# Identification of RAPD and ISSR Markers for Drought Stress in Some Egyptian Durum Varieties

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## Abstract

To develop crop plants with enhanced tolerance of drought stress, a basic understand of physiology and genetics is essential. Six durum wheat genotypes namely Bani-Swaif 1, 3, 4, 5, 6 and Sohag3 were first screened to get the most tolerant and sensitive genotypes. Then the tolerant, sensitive plants and their F1 and F2 were used for molecular indicators of drought tolerance using RAPD and ISSR techniques. Six RAPD and five ISSR primers were used to identify markers assisted selection (MAS) of drought tolerance. Under this study, RAPD technique exhibited 4 positive and 5 negative markers while ISSR revealed 6 positive and 6 negative markers.

## Keywords

Durum wheat, drought tolerance, RAPD and ISSR Markers.

## Introduction

Water stress is the main abiotic factor limiting plant production all over the world, the crop growth and economic yield being severely affected by water availability Araus et al., (2002). Severity and duration of drought determine physiological stress responses in plants Chaves et al., (2003).Wheat is one of the most abundant nutrition source of energy and protein for the world population, 95 % of wheat grown now a daysis a self-pollinating hexaploid (Triticumaestivum L.) and the remaining 5% is durum (tetraploid wheat) Patnaik and Khurana(2001). Wheat production is adversely affected by drought in 50 % of the area under cultivation in the developing countries Trethowan and Pfeiffer (2000).

The negative impact of drought depends on the developmental stage of plants, tissue and organ specificity, soil type and experimental conditions of stress application Gupta et al. (2001). The degree of plant drought tolerance is not only differing among the species, but also among different varieties of the same species Erdei et al., (2002) andRapacz et al. (2010).

Among the morph physiological traits that have been shown to influence the adaptive response of durum wheat to drought Reynolds et al., (2007), a number of these have been suggested as potentially useful for breeding purposes Ferrioet al., (2007).

Until now, durum wheat molecular genetics and genomics have largely relied on hexaploid wheat tools Gupta et al., (2008) and Varshneet al., (2007). Molecular markers (e.g. RFLP, SSR, AFLP, etc.) developed for hexaploid wheat are generally useful for genetic mapping experiments in tetraploid wheat based on the sharing of the two A and B genomes Korzunet al., (1999) and Lottiet al. (2000). However, markers

and polymorphism information content in durum wheat germplasm are differing from that observed in hexaploid wheat Maccaferri et al., (2003) and (2005).

DNA molecular markers have become excellent tools for plant breeders Lima-Britoet al., (2006). RAPD technique gives fast results but also has limitations, such as low reproducibility Fernandez et al., (2002). In contrast, ISSR markers are more reproducible and their distributions in the eukaryotic genome are highly informative with no prior information of the sequence Bornetet al., (2002). In cereals, ISSR markers have been used to study genetic diversity and phylogenetic relationships Matos et al., (2001), gene mapping Kojiamet al., (1998) and DNA fingerprinting Carvalhoet al., (2005).

The aims of this study were: determine RAPD and ISSR markers associated with drought tolerance in durum wheat and can be used in the wheat breeding programs.

## Materials and methods

### Plant material:

Six genotypes of durum wheat namely; Bani-Swaif1, 3, 4, 5, 6 and Sohag3 which were kindly provided by the Agriculture Research Centre, Giza, Egypt. Field experiments were carried out in the years 2011/2012, 2013/2014 and 2014/2015 in research plots and in a greenhouse of the Faculty of Agricultural, Zagazig University, Egypt.

### Field Experiment:

Seeds were grown at the beginning of November in 1-m rows spaced 0.2 m apart with 70 seeds per row. Three replications per treatment were used for each cultivar and arranged in a randomized complete block design under two irrigation treatments (irrigation every 21 days) and drought treatments (irrigation after 70 days from seedlings emergence).

### Greenhouse experiment:

15 plastic pots were used for each replicate. Pots were filled with mixture of sand/peat moss/vemoclit a ratio of 1:1:1 to each pot at the time of sowing and the quantity required for each pot was determined based on the weight. The parents Sohag3 (sensitive) and Bani-swaif4 (tolerant), their F1 and F2 grains were grown in a temperature -controlled greenhouse under 24/16 C˚, day/night cycle and mean RH was (80 %) and complete light hours to 12 h by artificial lamp.

The pots were arranged in a factorial randomize complete block design. Seven grains were sowed in each pot. The experiment was irrigated by Tap water. Supplement Hoagland stock solution (Hoagland and Arnon, 1950) was used as the base nutrient medium.

### Intervals of irrigation:

In three periods after 21, 28 and 42 days.

### Data recording:

Following data were recorded from all plants for the traits (which related to drought tolerance),plant height (cm),flag leaf area (cm2), number of leaves, number of tillers, spike length (cm) number of spikelet's, biological yield (g),grain yield (g) and number of kernels.

Data were recorded for six varieties after harvest for the grain yield trait (kg) and analyzed using analysis of variance (ANOVA) procedure according to Snedecor and Cochran, (1980). Susceptibility Index (SI) was calculated for six varieties according to Fischer and Maurer, (1978) to select the tolerant and sensitive parents for this study

SI= 1-(YD/YI)/D, D=1-(Mean of YD /Mean of YI)

Where: YD= grain yield (kg) with drought stress (one irrigation).

YI =grain yield (kg) irrigated (normal irrigation).

Mean of YD =mean of grain yield (kg) for six varieties under drought treatment.

Mean of YI = mean of grain yield (kg) for six varieties under irrigation treatment.

The difference among means was compared using Duncan's new multiple ranges tests (Duncan's 1955).

**DNA extraction:**

DNA of the tolerant and sensitive parents and their F1,the most five plants tolerant F2 and the most sensitive F2plantswere extracted from the leaves using EZ-10 Spin column Genome DNA Minipreps kit method.

**RAPD and ISSR reaction conditions:**

RAPD analysis was a performed using 6 RAPD primers (Metabion, Martinsried, Germany) and 5 ISSR primers were a produced from Integrated DNA Technologies Inc. (San Diego, CA, USA) based on core repeats anchored at the 5′ or 3′ end as shown in Table (1). Regarding to RAPD reaction, the mixture was standardized to 20µl (PCR buffer 1X, MgCl2 2.5mM, dNTPs1mM, Primer 50ng, Taq Polymerase1 unit, genomic DNA 25ng). PCR program was set as 45 cycles of 36 °C: 1 min annealing, 2 min extension at 72 °C and 7 min final extension at 72 °C). The products of RAPD–PCR were analyzed on 1.5 % (w/v) agarose gel.

Regarding to ISSR reaction, the mixture was standardized to 20µl (PCR buffer1X, MgCl22.5mM, dNTPs1mM, 10pMol of each primer, Taq Polymerase1unit, and genomic DNA50ng). PCR program was set as 40 cycles of 56 °C: 1 min annealing, 2 min extension and 10 min final extension at 72 °C). The products of ISSR–PCR were analyzed on 1.4 % (w/v) agarose gel. Gels were photographed under gel documentation system (Syngene™) and size of amplicons was detected using 1Kb DNA ladder (Ferments Life Sciences).

**Table 1**: Code names and sequences of RAPD and ISSR primers used for PCR analyses.

| Primers | Code name | Sequences |
|---|---|---|
| RAPD | OPE-26 | 5' AACGGTGACC 3' |
| | A-12 | 5' TCGGCGATAG 3' |
| | E-10 | 5' CACCAGGTGA 3 |
| | OPT-08 | 5' AACGGCGACA 3' |
| | OPC-19 | 5 'GTTGCCAGCC 3' |
| | OPX-17 | 5' GACACGGACC 3' |
| ISSR | M-1 | 5` (AC) 8 CG 3` |
| | UBC-811 | 5` (GA) 8 C 3` |
| | UBC-817 | 5` (CA) 8 A 3` |
| | UBC 814-32 | 5` (CT) 7CCTA 3` |

| Primers | Code name | Sequences |
|---|---|---|
| | UBC 876-32 | 5` (GATA) 2 (GACA) 2 3` |

## Results and Discussion

### Field experiment

Regarding to the grain yield (Kg), the statistical analysis showed that Bani-swaif4 was the most drought tolerant variety, while Sohag3 was the most drought sensitive one according to Drought Susceptibility Index (Fischer and Maurer, 1978) (Table 2).

DSI values for the yield (Table 2) ranged from 0.484 to 1.259. So, we selected the two varieties and grown them in the field and crossed to obtain their F1 grains. Some of F1 grains were sown in the field and selfed to obtain the F2.

### Greenhouse experiment

The two parents and their F1 and F2 plants were grown under the greenhouse conditions. The plants were subjected to drought stress (150 mm water) compared to their control (700mm water). Data were recorded for all plants for the nine traits as shown in Table (3).

For plant height trait, there was a significant difference between control and drought treatment. The tolerant parent Bani-Swaif4 exhibited a higher mean value (70.19cm) compared with the sensitive parent Sohag3 (66.59cm). While, the plant heightof F1plants were (66.44cm). There was a decrease in the value for the sensitive parent under treatment (57.7cm) compared with control (75.48cm). This criterion can be used as indicator for the effects of drought stress and plant growth. The obtained results were in agreement with Subhani and Chowdhry (2000) who observed that water limitation significantly decreased plant height in the early stage of water deficit Nabipouret al., (2002); Saleem, (2003); Moayediet al., (2010).

Regarding to the flag leaf area, there was a significant difference between control and drought treatment. The tolerant parent and F1 plants exhibited high values under drought stress (31.63 and 30.62cm2, respectively) compared with the sensitive parent which exhibited a low value (24.29 cm2) compared with control (31.55cm2). Similar results were obtained by Khaliqet al. (2008), who noticed that leaf area cause/more water losses due to more evapotranspiration from the surface.

Concerning number of leaves trait of the tolerant parent exhibited a high mean value (10.12) and had significant differences compared with the mean values of the sensitive parent and F1 plants (7.73 and 7.98, respectively).

Regarding to the number of tillers, the mean values had significant differences among the tolerant parent, the sensitive parent and F1 plants. This agreed with those of Sharif (1999) and Musaddiqueet al. (2000) who found that the maximum number of tillers were associated with greater number of irrigations.

Regarding to spike length (cm)/plant, under drought treatment there were significant differences between F1 (exhibited a high value 6.13cm) when compared with tolerant parent and F1 plants (5.86 and 5.56 cm, respectively). Our results were in agreements with those of Iqbalet al., (1999) who indicated that the highest reduction of spike length trait in durum wheat under water deficit conditions was in the flowering stage.

Bayoumi et al., (2002) observed that the reduction percentage due to water stress was 18.8% of spike length.

For Number of spikelets/spike, there were significant differences under drought between the tolerant parents which exhibited a value of (14.17) compared with the sensitive parent and F1 plants (13.80 and 13.9, respectively). For the mean value, there were no significances among the tolerant parent (15.08), the sensitive parent (15.8) and F1 plants (15.23). This result agreed with Denciset al., (2000) who concluded that spikelets/spike is more sensitive to drought stress in different cultivars of wheat.

Regarding to the biological yield trait, the tolerant parent gave a higher value (2.22gm) under drought than the sensitive parent and F1 plants (1.22 and 1.64gm, respectively). The same results were obtained by Rashedet al., (2010) and (2011) who noticed that drought treatment resulted to a significant reduction in biological yield in the sensitive parent than the tolerant parent.

For grain yield trait, there were no significant differences between the tolerant parent and F1 plants (0.72 and 0.72gm, respectively) when compared with the sensitive parent (0.38gm) under drought treatment. The mean values, showed significance between the tolerant parent (0.93gm) compared with the sensitive parent and F1 plants (0.67 and 0.91gm, respectively). The obtained results were in agreement with those of Hassan et al., (1987) who found that the final grain yield of spring wheat was reduced when the plants were subjected to water stress at any stage of development.

For the number of kernels/spike trait, there were significant differences between control and drought treatment. The F1 exhibited higher value (19.33) under drought comparing with the tolerant and sensitive parents (18.61 and 16.77, respectively). These results were in agreement with those of Qadir and Cheema (1999), Nabipouret al., (2002) and Saleem (2003) who found that water stress was significantly decreased the number of kernels/spike.

**Response of F2 plants:**

F2 plants represented by 151 individuals were classified into groups according to their performance under drought stress for each trait, and then each trait was classified according to its range as presented in (Table 4), which shows the minimum, the maximum, and the means ± standard error values of the nine studied traits.

According to these classifications, five F2 plants from each of the most tolerant and the most sensitive ones were selected to represent drought stress for each trait as shown in (Tables 5 and 6).
The comparisons between the means for the two groups of F2 plants on the basis of each trait and plant vigor visual ranks, as shown in Tables (5 and 6), indicated the marked differences between the two contrasting F2 genotypes.

**RAPD markers associated with drought tolerance:**

Genomic DNA was isolated from the two durum wheat genotypes tolerant parent Sohag3, sensitive parent Bani-Swaif4, their subsequent F1 plants,the most five tolerant F2 bulked plants and the most five sensitive F2 bulked plants.

A total of 44 amplified DNA fragments ranging in size from 159 to 870bp were observed using six random amplified polymorphic DNA (RAPD)primers (Fig 1 and

Table 7),a maximum of 9 fragments were revealed with primer OPX-17,however primer OPE-26 revealed a minimum of 5 fragments .

Primer OPC-19 exhibited one positive and one negative markers with molecular size 316 and 229bp respectively,primer OPX-17 exhibited one positive marker with molecular size 868bp and one negative marker with molecular size 668bp ,and also primer A-12 exhibited two markers one negative and one positive with molecular size 547 and 280bp, respectively.

On the other hand, two primers OPE-26 and OPT-08 exhibited two distinct markers negative and positive with molecular size 313 and 553bp respectively.

Primer E-10, two determined negative markers with molecular size 650 and 510bp.

The RAPD-PCR technique determined 10 molecular markers; four of them were positive and six negative associated drought stress.

These results indicated that these four positive and five negative RAPD markers could be considered as reliable markers for drought tolerance in durum wheat. These results agreed with many reports which detected RAPD markers for abiotic stress tolerance in wheat Abdel Tawabet al., (2003); Rashedet al., (2010) and El-Ameen, (2013).

**Table 2:** Reduction percentage and drought susceptibility index for means comparisons of grain yield of six durum wheat varieties under drought treatment.

| Varieties | Y(I)=Grain Yield(Irr) | Y(D)=Grain Yield(Str) | Y(D)/ Y(I) | 1- Y(D)/ Y(I) | D=(Drought Intensity) D=1-(Mean of Y(D)/Mean of Y(I)) | Susceptibility Index (SI) (SI)=(1-Y(D)/Y(I)/D |
|---|---|---|---|---|---|---|
| **Bani-swaif1** | 17.87 | 11.55 | 0.64 | 0.36 | 0.413 | 0.872 |
| **Bani-swaif3** | 43.33 | 26 | 0.60 | 0.4 | 0.413 | 0.969 |
| **Bani-swaif4** | 47.67 | 22.89 | 0.48 | 0.52 | 0.413 | 1.259 |
| **Bani-swaif5** | 34.26 | 17.07 | 0.50 | 0.50 | 0.413 | 1.211 |
| **Bani-swaif6** | 30.87 | 17.03 | 0.55 | 0.45 | 0.413 | 1.090 |
| **Sohag3** | 37.57 | 29.67 | 0.80 | 0.2 | 0.413 | 0.484 |
| **Mean** | 35.26 | 20.70 | - | - | - | - |

**Table 3:** Means of some drought-related traits of the two parents and their $F_1$ plants.

| Genotype | | Plant height | Flag leaf area | No of leaves | No of tillers | Spike length | No of spikelet | Biological Yield | Grain Yield | No of kernels |
|---|---|---|---|---|---|---|---|---|---|---|
| Bani-Swaif4 | Cont. | 78.48 | 33.98 | 11.95 | 2.71 | 6.79 | 15.99 | 3.55 | 1.14 | 36.18 |
| | Treat. | 61.90 | 31.63 | 8.29 | 2.19 | 5.86 | 14.17 | 2.22 | 0.72 | 18.61 |
| | Mean. | 70.19 | 32.80 | 10.12 | 2.45 | 6.32 | 15.08 | 2.88 | 0.93 | 27.39 |
| Sohag3 | Cont. | 75.48 | 31.55 | 8.72 | 1.52 | 6.82 | 17.8 | 3.18 | 0.96 | 33.5 |
| | Treat. | 57.7 | 24.29 | 6.75 | 1.09 | 5.56 | 13.80 | 1.22 | 0.38 | 16.77 |
| | Mean. | 66.59 | 27.92 | 7.73 | 1.30 | 6.19 | 15.8 | 2.2 | 0.67 | 25.13 |
| $F_1$ | Cont. | 74.97 | 40.54 | 9.25 | 1.76 | 7.26 | 16.56 | 3.48 | 1.11 | 34.02 |
| | Treat. | 57.91 | 30.62 | 6.72 | 1.37 | 6.13 | 13.9 | 1.64 | 0.72 | 19.33 |
| | Mean. | 66.44 | 35.58 | 7.98 | 1.56 | 6.69 | 15.23 | 2.56 | 0.91 | 26.67 |

**Table 4:** The minimum, the maximum values, and the means ± standard error of $F_2$ plants of the cross Sohag3 and Bani-Swaif4 for the nine studied traits.

| Character | Minimum value | Maximum value | Means ± standard error | Var. | Range |
|---|---|---|---|---|---|
| Plant height (cm) | 23.20 | 75 | 51.64 ± 0.889 | 120.88 | 51.80 |
| Flag Leaf Area ($cm^2$) | 6.00 | 68.45 | 37.05 ± 0.935 | 132.95 | 62.45 |
| No. of Leaves/plant | 2.00 | 15.00 | 5.66 ± 0.150 | 3.42 | 13.00 |
| No .of tillers | 1.00 | 4.00 | 1.43 ± 0.052 | 0.42 | 3.00 |
| Spike length (cm) | 4.90 | 16.00 | 7.85 ± 0.120 | 2.18 | 11.10 |
| No. of spikelet /spike | 9.00 | 29.00 | 16.67 ± 0.228 | 7.95 | 20.00 |
| Biological yield(gm.) | 1.25 | 3.35 | 2.20 ± 0.454 | 0.32 | 2.10 |
| Grain Yield(gm.) | 0.13 | 0.90 | 0.46 ± 0.018 | 0.05 | 0.78 |
| No. of kernels | 0.00 | 55.00 | 20.61 ± 0.685 | 71.82 | 55.00 |

**Table 5:** The most drought tolerant $F_2$ groups according to their performances in some yield-related traits.

| Code No. | Plant No. | Plant height | Flag leaf area | No of leaves | No of branches | Spike Length | No of spikelet | Biological yield | Grain yield | No of kernels | Visual Rank of Plant Vigor |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 75 | 68.45 | 15 | 4 | 16 | 29 | 3.35 | 0.90 | 55 | 1 |
| 2 | 18 | 75 | 61.50 | 11 | 3 | 11.5 | 25 | 3.35 | 0.83 | 39 | 1 |
| 3 | 151 | 72 | 49.4 | 10 | 3 | 9.5 | 19 | 3.35 | 0.83 | 36 | 1 |
| 4 | 139 | 70 | 43.68 | 9 | 3 | 7 | 14 | 3.35 | 0.80 | 22 | 1 |
| 5 | 150 | 69.06 | 42.04 | 7 | 2 | 6.5 | 14 | 2.91 | 0.69 | 22 | 1 |
| Mean | - | 72.21 | 53.01 | 10.4 | 3 | 10.1 | 20.2 | 3.26 | 0.81 | 34.8 | - |

**Table 6:** The most drought sensitive $F_2$ groups according to their performances in some yield–related traits.

| Code No. | Plant No. | Plant height | Flag leaf area | No of leaves | No of branches | Spike Length | No of spikelet | Biological yield | Grain yield | No of kernels | Visual Rank of Plant Vigor |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 118 | 23.2 | 6.00 | 2 | 1 | 4.90 | 9 | 1.25 | 0.13 | 0 | 10 |
| 2 | 73 | 27 | 13.65 | 3 | 1 | 5 | 11 | 1.33 | 0.14 | 2 | 10 |
| 3 | 89 | 27 | 14.43 | 3 | 1 | 5 | 12 | 1.33 | 0.12 | 2 | 10 |
| 4 | 69 | 30 | 15.56 | 3 | 1 | 5 | 14 | 2 | 0.2 | 4 | 10 |
| 5 | 53 | 30 | 16.00 | 5 | 1 | 6.5 | 15 | 2.14 | 0.43 | 4 | 10 |
| Mean | - | 27.44 | 13.13 | 3.2 | 1 | 5.28 | 12.2 | 1.61 | 0.20 | 2.4 | - |

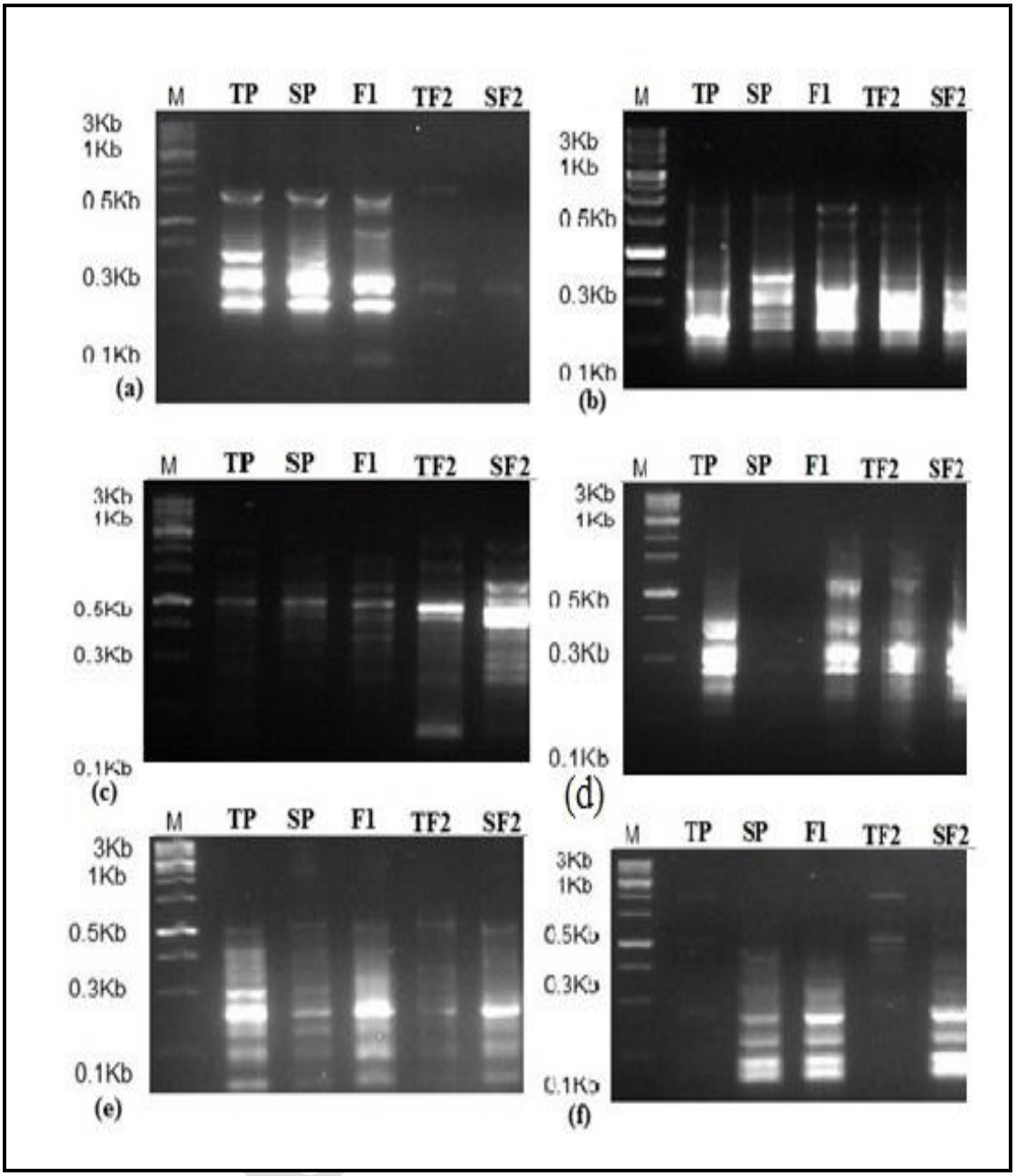


**Figure 1:** DNA fragments generated by RAPD-PCR with six arbitrary primers, (a) OPE-26, (b) A-12, (c) E-10, (d) OPT-08, (e)OPC-19 and (f) OPX-17 for the tolerant parent (TP), the sensitive parent(SP), F1 plants, the most five tolerant F2 bulk (TF2) and the most five sensitive F2 bulk (SF2).

**Table 7:** RAPD-PCR fragments of six tested primers with the two parents, their subsequent $F_1$ plants, the tolerant and the sensitive $F_2$ plants.

| Primer code | FN | MS | TP | SP | F1 | TF2 | SF2 | Marker type |
|---|---|---|---|---|---|---|---|---|
| OPE-26 | 1 | 788 | 0 | 0 | 1 | 0 | 0 | |
| | 2 | 438 | 1 | 1 | 1 | 1 | 1 | |
| | 3 | 313 | 0 | 1 | 1 | 0 | 1 | Negative |
| | 4 | 236 | 1 | 1 | 1 | 1 | 1 | |
| | 5 | 159 | 1 | 1 | 1 | 1 | 1 | |

| Primer code | FN | MS | TP | SP | F1 | TF2 | SF2 | Marker type |
|---|---|---|---|---|---|---|---|---|
| A-12 | 1 | 657 | 0 | 0 | 1 | 0 | 0 | |
| | 2 | 547 | 0 | 1 | 1 | 0 | 1 | Negative |
| | 3 | 432 | 1 | 1 | 1 | 1 | 1 | |
| | 4 | 374 | 1 | 1 | 1 | 1 | 1 | |
| | 5 | 280 | 1 | 0 | 1 | 1 | 0 | Positive |
| | 6 | 248 | 1 | 0 | 0 | 0 | 0 | |
| | 7 | 206 | 1 | 1 | 1 | 1 | 1 | |
| E-10 | 1 | 870 | 0 | 0 | 1 | 0 | 0 | |
| | 2 | 650 | 0 | 1 | 1 | 0 | 1 | Negative |
| | 3 | 590 | 1 | 1 | 1 | 1 | 1 | |
| | 4 | 540 | 1 | 1 | 1 | 1 | 1 | |
| | 5 | 510 | 0 | 1 | 1 | 0 | 1 | Negative |
| | 6 | 312 | 1 | 0 | 0 | 0 | 0 | |
| | 7 | 275 | 1 | 1 | 1 | 1 | 1 | |
| OPT-08 | 1 | 763 | 1 | 0 | 0 | 0 | 0 | |
| | 2 | 629 | 1 | 0 | 0 | 0 | 0 | |
| | 3 | 553 | 1 | 0 | 1 | 1 | 0 | Positive |
| | 4 | 453 | 0 | 0 | 0 | 0 | 1 | |
| | 5 | 394 | 1 | 1 | 1 | 1 | 1 | |
| | 6 | 275 | 1 | 1 | 1 | 1 | 1 | |
| | 7 | 198 | 1 | 1 | 1 | 1 | 1 | |
| | 8 | 175 | 0 | 1 | 0 | 0 | 0 | |
| OPC-19 | 1 | 636 | 0 | 1 | 0 | 0 | 0 | |
| | 2 | 524 | 0 | 1 | 0 | 0 | 0 | |
| | 3 | 449 | 0 | 0 | 1 | 0 | 0 | |
| | 4 | 396 | 1 | 1 | 1 | 1 | 1 | |
| | 5 | 316 | 1 | 0 | 1 | 1 | 0 | Positive |
| | 6 | 280 | 1 | 1 | 1 | 1 | 1 | |
| | 7 | 229 | 0 | 1 | 1 | 0 | 1 | Negative |
| | 8 | 182 | 1 | 1 | 1 | 1 | 1 | |
| OPX-17 | 1 | 812 | 1 | 0 | 0 | 0 | 0 | |
| | 2 | 668 | 1 | 0 | 1 | 1 | 0 | Positive |
| | 3 | 593 | 0 | 0 | 0 | 0 | 1 | |
| | 4 | 521 | 1 | 1 | 1 | 1 | 1 | |
| | 5 | 468 | 0 | 1 | 1 | 0 | 1 | Negative |
| | 6 | 426 | 1 | 1 | 1 | 1 | 1 | |
| | 7 | 379 | 1 | 1 | 0 | 0 | 0 | |
| | 8 | 341 | 1 | 1 | 1 | 1 | 1 | |
| | 9 | 274 | 0 | 1 | 0 | 0 | 0 | |

**ISSR assay and markers of regenerated drought tolerance:**

Five inter simple sequence repeats (ISSR) were used to detect DNA markers of drought stress. The primers M-1 exhibited one positive marker with molecular size of 510bp and one negative with molecular size of 175bp, primer UPC-867-32 exhibited one positive marker of 272bp and one negative marker with molecular size of 466bp.

Primer UBC-811 exhibited one negative marker of 589bp and two positive of 887, 258bp, also primer UBC-817 exhibited one negative 478bp marker and two positive markers of 358, 278bp.

Primer UPC814-32 exhibited two negative markers with molecular size 261, 157bp and one positive marker with molecular size 119bp. (Fig. 2 and Table 8).

In this context, Moghaieb et al. (2010) determined the genotype specific SSR markers in nine bread and pasta wheat genotypes. They reported that 13 markers can be considered as a useful marker for screening for salt tolerance in these wheat genotypes. Abd El-Hadi (2012) showed four genotype–specific markers for the drought tolerance in durum wheat. ISSR analysis showed a significant increase in grain yield/plant over their parents under drought stress conditions. Using ISSR analysis, we were able to identify seven unique bands in some drought tolerant wheat genotypes. These bands might be considered a useful marker linked with drought tolerance in bread wheat breeding programs.
Further experiments need to be done to determine the linkage between the genotype–specific ISSR markers used in the present study and gene(s) for drought tolerance in the studied durum wheat genotypes. The present results support the idea that ISSR analysis can provide a fast detection of species-specific markers linked to drought stress tolerance in wheat.

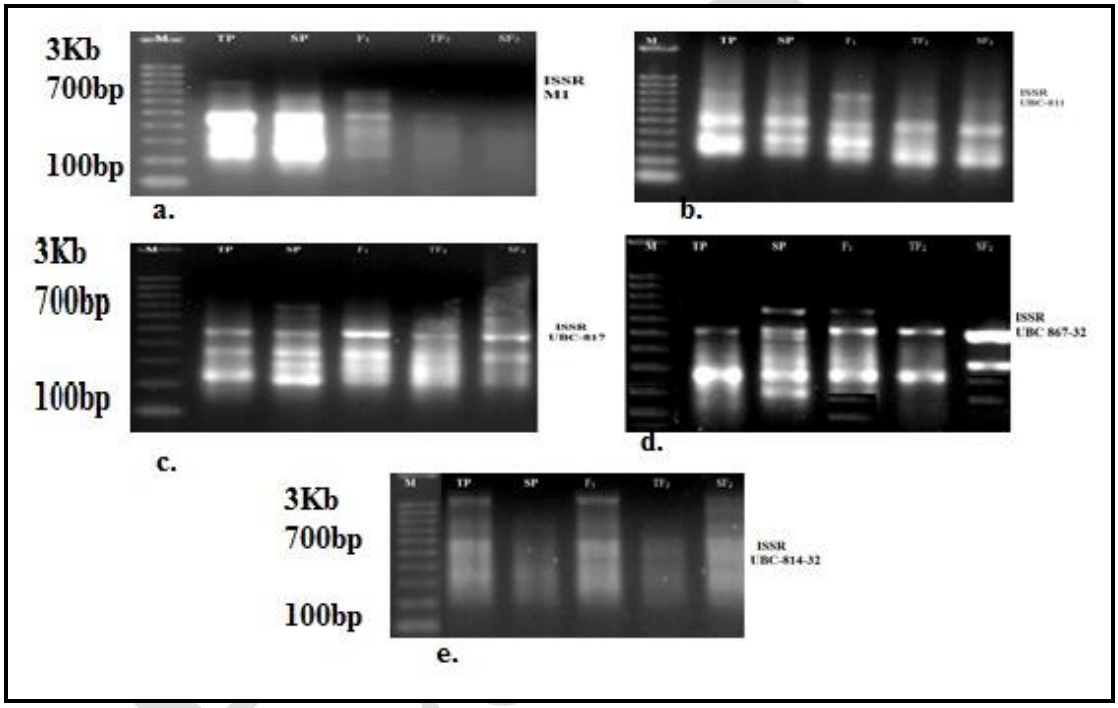


**Figure 2:** DNA fragments generated(FN) fragment no. by ISSR with primers (a) M1, (b)UPC 811, (c) UPC 817, (d) UPC 814-32 and (e) UPC 876-32 for the tolerant parent (TP), the sensitive parent (SP), the F1 plants, the most five tolerant F2 Bulk(TF2) and the most five sensitive F2 Bulk (SF2) plants.

**Table 8:** ISSR fragments of the five tested primers on the two parents, their subsequent $F_1$ plants, the tolerant $F_2$ bulk and the sensitive $F_2$ bulk plants.

| Primer code | FN | MS | TP | SP | F1 | TF2 | SF2 | Marker type |
|---|---|---|---|---|---|---|---|---|
| M -1 | 1 | 1240 | 1 | 0 | 1 | 0 | 1 | |
| | 2 | 771 | 1 | 0 | 1 | 0 | 1 | |
| | 3 | 510 | 1 | 0 | 1 | 1 | 0 | Positive |
| | 4 | 406 | 1 | 1 | 1 | 1 | 1 | |
| | 5 | 329 | 0 | 0 | 1 | 0 | 0 | |
| | 6 | 237 | 1 | 1 | 1 | 1 | 1 | |
| | 7 | 175 | 0 | 1 | 1 | 0 | 1 | Negative |
| UPC-811 | 1 | 887 | 1 | 0 | 1 | 1 | 0 | Positive |
| | 2 | 700 | 1 | 0 | 1 | 1 | 1 | |
| | 3 | 623 | 1 | 0 | 0 | 0 | 0 | |
| | 4 | 589 | 0 | 1 | 0 | 1 | 1 | Negative |
| | 5 | 437 | 1 | 1 | 1 | 1 | 1 | |
| | 6 | 387 | 1 | 1 | 1 | 1 | 1 | |
| | 7 | 258 | 1 | 0 | 1 | 1 | 0 | Positive |

| Primer code | FN | MS | TP | SP | F1 | TF2 | SF2 | Marker type |
|---|---|---|---|---|---|---|---|---|
| | 8 | 212 | 1 | 1 | 1 | 1 | 1 | |
| UPC-817 | 1 | 1101 | 1 | 0 | 1 | 0 | 0 | |
| | 2 | 832 | 0 | 0 | 0 | 0 | 1 | |
| | 3 | 715 | 1 | 1 | 1 | 1 | 1 | |
| | 4 | 478 | 0 | 1 | 1 | 0 | 1 | Negative |
| | 5 | 443 | 1 | 1 | 1 | 1 | 1 | |
| | 6 | 406 | 0 | 1 | 0 | 1 | 1 | |
| | 7 | 358 | 1 | 0 | 1 | 1 | 0 | Positive |
| | 8 | 278 | 1 | 0 | 1 | 1 | 0 | Positive |
| | 9 | 220 | 1 | 1 | 1 | 1 | 1 | |
| | 10 | 182 | 1 | 0 | 1 | 1 | 1 | |
| | 11 | 144 | 1 | 0 | 1 | 1 | 1 | |
| UPC 814-32 | 1 | 611 | 0 | 1 | 1 | 0 | 0 | |
| | 2 | 426 | 1 | 1 | 1 | 1 | 1 | |
| | 3 | 325 | 0 | 1 | 1 | 0 | 0 | |
| | 4 | 261 | 0 | 1 | 1 | 0 | 1 | Negative |
| | 5 | 207 | 1 | 1 | 1 | 1 | 1 | |
| | 6 | 175 | 0 | 1 | 1 | 0 | 0 | |
| | 7 | 157 | 0 | 1 | 1 | 0 | 1 | Negative |
| | 8 | 136 | 1 | 0 | 0 | 1 | 0 | |
| | 9 | 119 | 1 | 0 | 1 | 1 | 0 | Positive |
| UPC 867-32 | 1 | 693 | 1 | 1 | 1 | 1 | 1 | |
| | 2 | 466 | 0 | 1 | 1 | 0 | 1 | Negative |
| | 3 | 419 | 1 | 1 | 1 | 1 | 1 | |
| | 4 | 360 | 1 | 0 | 1 | 0 | 0 | |
| | 5 | 306 | 0 | 1 | 0 | 0 | 0 | |
| | 6 | 272 | 1 | 0 | 1 | 1 | 0 | Positive |
| | 7 | 214 | 1 | 1 | 1 | 1 | 1 | |
| | 8 | 144 | 1 | 0 | 1 | 0 | 1 | |

**Genetic polymorphism among the five wheat genotypes**

The RAPD primers produced multiple amplified bands (44 alleles), out of which 17 bands (38.64%) were monomorphic and 27 bands (61.36%) were polymorphic (Table 9).

The ISSR primers revealed discernible amplification profiles. Therefore, they were employed to investigate the genetic polymorphism among the genotypes. Five ISSR primers gave 43 different bands thirteen of them were monomorphic bands (30.23%) and 30 were polymorphic with polymorphism 69.77%.

**Table 9:** Total number, monomorphic, polymorphic, unique bands and percentage of polymorphism as revealed by RAPD and ISSR primers.

| Primer type | Primer | Total no. of loci | Monomorphic loci | Polymorphic loci | Unique loci | % polymorphism |
|---|---|---|---|---|---|---|
| RAPD | 1 | 5 | 3 | 2 | 1 | 40 |
| | 2 | 7 | 3 | 4 | 2 | 57.143 |
| | 3 | 7 | 2 | 5 | 3 | 57.143 |
| | 4 | 8 | 3 | 5 | 4 | 62.500 |
| | 5 | 8 | 3 | 5 | 1 | 62.500 |
| | 6 | 9 | 3 | 6 | 3 | 66.667 |
| *T.B. & % | | 44 | 17(38.64%) | 27(61.36%) | 14(31.18%) | 61.36% |
| ISSR | 1 | 7 | 2 | 5 | 1 | 71.43 |
| | 2 | 8 | 3 | 5 | 1 | 62.50 |
| | 3 | 11 | 3 | 8 | 1 | 72.72 |
| | 4 | 9 | 2 | 7 | 0 | 77.78 |
| | 5 | 8 | 3 | 5 | 1 | 62.50 |
| T.B. | | 43 | 13(30.23%) | 30(69.77%) | 4(9.03%) | 69.77% |

*T.B.: Total number of bands.

## Conclusions:

Drought stress is one of the major obstacles to wheat productivity. To develop crop plants with enhanced tolerance of drought stress, various genomics tools have helped to improve our understanding of stress signal perception and transduction, and of the linked molecular regulatory mechanism. These tools have revealed several stress inducible genes and various transcription factors that regulate the drought stress-inducible systems.

The present study used RAPD and ISSR to identify markers associated with drought tolerance. RAPD-PCR exhibited four positive and six negative markers, while ISSR exhibited seven positive and six negative markers.
RAPD and ISSR molecular markers which used in this work could be considered as reliable molecular markers assisted for drought tolerance in durum wheat and could be used in the wheat breeding programs.

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