

**AGRONOMIC AND MOLECULAR CHARACTERIZATION OF SOME MAIZE HYBRIDS
AS INFLUENCED BY WATER STRESS AND SOAKING LEVELS
OF SODIUM NITRO PEROXIDE**

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ABSTRACT

Seven hybrids of maize (*Zea mays* L.) included three single crosses *i.e.*, SC 120, SC 129, and SC 155 and four three-way crosses, *i.e.*, TWC 310, TWC 311, TWC 314 and TWC 352 were tested under two levels of irrigation intervals, *i.e.*, normal irrigation every 12 days and stress irrigation every 20 days and three levels of antioxidants, *i.e.*, soaking seeds in low (2.5 mM) and high (5mM) concentrations of sodium nitro peroxide solution for 24 h in addition to the control. Soaking treatments were activated by spraying maize plants by sodium nitro peroxide solution after 30 days from planting. Each irrigation treatment was planted in a separate experiment. A split-plot design was used, where soaking treatments were assigned to the main plots and hybrids in the sub-plots with four replications. The single cross SC 155 was the earliest in terms of the number of days to 50% tasselled and silking, but SC 120, SC 129, TWC 310, TWC 311, TWC 314, and TWC 352 were the latest under stress irrigation. The same single cross SC 155 had the shortest plant and ear height under normal irrigation, but the single crosses SC 120, SC 129, and TWC 310 had the tallest plants and the highest ear height under stress irrigation. The three way cross TWC 352 had the lowest grain yield followed by TWC 310, TWC 311 and TWC 314 while the single crosses SC 120, SC 129 and SC 155 were the most superior hybrids under stress irrigation in both seasons. Soaking seeds of maize hybrids in sodium nitro peroxide solution was associated with a reduction in plant height at stress irrigation and grain yield at normal irrigation in the first season only. No significant differences were detected among soaking treatments under normal and stress irrigation for other studied traits. Interaction between hybrids and soaking was significant for grain yield under normal irrigation in the first season and plant height besides silking under normal irrigation in the second season. Interaction between hybrids and soaking was not significant for other studied traits.

RAPD analysis represented a level of polymorphism of 84.8%. Cluster analysis grouped the selected five hybrids according to their pedigree and also, their drought tolerance. RAPD assay was successful in identifying each of the five hybrids with unique markers, except for TWC 314.

Key words: drought, genetic relationship, hybrids, irrigation intervals, maize, RAPD, sodium nitro peroxide

1. INTRODUCTION

Water deficit (commonly known as drought) can be defined as the absence of adequate moisture necessary for plant growth and to complete the life cycle (Zhu, 2002). Drought is one of the most important abiotic stresses that seriously decreases final grain yield in maize. It occurs in many areas of the world every year, often with devastating effects on crop production (Ludlow and Muchow, 1990). The environmental stress such as drought, temperature, salinity, air pollution, heavy metals, pesticides and soil pH are major limiting factors in crop production

(Hernandez *et al.*, 2001 and Lawlor, 2002). Since the occurrence of drought is not predictable, breeders have to produce new maize hybrids and varieties showing tolerance to stressful growing conditions. Genetic variability of maize hybrids grown in the fields is very important because better understanding of the genetic basis of this variability will improve the efficiency of maize improvement for biotic and abiotic stresses which cause great losses in yield. So, information on genetic diversity of maize hybrids is also very important for germplasm enhancement, hybrids breeding and in preventing environmental damage which may occur on large areas.

In recent years, a series of techniques and genetic markers have been developed to analyze and estimate genetic diversity, but no single technique is universally ideal; each available technique has both strengths and weaknesses (Mueller and Wolfenbarger, 1999). Different methodologies using molecular markers are widely used to analyze genetic variability in different crops. Among the various marker systems, RAPDs are one of the most popular DNA based approaches (Behera *et al.* 2008). Further, RAPDs are the least technically demanding and offer a fast method for providing information from a large number of loci, particularly in species where no study, has previously been undertaken. Moreover, RAPD markers offer a number of

intervals and soaking seeds in sodium nitro peroxide solution and (ii) to characterize some selected maize hybrids at the molecular level using RAPD analysis.

2. MATERIALS AND METHODS

Seven hybrids of maize developed by the Maize Research Program, Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Egypt were used. These plant materials were chosen according to preliminary evaluation experiments, which were conducted at different locations. Selected hybrids were: SC 120, SC 129, SC 155, TWC 310, TWC 311, TWC 314, and TWC 352. Their pedigree is presented in Table (1).

Table (1): Maize genotypes and their pedigree.

No.	Hybrids	Pedigree	No.	Hybrids	Pedigree
1	SC 120	Sd 34 x Sd 63	5	TWC 311	SC 21 x Sd 34
2	SC 129	Gz 612 x Gz 628	6	TWC 314	SC 24 x Sd 34
3	SC 155	Gm 1002 x Gm 1021	7	TWC 352	SC 52 x Gm 1021
4	TWC 310	SC 10 x Sd 34			

advantages in plant breeding program. The RAPD procedure requires extremely small amount of DNA, does not need any sequence information, suitable for work on anonymous genomes, efficient and low cost (Yoke-Kqueen and Radu, 2006).

Nitrite oxide (NO) is a lipophilic molecule that diffuses through membranes. Although at first described as a signal molecule in animals, accumulating evidence shows that NO is an important signal molecule involved in plant response to biotic and abiotic stresses (Uchida *et al.*, 2002 and Yang *et al.*, 2006)

Sodium nitro peroxide (SNP) was used as NO donor to alleviate oxidative damage of drought stress (Rashad and Abou-Elalla, 2009). They studied the changes of antioxidant enzyme activities (GR, SOD, CAT, and GPx.), the level of some antioxidant compounds (ascorbate and nitric oxide), osmolality and chlorophyll content after treatment with SNP in leaves of seven hybrids and inbreds of maize grown under water shortage followed by a rewatering in three different locations of Egypt.

The objectives of this study were (i) to investigate the response of some maize hybrids to different soil water levels represented in irrigation

2.1. Field study

Field experiments were conducted at Sids Research Station in Egypt in two successive growing seasons; 2008 and 2009 to study the agronomic and molecular characterization of some maize hybrids as influenced by water stress and soaking levels of sodium nitro peroxide. The irrigation interval treatments were two, every 12 days and every 20 days started at the third irrigation. Three levels of antioxidant treatments soaking grains in low (2.5mM) and high (5 mM) concentrations of sodium nitro peroxide solution for 24 h prior to planting, in addition to the control. Soaking treatments were activated by spraying maize plants by sodium nitro peroxide solution at 30 days from planting.

Concentration of antioxidant treatments (SNP) for the present investigation was 2.98 mg/l and 5.96 mg/l.

The preliminary green house experiment was conducted to detect the effect of SNP concentrations on the specific enzymes and several hybrids (18) and inbreds (22) supported from Maize Research Department, Field Crops Research Institute, Agricultural Research Center.

Each irrigation treatment was planted in a separate experiment. Split plot design with four

replications was used. Antioxidant treatments were located in the main plot, while hybrids were randomly distributed in sub plot. Plot size was two rows; 80 cm in width, 6 m in length and, 25 cm between hills. Eight and six irrigations were applied for 12 and 20 days, respectively. Proper mechanical weed control was applied by hoeing twice before the first and the second irrigations. Nitrogen was added at 120 kg N/fed. as urea (46%) in two equal doses, *i.e.*, before the first and the second irrigations, while calcium superphosphate (15.5 %) at the rate of 31 kg P₂O₅ /fed. was added at soil preparation. Other cultural protocols were applied as recommended. Data recorded were included days from planting to 50% tasselling and silking, plant and ear heights, and grain yield.

All plants per plot were harvested and the grain yield was adjusted to 15.5% moisture

Statistical analysis of the data was performed using Duncans Multiple Range Test to determine the significant differences of the means at 5% level, different letters (a-d) indicated significant differences at P ≤ 0.05 level (Duncan, 1955).

Random Amplified Polymorphic DNA (RAPD)

Assay: These experiments were conducted in the Molecular Laboratory at the Plant Physiology Division, Dept. of Agric. Botany, Faculty of Agriculture, Cairo University, Giza, Egypt.

Extraction of DNA: EZ-10 spin Column Genomic DNA Isolation Kit (BS425, 50 Preps) was used for the isolation of total genomic DNA from maize seedlings.

RAPD-PCR Reactions: Ten random primers

(Operon Technologies Inc., USA) were used. Names and sequences of the primers are listed in Table (2). Random Amplified Polymorphic DNA assay was performed as described by Williams *et al.* (1990) with some modifications. Polymerase Chain Reactions(PCR) were carried out in a volume of 25 ul containing 20 ng of genomic DNA, 25 pmoles primer, 2 mM dNTPs, 2.5 mM MgCl₂, and 2.5 unit Taq polymerase (Fermentas) with 1X buffer (NH₄)₂ SO₄.

Thermocycling Profile and Detection of PCR products:

PCR amplification was carried out in a TECHNE (TC3000) thermocycler programmed for 47 cycles as follows: an initial denaturation cycle for 5 min at 94° C, followed by 45 cycles. Each cycle consisted of a denaturation step at 94°C for 1 min., an annealing step at 36° C for 1 min., and an elongation step at 72° C for 2 min. The primer extension segment was extended to 7 min at 72° C in the final cycle.

Amplification products were separated by electrophoresis on 1.5 % agarose gel containing ethidium bromide (0.5 ug / ml) in 1X TAE buffer at 150 volts for one hour, and then the gels were photographed using Fuji camera (Gene Tooles Software) under UV light.

Size of bands was estimated by molecular weight against marker (100 bp ladder, Fermentas) using Gene Tools Software.

2.2. Data analysis

The banding patterns generated by RAPD were scored either as present (1) or absent (0). Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between each two genotypes was estimated according to Dice coefficient (Sneath and Sokal, 1973) using the SPSS-PC program version 12.

Dice Formula: $G_s(ij) = 2a / (2a + b + c)$ where; GS (ij) is the measure of genetic similarity between individuals i and j, (a) is the number of bands shared by i and j, (b) is the number of bands present in i and (c) is the number of bands absent in i and present in j.

3. RESULTS AND DISCUSSION

3.1. Hybrid effect

3.1.1. Number of days to 50% tasselling and silking

3.1.1.1. Normal irrigation

Hybrids SC 129, SC 155, and TWC 352 were the earliest, while SC 120, TWC 310, TWC 311, and TWC 314 were the latest in terms of number of days from planting to 50% tasselled in both growing seasons (Tables 3&4). These variations

Table (2): Sequence of the ten decamer arbitrary primers used in RAPD.

No.	Primer	Sequence (5' - 3')
1	OPB03	CATCCCCCTG
4	OPB05	TGCGCCCTTC
3	OPB07	GGTGACGCAG
4	OPG08	GTCCACACGG
5	OPG01	CTACGGAGGA
6	OPG02	GGCACTGAGG
7	OPG03	GAGCCCTCCA
8	OPG16	AGCGTCCTCC
9	OPG17	ACGACCGACA
10	OPZ01	TCTGTGCCAC

are mainly due to the differences in the genetic background of the tested hybrids.

3.1. 1.2. Irrigation stress

Single cross SC 155 was the earliest in terms of number of days to 50% tasselling and silking. However, SC 120, SC 129, TWC 310, TWC 311, TWC 314 and TWC 352 were the latest. Single crosses SC 129 and TWC 352 were the earliest under normal irrigation and the latest under irrigation stress. This might indicate that SC 129 and TWC 352 were susceptible to water stress. In this respect, similar results were reported by many investigators including Grzesiak (1991) Jeffery *et al.* (1997), Zaidi *et al.* (2004), El-Koomy *et al.* (2005), and Ibrahim and Kandil (2007) who reported that maize genotypes show different dates to silking. Villegas *et al.* (1985) reported that drought stress delayed the silking and anthesis dates. These results agreed with those obtained by Chapman and Edmeades (1999) who suggested that screening progenies under a severe drought stress that coincides with flowering is a robust and reliable methodology for improving tolerance to mid and late season drought in tropical maize populations.

3.1.2. Plant and ear heights

3.1.2.1. Normal irrigation

Single cross SC 155 had the shortest plant and ear heights, while SC 129 and TWC 310 had the highest plant and ear heights (Tables 3&4). There were no significant differences between SC 120 and SC129 and between TWC 310 and TWC 314 in plant and ear heights in the second growing season. Differences in plant height among hybrids might be attributed to differences in the number and /or length of internodes reflecting genetical make up of maize hybrids. However, these results are similar to those obtained by El-Morshidy *et al.* (2003) in Egypt who found that high genotypic variance in plant height was obtained under water stress in the two populations (Giza-2, white and pop-45, yellow).

3.1.2.2. Irrigation stress

Single crosses SC 120, SC 129, and three way cross TWC 310 had the tallest plants and the highest ear height under irrigation stress (Tables 3&4). There were no significant differences between TWC 310, TWC 311, and TWC 314 in ear height and between TWC 311 and TWC 314 in plant height in the second growing season. Similar results were obtained by Ragab *et al.* (1986), Human *et al.* (1990), Dhillon *et al.* (1995), Ibrahim *et al.* (2005), and El Kalla *et al.* (2007) who found that water stress significantly decreased maize plant height.

3.1.3. Grain yield

3.1.3. 1. Normal irrigation

Single cross SC 120 produced the highest grain yield followed by SC 129 and TWC 310. On the other hand, hybrid TWC 352 followed by SC 155, TWC 311, and TWC 314 showed the lowest grain yield in the first season, while, SC 120 had the highest grain yield followed by SC 129 and TWC 314. Hybrid TWC 352 followed by TWC 310, SC 155, and TWC 311 had the lowest grain yield in the second growing season.

3.1.3. 2. Irrigation stress

Three way cross TWC 352 had the lowest grain yield followed by TWC 310, 311, and 314. In contrast, single crosses SC 120, SC 129, and SC 155 were the most superior hybrids under stress irrigation in both seasons. Results of Fredrick *et al.*(1989), Essam (1992), Dass *et al.* (1999), Baser and Gencton (1999), and Abo-Shetaia *et al.* (2005) reported significant differences among maize genotypes under different drought treatments respecting grain yield. This suggests that the studied maize genotypes differed greatly under drought stress for grain yield.

3.2. Soaking effect

Soaking grains of maize hybrids in sodium nitro peroxide solution was associated with a reduction in plant height at irrigation stress and grain yield at normal irrigation in the first season only. No significant differences were detected among soaking treatments under normal and irrigation stress for the other studied traits. This may indicate that changing time and treatment application and/or treatment concentrations is required for improving the role of sodium nitro peroxide solution in improving drought tolerance of maize hybrids.

Owing to the ability of NO to reduce oxidative damage and simulate antioxidant compounds accumulation in order to get an insight into the function of NO in alleviating damage caused by drought stress, further research is needed to focus on the role of molecular biology approach to show the differences between maize hybrids in their response to drought and SNP treatment.

3. 3. Hybrids x soaking interaction

Interaction between hybrids and soaking were significant for grain yield under normal irrigation (Data are not shown) in the first season, also plant height and silking under normal irrigation in the second season. Interactions between hybrids and soaking were not significant for the other studied traits.

Table (3): Means of days to 50% tasselling, days to 50% silking, plant height (cm), ear height (cm), and grain yield (ard/fed) of maize hybrids under two water intervals and three soaking treatments in 2008 growing season.

Hybrids	2008																			
	Soaking treatment																			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
	Tasselling (days)				Silking (days)				Plant height (cm)				Ear height (cm)				Grain Yield (ard/fed)			
	12 days																			
SC120	63.8	63.5	63.8	63.7a	64.5	65.3	66.3	65.3a	269	266	263	266a	163	163	163	163a	32.23	32.69	27.76	30.89a
SC129	59.3	60.3	59.8	59.8c	62.5	62.0	62.8	62.4b	274	268	275	272a	165	160	170	165a	29.58	30.78	29.97	30.11b
SC155	59.3	59.0	58.8	59.0d	59.8	59.3	59.5	59.5c	256	248	256	253b	150	141	154	148b	27.36	29.39	26.21	27.65d
TWC310	63.5	63.3	63.5	63.4a	65.5	65.8	66.0	65.8a	273	276	269	273a	168	165	164	165a	29.66	30.82	24.89	28.46c
TWC311	63.5	62.8	62.8	63.0a	65.8	65.5	66.3	65.8a	268	273	264	268a	164	168	160	164a	26.71	29.05	24.37	26.71d
TWC314	63.8	63.0	63.0	63.3a	65.8	65.8	66.5	66.0a	271	276	265	271a	165	170	158	164a	24.59	31.09	23.17	26.28d
TWC352	60.5	61.5	61.3	61.1b	60.5	62.8	62.5	61.9b	238	245	246	243c	133	138	143	138c	18.39	20.53	18.39	19.10e
Mean	61.9a	61.9a	61.8a	61.9	63.5a	63.8a	64.3a	63.8	264a	264a	263a	264	158a	158a	159a	158	26.93ab	29.19a	24.96b	27.03
	20 days																			
SC120	65.8	65.5	65.8	65.7a	68.0	67.5	67.8	67.8a	238	220	220	226a	134	124	119	125b	19.89	20.06	19.72	19.89a
SC129	62.5	62.3	61.8	62.2b	65.3	64.5	65.8	65.2b	234	233	228	231a	128	125	123	125b	17.78	15.30	15.62	16.23b
SC155	60.5	60.3	60.5	60.4c	60.8	61.3	60.8	60.9c	226	211	215	218b	121	113	116	117c	19.29	18.03	17.95	18.42a
TWC310	65.5	65.5	65.5	65.5a	68.3	67.3	67.8	67.8a	233	226	228	229a	129	128	130	129a	14.99	13.02	14.00	14.00c
TWC311	65.8	65.5	65.3	65.5a	68.3	68.0	67.5	67.9a	231	229	224	228a	126	124	119	123b	16.13	15.66	14.87	15.55c
TWC314	65.5	65.8	64.8	65.3a	68.0	68.3	67.3	67.8a	229	224	226	226a	126	124	126	125b	13.35	13.78	14.10	13.74c
TWC352	62.3	63.0	62.3	62.5b	66.0	64.3	65.3	65.2b	209	214	204	209c	109	114	106	110d	9.72	10.98	11.43	10.71d
Mean	64.0a	64.0a	63.7a	63.9	66.4a	65.9a	66.0a	66.1	228a	222ab	221b	224	125a	121a	120	122	15.88a	15.26a	15.38	15.51

Vertical means (hybrids) with the same letter are not significantly different at 0.05 level of significance

Horizontal means (soaking) with the same letter are not significantly different at 0.05 level of significance

1 = low concentration of sodium nitro peroxide

2 = high concentration of sodium nitro peroxide

3 = control treatment

Table (4): Means of days to 50% tasselling, days to 50% silking, plant height (cm), ear height (cm) and grain yield (ard/fed) of maize hybrids under two water intervals and three soaking treatments in 2009 growing season.

Hybrids	2009																			
	Soaking treatment																			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
	Tasselling (days)				Silking (days)				Plant height (cm)				Ear height (cm)				Grain Yield (ard/fed)			
	12 days																			
SC120	67	66.3	65.8	66.4a	68	67.3	66.5	67.3	243	250	243	245a	134	141	131	135	23.35	26.73	27.61	25.90a
SC129	64	64.3	63.3	63.9b	66.8	65.8	65.5	66	248	251	236	245	134	133	121	129	22.02	20.23	20.78	21.01b
SC155	61.8	61.3	61.3	61.5c	62.3	62.8	62.3	62.5	218	226	218	220	115	118	113	115	18.23	18.43	17.67	18.11c
TWC310	67.5	66.3	66.5	66.8a	68.8	69	68.5	68.8	253	246	245	244	135	135	134	135	15.28	17.68	17.99	16.98c
TWC311	67	65.8	65.5	66.1a	69	67.8	67.8	68.2	238	243	246	242	130	131	133	131	20.27	20.24	20.33	20.28b
TWC314	66	66.3	66	66.1a	69.8	68	68.5	68.4	246	241	246	244	138	133	138	136	21.05	19.56	21.93	20.85b
TWC352	64.3	63.3	63.8	63.8b	66	63.8	65.8	65.2	226	216	214	219	121	114	114	116	13.65	12.99	12.88	13.17d
Mean	65.4a	64.8a	64.6a	64.9b	67.1a	66.3a	66.4a	66.6	239a	239a	235a	237	129a	129a	126a	128	19.12a	19.41a	19.88a	19.47
	20 days																			
SC120	69.3	69.5	68.8	69.2	72.5	71.8	70.5	71.6	200	210	200	203	106	111	108	108	9.62	11.5	12.21	11.11a
SC129	67	67	66	66.7	70	70.5	69.5	70	195	211	195	200	98	105	96	100	8.31	8.7	12.37	9.79b
SC155	66.8	66.5	65.3	66.2	68	67.5	67.8	67.8	188	180	180	183	104	98	93	98	8.15	8.94	8.71	8.60c
TWC310	71	70	70.3	70.4	74.5	74	74.8	74.4	208	205	209	207	109	99	105	105	5.25	7.5	5.65	6.13e
TWC311	68	70	68.3	68.7	72.5	74.3	73	73.3	204	204	204	204	103	105	105	105	8.91	6.55	7.9	7.79c
TWC314	69.8	69.5	70.8	70.0	74.5	74.3	74.5	74.4	195	213	201	203	103	109	105	106	8.05	7.19	7.97	7.74d
TWC352	68	69	68.8	68.6	69.8	70.8	70	70.02	188	173	176	179	98	90	91	93	6.23	6.16	6.07	6.15e
Mean	68.5a	68.8a	68.3a	68.5	71.7a	71.9a	71.4a	71.7	197a	199a	195a	197	103a	102a	100a	102	7.79a	8.08a	8.70a	8.19

Vertical means (hybrids) with the same letter are not significantly different at 0.05 level of significance
 Horizontal means (soaking) with the same letter are not significantly different at 0.05 level of significance
 1 = low concentration of sodium nitro peroxide
 2 = high concentration of sodium nitro peroxide
 3 = control treatment

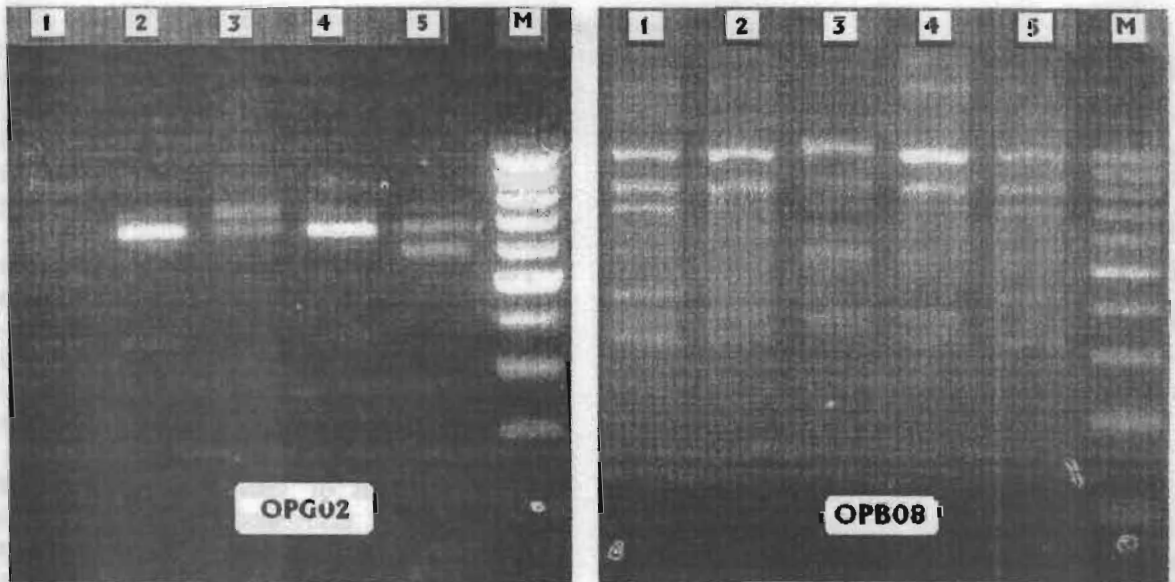


Fig. (1): RAPD profiles of the five maize genotypes using primers OPG02 and OpB08. 1: Sc129, 2:Twc310, 3: TWC311, 4:TWC314 and 5: TWC352, M: DNA molecular weight marker (100 bp ladder, Fermentas).

3.4. Analysis of genetic diversity using RAPD markers

3.4.1.Polymorphism detected by RAPD markers

Random Amplified Polymorphic DNAs (RAPDs) analyses are widely used for detecting genetic polymorphism between genotypes at the molecular level in many crops. During this study ten RAPD primers were used to estimate the genetic variability among five maize hybrids; SC 129, TWC 310, TWC 311, TWC 314 and TWC 352. These hybrids exhibited different levels of drought tolerance in the field experiments. The TWC 352 was the most susceptible hybrid in the two growing seasons. However, the other hybrids exhibited different degrees of drought tolerance and the best one of them was SC 129. Seven of the ten primers gave clear and reproducible banding patterns. Total number of amplified DNA fragments was, 66 bands and 56 of them were polymorphic. This represented a level of polymorphism of 84.8% as shown in Table (5). Different primers expressed different levels of polymorphism (Fig.1), ranging from 75% with primers OPB05 and OPB08 to 100% with primers OPG02 and OPG17 in the studied hybrids. The size of the amplified fragments varied with different primers, ranging from 260 to 1900 bp.

Similar results were reported by Lanza *et al.* (1997), Sun *et al.* (2001), Bauer *et al.* (2005), Drinic *et al.* (2005) and Hussein *et al.* (2006) who observed different levels of polymorphism and

number of amplified DNA fragments with the different tested primers.

Table (5): Number of amplified bands, polymorphic bands, and percentage of polymorphism as revealed by RAPD markers.

Primer	Hybrids		
	No. of bands	Polymorphic bands	Polymorphism %
OPB03	9	8	88.8
OPB05	8	6	75.0
OPB07	9	7	77.7
OPB08	12	9	75.0
OPG02	9	9	100
OPG17	4	4	100
OPZ01	15	13	86.6
Total	66	56	--
Average	9.4	8.0	84.8

3.4.2. Genetic relationships as revealed by RAPD data

Estimates of Dices genetic similarity in hybrids based on RAPD data are presented in Table (6). The highest genetic similarity value 79.5 was between TWC 310 and TWC 314, and the lowest value 50.7 was between SC 129 and TWC 314. These results are in good agreement with the pedigree information.

Table (6): Genetic similarity (GS) matrix among hybrids as revealed by RAPD data.

Hybrids	SC 129	TWC 310	TWC 311	TWC 314
TWC 310	56.7			
TWC 311	55.7	66.7		
TWC 314	50.7	79.5	58.3	
TWC 352	56.7	53.8	52.8	61.5

The dendrogram constructed from cluster analysis based on Dice similarity coefficients is presented in Fig. (2) The dendrogram clustered the five crosses in a good agreement with their pedigree as well as according to degrees of drought tolerance. The first cluster was divided into two subclusters, one included two hybrids, TWC 310 and TWC 314. While the second subcluster included only one hybrid TWC 311. All of these crosses can be considered moderate drought tolerant. The other hybrids distributed in two separate clusters, one included SC 129 considered drought tolerant and the second included the drought susceptible hybrid (TWC 352) as revealed from field experiments. This is in good accordance with Moeller and Schaal (1999), Sun *et al.* (2001), Drinic *et al.* (2005) and Hussein *et al.* (2006).

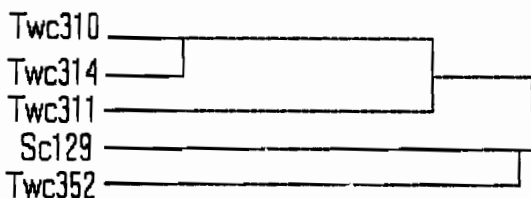


Fig. (2): Dendrogram for the five maize hybrids constructed from RAPD Data.

3.4.3. Unique markers as revealed by RAPD

Assay of RAPD was successful in identifying each of all of five hybrids except, TWC 314 as shown in (Table 7). The different primers revealed different numbers of unique positive and/or negative markers across the selected maize genotypes. Total number of unique markers in hybrids was 16 markers; 13 positive and 3 negative markers. The highest number of unique

markers (8) was exhibited by the hybrid SC 129. While, the lowest number of unique markers was revealed by the hybrid TWC 310 which was characterized by one positive marker. Similarly, Sun *et al.* (2001), El-Khishin *et al.* (2003) and Hussein *et al.* (2006) determined unique positive and/or negative RAPD markers characterizing different maize genotypes.

For this study it could be concluded that the hybrids SC 120 and SC 129 were the best hybrids for grain yield under moisture stress and non-stress conditions under the environmental condition so, it can be considered as drought tolerant. On the other hand, TWC 352 was the most susceptible hybrid to drought stress. Whereas, analysis of genetic relationship and cluster analysis distributed the hybrids into clusters according to degrees of drought tolerance as revealed in field experiments. Thus, investigate in depth the factors which are responsible for drought tolerance in the hybrids (SC120 and SC129) compared with the hybrid TWC 352 are needed to the deeply investigated.

In an effort to better understanding the drought stress tolerance of these different genotypes under sodium nitro peroxide treatments and different other environmental conditions, more experiments are proposed.

However, the new goal needs more advanced studies on the expression levels. To investigate the up and down regulated genes under drought conditions and under sodium nitro peroxide treatments as well. Besides, it is a useful goal to understand the regulation mechanisms of these drought tolerance responsible genes.

As a result, the following scheme is proposed to investigate the up and down regulated genes during drought conditions. Collecting total RNAs from different developmental stages and tissues of the genotypes of interest which are subjected to different drought stress levels and different sodium nitro peroxide treatments. Using their synthesized cDNAs in multiplexing and/or multiplexing quantitative real time PCR reactions targeting amplification of drought tolerance responsible genes, *e.g.*, the dehydration response elements (DREs), and the transcription factor DREB1A.

Moreover, it is assumed that sodium nitro peroxide regulates the dehydration responsive elements epigenetically. Thus, the target is to detect the gene expression level changes of dehydration response elements in order to detect structural changes in their promoters and regulating regions.

Table (7): Unique markers as revealed by RAPD.

Hybrids	Primer	UPM bp	UNM bp	Total	
				Primer	Genotypes
SC 129	OPB03	450, 600, 650		3	8
	OPB08	1400		1	
	OPG02	314, 500		2	
	OPZ01	480, 705		2	
TWC 310	OPZ01	289		1	1
TWC 311	OPB03	1025	1133	1	3
	OPB08	850		1	
	OPG17			1	
TWC 352	OPG02	600, 776	1400, 1600	2	4
	OPZ01			2	
Grand total		13	3		16

UPM= Unique positive markers.

UNM= Unique negative markers.

In conclusion, goals of determining the tolerant ten genotypes under different drought conditions and SNP treatments out of 40 genotypes at the beginning of preliminary seasons 2006 and 2007 were achieved. The present study is looking forward to better understanding the tolerance mechanisms and their regulation in maize hybrids.

Single crosses SC 120, SC 129, and SC 155 were the most drought tolerant varieties under water stress conditions. These findings were supported by molecular analysis results.

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التوصيف المحصولي والجزيني لبعض هجن الذرة الشامية تحت تأثير الإجهاد المائي ومعاملات النقع بمادة النيتروبروكسيد الصوديوم

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ملخص

يهدف هذا البحث إلى دراسة تأثير فترات الري ومستويات النقع بمادة نيتروبروكسيد الصوديوم على بعض هجن الذرة الشامية. أجريت التجارب الحقلية في محطة بحوث سدس بمحافظة بنى سويف خلال موسمي الزراعة ٢٠٠٨، ٢٠٠٩. استخدمت في هذه الدراسة الهجن الفردية البيضاء، هجين فردى ١٢٠، هجين فردى ١٢٩، الهجين الفردى الأصفر ١٥٥ والهجن الثلاثية البيضاء هجين ثلاثى ٣١٠، هجين ثلاثى ٣١١، هجين ثلاثى ٣١٤ والهجين الثلاثى الأصفر ٣٥٢. كانت معاملات الري هي الري كل ١٢ يوما، والري كل ٢٠ يوما وكذلك مستويات من النقع بمادة نيتروبروكسيد الصوديوم عبارة عن مستوى منخفض (٢,٥ مللى مول) وعالى (٥ مللى مول) وذلك لمدة ٢٤ ساعة بالإضافة إلى معاملة المقارنة وهي النقع بالماء لنفس المدة. تم تنشيط الحبوب المنقوعة بمادة نيتروبروكسيد الصوديوم خلال الموسم بالرش بغس المادة وذلك عند عمر ٣٠ يوما من الزراعة. اوضحت النتائج المتحصل عليها: أن الهجين الفردى ١٥٥ كان أكثر الهجن تكبيراً تحت ظروف الإجهاد المائي والهجن الفردية ١٢٩ و ١٥٥ كانت أكثر الهجن تكبيراً تحت ظروف الري العادى. وبالنسبة لصفة محصول الحبوب كانت الهجن الفردية هجين فردى ١٢٠ وهجين فردى ١٢٩ أعلى الهجن محصولاً تحت ظروف الري الطبيعى وظروف الإجهاد المائي، بينما كان الهجين الثلاثى ٣٥٢ أقل الهجن محصولاً تحت ظروف الري العادى وظروف الإجهاد المائي. كان تأثير نقع حبوب هجن الذرة الشامية البيضاء والصفراء فى مادة نيتروبروكسيد الصوديوم معنى فى صفتى ارتفاع النبات تحت ظروف الاجهاد المائي وصفة المحصول تحت ظروف الري العادى وذلك فى العام الاول فقط. بينما كانت باقى الصفات غير معنوية فى كل من الري العادى والاجهاد المائي، وتَـان التفاعل بين الهجن والنقع فى مادة نيتروبروكسيد الصوديوم معنويا لصفة المحصول وكذلك لصفة ارتفاع النبات وظهور ٥٠% من الحراير تحت ظروف الري العادى فى العامين الأول والثانى، على الترتيب بينما كان التفاعل غير معنوى للصفات الأخرى تحت الدراسة كما أظهرت نتائج التحليل الجزئى باستخدام تقنية الـ RAPD لخمس من هذه الهجن تختلف فى درجة تحملها للجفاف وهى: هجين فردى ١٢٩، هجين ثلاثى ٣١٠، هجين ثلاثى ٣١١، هجين ثلاثى ٣١٤، وهجين ثلاثى ٣٥٢ أن نسبة التباين كانت ٨٤.٨%. وأظهر التحليل العنقودى لهذه الهجن الخمسة انها توزعت فى مجاميع بما يتوافق مع الخلفية الوراثية الخاصة بها أيضاً بما يتوافق مع درجة تحملها للجفاف. بالإضافة إلى أن واسمات الـ RAPD الجزئية تمكنت من توصيف هذه الهجن بواسمات فريدة خاصة بكل هجين فيما عدا الهجين الثلاثى ٣١٤.

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