

Full Length Research Paper

Germination, seedling growth and ion accumulation of bitter vetch (*Vicia ervilia* (L.) Willd.) lines under NaCl stress

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This study was conducted to compare the effect of NaCl levels on germination and seedling growth, and ion accumulation in five bitter vetch lines. Germination percentage (%), mean germination time (MGT, day), emergence percentage (%), shoot and root length (mm), shoot and root fresh and dry weight (mg/plant) and the Na⁺, K⁺, Cl⁻, and Na⁺/K⁺ ratio for both root and shoot were determined at different NaCl salt concentrations (0, 5, 10, 15 and 20 dS m⁻¹). Results of this research showed that NaCl adversely affected germination time and the emergence percentage as compared to final germination percentage in all the lines. Seedling characters decreased with increasing NaCl levels, but the decrease was more acute in the shoots than in the roots. Salinity stress induced a significant increase in shoot and root Na⁺, Cl⁻ and Na⁺/K⁺ ratio. Results show that line 1 was relatively more tolerant than other lines and line 2 was more sensitive. Salinity affected seedling growth especially at 10 dS m⁻¹ and higher salinity levels.

Key words: Bitter vetch, NaCl stress, germination, seedling growth, ion accumulation.

INTRODUCTION

Germination is a crucial stage in the life cycle of plants. Tolerance to salinity during germination is critical for the establishment of plants growing in the saline soils of arid regions (Ungar, 1995; Khan and Gulzar, 2003; Gorai and Neffati, 2007). Salinity may influence the germination of seeds by creating an osmotic potential external to the seed, preventing water uptake through the toxic effects of Na⁺ and Cl⁻ ions on the germinating seed and disturbance of the uptake and translocation of nutritional ion (Khajeh-Hosseini et al., 2003).

Bitter vetch (*Vicia ervilia* (L.) Willd.) is an ancient legume of the Mediterranean region that has been used for grain and hay production. It is widely distributed throughout the southern half of Europe, Western and Central Asia, and North Africa (GRIN, 2008). It has a number of favorable characteristics, such as resistance to

drought and insects and good energy and protein content that make it a potential economically useful source for poultry diets. The crop is easy to cultivate and harvest and can be grown on very shallow, alkaline soils where other grains such as corn and soybean do not grow successfully (Sadeghi et al., 2009).

In the same saline environment, different plant species may exhibit different growth response. The ability to germinate seeds under salt stress would be useful in the reclamation of saline soils. Information on the germination and seedling stages of bitter vetch in saline conditions is not available. The aim of this study was to evaluate the effect of NaCl concentrations on the germination, emergence, young seedling growth and Na⁺, K⁺ and Cl⁻ content of bitter vetch lines.

MATERIALS AND METHODS

This study was conducted at the Department of Field Crops, Agricultural Faculty, Erciyes University, Kayseri, Turkey. The bitter vetch lines obtained from Prof. Dr. Hayrettin Ekiz, Department of

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Table 1. Effect of different NaCl levels on seed germination (%) of five bitter vetch lines.

Line	NaCl level					Mean
	0 dS m ⁻¹	5 dS m ⁻¹	10 dS m ⁻¹	15 dS m ⁻¹	20 dS m ⁻¹	
1	100 (90±0.00)	100 (90±0.00)	100 (90±0.00)	100 (90±0.00)	100 (90±0.00)	100 (90.00)
2	100 (90±0.00)	99 (87.1±5.77)	99 (87.1±5.77)	98 (84.2±6.66)	98 (84.2±6.66)	98.8(86.54)
8	100 (90±0.00)	100 (90±0.00)	100 (90±0.00)	100 (90±0.00)	100 (90±0.00)	100 (90.00)
9	100 (90±0.00)	100 (90±0.00)	100 (90±0.00)	99 (87.1±5.77)	97 (81.4±5.77)	99.2 (87.69)
10	100 (90±0.00)	100 (90±0.00)	100 (90±0.00)	99 (87.1±5.77)	99 (87.1±5.77)	99.6 (88.85)
Mean	100 (90.00)	99.8 (89.42)	99.8 (89.42)	99.2 (87.69)	98.8 (86.54)	

Data represent mean ± SD of four replicates; LSD values for bitter vetch lines and NaCl levels = 2.14 (P<0.05, df = 75).

Table 2. Effect of different NaCl levels on mean germination time (day) of five bitter vetch lines.

Line	NaCl level					Mean
	0 dS m ⁻¹	5 dS m ⁻¹	10 dS m ⁻¹	15 dS m ⁻¹	20 dS m ⁻¹	
1	1.69±0.06	1.75±0.088	1.82±0.05	1.96±0.06	2.08±0.06	1.86
2	1.10±0.08	1.20±0.09	1.66±0.02	1.86±0.07	1.93±0.07	1.55
8	1.24±0.10	1.27±0.11	1.68±0.08	1.89±0.04	2.00±0.00	1.62
9	1.59±0.12	1.70±0.07	2.01±0.05	2.06±0.05	2.18±0.02	1.90
10	1.47±0.11	1.72±0.07	1.77±0.07	1.97±0.04	2.00±0.00	1.79
Mean	1.42	1.53	1.79	1.95	2.04	

Data represent mean ± SD of four replicates; LSD values for interaction = 0.43 (P<0.05, df = 75).

Field Crops, Ankara University were used as materials.

The NaCl concentrations at electrical conductivity were arranged as 5, 10, 15 and 20 dS m⁻¹. Distilled water served as a control (0 dS m⁻¹). Four replicates of 25 seeds for each line (25 x 4) were germinated between three rolled filter papers (20 x 20 cm) with a 10 ml respective test solution to determine germination values (Rehman et al., 1996; Okcu et al., 2005; Kaya, 2009). Every sample was put into a sealed plastic bag to prevent evaporation. Seeds were allowed to germinate in the dark at 20 ± 1 °C for 10 days. A seed was considered to have germinated when the emerging radicle was over 1 mm long. The germination percentage was recorded every 24 h for 10 days. Mean germination time (MGT) was calculated for the rate of germination (Ellis and Roberts, 1980) with the following formula:

$$MGT = f_1.t_1 + f_2.t_2 + f_3.t_3 + \dots + F_n.t_n / f_1 + f_2 + f_3 \dots + f_n$$

Where f_1, f_2, \dots are the number of newly germinated seeds at times t_1, t_2, t_3, \dots (days of counting).

For the emergence experiment, each line was sown to a depth of 1 cm in a plastic tray containing sand and kept in a growth chamber at 20 ± 1 °C for 16/8 h photoperiods. Every two days, the plastic trays were watered with saline solutions with an excess of solution permitted to flush the trays, allowing draining of the excess solution to maintain the level of salinity. All the drained solution was collected to measure electrical conductivity and to verify that the salinity of the treatment solutions and the drained solutions were similar. Each experiment consisted of four replicates with 50 seeds. The emergence percentage was recorded every 24 h for 15 days. Root and shoot lengths and fresh weights were measured on the 15th day. Samples were then washed with distilled water to prevent salt contamination. Dry weights were measured after drying samples at 70 °C for 48 h in an oven. The oven-dried root and shoot tissues were ground to a fine powder and 500 mg of the sample

was transferred to a digestion flask containing a 6 ml acid mixture of HNO₃ and HClO₄ in the ratio of 4:1(v/v). The flask was heated gently on a heat block, cooled and diluted by adding distilled water. Sodium and potassium analysis was performed using the flame photometric method. Chloride contents were determined using a titrimetric method (Kacar and Inal, 2008).

The experiment was designed with two factors and arranged at random. The first factor was lines and the second factor was NaCl levels. Data given in percentages were subjected to arcsine transformation before statistical analysis. For all investigated parameters, an analysis of variance was performed using SPSS 16 for Windows. Significant differences among the mean values were compared by an LSD test (P<0.05).

RESULTS AND DISCUSSION

Germination was significantly affected by lines and salinity levels. The effect of increasing NaCl levels on the final germination percentage was nearly the same for all cultivars (Table 1). All lines germinated at all levels of NaCl, but MGT differed in relation to lines and NaCl (Table 2). Increasing NaCl levels delayed mean germination time rather than affecting the final germination percentage. Mean germination time increased as the NaCl levels increased. In the controls, all lines had a different MGT, the minimum MGT was obtained from line 2, and the maximum MGT was obtained from line 9. However, the increase in MGT at different NaCl levels was higher in line 2 than in line 9 as compared to the controls. In view of mean germination time, there was a

Table 3. Effect of different NaCl levels on emergence percentage of five bitter vetch lines.

Line	Emergence percentage (%)					Mean
	0 dS m ⁻¹	5 dS m ⁻¹	10 dS m ⁻¹	15 dS m ⁻¹	20 dS m ⁻¹	
1	98 (82±5.44)	98 (82±5.44)	98 (82±5.44)	95 (77±3.56)	93 (77±10.41)	96 (80)
2	100 (90±0.00)	99 (87±5.76)	99 (85±5.85)	96 (79±3.49)	87 (67±2.55)	96 (82)
8	96 (80±6.76)	92 (73±1.16)	92 (74±7.75)	91 (75±11.63)	88 (70±3.64)	91 (74)
9	94 (76±2.00)	93 (75±1.30)	91(73±4.14)	90 (72±4.83)	89 (71±3.45)	91 (73)
10	98 (84±7.50)	98 (84±7.86)	97 (83±8.19)	95 (79±8.44)	90 (72±6.26)	95 (80)
Mean	97 (82)	95.6 (80)	95 (79)	93.2 (76)	89 (72)	

Data represent mean (transformation value) ± SD of four replicates; LSD values for bitter vetch lines and NaCl levels = 3.79 (P<0.05, df = 75).

considerable increase in this character in lines 8 and 2 at 10, 15, and 20 dS m⁻¹ salinity levels as compared to the others. Line 1 was not significantly delayed in its mean germination time at 5, 10, and 15 dS m⁻¹ salinity levels as compared to the controls.

Emergence was significantly affected by lines and salinity levels. Increasing salinity levels of salt concentration reduced emergence in all lines (Table 3). The emergence percentage ranged between 87 (at line 2 for 20 dS m⁻¹) and 100% (at line 2 for the control).

In our study, NaCl adversely affected the germination time and emergence percentage as compared to the final germination percentage. This result agrees with those of others (Murillo et al., 2002; Ellis and Roberts, 1980; Kaya et al., 2008) who observed that NaCl delayed mean germination time but did not affect the final germination percentage. In the germination stage, radicle protrusion is required to confirm seed germinability, but in the emergence stage, seedlings are prone to hypocotyls salt injury that militates against seedling emergence in soil or sand (Assadian and Miyamoto, 1987; Esehiea et al., 2002).

The ANOVA for both lines and salinity levels with respect to seedling characteristics (shoot length, root length, shoot and root fresh weight and shoot and root dry weight) were found to be significant (P<0.05). The shoot and root length was suppressed by NaCl salinity with the exception of 5 dS m⁻¹ (Table 4). Nevertheless, the influence was more pronounced at 15 and 20 dS m⁻¹ salinity levels than at lower salinity levels. Root length decreased by an average of 32 to 51% at 15 dS m⁻¹ and 61 to 76% at 20 dS m⁻¹ NaCl as compared with the control. This decrease was more drastic in lines 2 and 10 than in lines 1, 9 and 8. Shoot and root length decreased with increasing NaCl levels and the decrease was more drastic in shoots as compared to roots. Similar observations have been reported in wheat (Saboor and Kiarostami, 2006) and triticale (Atak et al., 2006). Salinity stress of 15 and 20 dS m⁻¹ resulted in decrease in the fresh and dry weights of bitter vetch lines as compared to the control for both shoot and root. For all species, root and shoot fresh and dry weights decreased in response to increasing concentrations of NaCl; and this decrease depended to some extent on the decrease in the lengths.

The decrease in weight agrees with the reports of other studies (Atak et al., 2006; Ateş and Tekeli, 2007). NaCl reduced the seedling growth of bitter vetch lines. The reason for reduced shoot and root development may be due to the toxic effects of the NaCl used as well as to the seedlings unbalanced nutrient uptake or to a slowing down of the water uptake by the plant (Saboor and Kiarostami, 2006). In some plants, these phases may occur sequentially (Meloni et al., 2008).

According to the variance analysis results of shoot and root Na⁺, K⁺, Cl⁻, Na⁺/K⁺, an interaction was observed between the lines and NaCl concentrations (Figure 1). Higher NaCl levels resulted in a higher Na⁺ accumulation in the shoots and roots. Although shoots accumulated less Na⁺ than roots, since the plant was exposed to salinity, the roots came into contact with NaCl and absorbed it directly; the Na⁺ content of shoots increased much more than the roots at 10, 15 and 20 dS m⁻¹ salinity levels. Similar findings are in conformity with the reports of other studies (Quadir and Shams, 1997; Essa, 2002). Cl⁻ uptake increased with increasing NaCl levels. At all salinity levels, the maximum increase were obtained from line 2, and the minimum increase was found in the shoots of lines 1 and 8. In the study, Cl⁻ uptake increased more rapidly than Na⁺ uptake with increasing NaCl levels. This was observed by Renault et al. (2001) and Meloni et al. (2008). They observed that Cl⁻ may be more harmful than Na⁺, as Cl⁻ uptake and transport appears to be less controlled than Na⁺.

The concentration of K⁺ in shoots was lower than that in roots in the control treatment. Generally, K⁺ concentration in shoots was higher at 5, 10, 15 and 20 dS m⁻¹ salinity levels as compared to the control. There was an increase but not a high tendency. K⁺ concentration in roots decreased with increasing salinity levels, except at 5 dS m⁻¹. The ratio of Na⁺/K⁺ in the shoots and roots significantly increased with increasing salinity levels. Lines 2, 8 and 9 showed a drastic increase as compared to lines 1 and 10 at all salinity levels. Parallel with increasing NaCl, Na⁺ accumulation and a decline in K⁺ content resulted in increased Na⁺/K⁺ ratio; suggesting that K⁺ or its absorption decrease may occur in root media due to Na⁺ competition. Similar results were obtained by

Table 4. Effect of different NaCl levels on seedling characteristics of five bitter vetch lines.

Line	Salinity (dS m ⁻¹)	Length (mm)		Shoot weight (mg plant ⁻¹)		Root weight (mg plant ⁻¹)	
		Shoot	Root	Fresh	Dry	Fresh	Dry
1	Control	222.3±6.29	98.0±6.18	161.5±14.27	14.8±0.50	101.8±8.73	7.0±0.57
	5	219.0±6.64	96.2±11.32	161.0±5.03	14.3±0.50	130.3±9.50	6.5±0.80
	10	187.8±9.50	92.3±3.74	138.8±10.81	13.3±0.95	106.3±8.77	4.7±0.90
	15	128.0±9.92	69.3±1.06	86.0±4.69	7.0±0.81	52.3±3.30	2.8±0.50
	20	63.3±3.90	46.3±4.21	52.3±3.30	4.5±0.57	33.3±1.50	2.3±0.50
2	Control	224.8±9.07	111.9±4.68	162.5±7.94	16.5±1.29	103.5±8.39	6.3±0.50
	5	217.2±7.19	109.3±3.13	171.3±11.21	16.5±1.00	114.8±6.65	5.0±0.41
	10	173.8±3.57	85.9±3.35	126.3±4.99	13.0±0.82	71.0±5.10	3.3±0.50
	15	110.4±6.06	70.7±5.31	81.5±5.20	7.0±0.82	42.5±5.00	2.5±0.60
	20	59.6±5.52	26.9±5.66	54.0±5.48	4.8±0.50	20.5±2.65	2.0±0.00
8	Control	203.0±5.78	87.8±5.69	139.5±6.35	13.8±0.96	85.0±8.72	5.0±0.45
	5	200.9±4.79	89.2±8.36	145.0±5.83	14.2±0.13	91.5±9.33	4.3±0.69
	10	172.4±3.08	79.3±6.01	128.0±2.94	14.6±0.65	81.8±1.71	4.2±0.22
	15	136.3±2.83	68.9±8.19	104.0±5.77	9.4±0.48	57.0±6.68	2.5±0.58
	20	78.7±1.54	44.6±2.31	61.8±2.63	5.3±0.50	34.0±1.41	1.7±0.55
9	Control	217.3±2.50	89.0±8.75	158.5±6.46	14.8±0.96	82.5±9.33	5.5±0.58
	5	226.7±9.51	108.1±6.36	189.0±14.07	17.3±1.89	127.0±19.15	7.3±0.60
	10	182.4±4.11	89.9±3.67	146.5±6.40	13.5±0.56	102.3±3.40	5.0±0.00
	15	117.8±2.22	68.9±4.91	89.3±4.19	8.3±0.50	51.3±3.50	3.3±0.50
	20	53.1±4.24	40.8±5.85	50.3±4.43	4.3±0.50	28.3±1.50	2.3±0.50
10	Control	222.3±7.77	95.1±7.68	156.3±5.32	14.8±0.50	90.8±6.65	5.5±0.40
	5	221.3±4.15	98.3±7.78	156.5±7.33	15.0±0.82	97.8±7.37	4.3±0.48
	10	192.5±4.66	87.9±2.81	131.3±8.73	12.8±0.96	83.8±5.19	4.8±0.96
	15	120.7±5.62	61.7±2.44	79.8±1.26	7.3±0.50	45.3±3.59	1.8±0.96
	20	56.9±2.32	34.1±3.06	43.5±1.73	4.0±0.82	28.5±2.89	1.3±0.53
LSD (Int)		8.20	8.19	9.97	1.53	10.03	0.81

Data represent mean ±SD of four replicates (P<0.05. df = 75).

Meloni et al. (2008) who reported that the highest decrease in K⁺ took place in the roots but relatively high K⁺ levels were maintained in the leaves, possibly acting as a major monovalent cationic osmoticum in the presence of external salt. K⁺ is a beneficial and essential element for plant growth and in saline environments, K⁺ uptake is generally reduced under NaCl conditions (Kiliç et al., 2008). Several reports indicate that the ability of plants to maintain high internal K⁺ concentrations determines salt tolerance of plant (Rehman et al., 2000; Rejili et al., 2007; Kiliç et al., 2008). The Na⁺/K⁺ ratio plays an extremely important role in salinity tolerance because it has been reported that the Na/K ratio was related to higher salt tolerance (Alian et al., 2000).

In conclusion, in the germination, emergence and early seedling stages, the lines showed a slightly different response to NaCl stress. NaCl significantly reduced germination, emergence and seedling characteristics. The results indicate that line 1 was relatively more salt tolerant than the other lines. However, seedling growth was affected, especially at 10 dS m⁻¹ and higher salinity levels.

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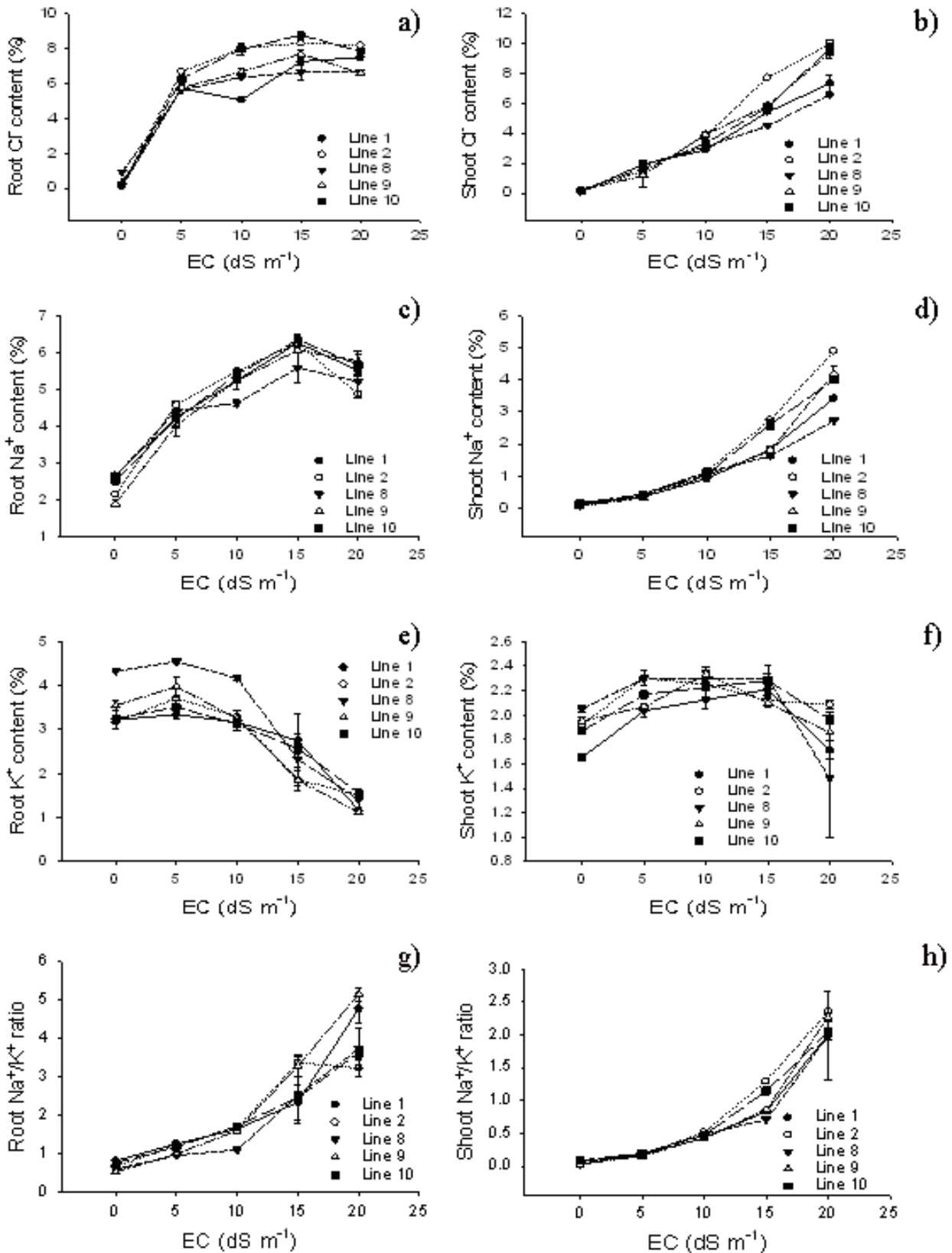


Figure 1. Interactive effect of bitter vetch lines and NaCl levels on ion accumulation. a) Cl⁻ in root (LSD_{int}:0.27); b) Cl⁻ in shoot (LSD_{int}:0.39); c) Na⁺ in root (LSD_{int}:0.32); d) Na⁺ in shoot (LSD_{int}:0.12); e) K⁺ in root (LSD_{int}:0.35); f) K⁺ in shoot (LSD_{int}:0.19); g) Na⁺/K⁺ in root (LSD_{int}:0.39); h) Na⁺/K⁺ in shoot (LSD_{int}:0.23). P < 0.05, df = 50.

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