Journal of Medicinal Plants Research Vol. 5(30), pp. 6609-6614, 16 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.034

Full Length Research Paper

Salinity effects on germination and growth of chamomile genotypes

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Accepted 22 August, 2011

Our objectives were to investigate the effects of salinity on germination and growth of two chamomile species by measuring yield and yield components of Four genotypes of *Matricaria recutita* and four genotypes of *Matricaria aurea*. The genotypes were cultivated in sand in a greenhouse and irrigated with additional nutrient solutions. The treatments included salinity (NaCl) levels of control, 0, 6, 12, and 18 dS m^{-1} and two salinity periods; one of them started at seedling stage (35 days after emergence with plants at 8 to 10 leaves) until the end of the experiment (about three months), the other on another part of plants began in stem elongation and seedlings emergence from rosette stage to harvest (vegetative to the end of the expriment) (about 1.5 month). This experiment was carried out as a split-split plot with three replications on the bases of Complete Randomized Design (CRD). The traits measured were plant height (PH), root length (RL), the number of leaves (LN) per plant, node numbers (IN), stem fresh weight and dry weight (SFW) and root fresh weight (RFW) and dry weight (RDW). The salt treatments indicated that dry matter yield decreased with increasing sodium chloride (NaCl) doses. The dry matter yields were higher in control than those in the 18 dS m^{-1} NaCl levels. Either the dry matter yields were much higher in stem formation than the early seedling stage period. *M. aurea* were superior to *M. recutita* genotypes based on dry matter. Simple correlation coefficient of dry matter (DM) yield components showed that positive and highly significant relationships existed between DM yield with PH, RL, IN, LN, SFW, RFW, RDW and RRW. Path analysis showed that plant height, root fresh weight and stem fresh weight, had strong positive direct effect, in that order node number, stem relative water (SRW) and root dry weight had strong negative direct effect. There was a significant difference between genotypes studied for all traits except for the root relative water (RRW) content. The *M. aurea* genotypes, especially in Isfahan and Mashhad, revealed more tolerance to salinity.

Key words: *Matricaria recutita, Matricaria aurea*, salinity, path analysis, salt tolerance.

INTRODUCTION

Chamomile *(Matricaria recutita)* is a very important medicinal plant species (Salamon, 1992; Ghanavati, 2007). *Matricaria aurea* (syn: *Chamomilla aurea*) is another species of chamomile (Ghanavati, 2007). The use of chamomile dates back 2500 years to ancient Egypt. In 500 B.C., Hippocrates, the founder of modern medicine in ancient Greece, recognized the therapeutic properties of chamomile. Ancient Egyptians, Romans, Greeks and Iran used chamomile flowers to relieve colic (Isaac, 1989). Pharmacological properties include anti-inflammatory, antiseptic, carminative, healing, sedative and spasmolytic activity (Salamon, 1992). About 120 chemical constituents have been identified in chamomile as secondary metabolites, including 28 terpenoids, 36 flavonoids and 52 additional compounds with potential pharmacological activity (Mann and Staba, 1992).

There is a serious concern that plant growth and yield are affected by water salinity. Based on salt tolerance studies under greenhouse conditions on a rice cultivar in California, the lowest effective salinity levels in nutrient solutions affects the seedling growth and survival. Substantial loss in plant growth and final yield reduction

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were also observed in the salt-affected rice fields (Scardaci et al., 1996; Shannon et al., 1998). Salinity problems in salt-affected fields might be relieved by developing appropriate management options for rice growers. Salinity is one of the major abiotic stresses that affects crop productivity and quality adversely. About 20% of irrigated agricultural land is adversely affected by salinity (Flowers and Yeo, 1995). The problem of soil salinity is further increasing due to the use of poor quality water for irrigation and poor drainage. The general effects of salinity are the results in both osmotic and ionic stresses. The initial and primary effect of salinity, especially on moderate salinity concentrations, is due to its osmotic effects (Jacoby, 1994). According to the USDA salinity laboratory, saline soil can be defined as soil having an electrical conductivity of the saturated paste extract of 4 dS m^{-1} or more. Most grain crops and vegetables are glycophytes and are highly susceptible to soil salinity even when the soil EC is <4 dS m^{-1} (Chinnusamy et al., 2005).

The measurable effects of salinity on plants can include reduced growth rate, reductions in yield components, or typical symptoms of nutritional disorders under osmotic and ionic stress (Heenan et al., 1988). Tolerance to a biotic stresses is associated with modifications of morphological and physiological traits (Edmeades et al., 2001). Soil salinity is one of the most significant a biotic stresses for crop plants, including legumes (Duzan et al., 2004). In general, high sodium chloride (NaCl) concentrations produce water deficit, ion toxicity, nutrient imbalance and oxidative stress (Vinocur and Altman, 2005). These adverse effects cause modifications of root morphology and inhibition of plant growth, and can result in plant death. For example, in winter wheat, changes in root growth and architecture in response to a biotic stresses in a resistant cultivar differ from those observed in a sensitive one (Abdrakhamanova et al., 2003).

The success of selection depends on the choice of selection criteria for improving plants yield. A correlation coefficient which measures the simple linear relationship between two traits clearly does not predict the success in selection. It is not sufficient to describe this relationship when the causal relationship among characteristics is needed (Seker and Serin, 2004). However, path analysis is used when we want to determine the amount of direct and indirect effect of the causal components on the effect component, In other words, for knowing causes. Path analysis has been increasingly utilized to define the best criteria for selection in biological and agronomic studies (Güler et al., 2001; Sengul and Sagsoz, 2004; Sengul, 2006). Little or no information has been reported on the interrelationships between vegetative yield and yield components at different seeding densities in chamomile under salinity. Our objectives were to investigate the effects of salinity levels on vegetative yield and yield components, analyze the relationships between yield components and final vegetative yields at different seeding densities under salinity, and determine if the yield loss under salinity would be compensated for by increasing seeding density above normal density levels.

MATERIALS AND METHODS

Plant material

Four accessions of *M. recutita* belong to Isfahan and Zabol (parts of Iran) and Hungry and Italy and four accessions of *M. aurea* collected in Isfahan, Tabriz, Shahrekord and Mashhad (different parts of Iran).

Salinity treatments at growth stage

The experiment was conducted in a greenhouse in Isfahan, Iran (32°40′ N latitude and 51°52′ E longitude) from 2005 August to 2006 January. Seeds were cultured in hougland nutrient solution in sand tanks (40 by 60 by 20 cm deep) filled with sand (#12, Cisco, Corona, CA). The rows were spaced 10 cm apart. Irrigation solutions were prepared in reservoirs of 20 L each and pumped to provide irrigation to sand tanks. Each reservoir irrigated three sand tanks (replicates). Air temperature was controlled between the ranges of 23 and 26°C during day and 12 and 15°C during night. The experimental design was a split-split plot randomized block. This factorial experiment was consisted of two salinity periods and four salinity levels and eight genotypes, replicating the whole experiment three times in the same contexts. Salinity periods were main plot and salt levels were sub-plot and genotypes were sub-sub-plot. Experiment was divided to 2 parts and each salinity periods were dispensed separately on each part and continued to the end of growth stage. All the plants were irrigated with unstressed water until seedling stage and then the first salinity duration was started at seedling stage, when the plants height were about 6 to 7 cm and the second duration was consisted of the stem formation on separated plants in another part of experiment. NaCl was added to the nutrient solutions of control, 6, 12, and 18 dS m^{-1} of the combined salts were determined. Electrical conductivities were measured with a Model METROHM 644 conductivity bridge (The Co. Swiss). At the end of experiment five plants were randomly collected from each of the inner-plots to measure shoot dry weights and yield components. The shoots and panicles of each of these plants were bagged individually and dried at 70°C for 48 h. The entire salinity from an individual plant were measured and averaged. Yield components were analyzed based on the measurements from these 5 plants to determine the stem and root length, leaves per plant, node number, stem fresh and dry weight, root fresh and dry weight, stem and root relative water content. Measurements of growth characteristics regarding plant height, fresh and dry weight, were taken in two physiological stages (seedling stage and stem formation). Data were averaged over the five sub samples. Plants were harvested in January 2006. All the plots were harvested in one week.

Determination of fresh and dry weight of plant stems and roots

Fresh and dry weight of plant stems and roots were measured at 30 to 45 day intervals after the highest salt concentration was reached. Dry mass was determined after drying for 48 h in a forced-draft oven at 75°C.

Determination of relative water content in stem and root

Water content of root and stem tissue was calculated on a tissue dry weight basis, that is, gram water per gram dry weight of tissues

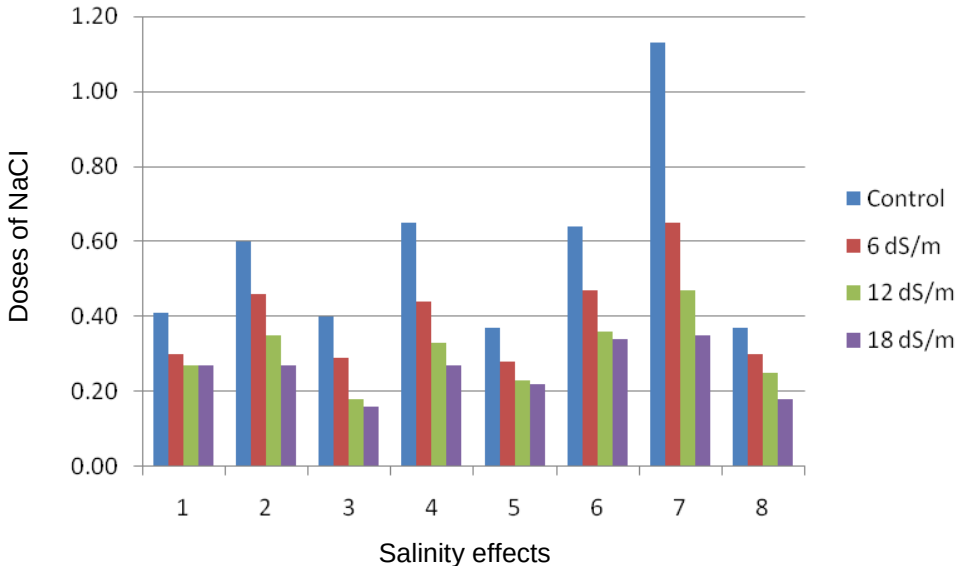

Figure 1. Change in DM yield per plant in relation to salinity effects on seedling stage.

by the following formula: (Hussain et al., 2003).

Water = F. wt - D. wt/ D. wt.

Where, F. wt. = fresh weight of tissue and D. wt. = dry weight of tissue.

The data were analyzed using general linear models with SAS (version 6.12) and the procedures were described by SAS (SAS Inst., 1994). The relative importance of yield components was analyzed using multiple factorial analyses. The relative importance of direct and indirect effect on dry matter yield was determined by path analysis. In path analysis DM yield was the dependent variable and plant characteristics were considered as independent variables.

RESULTS AND DISCUSSION

Dry matter yield varied significantly depending on treatments and period of salt application ($P<0.01$). Effect of salt treatment was higher in seedling stage (0.341 g plant^{-1}) than stem formation (0.423 g plant^{-1}). The effect of salt treatment on genotypes was homogenous in the stem formation; besides, it is highly varied in seedling stage (Figure 1), genotypes of *M. recutita* was influenced more than genotypes of *M. aurea* (Table 1). Salt application decreased the dry matter yield, when checked gradually, from 0.572 to 0.257 g plant^{-1} at 18 Ds m^{-1} NaCl application (Table 1). However, the highest salt effect were obtained from the yields of *M. recutita* Hungary genotypes (0.255 g plant^{-1}), and *M. aurea* Isfahan (0.274 g plant^{-1}), followed by 0.274 g plant^{-1} with *M. aurea* Mashhad genotypes. On the other hand, the highest yield was obtained from *M. aurea* Shahrekord genotypes (0.650 g plant^{-1}).

Salt application period was highly significant for all the characters evaluated, except for the root relative water. All of the examined characters were significantly affected by salinity. The period X salinity interaction was highly significant for PH, IN, and LN, DM and SRW were not significant. Almost all characters were highly significant for the genotype effect, except for the RRW. Period X genotype interaction was highly significant for DM, PH, and IN. On the other hand, the SRW was significant but there was not any significant relationship between RRW and LN. There were highly significant relationships examined with salinity X genotype interaction and also the period X salinity X genotypes interactions with exception of LN and RRW (Table 2). Sengul (2002) indicated that DM yield per plant on alfalfa ecotypes were negatively correlated with node number. There is significant correlation between plant height and node number.

Data showed from salt application trials indicated that dry matter yield were decreased with increasing NaCl doses. The dry matter yields were two times higher in check than that of the 18 dS m^{-1} NaCl levels. Salt application tolerances of genotypes were much higher in stem formation than the early seedling stage period (Figure 2). All the criteria investigated suggest, therefore, that *M. aurea* were superior to *M. recutita* genotypes (Table 1). There was a serious concern that plant growth and the development of yield components were affected by water salinity (Zeng and Shannon, 2000; Edmeades et al., 2001). Soil salinity is one of the most significant abiotic stresses for crop plants, including legumes (Duzan et al., 2004). In general, high NaCl concentrations produce water deficit, ion toxicity, nutrient imbalance and oxidative stress (Vinocur and Altman, 2005). These adverse effects cause modifications in root morphology and the inhibition of plant growth, and can result in plant death. It is well documented for abiyotik stress that a coordinated crosstalk amongst drought, cold and high

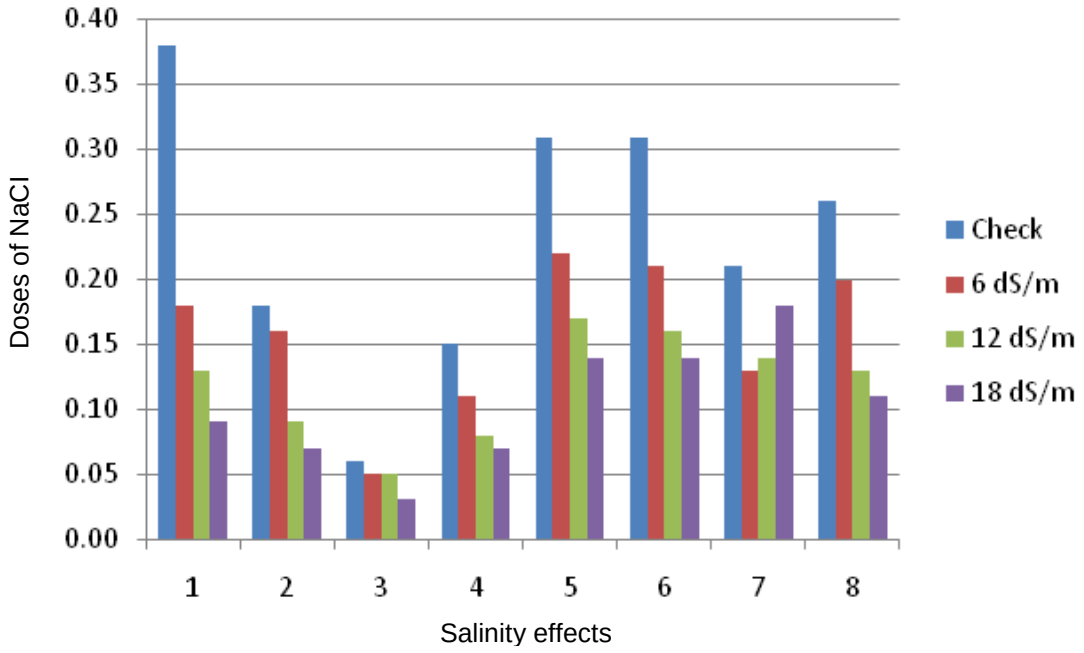

Figure 2. Change in DM yield per plant in relation to salinity effects on stem formation.

Table 1. Dry matter yield in *M. recutita* and *M. aurea* in relation to salinity on two different salinity expert (g plant^{-1}).

Treatment	Seedling stage	Stem formation	Total	Grand total
Check	0.57	0.58	0.58^{a}	
6 dS m^{-1} NaCl	0.33	0.46	0.40^{b}	
12 dS m^{-1} NaCl	0.25	0.35	0.30^{c}	
18 dS m^{-1} NaCl	0.21	0.30	0.26^{d}	
Total	0.34^{b}	0.42^{a}		
M. recutita				0.35
Isfahan	0.32	0.30	0.31^{c}	
Zabol	0.29	0.55	0.42^{b}	
Hungary	o.22	0.29	0.26^{d}	
Italy	0.27	0.57	0.42^{b}	
M aurea				0.41
Isfahan	0.23	0.32	0.27^{d}	
Tabriz	0.30	0.60	0.45^{b}	
Shahrekord	0.88	0.42	0.65^{a}	
Mashhad	0.22	0.33	0.28^{d}	

*The differences between the values with the same letters are insignificant at p<0.01.

salinity pathways exists (Mahajan and Tuteja, 2005; Merchan et al., 2007; Indorf et al., 2007).

In total factorial analysis showed that 70.8% of the variation in DM yield could be explained by the variation of the five independent variables (Table 3). The unexpected variation of the total 29.7% may be due to the variation in the other component under consideration. Starting this point, factorial analysis and path analysis had been concentrated on those trials.

Positive and highly significant relationships existed between DM yield and all its components with the exception of the stem relative water (r = 0.05). In general,

Table 2. Means and ANOVA mean squares for dry matter yield per plant and its components in factorial analysis.

Source of variation	df	DM	SRW	RRW	PH	IN	LN
Mean		0.38	74.3	79.5	15.5	23.8	28.2
Period	1	0.32**	1001.0*	15.1^{ns}	285.5**	147.5**	826.1**
Error (a)	2	0.00**	23.9	4.7	0.2	8.2	10.7
Salinity	3	0.93**	850.9*	538.6*	664.7**	124.6*	311.5 **
Error (b)	6	0.04	102.8	72.8	7.3	24.9	26.1
Period X Salinity	3	0.03^{ns}	314.0^{ns}	382.4*	1.9**	105.1**	200.5**
Error (c)	6	0.01**	67.2	57.1	2.6	4.6	5.2
Genotypes	7	0.42**	796.6**	130.4^{ns}	504. 6**	156.2**	211.7**
Period X Genotypes	7	0.38**	65.2*	127.8^{ns}	17.8**	46.2**	34.2^{ns}
Salinity X Genotypes	21	0.05**	131.2**	111.7*	20.9**	23.7**	29.0^{ns}
Period X Salinity X Genotypes	21	0.06**	55.0*	84.9^{ns}	10.0**	18.8**	47.7**
Error (d)	112	0.04**	30.2	63.2	4.7	8.3	18.3

Table 3. Summary of the total variance explained DM yield and its components in *M .recutita* and *M. aurea.*

Initial Eigenvalues			
Components	**Total**	**% of variance**	**Cumulative (%)**
Stem relative water	5.615	29.6	29.6
Root relative water	3.534	18.6	48.2
Plant height	1.744	9.2	57.8
Node number	1.358	7.1	64.9
Leaf number	1.123	5.9	70.8

Table 4. Simple correlation coefficient of dry matter yield components in *M. recutita and M. aurea* genotypes.

Traits	DM	PH	RL	IN	LN	SN	SFW	RFW	RDW	SRW
Dry weight (DM) g	-									
Plant height (PH) cm	0.36**	-								
Root length (RL) cm	0.31**	0.01^{ns}	-							
Node number (IN)	0.14*	0.52**	0.15*	-						
Leaf number (LN)	0.18**	0.57**	0.23**	0.76**	-					
Stem number (SN)	0.14 *	0.14*	0.26**	0.22**	0.48**	-				
Stem fresh weight (SFW) g	0.51 **	0.21**	0.69**	0.21**	0.22**	0.23**	-			
Root fresh weight (RFW) g	0.42**	-0.08^{ns}	0.79**	0.04 ^{ns}	0.07^{ns}	0.18**	0.82**	-		
Root dry weight (RDW) g	0.33**	0.16*	0.64**	0.18**	0.20**	0.20**	0.68**	0.79**	-	
Stem relative water (SRW)	0.05^{ns}	-0.16*	0.31**	-0.19**	-0.12^{ns}	0.09^{ns}	0.31**	0.37**	0.19**	-
Root relative water (RRW)	0.23**	0.16 *	0.21**	-0.12^{ns}	0.01^{ns}	0.05^{ns}	0.26**	0.25**	0.18*	0.38**

the components had significant positive and negative correlation with each other (Table 4). Path analysis showed that plant height, Root fresh weight, stem fresh weight and leaf number (LN) had strong positive direct effect; whereas, node number, stem relative water and root dry weight had strong negative direct effects.

The main effect of all components were significantly positive and resulted from the positive indirect effect via node number, leaf number, root length, root fresh water and root dry weight (Table 5). Comparing wild plants with cultivated forage crops, wild crops may have had some advantages. Some wild plants are more resistant to negative environmental condition (salinity, drought and cold resistant) diseases and pest damages (Tan and Yolcu, 2002). The wild plant of Turkey rangeland studies by Ayan et al. (2006) stated that plant height ranged from

Table 5. Path coefficient analysis for DM yield and its components for the *M. recutita* and *M. aurea* genotypes (n = 192). Direct effect (bold) and indirect effect are shown for each yield component pc (path-coefficient and its percentage).

Traits	PH (pc)	(%)	IN (pc)	(%)	LN (pc)	(%)	SF (pc)	(%)	SRW (pc)	(%)
Plant height (PH, cm)	0.43	36.7	0.22	26.3	0.24	27.4	0.09	5.4	0.05	4.0
Node number (IN)	-0.07	5.7	-0.13	15.2	-0.10	11.0	-0.03	1.7	0.05	6.2
Leaf number (LN)	0.02	1.3	0.02	2.5	0.03	3.1	0.01	0.4	0.03	3.8
Stem fresh weight (SFW, g)	0.05	4.7	0.06	6.7	0.06	6.5	0.27	16.5	-0.09	5.4
Stem relative water (SRW)	0.05	4.0	0.05	6.2	0.03	3.8	-0.09	5.4	-0.28	23.6

Traits	RL (pc)	(%)	RFW (pc)	(%)	RDW (pc)	(%)	RRW (pc)	(%)
Root length (RL cm)	-0.10	6.6	-0.08	4.6	-0.07	4.2	-0.02	2.9
Root fresh weight (RFW, g)	0.38	24.3	0.47	27.0	0.38	24.2	0.12	16.4
Root dry weight (RDW, g)	-0.14	9.2	-0.18	10.0	-0.22	14.3	-0.04	5.6
Root relative water (RRW)	0.02	1.0	0.02	1.1	0.01	0.9	0.08	10.6

14.46 to 100.06 cm DM yield was changed 14.8 to 25.19 g plant^{-1}. Total biomass yield increased when plant size (as stem yield) increased (r = 0.581**) (Şeker and Serin, 2004). Forage yield positively related to stem yield m^{-2} (r = 0.920**) and plant height (r = 0.921**) could be possible to develop to high forage yielding population, and also produce substantial amount of high quality yields on sweet bromegrass. Path coefficient analysis has been used successfully to determine the direct and indirect effect on various plant characteristics on yield and yield components *Triticum aestivum* (Korkut and Taser, 1993), in *Cicer arietinum L* (Güler et al., 2001), in *Medicago sativa L*. (Sengul, 2006).

Our result showed that *M. Aurea* was resistant against salinity. Our primary recommendation is that this experiment should done in field with another type of genotype.

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