

Genetic diversity and association analysis among Egyptian barley (*Hordeum vulgare L.*) genotypes with different adaptations to saline conditions analyzed by SSR markers.

Ammar Elakhdar, Mohamed Abd El-sattar, Khairy Amer and Toshihiro Kumamaru*

Supplementary Table 1. The name and origin of the 30 Egyptian barley genotypes used in the study.

No	Code	Pedigree	Salinity tolerance*
1	G 123	Giza 123(Giza 117/ FAO86)	M
2	C.M	California Mariout	S
3	Line 3	Giza118 /3/ Aths/Lignee 686//ACSAD 618	M
4	Line 4	Giza 123 /4/ LBIRAN / UNAB 271 // GLORIA - BAR / COME - B /3/.....	T
5	Line 5	Giza 125 /5/ Apm/HC1905// Robur/3/ Arar/4/ Baca'S'/3/AC253.....	T
6	Line 6	Giza 126 /4/ Acsad 1180 /3/ Mari / Aths *2 // M-Att-73-337-1	M
7	Line 7	Giza2000/4/LBIRAN / UNAB 271 // GLORIA - BAR / COME - B /3/..	T
8	Line 8	C.C 89 /5/ ACSAD 1182/4/ Arr/ ESP // Alger/ Ceres 362-1-1/3/ WI	S
9	Line 9	Alanda // Lignee 527 /Arar /5/Ager // Api / CM67 /3/ Cel / WI2269 /...	M
10	Line 10	QUINN // ARUPO *2 / JET /5/ 2505 / CI 5831 // SMA1 / ... /6/ M64 - 76 / ...	M
11	Line 11	ACSAD 1182 /4/ Arr/ ESP//Alger/Ceres362-1-1/3/WI /5/ API / CM 67- B //	M
12	Line 12	BLLU/PETUNIA1//CABUYA /3/ Alanda / Hamra -01 // Gloria 'S' / Copal 'S'	T
13	Line 13	Beecher/5/Baca'S'/3/AC253//CI08887/CI05761/4/Cen/Bglo'S'	T
14	Line 14	Lignee527/Aths/Lignee527/NK1272/5/Baca'S'/3/AC253//CI08887/CI05761/4/..	T
15	Line 15	AwBlack/Aths//Arar/3/9Cr279-07/Roho/4/CompCr229//As46/ Pro/3/ ..	T
16	Line 16	IPA7/4/AwBlack/Aths//Arar/3/9Cr279-07/Roho/5/CompCr229//As46...	T
17	Line 17	Acsad 1180 /3/ Mari / Aths *2 // M-Att-73-337-1/4/AwBlack/Aths//Rhn-08/...	M
18	Line 18	Soufara-02/3/RM1508/Por//WI2269/4/Hml-02/ArabiAbiad//ER/Apm/5/Sara	S
19	Line 19	Rhn-03/Saida	M
20	Line 20	SUMBARD400/BERMEJO//SEN/3/TOCTE/4/SUMBARD400/BERMEJO//...	T
21	Line 21	IPA7/4/AwBlack/Aths//Arar/3/9Cr279-07/Roho/5/Arar/Rhn-03	M
22	Line 22	Giza126/3/Lignee527/NK1272//Alanda/6/Lignee527//Bahtim/DL71/3/Api/...	M
23	Line 23	U.Sask.1766/Api//Cel/3/Weeah/4/Lignee527/NK1272/5/Express	S
24	Line 24	Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686/5/AwBlack/Aths//Arar/3/...	M
25	Line 25	Alanda/Hamra//Alanda-01	T

26	Line 26	Eldorado//Alanda/Hamra-01	S
27	Line 27	Badia	S
28	Line 28	Giza 117/4/Arar//Hr/Nopal/3/Alanda -01/Alanda-01	M
29	Line 29	Giza 121/4/Arar//Hr/Nopal/3//Alanda-01	T
30	Line 30	Giza 123/6/Alanda-01/5/CI01021/4/CM67/U.Sask. 1800 //Pro/ CM67/3/DL70	T

*Genotypes were classified into three categories: T, salt tolerant; M, moderately tolerant; S, salt sensitive, based on their agronomic performance in saline soils locutions from their evaluation trials at Field crops Research Institute, ARC, Egypt.

Supplementary Table 2. Mean performance of five field studied traits for all genotypes under salt affected soil over years.

Genotypes	GY	No. grain	1000- GW	LA	CHL
G 123	3.73	53.00	48.25	43.27	38.40
C.M	4.24	48.17	50.34	40.37	33.62
Line 3	4.43	45.50	40.93	38.14	38.32
Line 4	4.51	47.33	46.28	46.28	40.17
Line 5	4.16	45.50	44.03	72.62	39.67
Line 6	4.73	50.67	49.54	66.32	38.15
Line 7	4.15	47.00	49.01	73.03	39.58
Line 8	4.19	47.17	48.62	56.40	30.80
Line 9	4.29	57.67	42.52	61.33	38.32
Line 10	4.02	51.33	42.50	60.55	38.15
Line 11	4.53	57.00	43.48	47.27	37.47
Line 12	4.81	48.17	41.99	29.80	42.38
Line 13	5.04	47.50	50.68	51.15	40.72
Line 14	4.32	49.50	46.93	53.94	39.35
Line 15	4.67	57.00	40.74	45.06	40.62
Line 16	4.94	56.50	48.45	42.36	38.83
Line 17	3.64	63.83	42.47	56.71	36.82
Line 18	4.95	63.00	47.18	37.84	35.58
Line 19	4.31	59.00	46.22	48.09	38.32
Line 20	4.40	58.67	49.09	59.92	39.21
Line 21	3.46	60.00	49.83	60.66	36.62
Line 22	5.28	63.00	45.08	46.57	37.23
Line 23	4.45	61.00	41.39	48.87	36.42
Line 24	4.09	50.67	44.17	43.48	38.23
Line 25	4.22	63.00	41.49	42.51	39.20
Line 26	4.44	64.00	40.47	42.63	34.84
Line 27	4.66	54.00	43.73	34.94	32.25
Line 28	4.95	61.00	45.97	51.50	37.69
Line 29	4.17	58.00	46.44	52.61	42.38
Line 30	4.46	69.00	48.60	43.19	39.49
Mean	4.41	55.24	45.55	49.91	37.96
Inter.	**	n.s	*	**	**
LSD 0.05	0.78		5.07	11.61	5.55

G.Y; grain yield (ton/h), No. Grain; Number of grains per spike, 1000-G.W; 1000grain weight, LA; flag leaf area and CHL; total chlorophyll content.
*, **, indicate significance at 0.05 and 0.01 levels, respectively, n.s; not significantly different Inter.; interaction.

Supplementary Table 3. Means of germination percentage G% and six seedling characters of four salinity levels over twelve barley genotypes.

Salinity	R%	GER	SL	RL	SFW	RFW	SDW	RDW
L0: control	-	98.00	14.50	6.63	0.220	0.174	0.048	0.031
L1: Ec 12	16.29	81.71	10.83	4.07	0.162	0.075	0.038	0.016
L2: Ec 16	27.43	70.57	8.60	3.44	0.131	0.052	0.028	0.007
L3: Ec 20	43.14	54.86	6.44	2.98	0.120	0.034	0.010	0.005
f test		**	**	**	**	**	**	**
LSD 0.05		0.375	0.620	0.380			0.036	0.0018
Cultivars	1	4.300	8.466	3.920	0.543	0.079	0.017	0.006
	2	3.900	9.171	3.912	0.108	0.257	0.082	0.012
	3	4.500	10.047	3.755	0.152	0.054	0.022	0.010
	4	6.200	11.513	4.457	0.148	0.362	0.030	0.016
	5	6.400	11.300	3.688	0.337	0.062	0.032	0.012
	6	6.300	11.909	3.817	0.146	0.061	0.067	0.014
	7	5.300	10.058	3.777	0.147	0.072	0.025	0.029
	8	6.000	10.602	4.458	0.228	0.074	0.043	0.026
	9	5.200	10.487	5.015	0.250	0.083	0.025	0.015
	10	4.800	9.562	4.540	0.202	0.073	0.042	0.023
	11	5.800	10.168	4.169	0.119	0.073	0.050	0.010
	12	6.000	10.154	4.378	0.138	0.070	0.114	0.010
f test		**	**			**	**	
LSD 0.05		0.72	0.81	0.52				0.006

G %; germination %, R%; Reduction %, SL; Shoot length, RL; root length. STW; Shoot fresh weight, RFW; root fresh weight, SDW; shoot dry weight and RDW; root dry weight.

*, **; indicate significance at 0.05 and 0.01 levels, respectively.

Supplementary Table 4. Mean effects of salinity levels × genotypes interaction on germination percentage G % and six seedling traits for twelve barley genotypes.

Salinity	Genotype	G%	S L	R L	SFW	RFW	SDW	RDW
EC 0	12	100.00	15.47	5.47	0.155	0.298	0.028	0.011
	29	100.00	13.91	5.26	0.157	0.106	0.025	0.035
	13	100.00	14.47	5.39	0.237	0.392	0.036	0.015
	15	100.00	16.13	4.49	0.234	0.154	0.067	0.036
	4	100.00	15.75	5.69	0.209	0.105	0.038	0.020
	5	95.29	17.33	6.44	0.257	0.124	0.033	0.008
	7	95.29	14.35	6.30	0.247	0.156	0.056	0.076
	30	100.00	16.48	7.21	0.234	0.141	0.077	0.018
	14	95.29	15.28	8.45	0.240	0.155	0.037	0.024
	20	100.00	14.24	6.59	0.248	0.156	0.057	0.076
EC 12	27	95.29	10.42	6.68	0.180	0.136	0.077	0.019
	8	95.29	10.21	10.6	0.240	0.160	0.039	0.025
	12	52.43	8.56	3.59	0.113	0.044	0.015	0.007
	29	57.14	9.38	3.58	0.105	0.036	0.018	0.008
	13	76.14	10.54	3.75	0.122	0.039	0.017	0.008
	15	90.43	13.39	4.55	0.152	0.066	0.018	0.008
	4	95.29	11.52	3.17	0.119	0.043	0.015	0.008
	5	95.29	11.37	2.83	0.135	0.035	0.019	0.005
	7	85.71	10.27	3.39	0.132	0.058	0.017	0.005
	30	90.43	10.02	5.06	0.155	0.065	0.016	0.009
EC 16	14	85.71	12.52	4.34	0.155	0.087	0.016	0.008
	20	81.00	12.22	4.42	0.155	0.068	0.019	0.009
	27	95.29	9.51	3.37	0.121	0.043	0.011	0.008
	8	76.14	10.64	6.71	0.134	0.039	0.016	0.006
	12	52.43	6.33	3.72	0.066	0.034	0.032	0.004
	29	57.14	8.78	3.34	0.105	0.062	0.092	0.005
	13	52.43	8.79	3.21	0.105	0.025	0.051	0.007
	15	100.00	10.87	4.02	0.133	0.084	0.056	0.007
	4	95.29	11.4	3.63	0.119	0.061	0.051	0.013
	5	90.43	9.41	2.82	0.135	0.065	0.054	0.008
EC 20	7	81.00	9.39	2.70	0.124	0.071	0.051	0.008
	30	85.71	7.56	3.44	0.076	0.055	0.042	0.006
	14	66.71	9.57	3.68	0.135	0.056	0.050	0.009
	20	52.43	6.38	3.84	0.110	0.034	0.069	0.004
	27	75.14	7.42	3.55	0.080	0.057	0.062	0.005
	8	57.14	7.25	3.35	0.102	0.044	0.050	0.005
	12	42.86	6.18	3.56	0.082	0.050	0.009	0.004
	29	47.57	5.61	3.27	0.081	0.012	0.018	0.008
	13	29.57	6.53	2.57	0.080	0.023	0.008	0.004
	15	71.43	7.33	3.13	0.081	0.026	0.003	0.004
EC 20	4	71.43	6.19	2.27	0.085	0.016	0.008	0.004
	5	71.43	7.18	2.55	0.080	0.021	0.016	0.006
	7	47.57	6.56	2.38	0.091	0.028	0.008	0.006
	30	52.43	7.35	2.79	0.071	0.056	0.012	0.008
	14	57.14	5.57	2.70	0.088	0.026	0.006	0.003
	20	42.86	6.07	3.64	0.081	0.051	0.009	0.004
	27	71.43	6.31	3.34	0.082	0.055	0.018	0.005
	8	52.43	6.41	3.53	0.081	0.045	0.008	0.004
	f test		**	**	**	*	**	**
	L.S.D							
0.05		1.033	0.879	0.952	0.0173	0.171	0.016	0.016

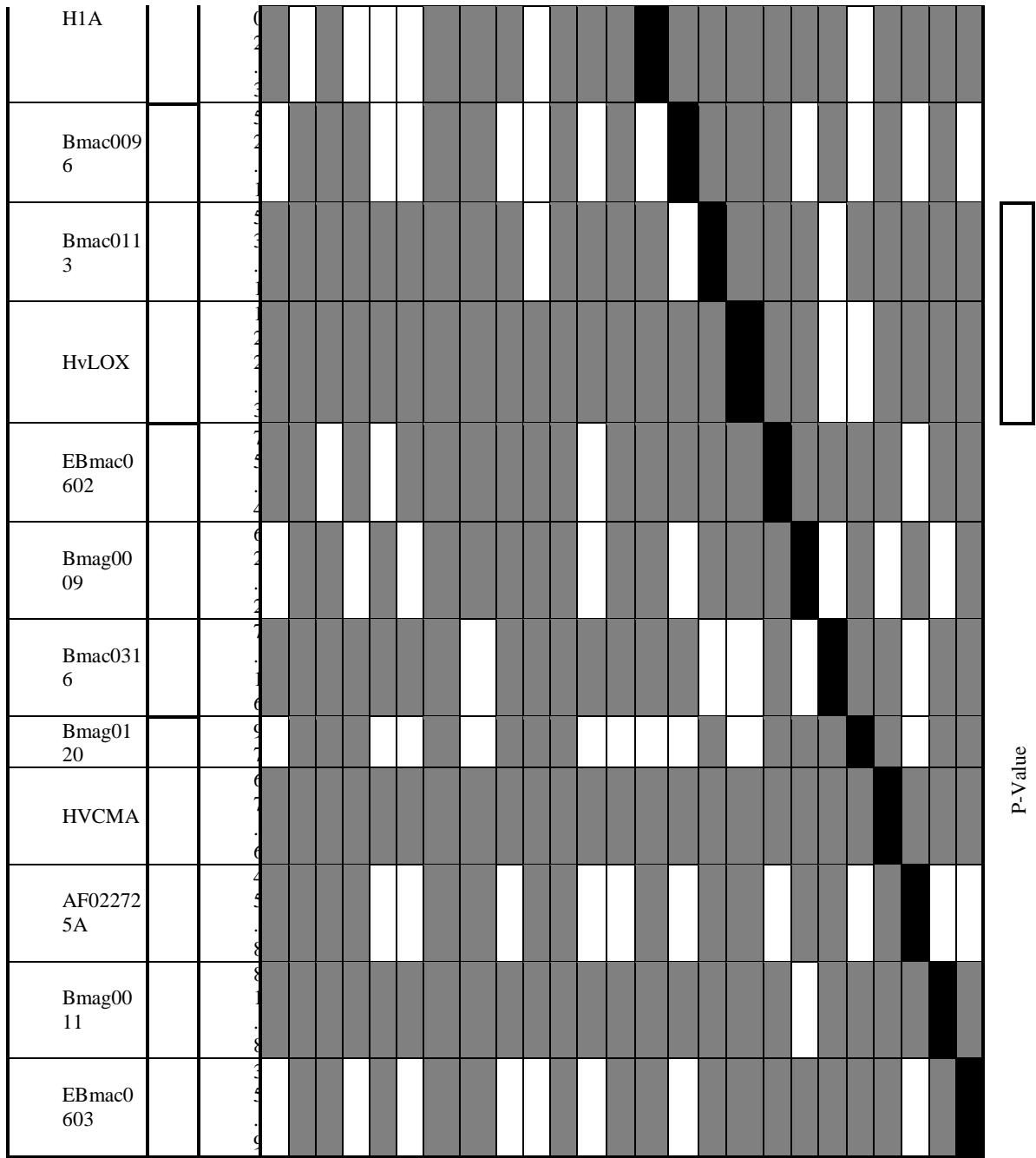
G %; germination %, SL; Shoot length, RL; root length. STW; Shoot fresh weight, RFW; root fresh weight, SDW; shoot dry weight and RDW; root dry weight. *, **, indicate significance at 0.05 and 0.01 levels, respectively.

Supplementary Table 5. Linkage disequilibrium of the 25 simple sequence repeat (SSR) markers.

	Chromosome	Position [cM]	EBmac0501	Bmac0154	Bmag770	Bmag382	Bmac0213	Bmag0125	EBmac0415	Bmag749	EBmac0871	HvLTTPB	Bmac0209	Bmac030	EBmac0701	HVMLO
Chromosome																
Position [cM]																
EBmac0501			1.0													
Bmac0154				1.0												
Bmag770					1.0											
Bmag382						1.0										
Bmac0213							1.0									
Bmag0125								1.0								
EBmac0415									1.0							
Bmag749										1.0						
EBmac0871											1.0					
HvLTTPB												1.0				
Bmac0209													1.0			
Bmac030														1.0		
EBmac0701															1.0	
HVMLO																1.0

$P < 0.05$

$P > 0.05$

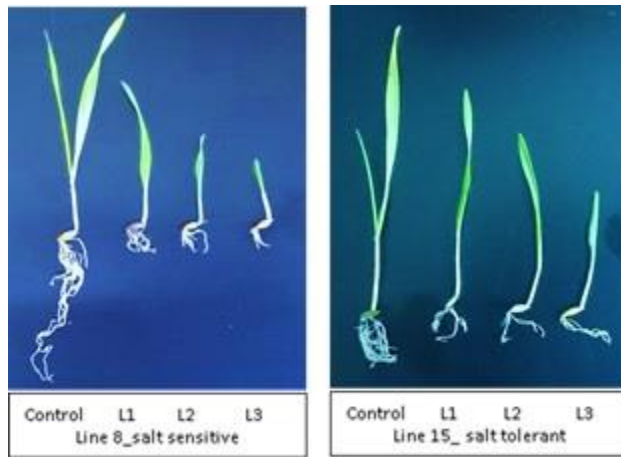


R Square

r^2

$r^2 > 0.05$

 $r^2 < 0.05$



Supplementary Fig 1. Effect of sodium chloride ($NaCl$) and Calcium chloride ($CaCl_2 \cdot 2H_2O$) (W/W) on early seedling growth of barley genotypes.