Acta Ecologica Sinica xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Acta Ecologica Sinica

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

Salicylic acid changes morpho-physiological attributes of feverfew (*Tanacetum parthenium* L.) under salinity stress

Tahereh Mallahi [a], Mohammad Jamal Saharkhiz [a,b,*], Jamal Javanmardi [a]

[a] Department of Horticultural Sciences, College of Agriculture, Shiraz University, Shiraz, Iran
[b] Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Article history:
Received 20 November 2017
Received in revised form 24 January 2018
Accepted 7 February 2018
Available online xxxx

Keywords:
Antioxidant enzymes
Dry weight
Essential oil
Proline
Protein
Salinity stress
Salicylic acid

ABSTRACT

Feverfew (*Tanacetum parthenium*) (TP) is a valuable medicinal plant from Asteraceae family with various pharmaceutical and therapeutic properties. A pot experiment was conducted to evaluate the effect of salicylic acid (SA) on the physiological and morphological responses of TP under salinity stress. Salinity was induced by NaCl and $CaCl_2$ (2:1) at 30, 60, 90, 120, 150 and 180 mM levels. SA was applied as foliar application at 0, 200 and 300 ppm concentrations. Plant height, leaf and shoot number, fresh and dry weight and essential oil, starch, sugar, protein, proline, catalase (CAT), peroxidase (POD), and ascorbic peroxidase (APX) contents were as measured morpho-physiological traits. The results showed that SA significantly ($P \leq 0.05$) improved the measured traits and caused higher tolerance in TP plants under salinity stress. The essential oil content increased with increasing the salinity level up to 90 mM, which was more significant when combined with SA application. All of the measured traits except proline content, antioxidant enzymes, essential oil and sugar decreased at high salinity levels.

1. Introduction

Feverfew (TP) is a perennial herbaceous plant, native to Kazakhstan, Central Asia and the Mediterranean region, with a wide distribution in Europe, Asia and America [1,2]. It is distributed in various regions of the north, west, east and central Iran, and is found in Golestan, Mazandaran, Gilan, East Azerbaijan, West Azerbaijan, Tehran, Hamedan, Markazi and Yazd Provinces as a wild herb [3]. TP has been reported to contain many sesquiterpene lactones as major secondary metabolites, of which parthenolide is considered the major active component of the plant [4]. Parthenolide has multiple pharmacological properties such as anticancer, anti-inflammatory and cardiotonic effects. It has been historically used for the therapy of headache [5]. In Iran, this medicinal plant is cultivated for various therapeutic purposes such as for migraine headaches [3].

Different environmental conditions affect the medicinal plants' quality. The environmental factors have a great effect on the phytochemical composition of TP including sesquiterpene lactones, flavonoid, volatile oil and lipid contents in different plant organs. The highest parthenolide content as the main active ingredient in TP is in the plants receiving low-water regimens and enhanced light conditions before harvest [6].

Salinity stress is an important environmental abiotic factor that limits the plant's growth, development and production [7]. Plants can respond and adapt to salinity stress by several physiological, biochemical and molecular processes [8]. Soil salinity enhances the formation of reactive oxygen species (ROS), which activates both protective mechanism and cellular damages. Adverse effects of excess NaCl on growth, water and mineral uptake, photosynthesis rate and ROS overproduction have been previously reported on German chamomile (*Matricaria recutita*) [9].

Application of plant growth regulators plays an important role in the plants' responses to stress [10]. SA as a strong candidate for stress ameliorators has been recognized as a plant hormone [11]. It plays diverse physiological roles in plants, including plant growth, thermogenesis, flower induction, nutrient uptake, ethylene biosynthesis, stomatal closure, photosynthesis, enzyme activities [11], disease resistance and abiotic stress tolerance [12]. Experiments by Eraslan [13] revealed that exogenous application of SA on carrot plants grown under combined stress of salinity and boron toxicity, enhanced the overall growth and sulfur concentration as well as carotenoids and anthocyanin contents with a concomitant enhancement of the total antioxidant activity of both shoot and root. However, Pancheva et al. [14] found that the SA application regulated the proline accumulation and decreased the toxic ion (Cl, B) accumulation in the plant's shoot and root. SA acts as an antioxidant defense agent in plants through regulating physiological/biochemical processes, photosynthesis and stomatal closure under salinity and drought stress conditions [15]. The studies regarding the

* Corresponding author at: Department of Horticultural Sciences, College of Agriculture, Shiraz University, Shiraz, Iran.
E-mail address: saharkhiz@shirazu.ac.ir (M.J. Saharkhiz).

https://doi.org/10.1016/j.chnaes.2018.02.003
1872-2032/

increase of salinity and salt response in medicinal and aromatic plants are of prime interest. However, little information exists about the effect of salinity on medicinal plants and yet no information is available on the effect of SA on the physiological and morphological responses of TP under salinity. This study evaluates the effects of SA foliar application at different salinity levels to determine the tolerance range of salinity and whether SA removes oxidative damage due to salt stress in TP. To our knowledge, SA's interaction with basic plant physiological functions has not been investigated under salinity stress in TP yet. Therefore, the objective of this study is to determine the morpho-physiological responses associated with enhanced tolerance resulting from the application of SA to TP plants grown under salinity conditions.

2. Materials and methods

2.1. Project location and specifications

The experiment was carried out in the Research Greenhouse of Shiraz University, Iran. The polyethylene covered greenhouse equipped with temperature and relative humidity (RH) control system. The temperature and RH were set to 25 °C and 50–60%, respectively.

2.2. Plant materials and growth conditions

TP seeds were soaked in running water under red light treatment for 24 h to hasten germination. Then they were sown in trays filled with peat moss and perlite (2:1 v/v). In the next step, two numbers of seedlings with 2–3 leaves were transferred to 4-liter pots filled with the same substrate mentioned above and fed with half strength Hoagland solution. After planting of the seedlings, SA solution (at 0, 200 and 300 ppm concentrations) was applied twice as a foliar spray 48 h before and after the salinity treatments. Salinity was induced by NaCl and $CaCl_2$ (2:1) both at 0, 30, 60, 90, 120, 150 and 180 mM concentrations. The salinity levels were selected based on a preliminary test of the salinity tolerance of the species. Eventually, 45 days after imposition of different SA and salinity treatments, the plant samples were harvested in mid-vegetative stage. The plant species was identified and authenticated by Ahmad Reza Khosravi, a plant taxonomist at Shiraz University Herbarium, Shiraz, Iran. Voucher specimen was deposited in the herbarium.

2.3. Determination of morphological traits

Plant height, leaf length, leaf number, shoot number, and fresh and dry weights of TP plants were measured. In order to dry weight measurement, the selected plants were dried in an oven at 70 °C for 24 h and weighed.

2.4. Assessment of biochemical traits

Essential oils were extracted by water distillation method using Clevenger apparatus. Briefly, 30 g of the air dried herb was boiled in 1000 ml distilled water in 2-liter Clevenger flask. The extraction process continued for 3 h and had three replications. The essential oil content was measured as weight per dried weight (W/W %) [16]. The obtained EO samples were dried over anhydrous sodium sulfate and stored in sealed vials in dark at 4 °C. Soluble sugars in the leaves were measured using phenol sulfuric acid at 490 nm by a spectrophotometer (Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., USA) as described by DuBois et al. [17]. Starch content from the remaining material of the sugar test was measured spectrophotometrically at 630 nm [17]. Protein concentration was measured by Bradford [18] assay spectrophotometrically at 595 nm. Leaf proline content assay was based on Bates et al. [19] at 540 nm.

2.5. Antioxidant enzymes assay

Catalase activity was estimated by the method described by Dhindsa et al. [20]. Peroxidase activity was determined by the method described by Chanes and Mahely [21] spectrophotometrically with slight modifications. The activity of ascorbic peroxidase enzyme was assayed spectrophotometrically as described by Asada [22] at 290 nm after 1 min [16].

2.6. Statistics

The present study was conducted in a factorial experiment based on a completely randomized design with three replications and three sub-replicates. Data were subjected to analysis of variance (ANOVA) using SAS 9.1 SAS (Institute, North Carolina State University). The significance of difference among the treatment means was determined by Duncan's multiple range test at $P < 0.05$.

3. Results and discussion

Salinity is known as the most challenging problem that adversely affects the growth and development of all plants. The findings of the present study showed that with the increase of salinity levels in the growth media of TP plants, the plant's growth and development decreased significantly ($P < 0.05$) (Table 1). The interaction effects of salinity and SA showed some positive significant effects on the evaluated morpho-physiological traits of TP plants (Tables 1 and 2). According to Table 1, with an increase in salinity level, there was a reduction in leaf length, plant height, dry and fresh weight, and shoot and leaf number ($P \leq 0.05$), may be due to the negative effect of sodium chloride solution on the rate of photosynthesis, enzyme activity change (that subsequently affects protein synthesis), and also decrease in the level of carbohydrates and growth hormones, both of which can lead to the inhibition of growth [23]. In this study, for all morphological traits, the control level had the highest value except for leaf number. The maximum value for leaf number belonged to 30 mM salinity in combination

Table 1
Effect of salinity and salicylic acid on different morphological traits in Feverfew (*Tanacetum parthenium*).

Salinity (mM*)	SA** (ppm***)	Leaf length (cm)	Leaf number	Plant height (cm)	Dry weight (gr)	Fresh weight (gr)	Shoot number
0	0	12.83^{a}[1]	139.33^{b}	20.87^{a}	33.04^{a}	151.99^{a}	15.33^{a}
	200	13.12^{a}	139.66^{b}	21.19^{a}	32.9^{a}	152.43^{a}	15.67^{a}
	300	13.29^{a}	140.33^{b}	21.24^{a}	32.77^{a}	152.67^{a}	16^{ab}
30	0	10.17$^{e–h}$	134.33^{c}	16.63^{d}	28.02^{d}	130.22^{d}	13.67^{c}
	200	11.40^{c}	141.00^{b}	18.27^{c}	29.19^{c}	135.79^{c}	14.67^{b}
	300	12.57^{b}	148.67^{a}	19^{b}	30.35^{b}	145.47^{b}	15.33^{ab}
60	0	9.87$^{g–j}$	117.33^{ef}	15.27^{f}	25.45^{f}	121.58^{fg}	12.33^{d}
	200	10.81^{d}	126.33^{d}	15.87^{e}	26.72^{e}	124.01^{ef}	13.67^{c}
	300	12.80^{a}	131.00^{c}	17.03^{d}	27.89^{d}	125.97^{e}	14.67^{b}
90	0	9.07^{kl}	106.00^{h}	14.10^{g}	24.13^{g}	116.86^{hi}	10.00^{f}
	200	10.10$^{f–i}$	120.00^{e}	15.13^{f}	24.82^{fg}	119.49^{gh}	13.00^{cd}
	300	11.73^{c}	126.33^{d}	15.97^{e}	25.03^{f}	120.43^{g}	13.67^{c}
120	0	9.60^{ij}	96.67^{j}	13.47^{h}	22.21^{ij}	113.09^{jk}	8.33^{h}
	200	10.33$^{d–g}$	112.33^{g}	14.1^{g}	22.49^{i}	114.14^{ij}	11.00^{e}
	300	10.66^{de}	115.33^{fg}	14.97^{f}	23.24^{h}	115.25^{ij}	13.00^{cd}
150	0	7.33^{m}	90.00^{k}	12.43^{ij}	20.24^{kl}	97.59^{m}	7.33^{i}
	200	9.53^{jk}	99.00^{ij}	12.9^{hi}	20.54^{k}	106.24^{l}	9.33^{fg}
	300	10.60$^{d–f}$	107.67^{h}	13.37^{h}	21.56^{j}	110.59^{k}	9.67^{f}
180	0	6.97^{m}	83.00^{l}	11.1^{k}	16.26^{n}	76.35^{o}	5.33^{j}
	200	8.60^{l}	96.00^{j}	12.03^{j}	18.22^{m}	90.37^{n}	8.33^{h}
	300	9.77$^{h–j}$	102.00^{i}	12.27^{j}	19.72^{l}	93.55^{n}	8.67^{gh}

[1] Columns with the same letter are not significantly different as indicated by Duncan's multiple range test ($P \leq 0.05$).
* mM: millimolar.
** SA: Salicylic acid.
*** ppm: part per million.

Please cite this article as: T. Mallahi, et al., Salicylic acid changes morpho-physiological attributes of feverfew (*Tanacetum parthenium* L.) under salinity stress, Acta Ecologica Sinica (2018), https://doi.org/10.1016/j.chnaes.2018.02.003