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Sodium Chloride Effects on Seed Germination, Growth and Ion Concentration in Chamomile (*Matricaria Chamomilla)*

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Abstract-*Matricaria chamomilla* is a medicinal plant that is widely cultivated in salt affected soils. This investigation was undertaken to study the effect of NaCl concentration on germination, and the physiological, biochemical and growth characteristics of chamomile. Seed germination and growth were studied at five NaCl concentration levels (0, 40, 80, 120 and 190 mM NaCl concentration). Increasing the level caused significant reduction in seed germination. Salt concentration up to 40 mM led to higher growth, but more than 40mM of NaCl caused significant growth limitation. Chloride and sodium ions increased significantly in various parts of the plant with salinity. Specifically, chloride ions were predominantly concentrated in the shoots whereas sodium ions were concentrated mostly in the roots. With increasing salinity rate, K and Ca concentrations significantly decreased in the shoot and root of *M. chamomilla* as compared to the control. There was a consistent decrease in K^+/Na^+ and Ca^{2+}/Na^+ ratios in the shoots and roots of *M. chamomilla*. Plants maintained considerably higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in the shoots than the roots, and the former ratio was significantly higher than 1 in a 40mM salinity level. No change in leaf proline concentration was observed up to 80 mM, but a sharp rise at higher salt levels occurred. Overall, based on the results, *M. Chamomilla* is a tolerant to moderately salt tolerant crop during its growth, and its response to salinity is associated with the maintenance of high K^+/Na^+ and Ca^{2+}/Na^+ ratios in shoots, the accumulation of Na^+ in roots, and proline accumulation in shoots.

Keywords: Chamomile, Germination, Growth, NaCl concentration, Physiology, Proline, salinity stress

INTRODUCTION

The interest in medicinal plant products has considerably increased all over the world due to the fact that many herbal medicines are free from side effects [7,37]. Among traditional potential herbs, *Matricaria chamomilla*, commonly referred to as the chamomile plant, is a member of the Asteraceae, and is native to Europe and

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Western Asia [49]. It is an annual herb that has escaped to the wild and is now naturalized on almost every continent [48]. Chamomile is known to be medically good for soothing, calming, relaxation, aching muscles, indigestion, acidity, hay fever, asthma, morning sickness, eczema, sore nipples, and exhaustion as well as being useful as a sedative, anti-inflammatory and anti-tenseness medicine [9,22-40].

Matricaria chamomilla has been cultivated in arid and semiarid regions of Iran. These areas are frequently affected by high salinity [33]. However, salt affected soils can be utilized by growing salt tolerant crops because such soil allows for the expansion of crop production to areas where conventional reclamation procedures are economically or technically limited [43]. Seed germination and early seedling growth are critical stages for the establishment of plant populations under saline conditions, although salt tolerance at germination seems to have no relation to the tolerance level during seedling growth [8,34]. Salinity reduces the ability of plants to take up water by lowering soil water potential [28]. Growth is also reduced due to the ion-specific effect such as high Na^+ and/or Cl^- concentrations in the soil solution resulting in 'ion toxicity' in plant tissues [28]. Inhibition of the uptake of nutrients such as K^+ and Ca^{2+} which cause nutrient imbalances in the plant might also impede growth [24].

Salt tolerance depends on a number of traits, expressed at several levels of organization [24, 28]. Sodium and K are two important ions involved in salt stress signaling. High concentrations of Na^+ cause nutrient imbalance, membrane disorganization, reduction in growth, inhibition of cell division and expansion [24, 30]. An apparent antagonistic relationship between Na^+ and K^+ has been distinguished [47]. Where sodium (Na^+) in high concentration is deleterious for plant growth, K^+ is one of the essential elements required by the plant in large quantities: potassium is required for maintaining osmotic balance, an essential co-factor for many enzymes, and it also has a role in the opening and closing of stomata [24, 30]. Calcium plays a vital nutritional and physiological role in plant metabolism [27]. Root growth and function may be restricted by high Na^+/Ca^{2+} [3].

Amino acid proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to the stabilization of sub-cellular structures (e.g. membranes and proteins), the scavenging of free radicals, and buffering cellular redox potential under stress conditions [6, 35].

In spite of reports on cultivating chamomile in saline soils, the performance of this plant on salinity conditions has not been well studied and documented. Thus, the effect of NaCl concentration on germination, and the physiological, biochemical and growth characteristics of chamomile were examined in this study.

MATERIALS AND METHODS

Seed Germination

This study was carried out in an experimental greenhouse at the Isfahan University of Technology, Isfahan, Iran. Seed germination percentage of chamomile was studied at five concentrations of NaCl (0, 40, 80, 120 and 190 mM NaCl concentration). Salinity levels were selected according to pretreatments. A total of 1000 chamomile seeds were sterilized with 0.5% sodium hypochlorite solution for 1 min and then, washed twice with distilled water. The experiment was arranged in a completely randomized design (CRD). Four replicates of 50 seeds were used for each NaCl concentration level. The seeds were placed on one layer of filter paper in 90 mm petri dishes. Germination was carried out in a germination chamber with a regime of 12 h light at 20°C. Distilled water or fresh salt solutions were added periodically keeping the filter paper wet during the 21 days of the experiment. Seeds with roots 2 mm long were considered as germinated. The number of germinated seeds was counted daily and expressed as the percentage of the total seeds.

Determination of Proline and Ions Concentration in Plant

Chamomile growth was investigated at five salinity levels achieved by using irrigation water with salinities of 0, 40, 80, 120 and 190 mM NaCl, respectively. The solutions were prepared by adding NaCl to half strength Johnson nutrient solution [17]. The final concentrations of NaCl in the nutrient solutions were achieved after two weeks and continued during 70 days. The pH of the nutrient solution was adjusted to 5.5 by adding KOH or HNO_3 as needed. The nutrient solutions were renewed every 15 days during the growing period. The experiment was set up in a completely randomized design with four replications. Plants were harvested at the flowering stage, separated into roots and shoots, and their fresh weights determined. The shoots and roots were washed twice with distilled water and dried at 85°C for 48 h to determine their dry weights. To determine shoot and root concentrations of Na, K and Ca, the dried samples were ground, ashed at 550^0C for 8 h, the ashed material then being dissolved in HCl [12]. Concentrations of Na and K in the digest solutions were measured on a flame photometer and Ca was measured by atomic absorption. The chloride concentration of Cl was determined by $AgNO_3$ titrimetric procedure [12].

Free proline was extracted from 0.1 g of fresh shoot samples in 3% (w/v) aqueous sulphosalicylic acid and was estimated by ninhydrin reagent [13].

Statistical Analysis

Data were analyzed by Analysis of Variance using the SAS software program. The mean values were compared using the least significant difference test (LSD).

RESULTS AND DISCUSSION

Increasing the salinity level resulted in significant decreases ($P<0.05$) in seed germination of *M. chamomilla* (Figure. 1). The lowest germination percentage was observed at the 190 mM NaCl treatment where less than 10% of the seeds were germinated. In all treatments, maximum seed germination was achieved 6 days after sowing (Figure. 1).

Plant growth significantly increased with increasing NaCl concentration up to 40 mM but decreased at higher NaCl levels (Table 1). In fact, the highest and lowest shoot and root dry weight were found in the 40 and 190 mM NaCl treatments, respectively. There was no significant difference in the root and shoot dry weights between the control and 80 mM NaCl treatments; however, the adverse effect of NaCl was more pronounced on root biomass as compared to shoot biomass (Table 1). Based on the results of a preliminary experiment, the growth of *Matricaria chamomilla* was totally inhibited at 250 mM NaCl concentration (data not shown).

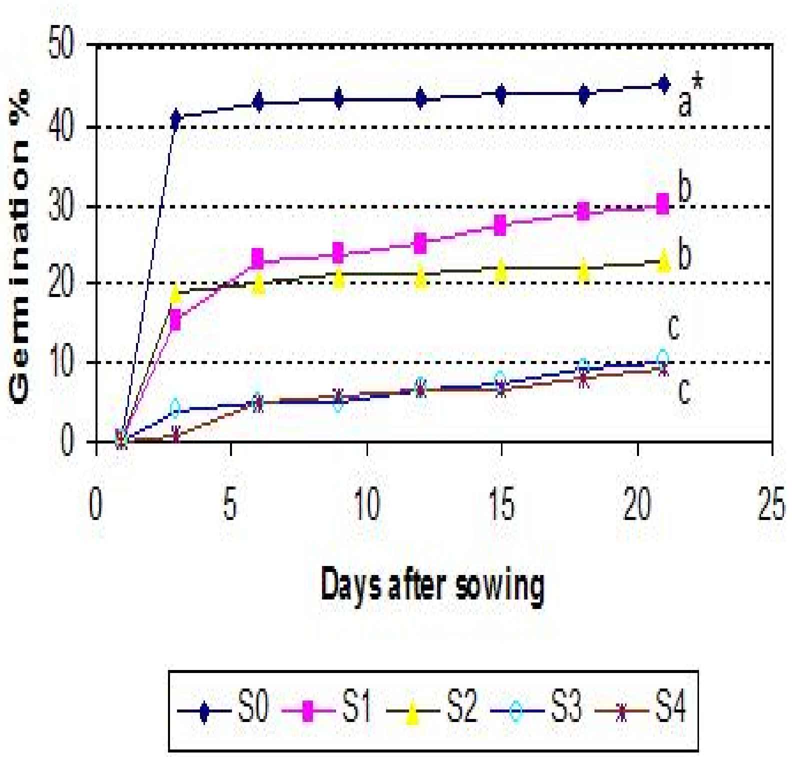

Fig. 1. Germination percentage of *Matricaria chamomilla* seeds and the seed germination versus time as influenced by salinity. S0 to S4 represent different levels of osmotic potentials of 0, -0.175, -0.358, -0.541, -0.716 MPa, respectively. * Treatments with the same letters, are not significantly different at $P < 0.05$

The chloride and sodium ions increased significantly in various parts of the plant with NaCl levels and were maximum at the highest NaCl concentration level, i.e. 190 mM (Table 1). Chloride ions predominantly concentrated in the shoots; whereas sodium ions concentrated mostly in the roots. A progressive decrease in the concentrations of K^+ and Ca^{2+} in both shoots and roots of *M. chamomilla* was found with an increase in the concentration of NaCl in the growth medium (Table 1). However, concentrations of these two ions were more in the shoots than in the roots at all external salt levels.

No significant difference was found in the shoot K concentration among different treatments when NaCl concentration increased (Table 1). The 190 mM NaCl treatment significantly decreased the root K concentration while no significant decrease was found at the lower salinity rates. Although Ca concentration decreased significantly in the shoot and root with the application of NaCl, the shoot Ca content between the control and the 40mM treatments was not significantly different. There was a consistent decrease in K^+/Na^+ and Ca^{2+}/Na^+ ratios in the shoots and roots of *M. chamomilla* with increasing NaCl concentrations of the rooting medium (Table 1). Plants maintained considerably higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in the shoots as compared to the roots, and the former ratio was significantly higher than 1 in the 40mM NaCl concentration level.

No change in leaf proline concentration was observed up to 80 mM, but there was a sharp rise in its concentration at the higher salt levels, i.e. 120 and 190 mM of NaCl (Table 1).

Table 1. Averages of root, shoot dry weights (g/plant), Na^+ (%), K^+ (%) , Ca^+(%), Cl^-(%), K^+/Na^+ and Ca^+/Na^+ ratios in root and shoot and proline (μm/g Fresh Weight) in shoots of M. chamomilla as influenced by salinity

	NaCl rate (mM)					MS^1
	0	40	80	120	190	
			Shoot			
Dry Weight(g/plant)	6.19^{b}	7.95^{a}	5.65^{bc}	5.08^{bc}	4.18^{c}	7.93^{*}
Na(%)	0.11^{c}	2.78^{b}	4.87^{a}	5.75^{a}	6.30^{a}	25.74^{*}
K(%)	4.11^{a}	2.95^{b}	2.82^{b}	2.84^{b}	2.62^{b}	1.14^{**}
Ca(%)	0.211^{a}	0.177^{ab}	0.133^{bc}	0.109^{bc}	0.094^{c}	0.01^{**}
Cl(%)	0.943^{c}	11.360^{b}	14.333^{ab}	19.037^{ab}	21.902^{a}	264.19^{***}
K/Na	45.55^{a}	1.06^{b}	0.66^{b}	0.50^{b}	0.41^{b}	1612.16^{***}
Ca/Na	2.585^{a}	0.064^{b}	0.033^{b}	0.019^{b}	0.014^{b}	5.215^{**}
Proline (μm/g F.Wt)	2.94^{c}	8.97^{c}	16.27^{bc}	30.48^{ab}	33.96^{a}	720.27^{***}
				Root		
Dry Weight (g/plant)	2.62^{bc}	4.68^{a}	3.35^{b}	3.06^{bc}	1.90^{c}	4.23^{*}
Na(%)	0.08^{c}	4.78^{b}	5.92^{ab}	6.12^{ab}	6.49^{a}	28.04^{*}
K(%)	3.64^{a}	3.29^{a}	3.09^{a}	2.80^{a}	1.33^{b}	3.20^{*}
Ca(%)	0.283^{a}	0.156^{b}	0.129^{b}	0.097^{b}	0.07^{b}	0.03^{***}
Cl(%)	0.433^{b}	9.851^{a}	11.360^{a}	14.162^{a}	14.801^{a}	133.7^{***}
K/Na	46.91^{a}	0.72^{b}	0.57^{b}	0.44^{b}	0.20^{b}	1724.32^{***}
Ca/Na	3.551^{a}	0.033^{b}	0.022^{b}	0.015^{b}	0.011^{b}	9.97^{***}

[1] Mean squares obtained from analyses of variance

A trend of decreasing germination percentage with higher NaCl concentrations was found. The germination was highly inhibited at 120 and 190mM NaCl concentrations (Figure. 1). Similar results were observed in many studies on Phaseolus species [1] and *Catharanthus roseus* [57]. Salinity stress can affect seed germination through osmotic effects and cause ion toxicity [15] or changes in metabolic activity [31]. Physiological studies to distinguish between the two effects are limited, but evidence suggests that low water potential of the germination medium is a major limiting factor [10].

Increasing the NaCl concentration up to 40 mM increased shoot and root dry matter yield (Table 1). It has been reported that the optimal growth of halophytic plants take places in low salt concentrations [21], while high salinity levels will cause a distinct decrease in plant growth. Such an adverse effect of salt stress on growth has been observed in a number of crops earlier, e.g. alfalfa [26], carrot [30], and ajwain [7]. The reduction in shoot dry biomass at the highest NaCl concentration level (190 mM) with respect to the control was about 32%. According to Maas and Hoffman [2] this crop can be categorized as tolerant to moderately salt tolerant.

As in most other glycophytes, a consistent increase in Na^+ and Cl^- and a decrease in K^+ and Ca^{2+} in both shoots and roots of *M. chamomilla* were found with an increase in the NaCl concentration of the growth medium. The increase in Na^+ and Cl^-concentrations in shoots and roots (as the result of NaCl-salinity) is a common and expected response, and has been reported many times in the literature [20,32,45].

The Na concentration increased in all plant parts with increasing the NaCl concentration of the growth medium (Table 1). The highest Na accumulation was observed in roots, especially for the 40 mM salinity which showed the highest growth rate. Investigations on salt-sensitive plants like *Pumica granatum* [44] and recently on red raspberry [20] revealed that Na accumulation in shoots could be inhibited by the accumulation of this ion in the lower part of the shoot and roots. Such plants that accumulate some Na^+ in their roots and exclude it from the shoots are referred to as Na^+ excluders. Regulation of Na^+ uptake by cells and long distance Na^+ transport seems to be a crucial adaptation of plants to salt stress [5, 51]. Such Na retention in the roots plays an important role in the adaptation of the glycophytes under salt conditions [24], even though the avoidance of Na accumulation in shoots of glycophytes is normally limited especially under long term conditions. This particular pattern may be suitable in our case as well. Strategies to reduce Na^+ accumulation in the shoot may be the control of xylem loading, the retrieval of Na^+ entering the xylem before reaching the bulk of the shoot and the recirculation of Na^+ back to the roots by the phloem [14]. Even though there is no study reporting on critical leaf Cl and Na concentration ranges in chamomile, in the present study the Na concentration remained at low, non-toxic levels in shoots. According to Bernstein and Hayward [42], for most crops the salts' toxic levels are similar. This points out that the salt injury effect, observed in the present study, could be attributed to Cl toxicity and Na could be a factor affecting water relation in the roots.

Chloride ions are absorbed at higher rates as compared to Na ions [20, 11]. As a result, the concentration of Cl is higher than that of Na in plant tissues, possibly causing leaf damage [24] in plants like citrus [54]. This seems to be the case in the current study with chamomile as well (Table 1). Chloride accumulation is probably the main factor for reduced growth and yield in citrus [54] and avocado [,23,53], supporting the results obtained in this study. In accordance, Feierabend et al. [50] reported that Cl ions at toxic levels can cause blockage in the electron transport within photosystem II, and Saied et al. [55] found that the dark reactions in photosynthesis were relatively more impaired by salinity than the light reactions.

Howevery Corresponding author author r, reasonable amounts of both K^+ and Ca^{2+} are required to maintain the integrity and functioning of cell membranes [11, 19, 16]. The underlying mechanism for the maintenance of adequate K^+ in plant tissues under salt stress seems to be dependent on selective K^+ uptake and selective cellular K^+ and Na^+ compartmentation and distribution in the shoots [5,39]. In fact, it is possible that a high K^+ / Na^+ ratio is more important for many species than simply maintaining a low concentration of Na^+ [56], which makes sense given that much of the basis for Na^+ toxicity is because of its competition with K^+ for K^+-binding sites. However, high K^+/Na^+ selectivity in plants under saline conditions is considered as one of the important selection criteria for salt tolerance [4,6,18,24,30,34,51,52]. Furthermore, the maintenance of Ca acquisition and transport under salt stress is also an important determinant of salinity tolerance [29]. The relationship between salt tolerance and Ca^{2+} retention among different plant species was investigated by Unno et al. [29]. Although both K^+/Na^+ and Ca^{2+}/Na^+ ratios decreased consistently in the shoots and roots of *M. Chamomilla* after increasing the NaCl concentration level of the growth medium, both ratios were markedly higher in shoots as compared to the roots. At the 40mM NaCl concentration level, the shoot K^+/Na^+ ratio was greater than 1, which is a minimum level suggested for the normal functioning of most mesophytes under saline conditions [4]. The maintenance of higher K^+/Na^+ and

Ca^{2+}/Na^{+} ratios in shoots by *M. chamomilla* could be an important component of its salt tolerance.

In the present study, proline concentration in the shoots of *M. chamomilla* increased, particularly at higher external NaCl concentration levels. In salt tolerant and relatively salt tolerant plants like, *Beta vulgaris* [46], and alfalafa [41] sharp increases in proline levels were reported under salinity conditions. This shows the positive role of proline in the salt toleration of this crop, in as much as proline is known to contribute to membrane stability [34] and mitigate the effect of NaCl on cell membrane disruption [58]. In addition, Greenway and Munns [24] were of the view that proline is related to survival rather than to growth maintenance. Increase in proline due to salinity has also been reported in some medicinal plants [25,7] .

Osmotic adjustment is an effective phenomenon of salinity tolerance in many crops. Ashraf et al. [6] found that salt tolerance of cowpea (*Vigna unguiculata*) was associated with its higher capacity of osmotic adjustment by accumulating proline and inorganic ions such as Na^{+}, K^{+} and Ca^{2+}. From the results obtained by increasing tissue Na^{+} and Cl^{-}, and proline concentrations in *M. Chamomilla* under saline conditions, it could be concluded that these osmotica very likely played a substantial role in the osmotic adjustment of the plants under saline conditions, although the contribution of organic osmotica to an osmotic adjustment other than proline was not determined in the present study.

In general , during growth, *M. Chamomilla* is a tolerant to moderately salt tolerant crop and its response to NaCl is associated with the maintenance of high K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios in shoots, accumulation of Na^{+} in roots, and proline accumulation in shoots.

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اثرات سدیم کلراید بر جوانه زنی، رشد و غلظت برخی از یون ها در گیاه داروئی بابونه(*Matricaria Chamomilla*)

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چکیده - کشت گیاه داروئی بابونه (*Matricaria chamomilla*) در زمین های شور بطور گسترده ای انجام شده است. این پژوهش به منظور بررسی تأثیر غلظت های مختلف سدیم کلراید بر جوانه زنی و پاسخ بیو شیمیائی، رشد و غلظت برخی از عناصر در بابونه صورت گرفت. مطالعه جوانه زنی و رشد در پنج سطح سدیم کلراید با غلظت های ۰ ، ٤٠، ۸۰، ۱۲۰ و ۱۹۰ مولار نمک انجام گردید. با افزایش میزان غلظت سدیم کلراید محلول، درصد جوانه زنی به طور معنی داری کاهش یافت. رشد گیاه بطور معنی داری تا غلظت ٤٠ میلی مولار نمک افزایش ولی در غلظت های بیشتر از آن کاهش یافت. غلظت یون های کلرید و سدیم در بخش های مختلف گیاه با افزایش شوری بطور معنی داری بیشتر شد. تجمع یون کلرید بطور غالب در شاخسار و یون سدیم بطور عمده در ریشه ها مشاهده گردید. با افزایش غلظت سدیم کلراید در محلول، غلظت یون های پتاسیم (K) و کلسیم (Ca) بطور معنی داری در ریشه و ساقه بابونه آلمانی نسبت به شاهد کاهش نشان دادند. همچنین، کاهش نسبت های K^+/Na^+ و Ca^{2+}/Na^+ در ریشه و ساقه بابونه مشاهده گردید. نسبت K^+/Na^+ و Ca^{2+}/Na^+ در اندام هوایی نسبت به ریشه ها افزایش قابل ملاحظه ای را نشان داد، بطوری که نسبت K^+/Na^+ بطور معنی داری در غلظت ٤٠ میلی مولار بیشتر از یک بوده است. غلظت پرولین برگ تا تیمار ۸۰ میلی مولار نمک تغییری را نشان نداد اما در غلظت های بالاتر نمک مثل ۱۲۰ و ۱۹۰ میلی مولار افزایش شدیدی نشان داده است. بطور کلی، بابونه آلمانی دارای درجه تحمل"متوسط" تا "مقاوم" به شوری در دوره رشد بوده که این مقاومت از طریق تامین غلظت های بالای K^+/Na^+ و Ca^{2+}/Na^+ در ساقه، تجمع Na^+ در ریشه و تجمع پرولین در ساقه می باشد.

واژه های کلیدی: بابونه، پرولین، تنش شوری، جوانه زنی، رشد، غلظت سدیم کلراید، فیزیولوژی

***به ترتیب استادیار، استاد و استاد**

****مکاتبه کننده**