Journal of Applied Research on Medicinal and Aromatic Plants xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

Morphological, physiological and phytochemical response of different *Satureja hortensis* L. accessions to salinity in a greenhouse experiment

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ARTICLE INFO

Keywords:
Accession
Essential oil
Phenolic compounds
Salinity
Satureja hortensis

ABSTRACT

Recently, water salinity is considered as an important factor affecting the quantity and quality of plant products. Plant response to stresses depends on the type of stress, stress intensity, plant species, etc. Many adaptation mechanisms have been developed in different accessions for coping with stress and completing the life cycle. This study is focusing on *Satureja hortensis* L., an annual herbal plant, which is accepted as a spice and traditional herbal medicine in Iran. The aim of this study is to investigate effects of salinity stress on the morphological, physiological, osmotic and phytochemical parameters of *S. hortensis* accessions. This experiment was arranged as factorial based on a completely randomized design with three replications. Factors were salinity stress, including 0 and 50 mM NaCl treatments and accessions including Rafsanjan, Zarand, Shahr-e Babak, Sirjan, Kerman, Baft, Jiroft, Bardsir and Kahnuj. All accessions showed significant reduction in their height, leaf area, shoot fresh weight (SFW), shoot dry weight (SDW), total chlorophyll (TChl), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids, K, Ca and significant enhancement in Na, Cl, proline, total soluble carbohydrate (TSC), total phenolic compounds (TPC), essential oil content and its main components under the salinity stress. Results also show that different accessions have different responses to the salinity stress. We also identified twenty five different compounds in this oil; the majority of them were carvacrol (19–46.6%), ϒ-terpinene (11.59–24.8%), p-cymene (9.84–34.56%), myrcene (1.4–2.78%) and β-pinene (1.20–1.91%). According to our results, Rafsanjan, Zarand and Kahnuj accessions showed more resistance to the salinity stress. Accumulating more osmolytes, essential oil, K and Ca, which caused more dry mass production, may cause increasing their resistancy toward the salinity stress.

1. Introduction

Summer savory (*Satureja hortensis* L.) is an annual plant, belongs to the Lamiaceae family and is native to the eastern Mediterranean region and western Asia (Silic, 1979). It is an annual medicinal plant which is regarded as a spice and traditional herb in Iran. Summer savory plants also have antispasmodic, antidiarrheal, antioxidant, sedative and antimicrobial properties (Gursoy et al., 2009). Beneficial effects of *S. hortensis* and its essential oil on the hypertension and carminative effects were also reported by Svoboda (2003). Thyme and carvacole are two major compounds of this essential oil, which have antiseptic, antifungal and anti-bacterial characteristics (Deans and Svoboda, 1989). Savory herb contains many vitamins, including B-complex group vitamins, vitamin-A, vitamin-C, niacin, thiamin and pyridoxine, which make it an excellent herb to be used for medicinal purposes (Jadczak, 2007).

Different factors like genetic and environmental condition can remarkably affect chemical constituents of medicinal plants and their morphological and physiological parameters (Heywood, 2002). Different environmental conditions can be the main reason of difference in morphological parameters, which is able to change plant's phenotype in a short term and plant's genotype in a long term (Saito and Matsuda, 2010). Abiotic environmental tensions such as salinity and drought stress, decrease plant growth and development significantly (Flowers and Muscolo, 2015; Khoyerdi et al., 2016). Over the last century, salinity has become a well-documented problem affecting agricultural production worldwide, particularly in the arid and semiarid regions (Alam et al., 2015b). Plant responses to salinity stress are complex and

Abbreviations: SDW, shoot dry weight; SFW, shoot fresh weight; TPC, total phenolic compounds; TSC, total soluble carbohydrate; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; TChl, total chlorophyll

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https://doi.org/10.1016/j.jarmap.2018.04.005
Received 27 August 2017; Received in revised form 11 April 2018; Accepted 24 April 2018
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highly depended to time and plant growth stage (Cramer et al., 2001). Salinity stress causes stomata closure and transpiration reduction. These changes will eventually decrease water potential in plant tissues; growth inhibition and reduction in the ion uptake, especially K and Ca. In response to these physiological changes, some morphological changes seem inevitable, such as reduction in leaf number and total leaf area (Vassileva et al., 2012). Salinity treatment has negative effects on morphological parameters and growth of *Mentha Canadensis* (Yu et al., 2015). Reduction of plant growth under salinity stress has been reported in lots of studies (Muscolo et al., 2015; Alam et al., 2015a). Najafi and Khavari-Nejad (2010) reported that the increased NaCl concentration reduced *S. hortensis* growth parameters significantly. This reduction was more remarkable in higher levels of salinity stress (75 and 100 mM NaCl).

Photosynthesis is one of the most important mechanisms affected by salinity stress. These effects happen as a result of changes in the enzyme activity and the reduction of chlorophyll and carotenoids (Stepien and Klbus, 2006). Chlorophyll content in stressed tissue could be considered as an indicator of salt tolerance. Therefore, genotypes with more photosynthetic pigments are more tolerant to salt stress (Ali et al., 2014). Turan and Tripathy, (2015) reported a significant reduction in chlorophyll and carotenoid synthesis in the rice plants under salinity condition.

Most plant species use the osmotic adjustment to reduce negative effects of salinity stress. Accumulation of the low molecular weight organic solutes, named osmoregulators, helps plant cells to maintain their turgor pressure (Chaves et al., 2003). Biosynthesis and accumulation of osmoregulators such as proline, soluble sugars, sucrose and phenolic compounds seem to contribute to the membrane stability (Javadi et al., 2008). Several studies have investigated the correlation between the accumulation of osmoregulators and salt tolerance (Ashraf and Tufail, 1995; Joe et al., 2016). Siddique et al (2015) reported that more stress tolerant species are able to store and produce more osmoregulators under stress condition. Salinity stress also has significant effects on the essential oil content and their composition in aromatic plants (Olfati et al., 2012). Several studies have been done on the effects of salt stress on essential oil and chemical composition (Baâtour et al., 2011; Mostajeran et al., 2014). Salinity stress causes salt tolerance and an increase in the crop yield under the salt stress condition through influencing biosynthesis and accumulation of terpenoid compounds (essential oil) (Witzel et al., 2014). Nowadays, superior species are being introduced according to their yield and resistance against different stresses (Uddin et al., 2011). Salt tolerant plants are determined by a number of morphological, physiological and biochemical parameters (Jacoby, 1999). These parameters can be used as a marker to determine resistant accessions toward salt stress. Therefore, the aim of this study is to evaluate the performance of *Satureja hortensis* L. accessions under salinity stress to select the tolerant accessions as an economical crop for savory cultivation. The savory tolerance screening was done based on some morphological, physiological and biochemical parameters.

2. Material and methods

2.1. Plant material and growth condition

Seeds of nine accessions of *S. hortensis* L. were provided from local gardeners around Kerman province, which located in the south-eastern of Iran (Table 1; Fig. 1). In order to determine the salt tolerant accessions, all accessions were planted in our research greenhouse (Tday: 23–25 °C; Tnight: 18-22 °C, RH: 50%). Seeds of 9 accessions were planted in plant culture boxes (45 × 32 × 23 cm) containing soil (45% sand, 24% clay and 31% silt) with pH 7.5, organic carbon 2.14% and EC1.69 ds m^{-1}. Twenty days after the germination 12 seedlings were kept in each box. During this period, seedlings were irrigated by distillated water when necessary. The salinity stress treatments were initiated 40 days after germination and continued until the end of the study. The NaCl (Merck, Darmstadt, Germany) treatment (salt stress) was applied with distillated water at two levels (0 and 50 mM). Control plants and plants under salinity condition were irrigated every 2 days with 500 ml distillated and saline water, respectively. Plants were harvested individually at flowering stage, at the end of salinity period.

Table 1
Geographical location collection site of accessions used in this study.

No	Location	latitude	Longitude	Altitude
1	Rafsanjan	30°37′09″	55°35′21″	1850
2	Zarand	30°48′40″	56°25′05″	2156
3	Shahr-e Babak	30°06′11″	55°06′11″	1845
4	Sirjan	29°26′11″	55°39′18″	1766
5	Kerman	29°58′53″	57°11′22″	2500
6	Baft	29°14′08″	56°36′42″	2040
7	Jiroft	29°16′45″	57°25′59″	2601
8	bardsir	29°48′38″	56°25′23″	3765
9	Kahnuj	27°31′34″	57°52′57″	2040

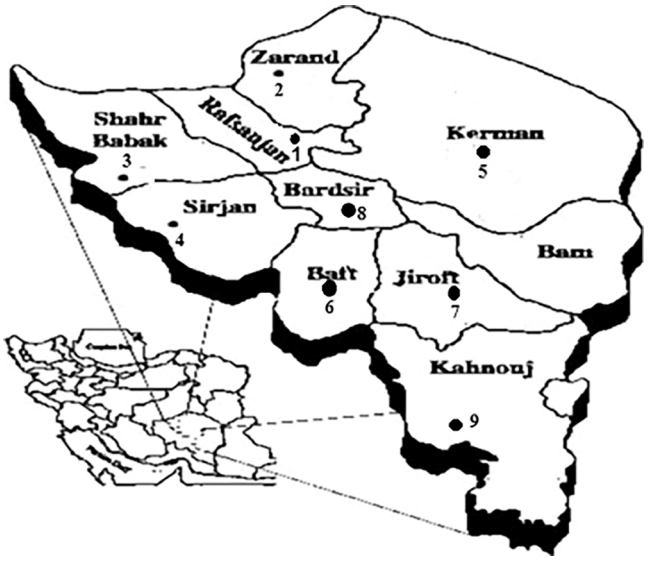

Fig. 1. The sites of *Satureja hortensis* L. accessions were collected from different parts of Kerman Province: 1) Rafsanjan, 2) Zarand, 3) Shahr-e Babak, 4) Sirjan, 5) Kerman, 6) Baft, 7) Jiroft, 8) Bardsir and 9) Kahnuj.

2.2. Plant growth and morphological characteristics

At the end of the experiment at flowering stage, the growth performance of *S. hortensis* plants was determined based on the plant height, stem diameters, leaf area, fresh and dry weight. Aerial parts of the plants were harvested from each plot and their fresh weight was recorded. All material was oven dried for 72 h at 50 °C for measuring dry weight. Leaf area was determined by the leaf area meter (CI-202, USA) before drying.

2.3. Total chlorophyll and carotenoids

In order to determine the chlorophyll (Chl *a, b* and total Chl) and carotenoid contents, 1 g of fresh extended leaves collected from the middle part of plants was homogenized with 10 ml of 80% aqueous acetone in mortar and pestle. After filtering, absorbance of the centrifuged extracts were measured at 480, 510, 645, 652, and 663 nm using the spectrophotometer model U-2000, Hitachi Instruments, Tokyo, Japan (Lichtenthaler, 1987).

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