# VARIATION IN SALT TOLERANCE OF CULTIVATED (HORDEUM VULGARE L.) AND WILD (H. SPONTANEUM C. KOCH) BARLEY GENOTYPES FROM IRAN

### H. PAKNIYAT, A. KAZEMIPOUR AND GH. A. MOHAMMADI<sup>1</sup>

Department of Crop Production and Plant Breeding, College of Agriculture, Shiraz University, Shiraz, I.R. Iran.
(Received: October 15, 2002)

#### ABSTRACT

Sixty three barley genotypes including 28 cultivated (Hordeum vulgare L.) and 35 wild (H. spontaneum C. Koch) genotypes collected from a wide geographic area of Iran were compared for salt tolerance. Plants were grown in 2-kg pots and subjected to three salinity (NaCl) treatments [0.97 (control), 9.2 and 17.3 dS m<sup>-1</sup>] in a greenhouse, in a factorial experiment with complete randomized design and three replications. During vegetative growth, shoot Na+, K+, K+/Na+ and proline contents were measured for all genotypes. Plant traits including the plant height, length of spikes, number of spikes per plant, fresh weight and dry matter and grain yield per plant were also measured. There was a very wide variation in salt tolerance of the genotypes with regard to Na+, K+/Na+ and proline content. In general, tolerant genotypes with better agronomic performance, contained lower Na+ and a higher amount of proline compared to non-tolerant ones and these two parameters were significantly and negatively correlated (r=-0.62, P<0.01). Salinity tolerance index (ratio of grain yield in saline media to grain yield in non-saline media) was highly negatively correlated with Na+ (r=-0.9, P<0.01). Therefore, Na<sup>+</sup> and proline content of the genotypes are two criteria which can be used for indirect selection for tolerant genotypes in breeding programs.

**<u>Key words:</u>** Barley, Iran, Na<sup>+</sup>, Proline, Salt tolerance, Wild barley.

<sup>1.</sup> Assistant Professor and former Graduate Students, respectively.

# تحقيقات كشاورزي ايران

Y1:40-8Y (1TA1)

تنوع در تحمیل بیه شیوری در ژنوتیپ هیای جیو زراعیی (H. spontaneum C. Koch) و وحشیی (Hordeum vulgare L.) ایران

### حسن پاک نیت، علیرضا کاظمی پور و غلامعباس محمدی

به ترتیب، استادیار و دانشجویان پیشین کارشناسی ارشد، بخش زراعت و اصلاح نباتات دانشکده کشاورزی. دانشگاه شیراز، شیراز، جمهوری اسلامی ایران.

# چکیده

شصت و سه ژنوتیپ جو شامل ۲۸ ژنوتیپ زراعی (Hordeum vulgare L.) و ۳۰ ژنوتیپ وحشی (H. spontaneum C. Koch) جمع آوری شده از یک منطقه وسیع جغرافیایی ایران، برای تحمل به شوری مقایسه شدند. گیاهان، در گلدان های ۲ کیلوگرمی، تحت تنش شوری (NaCl) به مقادیر  $/^4$  (شاهد)،  $/^4$  و  $/^4$  دسی زیمنس در متر در یک آزمایش فاکتوریل در قالب یک طرح کاملا تصادفی و در سه تکرار در گلخانه کاشته شدند. در دوران رشد رویشی میزان  $/^4$   $/^4$  الله  $/^4$  و پرولین اندازهگیری شد. ویژگی های گیاه شامل ارتفاع، طول سنبلهها، تعداد سنبله در بوته، وزن ماده تر و خشک و عملکرد دانه در بوته اندازهگیری شدند. تنوع زیادی از نظر مقادیر بوته، وزن ماده تر و خشک و عملکرد دانه در بوته اندازهگیری شدند. تنوع زیادی از نظر مقادیر ویژگی های زراعی برتر در مقایسه با ژنوتیپها مشاهده شد. به طور کلی، ژنوتیپهای مقاوم با ویژگی های زراعی برتر در مقایسه با ژنوتیپهای حساس، دارای مقدار کمتر  $/^4$  و مقدار بیشتری پرولین بودند و این دو معیار همبستگی منفی و معنیداری داشتند  $/^4$  و  $/^4$  و  $/^4$  اله سردی که نسبت محصول دانه در محیط شور به محصول دانه در محیط غیرشور است، با

میزان  $Na^+$  همبستگی منفی و معنیداری داشت  $P < \cdot / \cdot 1$  و  $P < \cdot / \cdot 1$ . بنابراین میزان  $Na^+$  برولین ژنوتیپها دو شاخص ارزشمند برای انتخاب غیر مستقیم بوده ومی توان از آن ها در برنامههای بهنژادی در شناخت ژنوتیپهای مقاوم به شوری بهره جست.

#### INTRODUCTION

Salinity of agricultural lands and irrigation water is the most limiting factor for plant growth in many dry parts of the world. Twenty five million ha of agricultural land are saline in Iran, and this is increasing due to poor irrigation management.

Barley is a relatively tolerant crop to soil salinity, and genetic variations exist among genotypes of cultivated barley (Hordeum vulgare L.) and its wild progenitor (H. spontaneum C. Koch). Wild barley has its center of diversity in the Fertile Crescent of the Middle East where it colonizes a wide range of habitats from high rainfall to desert, from cool to hot areas and from sub-sea levels to altitudes in excess of 1700 meters. Both H. vulgare and H. spontaneum are diploid species, with seven pairs of chromosomes, with no biological barriers to crossing or meiotic recombination. Consequently, there is great interest in exploiting the rich genetic variation of the wild species for crop improvement.

There is a general relationship between low Na accumulation and salt tolerance in barley (2, 5, 12). The amount of  $K^+$  and  $K^+/Na^+$  may be correlated with tolerance to salinity (4).

Other indices such as shoot  $\delta^{13}C$  (10, 11), and some molecular and biochemical factors like proline and glycinebetaine (3, 6, 7, 9) have been indicated to be associated with salt tolerance in many crops.

Here we used the shoot Na content, K<sup>+</sup>, K<sup>+</sup>/Na<sup>+</sup> and proline to screen and differentiate 63 genotypes of cultivated and wild barleys, including cultivars, breeding lines and different genotypes of *H. spontaneum* collected from different parts of Iran. The study was aimed at investigating the genetic variation in salt tolerance and to find the suitable selection criteria for differentiation of genetic variation for the trait. In a previous work we used AFLP markers for fingerprinting of 39 genotypes of *H. spontaneum* selected from three geographically separated areas of the Fertile Crescent:

Iran, Turkey and Israel and we observed a high level of variation among the genotypes with regard to AFLP fingerprints, shoot Na content and  $\delta^{13}$ C (11). The most salt tolerant genotype was from Ilam, province in west part of Iran. However, sharp genetic differences were detected between genotypes separated by relatively short distances.

Since the genotypes collected from Iran showed good tolerance to salt, the present experiment was performed to detect genetic variation for salt tolerance among a wide range of wild and cultivated barleys collected from different areas in this country.

#### MATERIALS AND METHODS

#### Plant Material

Sixty-three barley genotypes consisting of 35 wild (Hordeum spontaneum C. Koch) and 28 cultivated (H. vulgare L.) barley genotypes collected from different parts of Iran were tested for shoot Na<sup>+</sup> and K<sup>+</sup> analysis and proline content (Table 1) in the College of Agriculture, Shiraz University, Iran.

#### Planting

Before planting, the seeds of all genotypes were surface sterilized by 2.5% sodium hypochlorite solution for 15 min, and rinsed 3 times with distilled water. In order to break dormancy of wild genotypes, and to provide uniformity in germination, imbibed seeds were placed at 4-6°C for 5 days in the dark. All genotypes were planted in a greenhouse in pots containing 2 kg of soil.

### **Experimental Design and Salt Treatments**

A factorial experiment with 2 factors, genotypes (63 genotypes) and salt treatments (3 levels, 0, 2500 and 5000 mg NaCl  $kg^{-1}$  soil), was conducted in a completely randomized design with 3 replications.

Five germinated seed of each genotype were planted in a 2 kg-pot containing 2 kg of soil (sandy-clay-loam; 29% clay, 24% silt and 47% sand, pH 7.8), with moisture saturation of 54%, field capacity of 24% and electric conductivity (EC) of 0.57 dS m<sup>-1</sup>. The final salinity levels after irrigation were 0.97 (control) 9.2 and 17.3 dS m<sup>-1</sup>. The pots were put in plastic bags to

Variation in salt tolerance of cultivated and wild barley genotypes...

Table 1. Wild and cultivated barley genotypes used in the experiment.

Genotype	Genotyp	Collection	Gen	otype Genotype code	Collection	
no	e code	location	n	0.	location	
1 <sup>†</sup>	TN-02-199	Espand Isl.	33	Badjgah (Fars)	Shiraz Agric. Col	
2	TN-02-204	Arezoo Isl.	34	Badjgah (Fars)	Shiraz Agric. Col	
3	TN-02-206	Loreastan	35	Badjgah (Fars)	Shiraz Agric. Col	
4	TN-02-214	Loreastan	36	Asse/Karoon	BL (Zarghan)	
5	TN-02-295	Western	37	Torsh/9cr.279-071lbBgs	BL (Zarghan)	
		Azarbayjan				
6	TN-02-421	Ilam	38	Star/Jerusa/em/Rihane-03	BL (Zarghan)	
7	TN-02-422	Ilam	39	Zarjow/Rhiane/L.640	BL (Zarghan)	
8	TN-02-425	Ilam	40	73M47	BL (Zarghan)	
9	TN-02-430	West.	41	Kavir/Badia	BL (Zarghan)	
		Azarbayjan				
10	TN-02-343	West.	42	Karoon/Kavir	BL (Zarghan)	
		Azarbayjan				
11	Plot No. 1	Zarghan(Fars)	43	80-5010/Mona	BL (Zarghan)	
12	Plot No. 5	Zarghan(Fars)	44	Na-cc-4000-123/Walfajre	BL (Zarghan)	
13	Plot No. 16	Zarghan(Fars)	45	Walfajře/Apm/Hc-905/Roho	BL (Zarghan)	
14	Plot No. 18	Zarghan(Fars)	46	Zarjow/Bit/CM67	BL (Zarghan)	
15	Plot No. 19	Zarghan(Fars)	47	Zarjow/Hiproly	BL (Zarghan)	
16	Plot No. 20	Zarghan(Fars)	48	Kavir/Mch-4/3/Apm/Dwarf	BL (Zarghan)	
17	Plot No. 21	Zarghan(Fars)	49	Torsh/9cr.279-0711Bgs	BL (Zarghan)	
18	Plot No. 23	Zarghan(Fars)	50	Chat/Roho/Alger-ceres	BL (Zarghan)	
19	Plot No. 24	Zarghan(Fars)	51	Ligness527/NK1272	BL (Zarghan)	
20	Plot No. 25	Zarghan(Fars)	52	P12315/Maf/02/cossack/3/Li	BL (Zarghan)	
				gnee527		
21	Plot No. 29	Zarghan(Fars)	53	Rihane,s,/Deiralla106/Mzq/0	BL (Zarghan)	
				L71		
22	Plot No. 30	Zarghan(Fars)	54	C1717-9/ Deiralla 106/	Zarghan	
				Th.unk48		
23	Plot No. 31	Zarghan(Fars)	55	Black seed (2 row)	Zarghan	
24	Plot No. 34	Zarghan(Fars)	56	Black seed (6 row)	Zarghan	
25	Plot No. 35	Zarghan(Fars)	57	6 row Hooded	Zarghan	
26	Plot No. 38	Zarghan(Fars)	58	Himalya	Zarghan	
27	Plot No. 39	Zarghan(Fars)	59	Victoria	Zarghan	
28	Plot No. 41	Zarghan(Fars)	60	Probest dwarf	Zarghan	
29	Zarghan(vine yard)	Zarghan(Fars)	61	Reyhan	Zarghan	
30	Sarvestan	Zarghan(Fars)	62	Valfajr	Zarghan	
31	Zarghan(vine yard)	Zarghan(Fars)	63	Afzal	Zarghan	
32	Beyza	Zarghan(Fars)				

<sup>†</sup> Genotypes number 1 to 35 are wild and number 36 to 63 are cultivated.

prevent excess water drainage and hence to control salinity level of the pots.

Salt (NaCl) was applied by making a saline stock solution of 125 g NaCl<sup>-1</sup>

The salt treatments were applied at 4 stages, at one-week intervals. The first application was at the 2-leaf stage. For the first level of salinity at each stage, 10 ml of the stock solution and for the second level of salinity, 20 ml of the stock solution were applied into each pot and considering field capacity of the soil, water was added in adequate amounts while the pots were placed on a balance. Pots were placed in plastic bags in order to reuse drained water and maintain the applied salinity level.

#### Sampling and Measurements

Ten days after the last stage of salt treatment, shoots of a plant from each pot were harvested for proline measurement and kept at  $20^{\circ}$ C. For measuring Na<sup>+</sup> and K<sup>+</sup>, 4 weeks after the last stages of salt treatment, shoots of 3 plants of each pot were harvested and oven-dried at a 65°C for 48 hr, and then milled to a fine powder.

#### **Proline Measurement**

Plant extracts were used for proline measurement. Extracts were prepared by putting 0.5 g of plant shoot material in 10 ml of sulfosalicilic acid (3%). The mixture was homogenized in a mortar and then filtered by Watman filter paper No. 3. Proline content of the genotypes was measured according to Bates et al. method (1).

#### Na+ and K+ Measurement

A 0.5-g sample of millèd shoot was put in a crucible. The samples were then ashed by placing in a furnace at 500°C for 6 hr. 5 ml HCl (2N) was added into each crucible and mixed thoroughly. The mixture made up to 50 ml with boiling distilled water and filtered in a 50 ml volumetric flask. Na<sup>+</sup> and K<sup>+</sup> concentrations were measured using flame photometry.

#### Plant Morpho-physiological Measurements

Individual plant fresh weights (FW) were measured four wk after salt treatments. At harvest, other traits including the dried plant weight (DW)

Variation in salt tolerance of cultivated and wild barley genotypes...

plant grain yield (GY), plant height (PH), spike length of the main tiller, number of spikes per plant and number of tillers per plant were measured.

#### **Statistical Analysis**

Analysis of variances of the data was performed using MSTATC and SPSS computer program softwares. Harvard Graphic software was used for drawing diagrams and graphs. Cluster analysis of genotype for Na<sup>+</sup> content was performed for grouping of the genotypes using the hierarchial technique and Agglomeration method (8). Correlation coefficients between measured characters were determined.

#### RESULTS AND DISCUSSION

### Sodium Content of Genotypes

In general, increasing salinity level from 0.97 dS m<sup>-1</sup> to 17.3 dS m<sup>-1</sup> caused a significant increase in Na content in all genotypes (Fig. 1). There was a very wide variation between different genotypes with regard to shoot Na content (Table 2).

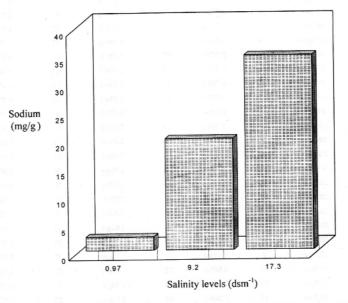


Fig. 1. Mean Na content of barley genotypes at different salinity levels.

Pakniyat et al.

Table 2. Shoot Na<sup>+</sup>, proline content, grain yield, and K<sup>+</sup>/Na<sup>+</sup> ratio of wild and cultivated barley genotypes.

Na⁺		Proline		Grain yield		K <sup>+</sup> /Na <sup>+</sup>	
Genotype	Na*	Genotype	Proline	Genotype	Grain plant <sup>1</sup>	Genotype	K*/Na
no.	(mg g <sup>-1</sup> )	no.	(mg g <sup>-i</sup> )	no.		no.	
29	10.8x	29	3.08a	29	2.77a	63	4.55
30	11.7wx	24	2.94a-b	41	2.64ab	29	4.01
63	12.2vwx	17	2.92a-b	30	2.63abc	10	3.10
41	13.3u-x	8	2.90a-c	63	2.62abc	8	2.81
7	13.6u-x	30	2.88a-d	7	2.54a-d	30	2.80
10	13.8t-x	62	2.86а-е	10	2.54a-d	17	2.67
6	14.5s-x	46	2.84a-f	6	2.48a-d	6	2.65
8	14.6s-x	43	2.83a-f	8	2.36a-e	24	2.57
59	14.83-x	7	2.83a-f	59	2.35a-e	59	2.55
17	15.5q-w	53	2.82a-f	17	2.35а-е	4	2.50
44	15.8p-w	37	2.81a-g	44	2.33а-е	62	2.48
3	16.0o-w	44	2.81a-g	3	2.34b-e	13	2.32
24	16.2n-w	39	2.80a-g	24	2.28b-e	55	2.31
62	16.9m-v	55	2.80a-h	62	2.26b-f	48	2.18
47	17.01-u	5	2.79a-h	47	2.26 b-g	44	2.16
55	17.7k-u	23	2.79a-h	55	2.26 b-g	3	2.13
43	17.8j-u	63	2.79a-h	43	2.24 b-g	7	2.13
20	17.9I-u	10	2.77a-i	20	2.22 b-g	51	2.01
56	18.5h-t	45	2.76a-i	56	2.21b-g	47	1.96
1	18.8g-s	59	2.76a-i	1	2.20c-g	57	1.95
27	18.8g-s	51	2.75b-i	27	2.19c-h	21	1.90
25	19.0f-s	54	2.75b-i	25	2.18d-I	43	1.88
53	19.5е-г	25	2.74b-i	53	2.14d-I	52	1.87
13	19.5е-г	13	2.73b-i	13	2.13d-I	54	1.87
54	19.5e-r	6	2.73b-i	54	2.12d-I	1	1.86
49	19.8d-p	16	2.74b-i	49	2.02e-j	14	1.84
60	20.2c-q	22	2.72b-i	60	2.01e-j	22	1.83
5	20.3c-q	40	2.710b-i	5	2.00e-j	34	1.83
58	20.3c-q	32	2.70b-i	58	2.00e-j	49	1.82
35	20.3c-q	56	2.70b-i	35	1.98e-j	20	1.82
21	20.5с-р	60	2.68b-i	21	1.97e-k	60	1.82
40	20.5с-р	57	2.68b-i	40	1.82f-l	2	1.82
14	20.5с-р	58	2.67b-i	14	1.82g-l	45	1.81
15	20.5с-р	41	2.67b-i	15	1.76j-m	56	1.80
4	20.7с-р	9	2.67b-i	4	1.76j-m	35	1.77
22	20.8c-o	26	2.66b-i	22	1.65j-n	31	1.75
51	20.8c-o	52	2.66b-i	51	1.63j-n	5	1.72
48	20.8c-o	15	2.66b-i	48	1.61j-o	27	1.71

Variation in salt tolerance of cultivated (Hordeum vulgare L.)...

Table 2	continued	1 ,8 9 100	imana amil				
52	21.0c-n	2	2.65b-i	52	1.56k-p	33	1.70
19	21.5c-m	21	2.65b-i	19	1.441-q	25	1.70
2	21.6c-m	12	2.65b-i	2	1.411-q	41	1.69
57	21.7c-m	3	2.64b-i	57	1.41m-r	46	1.67
37	21.7c-m	31	2.64b-i	37	1.34 n-r	9	1.66
46	21.8c-m	12	2.62b-i	46	1.31 n-r	15	1.65
33	21.9c-l	47	2.62b-i	33	1.30 n-r	50	1.61
12	22.2b-k	1	2.62b-i	12	1.28 n-r	19	1.56
45	22.8b-j	14	2.57c-j	45	1.25 n-r	42	1.56
34	22.8b-I	20	2.57c-j	23	1.24 n-r	32	1.55
23	22.8b-l	61	2.57c-j	34	1.19o-s	23	1.52
9	23.3a-h	38	2.56d-j	16	1.18o-s	40	1.50
16	23.7a-g	4	2.63d-j	9	1.183o-s	53	1.49
50	24.0-f	27	2.54e-j	50	1.67p-s	28	1.48
32	24.0-f	36	2.54e-j	32	1.63 p-s	26	1.45
42	24.2a-e	48	2:53e-j	42	1.14 p-s	37	1.41
18	24.2a-e	19	2.52f-j	18	1.14p-s	16	1.41
39	24.3а-е	49	2.51f-j	39	1.10 q-s	11	1.39
31	24.3а-е	34	2.48g-I	31	1.04 q-s	38	1.38
36	24.7a-d	18	2.47h-j	36	1.03 q-s	39	1.33
11	24.8a-d	11	2.46I-j	11	1.02q-s	58	1.26
26	24.8a-d	50	2.28j-k	26	0.94rs	12	1.23
61	25.2a-c	33	2.14k-l	61	0.92rs	36	1.20
38	27.0ab	28	2.11k-l	38	0.79rs	61	1.06
28	28.0a	35	1.911	28	0.77s	18	1.04

Genotypes collected from different and same areas showed high variation for Na content. For example, genotypes number 28 and 29 collected from the Zarghan area, had the maximum and minimum Na<sup>+</sup> content, respectively, and both genotypes were from the wild species gene pool. On the other hand, the two wild genotypes, 29 and 31 (collected from a vineyard in Zarghan) were completely different for their Na contents. Genotype number 29 was the least amount of Na (most tolerant) and genotype number 31 was among the most susceptible genotypes. This indicates high variability of the wild species which sometimes show great genetic differences over short distances. The wild genotype did not show any significant difference regarding Na content with Afzal (cultivar number 63) which is considered a salt tolerant cultivar. Reyhan (cultivar number 61) which is not a tolerant cultivar for salinity, ranked among the highest Na content and hence the most salt sensitive genotypes.

Wild genotypes collected from Ilam (numbers 6, 7 and 8) were among the more tolerant genotypes. It is interesting to mention that in a previous study (10) in which a number of wild barley genotypes collected from Iran, Turkey and Israel, were compared for salt tolerance, the most tolerant genotype was from Ilam, province in western Iran. Since this area is considered as a part of the Fertile Crescent (the center of origin for barley), it may be a good source for finding tolerant genotypes.

Among advanced breeding lines (numbers 36 to 54), lines 43 and 44 were tolerant lines, and lines 36 and 38 were susceptible for salt tolerance. Reyhan (salt susceptible cultivar) contributes to the pedigree of line 38, and Valfajr (salt tolerant; number 62) is in the pedigree of line 44. The response of some tolerant and susceptible cultivars under controlled conditions (0.97 dS m<sup>-1</sup>) and 2 levels of salinity (9.2 and 17.3 dS m<sup>-1</sup>) are shown in Fig. 2.

# Salinity Effects on Grain Yield and Yield Components of Genotypes

Salt stress had adverse effects on grain yield and yield components of all genotypes. These adverse effects were drastic in cultivated compared to wild genotypes. There were significant differences (P<0.05) among genotypes for grain yield per plant (Table 2) and a significant (P<0.01) negative correlation (r=-0.95) was observed between Na content and grain yield per plant (Table 3).

Likewise, effect of salinity on plant dry weight was significant (P<0.01) with negative correlation of r=-0.94 between dry matter production and Na<sup>+</sup> content (Table 3). The same negative correlation was observed between plant height, salinity index [grain yield in saline condition/grain yield in nonsaline (control)].

The salinity index for the genotypes is shown in Table 4. This index indicates the salt tolerance of each genotypes and stability of grain yield production in saline condition. The most tolerant genotypes having lower amount of Na' had higher salinity indices compared to salt susceptible genotypes having higher Na contents and lower salinity indices.

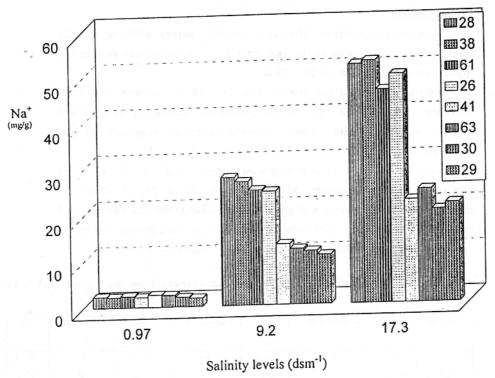


Fig. 2. Na content of tolerant (29, 30, 63, 41) and non-tolerant (28, 38, 61, 26) barley genotypes at different salinity levels.

Table 3. Correlation coefficients between different traits of genotypes.

	Dry	Seed yield	Plant	Salinity	K <sup>+</sup> /Na <sup>+</sup>	K <sup>+</sup>
	weight		height	index		
Dry weight	x16.51			1.7		17.7
Seed yield	0.99**	· -				
Plant height	0.93**	0.93**	-			
Salinity index	0.98**	0.99**	0.93**	-		
K+/Na+	0.54**	0.53**	0.59*	0.55*	-	
$K^{+}$	$0.10^{\mathrm{ns}}$	0.10 ns	$0.09^{\mathrm{ns}}$	$0.11^{\rm  ns}$	0.61*	-
Na <sup>+</sup>	-0.94**	-0.95**	-0.97*	-0.90**	-0.63**	-0.14 <sup>ns</sup>

<sup>\*\*, \*</sup> Significant at 1 and 5 % probability levels, respectively.

ns not significant.

#### **Proline Content**

There were significant differences (P<0.01) among different salinity levels for proline content of the genotypes (Fig. 3). Genotypes showed the highest amount of proline at 17.3 dS m<sup>-1</sup>

Proline content of the genotypes at 17.3 dS m<sup>-1</sup> showed significant ifference <0.01) (Table 2), and the interaction between genotypes and salinity was significant, which indicates different responses of the genotypes to salinity.

For the sum of all salinity treatments, genotypes 29, 24, 17, 8, 30, 62 and 46 contained the highest and genotypes 35, 28, 33, 50, 11, 18 and 34 contained the lowest amounts of proline. In tolerant genotypes, the response to increasing salinity level was an increase in free proline, while in susceptible lines no specific variation in proline content was observed.

Table 4. Salinity indices (SI) of barley genotypes ranked from the highest (41) to the lowest (28).

Genotype	SI	Genotype no.	SI	Genotype no.	SI	Genotype	SI
no.			3.5			no.	
41	0.87	43	0.68	14	0.46	23	0.33
29	0.87	20	0.67	15	0.46	9	0.31
63	0.86	56	0.66	4	0.44	16	0.28
30	0.82	1	0.66	22	0.44	50	0.26
7	0.77	27	0.64	51	0.44	32	0.25
10	0.77	25	0.64	48	0.44	42	0.24
8	0.74	53	0.61	52	0.43	18	0.24
6	0.74	13	0.61	19	0.42	39	0.23
59	0.73	54	0.59	2	0.41	31	0.23
17	0.73	49	0.59	57	0.40	36	0.22
44	0.72	60	0.58	37	0.39	11	0.2
3	0.72	5	0.58	46	0.39	26	0.19
24	0.71	58	0.58	33	0.38	61	0.16
62	0.71	35	0.57	12	0.38	38	0.14
55	0.69	21	0.50	45	0.35	28	0.12
47	0.68	40	0.50	34	0.33		

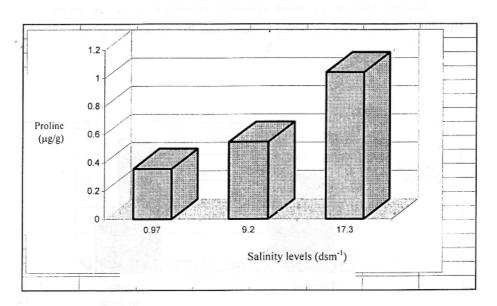


Fig. 3. Mean proline content of all genotypes at different salinity levels.

### Relation Between Proline and Na Content

There was a negative correlation (r=-0.62, P<0.01) between Na content and proline concentration of tolerant genotype. The comparison between tolerant and non-tolerant genotypes for Na and proline contents has been shown in Fig. 4.

### Salinity Index (SI)

Salinity index expresses as a measure of the tolerance of the genotypes to salinity was highly negatively correlated with Na content (r=-0.90, P<0.01) (Table 3). Genotypes 41, 30, 29 and 63 which are salt tolerant showed higher salinity indices, while genotypes 61, 38 and 28 which were classified as susceptible showed the lowest salinity indices.

### Cluster Analysis of the Genotypes

Cluster analysis in saline condition classified the 63 genotypes into 5 different groups according to their shoot Na contents (Fig. 5).

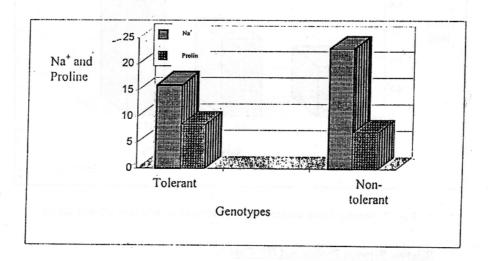


Fig. 4. Comparative Na and proline contents of 4 tolerant (29, 30, 41, 63) and 4 non-tolerant (26, 28, 38, 61) genotypes.

# CONCLUSIONS

Western Iran, which forms part of the Fertile Crescent (the center of origin for barley), is of value as a genetic resource for various important crops species including barley. This is why in a previous research in which 39 Hordeum spontaneum genotypes collected in Fertile Crescent (Iran, Turkey and Israel), the highest variation and the most salt tolerant genotypes were from Iran (11).

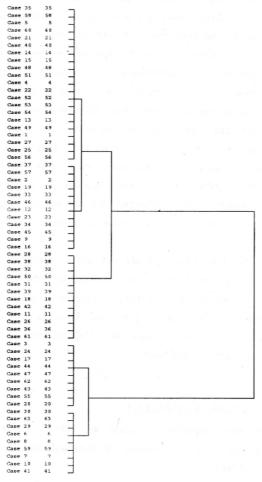


Fig. 5. Dendrogram from cluster analysis of barley genotypes based on Na contents.

#### LITERATURE CITED

- Bates, L.S., R.P. Warden and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. Plant Soil 39:205-207.
- Forster, B.P., H. Pakniyat, M. Macaulay, M. Matheson, M.S. Phillips, W.T.B. Thomas and W. Powell. 1994. Variation in leaf Na content of Hordeum vulgare (barley) cultivar Maythorpe and its derived mutant cv. Golden Promise. Heredity 73:249-253.
- Gary, V. and C.T Stewart. 1984. Steady state proline levels in salt shocked barley leaves. Plant Physiol. 76: 567-570.
- Gorham, J., A. Bristol, E.M. Young, R.G. Wyn Jones and G. Kashour. 1990. Salt tolerance in the triticeae: K<sup>+</sup>/Na<sup>+</sup> discrimination in barley. J. Exp. Bot. 41:1095-1102.
- Gorham, J., R. Papa and M. Aloy-Leonard. 1994. Varietal differences in Na uptake in barley cultivars exposed to soil salinity or salt spray. J. Expt. Bot. 45: 895-901.
- Grumet, R. and A.D. Hanson. 1986. Genetic evidence for an osmoregulatory function of glycinebetaine in barley. Aust. J. Plant Physiol. 13:353-364.
- Jain, S., H.S. Naiawatee, R.K. Jain, and E. Chowlhury. 1991. Proline status of genetically stable salt tolerant *Brassica juncea* L. somaclones and their parent cv. Plant Cell Rep. 9:684-687.
- 8. Johnson, A.R. and D.W. Wichern, 1996. Applied Multivariate Statistical Analysis (3rd ed.). Prentice Hall. India.
- Lin, C.C. and C.H. Kao. 1996. Proline accumulation is associated with inhibition of rice seedling root growth caused by NaCl. Plant Sci. 114:121-128.
- Pakniyat, H., L.L. Handley, W.T.B. Thomas, M. Connolly, M. Macaulay, P.D.S. Caligari, and B.P. Forster. 1997. Comparison of shoot dry weight, Na<sup>+</sup> content and δ<sup>13</sup>C values of ari-e and other semi-dwarf barley mutant under salt stress. Euphytica 94:7-14.

#### Pakniyat et al.

- 11. Pakniyat, H., W. Powell, E. Baird, L.L. Handley, D. Robinson, C.M. Scrimgeour, E. Nevo, C.A. Hackett, P.D.S. Calogari and B.P. Forster. 1997. AFLP variation in wild barley (Hordeum spontaneum C. Koch) with reference to salt tolerance and associated ecogeography. Genome 40:332-341.
- Pakniyat, H., W.T.B. Thomas, P.D.S. Caligari and B.P. Forster. 1997.
   Comparison of salt tolerance of GPert and non-GPert barleys. Plant Breed. 116:189-191.