antioxidants as phenolics and ascorbic acid and several antioxidant enzymes as well[17, 18, 19]. A negative correlation was recorded between the relative growth rate and the contents of malondialdehyde, H_2O_2, antioxidants including phenol content, thus indicating the importance of lipid peroxidation and consequently EL as a determinant of physiological processes in selecting tomato plants tolerant to water stress [20]. Therefore, it could be concluded that a better mechanism against oxidative damage would be achieved by inducing higher activities of antioxidant enzymes and levels of non enzymatic antioxidants. In fact, plants have evolved various molecular mechanisms to reduce their consumption of resources and adjust their growth to adapt to adverse environmental conditions[21,22,23,24,25]. Other workers [26, 27] concluded that different environmental conditions could modulate the contents of the phenol compounds and the antioxidant potential of rosemary extract. But, most attention in this respect has been driven to the changes taking place under stress conditions, perhaps because under normal conditions such modulations are either absent or very weak. For e.g., [28] showed that antioxidant enzymes were rarely enhanced under moderate water condition but often increased under low water levels.

Thus, in the present work, we intended to shed more light on the alterations that would take place under normal environmental conditions of rosemary plants grown in sandy loam soil and sandy clay soils and irrigated once or twice per week during two cuts (February and August). For this aim, the electrolyte leakage, and lipid peroxidation of roots and leaves and the concomitant contents of H_2O_2, non enzymatic antioxidants, and the activities of antioxidant and oxidative enzymes in leaves were estimated. Understanding these key factors under usual planting conditions, would enable to predict best conditions for the growth and productivity of rosemary plants.

Materials and Methods

Plant material

Uniform transplants of rosemary (*Rosmarinus officinalis* L.) were kindly provided by the Medicinal and Aromatic Plant Research Branch, El-Qanatir El-Khairiya, Horticulture Research Institute, Ministry of Agriculture, Cairo, Egypt.

Chemicals and Reagents

All chemicals employed in this study were of highest purity obtained from E. Merck (Darmstadt, Germany), except colorimetric assay kits for ascorbic acid and total antioxidant capacity that were purchased from Biodiagnostic Co., Cairo, Egypt. All organic solvents were of AR grade.

Time course experiment

A pot experiment was conducted at the green house of the Botanic garden, Faculty of Science, Ain Shams University, Cairo, Egypt, at November 2013 to August 2014. Sandy clay (SC) and sandy loam (SL) soils were used; each with two irrigation systems, i.e. once (I_1) or twice (I_2) per week. Each pot was filled with one type of soil, i.e. either SC or

SL soil. When the plants were well established, the irrigation system was applied as I_1 or I_2/ week with each soil type. Plastic pots (30cm diameter and 18 cm depth) were used. Three uniform transplants (60 day- old) of rosemary were planted in each pot. The irrigation system was applied (once or twice/ week) with each soil type after seven days from transplantation. The pots were arranged in complete randomized block designs with the different treatments. Two cuts (3 and 9 months from transplantation at February and August, respectively) were taken for experimentation.

During the experiment, the mean temperature and relative humidity were 16.8°C and 56.25 %, respectively, during November (start of experiment) and 23.8°C and 56.8%, during August (end of experiment).

Analysis of soil and irrigation water

Analyses of physical and chemical properties of the soil types used in this study were done as described by [29].

Measurement of soil water content

Soil water content was determined in 100g soil, where the reduction in mass by oven drying (105°C) was due to loss of water.

Electrolyte leakage percentage

Electrolyte leakage was used to assess cell membrane permeability according to a modified method described by [30]. Fresh leaves or roots (0.2g) were cut into 0.5cm segments, and then placed in 50 ml glass vials rinsed with distilled water to remove electrolytes released during leaf disc excision. The vials were then filled with 30 ml distilled water and allowed to stand in the dark for 24 hr at room temperature. Electrical conductivity (EC1) of the bathing solution was determined using a conductivity meter (Model Ohm-419). The vials were heated in a temperature-controlled water bath at 95 ºC for 20 min and then cooled to room temperature and the electrical conductivity (EC2) was again measured. The relative electrolyte leakage (REC) was calculated as a percentage of EC_1/ EC_2 using the following equation:

$$\text{Relative permeability (\%)} = \left(\frac{\text{EC1}}{\text{EC2}}\right) \text{X } 100$$

Lipid peroxidation

The level of lipid peroxidation was measured by thiobarbituric acid (TBA) test, which determines malondialdhyde (MDA) as an end product of lipid peroxidation according to[31]. Leaf and root samples (0.5g) were homogenized in 10 ml TBA reagent (18% TCA mixed with 0.45 % TBA; 1: 2 v/v). The mixture was incubated in a boiling water bath for 15 min, warm filtrated and the reaction stopped by transferring the reaction tubes to an ice bath. Then, the samples were centrifuged at 6000 rpm for 10 min, and the absorbance of supernatant was read at 532 nm. The value of non-specific absorption at 600 nm was subtracted. The MDA concentration was expressed as µmol g^{-1} d.wt. equivalent. After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient 155 mM^{-1} cm^{-1}.

MDA equivalents ($nmol.ml^{-1}$) = [(A_{532} - A_{600})/ 155 000] X 10^6.

Hydrogen peroxide content

Hydrogen peroxide level was determined as described by [32]. Leaf tissues (0.5g) were homogenized in an ice bath with 5 ml 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 6000 rpm for 30 min at 4°C; and then 0.5 ml of the supernatant was added to 0.5 ml potassium phosphate buffer (10mM at pH 7.0) and 1 ml of 1.0M potassium iodide. A blank solution containing 0.5 ml dist. H_2O instead of plant extract buffer was also prepared. The absorbance of the supernatant was read at 390 nm. The content of H_2O_2 was given from a standard curve and the results were expressed as µmol g^{-1} d.wt. equivalent.

Non enzymatic antioxidants

The non-enzymatic antioxidants analyzed in the air dried leaf tissue were total phenols and total flavonoids. Ascorbic acid and total antioxidants were estimated in fresh leaf tissue. Total phenol contents were determined according to the method described by [33], using Folin–Ciocalteu reagent and gallic acid as a

standard. Total flavonoid contents of rosemary extract were estimated using aluminum chloride colorimetric assay according to [34, 35].

Ascorbic acid and total antioxidant capacity were measured as described by[36,37], respectively, using colorimetric assay kits (purchased from Biodiagnostic Co., Cairo, Egypt).

Antioxidant and oxidative enzymes

Preparation of extracts

Fresh leaf tissue of rosemary was used for preparation of enzyme extracts according to [38] as follows: 0.5g was homogenized in mortar with 10 ml cold phosphate buffer (Na/ K phosphate 0.1M at pH 6.8). The homogenate was centrifuged for 10 minutes at 6000 rpm and 4°C. The supernatant was completed to a known volume and used for photometric assaying of enzyme activities.

Antioxidant enzymes

The antioxidant enzymes analyzed in the fresh leaves of rosemary were peroxidase and catalase. Peroxidase (EC 1.11.1.7) activity was assayed according to [39], with slight modifications; by recording changes in absorbance for 30 seconds up to 3 minutes at 436 nm. Catalase (EC 1.11.1.6) was assayed following the method of [39]. The reaction mixture was initiated by adding H_2O_2 and the residual of H_2O_2 was monitored spectrophotometrically at 405 nm.

Oxidative enzymes

The oxidative enzymes under study were polyphenol oxidase (PPO) (EC 1.14.18.1), ascorbic acid oxidase (ASAO) (EC 1.10.3.3) and IAA oxidase (IAAO) (EC 1.2.3.7.). The activity of PPO was assayed according to [40] by following change in color intensity at 495 nm for 30 seconds up to 5 minutes. The activity of ASAO was assayed using the method of [41] with some modification as described by [42], following the rate of disappearance of ascorbate by reading optical densities after 30 sec interval up to 3 minutes at 265 nm. IAAO activity was assayed by a modified method of [43] as described by [44]. The developed color was monitored spectrophotometrically at 530 nm.

The activities of different enzymes were expressed as changes in optical density g^{-1} d.wt. equiv. hr^{-1}.

Statistical analysis

The data were expressed as mean ± standard error (SE) of the triplicate values for all the chemical analysis. Statistical analysis was performed using one-way analysis of variance ANOVA followed by Duncan's Multiple Comparison Test using IBM Statistical Product and Service Solutions, SPSS Statistics for Windows, Version 21, and $P<0.05$ was denoted as being statistically significant for means compared, using least significant difference (LSD) at 0.05 P.

Results

It should be pointed herein that the soil moisture contents (%) under cultivation in sandy clay (SC) and sandy loam (SL) soils and irrigation once (I_1) or twice (I_2), with each soil type, at the two plant cuts (3 and 9 months from transplanting, respectively), were as follows:

Treatments	SC		SL	
	I_1	I_2	I_1	I_2
1st cut	72.80	83.70	68.70	76.03
2nd cut	64.35	71.17	56.31	61.75

Electrolyte leakage, lipid peroxidation, and H_2O_2 levels

Within the different applied treatments, the percentage of electrolyte leakage (EL) as a measure of cell membrane permeability and the concentration of malondialdhyde (MDA) as a product of lipid peroxidation damage by free radicals were higher in leaves and roots of rosemary plants at the second cut than the first cut (Table 1). Generally, cell membrane permeability and lipid peroxidation were higher in leaves than in roots during the two experimental cuts. The results in Table 1 also show a more or less similar trend in roots and leaves with higher values in the sandy clay (SC) soil and irrigation twice/ week (I_2) at the 1st cut and I_1 irrigation in the same soil type (SC) at the 2nd cut. In the sandy loam (SL) soil the cell membrane permeability and lipid peroxidation were lower in response to I_2 irrigation in both leaves and roots during the two cuts.

The results obtained with hydrogen peroxide levels in leaves showed also comparable trends with those mentioned above for membrane permeability and lipid peroxidation throughout the different treatments (Table 1).

Table 1: Effect of sandy clay soil (SC) or sandy loam soil (SL) and irrigation once/week (I_1) or twice/week (I_2), with each soil type, on lipid peroxidation (nmol g^{-1} d.wt. equiv.), electrolyte leakage percentage (d.wt. equivalent) in fresh leaves and roots of rosemary plants and the content of hydrogen peroxide (H_2O_2) in fresh leaf tissue at the 1st and the 2nd cuts (3 and 9 months from transplanting, respectively). The results are expressed as means of three replicates ±SE. Statistical analysis was carried out using Duncan test. Different letters show significant variation at 0.05 P.

Treatment		% Electrolyte leakage (d. wt. equiv.)				Lipid peroxidation (nmol g^{-1} d.wt. equiv.)				H_2O_2 (µM g^{-1} d.wt. equiv.)	
Soil type	Irrigation system	Leaves		Roots		Leaves		Roots		Leaves	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
SC	I_1	46.00 b ±0.58	54.60 a ±0..30	34.67 b ±0.33	42.33 a ±0.88	17.46 b ±0.09	21.15 a ±0.38	11.66 b ±0.02	15.86 a ±0.32	1.43 b ±0.01	7.28 a ±0.04
	I_2	48.00 a ±0.33	54.33 a ±0.33	38.00 a ±0.58	40.87 a ±0.58	18.51 a ±0.07	20.85 a ±0.65	12.62 a ±0.01	15.00 a ±0.11	1.77 a ±0.05	5.74 b ±0.03
SL	I_1	44.67 b ±0.33	54.12 a ±0.12	34.23 b ±0.07	38.00 b ±1.15	17.03 b ±0.24	20.19 a ±0. 24	11.03 ±0.01 c	13.83 b ±0.33	1.38 b ±0.01	5.37 c ±0.07
	I_2	40.00 c ±0..58	52.67 b ±0.33	33.63 b ±0.22	34.00 c ±0..58	16.82 b ±0.46	18.16 b ±0.21	10.99 c ±0.01	12.71 c ±0.31	1.29 c ±0.01	4.78 d ±0.07
LSD at 0.05 P		2.0	1.66	3.33	2.0	1.05	1.05	1.53	0.63	0.09	0.37

Non enzymatic antioxidants

The results presented in Tables 2 and 3 show that in most cases, the trends of variations in non-enzymatic antioxidants (under different soil types and water contents) were nearly similar during the two cuts under investigation. Despite the irrigation system and soil type, non-enzymatic antioxidants; i.e. total phenols and flavonoids (Table 2), ascorbic acid, and total antioxidant capacity (Table 3) were higher at the second cut, as compared with the first cut. The content of the above mentioned measured non-enzymatic antioxidants were higher in the leaves of the plants grown in the SL soil during the two cuts, as compared with those of corresponding plants in the SC soil. Despite the soil type (SC and SL), the levels of non-enzymatic antioxidants were higher with I_1 irrigation, as compared with those of corresponding plants irrigated twice every week.

Table 2: Effect of sandy clay soil (SC) or sandy loam soil (SL) and irrigation once/week (I_1) or twice/week (I_2), with each soil type, on total phenol compounds, expressed as mg gallic acid equivalents (GAE) g^{-1}d.wt., and total flavonoids, as mg quercetin equivalents (QE) g^{-1} d.wt., of air dried rosemary plants at the 1st and the 2nd cuts (3 and 9 months from transplanting, respectively). The results are expressed as means of three replicates ±SE. Statistical analysis was carried out using Duncan test. Different letters show significant variation at 0.05 P.

Treatment		Total phenol compounds (mg GAE g^{-1} d. wt.)		Total flavonoids (mg QE g^{-1} d. wt.)	
Soil type	Irrigation system	1st cut	2nd cut	1st cut	2nd cut
SC	I_1	171.13±0.45 c	237.91±1.53 c	111.35±2.97 b	139.33±0.24 c
	I_2	147.69±0.51 d	214.29±1.18 d	101.13±0.35 c	129.88±0.57 d
SL	I_1	193.07±0.05 a	301.50±0.46 a	124.34±1.94 a	149.71±0.11 a
	I_2	182.27±1.02 b	271.43±0.46 b	119.99±0.50 a	143.91±0.40 b
LSD at 0.05 P		10.80	23.62	8.64	4.58

Table 3: Changes in the content of ascorbic acid (ASA) and total antioxidant activity of fresh leaf extract of rosemary (*Rosmarinus officinalis* L.) plants at the 1st and the 2nd cuts (3 and 9 months from transplanting, respectively) as affected by cultivation in sandy clay (SC) or sandy loam (SL) soil and irrigation once/week (I_1) or twice/week (I_2) with each soil type. The results are expressed as means of three replicates ± SE; statistical analysis was carried out using Duncan. Different letters show significant variation at 0.05 P.

Treatment		ASA mg ml^{-1}		Total antioxidant activity of extract (mM L^{-1})	
Soil type	Irrigation system	1st cut	2nd cut	1st cut	2nd cut
SC	I_1	1.75±0.01 c	3.72±0.18 c	1.25±0.00 b	1.45±0.00 c
	I_2	1.47±0.03 d	3.23±0.29 d	0.95±0.01 c	1.42±0.00 d
SL	I_1	2.17±0.03 a	6.25±0. 27 a	1.36±0.01 a	2.05±0.00 a
	I_2	1.93±0.01 b	3.99±0.14 b	1.27±0.00 b	1.49±0.00 b
LSD at 0.05 P		0.17	0.28	0.09	0.03

Antioxidant enzymes

The activities of both the antioxidant enzymes peroxidase (POX) and catalase (CAT) were higher in the leaves of the plants grown in either the SC or SL soil at the 2nd cut, as compared with those at the 1st cut (Table 4).

The results showed that the activities of POX and CAT were higher in the SL soil and irrigation once per week (I_1) than those in case of I_2 irrigation at both cuts. Twice irrigation per week (I_2) of the plants grown in SC soil also, resulted in minimum values of the activities of POX and CAT during the 1st cut and the 2nd cuts.

Furthermore, the results obtained showed that I_1 irrigation system was more effective than I_2 irrigation in increasing the activities of POX and CAT in both SC and SL soil types during the two cuts (Table 4).

Table 4: Changes in the activities of the antioxidant enzymes peroxidase (POX) and catalase (CAT) (g^{-1} d.wt. equiv. hr^{-1}) of fresh rosemary (*Rosmarinus officinalis* L.) plants at the 1st cut and the 2nd cuts (3 and 9 months from transplanting, respectively) as affected by cultivation in sandy clay (SC) or sandy loam (SL) soil and irrigation once/week (I_1) or twice/week (I_2) with each soil type. The results are expressed as means of three replicates ± SE. Statistical analysis was carried out using Duncan test. Different letters show significant variation at 0.05 P.

Treatment		Enzyme activities (g^{-1} d.wt. equiv. hr^{-1})			
Soil type	Irrigation system	POX		CAT	
		1st cut	2nd cut	1st cut	2nd cut
SC	I_1	25.00±1.25 b	36.04±0.75 b	277.27±3.67 c	354.69±5.49 c
	I_2	24.85±2.64 b	25.56±1.55 c	115.11±4.63 d	262.78±7.22 d
SL	I_1	33.87±0.56 a	50.67±2.67 a	484.68±5.91 a	663.89±7.35 a
	I_2	32.43±0.94 a	40.43±0.94 b	370.97±1.86 b	416.94±0.70 b
LSD at 0.05 P		7.43	10.23	93.69	62.24

Oxidative enzymes

The data presented in Table 5 show that the activities of polyphenol oxidase (PPO), ascorbic oxidase (ASAO) and IAA oxidase (IAAO) enzymes of rosemary plants were higher at the 1st cut rather than the 2nd cut. The activities of these three enzymes were also higher in the leaves of the plants grown in the SC soil, compared to those grown in the SL soil. Maximum activities of both PPO and ASAO (at both the 1st and 2nd cuts) were observed in the leaves of the plants cultivated in the SC soil concomitant with the I_2 irrigation system. Generally, I_2 enhanced the activities of PPO and ASAO than I_1 irrigation. In case of IAAO enzyme, the activity of IAAO was increased under the I_1 irrigation than the I_2 system with the SL soil during both cuts and with the SC soil at the second cut, whereas a reverse situation was observed with the SC soil at the first cut, where the I_2 irrigation resulted in highest activity of IAAO than I_1 irrigation (Table 5).

Table 5: Changes in the activities of polyphenol oxidase (PPO), ascorbic acid oxidase (ASAO) and indoleacetic acid oxidase (IAAO) of fresh rosemary (*Rosmarinus officinalis* L.) plants at the 1st and the 2nd cuts (3 and 9 months from transplanting, respectively) as affected by cultivation in sandy clay (SC) or sandy loam (SL) soil and irrigation once/week (I_1) or twice/week (I_2) with each soil type. The results are means of three replicates ± SE. Statistical analysis was carried out using Duncan test. Different letters show significant variation at 0.05 P.

Treatment		Enzyme activities (g^{-1} d. wt. equiv. hr^{-1})					
Soil type	Irrigation system	PPO		ASAO		IAAO	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
SC	I_1	191.61±3.15 b	178.18±1.47 b	110.0±1.92 b	44.44±2.00 b	8.22±0.03 b	8.07±0.03 a
	I_2	218.40±3.23 a	197.81±2.91 a	180.0±3.85 a	89.90±2.68 a	8.90±0.02 a	7.24±0.09 b
SL	I_1	173.63±3.83 c	127.20±1.96 d	56.13±1.86 d	11.26±0.45 d	8.12±0.07 bc	7.06±0.06 b
	I_2	188.50±7.43 bc	167.68±1.43 c	64.59±1.99 c	18.05±0.70 c	7.94±0.10 c	5.94±0.16 c
LSD at 0.05 P		17.98	24.18	8.46	6.79	0. 27	0.84

Discussion

In our previous work [45], the results obtained concluded that irrigation twice per week (I_2) positively affected the growth and yield of essential oil (EO) of rosemary plants grown in sandy loam (SL) soil, but negatively affected those grown in sandy clay (SC) soil during the 1st cut (winter). On the other hand, I_2 irrigation was more fitting at the 2nd cut (summer), than irrigation once per week (I_1**)** in both soil types (SL and SC).

In the present work, it has been intended to elucidate some underlying biochemical components and enzyme activities concomitant with the applied treatments mentioned above. In this respect, since the cell membrane is maintaining cell turgor and physiological functions, lipid peroxidation was of prime importance, whereas electrolyte leakage (EL) has been widely used as a key parameter to estimate cell membrane stability [46,47]. The percentage of electrolyte leakage (EL) as a measure of cell membrane permeability (MP) and the concentration of malondialdhyde (MDA) as a product of lipid peroxidation damage by free radicals were found to be higher in leaves and roots of rosemary plants at the second cut than at the first cut (Table 1). The results obtained with hydrogen peroxide levels in leaves showed also comparable trends with those obtained with MP and MDA throughout the different treatments (Table 1). Thus, a negative correlation could be observed between rosemary growth rates and their EO yield (previous data of the present authors[45]), on one hand, and ML, MDA, H_2O_2 levels, on the other hand. Our results coincided to a wide extent with those obtained by other authors under different conditions as water stress [48, 28], salt stress [49,50, 51], and drought [52,15,53], where the growth rates and productivity of many plants were reversibly related with EL and MDA cellular levels. In our work, although the soil water contents during the two cuts (refer to page 6) could not be considered stressful in either the SC or the SL soil under any of the applied irrigation systems (I_1 or I_2), but the water in each case seemed to elicit different metabolic tunes in rosemary roots and leaves. In other words, for e.g., the increase in EL, MDA and H_2O_2 contents in the plants grown in the SL soil and subjected to I_1 irrigation at the second cut seemed likely to mimic a water stressful condition. According to [54, 55], the metabolic network of plants must be reconfigured under stress conditions in order to allow both the maintenance of metabolic homeostasis and the production of compounds that ameliorate the stress. To this extent we might conclude that even under non stressful conditions, information concerning EL, MDA or H_2O_2 would provide an immense prediction for determining the soil type and irrigation requirements for best growth and EO productivity of rosemary plants.

It should also be pointed that our results showed evidently higher levels of EL, MDA, and H_2O_2 in rosemary leaves than in roots, despite of the given treatment. In this respect, it should be tentatively prospected that plant leaves are predicted to display more metabolic adjustments, due to the crucial role of photosynthesis, than roots. Similar conclusions were attained by other authors although under stress conditions [55, 56, 57]. The activities of both the antioxidant enzymes peroxidase (POX) and catalase (CAT) showed: a) higher levels in the leaves of the plants grown in either the SC or SL soil at the 2nd cut than the 1st cut, b) higher values in the plants grown in the SL soil and irrigated once per week (I_1) than those irrigated twice per week (I_2) at both cuts, and c) minimum activities during the two cuts in the plants grown in SC soil with I_2 irrigation (Table 4). The results obtained showed that I_1 irrigation system (soil water content (%) =72.80, 64.35 in the SC soil at the 1st and 2nd cuts, respectively and 68.70, 56.31 in the SL soil at the 1st and 2nd cuts, respectively) led to higher enhancements of POX and CAT activities than I_2 irrigation in both the SC and the SL soils during the two cuts (Table 4). In this respect, in case of I_2 irrigation the soil water content (%) was 83.70, 71.17 in the SC soil at the 1st and 2nd cuts, respectively and 76.03, 61.75 in the SL soil at the 1st and 2nd cuts, respectively.

The activities of the oxidative enzymes (Table 5) showed trends that were generally opposite to those obtained with the antioxidant enzymes POX and CAT. Thus, whereas the activities of both enzymes were higher at the second cut, in the SL soil and I_1 irrigation system, those of oxidative enzymes were more enhanced at the 1st cut, in the SC soil, and I_2 irrigation system. Thus, during the two cuts, minimum activities of both polyphenol oxidase (PPO) and ascorbate oxidase (ASAO) were shown in the plants grown in the SC soil with I_2 irrigation, whereas maximum activities were recorded in the SC soil concomitant with the I_2 irrigation system. A more or less similar trend was also generally observed with the activity of indoleacetic acid oxidase (IAAO) that was higher under I_1 irrigation in the SL soil at both plant cuts and with the SC soil at the second cut, whereas a reverse situation was observed with the SC soil at the first cut, where the I_2 irrigation resulted in a higher activity of IAAO than I_1 irrigation (Table 5). Thus, it might be concluded that increased EL and MDA, i.e. enhanced membrane lipid peroxidation was concomitant with provoked activity of oxidative enzymes.

Therefore, it could be assumed that rosemary plants during either of the two cuts could modulate their antioxidant metabolites and antioxidant enzyme activities in order to counteract the activities of oxidizing enzymes and optimize plant growth and essential oil production, in response to soil types and soil water contents. Variations in the contents of phenol compounds and antioxidant potential of rosemary extracts under different environmental conditions have been also reported by many workers (e.g. [26, 27]). The increase in the accumulation of phenol compounds and ASA might be at least partially due to inhibiting their oxidation as a result of decreases in the activities of PPO and AAO [58]. However, such variations might be important to gain an insight to predict plant responses and strategies that cope with soil and water availability.

Conclusion

Rosemary (*Rosmarinus officinalis* L.) transplants were grown in sandy loam (SL) and sandy clay (SC) soils, each with irrigation once (I_1) or two (I_2) per week. Analyses were carried out at two cuts at February and August (3, 9 months from transplanting, respectively). Cell membrane leakage and lipid peroxidation were higher in leaves than in roots and in both roots and leaves at the second cut than at the first cut. The results obtained with hydrogen peroxide levels in leaves showed also comparable trends with those obtained with EL and MDA throughout the different treatments. A negative correlation was shown between rosemary growth rates and essential oil yield (Recorded previously [45]), on one hand, and ML, MDA, H_2O_2 levels, on the other hand. The contents of non enzymatic antioxidants (phenols, flavonoides, ascorbate) as well as the activities of antioxidant enzymes (peroxidase and catalase) were generally reversibly correlated with the activities of oxidation enzymes (polyphone oxidase, ascorbate oxidase, and indolacetic acid oxidase). Thus, it could be concluded that within the range of favorable environmental conditions, EL, MDA, and the network of antioxidant/ oxidant levels might be taken as key factors to speculate the best conditions for rosemary growth and productivity.

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