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# Assessment of salinity tolerance and SSR profile differentiation in nine maize genotypes (*Zea mays* L)

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## Abstract

The low productivity of maize in Iraq is resulting from many factors that relate to insufficient knowledge of the genetic sources of germplasm used for crop improvement. A major goal of this project was to find the phenotypic, and genotypic variations by using salinity stress and Simple sequence repeat markers among nine maize genotypes. Genetic variation for salt tolerance was assessed in nine maize genotypes using four salinity levels (control, 50, 100, and 150 mM NaCl). Seedling of each genotype was compared to their growth under control and saline conditions. Salt stress (sodium chloride) markedly reduced the germination percentage, shoot and root lengths and fresh and dry masses. However, Dhqan, Fajr 265, Talar were considered as tolerant genotypes while Medium 791 defined as sensitive genotype.

Genotypic analysis was carried out using 18 SSR primers. A total of 46 polymorphic alleles were perceived among the maize genotypes with a range of 1–7 polymorphic bands were detected with size ranging from (90 to 500 bp) along the different maize genotypes. Polymorphism information content, marker index and gene diversity ranged from 0.178 to 0.788, 8.900 to 68.800, and 0.198 to 0.815, respectively. Dendrograms were constructed based on total microsatellite polymorphism, and nine genotypes were grouped into three major clusters. The analysis of molecular variance results revealed 17 and 83% of the total variation between and within populations (local and Iranian). The results confirmed the efficiency of SSR (Simple Sequence Repeat) markers in the detection of genetic variation among maize genotypes.

**Keywords**: maize, salt stress, SSR, genetic diversity

## Introduction

Maize (*Zea mays* L), is considered as the third most important cereal crop after wheat and rice around the world. It is covered 4.8% of the total land area and attributed by 3.5% of global crops production (Ahmad et al, 2011). Many million people in the world consume maize as an outstanding food, and it is grown in many countries than any other crops, but 90% of the world's corn was cultivated in the United States. However, the cultivation lands and production of corn in Kurdistan is the limit. It is primarily utilized for livestock feed and some foods and industrial products. In general, the cultivated genotypes in Kurdistan have the low-quality nutrition. The maize protein is comprised of the zein fractions that is defective in methionine, lysine, and tryptophan (Vasal, 1993).

Abiotic stress like salt tolerance is another method used in discrimination of genotypes. Salinization is the aggregation of water soluble (sodium chloride) NaCl in the soil to a step that has a severe impact on crops production, environment and economic (Rengasamy, 2006). The salinity of soil is one of the serious problems which remarkably influence crops productivity. This grave problem is due to low precipitation and high evaporation causing disorder in the ground salt balance; this also delivers to ground water and has an unfavorable impact on plant growth and productivity (Rhoades and Loveday, 1990; Evans, 1998). Salinity affects and modifies the growth and the metabolisms of plants depending upon genotypes, period and level of stress (Khan et al, 2003; Munns and James, 2003).

As a consequence, breeders are concerned with new tools that can make this way more powerful. For this reason, molecular-marker techniques in combination with phenotypic characters are commonly being used to assess the diversity and genetic dissimilarity among and within maize populations (Ibitoye and Akin-Idow, 2010). Molecular markers have been confirmed to be very efficient for genome description and breeding, and they have been efficiently combined with classical methods (Araus et al, 2008). In this respect, several molecular markers methods viz., RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphisms), SSR (Simple Sequence Repeat), etc. own much usefulness over the agro-morphological markers for genotype estimation (Staub et al, 1997; Cholastova et al, 2011). Among these markers, the SSR markers are important in terms of a high degree of polymorphic

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bands, reliable and reproducible (Qi-Lun et al, 2008). The SSR loci composed of 2 to 6 base pair tandem repeats and are considered as co-dominant markers, multi-allelic, highly polymorphic, and randomly scattered over the genome, they are broadly used for analyzing of maize genetic distance (Messmer et al, 1993). Therefore, the plan of this project was: to screen the different genotypes to different concentration of salt and to develop a molecular fingerprint for identification and characterization of maize genotypes using SSR markers.

## Materials and Methods

### *Plant materials*

Nine genotypes of maize were used in the field and laboratory experiments. The nine genotypes were Es-Solito, Medium 791, Dhqan, Fajr 260, Btaris, Cantabpis, Talar, MSIxB, and ZP434xA. Es-Solito, Medium 791, Dhqan, Fajr 260, Btaris, and Cantabpis genotypes were obtained from College of Agriculture, the University of Kurdistan, Sanandaj, Iran, while the local genotypes: Talar, MSIxB, and ZP434xA were produced by Ministry of Agriculture in Kurdistan.

### *Salinity stress*

All of the experiment treatments and maize genotypes were arranged in a factorial experiment in an entirely randomized design (9 genotypes x 4 salinity levels x 3 replicates) with one Petri dish for each replicate. Treatment combinations included four salinities levels 0, 50, 100, and 150 mM and nine maize genotypes. Salt solutions of different NaCl (sodium chloride) concentrations were prepared by dissolving NaCl in distilled water. Kernels maize were firstly sterilized and then transferred to Petri dish containing two filter papers. All Petri dishes were incubated in the growth room for ten days under (25±2°C). Daily, 10 ml of distilled water and salt solutions were added to each Petri dish. After ten days of treatment, the following parameters were registered: germination percentage, the length of shoot and root, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight.

### *Sampling, grinding, and DNA isolation from maize leaves*

The leaf samples of the two-weeks-old plant were cut into small segments with a sterilized scissors and placed in a pre-chilled mortar. Liquid nitrogen was added to freeze and grind to fine powder. The crushed tissue has been put in a 10 ml polypropylene centrifuge tube and stored at -20°C until used as a source of DNA.

Genomic DNA was isolated by using CTAB (Cetyltrimethyl-ammonium bromide) methods as described by Doyle et al (1987) with some modifications. One gram of leaves of two weeks old plant (ten plants) was crushed in liquid nitrogen, and 6 ml of CTAB lysis buffer was added to lyse cellular and nuclear membranes. The samples were then incubated for 80 min at 60°C in a water bath with occasional mixing. The samples were centrifuged at 4,000 rpm for 25 min at 20°C. Solvent extraction was done by adding 5 ml of chloroform to each sample followed by thorough mixing by inverting the tubes two to seven times. The tubes were centrifuged at 4,000 rpm for 25 min at 24°C. The upper aqueous layer transferred into clean tubes and ethanol (2V) and 0.08 volume of 7.5 M ammonium acetate solution was added to each sample followed by incubation at -20°C for overnight to allow precipitation. The tubes were span at 4,000 rpm for 35 min at 4°C and the supernatant discarded. To wash the DNA pellet, 2 ml of 70% ethanol were added and centrifuged at 4,000 rpm for 30 min. The ethanol was removed, and the pellet was air-dried for 70 min. DNA pellets were dissolved in 1 ml $ddH_2O$. RNA was removed by adding 25 µl RNase A (10 mg $ml^{-1}$), and incubating at 50°C for 60 min. Protein contaminates from the cell lysate were removed using 30 µL of Proteinase K (20 mg $ml^{-1}$) at 45°C for 60 min. Two ml of chloroform were added. The samples were span at 4,000 rpm for 25 minutes at 20°C. The upper aqueous fase was transferred into clean tubes and two volumes of ethanol and 0.08 volume of 7.5 M ammonium acetate were added to each sample followed by incubation at -20°C for overnight to allow precipitation. The tubes were centrifuged at 4,000 rpm for 35 min at 4°C and the supernatant discarded. The DNA pellet was washed with 2 ml of 70% ethanol and centrifuged at 4,000 rpm for 35 min. The ethanol was discarded, and the pellet was air-dried for 60 min. DNA pellets were suspended in 100 µl $ddH_2O$. The quality of genomic DNA was assessed on 0.9% agarose gel. Five µl of DNA and two µl of loading dye were mixed and electrophoresed for 100 min at 87 volts in a 1x TBE buffer (0.1 M Tris-base, 0.1 M boric acid, and 0.02 M EDTA; pH 8.0). The DNA were visualized under UV light and photographed. The ratio 1:5 was used to dilute the stock DNA and use for PCR.

### *SSR analysis*

A total of 24 SSR primers were selected from the site of Maize GDB (http://www.maizegdb.org/data_center/ssr) based on chromosome location. PCR (Polymerase chain reaction) was performed in 25 µl reaction mixes consisting of 5 µl (70 ng) of template DNA, 3 µl (10 µM) of SSR primers (forward and reverse) and 12.5 µl of master mix (1.5 mM $MgCl_2$, 0.8 mM dNTP mix, 0.125 U Taq polymerase and 1 x PCR reaction buffer) in a 0.2 ml micro-PCR tube on ice. The tubes placed in a thermal cycler (GeneAmp PCR system 2700 from Applied Biosystems) with the following PCR program; Initial denaturation at 94°C for 7 min, followed by 41 cycles of 94°C for 60 seconds, 55, 58, and 60°C for 60 seconds and 72°C for 2 minutes. This step was followed by one final extension at 72°C for 8 min. The SSR amplification products were resolved on 2.5% agarose gel in 1x TBE (Tris/Borate/EDTA) buffer. Gels were run in a large layout horizontal gel system at 84 volts for 2 hours and were

Table 1 - Mean of different physiological traits of maize seedlings under different salinity levels.

| Interaction | Germination (%) | Shoot length seedling$^{-1}$ (cm) | Root length seedling$^{-1}$ (cm) | Vigor index | Shoot fresh weight seedling$^{-1}$ (g) | Root fresh weight seedling$^{-1}$ (g) | Shoot dry weight seedling$^{-1}$ (g) | Root dry weight seedling$^{-1}$ (g) |
|---|---|---|---|---|---|---|---|---|
| G1*C0 | 83.333$^{abc}$ | 3.672$^{cde}$ | 7.581$^{bc}$ | 944.567$^{bcd}$ | 3.327$^{abcde}$ | 0.160$^{fghij}$ | 1.653$^{abcde}$ | 0.067$^{cdefg}$ |
| G1*C1 | 76.667$^{abcde}$ | 2.273$^{fghi}$ | 2.964$^{ghij}$ | 394.340$^{fghij}$ | 2.753$^{cdefg}$ | 0.123$^{ghij}$ | 1.683$^{abcd}$ | 0.037$^{defghijk}$ |
| G1*C2 | 53.333$^{bcdefg}$ | 1.778$^{ghij}$ | 2.229$^{ijh}$ | 205.040$^{hij}$ | 2.243$^{fghi}$ | 0.087$^{hij}$ | 1.189$^{bcdefghijk}$ | 0.016$^{ghijk}$ |
| G1*C3 | 26.667$^{fg}$ | 1.022$^{ij}$ | 1.855$^{ij}$ | 75.433$^{ij}$ | 0.880$^{jk}$ | 0.037$^{j}$ | 0.554$^{mn}$ | 0.004$^{k}$ |
| G2*C0 | 56.667$^{abcdefg}$ | 3.821$^{cde}$ | 4.500$^{defg}$ | 477.567$^{efgh}$ | 2.163$^{fghi}$ | 0.150$^{fghij}$ | 1.013$^{fghijklm}$ | 0.058$^{defghij}$ |
| G2*C1 | 53.333$^{bcdefg}$ | 2.194$^{fghi}$ | 2.695$^{ghij}$ | 266.363$^{ghij}$ | 1.483$^{hijk}$ | 0.110$^{hij}$ | 0.766$^{klmn}$ | 0.023$^{fghijk}$ |
| G2*C2 | 53.333$^{bcdefg}$ | 2.002$^{fghij}$ | 1.704$^{ij}$ | 195.353$^{hij}$ | 1.570$^{hij}$ | 0.087$^{hij}$ | 0.872$^{ijklm}$ | 0.018$^{ghijk}$ |
| G2*C3 | 23.333$^{g}$ | 1.005$^{ij}$ | 1.243$^{j}$ | 53.460$^{j}$ | 0.557$^{k}$ | 0.057$^{hij}$ | 0.349$^{n}$ | 0.007$^{jk}$ |
| G3*C0 | 86.667$^{ab}$ | 5.553$^{a}$ | 9.996$^{a}$ | 1312.960$^{a}$ | 3.707$^{abc}$ | 0.607$^{abcd}$ | 1.198$^{bcdefghijk}$ | 0.157$^{a}$ |
| G3*C1 | 80.000$^{abcd}$ | 3.591$^{cde}$ | 5.477$^{def}$ | 792.826$^{cde}$ | 2.723$^{cdefg}$ | 0.257$^{efghij}$ | 1.447$^{abcdefgh}$ | 0.071$^{bcdef}$ |
| G3*C2 | 63.333$^{abcdefg}$ | 1.626$^{hij}$ | 2.949$^{ghij}$ | 296.627$^{fghij}$ | 2.053$^{fghi}$ | 0.093$^{hij}$ | 1.321$^{abcdefghij}$ | 0.019$^{fghijk}$ |
| G3*C3 | 63.333$^{abcdefg}$ | 0.817$^{j}$ | 1.718$^{ij}$ | 162.730$^{hij}$ | 1.307$^{ijk}$ | 0.047$^{ij}$ | 0.959$^{ghijklm}$ | 0.004$^{k}$ |
| G4*C0 | 96.667$^{a}$ | 4.374$^{bc}$ | 6.374$^{cde}$ | 1034.663$^{abc}$ | 4.263$^{a}$ | 0.623$^{abc}$ | 1.720$^{ab}$ | 0.135$^{a}$ |
| G4*C1 | 83.333$^{abc}$ | 3.053$^{defg}$ | 3.651$^{fghi}$ | 450.400$^{efghij}$ | 2.153$^{fghi}$ | 0.240$^{efghij}$ | 1.202$^{bcdefghijk}$ | 0.030$^{efghijk}$ |
| G4*C2 | 66.667$^{abcdef}$ | 2.280$^{fghi}$ | 2.528$^{ghij}$ | 406.216$^{fghij}$ | 2.683$^{defg}$ | 0.250$^{efghij}$ | 1.546$^{abcdef}$ | 0.041$^{defghijk}$ |
| G4*C3 | 53.333$^{bcdefg}$ | 1.385$^{hij}$ | 1.722$^{ij}$ | 173.130$^{hij}$ | 1.990$^{fghi}$ | 0.117$^{ghij}$ | 1.317$^{abcdefghij}$ | 0.003$^{k}$ |
| G5*C0 | 90.000$^{ab}$ | 4.084$^{cd}$ | 7.201$^{bcd}$ | 1027.820$^{abc}$ | 3.303$^{abcde}$ | 0.427$^{cdefg}$ | 1.442$^{abcdefgh}$ | 0.087$^{bcd}$ |
| G5*C1 | 70.000$^{abcde}$ | 2.254$^{fghi}$ | 2.845$^{ghij}$ | 353.980$^{fghij}$ | 2.727$^{cdefg}$ | 0.283$^{efghij}$ | 1.441$^{abcdefgh}$ | 0.045$^{defghijk}$ |
| G5*C2 | 53.333$^{bcdefg}$ | 1.782$^{ghij}$ | 1.727$^{ij}$ | 178.416$^{hij}$ | 1.393$^{ijk}$ | 0.207$^{fghij}$ | 0.841$^{jklmn}$ | 0.017$^{ghijk}$ |
| G5*C3 | 40.000$^{defg}$ | 1.072$^{ij}$ | 1.178$^{ij}$ | 91.316$^{hij}$ | 1.933$^{fghi}$ | 0.143$^{fghij}$ | 1.140$^{defghijkl}$ | 0.013$^{defghi}$ |
| G6*C0 | 76.667$^{abcde}$ | 3.750$^{cde}$ | 5.265$^{ef}$ | 666.017$^{def}$ | 2.707$^{cdefg}$ | 0.350$^{cdefghi}$ | 1.303$^{abcdefghijk}$ | 0.063$^{defghi}$ |
| G6*C1 | 66.667$^{abcdef}$ | 2.093$^{fghij}$ | 2.923$^{ghij}$ | 332.333$^{fghij}$ | 2.143$^{fghi}$ | 0.260$^{efghij}$ | 1.219$^{bcdefghijk}$ | 0.054$^{defghijk}$ |
| G6*C2 | 56.667$^{abcdefg}$ | 1.844$^{ghij}$ | 2.411$^{hij}$ | 238.670$^{ghij}$ | 1.933$^{fghi}$ | 0.233$^{efghij}$ | 1.168$^{cdefghijkl}$ | 0.037$^{defghijk}$ |
| G6*C3 | 43.333$^{cdefg}$ | 1.297$^{ij}$ | 1.421$^{ij}$ | 105.003$^{hij}$ | 1.813$^{ghij}$ | 0.157$^{fghij}$ | 1.116$^{efghijkl}$ | 0.008$^{ijk}$ |
| G7*C0 | 90.000$^{ab}$ | 5.628$^{a}$ | 7.849$^{bc}$ | 1175.987$^{ab}$ | 3.756$^{ab}$ | 0.777$^{ab}$ | 1.487$^{abcdefg}$ | 0.114$^{abc}$ |
| G7*C1 | 86.667$^{ab}$ | 2.630$^{efgh}$ | 4.143$^{fgh}$ | 607.897$^{defg}$ | 3.607$^{abcd}$ | 0.847$^{a}$ | 1.829$^{a}$ | 0.115$^{abc}$ |
| G7*C2 | 83.333$^{abc}$ | 1.831$^{ghij}$ | 1.802$^{ij}$ | 301.826$^{fghij}$ | 2.667$^{defg}$ | 0.343$^{cdefghij}$ | 1.579$^{abcde}$ | 0.068$^{cdefg}$ |
| G7*C3 | 60.000$^{abcdefg}$ | 0.828$^{ij}$ | 1.130$^{ij}$ | 122.483$^{hij}$ | 1.937$^{fghi}$ | 0.323$^{defghij}$ | 1.291$^{abcdefghijk}$ | 0.052$^{defghijk}$ |
| G8*C0 | 83.333$^{abd}$ | 5.555$^{a}$ | 8.518$^{ab}$ | 1183.800$^{ab}$ | 3.633$^{abcd}$ | 0.450$^{cdef}$ | 1.399$^{abcdefghi}$ | 0.120$^{ab}$ |
| G8*C1 | 76.667$^{abcde}$ | 2.594$^{efgh}$ | 3.113$^{ghij}$ | 461.643$^{efghi}$ | 3.267$^{bcde}$ | 0.307$^{efghij}$ | 1.697$^{abc}$ | 0.063$^{defgh}$ |
| G8*C2 | 63.333$^{abcdefg}$ | 1.307$^{ij}$ | 1.344$^{ij}$ | 180.286$^{hij}$ | 2.937$^{bcdef}$ | 0.243$^{efghij}$ | 1.774$^{a}$ | 0.054$^{defghijk}$ |
| G8*C3 | 36.667$^{efg}$ | 1.042$^{ij}$ | 1.377$^{ij}$ | 93.343$^{hij}$ | 1.240$^{ijk}$ | 0.177$^{fghij}$ | 0.651$^{lmn}$ | 0.010$^{ijk}$ |
| G9*C0 | 70.000$^{abcde}$ | 5.262$^{ab}$ | 7.414$^{bc}$ | 866.070$^{bcd}$ | 2.437$^{efgh}$ | 0.537$^{bcde}$ | 0.936$^{hijklm}$ | 0.079$^{bcde}$ |
| G9*C1 | 63.333$^{abcdefg}$ | 3.112$^{def}$ | 2.678$^{ghij}$ | 351.920$^{fghij}$ | 2.060$^{fghi}$ | 0.363$^{cdefgh}$ | 1.155$^{cdefghijkl}$ | 0.055$^{defghijk}$ |
| G9*C2 | 43.333$^{cdefg}$ | 1.835$^{ghij}$ | 1.937$^{ij}$ | 168.906$^{hij}$ | 1.757$^{ghij}$ | 0.297$^{efghij}$ | 0.958$^{ghijklm}$ | 0.030$^{defghijk}$ |
| G9*C3 | 26.667$^{fg}$ | 1.478$^{hij}$ | 1.296$^{ij}$ | 67.560$^{ij}$ | 1.413$^{hijk}$ | 0.243$^{efghij}$ | 0.777$^{jklmn}$ | 0.016$^{ghijk}$ |

G1: Es-Solito 635, G2: Medium 791, G3: Dhqan, G4: Fajr 260, G5: Btaris, G6: Cantabpis, G7: Talar, G8: MSIxB, and G9: ZP 434xA. C0: 0 mM NaCl, C1: 50 mM NaCl, C2: 100 mM NaCl and C3: 150 mM NaCl. Data superscripted by the same letter are not significantly different at the 0.05 level using Duncan multiple range test.

photographed under UV light. The PCR was repeated twice for accuracy.

### *Statistical data analysis*

For salt tolerant, the software XLSTAT version 11 was used for statistical analysis like two-way analysis of variance (ANOVA-RCBD) and Duncan multiple range test at 5% level. Euclidean distance and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) was used to create phylogenetic tree. The SSR gels were analyzed and converted into binary data: 1 for the presence of band and 0 for the absence of the band. Jaccard's coefficient was measured based on binary data (0 and 1) by using XLSTAT version 11. The Jaccard's coefficient was converted to dissimilarity matrix (Jaccard, 1908). The matrix of binary data (1 and 0) was used to create a tree by UPGMA analysis. Polymorphism information content (PIC) and gene diversity were computed by using PowerMarker version 3.25 software. The dissimilarity based on Jaccard matrix was used to produce the dendrogram using UPGMA method. GenAlEx version 6.5 software also used to estimate the molecular variance among and within populations (Peakall et al, 2012).

## Results and Discussion

### *Salinity stress*

Salinity tolerance is, of course, necessary during the entire life cycle of the plant. However, it has been shown in several species that tolerance at the seedling stage reflects the enhanced salinity tolerance at the adult plant level (Ashraf and McNeilly, 1990). However, salinity stress adversely affects plant growth and development and results in significant reduction in yield and quality. In the present study, variation in response to salinity was observed among nine maize genotypes basing on seedlings physiological parameters.

As mentioned in (Table 1), treatment average appeared that all the genotypes generated minimum germination percentage, shoot length per seedling, root length per seedling, shoot fresh weight per seedling, root fresh weight per seedling, shoot dry weight per seedling and root dry weight per seedling were observed at C150 mM salinity level. Interaction of salinity levels and genotype means (Table 1) indicated that germination percentage, shoot length per seedling, root length per seedling, shoot fresh weight per seedling, root fresh weight per seedling shoot dry weight per seedling and root dry weight per seedling decreased with augmentation of salt concentration

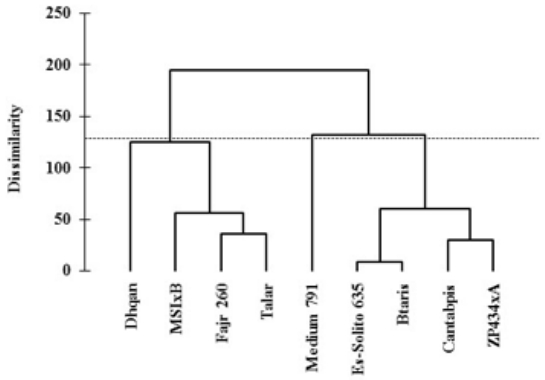


Figure 1 - Clustering of nine maize genotypes under salt condition using Euclidean distance and UPGMA method.

in all the genotypes. Talar gained maximum shoot length (5.628 cm at C0 mM) root fresh weight (0.847 g at C50 mM), and shoot dry weight per seedling (1.829 g at C50 mM), followed by Fajr 260 which recorded high value in percentage of germination (96.667%) and shoot fresh weight (4.263 g) at control condition (C0 mM). Whereas, minimum values of germination rate (23.333%), shoot fresh weight per seedling (0.557 g), and shoot dry weight per seedling (0.349 g) were acquired by Medium 791 (at C150 mM). This study showed the existence of an impressive variation in tolerance to NaCl during the early growth stages. The study also demonstrated that the maize genotypes responded varyingly under saline stress. Consequently, these traits would be very useful in salinity tolerance improvement programs. High salt level influences the kernel imbibition of water which would decrease the physiological activities, like convert of complex compounds to simple compounds, during the germination process. The progressive reducing in root and shoot length with increasing salinity concentration might be due to the restrictive issue of NaCl to root and shoot elongation by confining cell division and expansion in root and shoot. Moreover, the reduction in shoot and root length in maize genotypes under the salt condition is due to extra accumulation of NaCl in the cell wall elasticity. Further, the secondary cell appears sooner, and the wall becomes rigid as a consequence the turgor pressure efficiency in enlargement cell decreases. Salinity cause impeded growth in glycophytes which result in reduced shoot and root fresh weights (Parida and Das, 2005; Hager et al, 2006). It is confirmed that $Na^+$ with high concentration modifies various metabolic activities (Akram et al, 2007). It is also notified that NaCl tolerance is linked with $K^+$ contents due to its sharing in osmotic regulation (Ashraf et al, 2005). Further, decreasing the fresh weight of shoot and root might be caused by reduced uptake and aggregation of nutrients in the plant body (Dadkhah and Grrifiths, 2006). The lessening in seedlings dry weight of maize genotypes under salinity stress might be due to several factors like salinity response of root to downregulate shoot growth through a long distance signal (Alam et al, 2004).

Results from cluster analysis showed evident grouping (Figure 1). Clustering was accomplished at a standardized dissimilarity of 135 in the dendrogram and three distinct groups were detected. Talar, Fajr 260, Dhqan, and MSIxB combined into the first group which is considered as highly tolerant because of having the highest percentage of germination, average root length per seedling, average shoot length per seedling, vigor index, shoot fresh weight and root dry weight per seedling. Likewise, genotype Medium 791 was grouped under the sensitive category as it had the lowest value of germination percentage, root length per seedling, shoot fresh weight per seedling, root fresh weight per seedling, shoot dry weight, and root dry weight per seedling. The last group contained Es-Solito 265, ZP234xA, Cantabpis, and Btaris, which is considered as moderate salt tolerant.

Maiti et al (1996) reported that variation in maize at an early seedling stage in response to salinity reflects potential grain yield at maturity. This result signifies that for preliminary selection for salinity tolerance in maize, screening of seedling is a necessary and productive method because the variation at the early stage of growth is genetically based. The results from this study, are the results of Akram et al (2007), Giaveno et al (2007) and Hoque et al (2015) who reported that the morphological seedling traits of maize showed a decline with the increasing of salinity levels.

***Genotypic variation***

SSR markers were utilized in the study considering their high polymorphism, specificity, reproducibility and high variability (Brown et al, 1996; Pestova et al, 2000; Stachel et al, 2000). Several factors such as some SSR loci and repeat types influence allelic differences. Twenty-four SSR primers were used to estimate the genetic variation in maize genotypes. Out of 24 primers, 18 primers produced amplified fragments. The absence of amplification in some microsatellites primer submits that there might take place an annealing failure. This annealing defeat can be due to differences in base sequences of the loci between maize genotypes.

The polymorphisms were accounted according to the presence (1) and absence (0) of fragments. The lack of bands may be due to the defeat of primers to anneal at a location in some samples due to nucleotide sequence differences. (Clark and Lanigan, 1993). The monomorphic fragments were not giving any diversity issue, and they were removed from the analysis. The total numbers of amplified and polymorphic bands were recorded according to clarity and their molecular weight concerning the DNA Ladder. The allelic profile of maize genotypes representing the nine maize chromosomes is shown in Table 2. A total of 51 reproducible alleles were characterized with a molecular weight ranging from 90 to 500 bp along the different maize genotypes. Out of 51 bands, 46 bands were polymorphic. A range of 1–7 polymorphic

Table 2 - Information of 18 SSR loci, Allele divergence, PIC, Marker index and gene diversity values used in nine maize genotypes.

| Primers name | Motif | Bin | Annealing temperature (°C) | Number of amplified bands | Number of polymorphic bands | Size of amplified bands (bp) | PIC | Marker index | Gene diversity |
|---|---|---|---|---|---|---|---|---|---|
| Bnlg1108 | AG | 3.08 | 60 | 3 | 3 | 210-290 | 0.568 | 56.76 | 0.642 |
| Bnlg1189 | AG | 4.07 | 60 | 2 | 2 | 220-230 | 0.286 | 28.59 | 0.346 |
| Bnlg1194 | AG | 8.02 | 55 | 3 | 3 | 180-290 | 0.677 | 67.73 | 0.716 |
| Bnlg1429 | AG | 1.02 | 55 | 8 | 7 | 130-500 | 0.788 | 68.98 | 0.815 |
| Bnlg1810 | AG | 9.01 | 58 | 2 | 2 | 140-150 | 0.346 | 34.57 | 0.444 |
| Bnlg1867 | AG | 6.01 | 55 | 2 | 1 | 195-240 | 0.178 | 8.9 | 0.198 |
| Mmc401 | GGA and AG | 2.05 | 55 | 3 | 3 | 200-250 | 0.663 | 66.27 | 0.716 |
| Phi037 | AG | 1.08 | 55 | 3 | 3 | 130-150 | 0.505 | 50.48 | 0.593 |
| Phi069 | GAC | 7.05 | 55 | 2 | 2 | 195-205 | 0.505 | 50.48 | 0.593 |
| Phi075 | CT | 6.0 | 55 | 2 | 1 | 205-260 | 0.346 | 17.29 | 0.444 |
| Phi113 | GTCT | 5.03 | 55 | 2 | 2 | 290-300 | 0.438 | 43.77 | 0.494 |
| Phi126 | AG | 6.0 | 55 | 3 | 3 | 175-200 | 0.728 | 72.79 | 0.765 |
| Umc1038 | CT | 10.07 | 55 | 2 | 2 | 130-150 | 0.505 | 50.48 | 0.593 |
| Umc1069 | GGAGA | 8.08 | 58 | 2 | 2 | 100-110 | 0.286 | 28.59 | 0.346 |
| Umc1630 | ATGGG | 1.11 | 60 | 2 | 1 | 90-100 | 0.178 | 8.9 | 0.198 |
| Umc1653 | GAAA | 6.07 | 58 | 4 | 3 | 100-280 | 0.34 | 25.52 | 0.37 |
| Umc1946 | GCTGCT | 2.07 | 60 | 4 | 4 | 90-180 | 0.677 | 67.73 | 0.716 |
| Umc2013 | NA | 5.07 | 55 | 2 | 2 | 140-150 | 0.372 | 37.19 | 0.494 |
| Total | | | | 51 | 46 | | | | |
| Mean | | | | 2.833 | 2.556 | | 0.466 | | 0.527 |

bands with an average of 2.56 bands per primer were detected (Table 2). In similar studies, Li et al (2002) evaluated Chinese maize inbred lines by applying SSR markers, and they discovered several polymorphic bands with a range of molecular weight from 49 to 286 bp. Park et al (2008) stated that the molecular weight of microsatellite loci in 76 Korean waxy corn varied from 75 to 175 bp.

Regarding data presented in (Table 2) the highest number of polymorphic bands recorded for Bnlg1429 (7 bands) while the primers: Bnlg1867, Phi075, and Umc1630 had the minimum number of polymorphic bands (1 band). This difference may be due to diverse of genotypes and the selection of SSR primers. The considerable number of average detectable and polymorphic bands might be due to the number of GC of the primers used in this study. The variation in the number of bands amplified by different primers is affected by various factors such as primer sequence and less number of annealing locations in the genome. Some of SSR primers revealed one band during amplification. This difference indicated that the maize genotypes may be homozygous, had no mutation at specific SSR loci. Similar description has previously had been done by Warburton et al (2002) and Yen et al (2002) detected an average of 3.8 and 3.04 alleles per primer in nine Asian and thirteen Indian maize inbred lines, respectively. Vaz Patto et al (2004) studied maize genotypes by using 80 SSR primers, and they got 5.3 alleles per primer.

***Polymorphism information content, gene diversity, and Marker index***

The markers with many alleles consider as highly informative. The power of marker can be quantitatively measured by a method of statistics called the polymorphism information content (PIC). The PIC is the degree of polymorphism performed by a pair of primers and it is directly linked to the variation of the region of the chromosome and studied genotypes. Thus, a diminutive PIC may denote that the area is conserved in the group of genotypes. The PIC values range between 0 and 1. PIC depends on the number of polymorphic alleles and frequency distribution. PIC value range relies on the type of molecular markers (dominant or co-dominant marker). For dominant markers like RAPD, the PIC value is varied from 0.0 to 0.5, while the range of PIC value in a co-dominant marker like SSR is sited between 0.0 and 1.0 (Botstein et al, 1980). Primers with PIC value of zero or less than zero (negative value) should not be used for analysis since these are less informative regarding gene diversity. The greater value of PIC referred to a higher degree of polymorphism of the SSR markers and therefore assisted in selecting the best SSR markers in genetic divergence analysis.

PIC was calculated for each of the 18 markers by using PowerMarker software. The PIC values counted for the 18 SSR markers are detailed in Table 2. PIC value was varied from 0.178 (Bnlg1867 and Umc1630) to 0.788 (Bnlg1429). Primer Bnlg1429 was deemed very informative due to its great ability in detecting of polymorphisms among maize genotypes. The high level of polymorphism is due to various genotypes, and SSR loci applied in this study. In this study, it was noticed that SSR primers with dinucleotide repeats bided the grand PIC value. In contrast, trinucleotide motifs are more abundant in coding zones of a gene which contain a small number of the motif (Toth et al, 2000; Odeny et al, 2007). This finding here showed a great range of PIC compared to that reported by Legesse et al (2007) who declared a range of PIC value from 0.58 to 0.71. These results were aligned with those found by Van Etten et al (2008) who established an average of 0.62 of PIC values.

Marker index was determined as the product of the polymorphism percentage, and PIC was used to estimate the overall utility of each allele system and was calculated according to Sorkheh et al (2008). The

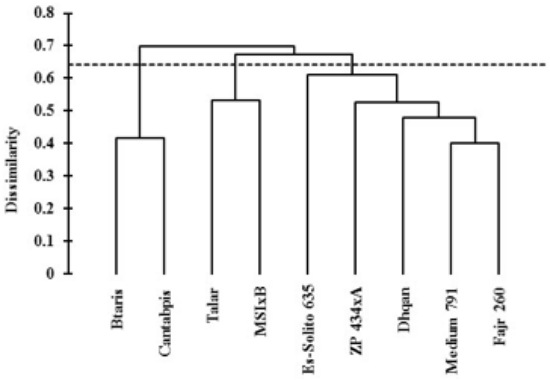


Figure 2 - Hierarchical clustering constructed for the nine maize genotypes using eighteen SSR markers based on Jaccard's coefficient and UPGMA method.

value of Marker index was ranged from 8.90 to 68.98. The value of Marker index confirmed the existence of a high number of polymorphic among maize genotypes.

The gene diversity revealed a great divergence across the genotypes varying from 0.198 (Bnlg1867) to 0.815 (Bnlg1429) with a mean of 0.527. The great difference between the minimum and the maximum value of gene diversity signifies the presence of high variation among maize genotypes.

*Genotypes clustering using UPGMA methods*

Unweighted pair-group method (UPGMA) was utilized to output the dendrogram on the foundation of 18 SSR primers and the nine genotypes to assess the overall genetic dissimilarity between the genotypes. The Jaccard's coefficient was also calculated to determine the genetic divergence among the maize genotypes. The dendrogram result clustered all maize genotypes into three significantly different groups (Figure 2). Cluster 1 composed of five genotypes Es-Solito 635, Medium 791, Dhqan, Fajr 260, and ZP 434xA. This cluster divided into four sub-clusters: sub-cluster 1 contained Fajr 260 and Medium 791. Dhqan, ZP 434xA, and Es-Solito 635 formed the sub-clusters 2, 3, and 4, respectively. Talar and MSIxB together produced the second cluster. The last cluster was created by Btaris and Cantabpis. The most diverse genotypes were Fajr 260 and MSIxB followed by Es-Solito 635 and MSIxB, which referees that the identity between them are depressed, and they were gathered from diverse origins. Medium 791 and Fajr 260 were closely related, which signifies that the similarity between these two genotypes had a high degree through SSR markers and probably they were from the same origin (Iran). These results are in alignment with investigators (Smith et al, 1997; Senior et al, 1998; Reif et al, 2003), who reported the correspondence of SSR marker dissimilarity with pedigree information in maize genotypes.

*Analysis of molecular variance (AMOVA)*

AMOVA is a method to evaluate population variation depending on molecular markers data (Excoffier et al, 1992). The analysis of molecular variance occurred 17 and 83% of the variation between and within populations (Local and Iranian populations), respectively (Table 3). The estimated differences extended from 1.802 between populations (local and Iranian) to 8.905 within populations. The variance within populations was significant at $P < 0.05$ level. AMOVA result suggested that the divergence was mostly within populations rather than between populations. This indicates high intra-population variation among maize genotypes was presented. This affirms the existence of high level of genetic distance among maize genotypes. This result is in harmony with the broad genetic base of the materials used in this study, and this significant variation is as expected from the genetic base and origin. The result of AMOVA in this study is not in consistence with the study of Reif et al (2003) who found 89.8 and 10.2% of the total variation between and within populations of maize. Terra et al (2011) detected a change of 35.5 and 64.5% between and within populations of maize. The variance within a population (83%) in this study was higher than obtained (59%) by Hoxha et al (2004).

*Conclusion*

In this study, high variation among maize genotypes was stated in term of salt tolerance. Dhqan, Fajr 265, Talar were considered as tolerant genotypes while Medium 791 defined as sensitive genotype. The results of salinity documented that the response to salinity levels depends on genotypes. In the present study, SSR was a powerful tool for simplifying the genotypic correlation within populations. All nine genotypes participated in this study exhibited wide ranges of genetic differences due to various sources of origin and genetic background. Cluster analysis and principal component analysis divided nine maize genotypes into three clusters.

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Table 3 - Analysis of molecular variance (AMOVA) of 18 microsatellite loci in nine maize genotypes of two populations (local and Iranian).

| Source | DF | SS | MS | Est. Var. | Variance % | P < 0.05 |
|---|---|---|---|---|---|---|
| Between populations | 1 | 16.111 | 16.111 | 1.802$^{ns}$ | 17% | 0.07 |
| Within populations | 7 | 62.333 | 8.905 | 8.905** | 83% | 0.003 |
| Total | 8 | 78.444 | | 10.706 | 100% | |

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