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Preliminary evidence of the associations between DNA markers and morphological characters in sunflower under natural and salt stress conditions

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Abstract

Soil salinity is a serious threat to agricultural products worldwide. Agricultural biotechnology mainly aims at developing plants with higher tolerance in order to face the challenging environmental conditions, such as drought and salinity. Identification of marker-character associations is the first step towards marker-aided selection in plant improvement programs. In the current study, quantitative trait loci (QTLs), associated with salt tolerance, were identified using 84 common sunflower (*Helianthus annuus* L.) inbred lines, collected from different geographical origins. The lines were fingerprinted with 30 simple sequence repeat (SSR) markers. This generated 71 clear and scorable bands, 87% of which were polymorphic. Associations between SSR markers and 18 agronomic characters were analysed using mixed linear model (MLM). Based on SSR markers data, the association panel was subdivided into two subpopulations (K = 2). About 2.06% of the 435 possible locus pairs of the studied SSRs represented significant linkage disequilibrium (LD). Six and 13 SSR loci showed significant ($P \leq 0.01$) association with the assessed characters under natural and salt stress conditions, respectively. Several molecular markers were significantly associated with more than one phenotypic character, suggesting the possible presence of genetic linkage or pleiotropic effects. The identified and associated markers are expected to be helpful in marker-aided selection in sunflower breeding programmes.

Key words: abiotic stress, linkage disequilibrium, molecular markers, oil crops, quantitative trait loci mapping.

Introduction

Soil and water salinity is the main factor limiting growth and performance of field crops worldwide (Shahbaz, Ashraf, 2013). About 6% (equivalent to 800 million hectares) of world's lands are affected by salt (Yang et al., 2011). It is estimated that from 230 million hectares of agricultural lands under irrigation, approximately 20% (equivalent to 45 million ha) are saline (Arzani, 2008). About 0.2–0.4% of the total cultivable lands are removed from plantation every year due to water logging and salinity problems (Makhdum, Ashfaq, 2008). Of the total land in Iran (162.2 million hectares), 23.8 million hectares are affected by salt (Arzani, 2008). Salt stress seriously affects the economy of country by limiting distribution of agricultural systems and crop productivity. The problem is increasing annually due to climatic change and poor irrigation management. Most cultivated plants are salt sensitive and, therefore, salinity is an ever-present threat to agricultural activity (Flowers, Flowers, 2005). Most important effect of salt stress includes limiting water availability, disrupting ions balance that results in reduced growth and photosynthesis rate, increased free radicals, and discontinuance membrane actions, enzymes and cell metabolism activities (Ashraf, 2004).

Osmotic stress occurs rapidly in the initial phase of salt stress and ionic stress occurs slowly in the second phase at high levels of NaCl, which results in plant cells death (Horie et al., 2012). The accumulation of salt around the root causes a very negative potential in soil, reducing water absorption by roots.

Cultivated common sunflower (*Helianthus annuus* L.) is a diploid plant (2n = 2x = 34) with a genome size of 2871 to 3189 Mbp (Schilling, 2006). It is one of the most ancient oilseed species in North America and the fifth largest among the oilseed crops after soybean, rapeseed, cotton, and groundnut. Consumers prefer its oil due to high content of unsaturated fatty acids.

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Although sunflower is moderately tolerant to salt stress, its production is greatly affected by saline conditions (Pasda, Diepenbrock, 1990). Salinity induces an adverse effect on all growth parameters of sunflower (Hussain, Rehman, 1993). Plant height, leaf number and leaf area of sunflower decrease with increasing salinity and show a reduction of 22.9% and 37% in the above-mentioned characters at 10 dS m^{-1} electrical conductivity (EC) of media, respectively. Similarly, salinity stress significantly reduces yield and yield components of sunflower (Rehman, Hussain, 1998). Agricultural biotechnology mainly attempts to develop more tolerant crops in the challenging environmental conditions like salinity, drought, extreme temperature, and oxidative stress. Salt tolerance is a complex character controlled by several quantitative trait loci (QTLs) (Flowers, 2004). Moreover, identification of marker and character associations is considered as an important step towards marker-aided selection for salt tolerant breeding programs.

Microsatellite or simple sequence repeat (SSR) is found in the genomes of most eukaryotes. Their constant units mostly consist of two, three or four nucleotides. SSRs technology is based on amplification of repeated motif, via primers designed for flanking area. Their polymerase chain reaction (PCR) outputs are replicable in different labs around the world. SSRs – due to their co-dominant and multi-allelic behaviour, high polymorphic information content and random distribution in the genome, are suitable tools for genomic variation assessment and QTL mapping activity (Snowdon, Fried, 2004). Association analysis, as an alternative to linkage mapping, is a valuable tool for the dissection of QTLs controlling complex characters in crop plants. This approach detects associations between phenotype and genotype on the basis of linkage disequilibrium (LD) (Gajardo et al., 2015). Indeed, association analysis relies on LD between markers and QTLs present in the collections of diverse germplasm (Pritchard et al., 2000). It exploits the recombination events that occurred during the long evolutionary history (Nordborg, Tavare, 2002). Mandel et al. (2013) investigated population differentiation and LD structure in sunflower by single nucleotide polymorphism (SNP) markers. In attempts to analyse their association, numerous informative markers were identified for characters related to plant architecture and flowering time. There are a few reports on application of molecular markers in salt tolerance assessment in sunflower. Morsali et al. (2016) evaluated a population of sunflower recombinant inbred lines (RILs) for yield and yield components under saline and normal conditions. They identified 14 and 17 QTLs for the studied characters in normal and salt stress conditions, respectively. In their QTL mapping attempts the highest amount of R^2 (7.33%), related to QTL, was identified for number of days to flowering character under salt stress conditions. They detected a number of co-localized QTLs for some studied traits under normal and salt stress conditions, which augment the efficiency of marker-aided selection in plant breeding programs. Zhao et al. (2016) performed association analysis for salt tolerance related characters in cotton (*Gossypium hirsutum* L.) using 74 SSR markers. They found eight markers significantly associated with characters with R^2 values, ranging from 2.91% to 7.82% with an average of 4.32%. Darvishzadeh (2016) investigated the population structure and LD among 106 dispersed sunflower genotypes by microsatellite, inter-retrotransposon amplified polymorphism (IRAP) and retrotransposon-microsatellite amplified polymorphism (REMAP) markers. He identified some informative markers for agro-morphological characters via mixed liner modelling analysis. In his study, high genetic variability was observed among sunflower genotypes in view of studied agro-morphological characters and amplified molecular markers.

Microsatellite markers due to the above mentioned advantage have high potential for improving salt tolerance in sunflower. The aim of the present study was to identify microsatellite markers linked to morphological characters in sunflower in natural and salt stress conditions. The study would be based on two consecutive years' data on 84 oily sunflower lines collected from different geographical regions of the world. Introduction and identification of specific DNA markers associated with salt tolerance-related characters will assist sunflower breeders in the selection of salt tolerant genotypes, accelerating and facilitating breeding activities under saline conditions.

Materials and methods

Plant materials and phenotyping. A total of 84 inbred lines of common sunflower (*Helianthus annuus* L.) from different geographical origins (Table 1) were evaluated in randomized complete block designs with three replications in pot conditions. The lines were evaluated during the period of 2014–2015 under natural and salt stress (8 dS m^{-1}) conditions outside the greenhouse in an open air area in Urmia University, Iran. Lines were individually grown in 24 × 24 cm^2 plastic pots, containing a mixture of 40% soil, 40% compost and 20% sand, as described in Poormohammad Kiani et al. (2007). Salinity stress was applied with NaCl. Based on the primary amount of soil salinity, a solution with 0.4 molar NaCl was prepared in order to achieve 8 dS m^{-1} NaCl. About 500 ml of solution was added to each pot at 8-leaf stage / code 18 on the BBCH scale. To avoid osmotic stress, the saline solution was added in two steps: 250 ml of solution was added in the morning and the rest applied in the afternoon. Soil salinity was controlled by electrical conductivity-meter during the experiment. A drip irrigation system was used and fertilization was carried out several times during the vegetative growth period. During irrigation, care was taken so that water did not drain out from the pots.

After flowering and harvesting of capitulum, some agro-morphological characters per plant were measured. The characters included leaf number, stem diameter, plant height, head diameter, upper leaf length, upper leaf width, upper petiole length, middle leaf length, middle leaf width, middle petiole length, bottom leaf length, bottom leaf width, bottom petiole length, chlorophyll index (SPAD), head dried weight, one hundred seed weight and grain yield. Number of days from planting to flowering was also recorded in each pot. Plants were harvested at physiological maturity stage. The chlorophyll concentrations were measured from the middle of the youngest fully expanded leaves. For the measurement of chlorophyll at the mentioned locations, a chlorophyll meter SPAD-502 (Minolta, Japan) was used. Furthermore, the values were averaged before entering them into the statistical analysis. The measurements were performed for both natural and salt stress conditions.

Simple sequence repeat (SSR) data used in the present study were provided by Sahranavard et al. (2015). Genomic DNA was extracted from the young leaves of 15-day-old seedlings. DNA quality was

Table 1. The origin and site description of oily sunflower inbred lines used in the present study

Code	Line name	Origin	Research centre	Code	Line name	Origin	Research centre
1	H100A/83HR4	France	ASGROW	43	CSWW2X	France	IFVC
2	H209A/LC1064	France	ASGROW	44	1009370-3(100K)	France	ENSAT
3	H205A/H543R	France	ASGROW	45	H100A	France	ASGROW
4	AS5306	France	ENSAT	46	15031	France	ASGROW
5	RHA858	USA	USDA	47	H205A/83HR4	France	ASGROW
6	H209A/83HR4	France	ASGROW	48	RHA265	USA	USDA
7	as3211	France	ENSAT	49	PM1-3	USA	USDA
8	254-ENSAT	France	ENSAT	50	RT948	France	RUSTICA
9	AS5304	France	ASGROW	51	283-ENSAT	–	–
10	1009329.2(100K)	France	ENSAT	52	QHP-1	France	INRAMONT
11	270-ENSAT	France	ENSAT	53	SDR19	USA	USDA
12	AS613	France	ASGROW	54	HA337B	USA	USDA
13	A-F1POPA	France	NOVARTIS	55	H100B	France	ASGROW
14	OES	France	INRAMONT	56	B454/03	Hungary	–
15	H100A/LC1064	France	ASGROW	57	HA304	USA	USDA
16	RHA266	USA	USDA	58	RT931	France	RUSTICA
17	PAC2	France	ENSAT	59	HA335B	USA	USDA
18	H157A/LC1064	France	ASGROW	60	SDB3	USA	USDA
19	5DES20QR	France	BRN	61	LC1064C	France	ASGROW
20	1009337(100K)	France	ENSAT	62	NS-R5	France	NOVARTIS
21	AS3232	France	ENSAT	63	H156A/RHA274	France	ASGROW
22	12ASB3	France	ASGROW	64	SDB1	USA	USDA
23	8ASB2	France	ASGROW	65	HAR-4	USA	USDA
24	9CSA3	France	Caussade	66	AS5305	France	ASGROW
25	H049+FSB	France	–	67	RHA274	USA	USDA
26	5AS-F1/A2×R2	France	ASGROW	68	H100A/RHA274	France	ASGROW
27	7CR16=PRH6	France	ASGROW	69	H209A/H566R	France	ASGROW
28	ENSAT699	France	C.F	70	ASO-1-POP-A	France	ENSAT
29	SSD-581	France	ENSAT	71	AS6305	France	ENSAT
30	TMB-51	France	ASGROW	72	D34	USA	USDA
31	110	Iran	INRAMONT	73	CAY	France	ENSAT
32	H603R	France	SPII	74	346	Iran	SPII
33	4	Iran	SPII	75	NS-F1-A5×R5	France	NOVARTIS
34	703-CHLORINA	France	INRAMONT	76	36	Iran	SPII
35	NSF1-A4×R5	France	SPII	77	38	Iran	SPII
36	28	Iran	ENSAT	78	SDB2	France	INRAMONT
37	30	Iran	NOVARTIS	79	H158A/LC1064	–	–
38	F1250/03	Hungary	SPII	80	H156A/H543R	France	ASGROW
39	SDR18	USA	SPII	81	H543R/H543R	France	ASGROW
40	LP-CSYB	France	–	82	H543R	France	–
41	803-1	Serbia	USDA	83	15038	France	ASGROW
42	1009370-1(100K)	France	ENSAT				

checked by running 1 mL DNA in 0.8% (w/v) agarose gels in 0.5× TBE (Tris-borate-EDTA) buffer: 45 mmol L^{-1} Tris base, 45 mmol L^{-1} boric acid, 1 mmol L^{-1} EDTA (ethylenediaminetetraacetic acid), pH 8.0, at 80 V for 45 minutes. DNA samples that gave a smear in the gel were discarded. A total of 30 microsatellite markers (out of 339 ORS (Oregon State University) SSR markers) were used for DNA fingerprinting (Table 2). Polymerase chain reaction (PCR) for SSR markers was performed in a volume of 15 µl. The reaction mixture contained the following: 2.5 mmol L^{-1} of each primer, 0.4 U of Taq DNA polymerase (Cinagene Co., Iran), 0.8 µl of each dNTP (deoxyribonucleoside triphosphate) (BioFluxbiotech), 2 µl of 10× PCR buffer, 0.16 µl $MgCl_2$ (Cinagene Co.), ddH_2O, and 5 ng of template DNA. Touchdown PCR was used for amplification of SSRs, which is as follows: 94°C (3′) + 1 cycle [(94°C (30″) + 64°C (30″) + 72°C (30″)]. This was followed by 10 cycles, with a decrease in annealing temperature with 1°C per cycle + 30 cycles [(94°C (30″) + 54°C (30″) + 72°C (45″)] + 72°C (10′). The reaction products were mixed with an equal volume of formamide dyes and resolved in a 3% (w/v) agarose gel (Invitrogen, France) in 0.5× TBE buffer. Those were stained with 1.0 mg mL^{-1} ethidium bromide and photographed under UV light, using an image analysis system Gel-Doc (Gel Logic 212 PRO, USA).

Data analysis and association mapping. Normality test were done by procedure *Proc Univariate* in the *SAS*, version 9.2 and there was no need to transform the data. Descriptive statistics, such as mean, standard error, and coefficient of variation, for each studied character were calculated by using software *SAS*.

The effective analysis of population structure and detection of mixed genotypes were carried out using Bayesian method implemented in the software package of *STRUCTURE*, version 2.3.4 (Pritchard et al., 2000). The number of initial subpopulations (K) was considered from 1–20 and with 10 replications for each run. Length of the burn-in period and Markov chain Monte Carlo (MCMC) replication number were set to 100,000. The admixture model and correlated allele frequencies were chosen. The actual number of subpopulations was determined by: 1) the logarithm of likelihood for each K; Ln P (D) = L (K) (Rosenberg et al., 2002), and 2) the

Table 2. Primer sequences of 30 simple sequence repeat (SSR) loci and their positions on sunflower genetic linkage map used for fingerprinting of sunflower lines

Primer	Forward sequence 5′→3′	Reverse sequence 5′→3′	Linkage group	Position (cM)
ORS785	CAAAATACCCAGGTCAAAGCA	CCTAGCTTATGGGACGTATGGA	LG4	53.8
ORS807	CCGATATTTTGACCGATATTTTGC	TCTCACCCTTCATCTCCTTCC	LG16	67.9
ORS608	CATGGAAAGCCGAGTTCTCT	CGTGCGTGATTAACATACCC	LG6	44.7
ORS609	GCGAAGGAACTGAACCGATA	GGATTTTAGTCCGCCAATCA	LG12	56.0
ORS1079	TACGACTGACGATTCCATTTCTC	AACTGGATTTCACAGGGAGTGTT	LG14	14.4
ORS718	CACTTTACGCACACCAAACC	ATGCAACACCCGAATCAAA G	LG3	30.2
ORS1265	GGGTTTAGCAAATAATAGGCACA	ACCCTTGGAGTTTAGGGATCA	LG9	25.0
ORS949	TGCAAGGTATCCATATTCCACAA	TATACGCACCGGAAAGAAAGTC	LG3	38.7
ORS378	GTGAAACCTTCGGACCTCTG	GTACAAAACTTATAAATAAAACAATA	LG16	86.3
ORS694	CCTGGAACTGAACCGAGAAC	GCCGTGAAACAGAGAGAGGA	LG14	35.8
ORS621	CGCCTTATGCTGAGAGGAAA	CCTGAAGCGAAGAAGAATCG	LG11	1.1
ORS488	CCCATTCACTCCTGTTTCCA	CTCCGGTGAGGATTTGGAT T	LG3	67.2
ORS728	CTCCATAGCAACCACCTGAAA	CCAAACTCTGAATGATACTTGTGAC	LG1	25.2
ORS844	ACGATGCAAAGAATATACTGCAC	CATGTTTAATAGGTTTTAATTCTAGGG	LG9	75.5
ORS878	TGCAAGGTATCCATATTCCACAA	TATACGCACCGGAAAGAAAGTC	LG10	29.9
ORS1179	GATTCGGAGCTGTTAGGAGGTAG	AAACGGGAAGCAAGAATAGAACA	LG13	60.1
ORS1215	ATACTCTTCCACCCTCAAATCCA	GGTTGCGGTAGTGGTCTGTAGT	LG15	74.8
ORS822	CAATGCCATCTGTCATCAGCTAC	AAACAAACCTTTGGACGAAACTC	LG3	69.0
ORS1256	GATGTTGATGTTGGTGAAGTTGC	CTCCGTCACCTTAAGCACTTGTA	LG6	68.4
ORS1088	ACTATCGAACCTCCCTCCAAAC	GGATTTCTTTCATCTTTGTGGTG	LG10	49.0
ORS617	GGTACTTGGTATTCATGGGTCAT	GACACCGCCAACTTAACACTT	LG9	92.3
ORS1064	TGAATGATCTATGAGTGGTGATGG	ACTCGCAGTGGTAAGTCGTTAGG	LG16	13.7
ORS1209	AACAAGCAAGCAAATCAACCATA	AGAATTAAACCCAACCCGGAAC	LG10	27.0
ORS1264	TAGAAGCGGTTGGGTTGACAGTA	TGAACTCGGTTGATTCTCTAGCC	LG2	15.7
ORS1242	GCAATCGTTTCACTCTTCCATTC	TGGTCGTAGAATTGTCGGTCAT	LG15	63.3
ORS630	TGTGCTGAGGATGATATGCAG	GCACGACCCGGATATGTAAC	LG13	44.2
ORS733	TATGAGTTGGCAAGGGCTTC	GGACTCCAACGAGAGAATCAGT	LG11	0.0
ORS565	TGGTCAACGGATTTAGAGTCAA	TCCAGTTTGGTCTTGATTTGG	–	–
HA3040	GACCCGAACCACACT	GTTCTTGCTTCGATCC	–	–
HS3070	GGGATGAGCTCTGTC	CTTTTCAATTCCCGCT	–	–

LG – linkage group, cM – centimorgan

statistic Delta K (ΔK), which is based on secondary rate of changes in likelihood; ΔK = [L" (K)]/ Stdev (Evanno et al., 2005). In this method, the probability of slope breaks at the point where the number of hypothetical K is at the maximum point of likelihood. Association analysis was performed in TASSEL 2.1, using kinship (K-matrix) and ancestry coefficients (Q values) as covariates in mixed linear model (MLM).

Results and discussion

Phenotypic diversity. The basic statistics such as mean, range, standard deviation and coefficient of variation for studied characters in sunflower lines were summarized in Table 3. Maximum reduction was observed in the plant height, chlorophyll index, leaf number and grain yield. Reduction of leaf area and consequent decrease in the chlorophyll content of sunflower leaves under saline state were reported by other researchers (Anwar-ul-Haq et al., 2013). The maximum genotypic and phenotypic coefficients of variation were observed for head (capitulum) dried weight (0.41, 0.67) and upper petiole length (0.20, 0.44) in natural and salt stress conditions, respectively. The minimum genotypic and phenotypic coefficients of variation were observed for days to flowering as 0.03, 0.07 and 0.05, 0.08, respectively, both in natural and salt stress conditions (Table 3).

The highest heritability value in natural conditions was observed for 100-seed weight (79%) and plant height (79%) and the lowest one was observed for bottom leaf width. The highest heritability value in salt stress states was observed for stem diameter (0.7) and the lowest one was observed for bottom leaf length (0.21) (Table 3). Heritability is the ratio of genotypic to phenotypic variations. Characters with higher heritability can be more easily changed through selection. When heritability is high, phenotypic value is a good estimator of genotypic value. Overall, the heritability values were lower under salt stress conditions than under natural conditions. This shows that heritability increases in a better environment. Probably, this results from the effect of environment on genotypes under salt stress states. Similar results were reported regarding yield components' heritabilities in faba bean under drought stress conditions (Toker, 2004). The high phenotypic variations among studied sunflower lines indicated the suitability of present population for association analysis of salt related characters.

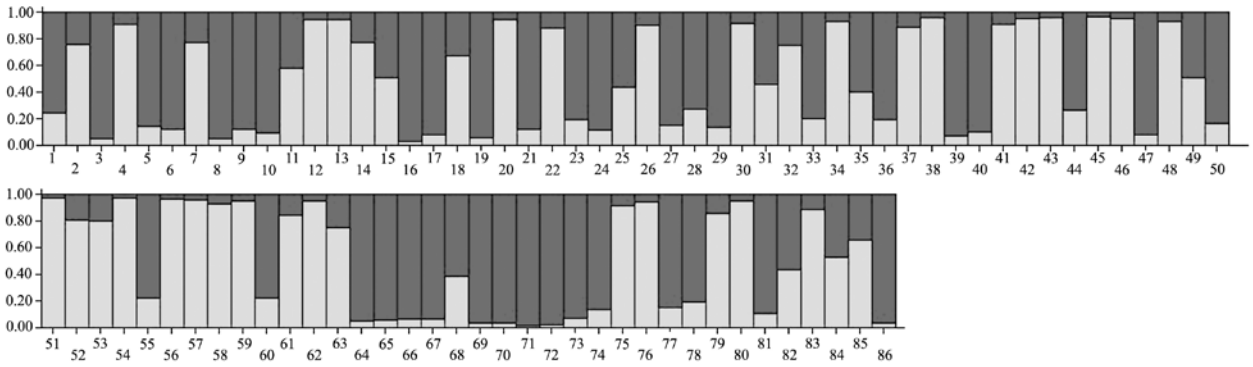
Genomic diversity and population structure. Using 30 SSR primer pairs, 71 alleles were generated, of which 87% were polymorphic. In the collection under investigation (association panel), 2.06% of the possible SSR locus pairs ([n (n-1)/2] = [30(30-1)/2] = 435 pairs) displayed a significant linkage disequilibrium (LD) ($P < 0.01$) (data not shown). LD presents the non-random correlation of alleles at different genetic loci on a single chromosome (Mackay, Powell, 2007). Several factors play important role in establishing the haplotype LD blocks in genome (Stich et al., 2005; Oraguzie et al., 2007). Out of which, mutation and recombination are the key factors affecting LD significantly. Effective analysis of the population structure and accurate classification of individuals into appropriate subpopulations were performed via Bayesian method implemented in the software *STRUCTURE*. This model-based clustering

Table 3. Descriptive statistics for agronomic characters in sunflower inbred lines evaluated under natural (NC) and salt stress (SSC) conditions

Trait	Item													
	Mean		SD		Vg		Vphe		H^2		CVg		CVphe	
	NC	SSC	NC	SSC	NC	SSC	NC	SSC	NC	SSC	NC	SSC	NC	SSC
Days to flowering	78.1	77.6	5.5	6.3	6.6	14.4	28.3	40.3	0.46	0.61	0.0	0.1	0.1	0.1
Leaf number	20.4	18.0	4.6	4.6	6.0	5.8	16.6	16.9	0.61	0.6	0.1	0.1	0.2	0.2
Stem diameter	11.8	11.3	2.4	1.9	2.5	1.6	5.5	3.5	0.7	0.7	0.1	0.1	0.2	0.2
Head diameter	9.1	8.5	2.5	2.2	2.8	1.4	6.4	4.7	0.69	0.54	0.2	0.1	0.3	0.3
Plant height	92.6	80.2	18.8	16.2	186	78.9	324	242	0.79	0.58	0.2	0.1	0.2	0.2
Upper leaf length	8.3	7.4	2.3	2.1	1.1	0.7	4.6	3.9	0.47	0.38	0.1	0.1	0.3	0.3
Upper leaf width	5.2	4.8	2.0	2.0	0.7	0.4	3.6	3.4	0.43	0.27	0.2	0.1	0.4	0.4
Upper petiole length	3.1	2.6	1.3	1.2	0.4	0.3	1.4	1.4	0.53	0.42	0.2	0.2	0.4	0.4
Central leaf length	13.0	12.2	2.8	2.3	2.5	0.9	7.3	4.9	0.59	0.38	0.1	0.1	0.2	0.2
Central leaf width	10.8	10.2	2.9	2.3	2.7	0.6	7.1	5.1	0.64	0.26	0.2	0.1	0.3	0.2
Central petioles length	8.3	7.4	2.0	1.9	1.5	0.6	4.1	3.5	0.61	0.38	0.2	0.1	0.3	0.3
Bottom leaf length	7.8	7.9	2.1	2.4	0.3	0.4	3.3	5.0	0.23	0.21	0.1	0.1	0.2	0.3
Bottom leaf width	5.1	5.4	2.1	2.5	0.1	0.5	3.2	5.1	0.1	0.24	0.1	0.1	0.4	0.4
Bottom petiole length	6.4	6.5	2.0	2.2	0.9	0.9	3.6	4.7	0.47	0.41	0.1	0.2	0.3	0.3
Chlorophyll index	38.2	28.6	5.3	4.9	9.1	7.6	28.4	23.4	0.57	0.58	0.1	0.1	0.1	0.2
Head dried weight	11.8	12.4	7.9	7.5	23.0	17.8	61.9	53.4	0.63	0.59	0.4	0.3	0.7	0.6
Grain yield per plant	25.9	24.0	9.5	8.1	34.1	17.7	88.7	65.5	0.64	0.51	0.2	0.2	0.4	0.3
100-seed weight	4.6	3.9	1.7	1.6	1.7	0.8	2.9	2.5	0.79	0.59	0.3	0.2	0.4	0.3

SD – standard deviation, Vg – genotypic variance, Vphe – phenotypic variance, H^2 – heritability, CVg – genotypic coefficient of variation, CVphe – phenotypic coefficient of variation

method is based on the allocation of individual to K clusters in such a way that Hardy-Weinberg equilibrium and linkage equilibrium are established within clusters. However, these kinds of equilibrium are absent between clusters. Maximum value of ΔK was observed in K = 2. Therefore, the studied panel probably consisted of two subpopulations (Fig.). Out of 84 studied sunflower lines, 12 lines showed mixed structure and 72 lines belonged to each one of first (36) and second (36) subgroups.



Note. Numbers on y-axis show the membership coefficient to sub-populations and numbers on x-axis show the individual code that belongs to sunflower populations.

Figure. Genetic relatedness of sunflower lines identified in software *STRUCTURE*

Based on the data obtained from the genetic diversity and population structure analysis, the studied association panel had a diverse genetic variation and was, thus, suitable for the association analysis.

Association analysis. The significant level of LD found in this study supported the idea of using natural populations for genomic studies. Association studies are affected by the problem of false positive rates coming from population structure and genetic relationships among individuals. This results from various factors such as admixture, migration and historical pedigree relationships. Theoretically, kinship creates LD not only between genetically linked loci but also between genetically unlinked loci when predominant parents are included in the population. In maize, kinship equally generated LD between genetically linked and unlinked loci (Stich et al., 2005). One of the main advantages of linear models is the promise of improved resolution by considering subpopulation and kinship effects in association model (Breseghello, Sorrells, 2006). Association tests between SSR markers and studied traits were performed using mixed linear models implemented in TASSEL software (Bradbury et al., 2007). The *P*-value determined whether a character was linked with a marker significantly or not. The MLM could remove most of the false positives by incorporation kinship coefficients (K-matrix) and ancestry coefficient (Q values) estimates as covariates in the association model (Saeed et al., 2014). Several SSR loci associated with genes controlling character were identified. A total of 6 and 13

significant ($P < 0.01$) marker-character associations were detected by MLM in natural and salt stress conditions, respectively (Table 4). However, P822 was associated with head diameter, P878 and P844 with plant height, P822 with chlorophyll and P949 with 100-seed weight.

Some markers were associated with characters under both natural and salt stress conditions. For instance, the SSR marker P608 was associated with more than one character such as days to flowering, leaf number, central leaf width, head dried weight, and 100-seed weight under both natural and salt stress conditions. Marker P718 was associated with days to flowering and leaf number, P1179 – with central petioles length and upper petiole length both in natural and salt stress conditions. Common markers between characters can be due to pleiotropic effects or linkage between genomic regions, involved in controlling characters (Jun et al., 2008). Identifying common markers is of great importance in plant breeding because it makes possible simultaneous selection of multiple characters. Basirnia et al. (2014) used MLM procedure to determine the SSR associated with chloride accumulation rate in oriental-type tobacco genotypes. Morsali et al. (2015) used a linkage map comprising 221 molecular markers (210 SSR/11 SNP) to identify genomic regions associated with physiological traits in sunflower under salinity stress conditions. Sahranavard et al. (2015) used general linear model (GLM) and mixed linear model (MLM) association models to

Table 4. Simple sequence repeat (SSR) loci identified for studied characters in sunflower inbred lines under natural and salt stress conditions using mixed linear model (MLM)

Character	SSR marker	Natural conditions		Salt stress conditions	
		P-value	*F*-value	*P*-value	*F*-value
Days to flowering	P608	0.0184	5.8197	0.0053	8.2832
	P718	0.0262	5.1677	0.0097	7.0859
	P694	–	–	0.0052	5.6676
Leaf number	P608	0.0093	7.1542	0.000047	18.7964
	P718	0.0106	6.9066	0.0128	6.5413
Stem diameter	P608	–	–	0.000126	16.4525
	P694	0.047	3.19430	–	–
	P822	–	–	0.0223	2.8119
	P1079	–	–	0.0421	3.3138
Head diameter	P728	0.0105	4.8929	–	–
	P630	0.0423	2.6118	–	–
	P304	–	–	0.0495	2.1105
	P694	–	–	0.0498	3.132
Plant height	P878	0.0302	3.6934	–	–
	P630	–	–	0.0493	2.5105
	P378	–	–	0.0246	3.9465
	P844	–	–	0.0112	4.7826
Upper leaf length	P718	–	–	0.043	4.2546
Upper leaf width	P488	–	–	0.0098	7.1035
	P378	–	–	0.0065	5.4827
	P996	–	–	0.0428	3.3084
Upper petiole length	P1179	0.0133	6.4599	0.0379	4.4842
	P694	0.0378	3.4315	0.0114	4.7727
	P378	–	–	0.0338	3.59
Central leaf length	P608	0.0025	9.7988	–	–
	P1088	0.0402	4.3565	–	–
	P718	–	–	0.0391	4.4275
Central leaf width	P608	0.0011	11.4753	0.0479	4.0531
	P565	–	–	0.027	3.8879
Central leaf width	P608	0.0011	11.4753	0.0479	4.0531
	P565	–	–	0.027	3.8879
Central petioles length	P1179	0.0454	4.1522	0.000883	12.1018
	P378	0.0495	3.1624	–	–
	P1265	–	–	0.0096	4.9418
Bottom leaf length	P996	0.0468	3.2082	–	–
Bottom leaf width	P304	–	–	0.03	2.3399
	P565	–	–	0.0473	3.2452
Bottom petiole length	P1265	0.0454	3.2221	0.0342	3.5325
	P996	0.0246	3.9216	–	–
	P378	–	–	0.0327	3.625
	P718	–	–	0.041	4.3429
Chlorophyll	P822	0.0170	2.9676	–	–
	P630	0.0016	3.4005	0.0016	4.8494
Head dried weight	P608	0.0037	8.9907	0.0014	11.1238
	P1256	–	–	0.0113	6.7372
	P621	–	–	0.0337	3.5667
Grain yield per plant	P630	–	–	0.0048	4.092
	P1079	–	–	0.0199	4.1395
100 seed weight	P608	0.0002154	15.239	0.0000063	23.8091
	P949	0.0286	3.7373	–	–
	P307	–	–	0.0304	2.8399

identify some microsatellite markers associated with agro-morphological characters in 106 sunflower lines under normal conditions. These studies accelerated the application of molecular markers in sunflower improvement programs to some extent. Association analysis was also used in other crops, such as wheat (Liu et al., 2010), barley (Wang et al., 2012), sorghum (Shehzad et al., 2009) and corn (Andersen et al., 2007). For salt tolerance related characters, different QTLs were identified in rice, including those on linkage group 1 – such as Saltol QTL, QNa, and SKC1/ OsHKT8 – along with QNa:K on linkage group 4. Saltol was involved in ion uptake during salinity stress (Bonilla et al., 2002).

Conclusion

Salt tolerance is a quantitative character governed by several genes. Poor knowledge about mechanism of its inheritance makes slow progress in its introgression into target crops. Our study showed extensive genetic variation in salt tolerance. Several simple sequence repeat (SSR) markers were identified for salt tolerance related characters, which can be potentially exploited in common sunflower (*Helianthus annuus* L.) improvement programs via marker-aided selection. Among the various molecular markers, SSR and single nucleotide polymorphism (SNP) markers have received considerable attention, because of co-dominant inheritance. Structure analysis divided the studied population of sunflower into two subpopulations. We presented numerous significant maker-characters associations over the whole sunflower genome. Some markers were associated with characters under both natural and salt stress states. For instance, the SSR markers such as P608, P718, P1265 and P1179 were associated with characters both in natural and salt stress conditions. The genetic relationships between any characters under different conditions were indicative of the presence of common quantitative trait loci (QTL). This further suggested that improving a character under one condition might result in offspring with improved character in other states. Markers with the highest association could be used for saturating linkage maps. Moreover, other physiological and agronomic characters could be studied with SSR markers to make a robust and tolerant plant with high yield.

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Nustatytos preliminarios asociacijos tarp paprastosios saulėgrąžos DNR žymeklių ir morfologinių požymių natūraliomis ir druskos streso sąlygomis

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Santrauka

Dirvožemio druskingumas yra rimta grėsmė žemės ūkio produktyvumui visame pasaulyje. Žemės ūkio biotechnologijų pagrindinis tikslas yra sukurti augalus, pasižyminčius didesniu atsparumu sudėtingoms aplinkos sąlygoms, pavyzdžiui, sausrai ir druskingumui. Siekiant pagerinti augalų savybes, pirmasis žingsnis žymekliais pagrįstose selekcijos programose yra asociacijos tarp žymeklio ir požymio nustatymas. Tyrimo metu kiekybiniai požymių lokusai, susiję su atsparumu druskingumui, buvo nustatyti naudojant 84 skirtingos geografinės kilmės paprastosios saulėgrąžos (*Helianthus annuus* L.) inbredines linijas. Linijos buvo identifikuotos su 30 paprastosios pasikartojančios sekos žymeklių. Buvo nustatyta ir įvertinta 71 aiškus fragmentas, iš kurių 87 % buvo polimorfiški. Asociacijos tarp paprastųjų pasikartojančių sekų (PPS) žymeklių ir 18 agronominių savybių analizuoti taikant mišrų linijinį modelį. PPS žymeklių analizės duomenimis, asociacijų grupė pasidalijo į dvi subpopuliacijas (K = 2). Iš tirtų PPS žymeklių 435 galimų lokusų porų maždaug 2,06 % parodė esminį sąsajų nesubalansuotumą. Esant natūralioms ir druskos streso sąlygoms, atitinkamai 6 bei 13 PPS lokusų ir vertintų agronominių savybių asociacijos buvo esminės ($P \leq 0,01$). Keletas molekulinių žymeklių buvo esmingai susiję su daugiau nei viena fenotipine savybe; tai rodo galimą genetinės sankibos, arba pleiotropinę, įtaką. Tikimasi, kad nustatyti asociacijas turintys žymekliai bus naudingi vykdant jais pagrįstą atranką paprastosios saulėgrąžos selekcijos programose.

Reikšminiai žodžiai: abiotinis stresas, aliejiniai augalai, jungčių nesubalansuotumas, kiekybinis požymių kartografavimas, molekuliniai žymekliai.