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The effects of mannitol and salinity stresses on growth and biochemical accumulations in lemon balm

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ABSTRACT

The effects of saline irrigation water and mannitol on the growth and content of essential oil, photosynthetic pigments, soluble sugars, proline, Na, macroelements (N–P–K) and microelements (Mg–Zn–Fe–Mn) of lemon balm (*Melissa officinalis* L.) plants were investigated. Saline irrigation water decreased certain growth characters (total leaf area, leaf number, and total fresh and dry mass). The photosynthetic pigments (chlorophylls *a* and *b*, total carotenoids) and mineral content (N–P–K–Mg–Zn–Fe–Mn) also decreased as saline irrigation water level increased. Saline irrigation water promoted the accumulation of essential oil content and its main components (citronellal, citronellol and geranyl acetate) as well as total soluble sugars, proline and Na^+ contents. The plants treated with saline irrigation water × mannitol resulted in higher plant growth, essential oil, total soluble sugars, proline, macro and micronutrient values than those treated with saline irrigation water alone, photosynthetic pigments and Na demonstrated an opposite trend.

1. Introduction

Saline soil can be defined as soil having an electrical conductivity of the saturated paste extract (ECe) of 4 dS m^{-1} (4 dS m^{-1}–40 mM NaCl) or more. Salinity is a major factor reducing plant growth and productivity worldwide; it affects about 7% of the world's total land area [1,2] and is the major environmental factor limiting plant growth and productivity [3]. The detrimental effects of high salinity on plants can be observed at the whole-plant level such as the death of plants or necrosis of plant organs and/or decreases in productivity. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within cells [4]. During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected [5]. The earliest response is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies [5]. Growth often resumes when the stress is relieved. Carbohydrates, which among other substrates are needed for cell growth, are supplied mainly through the process of photosynthesis, and photosynthetic rates are usually lower in plants exposed to salinity, especially to NaCl [5].

Many researchers have studied the effect of soil salinity on growth, essential oil (EO) and chemical composition of several plants. For example, Abou El-Fadl [6] indicated that even though soil salinity more than 2000 ppm decreased peppermint (*Mentha arvensis*) plant growth, the EO yield and that of its components increased. By increasing the levels of soil salinity plant growth of *Ocimum basilicum* (basil) was significantly decreased but EO and its main components increased [7]. The same trend was found for the EO and its components of damsesea (*Artemisia absinthium*) plant which increased with increasing salinity levels [8]. Khalid [9] demonstrated that increasing salinity decreased plant growth of black cumin (*Nigella sativa*). Salinity significantly increased sage (*Salvia officinalis*) EO yield and its main components [10]. Parida [11] indicated that salt significantly decreased the carotenoids content of mangrove (*Aegiceras corniculatum*). *Salvinia natans* pigments were significantly reduced by 50 mM NaCl and higher [12]. Increasing soil salinity strongly increased *Rosmarinus offfcinalis* (rosemary) EO biosynthesis [13]. Nevertheless, investigations dealing with the effect of this stress on EO production are scarce although in general, it affects the composition and causes a reduction in yield of the EOs of medicinal and aromatic species [14,15].

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However, not all studies have shown a positive effect of salt stress on EO parameters: it significantly reduced the EO yield of lemon balm (*Melissa officinalis* L.) [16] and sage (*S. officinalis*) [17]. The highest dry weight and EO content of chamomile (*Matricaria chamomila*) plant were observed under non-salinity stress [18]. The major volatile compounds in *Coriandrum sativum* L. (coriander) leaves and the content of these compounds were affected differently by the saline levels [19]. These results from the literature indicate that in fact there is no safe-fast trend and that the effects of salinity on EO yield needs to be tested on an individual plant or species basis. The EO concentration in plant tissue under salt stress increased compared with untreated controls, suggesting that oil synthesis and/or oil degradation processes were less sensitive to salt stress than similar processes in peppermint (*Mentha* × *piperita* L.), pennyroyal (*Mentha pulegium* L.) and apple mint (*Mentha suaveolens* Ehrh.) [20]. Within the EO, the relative level of various constituents increased, decreased, or did not change in mint (*Mentha* sp.) plants under salt stress compared with non-stressed control plants [20].

Moreover, mannitol treatments differently affected the accumulation of some mineral and organic matter, mannitol treatments resulted (i) in a decrease in K^+ content, while (ii) the contents in Na^+ and in soluble sugars were modified, and (iii) in an increase in proline content of *Sesuvium portulacastrum* plant. It is reasonable to hypothesize that the observed restriction in K^+ acquisition by the whole plant and in K^+ accumulation in leaves was the result of some detrimental effect of the osmotic stress on the plant physiology [21].

Lemon balm (*Melissa officinalis* L.) belongs to family *Lamiaceae*, is a well known herb used to give fragrance to different food and beverage products. It has also been used as a medicinal plant for treatment of headaches, gastrointestinal disorders, nervousness, and rheumatism [22,23]. The essential oil is a well-known antibacterial and antifungal agent, and it is also responsible for the mild depressive and spasmolytic properties of the plant [24].

In this study, we investigate the possible effect of saline irrigation water and / or mannitol on the growth, EO content, photosynthetic pigments, soluble sugars, proline, Na, macroelements (N–P–K) and microelements (Mg–Zn–Fe–Mn) of lemon balm (*M. officinalis* L.), an important medicinal and aromatic plant.

2. Materials and methods

2.1. Experimental

Experiments were carried out in a greenhouse at the Shanghai Institute of Plant Physiology and Ecology (SIPPE), Shanghai, China, during 2007 and 2008. *M. officinalis* L. seedlings were obtained from the Ministry of Agriculture, China through the SIPPE. Uniform seedlings were transplanted into plastic pots (30 cm diameter and 50 cm height). In the first week of November 2007, the pots were transferred to a greenhouse adjusted to 35/24 °C, 90/60% RH day/night and light intensity ~3700 μmol m^{-2} s^{-1}. Each pot was filled with 10 kg of air-dried Typic Torrifluvents soil [25]. Physical and chemical properties of the soil used in this study were determined according to Jackson [26,27] and are presented in Table 1. Three weeks after transplanting, the seedlings were thinned to three plants per pot. *M. officinalis* L. plants were divided into two main groups. The first group was subjected to different levels of saline irrigation water: After 45 days from sowing plants were subjected to different levels of saline irrigation water, 0.39 (tap water as control), 1.56, 3.13 and 4.69 dS m^{-1}. To prepare irrigation water with different salinity levels, highly soluble NaCl salt were used. The salinity levels were obtained by addition of appropriate amount of NaCl to water and were adjusted by a portable Ec meter instrument. The second group was subjected to the same treatments but mannitol was added at 20 g l^{-1} saline irrigation water. Plants subjected to saline irrigation water or saline irrigation water + mannitol every 7 days however all pots were leached by tap water every 28 days (if there was no leaching when irrigating with saline water, it may induce salt build up in pots).

2.2. Harvesting

At the full bloom stage, all plants were harvested twice (first and second harvest) during the growing season, by cutting the plants 5 cm above the soil surface (after 45 and 90 days from transplanting, respectively). Total leaf area (cm^2), leaf number $plant^{-1}$, total fresh and dry mass (g $plant^{-1}$) were recorded.

2.3. Essential oil isolation

Fresh mass was collected from each treatment during the first and second harvest, and then 300 g from each replicate of all treatments was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus [28]. The essential oil content was calculated as a relative percentage (v/w). In addition, total essential oil g $plant^{-1}$ was calculated by using the fresh mass. The essential oils extracted from *M. officinalis* L. were collected during the first and second harvests from each treatment and dried over anhydrous sodium sulphate to identify the chemical constituents of the essential oil.

2.4. Gas chromatography–mass spectrophotometric (GC–MS) analysis

The ADELSIGLC MS system, equipped with a BPX5 capillary column (0.22 mm id × 25 m, film thickness 0.25 μm) was used. Analysis was carried out using He as the carrier gas, with a flow rate of 1.0 ml/min. The column temperature was programmed from 60 to 240 °C at 3 °C/min. The sample size was 2 μl, the split ratio 1:20; injector temperature was 250 °C; ionization voltage applied was 70 eV, mass range *m*/*z* 41–400 amu. Kovat's indices were determined by co-injection of the sample with a solution containing a homologous series of *n*-hydrocarbons in a temperature run identical to that described above.

2.5. Identification of essential oil components

The separated components of the essential oil were identified by matching with the National Institute of Standards and Technology

Table 1
Physical and chemical properties of the soil.

Sand (g kg^{-1})			Silt (g kg^{-1})			Clay (g kg^{-1})							Texture class
260			360			380							Clay loam
$CaCo_3$ (g kg^{-1})	Organic matter (g kg^{-1})	pH1. 2.5	Electronic conductivity (dS m^{-1})	Total (mg g^{-2})		Soluble cations and anions ($Cmol_c$)							
				P	N	Na^+	Mg^{++}	Ca^{++}	K^+	HCO_3^{-1}	Co_3^+	Cl^{-1}	SO_4^{-2}
45	13	77	0.57	9.35	90	2.23	0.88	1.11	1.48	1.12	0.73	2.1	1.62