

Soil type and irrigation water contents affect carbohydrates, total soluble protein, mineral ion contents and phytohormone levels in rosemary (*Rosmarinus officinalis* L.)

Fatma Gharib¹, Safia Ghazi¹, Hebatallah Aly¹, and Seham Moustafa²

¹Department of Botany and Microbiology, Faculty of Science, Helwan University, Cairo, Egypt

²Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt.

*Corresponding author, E-mail: sehammoustafa@yahoo.com

Abstract: Cultivation in sandy loam (SL) soil and twice irrigation/ week (I₂) followed by SL and once irrigation /week (I₁) significantly increased the contents of total carbohydrate (TC) in leaves of rosemary (*Rosmarinus officinalis* L.) plants and the levels of the growth hormones indoleacetic acid (IAA), gibberellic acid (GA₃) and cytokinins (CKs), as compared to their respective levels in the plants cultivated in sandy clay (SC) soil. Generally, the TC and different minerals were higher at the 2nd cut (August), compared to the 1st cut (February), especially in the SL soil. Furthermore, the SC soil combined with I₂ irrigation system was more effective in increasing the total soluble sugars (TSS) and total soluble proteins (TSP) of rosemary leaves during the 1st cut, with a relatively higher abscisic acid (ABA) content and lower GA₃/ ABA ratios, as compared to corresponding plants supplied with I₁ irrigation system. The majority of mineral ions were higher in leaves of the plants grown in the SL soil during the two cuts, as compared with the SC soil. Decreasing the irrigation water to I₁, increased the Na⁺/K⁺ ratio in rosemary leaves grown in both SC and SL soils during the two cuts. In conclusion, rosemary plants could maximize their TC content and nutritive state in the SL soil combined with I₂ irrigation.

[Fatma Gharib, Safia Ghazi, Hebatallah Aly, and Seham Moustafa. **Soil type and irrigation water contents affect carbohydrates, total soluble protein, mineral ion contents and phytohormone levels in rosemary (*Rosmarinus officinalis* L.).** *World Rural Observ* 2016; 8(4): 1-9]. ISSN: 1944-6543 (Print); ISSN: 1944-6551 (Online). <http://www.sciencepub.net/rural>.1. doi:[10.7537/marswro080416.01](https://doi.org/10.7537/marswro080416.01).

Key words: Rosemary, Soil type, Irrigation system, Carbohydrates, Protein, Mineral ions, Phytohormones

1. Introduction

Rosemary (*Rosmarinus officinalis* L.) is one of the essential aromatic plants devoted to family Lamiaceae (Labiatae). It is an evergreen, perennial herb well cultivated in Egypt, available throughout the year and widely used in food processing as well as for pharmaceutical plant-based products (Moreno et al., 2006; Atsumi and Tonosaki., 2007). As a cultivated plant, rosemary growth and yield is mainly affected by both soil type (Abou-Leila et al., 1993; Devkota and Jha, 2009) and irrigation water content (Nicolás et al., 2008; Hassan et al., 2013). In our previous work by Gharib et al. (2016), variations in growth traits and yield of essential oil of rosemary have been studied in the plants grown in sandy clay (SC) and sandy loam (SL) soil types under irrigation once (I₁) or two (I₂) per week during two plant cuts; one in winter (February) and the other in summer (August). Consequently, a strategy for minimum irrigation water, in a certain soil type (particularly sandy soil), has been intended for maximum productivity.

Metabolic control in response to environmental conditions involves fine adjustments of carbohydrate and nitrogen fractions. Such responses help cells to restore chemical and energetic potential and are crucial to acclimation and survival (Fraire-Velázquez and Balderas-Hernández, 2013).

The photosynthetic efficiency, usually resulted from enhancement of photosynthetic pigments, and increase in carbohydrate levels are closely related to

the irrigation water quantity (Yousef et al., 2013; Mohamed et al., 2014; Gharib et al., 2016). But, this also depends on the irrigation water content, where a balanced amount is required to maximize the carbohydrate level of plants in different types of soils and at different ratios of water deficits (Hassan et al., 2013). Under different conditions, the ratio of soluble sugars to the value of total carbohydrate is important for plant adaptation in its growth medium (Yang et al., 2013; Nohong and Nampo, 2015). Total soluble protein content in roots and leaves of two maize varieties under drought stress first increased due to the expression of new stress proteins and then decreased due to a severe decrease in photosynthesis (Mohammadkhani and Heidari, 2008). Similarly, the content of soluble proteins in roots and leaves of summer maize decreased with increasing drought stress (Ti-da et al., 2006).

Plant growth and development also largely depend on the combination and concentration of mineral nutrients available in the soil, and changes in the climate and atmosphere can have serious effects in this respect, including changes in the availability of certain nutrients (Morgan and Connolly, 2013).

Soil plays a major role in determining the sustainable productivity of an agro-ecosystem and soil properties are responsible for the difference in nutrient status (Begum et al., 2015).

Water supply also influences the plant indirectly through its influence on the proportions of nutrients in the soil solutions; where the total amounts of elements increases with the water content (Metwally and Pollard, 2006). Mass flow of nutrients, in response to soil condition, have been studied by many workers from different points of views (Pirzadet al., 2012; Raza et al., 2013; He and Dijkstra, 2014). Under low water content, increase in Na^+/K^+ ratio is associated with drought tolerance in seedlings of sugar beet (*Beta vulgaris* L.) cultivars (Wu et al., 2014). In this respect, calcium (Ca^{2+}) could regulate K^+/Na^+ homeostasis in rice at low salinity by enhancing the selectivity for K^+ over Na^+ , reducing the Na^+ influx and efflux, and lowering the futile cycling of Na^+ . The N, P, K contents in rosemary herb were also affected by water content (Leithy et al., 2006; Hassan et al., 2013).

Phytohormones are molecules produced in very low concentrations and are important regulators of numerous developmental and physiological processes in plants (Wani et al., 2016).

Phytohormones are also known to play vital roles in the ability of plants to acclimatize to changing environments, by mediating growth, development, source/sink transitions and nutrient allocation (Morkunas et al., 2014; Fahad et al., 2015-b). In this respect, auxin is well-known to be involved in multiple plant growth processes and stress responses (Shi et al., 2014). In this respect, IAA signaling is able to induce a response to the change(s) in developmental or environmental stimuli (Iglesias et al., 2011; Peer et al., 2013).

Gibberellins (GAs) are important plant hormones that regulate diverse aspects of plant growth and development, probably through modulating the ABA signaling pathway (Dong Lei et al., 2013). More recently, GAs were shown to function via the regulation of DELLA proteins, which are negative regulators of GA signaling, and these proteins appeared to regulate ROS levels by controlling the expression of a subset of antioxidant genes (Xia et al., 2015). Reduction of GA levels and signaling has been shown to contribute to plant growth restriction on exposure to several stresses, including low water content (Colebrook et al., 2014). The GA_3/ABA ratio might represent the primary hormonal signal; the increase in GA_3/ABA ratio seemed to correlate with the improved metabolic activity and subsequent growth (Gharib et al., 2014).

Cytokinins may be involved in the regulation of plant adaptation to drought stress (Merewitz et al., 2011). Endogenous cytokinin (CK) biosynthesis, content, translocation, and activity decline in

response to lower water contents (Peleg and Blumwald, 2011; O'Brien and Benkova, 2013).

Abscisic acid (ABA) controls one of the fastest responses of plants to abiotic stress (Wilkinson et al., 2010; Peleg and Blumwald, 2011; Mehrotra et al., 2014; Sah et al., 2016). Under osmotic stress conditions ABA regulates root growth via an interacting hormonal network with cytokinin, ethylene and auxin (Rowe et al., 2016).

Thus, the present study aimed to evaluate the levels of total carbohydrates (TC), total soluble sugars (TSS), total soluble protein (TSP), mineral elements, and phytohormones in leaves of rosemary (*Rosmarinus officinalis* L.) under the influence of different soil types and irrigation water contents at the 1st and 2nd plant cuts (February and August, respectively).

2. Materials and Methods

Plant material

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

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. Rosemary was grown under two soil types and two irrigation water levels throughout the two cuts of the experiment. During the experiment, the minimum and maximum temperatures inside the greenhouse were 14.6°C and 32.1°C, respectively. Mean temperature and relative humidity were, 16.8°C and 56.25 %, during November and 23.8°C and 56.8%, during August. The pots were divided into four groups; each including thirty plastic pots (30 cm diameter and 18 cm in depth). Three uniform transplants (60 day- old) of rosemary were planted in each pot. Each pot was filled with one type of soil, i.e. either sandy clay (SC) or sandy loam (SL) soil. When the plants were well established, the irrigation system was applied once (I_1) or twice (I_2) / week with each soil type.

The pots were arranged in complete randomized block designs with the different treatments. Two cuts (3 and 9 months from transplantation) were taken for experimentation.

Determination of carbohydrate content

Total soluble sugars

A Known weight of dry powdered rosemary leaves was ground in 5 ml ethanol 70%, after centrifugation at 4000 rpm for 10 minutes, the supernatant was completed to a known volume by distilled water. Total soluble sugars were determined using anthrone technique as described by Umbrietet al. (1959). Six ml anthrone solution ($2 \text{ g L}^{-1} \text{ H}_2\text{SO}_4$

95%) were added to 3 ml sample and maintained on a boiling water-bath for 3 min. After cooling, the developed color was measured spectrophotometrically at 620 nm using spectrophotometer (Cecil CE. 1010). Standard curve of glucose was prepared and used for calculating the content of TSS in samples.

Total carbohydrates

Briefly, 30 mg of dry powdered leaves were hydrolyzed in 10 ml of 1N H₂SO₄, in digestion tubes, (80-90°C) for 8 hr. This was made up to a definite volume. Then the total soluble saccharides were determined as described above.

Total soluble proteins

A Known weight of dry powdered rosemary leaves was ground in 5 ml ethanol 70%, after centrifugation at 4000 rpm for 10 minutes, the supernatant was completed to a known volume by distilled water.

The procedure of Lowry et al.(1951) was followed. One ml rosemary extract was mixed with 5 ml freshly mixed solution (50:1 v/v) of 2% sodium carbonate in 4% sodium hydroxide and 0.5% copper sulphate in 1% sodium tartrate. The mixture was left 10 minutes before addition of 0.5 ml Folin and made up to a definite volume. The optical density of the mixture was measured spectrophotometrically after 30 minutes at 750 nm using Cecil CE 1010 spectrophotometer. Standard curve of bovine serum albumin was prepared and used for determination of the protein content in samples.

Determination of macro and micro elements

Mineral ion contents in air dry leaves of rosemary plants were estimated in the environment research Institute (SWERI), Agriculture Research Center, Giza.

Digestion of samples

The dried powder samples were wet digested as described in the method of Jackson (1973). The acid digest of the plant matter was analyzed for determination of nitrogen, phosphorus, potassium, sodium, calcium, iron and chlorine, according to the following methods:

Total nitrogen

Total nitrogen was determined using the modified Micro-Kjeldahl method according to AOAC (1980).

Phosphorus

Phosphorus was determined, using the vanadate molybdate method (Jackson, 1973). Phosphorus content was estimated by using molybdic acid to form phosphomolybdate complex, and then reduced with amino naphthol sulphonic acid to the complex molybdenum blue which was measured colourimetrically at 660 nm and calculated using a

standard curve of dihydrogen phosphate as recommended by Woods and Mellon (1941).

Potassium and Sodium

Potassium and sodium were measured using flame photometer (Atomic spectra AAS vario 6 according to Williams and Twine (1960).

Calcium, Iron and Magnesium

Estimation was carried out using Inductively Coupled Spectrometry Plasma (ICP) Model Ultima 2-Jobin Yvon.

Chlorine

Chlorine was measured by titration with 0.05 N silver nitrate using potassium chromate indicator (Boyle's Method).

Endogenous phytohormones

Phytohormones were analyzed at the Arid Land Agriculture Research (ALAR) and Services Center, Faculty of Agriculture, Ain Shams University. Ten grams fresh young leaves (following the top) of rosemary developed from different treatments at the first cut were used for the extraction of phytohormones according to a modified method described by Wasfy and Orrin (1975). The samples were ground in cold 80% ethanol, the macerated tissues were transferred to a flask with fresh ethanol and the volume was adjusted to 20 ml ethanol for 24 hours at 0°C and then was vacuum filtered through filter paper Whatman No 42. The residue was returned to the flask with a fresh volume of ethanol and stirred for 30 minutes with a magnetic stirrer, then filtered again. The procedure was repeated once more and the combined extracts were evaporated to the aqueous phase in a rotator flash evaporator.

To estimate the amounts of acidic hormones (fraction I), the aqueous phase (10-30 min) was adjusted to pH 8.6 with 1% NaOH and partitioned three times with equal volumes of ethyl acetate. The combined ethyl acetate fraction was evaporated to dryness and held for further purification. The aqueous phase was adjusted to pH 2.8 with 1% HCl and portioned three times with equal volumes of ethyl acetate. The remaining aqueous phase was discarded. The combined acidic ethyl acetate was reduced in volume (fraction 1), ready to high performance liquid chromatography (HPLC) for determination of acidic hormones (IAA, ABA, GA₃). The dried basic ethyl acetate fraction was dissolved in 80% ethanol. The ethanol was evaporated under vacuum, leaving an aqueous phase which was adjusted to pH 2.8 with 1% HCl and partitioned three times with 25-50 ml ethyl acetate. The ethyl acetate phases were combined and discarded. The remaining aqueous phase was adjusted to pH 5.5 with 1% NaOH and portioned three times with 50-100 ml water saturated n-butanol. All butanol phases were combined (fraction 2),

reduced in volume to 5 ml and stored at -20 °C until HPLC analysis for cytokinins. Injection of 10 µl into HPLC 510 was used for identification and determination of hormones using data model (Waters 746), detector (U.V Tumble Absorbance), and pump (HPLC 510). The chromatography was fitted (equipped with 3.9 × 300 µm Bond pack C18 capillary column). The HPLC was operated under temp 25°C. Standards of IAA, GA₃, ABA, benzyl adenine and kinetin were used. The retention time (RT) and the area of peaks of different phytohormones of authentic standards were used for the identification and characterization of peaks of samples under investigation.

Statistical 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 at P<0.05 that was denoted as being statistically significant for the means compared, using least significant difference (LSD) at p0.05.

Table 1: Effect of soil type (sandy clay soil (SC) or sandy loam soil (SL)) and irrigation once/week (I₁) or twice/week (I₂) with each soil type on total carbohydrate (TC), total soluble sugars (TSS) and total soluble protein (TSP) (mg g⁻¹ dwt equivalent) in leaves of rosemary (*Rosmarinus officinalis* L.) plants at the 1st and the 2nd cuts (3 and 9 months from transplanting, respectively). Statistical analysis was carried out using Duncan. Different letters show significant variation at p0.05.

Treatment		mg g. dwt. ⁻¹					
Soil type	Irrigation water content	TC		TSS		TSP	
		1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
SC	I ₁	81.07±0.71 ^c	98.42±0.42 ^d	26.76±0.10 ^b	37.10±0.37 ^a	331.80±0.81 ^b	765.61±1.78 ^a
	I ₂	67.18±0.54 ^d	102.19±0.71 ^c	28.50±0.32 ^a	36.10±0.22 ^b	343.86±3.01 ^a	755.58±1.94 ^b
SL	I ₁	99.60±0.42 ^b	130.94±0.10 ^b	25.98±0.55 ^c	29.49±0.18 ^c	288.40±1.64 ^c	754.38±1.72 ^b
	I ₂	115.15±0.19 ^a	135.64±0.13 ^a	25.01±0.31 ^d	27.19±0.06 ^d	227.95±2.42 ^d	676.62±4.71 ^c
LSD at p0.05		13.89	3.77	0.78	1.00	12.06	10.03

Mineral element contents (Figure 1)

Based on our experimental data in Figure 1, different minerals (N, K, Na and Ca as mg l⁻¹) were higher in the plants grown in SL soil during the two cuts, as compared with the SC soil. The highest values of K, Na, Ca and Cl were recorded by applying the I₁ irrigation system in SL soil, while the I₂ irrigation gave the highest value of N, P and Fe during the two cuts. In case of the SC soil, the levels of most measured minerals were lower with I₂ irrigation during the two cuts, while I₁ irrigation showed higher enhancement of minerals. Decreasing irrigation water to I₁, increased Na⁺/K⁺ ratio in rosemary leaves grown in both SC and SL soils during the two cuts.

Phytohormone concentrations (Figure 2)

The results presented in Figure 2 show the effect of soil types; i.e. sandy clay (SC) and sandy loam (SL) and their combinations with the irrigation

3. Results

Total carbohydrates, total sugars and soluble proteins (Table 1)

In both soil types (SC, SL) and irrigation systems (I₁ and I₂), the levels of total carbohydrates (TC), total soluble sugars (TSS) and total soluble proteins (TSP) in rosemary leaves were higher at the 2nd cut (August) rather than the 1st cut (February). Despite of the applied treatment, both TSS and TSP contents were reversibly correlated with the TC content. In the SL soil combined with I₁ and I₂ irrigation systems, the total carbohydrates were significantly increased accompanied by decreases in TSS and TSP in the leaves during the two cuts, as compared with corresponding plants in the SC soil. The data presented in Table 8 show that TC contents (at the 1st and 2nd cuts) were higher in the SL soil and I₂ irrigation system than those in case of I₁ irrigation. In case of the SC soil, TC content was higher with I₁ irrigation at the 1st cut but the reverse was observed at the 2nd cut, where I₂ irrigation showed higher enhancement of TC than I₁ irrigation and vice versa for TSS and TSP content during the two cuts.

systems as once or twice per week (I₁ or I₂, respectively) on endogenous phytohormones of rosemary (*Rosmarinus officinalis* L.) plants at the 1st cut (February).

The obtained data show that indole acetic acid (IAA), gibberellins (GA₃), kinetin (Kin), benzyl adenine (BA), were higher in the leaves of the plants grown in the SL soil, as compared with those of corresponding plants in the SC soil at the 1st cut. On the other hand, a reverse situation was observed with abscisic acid (ABA). Moreover, the above mentioned hormones as well as the GA₃/ABA ratios (59.20, 22.02, 81.78, 157.44 for SC+I₁, SC+I₂, SL+ I₁, SL+ I₂, respectively) were markedly higher in the SL soil, particularly with I₂ irrigation than those in case of I₁ irrigation, whereas a reverse trend was observed with ABA. In case of the SC soil, the different hormone fractions (IAA, GA₃, K and BA) and GA₃/ABA ratio were lower with I₂ than I₁ irrigation.

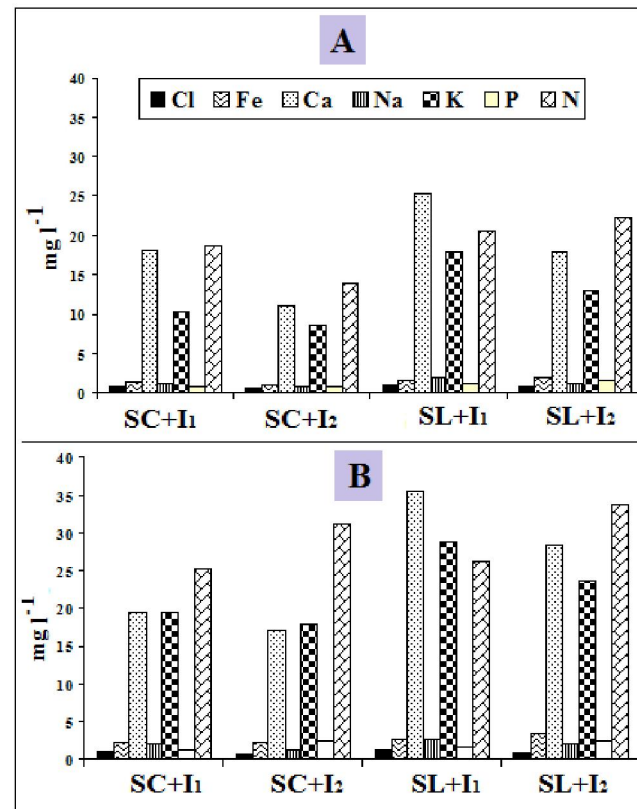


Fig 1: The element content of air dried leaf tissue of rosemary (*Rosmarinus officinalis* L.) plants at the 1st (A) and the 2nd cuts (B) (3 and 9 months from transplanting, respectively).

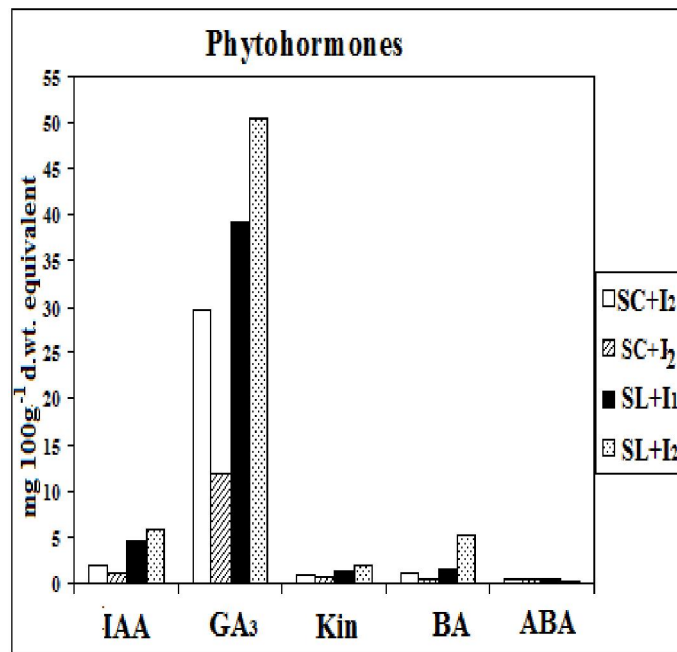


Fig 2 : Endogenous concentration ($\text{mg}/100\text{g}^{-1}$ dwt. equivalent) of indole acetic acid (IAA), gibberellic acid (GA_3), kinetin (Kin), benzyl adenine (BA), and abscisic acid (ABA) in young leaves of rosemary (*Rosmarinus officinalis* L.) plants at the 1st cut (5 month-old- plants) as affected by growing in sandy clay (SC) or sandy loam (SL) soil and irrigation once/ week (I_1) or twice/week (I_2) with each soil type.

4. Discussion

In this study, the levels of total carbohydrates (TC) were significantly enhanced in the sandy loam (SL) more than the sandy clay (SC) soil during the two plant cuts (February and August). In this respect, the SL soil combined with I_2 was the most effective in increasing the total carbohydrate contents of rosemary leaves at both cuts (Table 1). The increase in TC content might be explained on the bases of enhancement of photosynthetic capacity, predicted to result from increased carotenoids and total photosynthetic pigments as have been recorded in our previous work (Gharib 2016). In this connection, Mohamed et al. (2014) found a positive relation between the percentage of total carbohydrates and the content of irrigation water supply in two *Curcuma spp.* rhizomes grown in sandy loam soil. Similar conclusions were also attained by Yousef et al. (2013) in *Echinacea purpurea*. In the present work, SC combined with I_2 irrigation at the first cut (83.70% SWC) and combined with I_1 irrigation at the 2nd cut (64.35% SWC) caused a decrease in the total carbohydrate content. This could be attributed to minimization of the values of Chl a, b, Chl a/b ratio, carotenoids and total photosynthetic pigments in rosemary leaves (Gharib et al., 2016), where a declined photosynthetic efficiency would have been predicted. According to Hassan et al. (2013), carbohydrate percentage was increased by deficit irrigation treatments (decreasing irrigation frequency from 100 to 60% in rosemary plants grown in sandy soil. The contents of total soluble sugars (TSS) and total soluble protein (TSP) (Table 1) were higher in the SC soil, as compared with corresponding values in the SL soil during the 1st and 2nd cuts. The irrigation system I_1 increased the contents of TSS and TSP with SL soil type at the two cuts. This might be attributed to the speculated relation between the soil water content, TSS and TSP that are required for the plant osmotic adjustment. Our results could be reinforced by those of other workers who found that water stress treatments increased the soluble sugar contents of two grass species (Nohong and Nompo, 2015) and two Kentucky Blue grass cultivars (Yang et al., 2013), which would conclude that carbohydrate metabolism is important for plant adaptation.

The impact of soil types and irrigation on nutrient content of rosemary is not well documented; however, there are general conflicting reports about the effect of drought stress on nutrient absorption of different plant species (Ardakani et al., 2014). In the present study, the leaf contents of N, P, K, Na, Ca, Fe mg l⁻¹ and Cl (% ions) were higher at the 2nd than the 1st cut. This might be due to deeper and better proliferation of root biomass at the 2nd cut (Figure 1). Sandy loam soil was more effective in enhancing the

absorption of minerals than the SC soil, which might be attributed to the availability of sufficient aeration and moisture around the root in the SL soil. This would further enable better proliferation and absorption of root, reflected as better growth of rosemary in the SL than the SC soil as recorded in our previous work by Gharib et al. (2016). Decreasing the irrigation system to I_1 enhanced the content of K, Na, Ca and Cl, but decreased N, P and Fe contents in the leaves of rosemary plants grown in the SL soil at both cuts and in the SC soil at the 1st cut (Figure 1). These results might be partially reinforced by those of other workers who revealed increased K under lower water contents (Bahreinnejad et al., 2013), Na (Slama et al., 2007; Ma et al., 2012), Ca (Dogan and Akinci, 2011), and Cl (Peuke and Rennenberg, 2011), but reduced N, P (Raza et al., 2013). In well irrigated soil, Fe³⁺ was found to be reduced to the most available Fe²⁺ form for the plant, whereas under lower water content the soil relatively dries, the oxygen concentration increases, and Fe is oxidized to the insoluble ferric form (Pirzad et al., 2012). Additionally, under low soil moisture plant nutrient uptake is disturbed by reducing nutrient supply through mineralization (Schimel et al., 2007; Sanaullah et al., 2012). Nutrient diffusion and mass flow in the soil were also reduced in dry soil (Rouphael et al., 2012). Our results showed that in case of the SC soil, the level of most measured minerals were lower with I_2 irrigation during the two cuts that might be corresponding to over-irrigation at the first cut (February) and partial removal of nutrients from the soil by leaching, followed by reduced plant growth. On the bases of this conclusion, I_2 appeared to be an improper irrigation strategy for rosemary plants grown in the SC soil during winter. According to de Oliveira et al. (2013), plant growth can be limited by either water deficit or excess water. Excess water causes oxygen deficiency in soil, which affects nutrient and water uptake (Sairam et al., 2008).

The results of the present work also showed that in both soil types (SC and SL), decreasing irrigation water to I_1 , increased Na⁺/K⁺ ratio in rosemary leaves during the two cuts, which might be associated with osmotic adjustment and drought tolerance (Ma et al., 2012; Wu et al., 2014).

In the present study, at the first cut, the SL soil combined with I_1 or I_2 irrigation was much better than SC soil in enhancing the growth promoting hormones (Figure 2), expressed as indole acetic acid (IAA), gibberellic acid (GA₃), kinetin (Kin), benzyl adenine (BA), as well as GA₃/ABA ratios in the leaves of rosemary plants, as compared with corresponding plants in the SC soil, whereas a reverse situation was

observed with abscisic acid (ABA). Generally, increasing the irrigation level (I_2) in SL soil improved rosemary growth by increasing the GA_3/ABA ratios than in those with I_1 irrigation. The same base could be also applied with IAA under low water content, where IAA concentration was decreased but that was beneficial to inhibit vertical growth and avoid lodging for shade soybean (Zhang et al., 2011). In case of the SC soil, I_2 irrigation increased the level of ABA than I_1 irrigation at the 1st cut. This was also concomitant with decreasing the ratio of GA_3/ABA , which might represent the primary hormonal signal correlated with the decreased metabolic activity and subsequent growth of these plants. The difference in the mean values of growth hormones at the two types of soils (SC, SL) might be interpreted on the bases that at the first cut during winter (February), the water content was at a surplus in the SC soil through the I_2 system, which would allow excess water in such a non-porous soil with poor aeration. In accordance, under environmental stresses, ABA is an important messenger that acts as a signaling mediator for regulating the adaptive response of plants to different environmental stress conditions (Sah et al., 2016) and it might induce antioxidant defense systems and suppress toxicity of reactive oxygen species (ROS) under drought stress (Hu et al., 2010).

Conclusion

In the present work, soil types (SC and SL) and different irrigation water strategies (I_1 and I_2) were found to be important management techniques to cope with water scarcity. Generally, rosemary showed trends in the carbohydrate fractions, soluble protein, nutrient elements and different phytohormones that were more suitable in the SL type of soil combined with I_2 irrigation, particularly at the second cut, and seemed to positively affect plant efficiency rather than in the SC soil, during the two plant cuts.

References

Abou-Leila BH, Hussein MS, El-Sherbeny SE 1993. A comparative study on growth, yield and chemical composition of *Daturametel* L. grown in different soil types. Egypt. J. physiol. Sci. 17: 323-333.

AOAC 1980. Official Methods of Analysis of Anal. Chem., AmerChem Soc. 52(2), pp 148A. DOI: 10.1021/ac50052a726

Ardakani MR, Abbaszadeh B and Haghhigh ML 2014. The effect of drought stress on three clary sage (*Salvia sclarea* L.) populations from different habitats. J Biodiver Environ Sci (JBES). 5 (4):133-142.

Atsumi T and Tonosaki K 2007. Smelling lavender and rosemary increases free radical scavenging activity

and decreases cortisol level in saliva. Psychiatry Res. 150 (1): 89-96.

Bahreinejad B, Razmjoo J, Mirza M 2013. Influence of water stress on morpho-physiological flowering and some yield traits of coriander and phytochemical traits in *Thymus daenensis*. Int J Plant Produc. 7(1): 151-166.

Begum K, Sikder AHF, Khanom S, Hossain MF, Parveen Z 2015. Nutrient uptake by plants from different land types of Madhupur soils. Banglad J Sci Indust Res. 28(2): 113-121.

Colebrook EH, Thomas SG, Phillips AL, Hedden P 2014. The role of gibberellins in plant responses to abiotic stress. J Exp Biol. 217: 67- 75.

Devkota A and Jha PK. 2009. Variation in growth of *Centella asiatica* along different soil composition. Bot Res Int. 2 (1): 55-60.

Dogan, N and Akinci S (2011). Effects of water stress on the uptake of nutrients by bean seedlings (*Phaseolus vulgaris* L.). Fresenius Environ Bull. 20 (8a): 2163-2173.

Dong Lei Y, Wei Xin D, Ying Z, Zu Hua H 2013. Gibberellins modulate abiotic stress tolerance in plants. Scientia Sinica Vitae. 43 (12): 1119-1126.

Fahad S, Hussain S, Matloob A, Khan FA, Khaliq A, Saud S, Hassan S, Shan D, Khan F, Ullah N, Faiq M, Khan MR, Tareen AK, Khan A, Ullah A, Ullah N and Huang J 2015-b. Phytohormones and plant responses to salinity stress: A review-Plant Growth Regul. 75: 391-404.

Fraire- Velazquez S and Balderas- Hernandez VE 2013. Abiotic stress in plants and metabolic responses. In: Abiotic stress plant responses and applications in agriculture, K Vahdati and C Leslie (Eds.), Int Tech, Croatia. Pp.25- 48.

Gharib F, Ghazi S, Aly H, El-Araby M, and Moustafa S 2016. Effect of soil type and water content on rosemary growth and essential oil yield. Int J SciEngin Res. (IJSER). 7(6): 183-189.

Hassan FAS, Bazaid S, Ali EF 2013. Effect of deficit irrigation on growth, yield and volatile oil content on *Rosmarinus officinalis* L. Plant J Med Plant Stud. 1 (3): 12-21.

Hassan FAS, Bazaid S, Ali EF 2013. Effect of deficit irrigation on growth, yield and volatile oil content on *Rosmarinus officinalis* L. Plant. Journal of Medicinal Plants Studies. 1(3): 12-21.

He M and Dijkstra FA 2014. Drought effect on plant nitrogen and phosphorus: a meta analysis. New Phytol. 204: 924-931.

Hu XL, Liu RX, Li YH, Wang W, Tai FJ, Xue RL, Li CH 2010. Heat shock protein 70 regulates the abscisic acid-induced antioxidant response of

- maize to combined drought and heat stress. *Plant Growth Regul.* 60: 225–235.
- Iglesias MJ, Terrile MC, Casalagué CA 2011. Auxin and salicylic acid signaling counteract during the adaptive response to stress. *Plant Signaling and Behavior.* 6 (3): 452–454.
- Jackson ML 1973. *Soil Chemical Analysis*. Prentice Hall Indian Private Limited; M.97, Connght Citrus, New Delhi-1. Pp. 200–250.
- Leithy S, EL-Meseiry TA, Abdallah EF 2006. Effect of biofertilizer, cell stabilizer and irrigation regime on rosemary herbage oil yield and quality. *J Appl Sci Res.* 2 (10): 773- 779.
- Lowry OH, Rosenbrough NJ, Farr A. Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 193: 265-75.
- Ma Q, Yue LJ, Zhang JL, Wu GQ, Bao AK, Wang SM 2012. Sodium chloride improves photosynthesis and water status in the succulent xerophyte *Zygophyllum xanthoxylum*. *Tree Physiol.* 32: 4–13.
- Mehrotra R, Bhalothia P, Bansal P, Basantani MK, Bharti V, Mehrotra S 2014. Abscisic acid and abiotic stress tolerance- different tiers of regulation. *J Plant Physiol.* 171(17): 486–496.
- Merewitz EB, Gianfagna T, Huang B 2011. Protein accumulation in leaves and roots associated with improved drought tolerance in creeping bent grass expressing an ipt gene for cytokinin synthesis. *J Exp Bot.* 62 (15): 5311-5333.
- Mohamed MA, Wahba HE, Ibrahim ME, and Yousef AA 2014. Effect of irrigation intervals on growth and chemical composition of some *Curcuma* spp. plants. *Nusantara Biosci.* 6: 140-145.
- Mohammadkhani N and Heidari R 2008. Effects of drought stress on soluble proteins in two maize varieties. *Turk J Biol.* 32: 23-30.
- Moreno S, Scheyer T, Romano CS, Vojnov AA 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Res.* 40: 223–231.
- Morgan J and Connolly EL 2013. Plant-Soil Interactions: Nutrient Uptake. *Nature Educ Knowl.* 4(8): 2- 9.
- Morkunas I, Mai VC, Waśkiewicz A, Formela M, Goliński P 2014. Major phytohormones under abiotic stress. In: *Physiological Mechanisms and Adaptation Strategies in Plants under Changing Environment*. P Ahmad and MR Wani (Eds.), Springer. Pp.87-135.
- Nicolaś E, Ferrandez T, Rubio JS, Alarcoń JJ, Sańchez-Blanco M 2008. Annual water status, development, and flowering patterns for *Rosmarinus officinalis* plants under different irrigation conditions. *Hort. Sci.* 43 (5): 1580-1585.
- Nohong B and Nompo S 2015. Effect of water stress on growth, yield, proline and soluble sugar contents of Signal grass and Napier grass species. *Amer-Eurasian J Sustain Agric.* 9 (5): 14-21.
- O'Brien JA and Benkova E 2013. Cytokinin cross-talking during biotic and abiotic stress responses. *Front Plant Sci.* 4: 451-462.
- Peer WA, Cheng Y, Murphy AS 2013. Evidence of oxidative attenuation of auxin signaling. *J Exp Bot.* 64(9): 2629–2639.
- Peleg Z and Blumwald E 2011. Hormone balance and abiotic stress tolerance in crop plants. *Curr Opin Plant Biol.* 14: 290–295.
- Peuke AD and Rennenberg H 2011. Impacts of drought on mineral macro- and microelements in provenances of beech (*Fagus sylvatica* L.) seedlings. *Tree Physiol.* 31: 196–207.
- Pirzad A, Darvishzadeh R, Bernousi I, Hassani A, and Sivritepe N 2012. Influence of water deficit on iron and zinc uptake by *Matricaria chamomilla* L. *Chilean J Agric Res.* 72 (2): 232-236.
- Raza MAS, Saleem MF, Shah GM, Jamil M, Khan I H 2013. Potassium applied under drought improves physiological and nutrient uptake performances of wheat (*Triticum Aestivum* L.). *J Soil Sci Plant Nutr.* 13(1): 175-185.
- Rouphael Y, Cardarelli M, Schwarz D, Franken P, Colla G 2012. Effects of drought on nutrient uptake and assimilation in vegetable crops. In: *Plant Responses to Drought Stress*, R Aroca (Ed.), Berlin, Heidelberg, Germany: Springer. Pp. 171–195.
- Rouphael Y, Cardarelli M, Schwarz D, Franken P, Colla G 2012. Effects of drought on nutrient uptake and assimilation in vegetable crops. In: *Plant Responses to Drought Stress*, R Aroca (Ed.), Berlin, Heidelberg, Germany, Springer, 171–195.
- Rowe JH, Topping JF, Liu J, Lindsey K 2016. Abscisic acid regulates root growth under osmotic stress conditions via an interacting hormonal network with cytokinin, ethylene and auxin. *New Phytol.* 211(1): 225-39.
- Sah SK, Reddy KR, Li J 2016. Abscisic acid and abiotic stress tolerance in crop plants. *Front Plant Sci.* 7: (Article 571): 1-26.
- Sairam RK, Kumutha D, Ezhilmathi K, Deshmukh PS, Srivastava GC. 2008. Physiology and biochemistry of waterlogging tolerance in plants. *Biol Plant.* 52 (3): 401-412.
- Sanaullah M, Rumpel C, Charrier X, Chabbi A 2012. How does drought stress influence the decomposition of plant litter with contrasting

- quality in a grassland ecosystem? *Plant and Soil*. 352: 277–288.
- Schimel J, Balsler TC, Wallenstein M 2007. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88: 1386–1394.
- Shi H, Chen L, Ye T, Liu X, Ding K, Chan Z 2014. Modulation of auxin content in *Arabidopsis* confers improved drought stress resistance. *Plant PhysiolBiochem*. 82: 209–217.
- Slama I, Ghnaya T, Messedi D, Hessini K, Labidi N, Savoure A, Abdely C 2007. Effect of sodium chloride on the response of the halophyte species *Sesuvium portulacastrum* grown in mannitol-induced water stress. *J. Plant Res.*, 120, 291–299.
- Ti-da G, Fang-gong S, Li-ping B, Yin-van L, Guang-Sheng Z 2006. Effects of water stress on the protective enzyme activities and lipid peroxidation in roots and leaves of summer maize. *Agric Sci China*. 5(4): 101- 105.
- Umbreit WW, Burris RH, Statjfer JF 1959. *Manometric Techniques*, Burgess Publishing Co, Minneapolis. P. 173.
- Wani SH, Kumar V, Shriram V and Sah SK 2016. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *Crop J*. 4 (3): 162-176.
- Wasfy WS and Orrin ES 1975. Identification of plant hormones from cotton ovules. *Plant Physiol*. 55: 550–554.
- Wilkinson S and Davies WJ 2010. Drought, ozone, ABA and ethylene: New insights from cell to plant to community. *Plant, Cell and Environment*. 33: 510-525.
- Williams V and Twine S 1960. Flame Photometric Method for Sodium Potassium and Calcium. In: *Modern Methods of Plant Analysis*, K Peach and MV Tracey (Eds.), Springer-Verlag, Berlin, 3-5.
- Woods JT and Mellon MG 1941. Chlorostannous-reduced molybdophosphoric blue color method in sulfuric acid system. In: *Soil Chemical Analysis*, edited by Jackson M.L., 1958. Prentice-Hall International, Inc., London, UK. Pp 141–144.
- Wu G-Q, Wang C-M, Su Y-Y, Zhang J-J, Feng R-J, Liang N 2014. Assessment of drought tolerance in seedlings of sugar beet (*Beta vulgaris* L.) cultivars using inorganic and organic solutes accumulation criteria. *Soil Sci Plant Nutr*. 60 (4): 565-576.
- Xia X, Zhou Y, Shi K, Zhou J, Foyer CH, Yu J 2015. Interplay between reactive oxygen species and hormones in the control of plant development. *J Exp Bot*. 66 (10): 2839-2856.
- Yang Z, Xu L, Yu J, da Costa M, Huang B 2013. Changes in carbohydrate metabolism in two Kentucky blue grass cultivars during drought stress and recovery. *J Amer Soc Hort Sci*. 138(1): 24–30.
- Yousef RMM, Khalil SE, Said NAM 2013. Response of *Echinacea purpurea* L. to irrigation water regime and biofertilization in sandy soils. *World Appl Sci J*. 26 (6): 771- 782.
- Zhang J, Smith LD, Liu W, Xinfu Chen X, Yang W 2011. Effects of shade and drought stress on soybean hormones and yield of main-stem and branch. *Afr J Biotech*. 10(65): 14392-14398.

10/22/2016