PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.1728

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Determination of maize genotypes performance under water deficit using ISSR molecular index

Zeyad A. Abdulhamed[1], Mudhir I. Hwaidi[2] & Mustafa R. M. Alqaisi[3]*

[1] Department of Field Crops, College of Agriculture, University of Anbar, Anbar, Iraq
[2]Department of Field Crops, College of Agriculture,Tikrit University, Salah Al Deen, Iraq
[3]Department of Horticulture, College of Agriculture, Tikrit University, Salah Al Deen, Iraq

*Email: dr.mustafa.r.m@tu.edu.iq

ARTICLE HISTORY

Received: 13 February 2022
Accepted: 26 August 2022

Available online
Version 1.0 : 29 October 2022

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS etc.
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Abdulhamed Z A, Hwaidi M I, Alqaisi M R M. Determination of maize genotypes performance under water deficit using ISSR molecular index. Plant Science Today (Early Access). https://doi.org/10.14719/pst.1728

Abstract

Shortage of water is disastrous for crop production. The aim of this work is to obtain a group of maize inbred lines and hybrids that tolerates water deficit and collecting molecular characterizations for selection. This work was conducted in the 2019 and 2020. Five maize pure inbred lines B73, A132, NA30, S7 and DR-B1 were planted according to the design of half-diallel cross-breeding of the second Griffing method, and then 10 hybrids and 5 inbred lines were planted under the impact of irrigation intervals of 5, 10 and 15 days according to the Randomized Complete Block Design (RCBD) in split plot with 3 replicates. Results showed significance genotypes responses. The hybrid DR-B1 × S7 recorded the highest means of seed number per row (34.49 seed row^{-1}), weight of 300 seed (80.77 g), and single plant yield (167.2 g). The shortest irrigation interval of 5 days recorded the highest number of seeds per row (35.39 seed row^{-1}) and single plant yield (162.3 g). Similarity analysis showed a highest genetic diversity (lowest genetic similarity) was 0.83 between inbred 4 and 5. The highest genetic divergence (the least genetic similarity) between hybrids and inbred lines was 0.78 in inbred 4 and hybrid 3 x 2, while the highest genetic divergence of 0.92 was found between hybrids of 3 x 1 and 5 x 4. UPGMA analysis showed that inbred 5 (DR-B1) and inbred 4 (S7) in addition to 1×3, 1×2 and 1×4 hybrids high divergence. Therefore, yield components characteristics can be used for selection in breeding programmes and ISSR (inter simple sequence repeats) is a promising and efficient technique in identification of maize genotypes DNA molecular markers for water deficit tolerance selection.

Keywords

Maize, water deficit, plant breeding, ISSR

Introduction

Maize is considered as one of sensitive crops to water deficit, especially at the reproductive stage, and 45-60% of its total yield could be lost (1, 2). Millions of people around the world value maize as one of the high-profit crops. It is considered as the main source of food and feed (3), the good content of starch, protein, oil, vitamins and minerals in grain in addition to being industrial crop for bio fuel production (4). It is one of the crops that have long been associated with food security (5). According to UNESCO and by 2025, a significant % amounted to be more than 60% of people all over the world will face the danger of water shortage because of the predicted dramatic decrease in crop production (6).

Water shortage is a crucial factor in crop production. It limits the expansion and development of all activities of social and economic life in various fields, especially in the field of agriculture. Most Iraqi agricultural crops are located in semi-arid areas, and currently, the scarcity of irrigation water in terms of the quantity and quality of water flowing into the Tigris and Euphrates rivers is decreased. Therefore, their levels fluctuate from one season to another. Moreover, low falling rates of rain out of growing season, high temperatures and evaporation rates in addition to lack of water and soil resources management has increased water deficit. The demand for food is as normally increased as the population growth rate increases. As a result, improving drought-resistant genotypes is one of the most important approaches that can be applied in response to contemporary climatic changes. Water deficit is predicted to impact the growth of the world population significantly, which indicates a real danger in the distribution of available water resources. Therefore, there were suggestions and research by plant breeders to obtain hybrids that will be well adapted to tolerate drought and have an important role in modulating the physiological reactions of the plant leading to a rapid adaptation to adverse environmental conditions.

All that may help explain how characteristics can be inherited from one generation to another. One of these methods is the second method of half diallel cross that was designed and it involves parents and all the possible crosses. Although practicing field services immaculately explain about 30-50% of yield increases, the remaining percentage could be described by genetic methods (7, 8). Therefore, plant breeders through selection try to nominate the good parents for their aimed characteristics and then obtain the possible enhanced hybrids, especially for high yield. The most important concern of plant breeders is the rate of decrease or loss in yield as a result of stress, meaning that they thus emphasize the yield under stress conditions. However, the decrease in production per unit of the area could be explained by factors related to the adaptation of the crop rather than stress conditions. Number of methods was used to distinguish lines or genotypes that have a good productive capacity under conditions of stress or drought. Some of them used mathematical equations to compare the outcome under conditions of stress and non-stress. Several reports have been documented on the correlation of drought indices with drought tolerance of different crops (9-11).

Nowadays, the science of biotechnology is one of the vital elements that are widely applied for improving crop performance and production, especially the molecular aspects that deal with DNA because of its role in shortening the time the improvement (12-14). Molecular marker is independent of environmental effects and contributed exploration in plant breeding significantly. Inter Simple Sequence Repeats (ISSR) is a technology of detecting large number of polymorphic bundles with high level of efficiency of DNA sequencing determination. The aim of this work is to evaluate the tolerance of maize inbred lines and their hybrids to water deficit, and collecting genetic information and molecular characterization from DNA using ISSR index of genetic similarity and diversity for selection purposes.

Materials and Methods

This work had 5 locally derived pure inbred lines, and their details are listed in (Table 1).

Table 1. Inbred lines, their codes and origins

Inbred number	Inbred code	Inbred origin
1	B73	locally derived
2	A132	locally derived
3	NA30	locally derived
4	S7	locally derived
5	DR-B1	locally derived

Inbred lines experiment

The inbred lines seeds were planted in 2019. For obtaining pollen grains, inbred lines were planted into two separated plots. Firs plot was planted on 15th of July, and then around a week after another plot was planted for synchronized flowering. Each plot had the 5 inbred lines with 6 planted lines. Each planted line was 4 m in length and the distances between planted lines was 90 cm and 2-3 seeds were planted in distance of 2-3 cm hills. Crosses of inbred lines according to the half-diallel of second method of Griffing were carried out for all the possible hybridizations, and 10 hybrids were obtained according to the equation of P(P-1)/2.

Performance experiment

This experiment was carried out in 2020 to investigate the performance of hybrids and the inbred lines under water deficit. Randomized complete blocks design (RCBD) in split plot with three replicates was applied. Irrigation intervals of 5, 10 and 15 days between irrigations were in main plots. However, 15 genotypes (5 inbred lines and 10 hybrids) were in sub plots. The experimental unit had 15 lines each of 2 m length and the distance between lines was 75 cm and between hills was 25 cm. Seeds were planted on 20th of March and characteristics of days until 50% silking, plant leaf area (cm^2 $plant^{-1}$), grains number per row, weight of 300 grain (g) and single plant yield (g) were studied.

Molecular estimation

The DNA was extracted from actively growing leaves and the quantity of DNA was determined by a spectrophotometer at 260 nm. The ratio of 260 nm/280 nm of absorbance was applied to determine DNA purity. For PCR, a ratio of 1.8-2.1 in a 10 ng L^{-1} dilution was utilized. For measuring genetic diversity, 10 random ISSR markers were used (Table 2). 10 ng of DNA template, PCR buffer [50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM MgC_{l2} and 0.001 % (wt/vol) gelatin], 30 pmol primer, 200 mM of each dNTP, 1.25 U of Taq DNA polymerase, and PCR grade dH_2O were included in the optimal amplification reaction mixture (25 µl). DNA reaction mixtures were amplified for 10 min at 94 °C, then for 45 cycles of 1 min at 94 °C, 1 min at 45 °C and 2 min at 72 °C. After a final extension at 72 °C for 10 min, the reaction was terminated and the sample was kept at 48 °C for analysis. 1.5 % agarose gel electrophoresis was used to separate amplified fragments. The scored ISSR markers were represented by a binomial (0/1) matrix, which was the basic data structure. By using the un-weighted pair-group approach using arithmetic averages, the similarity matrix was employed as input data for cluster analysis (UPGMA).

Table 2. Nucleotide sequences of the primers in ISSR technology.

Primer	Nucleotide sequences 5-3	Temperature	Reference
UBC734	GCGCGTGTGTGTGTCT	45.4	?
UBC738	AGAGAGAGAGAGAGCT	30.6	?
UBC744	GAGACCGAGAGAGACCCC	36.3	?
UBC746	AGCAGCAGCAGCAGCAGA	40.8	?
UBC850	AGAGAGAGAGAGAGCTT	32.4	?
UBC856	GAGAGAGAGAGAGAGACT	56.2	?
UBC834	AGAGAGAGAGAGAGAGTT	0	?
B-76	CTCTCTCTCTCTCTGTG	25	?
N-41	CTCTCTTTCTCTCTCTT	30.5	?
A37	TGTGTGTGTGTGTGTGCT	32.8	?

Results and Discussion

Days until 50% silking

The plants of inbred 3 were the shortest until 50% of silking, which amounted to 68.18 days. While, the plants of inbred 4 was the longest, 73.75 days until 50% of silking. The impact of the differences between parents was clearly reflected in their hybrids obtained from the diallel crosses. The hybrid 5x1 was the shortest until 50% of silking (63.75 days) and did not significantly differ from 4x1 and 3x2. While, the hybrid 3×1 was the longest until reaching 50% silking and was recorded to 68.21 days (Table 3). Identical results (16, 17) were recorded and indicated clear differences between genotypes in the characteristic of until 50% of silking. The significant influence of irrigation periods was obvious. A 15-day irrigation interval recorded fewer days until 50% of silking (63.17 days) and it did not significantly differ from 10-days. While the 5-days irrigation interval gave the longest female flowering period of 73.63 days. Perhaps the reason for the short time until 50% of silking in 15 and 10 days irrigation intervals is because of the shortage of soil water availability for plants, high temperature and low relative humidity and thus hormonal changes in plant occurred that led to moving the plant process toward reproductive stages (18). These results are consistent with what was reported (19-21) who indicated that plants subjected to water stress reach the flowering stage earlier. Significant differences were noticed between means of interaction between genotypes and irrigation intervals which is considered as a good sign of breeding plants as it encourages plants to the short period of silking (Table 3).

Table 3. Performance of maize genotypes silking (days until 50%) under irrigation intervals of 5, 10 and 15 days.

Genotypes	Irrigation intervals (days)			Mean
	5	10	15	
1	72.79	67.87	69.21	69.96
2	75.42	65.64	65.97	69.01
3	74.77	65.03	64.73	68.18
4	80.11	70.31	70.82	73.75 a
5	79.77	69.32	67.58	72.22 b
2×1	72.01	68.73	59.55	66.76
3×1	74.65	66.73	63.26	68.21
4×1	71.34	64.47	57.31	64.37
5×1	70.08	61.94	59.22	63.75
3×2	72.30	60.42	58.87	63.86
4×2	74.05	65.94	60.35	66.78
5×2	74.00	62.93	62.51	66.48
4×3	69.84	62.41	65.02	65.76
5×3	72.04	65.36	60.02	65.81
5×4	71.21	64.17	63.13	66.17
Mean	73.63	65.42	63.17	
L.S.D		Irrigation		4.28
		Genotypes		1.04
		Interaction		1.80

Leaf area (cm^2)

Inbred 5 recorded the highest leaf area 3577 cm^2. While inbred 1 was the lowest in leaf area 3099 cm^2. The way parents responded to water shortage was very clear in the hybrids responses. The hybrid 5×1 was superior and showed the highest leaf area 4686 cm^2 compared to the hybrid 2×3 which gave the lowest leaf area 3845 cm^2 (Table 4). It was recorded similar results when they indicated significant differences between genotypes in leaf area (16, 22, 23). Results of this study indicated a clear impact of irrigation intervals on leaf area of maize and it was the highest (4439 cm^2) when the irrigation interval between irrigations

Table 4. Performance of maize genotypes leaf area (cm^2) under irrigation intervals of 5, 10 and 15 days.

Genotypes	Irrigation interval (days)			Mean
	5	10	15	
1	3825	3345	2127	3099
2	3646	3332	2321	3100
3	3897	3658	2282	3279
4	3898	3717	2773	3463
5	4021	3865	2844	3577
2×1	4278	4564	2996	3946
3×1	5127	4971	3231	4443
4×1	4615	4551	3112	4093
5×1	5529	4981	3548	4686
3×2	4034	4463	3038	3845
4×2	5111	4151	2974	4079
5×2	4711	4818	3653	4394
4×3	4553	3652	3249	3818
5×3	4826	4291	3336	4151
5×4	4521	5151	3178	4283
Mean	4439	4234	2977	
L.S.D		Irrigation		233.2
		Genotypes		157.4
		Interaction		291.9

was 5 days. However, the plants recorded a reduction in leaf area of about 29.68% and 32.93% when the irrigation intervals were 10 and 15 days between irrigation intervals respectively.

Probably the reduction in leaf area with increasing the irrigation interval was attributed to the reduction in the number of days from planting to silking Table 3, when leaf growth and expansion normally occurs. In addition to the decrease in the number of plant leaves, it is certain that the relative water content of the leaves would be decreased. As a result, a reduction in leaf growth rate occurred and the inability to elongate and expand reduced as well. The reason for the decrease may be attributed to the water tension by reducing the transport of auxin responsible for regulating the growth in the leaves and inducing the division and cellular elongation in the leaf sheath, or due to the decomposition of auxin due to the increase in the activity of the IAA-oxidase enzyme as a result of water tension, which negatively affected its area (24). This result agrees with the earlier study where it was found that plants exposed to water stress caused a reduction in leaf area (20, 21, 25-27).

The interaction was significant between irrigation treatments and genotypes, as it was shown in table 3, that the 5×1 hybrid plants grown under irrigation treatment for 5 days gave the highest leaf area of 5529 cm^2, with an increase of 159.9% and 85.9% for inbred 1 and hybrid 4×2 for 15 days irrigation interval.

Seeds number per row (seed row^{-1})

Inbred 3 recorded the highest number of seeds per row, 28.73 seed row^{-1} (Table 5) and did not show significant difference from inbred 4. However, inbred 2 gave the lowest number of seeds per row, 27.10 seed row^{-1}. The tolerance of hybrids seems to be genetically inherited from the inbred lines. The hybrid 4×2 recorded highest number of seeds per row, 34.40 seed row^{-1} and did not significantly differ from the hybrid 3x2. While, the hybrid 5×1 gave the lowest number of seeds per row, 30.47 seed row^{-1}. Records are on the identical results when they indicated the difference in the number of seeds per row between the genotypes (28, 29). It is well documented that water shortage decreases the rates of photosynthesis products that are necessary for producing significant numbers of seeds. And thus, shortage of water availability at different growth stages of plant especially at seed filling greatly reduces the number of seeds per row. Water impacts nutrients available for absorption significantly in addition to the activity of enzymes and regulating hormones, which means a weak growth of reproductive organs and probably the viability and that was reflected on the number of seeds per row. Similar results were previously mentioned where it was clearly showed that the negative impact of water deficit in the number of grains per row in maize ear (20, 21, 30). The interaction between genotypes and irrigation intervals was significant. It was clear that the reduction in the number of seeds per row was in term of water deficit, and all genotypes had a significant decrease in the number of seeds per row at 15 day irrigation interval compared 5 and 10 days. The highest percentage of decrease in the number of seeds per row was 59% in the hybrid 5×4 for irrigation interval of 15 day compared to inbred line number 1 at irrigation interval of 15 days.

Table 5. Performance of maize genotypes seeds number per row (grain row^{-1}) irrigation intervals of 5, 10 and 15 days.

Genotypes	Irrigation interval (days)			Mean
	5	10	15	
1	33.3	33.1	16.8	27.73
2	30.2	32.2	18.9	27.10
3	34.4	31.6	20.2	28.73
4	31.9	30.7	23.4	28.67
5	32.4	28.9	22.5	27.93
2×1	37.6	34.7	27.4	33.23
3×1	38.4	34.8	25.5	32.90
4×1	33.5	35.4	28.8	32.57
5×1	32.8	33.9	24.7	30.47
3×2	40.8	33.7	28.6	34.37
4×2	36.7	36.7	29.8	34.40
5×2	33.6	38.9	26.3	32.93
4×3	39.5	31.7	22.4	31.20
5×3	34.7	37.4	23.6	31.90
5×4	41.0	33.2	27.5	33.90
Mean	35.39	33.79	24.43	
L.S.D		Irrigation		3.41
		Genotypes		1.60
		Interaction		2.75

Weight of 300 seed (g)

Results of the study indicated that inbred 3 recorded the highest weight of 300 seed, 69.53 g. However, inbred 1 gave the lowest weight. The response of hybrids was impacted in the way that inbred lines behaved. Water deficit conditions impact was very clear in hybrids as well. The hybrid 5×4 gave the highest weight of 300 seed, 80.77 g and it did not show a significant difference from the hybrid 5x1. Hence, the hybrid 3×2 gave the lowest weight of 300 seed, 62.97 g (Table 6). Reports are on the same results of this work and indicated a difference in seed weight of different genotypes (7, 31). The 15 day irrigation interval recorded the lowest weight of 300 seed, 62.75 g, with a decrease of 16.8% and 20.6% compared to the 5 and 10 day irrigation intervals. The reason for the low weight may be due to the increase in water tension and its adverse impact in silking and the leaf area and then a decrease in the amounts of manufactured and stored nutrients, which determines the efficiency of the source capacity in delivering water and nutrients to seed during the gain filling period. As a result, the produced seed was shrunken, small and finally low weight. This result is consistent with the findings of an earlier study (9, 20, 21, 32). Results indicated significant difference in the interactions between factors and that probably because of the difference in the behavior of genotypes response under different irrigation intervals.

Table 6. Performance of maize genotypes in weight of 300 seed (g) under irrigation intervals of 5, 10 and 15 days.

Genotypes	Irrigation interval (days)			Mean
	5	10	15	
1	68.4	69.3	53.3	63.67
2	74.2	74.8	58.1	69.03
3	77.6	73.3	57.7	69.53
4	75.0	70.4	51.5	65.63
5	79.1	69.1	55.6	67.93
2×1	73.3	79.0	65.6	72.63
3×1	85.9	78.8	70.3	78.33
4×1	79.3	84.4	68.2	77.30
5×1	86.5	83.4	71.1	80.33
3×2	75.3	63.8	49.8	62.97
4×2	87.1	79.4	59.6	75.37
5×2	78.6	82.2	70.1	76.97
4×3	76.5	73.0	66.4	71.97
5×3	80.5	68.9	72.8	74.07
5×4	88.8	82.4	71.1	80.77
Mean	68.4	69.3	53.3	
L.S.D		Irrigation		5.09
		Genotypes		3.25
		Interaction		6.09

Single plant yield (g)

Results in Table 7 showed significant differences between genotyped in single plant yield. Inbred 5 recorded the highest mean, 107.1 g and did not show significant difference with inbred 2 and 3. Hence, inbred 4 gave the lowest mean of 75.23 g. The inbred lines inherited the heterogeneity to their reciprocal hybrids. The hybrid 5 × 4 gave the highest mean of single plant yield of 167.2 g and did not show a significant difference with hybrids 4 × 3 and 3 × 2. This significant difference between inbred lines and hybrids in single plant was relayed to the rain weight Table 6. Our results in this work had previously been confirmed and those results indicated significant differences between genotypes in single plant yield (7, 29, 33). The results indicate significant difference in single plant yield under irrigation intervals. The intervals of 5 and 10 days showed high means in single plant yield and gave 162.3 and 151.8 g, respectively. The reduction in single plant yield was around a half when plants irrigated each 15 days by giving a single plant yield of 73.84 g. The adverse conditions of abiotic stress during plant growth, especially water stress, could cause a huge adverse in the means of other plant characteristics and thus the single plant yield significantly reduced. Our results in Tables 3, 4 and 5 indicated significant decrease in leaf area, number of grains per row and 300 grain weight, and that was clear in the decrease in single plant yield Table 7. These results were in agreement with what was found earlier and indicated significant decrease in single plant yield due to exposing water stress conditions (20, 21, 30, 31, 34, 35). Single plant yield, as indicated by results in Table 7 significantly impacted by interaction between genotypes and irrigation interval. When genotypes exposed to irrigation water, the behavior and expression of genotypes was significantly different. The inbred 5 gave the highest single plant yield 133.8 g at 5 days irrigation interval. Moreover, the hybrid 5 x 4 recorded the highest mean 222.4 g at the same irrigation interval showing the high efficiency of water use. However, the hybrid 5 x 3 recorded the lowest mean of single plant yield 55.1 g at the irrigation interval of 15 days, showing the significant change in the behavior hybrids to water stress.

Table 7. Performance of maize genotypes single plant yield (g) under irrigation intervals of 5, 10 and 15 days.

Genotypes	Irrigation interval (days)			Mean
	5	10	15	
1	106.4	119.1	47.1	90.87
2	122.8	121.2	50.8	98.27
3	129.2	115.2	57.1	100.5
4	98.9	79.2	47.6	75.23
5	133.8	132.1	55.3	107.1
2×1	155.5	141.2	89.7	128.8
3×1	206.4	187.3	73.2	155.6
4×1	169.9	173.1	86.1	143.0
5×1	197.8	164.9	82.2	148.3
3×2	191.5	194.6	99.1	161.7
4×2	182.5	170.1	94.9	149.2
5×2	148.3	172.5	76.8	132.5
4×3	212.9	191.4	95.1	166.5
5×3	155.8	133.1	55.1	114.7
5×4	222.4	181.7	97.5	167.2
Mean	162.3	151.8	73.84	
		Irrigation		10.5
L.S.D		Genotypes		11.1
		Interaction		19.2

Diversity

The primer UBC 738 showed the highest number of bands of (14). However, both of UBC 850 and B 76 primers gave the lowest number of bands (10). The total number of bands was 109 and the total number of Polymorphic bands was 97 with a ratio of 89%. Primers of UBC 744, UBC 746 and A31 recorded a number of Polymorphic bands, amounted to 10, 12 and 11 bands respectively, with a percentage of 100%. Hence, primer UBC850 gave a number of Polymorphic bands of 6 with a percentage of 66.7% (Table 8). The primers used were characterized by a length of amplicons of 300-2100 bp.

Similarity

When the genetic dimension coefficient between inbred lines and their hybrids of maize plants was calculated, the obtained results showed 109 bundles with polymorphism using PAST program and based on the hamming similarity index (Table 9). The highest genetic diversity (lowest genetic similarity) was 0.83 between inbred lines 4 and 5, while the lowest genetic divergence was between inbred lines 1 and 2. We note from the same table that the highest

Table 8. Primers, total bands, Polymorphic bands percentage and amplicons length (bp).

Primer	Total bands	Polymorphic bands	Polymorphic bands %	Amplicons length bp
UBC734	12	11	91.7	450 - 1850
UBC738	14	12	85.7	350 - 2000
UBC744	10	10	100.0	600 - 1800
UBC746	12	12	100.0	500 - 1900
UBC850	9	6	66.7	600 - 1650
UBC856	11	10	90.9	300 - 1200
A31	11	11	100.0	400 - 1900
B76	9	7	77.8	450 - 950
N41	11	9	81.8	650 - 2100
A37	10	9	90.0	600 – 1850
Total	109	97		300 - 2100
mean	10.90	9.7	89.00	

Table 9. Genetic divergence values of genotypes using ISSR technology.

	1	2	3	4	5	2×1	3×1	4×1	5×1	3×2	4×2	5×2	4×3	5×3	5×4
1	**0.00**														
2	0.12	**0.00**													
3	0.30	0.57	**0.00**												
4	0.28	0.31	0.48	**0.00**											
5	0.41	0.13	0.62	0.83	**0.00**										
2×1	0.38	0.73	0.44	0.13	0.32	**0.00**									
3×1	0.47	0.13	0.23	0.22	0.55	0.24	**0.00**								
4×1	0.32	0.22	0.17	0.61	0.31	0.21	0.16	**0.00**							
5×1	0.53	0.32	0.46	0.33	0.18	0.24	0.16	0.42	**0.00**						
3×2	0.44	0.26	0.25	0.78	0.21	0.11	0.25	0.41	0.23	**0.00**					
4×2	0.60	0.14	0.51	0.66	0.19	0.87	0.60	0.32	0.21	0.50	**0.00**				
5×2	0.21	0.32	0.41	0.22	0.52	0.27	0.04	0.10	0.63	0.26	0.19	**0.00**			
4×3	0.67	0.15	0.14	0.61	0.32	0.81	0.63	0.71	0.26	0.44	0.61	0.40	**0.00**		
5×3	0.78	0.16	0.12	0.23	0.34	0.41	0.45	0.56	0.73	0.55	0.21	0.43	0.23	**0.00**	
5×4	0.72	0.75	0.68	0.28	0.34	0.78	0.92	070	0.52	0.62	0.11	0.32	0.36	0.32	**0.00**

genetic divergence (the least genetic similarity) between hybrids and inbred lines was 0.78 in inbred 4 and hybrid 3 x 2 and this predicts the development of a triple hybrid, while the highest genetic divergence of 0.92 was found between hybrids of 3 x 1 and 5 x 4 as well as between 2 x 1 and 4 × 2. The genetic divergence was 0.87 and this predicts that these crosses can be used to derive a double cross hybrid. Previous studies indicated similar to our results (4, 36). Results of this study and previous studies provide crucial information of the importance of genetic diversity and its utilizations in predicting the performance of maize genotypes in field under water deficit. Moreover, results of this study are useful in finding a group of inbred lines and hybrids compared to previous studies.

Organisms that are phenotypically similar to each other are more likely to be closer to each other than organisms of different sequences. The importance of determining genetic similarity is due to the possibility of organizing the germplasm, selecting the parents involved in the breeding programme, predicting the best hybrids and knowing the least possible number of genotypes that contain the largest possible number of genetic diversities in breeding programmes (37). Individuals may be different from each other phenotypically, but they are genetically close to each other, and then these differences may be due to environmental influences only, which are not taken into account in determining the degree of similarity between individuals. The cluster analysis of the studied inbred lines and hybrids using ISSR markers and based on the (UPGMA) method according to the nearest neighbor showed that the characteristic behavior of inbred 5 (DR-B1) can be observed with inbred 4 (S7) in addition to 1×3, 1×2 and 1×4 hybrids, which are the most divergent at the level of DNA (Fig. 1). By joining the 2 most heterogeneous groups on the path of the hierarchical shape, which predicts an increase in the size of the total heterogeneity between such inbred,

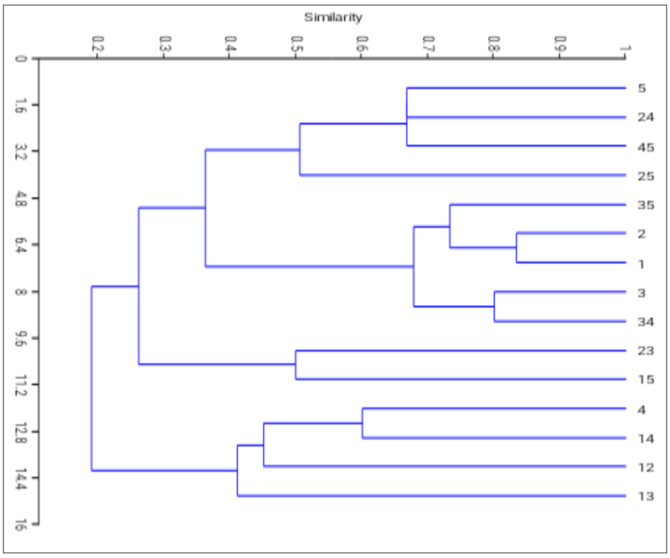

Fig. 1. Cluster analysis of genotypes based on the results of 109 bands.

may contribute to increasing the hybrid vigor, as the rest of the inbred was included with all the hybrids in another major group. The 5 breeds and the 10 hybrids were divided into 2 groups; the first group included breed 4 and 3 × 1, 2 × 1 and 4 × 1 hybrids, while the second group included the remaining of inbred lines and hybrids. The diversity values were invested in cluster analysis based on the results of using 12 primers in the ISSR technique to find the genetic relationship between the genotypes under the study. Similar results were reported where the genotypes were phenotypically divided into several groups according to the variance of the means of the studied characteristics (36, 38).

Conclusion

Water deficit impacts the performance of maize, and all genotypes characteristics showed different responses. Although there were within the limits and acceptable variations between genotypes, yield components of maize are useful tools for studying tolerance to water deficit, as variation indicates the adaptation of maize to water deficit. Good genetic diversity (low genetic similarity) and as approved of this study, can be practiced in improving maize and basic material in breeding programmes can be established for obtaining hybrids from different crosses. ISSR DNA markers are an efficient approach for the determination of the genetic diversity of maize genotypes. These molecular markers can be used for the selection and increasing the efficiency and precision of breeding programmes.

Authors contributions

ZAA designed and performed the study, derived the models and genetic analysis. MIH analyzed data statistically and wrote the manuscript. MA participated in writing the research paper and review process. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. Ramya S, Arulbalachandran D, Ramachandran M. Influence of exogenous abscisic acid on morpho-physiological and yield of maize (*Zea mays L.) under drought stress. Plant* Science Today (Early Access). https://doi.org/10.14719/pst.1413
2. Iedan SF. Effect of seed soaking by salicylic acid or cobalt spraying on growth, yield and quality of sweet corn under water stress. Ph.D. Dissertation. Dept. of Field Crop Sci. College of Agric. Univ. of Baghdad. Iraq. 2020; pp.189.
3. Dei HK. Assessment of maize (*Zea mays*) as feed resource for poultry. Poultry science. 2017; 1-32. https://doi.org/10.5772/65363
4. Amoon MH, Abdulhamed ZA. Determination genetic diversity of inbred lines and hybrids of maize using ISSR technic. Iraqi J of Agri Sci. 2020; 51(1):269-77. https://doi.org/10.36103/ijas.v51i1.925
5. Yra A J. Factors affecting food security of children from corn farming households in Cagayan valley, Philippines. Plant Science Today. 2021;8(2):352-56. https://doi.org/10.14719/pst.2021.8.2.1061
6. Vieno N, Hallgren P, Wallberg P, Pyhälä M, Zandaryaa S. Baltic Marine Environment Protection Commission. Pharmaceuticals in the aquatic environment of the Baltic Sea region: a status report. UNESCO Publishing. 2017; Sep 25.
7. Abdulhamed ZA, Faiath SE, Ajaj HA, Mohammed YA. Genetic parameters estimates through line× tester analysis for the stay green, yield and quality in maize (*Zea mays* L.). Annals of the Romanian Society for Cell Biology. 2021;8597-61.
8. Gazala P, Gangappa E, Ramesh S, Swamy D. Comparative breeding potential of two crosses for response to late wilt disease (LWD) in maize (*Zea mays* L.). Genetic Resources and Crop Evolution. 2021; 68(5):1751-57. https://doi.org/10.1007/s10722-021-01150-3
9. Ahmed MS. Efficiency of selection indices for drought tolerance to lerance in maize (*Zea mays* L.). Ph.D. Dissertation. Dept. Of Field Crop Sci. College of Agric. Univ. of Baghdad. Iraq. 2017;pp.146.
10. Nadali F, Asghari HR, Abbasdokht H, Dorostkar V, Bagheri M. Improved quinoa growth, physiological response and yield by hydropriming under drought stress conditions. Gesunde Pflanzen. 2021; 73(1):53-66. https://doi.org/10.1007/s10343-020-00527-1
11. Oloyede-Kamiyo QO, Olaniyan AB, Abdul-Waheed JA, Akinseye BA. Sources of tolerance to low soil nitrogen in some Striga resistant and quality protein maize (*Zea mays* L.) varieties. Journal of Crop Science and Biotechnology. 2021;8:1-8. https://doi.org/10.1007/s12892-021-00098-1
12. Guo X, Sun X, Liu S, Gong C, Feng C, Han X et al. Screening and application of SSR markers related to seed storability traits in maize (*Zea mays* L.). Genetic Resources and Crop Evolution. 2021;4:1-5.
13. Sreewongchai T, Sripichitt P, Matthayatthaworn W. Parental genetic distance and combining ability analyses in relation to heterosis in various rice origins. Journal of Crop Science and Biotechnology. 2021;24(3):327-36. https://doi.org/10.1007/s12892-020-00081-2
14. Rajarajan K, Ganesamurthy K, Raveendran M, Jeyakumar P, Yuvaraja A, Sampath P et al. Differential responses of sorghum genotypes to drought stress revealed by physio-chemical and transcriptional analysis. Molecular Biology Reports. 2021; (3):2453-62. https://doi.org/10.1007/s11033-021-06279-z
15. Ghomi K, Rabiei B, Sabouri H, Gholamalipour Alamdari E. Association analysis, genetic diversity and population structure of barley (*Hordeum vulgare* L.) under heat stress conditions using SSR and ISSR markers linked to primary and secondary metabolites. Molecular Biology Reports. 2021;1-22. https://doi.org/10.1007/s11033-021-06652-y
16. Matin MQ, Rasul MG, Islam AA, Mian MK, Ivy NA, Ahmed JU. Study of genetic diversity in maize (*Zea mays* L.) inbreds. Science Publishing Group. 2017; 5(2):31-35. https://doi.org/10.11648/j.plant.20170502.11
17. Ramadan AS, Mukhlif FH, Abdulhamed ZA. Performance and heterosis for the yield traits and components of maize (*Zea mays* L.) Using the Full Diallel Cross Method. Annals of the Romanian Society for Cell Biology. 2021;1270-81.
18. Fang Y, Xiong L. General mechanisms of drought response and their application drought resistance improvement in plants. Cell Mol Life Sci. 2015; 2:673-89. https://doi.org/10.1007/s00018-014-1767-0

19. Murtadha MA, Ariyo OJ, Alghamdi SS. Analysis of combining ability over environments in diallel crosses of maize *Zea mays* L. J of Saudi Soci of Agri Sci. 2018; 17:69-78. https://doi.org/10.1016/j.jssas.2016.01.004
20. Abdul Amir OQ. Effect of activating seeds with salicylic acid and spraying plants with nano-nitrogen on the growth and yield of three cultivars of maize. PhD thesis. College of Agriculture, University of Baghdad, Iraq. 2018, p.p.: 130.
21. Hassan AAH. Study of the physiological and chemical effects of growth stimulants and biofertilizers on the growth of maize yield under different irrigation levels. PhD thesis. College of Agriculture, University of Baghdad, Iraq. 2019; A.S:124.
22. Bello OB, Olawuyi OJ. Gene action, heterosis, correlation and regression estimates in developing hybrid cultivars in maize. Trop Agric. 2015; 92(2):102-17.
23. Abdulhamed, ZA, Abas SA, Abed AA. Studying some genetic in maize by line × tester analysis. Int J Agricult Stat Sci**.** 2020; (16), Supplement 1, pp. 1421-26.
24. Majda M, Robert S. The Role of auxin in cell wall expansion. J Mol Sci. 2018; 19(6):95-111. https://doi.org/10.3390/ijms19040951
25. Huang S, Lv L, Zhu J, Li Y, Tao H, Wang P. Extending growing period is limited to offsetting negative effects of climate changes on maize yield in the north china plain. Field Crops Res. 2018; 21(5):66-73. https://doi.org/10.1016/j.fcr.2017.09.015
26. Peng B, Guan K, Chen M, Lawrence DM, Pokhrel Y, Suyker A et al. Improving maize growth processes in the community land model: Implementation and evaluation. Agri and Forest Meteorology. 2018; 2(5):64-89. https://doi.org/10.1016/j.agrformet.2017.11.012
27. Song H, Li Y, Zhou L, Xu Z, Zhou G. Maize leaf functional responses to drought episode and rewatering. Agri. and Forest Meteorology. 2018; 2(4):57-70. https://doi.org/10.1016/j.agrformet.2017.11.023
28. Wuhiab KM, Hadi BH, Hassan WA. Hybrid vigor, heterosis and genetic parameters in maize by diallel cross analysis. Inter J Appl Agri Sci. 2016; 2(1):1-11. https://doi.org/10.11648/j.ijaas.20160201.11
29. Pheirim R, Niyaria R, Singh PK. Heterosis prediction through molecular markers. Rising A J Res. 2017; 1(1):45-50.
30. Al-Qaisi QA. The role of wheat residues and tillage in the growth and yield of maize under different irrigation levels. Master's Thesis. College of Agriculture. Al-Qasim Green University. 2017; pp: 107.
31. Ali F, Ahsan M, Ali Q, Kanwal N. Phenotypic stability of *Zea mays* L. grain yield and its attributing traits under drought stress. Frontiers in Plant Sci. 2017; 8:1397. https://doi.org/10.3389/fpls.2017.01397
32. Jin Z, XUE QW, Jessup KE, HOU XB, HAO BZ, Marek TH et al. Shoot and root traits in drought tolerant maize *Zea mays* L. hybrids. J of Int Agri. 2018; 17(5):1093-105. https://doi.org/10.1016/S2095-3119(17)61869-0
33. Dhoot M, Dubey RB, Ameta KD, Dhoot R, Kumar R, Badaya VK. Estimation of heterosis for grain yield and architectural traits in yellow seeded maize (*Zea mays* L.). Int J Curr Microbio App Sci. 2017; 6(7):4536-42. https://doi.org/10.20546/ijcmas.2017.607.473
34. Ogutu GE, Franssen WH, Supit I, Omondi P, Hutjes RW. Probabilistic maize yield prediction over East Africa using dynamic ensemble seasonal climate forecasts. Agri and For Met. 2018; 250: 243-61. https://doi.org/10.1016/j.agrformet.2017.12.256
35. Yuan C, Feng S, Huo Z, Ji Q. Effects of deficit irrigation with saline water on soil water salt distribution and water use efficiency of maize seed production in arid Northwest China. J Agr Wat Man. 2019; 212:424-32. https://doi.org/10.1016/j.agwat.2018.09.019
36. Dehghanisanij H, Kouhi N. Interactive effects of nitrogen and drip irrigation rates on root development of corn (*Zea Mays* L.) and residual soil moisture. Gesunde Pflanzen. 2020; Dec;72 (4):335-49. https://doi.org/10.1007/s10343-020-00516-4
37. Pahadi P, Sapkota M, Thapa DB, Pradhan S. Cluster and principal component analysis for the selection of maize (*Zea mays* L.) genotypes. Int J Exp Res Rev. 2017; 9:5-10.
38. Dwivedi SL, Gurtu S, Chandra S, Yuejin W, Nigam SN. Assessment of Genetic diversity among selected groundnut germplasm. I: RAPD analysis. Plant Breeding. 2001; 120:345-49. https://doi.org/10.1046/j.1439-0523.2001.00613.x
39. Al-Obaidi SSM. Morphological and molecular evaluation of genetic variation for number of maize *Zea mays* L. inbred line. Ph.D. Dissertation. Dept. of Field Crop Sci. College of Agric. Univ. of Anbar. Iraq. 2018; pp.141.

§§§