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# 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 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, efficienct 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 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 | | | |

### 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 $P_2O_5$ /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 $P \leq 0.05$ 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 $MgCl_2$, and 2.5 unit Taq polymerase (Fermentas) with 1X buffer $(NH_4)_2$ $SO_4$.

**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 |

**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: $Gs (ij) = 2a/ (2a + b + c)$ 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 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.**

| | 2009 | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soaking treatment | | | | | | | | | | | | | | | | | | | |
| | 1 | 2 | 3 | Mean | 1 | 2 | 3 | Mean | 1 | 2 | 3 | Mean | 1 | 2 | 3 | Mean | 1 | 2 | 3 | Mean |
| Hybrids | 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 | 68.8 | 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

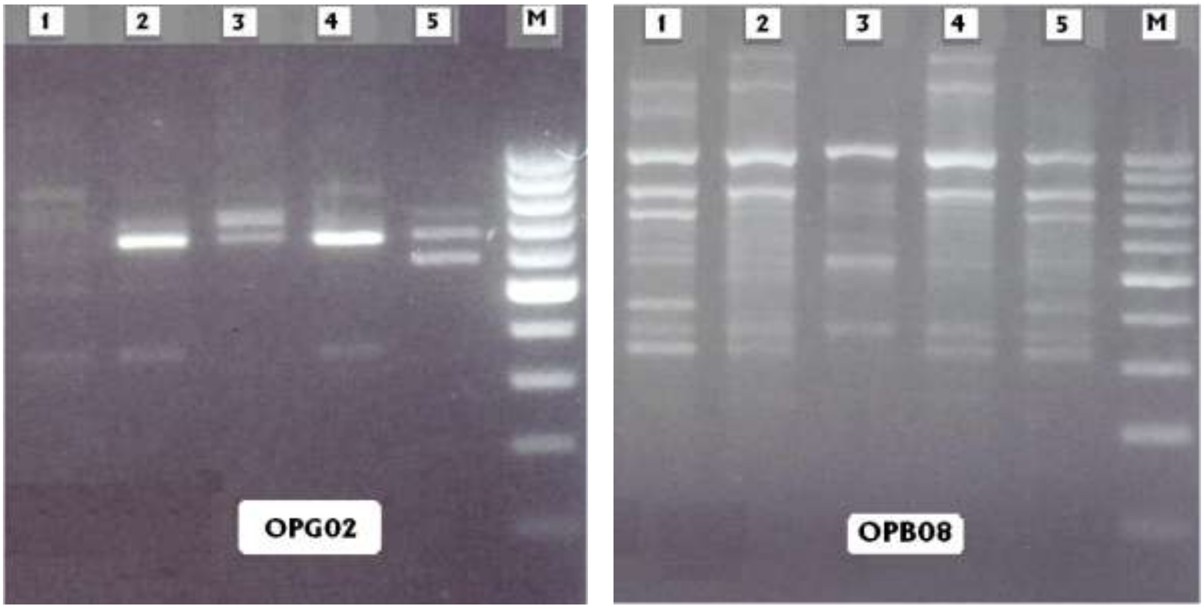


**Fig. (1): RAPD profiles of the five maize genotypes using primers OPG02 and OpB08. 1: Sc129, 2:Twc310, 3: TWC311, 4:TWC314 and 5: TWC352, M: DNA molecular weight marker (100 bp ladder, Fermentas).**

### 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).

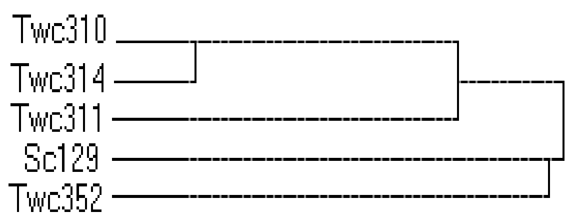


**Fig. (2): Dendrogram for the five maize hybrids constructed from RAPD Data.**

### 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 expermints. 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 invistigate 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 invistigate 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 monoplexing 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 promotors and regulating regions.

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

| Hybrids | Primer | UPM bp | UNM bp | Total | |
|---|---|---|---|---|---|
| | | | | Primer | Genotypes |
| SC 129 | OPB03<br>OPB08<br>OPG02<br>OPZ01 | 450, 600, 650<br>1400<br>314, 500<br>480, 705 | | 3<br>1<br>2<br>2 | 8 |
| TWC 310 | OPZ01 | 289 | | 1 | 1 |
| TWC 311 | OPB03<br>OPB08<br>OPG17 | 1025<br>850 | <br><br>1133 | 1<br>1<br>1 | 3 |
| TWC 352 | OPG02<br>OPZ01 | 600, 776 | <br>1400, 1600 | 2<br>2 | 4 |
| 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 acheived. 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 المحصولى والجزيئى لبعض هجن الذرة الشامية تحت تأثيرالإجهاد المائى 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 فى محطة بحوث سدس بمحافظة بنى سويف خلال موسمى الزراعة 2008، 2009. استخدمت فى هذة الدراسة الهجن الفردية البيضاء ، هجين فردى 120، هجين فردى 129، الهجين الفردى الأصفر 155 والهجن الثلاثية البيضاء هجين ثلاثى 310، هجين ثلاثى 311، هجين ثلاثى 314 والهجين الثلاثى الأصفر 352. كانت معاملات الرى هى الرى كل 12 يوما، والرى كل 20 يوما وكذلك مستويات من النقع بمادة نيتروبروكسيد الصوديوم عبارة عن مستوى منخفض(2,5ملى مول) وعالى (5 ملى مول) وذلك لمدة 24 ساعة بالإضافة إلى معاملة المقارنة وهى النقع بالماء لنفس المده. تم تنشيط الحبوب المنقوعة بمادة نيتروبروكسيد الصوديوم خلال الموسم بالرش بنفس المادة وذلك عند عمر 30 يوما من الزراعة. اوضحت النتائج المتحصل عليها:أن الهجين الفردى 155 كان أكثر الهجن تبكيرا تحت ظروف الإجهاد المائى والهجن الفردية 129 و155كانت أكثر الهجن تبكيرا تحت ظروف الرى العادى. وبالنسبة لصفة محصول الحبوب كانت الهجن الفردية هجين فردى 120 وهجين فردى 129 أعلي الهجن محصولا تحت ظروف الرى الطبيعى وظروف الإجهاد المائى، بينما كان الهجين الثلاثى 352 اقل الهجن محصولا تحت ظروف الرى العادى وظروف الإجهاد المائى. كان تأثير نقع حبوب هجن الذرة الشامية البيضاء والصفراء فى مادة نيتروبروكسيد الصوديوم معنوى فى صفتى ارتفاع النبات تحت ظروف الاجهاد المائى وصفة المحصول تحت ظروف الرى العادى وذلك فى العام الاول فقط.بينما كانت باقى الصفات غير معنوية فى كل من الرى العادى والاجهاد المائى، وكان التفاعل بين الهجن والنقع فى مادة نيتروبروكسيد الصوديوم معنويا لصفة المحصول وكذلك لصفة ارتفاع النبات وظهور 50% من الحراير تحت ظروف الرى العادى فى العامين الأول والثانى، على الترتيب بينما كان التفاعل غير معنوى للصفات الأخرى تحت الدراسة

كما أظهرت نتائج التحليل الجزيئى باستخدام تقنية الـ RAPD لخمسة من هذه الهجن تختلف فى درجة تحملها للجفاف وهى: هجين فردى 129، هجين ثلاثى 310، هجين ثلاثى 311، هجين ثلاثى 314، وهجين ثلاثى 352 أن نسبة التباين كانت 84.8%. وأظهر التحليل العنقودى لهذه الهجن الخمسة انها توزعت فى مجاميع بما يتوافق مع الخلفية الوراثية الخاصة بهاوأيضا بما يتوافق مع درجة تحملها للجفاف. بالإضافة إلى أن واسمات الـ RAPD الجزيئية تمكنت من توصيف هذه الهجن بواسمات فريدة خاصة بكل هجين فيما عدا الهجين الثلاثى 314.

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