

Salinity stress evaluation on Moldavian balm (*Dracocephalum moldavica* L.) under aeroponic system condition

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ABSTRACT: Salinity stress effect especially the highest concentration (2 dS m⁻²), was significant for all traits including plant height (PH), number of flowering branches (NFB), number of leaves per plant (NLP), stem diameter (SD), fresh shoot weight (FSW), fresh root weight (FRW), dry shoot weight (DSW), dry root weight (DRW), root length (RL), number of hairy roots (NHR), number of main roots (NMR), root diameter (RD), total chlorophyll content (TCC), chlorophyll a (Cha), chlorophyll b (Chb), carotenoid content (CC), root sodium content (RSC), leaf sodium content (LSC), leaf potassium content (LPC), protein amount (PA), proline magnitude (PM), peroxidase (POD), catalase (CAT) and decreased the measured traits compared to the control. NaCl at 2 dS m⁻² induced salinity reduced the number of leaves per plant by 43.58% compared to the control. The highest number of hairy roots (19.78) was observed in salinity treatment with 0.25 dS m⁻², which was accompanied by a significant decrease with increasing sodium chloride concentration to 2 dS m⁻². The total leaf protein content, proline accumulation and antioxidant activity of catalase and peroxidase at the highest salt concentration (2 dS m⁻²) showed a significant increase compared to control. The results of this experiment indicate that the tolerance of the herbaceous medicinal plant to salt stress is induced by increasing the accumulation of proline, soluble proteins and antioxidant enzymes activity.

KEYWORDS: *Dracocephalum moldavica*, Photosynthetic pigments, Salinity, aeroponics, Physiological traits

INTRODUCTION

Moldavian balm (*Dracocephalum moldavica* L.) as a perennial herb from the *Lamiaceae* is native to central Asia which is used in stomach and liver disorders, headache and congestion. Its chromosome number is $2n=2x=10$ and the active substances have medicinal properties [29]. Moldavian balm has been cultivated in eastern and central Europe and is frequently used as a food ingredient and a carditonic agent [8]. It is a hardy annual plant with aromatic, green foliage, the volatile oil content and its composition indicates variation in different regions. Moldavian balm is used in traditional medicine as a painkiller and for the treatment of kidney

complaints and plant extract is used in toothache, colds and antitumor. Crude extracts of the aerial parts of Moldavian balm are a number of constituents including flavonoids, iridoids and oleanolic acids and due to these medicinal components, its domestication and breeding is performing in many countries [28]. Dragonhead is used in folk medicine, as extracts and essential oil are used painkiller, toothache, anti-inflammatory, anticonvulsive and sedative properties as well as are used as a special beverage and food ingredient [32].

High concentration of salts suppresses the crop growth, the growth stages and their interaction and to overcoming

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Received: 14 January 2021/ Revised: 21 February 2021

Accepted: 27 April 2021

this problem, some characteristics such as seed germination is used for identification of tolerant plants [21]. Salinity tolerance is physiological and genetical process, but it has been suggested that emphasizing on trait-based selection is more efficient [9]. Pirzad et al. [22] showed the Moldavian balm salt tolerant plants under 0, 4, 8, 12, 16 and 20 dS m⁻² treatments had minimum decrease in index of germination, stalk and rootlet length, and wet weight of plantlet as well as dry weight of plantlet. Saberali and Moradi [22], naturally grew Moldavian balm in arid and saline soils and concluded it was tolerant to salinity up to 40 mM NaCl in seed germination and was suitable for cultivation in salt-affected areas. Shaikh-Abol-Hasani and Roshandel, [26] evaluated the effects of salicylic acid seed priming to increase salt tolerance in Moldavian balm under 0, 50, 100, 150, and 200 mM NaCl treatments and found that seed germination and antioxidant enzymes activities were increased at 100 and 150 mM NaCl treatments. Information about the level of Moldavian balm salt tolerance is restricted especially in antioxidant enzymes activities while such knowledge required as an aid in selecting medicinal species for cultivation in salt soils. The present study was performed to determine the effect of salinity stress on seedling growth of Moldavian balm in aeroponics system.

MATERIALS AND METHODS

Seeds of Moldavian balm (*Dracocephalum moldavica* L.), were obtained from Pakan Bazr Isfahan commercial supplier and were surface-sterilized for 5 min in 5% (v/v) sodium hypochlorite solution, and rinsed three times with deionized water. These seeds were planted in the plastic pots by 18×23 cm with 3 cm depth in mixed bed as 1:1:1 v/v by pit mass, perlite and sand. Similar and homage seedlings were transferred from the pot to aeroponics system as described by details in Movahedi and Rostami [18] at the eight to ten leaf stage after root cleaning and washing for salinity treatment application. Rooted seedlings were grown in an aeroponic cultivation system due to the opportunity of growing plants using small amounts of nutrient solution in a closed system and preparing easy procedures of sample collection. The plants were supplied with 1/3 Hoagland solution [13], when the average length of the roots reached 5-6 cm, the plants were supplied with a full Hoagland solution with the addition of salinity treatments (0.0, 0.25, 1.0 and 2.0

dS m⁻² NaCl) for 30 days. The computer controlled spraying schedule was: 20 s of spray at 20 min-intervals. This experiment was performed according to completely randomized design with three replicates. Plants were harvested and some traits measured consist on plant height (PH), number of flowering branches (NFB), number of leaves per plant (NLP), stem diameter (SD), fresh shoot weight (FSW), fresh root weight (FRW), dry shoot weight (DSW), dry root weight (DRW), root length (RL), number of hairy roots (NHR), number of main roots (NMR), root diameter (RD), total chlorophyll content (TCC), chlorophyll a (Cha), chlorophyll b (Chb), carotenoid content (CC), root sodium content (RSC), leaf sodium content (LSC), leaf potassium content (LPC), protein amount (PA), proline magnitude (PM), peroxidase (POD), and catalase (CAT). For units of the measured traits, refer to the tables.

Morphological traits were measured at 50 % flowering stage from 5 plants were randomly picked. Total chlorophyll (chlorophyll a+b) was measured according to the method of Lichtenthaler and Wellburn [14] while both sodium and potassium contents of tissue were determinations using extracted dry matter with hot water and measured by flame photometer (Fater electronic 405). Protein and Proline magnitude content were determined according to Bradford [5] and POD activity was performed and CAT activity was measured following the change of absorbance at 240 nm for 1 min due to H₂O₂ and both activities were expressed as unit per gram fresh weight (U g⁻¹ FW). Analysis of variance was performed by the statistical program SPSS version 18 and means statistical significance was compared with the Student's t-test (LSD, least significant differences), and differences were considered significant if probability values were 0.05.

RESULTS and DISCUSSION

The analysis of variance in morphological traits indicated salinity had significant effect on growth indices of Moldavian balm and all traits were significant with probability values as 0.01 (Results are not shown). Mean comparison of plant height (PH) showed different salinity concentrations decreased PH regarding control treatment as the least height (49.27 cm) was observed in 2.0 dS m⁻² salinity while the tallest plants (83.35 cm) were seen in control treatment (Table 1). In high concentration of salt, the growth process is suppressed due to osmotic effects of high salt which restricts the water absorption via roots [2].

Table 1. Mean comparison of morphological traits of Moldavian balm under salinity treatments

Salinity	PH (cm)	NFB (No)	NLP (No)	SD (mm)	FSW (g)	FRW (g)	DSW (g)	DRW (g)
Control	83.35 ^a	9.44 ^b	25.67 ^a	8.48 ^a	8.25 ^a	1.87 ^a	6.89 ^a	0.82 ^a
0.25 dS m ⁻²	70.02 ^b	11.76 ^a	19.67 ^b	5.34 ^b	5.07 ^b	0.97 ^b	6.29 ^a	0.59 ^b
1 dS m ⁻²	60.26 ^c	6.82 ^c	17.33 ^b	6.87 ^b	3.71 ^c	0.43 ^c	5.25 ^b	0.42 ^c
2 dS m ⁻²	49.27 ^d	6.24 ^c	10.67 ^c	4.11 ^d	1.36 ^d	0.19 ^c	2.84 ^c	0.15 ^d

Traits are: PH, plant height; NFB, number of flowering branches; NLP, number of leaves per plant; SD, stem diameter; FSW, fresh shoot weight; FRW, fresh root weight; DSW, dry shoot weight and DRW, dry root weight.

Different letters within columns indicate significant differences at $P < 0.05$ (LSD, $n = 4$).

Table 2. Mean comparison of some biochemical and morphological traits of Moldavian balm under salinity treatments

Salinity	RL (cm)	RD (mm)	NMR (No)	NHR (No)	Chla (mg g ⁻¹ FW)	Chlb (mg g ⁻¹ FW)	TCC (mg g ⁻¹ FW)	CC (mg g ⁻¹ FW)
Control	58.22 ^a	1.41 ^a	1.91 ^a	16.95 ^b	2.55 ^a	1.95 ^a	4.50 ^a	1.7 ^a
0.25 dS m ⁻²	55.79 ^b	1.19 ^b	1.59 ^c	19.78 ^a	2.19 ^a	1.64 ^b	3.83 ^b	1.3 ^b
1 dS m ⁻²	55.69 ^b	1.01 ^c	1.77 ^b	11.58 ^c	1.53 ^b	1.30 ^c	2.83 ^c	0.9 ^c
2 dS m ⁻²	35.45 ^c	0.97 ^c	1.56 ^c	9.29 ^c	1.01 ^c	1.09 ^d	2.10 ^d	0.7 ^c

Traits are: RL, root length; RD, root diameter; NMR, number of main roots; NHR, number of hairy roots; TCC, total chlorophyll content; Chla, chlorophyll a; Chlb, chlorophyll b and CC, carotenoid content.

Different letters within columns indicate significant differences at $P < 0.05$ (LSD, $n = 4$).

Salt existence in root zone causes the osmotic stress and disrupts cell ion homeostasis and toxic accumulation of sodium within cells cause hormonal changes and finally decreases the plant growth [6]. Our results are in good agreement with the report of Arzhe et al. [4], which indicated the plant height of Moldavian balm can meaningfully be affected by salinity and results in short plants.

The high number of flowering branches (NFB) was recorded 0.25 dS m⁻² salinity with 11.76 and the low NFB was seen in 1.0 and 2.0 dS m⁻² salinity treatments (6.82 and 6.24) (Table 1). It is clear that salinity stress encourages plants to using escape mechanism and early entering to reproductive phase, thus there is not proper time to produce normal number of flowering branches [20]. Comparison of number of leaves per plant (NLP) showed control with 12.67 leaves had the more NLP but the 2.0 dS m⁻² salinity treatment decreased it about 58% (Table 1). Daneshvar-Rad et al. [7] showed salinity stress decreased the number of leaves per plant and plant height in Moldavian balm under salinity stress. The thickest stem diameter (SD) with mean 8.48 mm was belong to control while the thinnest with mean 4.11 mm was belong to 2.0 dS m⁻² salinity treatment (Table 1).

The shoot and root biomass characteristics including fresh shoot weight (FSW), fresh root weight (FRW), dry shoot weight (DSW), dry root weight (DRW) significantly decreased with salinity increase similar to studies of

Akbari-Quzhi et al. [2] and Arzhe et al. [4]. Biomass characteristics are the most important indices of growth which is processed via the photosynthesis but in high levels of salt, the photosynthesis is decreased and reduction in photoassimilates transfer results in decreasing of total dry biomass. Mean comparison indicated that FSW, FRW, DSW and DRW were decreased from 0.25 to 2.0 dS m⁻² salinity treatments and most decrease was observed in 2.0 dS m⁻² salinity (Table 1). In other word, with increasing salinity level, the magnitude of the mentioned traits which related to biomass production is decreased significantly and statistical mean comparison verified this decrease properly.

The longest root length (58.22 cm) was seen in control and the shortest root length (35.45 cm) was seen in 2.0 dS m⁻² salinity treatment (Table 2). Relatively low decrease in 0.25 to 2.0 dS m⁻² salinity treatments can be result in natural defence mechanism of plant in salinity stress to avoid root shorten and maintain the ability to absorb water and nutrients. The shoot and root are the most important traits in salinity evaluation because the root takes water and nutrients and the shoot transfer them to the other parts, so the toxic effects of salinity can directly affect these sections [3]. Mean comparison of number of hairy roots (NHR) showed that the more NHR were seen in 0.25 dS m⁻² salinity and it decreased with increasing of salt concentration (Table 2).

Table 3. Mean comparison of some antioxidant enzymes and biochemical traits of Moldavian balm under salinity treatments

Salinity	RSC (mg kg ⁻¹ DW)	LSC (mg kg ⁻¹ DW)	LPC (mg kg ⁻¹ DW)	PA (mg kg ⁻¹ FW)	PM (mg kg ⁻¹ FW)	POD (U mg ⁻¹ protein)	CAT (mg g ⁻¹ protein)
Control	25.33 ^{ab}	13.33 ^d	20.2 ^c	0.61 ^b	0.40 ^c	0.17 ^d	0.04 ^c
0.25 dS m ⁻²	27.33 ^a	19.33 ^c	25.5 ^b	0.89 ^a	0.57 ^{bc}	0.28 ^c	0.03 ^c
1 dS m ⁻²	21.67 ^b	23.67 ^b	30.2 ^a	0.85 ^a	0.87 ^b	0.41 ^b	0.06 ^b
2 dS m ⁻²	23.12 ^{ab}	29.2 ^{ab}	14.3 ^{cd}	0.95 ^a	1.48 ^a	0.55 ^a	0.08 ^a

RSC, root sodium content; LSC, leaf sodium content; LPC, leaf potassium content; PA, protein amount; PM, proline magnitude; POD, peroxidase and CAT, catalase.

Different letters within columns indicate significant differences at $P < 0.05$ (LSD, $n = 4$).

It was interesting that, NHR of control was less than 0.25 dS m⁻² salinity and this temporary increase in NHR is a natural defence mechanism to overcome of plant to stress because in salinity conditions plants try to maintain them in ideal situation via increasing their abilities like root power. However, such effort causes to produce secondary metabolites and increasing essential oil quality [10]. The high number of main roots (NMR) with mean 1.91 was observed in control followed by 0.25 and 1 dS m⁻² salinity and the low NMR was seen in 0.25 dS m⁻² salinity (Table 2). In general, Moldavian balm tried to stand salinity stress by increasing its ability especially in root traits but with increasing in salt concentration this strategy was not effective and plant is affected by stress [31], so in this study the aeroponics system with preparing homogeny salinity caused to increase the Moldavian balm potential for salt tolerance but in high concentration and with accumulation of salt, this facility is gone or this advantage is lost. The control treatment had the thickest root diameter (RD) with mean 1.41 mm but 1.0 and 2.0 dS m⁻² salinity treatments had the thinnest RD (1.01 and 0.97 mm, respectively) (Table 2).

The mean comparison of total chlorophyll content (TCC), chlorophyll a (Chla), chlorophyll b (Chlb) and carotenoid content (CC) indicated that large magnitude of TCC (4.5 mg per fresh weight g), Chla (2.55 mg per fresh weight g), Chlb (1.95 mg per fresh weight g) and CC (1.7 mg per fresh weight g) were recorded in control and with salinity increase, remarkable decrease was seen in all of them (Table 2). The results are in agreement with reports of Yasar et al. [30] and Noreen and Ashraf [19] as salinity stress with increasing of chlorophyll degrading enzymes activity induce chlorophyll destruction and cause to loosing equilibrium of protein-pigment thus reduce chlorophyll and other pigments. According to Parida and Das [20], magnitude of photosynthesis related pigments such as chlorophyll and carotenoid are reduced under

salinity stress and we found similar results. Generally, chlorophyll reduction is a criterion for showing salinity stress damage to plant growth and development and its concentration variation is a quick reaction index for stress response. Also, in salinity stress, increasing sodium ion cause to chlorophyll degradation as well as magnesium shortage which cause to shortage in chlorophyll production [27]. Carotenoids act as light auxiliary receptors in photosynthesis and with blue light absorbing protect chlorophyll from oxidation as well as play an important role in the xanthophyll cycle and prevent chlorophyll degradation [25]. Another reason for the chlorophyll reduction due to salinity can be attributed to the limitation of nitrogen uptake under salinity stress by plant roots and restricts nitrogen amounts which essential for chlorophyll biosynthesis. Finally, carotenoid as biological antioxidants have important role in plant tissue protection and its increase with salinity increase can be part of plant defence protocols under salinity stress.

Results of mean comparison for sodium accumulation in leaves of Moldavian balm revealed that with increasing NaCl concentration from 0.25 to 2.0 , sodium accumulation is increased significantly in leaves and high amounts (29.2 mg per kg of dry matter) was seen in 2.0 treatment (Table 3). In contrast, sodium magnitude in roots was not affected by salinity stress while potassium amount of root was affected significantly by salinity treatments (Table 3). Also, potassium concentration in leaves was increased from 0.25 to 1.0 , but it decreased from 1.0 to 2.0 (Table 3). Relatively, similar results were reported by Heydarnezhad et al. [12] in evaluation of saltwort (*Seidlitzia Rosmarinus* L.) under salinity stress which they declared there was not a strong association between sodium and potassium amounts of shoot with root. Some of growth decrease can be devoted to sodium accumulation in leaves and this finding is verified in *Salvinia auriculata* under salinity stress [15]. According

to Saberali and Moradi [23], dragonhead has the most potential for successful germination and establishment in the saline environments in comparison to *Trigonella foenum-graecum*, *Satureja hortensis* and *Anethum graveolens* species. It seems that the decrease in potassium concentration in higher salt concentration is the abundance of sodium which cause to replacement of potassium with sodium and due to high absorption of sodium via roots, the shortage of potassium is happen. Also, the antagonistic relation between sodium and potassium can be regarded as another reason for this phenomenon.

The low amounts of protein amount (PA) with mean 0.61 mg g⁻¹ FW and proline magnitude (PM) with 0.40 mg g⁻¹ FW was seen in control and with salinity stress induction (from 0.25 to 2.0), PA and PM increased significantly but there are not any significant differences among salinity levels (Table 3). One of the plants' strategies in stress conditions is increasing defence components such as secondary metabolites and stressed plants have good tendency to metabolism of secondary metabolites whereas with biosynthesis of stress amino acids cause to accumulation of alkaloids [11]. Our results indicate that a significant increase in PA and PM of Moldavian balm can verify pervious findings. Also, with high concentration of PA and PM can prevent more salt absorption due to osmotic role of these components. The proline increase can be act as nitrogen source of osmotic components and is reported by many researches [22, 1, 16].

Mean comparison results of peroxidase (POD) and catalase (CAT) indicated that the highest POD activity (0.55 unit per mg protein) was seen in 2.0 and its lowest activity (0.17 unit per mg protein) was seen in control treatment and similarly the highest CAT activity (0.08 unit per mg protein) was seen in 2.0 and its lowest activity (0.04 unit per mg protein) was seen in control treatment (Table 3). Also, CAT activity was increased remarkably from control to 0.25 but it did not alter from 1.0 to 0.25 (Table 3). While under normal conditions, the production of reactive oxygen intermediates (ROIs) in plants is low salinity stress enhance them production which act as signals for the activation of stress-response and defense mechanism [16]. According to Shaikh-Abol-Hasani and Roshandel [26], salinity stress cause to increase in activity of some antioxidant enzymes like POD and CAT in Moldavian balm to aiding plant to undo the harmful effect of produced ROIs under stress condition. Understanding the mechanisms of salinity tolerance of Moldavian balm is crucial to detect tolerant genotypes and the

characterization of traits that contribute to tolerance could lead to the determination of specific traits for salt tolerance.

CONCLUSION

Overall, our results suggest that there are significant differences in all morphological and biochemical traits like sodium and potassium in Moldavian balm as well as significant differences between antioxidant defense enzymes when exposed to increasing salt concentrations. Increased POD and CAT activities suggest the tolerance capacity of the Moldavian balm to protect itself from oxidative damage which may be useful for elucidating the salinity tolerance mechanisms, and may provide useful strategies for reducing the salinity toxicity, especially when they are combined with field experiments.

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بررسی تنش شوری بر گیاه بادرشبو (*Dracocephalum moldavica*) در شرایط سیستم هواکشت

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چکیده

مطالعه حاضر به منظور بررسی اثر سطوح مختلف تنش شوری (۰، ۰/۲۵، ۱، ۲ دسی زیمنس بر متر مربع) بر خصوصیات مورفولوژیکی و فیزیولوژیکی گیاه بادرشبو (*Dracocephalum moldavica* L.) تحت شرایط سیستم هواکشت در قالب طرح کاملاً تصادفی با سه تکرار انجام شد. اثر تنش شوری به ویژه در بیشترین غلظت (۲ دسی زیمنس بر مترمربع) برای کلیه صفات شامل ارتفاع بوته، تعداد شاخه گلدار، تعداد برگ در گیاه، قطر ساقه وزن تر اندام هوایی، وزن تر ریشه، وزن خشک ساقه، وزن خشک ریشه، طول ریشه، تعداد ریشه‌های مویین، تعداد ریشه‌های اصلی، قطر ریشه، محتوای کلروفیل کل، کلروفیل a، کلروفیل b، محتوای کاروتنوئید، محتوای سدیم ریشه، محتوای سدیم برگ، محتوای پتاسیم برگ، مقدار پروتئین، مقدار پرولین، پراکسیداز و کاتالاز معنی‌دار بود و صفات اندازه‌گیری شده را نسبت به شاهد کاهش داد. کلرید سدیم در غلظت ۲ دسی زیمنس بر مترمربع باعث کاهش ۴۳/۵۸ درصدی تعداد برگ در بوته نسبت به شاهد شد. بیشترین تعداد ریشه‌های مویین (۱۹/۷۸) در تیمار شوری با غلظت ۰/۲۵ دسی زیمنس بر متر مربع مشاهده شد که با افزایش غلظت کلرید سدیم به ۲ دسی زیمنس بر متر مربع با کاهش معنی‌داری همراه بود. محتوای پروتئین کل برگ، تجمع پرولین و فعالیت آن‌تی‌اکسیدانی کاتالاز و پراکسیداز در بالاترین غلظت کلرید سدیم (۲ دسی زیمنس بر متر مربع) نسبت به شاهد افزایش معنی‌داری نشان داد. نتایج این آزمایش نشان می‌دهد که تحمل گیاه دارویی بادرشبو به تنش شوری با افزایش تجمع پرولین، پروتئین‌های محلول و فعالیت آنزیم‌های آن‌تی‌اکسیدانی القا می‌شود.

کلمات کلیدی: *Dracocephalum moldavica*، رنگیزه‌های فتوسنتزی، شوری، هواکشت، صفات فیزیولوژیکی