MARKER TRAITS ASSOCIATION OF SOME BARLEY GENOTYPES UNDER SOIL SALINITY CONDITION USING SSR MARKERS

M. E. EL-DENARY[1], M. N. NOAMAN[2], A. F. ABDELKHALEK[3] AND S. A. MARIEY[2]

1. *Faculty of Agriculture, Tanta University, Genetics Department*
2. *Barley Res. Dept., Field Crops Research Institute, Agricultural Res. Center, Giza, 12619, Egypt*
3. *Rice Research and Training Center, Sakha, Kafr El-Sheikh, Egypt*

Barley (*Hordeum vulgare* L.) 2n = 2x = 14 is a crop with a great adaptation potential in many regions of the world. Growers can obtain a harvest in areas with low precipitations, mainly because this crop has advantages in aspects such as salt, drought, frost tolerance and the early period of development (Bennett and Smith, 1976). It is ranking the fourth crop in terms of production after wheat, rice and maize (Bengtsson, 1992). In terms of importance, barley is used mainly for animal feed, brewing malts and for human consumption in several countries. It is one of the most economic and important cereals grown under saline or partially reclaimed alkaline soils.

Salinity is a major abiotic stress affecting crops in Egypt and throughout the world. More than 800 million hectares of land are salt affected globally, accounting for more than 6% of the total land area (Munns and Tester, 2008). Egypt is one of the countries that suffer severe salinity problems in some areas. For example, 33% of the cultivated land (Ghassemi *et al.*, 1995), which comprises about 3% of total land area in Egypt, is already salinized. The reduction in production of soils affected by salinity is about 30% (El-Lakany *et al.*, 1986).

Ashraf and Haris (2004) found that salt tolerance is a complex trait and is affected by large number of mechanisms. Therefore, the identification of a single criterion for ranking genotypes for their tolerance to salt stress is extremely difficult. Thus, by manipulating the heritable variation present in the germplasm, they concluded the possibility of developing saline-tolerant cultivars through breeding technique, but it is a cumbersome and time-consuming process. Flowers (2004) found that there was sufficient evidence that salt tolerance is a multi-genic trait, which suggested that the overall trait is determined by a number of sub-traits any of which might, in turn, be determined by any number of genes. These sub-traits generally include the ability to minimize the net accumulation of sodium and/or chloride ions and to select potassium from a background of high sodium concentration. Ahmed *et al.* (2001) found that bar-

ley genotypes significantly differed in plant height, biological yield and grain yield. They added that it was possible to identify some barley genotypes that could survive under salt stress conditions.

Ahmad *et al.* (2003) stated that increasing sodium chloride and sodium sulfate concentration resulted in the reduction of number of tillers, length of spike, number of spikelets per spike, biomass per plant and grain yield per plant. They also found that increasing sodium chloride concentration resulted in greater damage to all cultivars than sodium sulfate. Taghipour and Salehi (2008) studying salt tolerance of Iranian barley (*Hordeum vulgare* L.) genotypes in seedling growth stages found significant differences among the genotype x stress interaction for all characteristics studied. Their results showed that seedling growth stages were decreased in all 12 barley varieties they have studied with increasing salinity level.

However, the advent of the Polymerase Chain Reaction (PCR) favored the development of different molecular techniques. Simple sequence repeats (SSRs) are at the moment the most popular and widely used PCR-based marker systems in Marker-Assisted Selection (MAS). SSR markers combine a number of advantages for practical applications, as they are codominant and multi-allelic, stably inherited, amenable to automation and high-throughput analysis, highly variable, and detect the highest level of polymorphism per locus (Röder *et al.,* 2004). They are highly reproducible, highly polymorphic, PCR-based and readily portable within a species (Edwards *et al.,* 1996). SSRs polymorphism is easily assayed by PCR. Finally SSRs marker is technically efficient, cost-effective to use and are available for barley (Petersen and Seberg, 1998). According to Pupko and Graur (1999), any number of tandem repeats of a certain nucleotide combination may be regarded as a microsatellite. In addition, SSR markers are distributed all over the genomes (Varshney *et al.,* 2007). All these factors make them the markers of choice for genetic research. Barley is one species in which SSRs have been developed and there are now over 500 mapped (Waugh *et al.,* 1997). Initial work using SSRs in wild barley diversity studies involved just 11 mapped SSRs (Forster *et al*., 1997). The variation of SSRs in cultivars, landraces and wild barley shows that landrace and wild barley have unique alleles not found in the cultivated gene pool (Powell, 1997). The use of molecular markers accelerates the breeding process and offers a straightforward aid in the selection of resistant genotypes.

Eleuch *et al.* (2008) investigated genetic diversity of barley accessions for grain yield, heading date and plant height under salinity. They used 48 barley genotypes with 22 microsatellite simple sequence repeat (SSR) markers. Four of the 22 markers (Bmac316, scssr03907, HVM67 and Bmag770) were able to differentiate all barley genotypes. Cluster and principal coordinate analysis allowed clear grouping between countries from the same region. The genotypes used in this

study have been evaluated for agronomic performance at different environments. Also their study revealed a close association of the marker Bmag749 (2H and bin 13) in two different environments with common significant alleles (175 and 177), whereas the HVHOTR1 marker (2H and bin 3) was only significant at Sakha-Egypt with alleles size being 158 and 161 bp. Heading date also showed an association with scssr03907 through the common significant specific allele 111 and EBmac0415 markers at three different agro-climatic locations, whereas HVCMA, Scssr00103 and HVM67 were linked to heading date in the Egyptian environment only. The plant height association analysis revealed significant markers Bmag770 via the significant allele 152 and Scssr09398.

Therefore, the main objectives of the present study were to: 1) study the genetics of yield and yield components in some barley genotypes under salinity stress, 2) detect the best genotypes, which are salt tolerant and 3) establish specific DNA markers associated with salt tolerance in barley genotypes using SSR patterns to be useful in barley breeding programs.

MATERIALS AND METHODS

The present field investigation was carried out in the Sakha Research Farm, Barley Research Department, Field Crops Research Institute; Agricultural Research Center during two growing seasons; 2009/2010 and 2010/2011. Laboratory work was carried out at the Central laboratory for environmental studies, Kafr El-Sheikh University, Egypt. Two field experiments were carried out in this study; the first experiment was carried out during 2009/2010 season at two locations; El-Serw (as a saline soil) and Sakha (as a control) using 20 genotypes varied in their tolerance/sensitivity to salinity stress and were sown in a small scale as individual plants. The second experiment was carried out during 2010/2011 season at the same two locations; El-Serw and Sakha using the same twenty genotypes but sown in a large scale in bigger plots (1.6 m^2).

The selection criteria of these genotypes were based on pedigrees, origin of each genotype and the genotype performance, yield and its components, heading date and plant height (Eleuch *et al.*, 2008), based on normal distribution curve, while the focus was on those traits which are associated with salt tolerance. The present investigation also intended to study molecular markers associated with salt tolerance to be useful in barley future breeding programs. Moreover, to study the genetics of yield and yield components in the studied barley genotypes in order to detect the best genotypes, which are expected to be salt tolerant and to understand the genetic basis of key agronomic traits for the development of molecular markers.

Barley Genotypes

Twenty genotypes of barley (*Hordeum vulgare* L.) were selected from 48 genotypes based on their tolerance/sensitivity to salinity stress (Table 1). Barley genotypes were kindly provided by

Sakha Barley Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

Field experiments

Twenty genotypes selected from 48 genotypes were grown in the field at two locations (Sakha non-saline and El-Serw saline soil) in two cropping season 2009/2010 and 2010/2011 after taking soil samples from the experimental site at El-Serw to measure salinity level (EC). The twenty genotypes were planted in a randomized complete block design (RCBD) with three replicates each plot consisted of a genotype, which was planted in one row 2.5 m long and 30 cm apart in 2009/10 season and in plots of four rows 2.0 m long and 20 cm apart (plot area = 1.6 m^2) with three replications in the 2010/11 growing season.

Soil samples

Soil samples were taken before land preparation in two depths from the soil surface; i.e. 0-15 cm and 15-30 cm. Chemical properties of the soil in El-Serw and Sakha locations for the two seasons; 2009/2010 and 2010/2011 are shown in Table (2) and irrigation water for the two seasons at El-Serw are shown in Table (3). Field experimental samples were analyzed according to Piper (1950) and Black *et al.* (1965).

Studied Characteristics

Five growth measurements for the twenty barley genotypes were taken on ten individual plants which had been randomly taken from the central rows of each plot are seedling rate (%), days to 50% heading (days) (DH), plant height (cm), number of tillers/m^2 and grain yield (kg/m^2)

Statistical Analysis

Data collected from the two seasons were statistically analyzed as a randomized complete block design (RCBD) using analysis of variance (ANOVA) for each season and over all the two locations in the two seasons 2009/10 and 2010/11 as a combined analysis. The means of genotypes and cultivars included in this trial were compared using Duncan's New Multiple Range Test (Duncan, 1955) (LSD) at 0.05 level of probability. All statistical analyses were performed using the computer software MSTAT-C Computer Program according to (Snedecor and Cochran, 1969).

Microsatellite markers, DNA extraction and PCR amplification

Ten microsatellite primers from the published sequences of (Saghai-Maroof *et al.*, 1994; Pillen *et al.*, 2000; Ramsay *et al.*, 2000; Karakousis, 2002) have been used for this study. They were on average 18-24 bp in length. Primers' sequences, chromosomal location, size range, marker type, motif and the reference are listed in Table (4). Genotyped markers were assigned using the Grain Genes data base (*http://grain.jouy.inra.fr/cgibin/graingenes/browse.cgi*) (Kleinhofs and Graner, 2001).

Genomic DNA of the 20 barley genotypes was extracted from leaves isolated using CTAB method adapted by (Doyle and Doyle, 1990). The quantification of DNA was confirmed by agarose gel electrophoresis (2%) in 1 x TBE buffer against 100 bp DNA Ladder as a size marker. Polymerase chain reaction (PCR) amplification was prepared in volume of 25 µl using 40ng genomic DNA, 2 µmol dNTP, 25 mM $MgCl_2$, 10 pmol of each primer (forward and reverse), 5 U *Taq* polymerase.). PCR cycling was carried out as the following program; one cycle at 95°C for 5 min., then 35 cycles were performed as follows: 1min. at 95°C for denaturation, 45 sec. at (based on primer almost 54~56°C) for annealing and 30 sec. at 72°C for extension. Reaction was incubated at 72°C for 7 min then at 4°C for keeping.

Statistical analysis and data scoring

The amplified bands from SSR were scored under the heading of total scorable fragments. Amplification profiles of the 20 barley genotypes were compared with each other and bands of DNA fragments were scored as a binary data where presence (1) or absences (0), for all accessions and the marker-traits associations was investigated for the five characteristics under salt conditions in the two seasons 2009/10 and 2010/11. For identification of associations between SSRs and agronomic traits, ANOVA analyses were performed using COSTAT software to examine associations that were more likely could be based on repeat variation of SSRs. Through F-test using binary data, these specific significant alleles per significant marker have been disclosed according to Ivandic *et al.* (2002).

RESULTS AND DISCUSSION

Data were classified into two major topics; field screening and molecular analysis:

Field screening

Generally, field screening for salinity tolerance remains the main tool, despite its limitation of time required and environmental dependency. However, many potential criteria or traits have been proposed for field screening. The significant and the mean performance of the 20 barley genotypes were calculated for the five studied characteristics for the 20 barley genotypes, which was selected from 48 genotypes and were grown in the field at two locations (Sakha, non-saline and El-Serw, saline soil) in two cropping seasons 2009/10 and 2010/11.

Seedling rate (SR)

Seedling growth rate of each genotype was estimated and data were analyzed and tabulated in Tables (6 & 8). The data showed high significant differences among all 20 genotypes at seedling stage in locations and their combined during the two cropping seasons 2009/10 and 2010/11. Data indicate that the mean values of the highest germination of seedling stage were obtained from barley cultivar no.2 (Giza 123) and barley cultivar no. 8

(California Mariout) giving 100% germination in both locations and in their combined during the two cropping seasons. On the other hand, data in Table (7) indicate that barley genotype no.18 gave the lowest mean value of the germination under El-Serw (26.7%), (66.7%) under Sakha and about (46.7%) in the combined between the two locations followed by barley cultivar no.5 (Giza 132) giving (26.7%) under El-Serw conditions, (73.3%) under Sakha and (50.0%) in their combined in 2009/10 season. Moreover**,** the data in Table (9) showed barley genotype no.17 giving the lowest mean value of the germination at El-Serw (8.3%), and about (47.5%) in the combined between the two locations during 2010/11, followed by barley cultivar genotype no.5 (Giza 132) and barley genotype no.11 (Dier Alla) both gave the same value (80.0%) at Sakha.

High significant interaction (GxL) between the two locations (L) and genotypes (g) for seedling growth rate was detected in both seasons (Tables 6 & 8). These results were similar to those reported by Naseer *et al.* (2001) who reported that salt tolerance at the seedling stage is important because the initial plant stand affects the final production in growth stages, and Taghipour and Salehi (2008) who found that there were significant differences among the genotype × stress interaction for seedling growth.

Days to heading (DH)

Data of the appearance of 50% of spikes from the sheath (known as days to heading) are presented in Tables (6&8) demonstrating high significant differences for this characteristic among barley genotypes and between the two locations and their combined during the two cropping seasons. Results in Table (7) show the mean values for DH of the 20 barley genotypes under study at the two locations during the first season. Genotype no. 12 was the earliest at the two locations El-Serw and Sakha (79.3 and 89.3 days), respectively. In addition, this genotype was the earliest across the two locations having an average of 84.3 days and in the second season 2010/11 (Table 9) show that barley cultivar no.9 (Saiko) was the earliest at the two locations; El-Serw and Sakha (87.7 and 96.3 days), respectively. In addition, this genotype was the earliest across the two locations having an average of 92.0 days.

On the other hand, in the first season the latest barley cultivar was no.11. (Dier Alla) with average values of (89.3 days) at El-Serw, while at Sakha barley cultivar no.5 (Giza 132) was the latest (96.0 days). However, in the second season the latest barley genotype was no.17 with an average of 101.7 days at El-Serw, while at Sakha barley genotype no.10 (Beecher) and no.11 (Dier Alla) were both the latest genotypes and had the same value recording (105.7 days). Data in Tables (6 & 8) show significant interaction (GxL) between the two locations (L) and genotypes (G) for heading date in the two cropping seasons. These results are in agreement with those reported by (Ellis *et al.*, 2000; Mariey, 2004; Oraby *et al.*,

2005; Eleuch *et al.*, 2008).

Plant height

Data in Tables (6&8) showed high significant differences among the 20 barley genotypes for plant height at the two locations and their combined in both cropping seasons. Results in the first season, 2009/10 in Table (7) indicate that barley genotype no. 12 was ranked first for plant height (62.2 cm) under El-Serw conditions, and means of this trait clearly indicated that the Egyptian barley cultivar genotype Giza 123 was the tallest at each individual location and their combined recording 61.3, 100.5 and 80.9 cm, respectively. However, in the second season the results indicate that barley genotype no. 19 was ranked first for plant height (89.3 and 105.8 cm), at El-Serw location and the combined, respectively. Mean values of this trait clearly indicate that the Egyptian barley cultivar no.2 (Giza 123) was the tallest at Sakha location recording 124.3 cm.

On the other hand, in the first season (Table 7) the shortest genotype was recorded by genotype no.5 at each individual location and their combined recording 46.5, 58.9 and 52.7 cm, respectively, followed by genotype no.18 at each individual location and their combined recording 47.3, 66.7 and 57.6 cm, respectively. However, in the second season 2010/11 (Table 9) data indicate that the shortest genotype was recorded by barley cultivar no.6 (CC 89) at El-Serw location (67.3 cm), while barley genotype no.17 was the shortest at each of Sakha location and in the combined recording 100.7 and 87.6 cm, respectively. High Significant interaction (GxL) between the two locations (L) and genotypes (G) for plant height was detected (Tables 6 & 8). These results are in agreement with those recorded by (Ahmed *et al.*, 2001; Mariey, 2004; Singh, 2011).

Number of tillers plant^{-1}

Regarding number of tillers plant^{-1} in the first and second seasons, the data in Tables (6&8) indicate that there were high significant differences among the 20 barley genotypes at the two locations; El-Serw and Sakha and their combined in the two cropping seasons. The mean performance in the first season as shown in Table (7) reveal that barley cultivar no.1 (Giza 121) ranked first for number of tillers plant^{-1} at both locations and in their combined in the first season. However, data in Table (9) show that the same genotype no.1 (Giza 121) gave the highest value for number of tillers m^{-2} at Sakha and in their combined (597.0 and 517.0 tillers/m^2, respectively), while barley cultivar no. 2 (Giza 123) gave the highest value for number of tillers m^{-2} at El-Serw location (467.0 tillers/m^2) in the second season.

On the other hand, at El-Serw location and in the combined analysis (Table 7) data showed that barley cultivar no. 6 (CC89) had the lowest value for number of tillers plant^{-1} (6.4 and 8.1 tillers/plant^{-1}), respectively, and barely genotype no.17 was recorded as having the lowest genotype for number of tillers plant^{-1} (8.2 tillers/plant^{-1}) at Sakha location, while in

Table (9), in the second season, data revealed that barley genotype no.5 (Giza 132) had the lowest value for number of tillers m^2 (243.0 and 313.5 tillers/m^2) at El-Serw location and in their combined, respectively, and barely genotype no. 18 recorded the lowest value of number of tillers m^2 (353.0 tillers/m^2) at Sakha location. The combined analysis (Tables 6 & 8) showed high significant effect of the interaction between locations (L) and genotypes G (GxL) for the number of tillers/plant^{-1}, in both seasons. These results were supported by the results reported by (Ahmed *et al.*, 2003; Mariey, 2004; Singh, 2011).

Grain yield

Regarding grain yield and its response to salinity stress, high significant differences for grain yield among all 20 barley genotypes were detected as shown in Tables (6 & 8) at the two locations; El-Serw and Sakha and their combined in the two cropping seasons. Mean values of grain yield per plant under study are presented in Table (7). The maximum grain yield per plant (36.1 g) was obtained by barley cultivar no.1 (Giza 121) at Sakha followed by barley cultivar no.2 (Giza 123) at El-Serw and the combined (18.7 and 25.5 g), respectively, whereas the minimum value (5.8 g) was obtained by genotype no. 10 at El-Serw location, whereas barley genotype no. 14 gave the lowest value at Sakha and the combined recording 10.7 and 8.9 g, respectively. Moreover, data in Table (9) indicate that the maximum grain yield (Kg m^2) was obtained by barley cultivar no. 2 (Giza 123) at El-Serw and combined (0.98 and 1.11 Kg/m^2), while genotype no.20 gave the maximum grain yield (1.60 Kg m^2) at Sakha. On the other hand, the minimum value (0.17 Kg/m^2) was obtained by genotype no.17 at El-Serw location, while barley genotype no.5 (Giza 132) gave the lowest value at Sakha and combined recording (0.97 and 0.66 Kg/m^2), respectively. The combined analysis (Tables 6 & 8) showed high significant effect of the interaction (GxL) between locations (L) and genotypes (G) for grain yield per plant in the first season and grain yield in Kg/m^{-2} in the second season. These results are in agreement with those reported by (Ahmed *et al.*, 2001; Ahmed *et al.*, 2003; Mariey, 2004; Oraby *et al*., 2005; Singh, 2011).

It was concluded from the abovementioned information that there was an interaction between genotypes and environment, and there were two barley genotypes namely; genotypes no. 9 (Saiko) from (France) and barley genotype no.12 (line from Cyprus), out yielded the check cultivars (Giza 123 and Giza 124) in grain yield, significantly. They also have some other advantages such as earliness, plant height, and number of tillers/m^{-2}. Therefore, it is suggested that these two genotypes need more genetic stability studies to be grown in such saline soils and could be used as new tolerant genotypes for the saline breeding programs. We also consider barley genotype no. 17 (from ACSAD) as sensitive cultivar for salinity stress and can be used in barley breeding program and molecular studies as well.

Molecular Analysis.

Out of 10 SSR primer pairs used, only six primers (Bmac209, Bmac316, SCssr03907, Bmag770, HVM67 and HVHOTRI) generated clear patterns with high polymorphism. Three of them showed monomorphic band profiles (Scssr 0013, Bmag 387 and HVHvA1).

Association analysis

Marker traits associations with salinity for the genotypes were tested on the saline soil in the two cropping seasons as shown in Table (5). This is a novel way to associate between individual lines and DNA markers. This method allows us to screen as many as we can of barely lines for salinity tolerance. The six discriminatory primer pairs were used to evaluate marker traits association with salt tolerance in the 20 barley genotypes. These primer pairs revealed a total of 20 alleles ranging from two (HVHOTRI) to five (Bmac316) alleles per locus with a mean value of 3.5 alleles per locus. Moreover, primer pairs, Bmac0209 showed ambiguous scorable band with the 20 barely genotypes with varying response to salinity stress, which gave fewer band numbers, three alleles per locus but have high polymorphic percentage with 100% polymorphism as well as the marker HVHOTRI giving fewer band numbers, two alleles per locus with 50% polymorphism and showed obvious scorable band with the 20 barely genotypes with reliable response to salinity stress.

Out of the six primers, just two markers be evidenced for the marker traits association with salinity for three important traits (days to heading, plant height and grain yield), which was oriented as the agronomic traits association with the salt tolerance from the five studied traits under saline conditions in the two cropping seasons.

Heading date (HD)

Association analysis along with specific significant alleles was conducted for HD in the two cropping seasons 2009/10 and 2010/11. Data in Table (5) show a significant association of the marker HVHOTRI (chromosome 2H) in the two seasons under saline condition with common significant specific allele size 210 bp, and the marker Bmac0209 (3H) was significantly associated with HD in the first season under saline soil with common significant specific allele size 129 bp.

Plant Height (PH)

For plant height association analysis along with significance of that trait in Table (5) provided a significant association of the marker HVHOTRI (2H) with specific common allele size 210 bp in the first season under saline condition. Marker Bmag770 that have been reported by Eleuch *et al.* (2008) was associated with plant height but did not show any association with salinity tolerance in our study. Therefore, the marker Bmag770 should be further investigated in salinity programs of Egyptian barley genotypes for confirmation.

Grain Yield

Regarding, the association analysis along with significance of grain yield (Table 5) data showed a significant association of the marker HVHOTRI (2H) with specific common allele size 210 bp under saline condition in both seasons.

As a result, we can consider that marker HVHOTRI as shown in Fig. (1) is a marker-assisted selection for grain yield and days to heading under saline condition and we can also exercise this marker as specific marker for salt tolerance in Egyptian barely genotypes. This result was in a good harmony with those reported by Eleuch *et al.* (2008) who established similar data and found that the HVHOTRI marker (chromosome 2H) was only significant at Sakha, Egypt. We could also use the marker Bmac0209 (3H) shown in Fig. (1) as the specific marker for days to heading under saline condition for Egyptian barley genotypes.

Interestingly, our findings indicated that the potential efficacy of highly informative SSR markers were efficient screening for brewing genotypes in barley. Genetic relationships between barley cultivars revealed by genetic similarity at SSR levels were in agreement with their roles in agricultural production and breeding **(**Qian *et al.*, 2011**).** As a good confirmation, (Karakousis *et al.*, 2003**)** argued the usefulness of polymorphic SSR markers for the discrimination of breeding material in Australian barley. In barley, important traits such as salt tolerance are controlled by polygenes with additive and dominant effects that are described by quantitative trait loci (QTLs) **(**Eilles *et al.*, 2000) as salt tolerance is controlled by a variety of mechanisms.

For the present study we can consider that these genotypes which showed salt tolerance could serve as potentially novel germplasm that could be exploited for the development of new breeding lines with high level of salinity tolerance and to accelerate genetic advancement in barley and cost-efficient than conventional screening under saline field conditions. And we can indicate two markers which were more suitable for use in marker-assisted breeding than the others, the marker HVHOTRI (2H) was best in marker-assisted selection for most of the traits for salinity in Egyptian barley genotypes, also the marker Bmac0209 (3H) which can substantiated that the marker-assisted selection for days to heading in barley genotypes. These results are in a good harmony with those reported by (Eleuch *et al.*, 2008; Aliyu *et al.*, 2011). We can also conclude that these two markers were important and useful compared to the other markers, which need further invitations on Egyptian barley genotypes for salinity tolerance.

SUMMARY

The present study was conducted during two consecutive seasons; 2009/10 and 2010/11 to evaluate the performance of some barley genotypes under salt stress and to figure out the genetic pattern related to salt stress. Twenty barley genotypes differed in their tolerance potentiality

against salinity were planted in two screening field experiments at two locations; Sakha (as a control) and El-Serw (as saline condition) to detect their tolerance to salt stress. Moreover, molecular analyses were carried out using SSR-markers technique that could be associated with salt stress. The twenty barley genotypes were planted in a randomized complete block design with three replicates; each plot consisted of a genotype planted in one row 2.5 m long and 30 cm row spacing. The other field screening experiment was executed during 2010-2011 using the same 20 genotypes at the same two locations in a randomized complete block design in bigger plots of four rows 2 m long and 20 cm apart with three replicates. In the first experiment, Egyptian barley cv. no. 2 (Giza 123) and genotype no. 12 showed the highest mean values for most of the studied traits under saline conditions, and both of barley cultivars no. 8 (California Mariout) and no. 7 (Rihane-03) gave the highest mean values for some agronomic traits, while barley cultivars no.5 (Giza 132), no. 10 (Beecher) and no.18 showed the lowest mean performance values for most of the studied characteristics. Results from the second experiment showed that genotype no.9 (Saiko) gave the highest mean values for some traits such as heading date under saline condition. Out of the used ten SSR primer pairs, only six primers (Bmac0209, Bmac316, SCssr0397, Bmag770, HVM67 and HVHOTRI) generated clear patterns with high polymorphism. The six discriminatory primer pairs were used to evaluate the marker traits association with salinity under the saline soil, marker HVHOTRI (2H) had significant analysis with days to heading, plant height and grain yield with specific common allele size 210 bp and the marker Bmac0209 (3H) with specific common allele size 135 bp was specific marker for days to heading. It was concluded that those genotypes which showed salt tolerance could serve as potentially novel germplasm that could be exploited for the development of new breeding lines with high level of salinity tolerance and to accelerate genetic advancement in barley and cost efficient compared to conventional screening under saline field conditions.

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Table (1): Name, pedigree and origin and of 20 barley cultivars and lines included in the second and third filed experiments.

No.	Genotype		Origin	Pedigree
1	Giza 121		Egypt	Baladi16/Gem.
2	Giza 123		Egypt	Giza 117/FAO 86
3	Giza 124		Egypt	Giza 117/Bahteem 52// Giza 118/FAO 86
4	Giza 2000		Egypt	Giza117/Bahteem52// Giza118/ FAO86 / 3/ Baladi16/ Gem.
5	Giza 132		Egypt	Rihane-05//AS 46/Aths*2Athe/ Lignee 686
6	CC89		Egypt	Selected from composite crosses
7	Rihane3 (R3		ICARDA	As 46//Avt/Aths
8	California Mariout (CM)		Egypt	Selected landrace
9	Saiko		FRANCE	
10	Beecher		USA	Introduced to Egypt and named Giza 118
11	Dier Alla		Jordan	
12	Mr 25-84/Att/3/Mari/Aths//Bc	Line 1	Cyprus	CYB-5235-0AP
13	Alanda//Lignee527/Arar	Line 2	ICARDA	ICB89-0829-2LAP-3AP-0TR-3AP-0AP
14	Aths/Lignee686/5/Apm/RL/4/API/EB489-8-2-15-4//POR/U.SASK1766/3/ CEL/CL	Line 3	ACSAD	ACS-B-10328-5IZ-3IZ-IIZ-0IZ
15	CM67/4/Hma-02//11012-2/cm67/3/Arar	Line 4	ICARDA	ICB98-0238-0AP-7AP-0AP
16	Alanda01/5/c101021/4/CM67/U.Sask.1800//pro/CM67/3/dl70	Line 5	ICARDA	ICB890775-7AP-0AP-0AP-10AP-0AP-1AP-0AP
17	Panniy/Salmas/5/Baca"s"/3/AC253//C108887/C105761/4/JLB70-01	Line 6	ACSAD	ACS-B-10824-10IZ-3IZ-1IZ-0IZ
18	Lignee527//NK1272/3/Nacha2//Lignee640/Hma-01	Line 7	ICARDA	ICB95-0281-0AP-6AP-0AP-7TR-1TR-0AP
19	M6476/Bon//JO/York/3/M5/Galt//As 46/4/Hj34-80/Astrix/5/Nk1272	Line 8	ICARDA	ICB84-0156-0AP
20	ACSAD618//Aths/Lignee686	Line 9	ACSAD	ACS-B-9988-42IZ-1IZ-1IZ-0IZ

Table (2): Chemical properties of soil samples from the field experiments site at El-Serw and Sakha locations during the two consecutive seasons, 2009/10 and 2010/11.

Chemical properties	2009/10		2010/11	
	El-Serw	Sakha	El-Serw	Sakha
pH	8.3	7.2	8.6	7.9
ECe (dsm-^{1})	11.6	2.1	12.8	3.7
$CaCO_3$ %	0.73	0.0	0.88	0.0
SP †	100.0	7.6	100.0	7.8
SAR ‡	11.70	-	12.77	-
Soluble cations meq100^{-1} g soil				
Ca^{++}	7.8	4.6	10.7	4.7
Mg^{++}	12.5	2.5	14.7	5.7
Na^{++}	95.0	14.4	45.6	14.8
K^{+}	0.75	0.2	0.6	0.3
Soluble anions meq100^{-1} g soil				
SO_4	18.0	6.2	36.3	7.1
Cl^{-}	88.0	10.1	21.9	10.3
HCO_3	11.0	5.5	5.3	4.1
CO_3	-	-	-	-

† SP : Soil Paste ‡ SAR: Sodium Absorpation Ratio

Table (3): Chemical properties of irrigation water used at El-Serw location during the two consecutive seasons 2009/10 and 2010/11.

Chemical properties	2009/10	2010/11
ECw (dsm^{-1})	1.500	2.000
Ph	8.240	9.000
Fe	0.147	0.147
Zn	0.030	0.030
Mn	0.004	0.004
Cu	-	-
Pb	-	-
Ni	0.003	0.003
Mo	-	-
Cr	0.003	0.003
Cd	-	-
Br	0.067	0.067
$N\text{-}NO_3$ (mgI^{-1})	3.100	3.100
$N\text{-}NH_4$	13.60	13.600
P	3.180	3.180
SAR	3.950	3.950
Soluble anions ($meq100^{-1}$ g soil)		
CO_3	-	-
HCO^{--}	5.300	5.500
Cl^{-}	10.630	12.360
SO_4	13.260	13.260
Soluble cations ($meq100^{-1}$ g soil)		
Ca^{++}	5.070	5.250
Mg^{++}	12.140	13.000
Na^{+}	11.60	12.220
K^{+}	0.580	0.650

Table (4): Barley SSRs primers and their sequences, the chromosomal location of derived loci, size range, marker type, motif and the reference.

No.	Marker	Primers sequence	Chromosome location	Size	Type	Motif	Reference
1	HVHOTR1	†F:ATGAGCAGTCTTGTCTTAACC ‡R:AGTTGGTCGCTAGATCTTATG	2H	165	SSR	(CAA)6	Hayden *et al.*, (2006)
2	HVM67	F:GTCGGGCTCCATTGCTCT R:CCGGTACCCAGTGACGAC	4H	116	SSR	(GA)11	Sato K *et al.*, (2009)
3	HVAMY2	F:CTGTAAGTGAGACAATCGACA R:CAGTTGAACCCCTGAAAG	7H	134	SSR	(GCT)5	Ramsy *et al.*, (2000)
4	HVHVA1	F:CATGGGAGGGGACAACAC R:CGACCAAACACGACTAAAGGA	1H	136	SSR	(ACC)5	Ramsy *et al.*, (2000)
5	scssr0013	F: GGTAAGGAGTGGGTCTCAGG R:CAAGCAGATGCAACTACACC	6H	168	SSR, SNP	–	Hearnden etal, (2007)
6	scssr0397	F: CTCCCATCACACCATCTGTC R: GACATGGTTCCCTTCTTCTT	5H	unknown	SSR, SNP	–	Hearnden *et al.*, (2007)
7	Bmac0316	F': ATGGTAGAGGTCCCAACTG R :ATCACTGCTGTGCCTAGC	6H	135	SSR	(AC)19	Ramsy *et al.*, (2000)
8	Bmac0209	F: CTAGCAACTTCCCAACCGAC R:ATGCCTGTGTGTGGACCAT	3H	176	SSR	(AC)13	Varshney *et al.*, (2007)
9	Bmag770	F:AAGCTCTTTCTTGTATTCGTG R:GTCCATACTCTTTAACATCCG	1H	158	SSR	(GT)13, (AG)19	Ramsy *et al.*, (2000)
10	Bmag0387	F:CGATGACCATTGTATTGAAG R:CTCATGTTGATGTGTGGTTAG	5H	123	SSR	(AG)16	Varshney *et al.*, (2007)

†F = Forward ‡R = Reverse

Table (5): Marker-traits association analysis with important significant SSR markers under saline soil.

Marker association analysis	Chromosomal location	Traits association with marker	P value≤(0.05)	
			2009/2010 season	2010/2011 season
Bmac 0209	3H	Days to heading	0.0103*	-------
HVHOTRI	2H	Days to heading Plant height Grain yield	0.0169 * 0.0028** 0.0004***	0.0425* ---------- 0.0021**

Table (6): Mean squares of the five traits for 20 barley genotypes under El-Serw, Sakha conditions and their combined in the first experiment during 2009/2010 growing season.

S.O.V.	DF	Seedling rate (days)			Days to heading (days)			Plant height (cm)			No. Tillers plant^{-1}			Grain yield plant^{-1}		
		Location		combined	Location		combined	Location		combined	Location		combined	Location		combined
		Serw	Sakha		Serw	Sakha		Serw	Sakha		Serw	Sakha		Serw	Sakha	
Rep.	2	206.6	46.66	210	125	6.87 ***	3.56 *	75.25	4.74	29.72	2.53	0.68	0.38	0.81	4.014	1.17
Genotype	19	1730.17 ***	394.39 ***	1749.29 ***	22.203 ***	48.76 ***	22.885 ***	66.107 **	365.828 ***	330.81 ***	14.832 ***	25.49 ***	33.54 ***	45.5913 ***	134.3 ***	141.45 ***
Location	1			16803.3 ***			2585.40 ***			19364.6 ***			259.89 ***			894.3 ***
Gen. x Loc.	19			375.26 ***			11.075 ***			101.119 ***			6.7889 ***			38.51 ***
Error	78			76.666			0.9002			16.121			1.2349			2.4580

*, ** and *** indicate significance at $P \leq 0.05$, 0.01 and 0.001.

Table (7): Mean performance of five traits as affected by 20 barley genotypes under El-Serw and Sakha conditions and their combined in the first experiment during 2009/10 growing season.

Genotype	Seedling rate (%)			Days to heading (days)			Plant height (cm)			No. Tillers plant^{-1}			Grain yield plant^{-1}		
	Serw	Sakha	Combined	Serw	Sakha	Combined	Serw	Sakha	Combined	Serw	Sakha	Combined	Serw	Sakha	Com-bined
1	86.7	100.0	93.3	81.0	91.3	86.2	58.2	96.7	77.4	16.3	16.4	16.3	14.4	36.1	25.3
2	100.0	100.0	100.0	79.7	89.3	84.5	61.3	100.5	80.9	13.1	17.4	15.3	18.7	32.3	25.5
3	60.0	86.7	73.3	80.0	90.7	85.3	59.4	86.2	72.8	7.9	9.6	8.7	12.6	15.8	14.2
4	86.7	100.0	93.3	81.3	93.0	87.2	60.4	77.3	68.8	8.3	10.6	9.4	13.8	14.5	14.1
5	26.7	73.3	50.0	80.3	96.0	88.2	46.5	58.9	52.7	9.1	9.6	9.4	13.0	13.6	13.3
6	73.3	86.7	80.0	80.7	90.7	85.7	49.7	75.3	62.5	6.4	9.7	8.1	7.2	12.2	9.7
7	86.7	100.0	93.3	81.7	92.0	86.8	57.2	79.0	68.1	9.7	14.9	12.3	11.6	17.0	14.3
8	100.0	100.0	100.0	80.0	90.0	85.0	58.7	88.3	73.5	10.5	13.7	12.1	13.6	15.0	14.3
9	93.3	100.0	96.7	82.3	93.7	88.0	55.8	90.7	73.2	11.1	11.3	11.2	10.6	12.1	11.4
10	40.0	86.7	63.3	88.0	93.7	90.8	49.5	74.1	61.8	10.2	11.0	10.6	5.8	12.1	9.0
11	46.7	73.3	60.0	89.3	94.0	91.7	54.5	82.0	68.2	9.3	10.5	9.9	6.8	12.3	9.5
12	93.3	100.0	96.7	79.3	89.3	84.3	62.2	86.9	74.5	12.2	18.8	15.5	15.3	18.8	17.1
13	73.3	100.0	86.7	83.3	91.0	87.2	56.4	82.1	69.2	10.0	14.6	12.3	12.9	13.1	13.0
14	66.7	93.3	80.0	81.0	90.0	85.5	59.3	79.0	69.2	10.6	15.7	13.1	7.2	10.7	8.9
15	53.3	100.0	76.7	81.0	91.0	86.0	53.7	68.6	61.1	9.9	14.1	12.0	6.0	15.4	10.7
16	46.7	73.3	60.0	87.7	90.7	89.2	52.0	69.7	60.8	9.9	13.0	11.5	6.4	13.3	9.8
17	40.0	86.7	63.3	82.0	91.7	86.8	51.3	73.1	62.2	8.1	8.2	8.1	6.9	12.1	9.5
18	26.7	66.7	46.7	84.0	92.3	88.2	47.3	66.7	57.0	6.9	14.0	10.5	6.4	11.5	9.0
19	86.7	100.0	93.3	84.7	92.0	88.3	56.8	99.4	78.1	8.2	10.7	9.4	6.0	14.3	10.1
20	66.7	100.0	83.3	81.7	92.3	87.0	58.7	82.6	70.7	9.8	12.6	11.2	9.3	11.3	10.3
Average	67.7	91.3	79.5	82.5	91.7	87.1	55.4	80.9	68.1	9.9	12.8	11.3	10.2	15.7	12.9
LSD 0.05	16.06	12.87	10.06	1.59	1.37	1.09	7.71	5.12	4.62	9.9	1.72	1.27	1.71	3.21	1.80
CV%	14.36	8.53	11.01	1.17	0.89	1.08	8.41	3.84	5.89	11.56	8.13	9.79	10.14	12.41	12.11

Table (8): Mean squares of the five traits for 20 barley genotypes under El-Serw, Sakha conditions and their combined in the second experiment during 2010/2011 growing season.

S.O.V.	DF	Seedling rate (days)			Days to heading (days)			Plant height (cm)			No. Tillers plant^{-1}			Grain yield plant^{-1}		
		Location		combined	Location		combined	Location		combined	Location		combined	Location		combined
		Serw	Sakha		Serw	Sakha		Serw	Sakha		Serw	Sakha		Serw	Sakha	
Rep.	2	386.25 **	21.66	193.95 *	59.81 **	33.616 ***	91.52 ***	43.1166	177.45 *	192.508 **	12196.8 **	894.066	3259.9	0.0125	0.048 *	0.053 **
Genotype	19	738.14 ***	171.4 ***	588.8 ***	27.59 ***	17.389 ***	32.03 ***	96.8026 ***	134.34 ***	134.408 ***	10266.1 ***	9537.9 ***	16137 ***	0.1266 ***	0.101 ***	0.124 ***
Location	1			22550.2 ***			837.40 ***			1159.40 ***			124163.3 ***			13.01 ***
Gen. x Loc.	19			320.82 ***			12.95 **			96.741 ***			3666.9 ***			0.104 ***
Error	78			51.6506			5.6019			34.722			1461.609			0.00905

*, ** and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001.

Table (9): Mean performance of five traits as affected by 20 barley genotypes under El-Serw and Sakha conditions and their combined in the first experiment during 2010/2011 growing season.

Genotype	Seedling rate (%)			Days to heading (days)			Plant height (cm)			No. Tillers plant^{-1}			Grain yield plant^{-1}		
	Serw	Sakha	Combined	Serw	Sakha	Combined	Serw	Sakha	Combined	Serw	Sakha	Combined	Serw	Sakha	Combined
1	76.7	100.0	88.30	93.0	101.7	97.3	78.0	116.0	97.0	437.0	597.0	517.0	0.55	1.53	1.04
2	78.3	100.0	89.20	91.3	101.3	96.3	81.0	124.3	102.5	467.0	479.0	473.0	0.98	1.23	1.11
3	75.0	93.3	84.20	98.0	104.3	101.2	71.3	123.0	97.2	370.0	461.0	415.5	0.62	1.27	0.94
4	66.7	100.0	83.30	95.3	101.0	98.2	85.3	118.7	102.0	297.0	400.0	348.5	0.58	1.33	0.96
5	50.0	80.0	65.00	97.0	102.3	99.7	86.0	115.7	92.2	243.0	384.0	313.5	0.35	0.97	0.66
6	71.7	90.0	80.80	97.7	102.7	100.2	67.3	110.7	89.0	379.0	379.0	379.0	0.63	1.23	0.93
7	73.3	100.0	86.70	97.3	99.0	98.2	79.7	112.0	95.8	367.0	423.0	395.0	0.98	1.17	1.08
8	78.3	100.0	89.20	97.3	100.0	98.7	80.7	120.7	100.7	430.0	453.0	441.5	0.42	1.10	0.76
9	65.0	100.0	82.50	87.7	96.3	92.0	82.3	116.7	99.5	297.0	451.0	374.0	0.35	1.10	0.73
10	73.3	86.7	80.00	98.7	105.7	102.2	81.7	103.0	92.3	317.0	376.0	346.5	0.43	1.00	0.72
11	75.0	80.0	77.50	99.0	105.7	102.3	83.7	113.3	98.5	333.0	373.0	353.0	0.58	1.15	0.87
12	78.3	100.0	89.20	97.0	99.0	98.0	68.7	113.7	99.8	453.0	456.0	454.5	0.63	1.57	1.10
13	70.0	100.0	85.00	93.3	99.3	96.3	75.3	121.7	98.5	390.0	395.0	392.5	0.68	1.00	0.84
14	63.3	96.7	80.00	94.3	100.3	97.3	77.3	104.0	90.7	380.0	419.0	399.5	0.57	1.10	0.83
15	68.3	100.0	84.20	96.0	103.0	99.5	86.0	114.0	95.5	395.0	477.0	436.0	0.73	1.10	0.92
16	70.0	83.3	76.70	95.3	103.7	99.5	81.3	107.7	94.5	343.0	401.0	372.0	0.58	1.07	0.83
17	8.3	86.7	47.50	101.7	99.3	100.5	73.3	100.7	87.0	327.0	367.0	347.0	0.17	1.23	0.70
18	56.7	86.7	71.70	97.3	101.3	99.3	76.3	113.0	94.7	299.0	353.0	326.0	0.33	1.18	0.76
19	61.7	100.0	80.80	95.3	100.0	97.7	89.3	122.3	105.8	343.0	451.0	397.0	0.33	1.33	0.83
20	75.0	100.0	87.50	97.0	99.3	98.2	76.3	115.0	95.7	397.0	463.0	430.0	0.58	1.60	1.09
Average	66.75	94.17	80.47	95.98	101.26	98.63	79.04	114.4	96.45	363.2	427.9	395.6	0.55	1.21	0.89
LSD 0.05	13.25	9.12	8.26	4.69	3.03	2.72	6.52	12.18	6.77	63.3	52.7	43.9	0.115	0.191	0.109
CV%	12.02	5.86	8..93	2.95	1.81	2.30	5.01	6.44	6.12	10.53	7.45	9.66	12.5	9.53	10.76

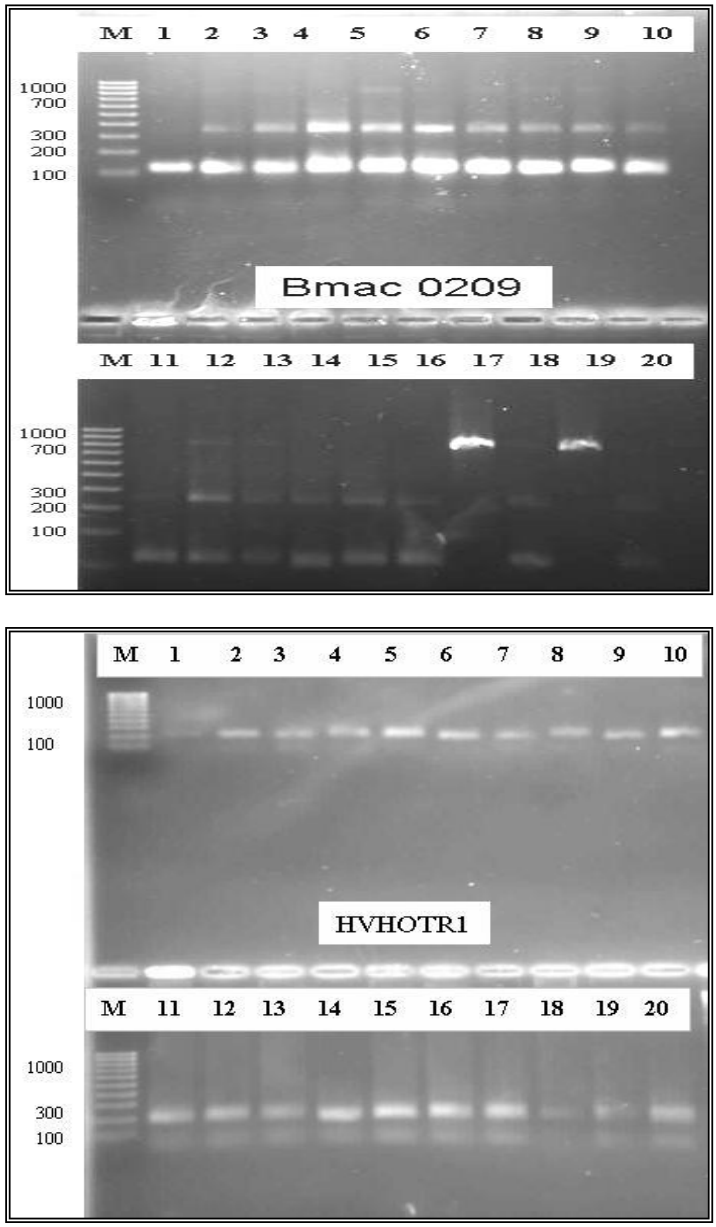

Fig. (1): Agarose gel showing the allelic segregation of the SSRs marker in the analyzed barley germplasm. 1:G.121, 2:G123, 3: California Mariot, 4:Rihane03, 5:Line1 6:G124, 7:G.2000, 8: Saiko, 9:Line2, 10:Line4, 11:Line8, 12:Line3. 13:Line9, 14:Line7, 15: CC89, 17:Line6, 18:G.132, 19: Beecher, 20: Dier Alla. M: Molecular size standard 100 bp DNA ladder.