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SRAP Markers Associated with Water Use Efficiency and Some Agronomic Traits in Wheat under Different Irrigation Regimes

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FIFTY bread wheat lines were evaluated for drought tolerance and compared to six local cultivars under three water regimes (Well-watered = 0.8 Evapotranspiration (ETp), Mild drought stress = 0.6 ETp and severe drought stress = 0.4 ETp.). Eight agronomic traits were evaluated, *i.e.* days to heading, number of spikes/plant, 100-kernel weight (g), relative water content (%), chlorophyll concentration (µg cm^{-2}), grain yield/plant (g), harvest index and water use efficiency (kg/m^3) under normal and stress conditions. Analysis of variance showed highly significant variations among the tested lines. As an average of all tested lines chlorophyll concentration was the most affected trait by drought followed by grain yield per plant and WUE, while harvest index showed the lowest reduction due to drought stress. Five lines (1, 5, 11, 41 and 42) showed high performance in grain yield/plant and surpassed all local varieties under all conditions. The sequence related amplified polymorphism (SRAP) technique was used for the detection of markers associated with drought tolerance. SRAP was able to discriminate between the bulked-DNA of high and low performance lines in some evaluated traits under drought. Moreover, several unique and specific bands for high- and low-bulked lines were generated exposing the efficiency of SRAP in genotyping and diversity analysis. Evaluation of WUE showed its efficiency in differentiating among the tested lines and was in agreement with SRAP analysis which showed the maximum number of specific markers when the high- and low-WUE bulks were compared unlike the other traits.The generated bands could serve preliminarily as selectable markers for drought tolerance in wheat.

Keywords: *Triticum aestivum*, Water deficit, Molecular markers, Grain yield.

Wheat (*Triticum aestivum* L.) is one of the most important crops in the world especially in developing countries as it can be considered as the main source of carbohydrates. In Egypt, there is a big gap between consumption and production in wheat. To fill up this gap, wheat import reached about 57 % of the total

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amount of wheat consumption (FAO, 2012). The total area of Egypt is about one million square kilometers, however out of this area only 3.7% can be used for living and farming due to limited water resources. In the last two decades Egypt population increased by about 84% (FAO, 2010), while the cultivated land and water resources still the same. To cover this increasing demand of wheat production, wheat could be cultivated under the limited water resources or even under drought condition

Stress conditions, such as drought, heat and salinity, are major problems that adversely affect wheat production. Drought is the main environmental abiotic stress, which has devastating effects on wheat productivity. Wheat production is adversely affected by drought in 50% and 70% of the area of the developed and the developing countries, respectively (Trethowan & Pfeiffer, 2000). Hence, the introduction of varieties with improved tolerance to drought stress has been considered as one of the most important goals of crop improvement programs (Ludlow & Muchow, 1990).

Drought tolerance is not a simple response, but is mostly conditioned by many components responses (Nazari & Pakniyat, 2010). Most of agronomical characters are expressed differently in normal and stress conditions and are known to be affected by environmental factors. Therefore, selection based only on the phenotype would be difficult for such traits (Hittalmani *et al*., 2003).

Recently biotechnology provided powerful techniques to detect the molecular basis of plant adaptation to its environment and phenotype. The time has come to identify new strategies that combine advanced molecular technologies with conventional breeding and physiological techniques to achieve this goal. One of the most effective molecular markers is the sequence-related amplified polymorphism (SRAP) which is based on the amplification of open reading frames (ORFs) by targeting the exonic regions, intronic regions and regions with promoters (Li & Quiros, 2001). SRAP markers are more reproducible, stable, simple, and more informative than other molecular markers.

In this study, we conducted two experiments to identify the effect of drought stress on yield and its components on some bread wheat genotypes. The first experiment was performed in the field under three levels of water stress and the second experiment was to differentiate between the highest and the lowest performance lines under molecular level using SRAP technique to detect markers associated with drought tolerance and then we can find an accurate tool for selection for drought tolerance at seedling stage, which will have a great impact on breeding programs for drought tolerance in wheat.

Material and Methods

Plant material

Fifty promising bread wheat lines were evaluated under different irrigation regimes. These lines were derived from two crosses as follow; 24 lines were derived from a cross between a high yielding local variety “Sids-4” with a drought tolerant variety "Tokwie" (South Africa) and 26 lines were derived from a cross between "Sids-4" and "Kasyon/glennson-81" (ICARDA). In addition, some local commercial varieties were used for agronomic evaluation comparison; those were Giza-164, Gemmiza-11, Sids-12, Shandawil-1, Masr-1 and Sahel-1.

Phenotypic evaluation

Two field experiments were carried out in the Experimental Farm of Faculty of Agriculture, Sohag University, Egypt, during 2012/2013 and 2013/2014 winter seasons. The experimental design was performed as randomized complete block combined over environments (irrigation treatments) and seasons with three replicates. The irrigation treatments were determined as different fractions of calculated potential evapotranspiration (ETp) in the experimental site, namely: Well-watered = 0.8 ETp, Mild drought stress = 0.6 ETp and severe drought stress = 0.4 ETp. The experimental plot was consisted of two rows with two meters in length and 20 cm in between. Plants were individually spaced at 10 cm within each row. All cultural practices of growing wheat in the experimental location were followed as recommended. At harvesting, 10 guarded plants from each experimental plot were chosen at random and the following data were recorded: days to heading, No. of spikes/plant, 100-kernel weight, relative water content (RWC), chlorophyll concentration (mg cm^{-2}) using SPAD chlorophyll meter and convert its reading using the formula ($y = 0.118x^2 + 0.919x + 7.925$) described by Dash *et al.* (2007) as y= chlorophyll concentration (mg cm^{-2}) and x= SPAD reading, grain yield/plant, harvest index and water use efficiency (WUE).

Climatic characteristics prevailing

Monthly means of maximum and minimum temperature (C^o), relative humidity (RH) %, wind speed (WS) m/sec, daily sunshine (DS) hours/day and evapotranspiration (ETo) values were computed using ETo_Calculator_V3.2. FAO 2012 (Table 1).

Soil characteristics of the experimental site

Basic relevant physical and chemical characteristics of the experimental soil were determined according to Klute (1986) and Page (1982), respectively. Infiltration rate was determined by means of a double-ring infiltrometer (Model ASTM-D5093). The values are presented in Table 2.

Irrigation requirement consumption and water supply

The experimental plots were given volumes of water to raise the moisture of the top 45 cm layer to the field capacity. Water applied to the plots at each irrigation was equal to the difference between moisture at the field capacity and the soil moisture content at irrigation time of each irrigation (for each irrigation treatment) plus 10% of quantity to ensure a good uniform distribution of water through the plots (Table 3).

TABLE 1. Averages of meteorological data and evapotranspiration reference (ETo) of the two growing seasons (2012/2013 and 2013/2014) .

Measurement	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Mean.
Max. Temp. (C^o)	34.25	27.27	24.95	27.27	30.00	34.68	29.73
Min. Temp. (C^o)	6.38	2.79	5.00	5.43	7.00	8.82	5.90
RH (%)	58	63	65	64	51	37	56.33
WS (m/sec)	1.6	1.4	1.3	1.6	1.9	1.9	1.62
DS (hours/day)	9.3	9	8.9	9.8	9.9	10.3	9.53
ETo (mm/day)	3.37	2.49	2.43	3.43	4.96	6.26	3.82

TABLE 2. Physical and chemical properties of the experimental soil.

Physical properties					
Depth (cm)	**Bulk density ($Mg\ m^{-3}$)**	**Field capacity (%)**	**Permanent wilting point (%)**	**Available water (%)**	**Soil texture**
0-15	1.4	25	10	15	Sandy clay loam
15-30	1.4	24	9	15	Sandy clay loam
30-45	1.5	15	6	9	Sandy loam

Chemical properties		
Properties	Depth (cm)	
	00-30	30-60
Soil pH	7.5	8.2
ECe (dS/ m at 25°C)	2.1	2.5
Available nitrogen (ppm)	50	20
Available phosphorus (ppm)	20	22
Available potassium (ppm)	69	62
$CaCO_3$ %	3.5	4.1
Organic matter %	1.9	1.4

TABLE 3. Irrigation numbers, seasonal irrigation requirement and seasonal evapo-transpiration for treatments in two seasons (2011/2012 and 2012/2013).

Treatments	Number of irrigations			Seasonal irrigation requirement (m^3/fed)			Seasonal evapo-transpiration (mm)		
	2012	**2013**	**mean**	**2012**	**2013**	**mean**	**2012**	**2013**	**mean**
Well-watered	12	12	12	2291	2302	2296.5	545.37	547.99	546.68
Mild stress	9	9	9	2128	2125	2126.5	506.57	505.86	506.21
Severe stress	6	6	6	1477	1487	1482.0	351.60	353.98	352.79

Time of irrigation

Daily evaporation data of pan (mm/day) were obtained from a standard Class-A-Pan located in the experimental field, and recorded. Cumulative pan evaporation data for each irrigation treatment were calculated by: (multiplying daily evaporation by the studied evaporation pan coefficient) as following:

- Irrigation using 0.8 pan evaporation coefficient
- irrigation using 0.6 pan evaporation coefficient
- irrigation using 0.4 pan evaporation coefficient

Determination irrigation time was performed by setting the cumulative pan evaporation to be equal to the allowable available soil moisture depletion (50%).

Irrigation requirement computation and water supply

Soil samples at three depths were collected directly before irrigation and after 48 hr from irrigation. The quantity of water for each irrigation treatment was computed according to the following formula:

$$Q = R \times D \times Bd. \times (F.C. - S.M.I.)/ 100$$

where :

Q = the quantity of water in cubic meter, R= area that would be irrigated in square meter, D= the soil depth required to be irrigated in meter, Bd= bulk density of the soil (gm/ cm^3), F.C= field capacity of the experimental field in percent and S.M.I= the soil moisture percentage before irrigation.

Applied water was measured and delivered for each plot using water meter (Table 3).

Evapotranspiration (ET), amount and rates

The amount of evapotranspiration during irrigation cycle is assumed to be equal to the difference between both soil moisture contents after irrigation and before the next irrigation. The quantities of ET were calculated for 45 cm soil depth. For an area of 4200.8 m^2 (one fed), evapotranspiration can be obtained by the following equation:

$$ET = \theta_2 - \theta_1 / 100 \times Bd \times D / 100 \times 4200.8$$

where:

ET= evapotranspiration in m^3, θ_2=soil moisture percent after irrigation, θ_1= soil moisture percent before next irrigation, Bd = bulk density in gm/ cm^3 and D= soil depth in cm.

Water use efficiency

WUE = grain yield (kg/ fed)/ Seasonal ET (m^3/ fed), (Vites, 1965)

Crop coefficient

Kc= ETc / ETo (Allen *et al.,* 1998)

Statistical analysis

Analysis of variance (ANOVA), appropriate for the specified experimental design, was performed with MSTAT-C software to evaluate the genetic difference among the wheat genotypes. Statistical significance was assumed at 5 and 1% levels of probability. Differences among means were tested by least significant difference (LSD) test at 5% probability level.

Molecular analysis

DNA extraction

Genomic DNA from the fifty lines was extracted at seedling stage using Dellaporta *et al.* (1983) method with some modifications (Youssef, 2012). DNA concentration and purity were measured using spectrophotometer according to Stulnig & Amberger (1994) and Khirshyat 1.0 micro-program (Youssef, 2012). Ten DNA samples of the highest and lowest lines in four agronomic traits evaluated under drought conditions were bulked and used for molecular analysis.

SRAP-PCR amplification

Ten SRAP primer combinations were selected and used for the molecular analysis (Table 4). The method of Li & Quiros (2001) was followed for the SRAP marker system. Each 20 µl SRAP amplification reaction consisted of 2 µl of 10× PCR buffer, 1.6 µl of 50 mM $MgCl_2$, 1.6 µl of 10 µM of each forward and reverse primer, 2.5 µl of 2 mMdNTPs, 25 ng template DNA and 0.25 µl of 5U Taq-DNA polymerase. The PCR was carried out with the initial cycle at 94°C for 2 min, 5 cycles of 94°C for 30 s, 35°C for 30 s and 72°C for 1 min, another 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1 min, and the final extension at 72°C for 5 min. SRAP-PCR products were separated on 8% polyacrylamid gel (PAGE) and visualized by ethidium bromide.

TABLE 4. SRAP primer combinations used in molecular analysis.

Forward (5`-3`)		Reverse (5`-3`)
1	Me-1: TGAGTCCAAACCGGATA	Em-3: GACTGCGTACGAATTGAC
2	Me-3: TGAGTCCAAACCGGAAT	Em-2: GACTGCGTACGAATTTGC
3	Me-3: TGAGTCCAAACCGGAAT	Em-3:GACTGCGTACGAATTGAC
4	Me-4: TGAGTCCAAACCGGACC	Em-2:GACTGCGTACGAATTTGC
5	Me-4: TGAGTCCAAACCGGACC	Em-3:GACTGCGTACGAATTGAC
6	Me-5: TGAGTCCAAACCGGAAG	Em-5:GACTGCGTACGAATTAAC
7	Me-3: TGAGTCCAAACCGGAAT	Em-5:GACTGCGTACGAATTAAC
8	Me-4: TGAGTCCAAACCGGACC	Em-5:GACTGCGTACGAATTAAC
9	Me-5: TGAGTCCAAACCGGAAG	Em-3:GACTGCGTACGAATTGAC
10	Me-5: TGAGTCCAAACCGGAAG	Em-4:GACTGCGTACGAATTTGA

Molecular data analysis

SRAP profiles were converted to binary data matrices by detecting the presence (1) or the absence (0) of the strong, reproducible and clearly distinguished bands. The number of unique and specific bands for each agronomic trait was registered. The percentage of polymorphism was calculated by dividing the total number of polymorphic bands on the total number of bands.

Results

Analysis of variance

The combined analysis of variance for days to heading, number of spikes/plant, 100-kernel weight (g), relative water content (%) , chlorophyll concentration (µg cm^{-2}), grain yield/plant (g), harvest index and water use efficiency (kg/m) revealed that the effect of highly significant affected by years, water stress treatments and genotypes.

TABLE 5. Mean squares of the combined analysis of variance for all studied traits.

S.O.V	D.F	Mean Squares							
		Heading date	Number of spikes / plant	100 kernel weight	RWC	Chlorophyll content	Grain yield / plant	Harvest index	WUE
Year (Y)	1	765.35**	19.86**	5.99**	697.22**	91802.82**	670.49**	256.58**	1.09**
Drought (D)	2	8824.12**	412.11**	303.13**	16302.02**	2957028.13**	4819.56**	392.10**	8.90**
Y x D	2	2.49	1.45	1.33**	7.25	3758.59*	21.69	41.96*	0.007
Error a	12	4.2	0.748	0.473	10.89	129.06	7.39	9.14	0.0033
Genotype (G)	55	156.40**	26.46**	1.59**	152.55**	125698.92**	73.84**	178.09**	0.131**
Y x G	55	3.43**	0.616**	0.520**	0.512	810.01**	6.93**	22.55**	0.0069**
D x G	110	12.14**	2.59**	0.533**	40.70**	14344.37**	18.35**	33.71**	0.029**
Y x D x G	110	1.22**	0.501**	0.207**	0.407	778.17**	1.88**	6.92**	0.0016
Error b	660	0.412	0.23	0.02	2.27	150.1	0.629	3.28	0.00097

* & **Significant at 5 % and 1 % levels of probability, respectively.

The results revealed that wheat genotypes varied from each other for all traits under the three water applications (Table 6).

TABLE 6. The range and mean values for all studied traits under well-watered, mild stress and severe stress conditions.

Traits	Well-watered		Mild stress		Sever stress	
	Range	Mean ± S.E	Range	Mean ± S.E	Range	Mean ± S.E
Heading date	75.83 – 91.50	85.71 ± 0.20	72.83 – 84.50	79.72 ± 0.22	70.50 – 79.00	75.67 ± 0.20
No. of spikes/plant	6.63 – 14.64	9.69 ± 0.20	5.86 – 10.58	8.13 ± 0.22	4.99 – 9.65	7.51 ± 0.20
100 kernel weight (g)	4.78 – 6.16	5.39 ± 0.02	3.82 – 5.81	4.65 ± 0.02	2.64 – 4.31	3.50 ± 0.02
RWC %	75.47 – 88.64	84.76 ± 0.07	69.84 – 81.61	76.82 ± 0.08	57.14 – 78.53	70.88 ± 0.07
Total chlorophyll content (µg cm-2)	32.20 – 68.02	47.09 ± 0.12	24.17 – 55.24	37.12 ± 0.12	19.29 – 47.74	30.61 ± 0.12
Grain yield/plant (g)	11.54 – 28.50	18.51 ± 0.02	9.77 – 17.72	12.98 ± 0.02	7.43 – 14.84	11.10 ± 0.02
Harvest index	28.15 – 46.35	33.71 ± 0.12	26.22 – 40.64	31.71 ± 0.12	24.07 – 41.05	31.80 ± 0.12
WUE (kg/m)	0.52 – 1.21	0.82 ± 0.12	0.44 – 0.77	0.59 ± 0.12	0.33 – 0.67	0.50 ± 0.12

Performance of wheat genotypes

The average number of days to heading in the two years ranged from 70.50 days for line No. 47 in severe drought condition to 91.50 days for line No. 28 in well-watered condition. The average of overall tested genotypes for number of days to heading was reduced from 85.66 days in well-watered condition to 79.92 days in mild stress condition and down to 75.44 days in severe drought condition. The earliest lines were No. 18, 19, 29, 37, 45, 46, 47 and 49 as compared with the earliest local variety under all stress conditions (Table 7).

Severe stress reduced the number of spikes/plant by 22% as an average for all tested genotypes when compared with well-watered conditions (Table 7). Results showed that lines No. 26 and 41 surpassed the check (Sahel 1) and local commercial varieties in No. of spikes/plant under all conditions.

Drought stress at grain filling adversely affected 100-kernel weight (g). As an average for all tested genotypes, the reduction ranged from 14 % to 35% due to mild and severe drought conditions, respectively. Only line No. 28 was significantly higher than the check (Sahel 1) and local commercial varieties under all conditions. While, lines No. 1, 4, 7, 8, 9, 11, 30, 35 and 39 were significantly surpassed the check (Sahel 1) and local commercial varieties under severe stress conditions.

Water stress cases a reduction on relative water content (RWC). In this regard, as overall average of all genotypes RWC was reduced from 84.90 % in well-watered conditions to 76.88 % in mild stress and the severe conditions recorded the lowest value 71.11 % (Table 7). Lines No. 1, 41 and 42 were significantly exceeded the check (Sahel 1) and local commercial varieties under all conditions. While, nine lines, *i.e.,* line No. 5, 10, 11, 16, 19, 22, 23, 34 and 43 surpassed the check (Sahel 1) and local commercial varieties in RWC under mild and severe stresses conditions.

Under well-watered condition the average chlorophyll concentration (mg cm^{-2}) was 344.33 with a range from 616.43 to 160.02 for lines No. 11 and 6, respectively. Meanwhile, there was a reduction in chlorophyll concentration about 36% and 54% caused by mild and severe conditions, respectively (Table 8). Results showed that seven lines, *i.e.,* No. 1, 2, 11, 37, 41, 42 and 43 surpassed the check (Sahel 1) and local commercial varieties in chlorophyll concentration over all conditions.

TABLE 7. Mean performance of heading date, number of spikes/plant, 100-kernel weight and relative water content .

Genotypes	Heading date			Number of spikes / plant			100-kernel weight			Relative water content		
	Well-watered	Mild stress	Sever stress	Well-watered	Mild stress	Sever stress	Well-watered	Mild stress	Sever stress	Well-watered	Mild stress	Sever stress
1	89.00	83.50	79.00	10.81	8.57	7.71	5.73	5.01	4.11	88.22	79.81	75.56
2	86.50	82.33	77.17	10.97	9.60	8.72	5.21	4.81	3.83	87.05	79.70	75.11
3	86.17	80.50	75.67	10.35	7.85	7.26	5.30	4.17	3.46	84.70	73.34	69.59
4	90.00	82.00	79.00	11.73	9.79	9.14	5.61	4.77	4.06	86.23	76.61	67.53
5	89.83	81.83	77.67	9.54	7.69	7.47	5.25	4.65	3.47	86.27	78.99	75.67
6	85.50	79.50	77.33	8.49	6.86	6.68	5.43	4.61	3.78	81.29	76.28	66.87
7	88.83	84.17	77.17	9.83	7.86	7.58	5.25	4.84	3.99	87.85	76.60	68.19
8	87.00	83.50	78.33	8.93	8.66	8.08	5.31	4.59	4.02	82.44	78.61	70.19
9	86.00	80.00	73.67	9.07	7.60	6.80	5.71	4.98	4.00	87.46	72.76	59.34
10	84.11	77.17	74.00	7.80	7.96	7.50	5.37	4.64	3.58	83.39	80.95	77.54
11	90.17	83.00	78.00	10.29	8.56	8.03	5.57	5.02	4.15	87.48	80.07	76.32
12	85.00	79.67	74.67	10.61	7.08	6.91	5.59	4.99	3.82	88.60	74.48	70.06
13	83.17	77.33	73.00	9.24	8.67	8.19	5.30	4.41	2.67	83.09	72.15	67.73
14	85.50	81.50	73.67	11.21	10.08	9.45	5.21	4.50	3.47	85.70	79.70	73.95
15	85.67	82.17	78.50	11.38	9.83	9.08	5.34	4.75	3.50	85.52	76.83	72.74
16	90.83	83.83	77.00	7.97	6.33	6.05	5.20	4.87	3.73	85.35	78.90	76.32
17	87.50	81.83	78.33	11.63	9.48	8.48	5.28	4.61	3.77	85.39	74.22	68.47
18	80.50	75.67	72.33	8.44	7.38	6.24	4.99	4.46	3.56	83.73	75.04	60.95
19	79.83	74.50	73.00	9.30	8.49	7.88	5.59	5.17	2.77	84.29	80.34	76.59
20	82.50	77.83	74.33	7.64	8.08	7.40	5.31	4.05	3.40	83.87	79.59	72.84
21	83.83	78.33	73.67	8.19	6.57	5.74	5.55	4.94	2.64	82.60	71.22	57.14
22	88.50	83.50	79.00	10.48	8.54	8.00	5.43	4.70	3.64	86.67	80.51	77.43
23	88.50	81.17	76.67	10.25	7.44	7.26	5.89	5.01	2.68	85.63	78.81	76.72
24	88.83	84.50	77.00	9.21	8.40	7.13	4.78	4.16	3.81	82.81	71.97	66.55
25	85.50	78.67	75.00	10.78	9.74	7.52	5.04	4.00	3.13	81.88	76.83	72.08
26	91.17	80.50	74.67	13.04	9.48	8.80	5.70	4.95	3.75	88.51	75.91	71.16
27	87.33	78.50	74.00	10.57	7.47	6.88	5.44	4.83	3.83	87.88	75.65	69.57
28	91.50	80.67	76.67	12.30	9.09	7.28	6.12	5.81	4.27	87.21	70.82	66.73
29	79.83	76.50	72.83	8.15	8.00	7.74	5.42	4.27	3.04	85.52	78.26	74.98
30	89.17	79.00	75.00	10.46	7.33	6.91	5.46	5.11	4.31	81.95	69.84	64.09
31	88.17	81.50	77.00	10.52	8.23	8.22	5.44	4.73	3.07	86.59	77.81	69.39
32	91.17	81.50	77.33	7.81	7.02	7.00	5.34	3.96	3.71	82.11	75.19	71.11
33	88.67	84.00	78.67	9.22	9.22	7.83	5.06	4.43	3.31	81.35	76.83	71.41
34	84.67	80.67	74.33	9.01	8.98	8.44	5.02	4.40	3.30	87.26	80.87	76.79
35	85.17	79.50	74.67	10.74	8.72	7.83	5.63	5.23	4.33	83.30	73.81	67.06
36	82.83	76.17	72.67	8.52	7.44	7.04	5.27	4.37	3.10	79.47	73.97	63.88
37	79.83	75.33	73.00	10.34	8.71	8.49	5.49	4.69	3.68	86.61	79.80	74.54
38	90.17	81.50	77.00	8.71	8.36	7.93	5.62	4.52	2.80	81.78	76.76	71.68
39	84.50	78.33	73.67	9.98	7.19	6.89	5.33	4.82	4.16	86.57	73.88	64.13
40	86.50	78.83	75.67	9.35	6.95	6.86	5.49	4.24	3.57	85.93	77.81	73.06
41	90.00	83.50	77.67	14.64	10.58	9.65	5.81	5.36	3.95	88.64	81.40	76.75
42	86.50	79.83	74.33	12.27	9.71	8.28	5.40	4.02	3.13	87.56	79.83	76.25
43	86.50	80.33	75.00	7.60	6.61	6.93	5.70	4.40	3.28	86.35	81.61	78.53
44	80.89	76.50	73.00	9.28	7.91	7.99	5.18	4.86	3.05	80.40	75.45	71.95
45	77.83	72.83	70.67	7.53	7.77	7.26	5.47	4.47	2.95	80.58	76.16	73.08
46	78.83	73.67	71.00	7.78	6.74	6.02	5.37	4.51	3.48	85.91	80.02	66.60
47	75.83	72.83	70.50	6.63	5.86	4.99	4.80	3.82	2.59	75.47	73.17	65.53
48	87.17	82.50	77.17	8.28	6.80	6.68	5.03	4.23	3.27	84.42	77.25	70.84
49	76.50	73.50	72.67	8.15	7.13	5.85	4.91	4.51	3.16	84.52	75.70	69.29
50	85.83	79.83	74.67	9.66	7.95	7.43	6.01	5.13	2.88	84.45	79.08	74.22
Mean	85.71	79.72	75.34	9.69	8.13	7.51	5.40	4.65	3.50	84.76	76.82	70.88
Giza 168	85.50	83.50	77.00	9.32	8.57	7.80	5.42	4.57	3.55	86.51	77.84	73.42
Gemmiza 11	89.83	84.83	79.00	9.91	8.88	8.68	5.23	3.94	3.42	85.97	75.42	71.67
Sids 12	86.50	82.83	78.17	5.46	4.52	3.98	5.82	5.05	3.96	87.35	78.62	71.36
Shandawel 1	85.33	79.17	74.67	12.38	9.46	8.74	5.20	4.46	3.56	87.56	78.28	74.71
Masr 1	80.83	78.83	74.17	9.78	7.67	7.26	5.42	4.31	3.39	84.73	75.04	66.29
Sahel 1	83.83	80.50	75.00	8.85	8.22	7.71	5.26	4.88	3.59	84.18	78.87	75.45
LSD0.05		0.73			0.54			0.16			1.70	

TABLE 8. Mean performance of Chlorophyll concentration, WUE, grain yield / plant and Harvest index.

Genotypes	Chlorophyll content			WUE			Grain yield / plant			Harvest index		
	Well-watered	Mild stress	Sever stress	Well-watered	Mild stress	Sever stress	Well-watered	Mild stress	Sever stress	Well-watered	Mild stress	Sever stress
1	465.33	328.51	249.42	1.11	0.79	0.66	24.71	17.51	14.76	37.63	34.95	34.84
2	474.62	319.92	241.28	0.83	0.69	0.60	18.46	15.41	13.30	31.42	31.37	34.27
3	322.94	193.15	147.28	0.73	0.57	0.53	16.34	12.78	11.85	28.47	32.02	32.82
4	416.78	257.76	125.97	1.06	0.62	0.44	23.54	13.81	9.78	37.58	31.14	26.40
5	409.93	298.21	211.40	0.90	0.70	0.62	20.04	15.48	13.84	40.82	37.76	37.61
6	160.02	108.09	78.97	0.68	0.47	0.43	15.10	10.50	9.55	39.11	32.21	33.76
7	491.27	276.84	162.43	0.79	0.54	0.47	17.56	11.94	10.44	36.16	33.08	32.65
8	172.39	138.75	99.02	0.64	0.56	0.46	14.36	12.41	10.27	30.72	29.19	28.29
9	402.78	212.25	100.31	1.01	0.61	0.39	22.48	13.57	8.60	35.94	33.15	27.46
10	418.65	289.88	227.98	0.74	0.62	0.57	16.37	13.86	12.65	32.00	33.99	34.55
11	616.43	418.98	320.75	1.16	0.72	0.64	25.91	16.13	14.29	39.55	37.29	38.00
12	432.64	270.83	199.03	1.03	0.54	0.50	22.86	12.09	11.12	35.58	27.74	29.75
13	217.23	126.64	109.10	0.80	0.51	0.48	17.90	11.31	10.68	35.37	30.72	33.03
14	246.81	190.45	107.55	0.75	0.59	0.52	16.62	13.04	11.68	28.89	27.15	27.78
15	353.45	232.09	176.37	0.81	0.53	0.48	17.98	11.82	10.62	29.74	29.02	31.02
16	280.51	223.86	193.92	0.86	0.62	0.59	19.21	13.73	13.21	36.32	32.35	35.42
17	255.67	161.32	119.85	0.83	0.53	0.47	18.57	11.83	10.53	33.63	28.90	30.91
18	167.80	114.05	84.22	0.79	0.59	0.39	17.58	13.04	8.63	33.36	29.21	25.12
19	453.17	301.58	232.47	0.78	0.69	0.62	17.28	15.46	13.90	29.44	27.71	29.41
20	263.15	198.59	163.41	0.69	0.59	0.51	15.39	13.16	11.25	33.78	34.53	35.74
21	215.46	136.71	80.65	0.70	0.46	0.35	15.58	10.25	7.82	33.18	28.49	25.91
22	384.39	300.74	219.34	0.85	0.69	0.61	18.84	15.30	13.55	35.20	32.68	36.53
23	378.04	264.89	230.46	0.79	0.64	0.60	17.68	14.34	13.45	34.79	32.28	34.63
24	176.45	131.68	88.74	0.72	0.55	0.42	16.13	12.25	9.30	29.85	30.10	28.01
25	195.46	136.41	101.65	0.73	0.60	0.46	16.35	13.28	10.31	32.83	32.14	30.37
26	408.83	261.38	204.18	1.07	0.60	0.52	23.72	13.36	11.51	38.04	35.72	34.07
27	473.11	234.91	153.05	1.00	0.54	0.42	22.21	12.06	9.29	39.38	28.26	28.67
28	530.51	110.97	119.64	1.05	0.46	0.42	23.33	10.27	9.39	35.41	26.75	30.38
29	301.40	193.68	156.09	0.77	0.58	0.56	17.04	12.88	12.29	28.41	27.56	30.36
30	265.84	164.60	84.13	0.82	0.51	0.39	18.31	11.25	8.70	30.91	27.05	24.07
31	413.13	256.86	186.90	0.82	0.56	0.42	18.31	12.55	9.37	33.45	32.21	28.72
32	232.16	118.06	90.72	0.70	0.46	0.43	15.49	10.15	9.64	33.15	30.87	32.09
33	174.13	125.15	95.52	0.70	0.57	0.42	15.52	12.73	9.41	30.38	30.97	29.64
34	426.00	300.97	249.67	0.88	0.71	0.62	19.68	15.81	13.74	33.06	32.42	33.31
35	194.72	129.94	84.03	0.87	0.53	0.48	19.35	11.78	10.64	35.55	31.42	31.63
36	168.79	115.24	73.24	0.61	0.51	0.46	13.61	11.34	10.28	28.15	30.73	32.27
37	462.19	311.97	235.52	0.96	0.61	0.56	21.39	13.69	12.41	38.43	35.09	36.00
38	183.93	143.05	86.83	0.76	0.56	0.53	16.83	12.49	11.78	29.62	29.63	32.46
39	576.92	121.45	76.36	0.96	0.46	0.40	21.46	10.18	8.88	34.81	29.51	29.34
40	353.59	235.64	163.77	0.82	0.55	0.45	18.28	12.25	10.12	31.02	29.96	30.17
41	496.08	356.63	270.03	1.28	0.71	0.66	28.50	15.84	14.60	40.85	40.14	40.55
42	505.56	332.28	282.04	1.09	0.80	0.67	24.17	17.72	14.84	36.80	36.94	38.74
43	476.86	291.84	281.74	0.88	0.73	0.62	19.69	16.28	13.71	33.39	31.78	33.74
44	222.18	153.56	113.29	0.61	0.48	0.45	13.55	10.79	10.12	29.34	26.22	27.24
45	173.99	114.67	85.58	0.63	0.48	0.45	14.04	10.66	10.00	30.73	28.65	30.89
46	347.48	247.45	98.42	0.75	0.60	0.40	16.60	13.25	8.86	29.70	28.40	24.90
47	163.64	99.33	69.63	0.52	0.44	0.33	11.54	9.77	7.43	29.18	30.81	29.62
48	352.72	227.07	140.36	0.69	0.54	0.45	15.40	12.02	9.97	33.52	32.37	34.99
49	222.92	158.07	110.05	0.69	0.53	0.49	15.34	11.89	10.90	30.39	29.31	39.31
50	277.18	209.27	144.80	0.86	0.62	0.54	19.25	13.92	11.92	33.75	32.05	32.23
Mean	335.50	212.92	154.54	0.83	0.58	0.50	18.51	12.98	11.10	33.58	31.28	31.79
Giza 168	408.54	279.79	194.30	0.80	0.63	0.57	17.78	14.13	12.67	30.15	27.73	30.25
Gemmiza 11	414.57	281.48	189.57	0.78	0.56	0.46	17.28	12.38	10.13	36.03	31.20	29.67
Sids 12	459.55	295.37	194.62	0.89	0.60	0.55	19.92	13.35	12.14	37.26	33.97	36.08
Shandawel 1	424.60	304.75	218.47	0.84	0.59	0.55	18.64	13.09	12.30	30.97	30.30	35.13
Masr 1	424.60	199.22	114.00	0.74	0.54	0.42	16.40	11.94	9.25	32.39	29.36	26.42
Sahel 1	375.12	248.89	212.38	0.76	0.62	0.57	16.88	13.78	12.69	34.02	32.11	35.15
LSD0.05		13.86			0.04			0.90			1.96	

As an average for all genotypes WUE reduced by 29% and 39% under mild and severe drought conditions, respectively (Table 8). Results showed that line No. 19 recorded the lowest reduction in WUE (12 % and 21%, respectively) under mild and severe drought conditions. On the other hand Line No. 28 was the most sensitive one (56% reduction) under mild drought conditions followed by line No. 9 under severe drought conditions (61% reduction). Six Lines (No. 1, 5, 11, 41, 42 and 43) surpassed the check (Sahel 1) and local commercial varieties in WUE over all conditions. Meanwhile, seven lines, *i.e.,* No. 2, 10, 16, 19, 22, 23 and 34 surpassed the check (Sahel 1) and local commercial varieties in WUE under mild and severe stresses conditions (Table 8).

Manifestly, the evaluated wheat genotypes in this study showed significant differences in their grain yield/ plant (Table 1). As an average for overall tested genotypes, grain yield/plant (g) was reduced from 18.51 in well-watered conditions to 12.98 in mild conditions and further down to 11.1 in severe conditions. Clearly, severe drought stress was strong for grain yield / plant to be reduced by 40% average reduction (Table 8). The highest grain yield/ plant (g) was obtained from lines No. 1, 5, 11, 41 and 42 which were significantly out-yielded the check (Sahel 1) and local commercial varieties under all conditions (Table 8).

Harvest index ranged from 28.15% to 40.85% with an average of 33.56% under well-watered condition (Table 8). While, under mild stress it ranged from 26.22% to 40.14% with a reduction on general mean by 6.97% as compared with well-watered condition. Moreover, under severe condition the mean harvest index for all genotypes was 31.83% recording the reduction by 5.17% as compared with well-watered condition. Results revealed that lines, *i.e.,* No. 1, 4, 5, 6, 11, 26, 27, 37 and 41 surpassed the check (Sahel 1) and local commercial varieties in harvest index under all conditions (Table 8).

The drought susceptibility index (DSI) for the tested lines shown in Table 9. DSI over mild stress condition ranged from 0.35 for line No. 19 to 1.88 for line No. 28 and twenty nine lines gave low values of drought susceptibility index (DSI < 1). Meanwhile, under severe stress condition the values of DSI ranged from 0.49 for line No. 19 to 1.55 for line No. 9 and twenty seven lines have DSI < 1. Finally, results indicated that eight lines, *i.e.,* No. 2, 5, 19, 22, 23, 34, 42 and 43 produced relatively high grain yield compared with the check (Sahel 1) and local commercial varieties under drought stress environments due to high yield potential, rather than having DSI<1 (Table 9).

TABLE 9. Mean performance of grain yield / plant and drought susceptibility index.

Genotypes	Grain yield / plant		DSI_{mild}	Grain yield / plant		DSI_{sever}
	Well-watered	Mild stress		Well-watered	Sever stress	
1	24.71	17.51	0.98	24.71	14.76	1.01
2	18.46	15.41	0.56	18.46	13.30	0.70
3	16.34	12.78	0.73	16.34	11.85	0.69
4	23.54	13.81	1.39	23.54	9.78	1.47
5	20.04	15.48	0.76	20.04	13.84	0.78
6	15.10	10.50	1.02	15.10	9.55	0.92
7	17.56	11.94	1.07	17.56	10.44	1.02
8	14.36	12.41	0.46	14.36	10.27	0.72
9	22.48	13.57	1.33	22.48	8.60	1.55
10	16.37	13.86	0.51	16.37	12.65	0.57
11	25.91	16.13	1.27	25.91	14.29	1.13
12	22.86	12.09	1.58	22.86	11.12	1.29
13	17.90	11.31	1.24	17.90	10.68	1.01
14	16.62	13.04	0.72	16.62	11.68	0.75
15	17.98	11.82	1.15	17.98	10.62	1.03
16	19.21	13.73	0.96	19.21	13.21	0.78
17	18.57	11.83	1.22	18.57	10.53	1.09
18	17.58	13.04	0.87	17.58	8.63	1.28
19	17.28	15.46	0.35	17.28	13.90	0.49
20	15.39	13.16	0.49	15.39	11.25	0.68
21	15.58	10.25	1.15	15.58	7.82	1.25
22	18.84	15.30	0.63	18.84	13.55	0.71
23	17.68	14.34	0.63	17.68	13.45	0.60
24	16.13	12.25	0.81	16.13	9.30	1.06
25	16.35	13.28	0.63	16.35	10.31	0.93
26	23.72	13.36	1.47	23.72	11.51	1.29
27	22.21	12.06	1.53	22.21	9.29	1.46
28	23.33	10.27	1.88	23.33	9.39	1.50
29	17.04	12.88	0.82	17.04	12.29	0.70
30	18.31	11.25	1.29	18.31	8.70	1.32
31	18.31	12.55	1.06	18.31	9.37	1.23
32	15.49	10.15	1.16	15.49	9.64	0.95
33	15.52	12.73	0.60	15.52	9.41	0.99
34	19.68	15.81	0.66	19.68	13.74	0.76
35	19.35	11.78	1.31	19.35	10.64	1.13
36	13.61	11.34	0.56	13.61	10.28	0.61
37	21.39	13.69	1.21	21.39	12.41	1.06
38	16.83	12.49	0.87	16.83	11.78	0.75
39	21.46	10.18	1.76	21.46	8.88	1.47
40	18.28	12.25	1.11	18.28	10.12	1.12
41	28.50	15.84	1.49	28.50	14.60	1.23
42	24.17	17.72	0.90	24.17	14.84	0.97
43	19.69	16.28	0.58	19.69	13.71	0.76
44	13.55	10.79	0.68	13.55	10.12	0.64
45	14.04	10.66	0.81	14.04	10.00	0.72
46	16.60	13.25	0.68	16.60	8.86	1.17
47	11.54	9.77	0.51	11.54	7.43	0.89
48	15.40	12.02	0.74	15.40	9.97	0.89
49	15.34	11.89	0.75	15.34	10.90	0.73
50	19.25	13.92	0.93	19.25	11.92	0.96

Molecular analysis of wheat lines under drought

A comparison between the highest and lowest 10 bulked-lines in some agronomic traits performance evaluated under drought conditions was achieved. Ten SRAP primers used in the analysis showed the difference between the highest and lowest lines by generating unique and specific bands for each bulk (Table 10, Fig. 1). The total number of unique and specific bands ranged from 5 to 14 bands per trait, while the average percentage of polymorphism (%P) ranged from 4.07 to 10.37% for yield and water use efficiency (WUE), respectively (Table 10).

TABLE 10. Level of polymorphism and number of specific bands of 10 lines showed highest and lowest performance in yield, WUE, RWC and chlorophyll concentration under drought stress.

Primer*	Traits																TSB
	Yield				WUE				RWC				Chlorophyll concentration				
	TNB	%P	Low	High	TNB	%P	Low	High	TNB	%P	Low	High	TNB	%P	Low	High	
1	8	0.00	0	0	8	0.00	0	0	8	0.00	0	0	8	0.00	0	0	0
2	10	10.00	1	0	10	10.00	1	0	12	0.00	0	0	10	20.00	2	0	4
3	10	0.00	0	0	15	20.00	2	1	9	0.00	0	0	14	21.43	3	0	6
4	20	10.00	2	0	20	20.00	4	0	24	12.50	0	3	20	0.00	0	0	9
5	12	16.67	0	2	19	31.58	6	0	11	0.00	0	0	10	20.00	2	0	10
6	16	0.00	0	0	17	0.00	0	0	18	0.00	0	0	11	0.00	0	0	0
7	14	0.00	0	0	16	0.00	0	0	10	10.00	1	0	16	0.00	0	0	1
8	8	0.00	0	0	9	0.00	0	0	12	0.00	0	0	14	0.00	0	0	0
9	12	0.00	0	0	7	0.00	0	0	12	41.67	0	5	12	0.00	0	0	5
10	13	0.00	0	0	14	0.00	0	0	14	0.00	0	0	16	6.25	1	0	1
Total	123	4.065	3	2	135	10.37	13	1	130	6.92	1	8	131	6.11	8	0	36
G-Total			5				14				9				8		

* Primer numbers related to Table 4, TNB: Total number of bands, %P: Percentage of polymorphism, L: Lines with low performance, H: Lines with high performance, TSB: Total number of specific bands, G-total: Grand total.

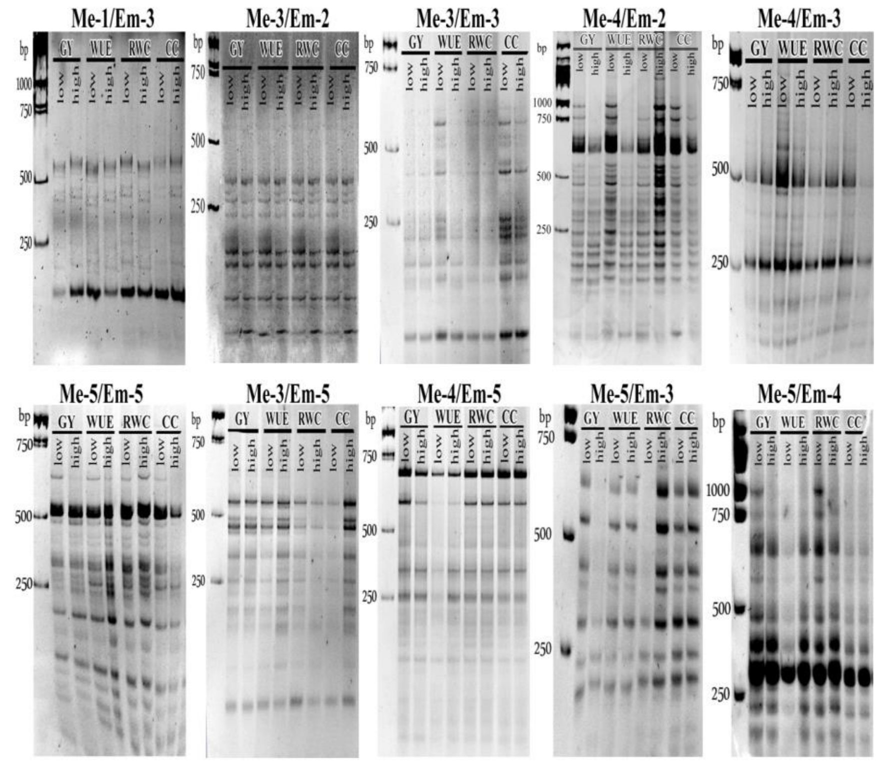

Fig. 1. SRAP profile of 10 primer combinations showing the difference between two bulks of 10-high and low performance wheat lines in some traits evaluated under drought stress; GY: Grain yield per plant, WUE: Water use efficiency, RWC: Relative water content, CC: Chlorophyll concentration, high: Bulk of the highest 10 lines and low: Bulk of the lowest 10 lines.

The total number of bands generated for high- and low- bulked lines for yield, water use efficiency (WUE), relative water content (RWC) and chlorophyll concentration was 123, 135, 130 and 131 bands, respectively. A total of 36 bands were generated uniquely and specific for the evaluated traits (Tables 10 and 11). For instance, 5 bands were generated for high- and low-yield bulked lines (2 and 3, respectively) with 4.07% polymorphism. The total number of specific bands in the case of WUE was 14, out of them 13 were specific for bulked-lines with low WUE and one band for bulked-lines with high WUE with 10.37% polymorphism. In addition, nine specific bands were generated for WRC lines, out of them 8 were specific for high WRC bulked-lines, while one band was specific for low WRC with 6.92% polymorphism. Furthermore, eight bands were specific for lines with high chlorophyll concentration with an average of 6.11% polymorphism, while no specific bands were generated for low chlorophyll concentration lines (Table 10).

TABLE 11. Size in base pair of specific bands for tow bulks of 10 lines showed highest and lowest performance in Yield, WUE, RWC and chlorophyll concentration under drought stress.

Primer *	Traits								NSB
	Yield		WUE		RWC		Chlorophyll concentration		
	Low	High	Low	High	Low	High	Low	High	
2	295	-	295	-	-	-	295 230	-	4
3	-	-	560 595	140	-	-	380 530 650	-	6
4	750 920	-	400 750 920 1100	-	-	580 920 1050	-	-	9
5	-	305 340	775 735 705 655 640 615	-	-	-	305 380	-	10
7	-	-	-	-	185	-	-	-	1
9	-	-	-	-	-	365 400 450 530 655	-	-	5
10	-	-	-	-	-	-	600	-	1
NSB	3	2	13	1	1	8	8	0	36

* Primer numbers related to table 4, NSB: number of specific bands

Discussion

The selection for some physiological traits such as water use efficiency (WUE), relative water content (RWC), chlorophyll concentration in drought conditions studies are important for improving drought tolerance in wheat (Larbi, 2004 and Shirazi *et al.,* 2014). In the present study, drought caused significant reduction in all evaluated agronomic traits. In this regard, drought stress at grain filling period adversely affected 100-kernel weight, as it is more critical phase and results in substantial yield losses (Muhammad *et al.*, 2014). Moreover, in our study, water stress caused a reduction on RWC which reflected the effect of drought stress and exposed the difference among the evaluated wheat genotypes. In this regard, Shamsi (2010) reported that plants which can reserve more water content on its tissues have a good performance in drought conditions. In addition, the tested genotypes showed significant differences in chlorophyll concentration which considered as an indicator for the yield stability under drought conditions (Sairam, 1994 and Shamsi, 2010).

To get the maximum output from wheat genotypes,we should evaluate the genetically improved varieties for growth performance and water use efficiency (WUE) under drought conditions (Shirazi *et al.*, 2014). In the present study, WUE was used to evaluate the tested lines and showed higher reduction under both mild and severe drought conditions. Additionally, it was reported that genotype which can use water more efficiently produce maximum biomass (Shirazi *et al.,* 2014).

Grain yield is frequently used in crops such as wheat as the main criteria for drought resistance (Li *et al.,* 2011). The reduction showed in the present study in grain yield due to water deficit is supported by that such trait is affected by a complex of different morphological and physiological characters which are in turn influenced by the soil moisture (Sodabeh *et al.*, 2013). Moreover, under water limited conditions, genotypes which show the highest harvest index and yield stability were reported as drought tolerant (Rathore, 2005).

In general, our results are in agreement with those of Li *et al.* (2011) who studied the effect of drought stress on some agronomic characteristics and grain yield in spring wheat. They found that drought caused obvious reductions in several traits including plant height, grain volume weight, kernel weight and diameter and grain yield. Furthermore, El-Rawy & Youssef (2014) found that drought conditions generated by the treatment of polyethylene glycol caused high reduction in shoot and root lengths in some wheat lines evaluated at seedling stage.

On the other hand, the association of molecular markers with phenotypic evaluationis one of important factors to understand and investigate the genetic role of tolerance by prediction the genomic regions that affect the plant's response (Roy *et al.,* 2011). Therefore, identification of molecular bands associated with some traits evaluated under stress is the most important step in selecting genotypes having tolerance to such trait at the early stages of growth. In addition, molecular markers can improve the efficiency of breeding by allowing manipulation of the genome through marker-assisted selection (Prerna *et al.,* 2013).

In the present study, SRAP marker was able to differentiate among different bulked-DNA of high and low performance in all agronomic traits evaluated under drought stress. The dissimilarities within each group was reduced by gathering their DNA samples, therefore the difference between the high and low bulks was mainly due to the trait of interest. Moreover, SRAP showed its effectiveness by generating several specific bands for the tolerant and susceptible bulked-lines. The generated bands could serve preliminarily as selectable markers for drought tolerance in wheat; however purification, sequencing and analysis of these bands might be necessary in the proximate research work.

The previous studies on wheat diversity and genotyping indicated that SRAP was an efficient technique for wheat diversity evaluation. In this regard, Zaefizadeh & Goleiv (2009) investigated the genetic diversity and relationships among durum wheat landraces from the region of North West Iran and Azerbaijan by SRAP marker and phenotypic differences. They found that 12 combinations of SRAP markers were distinguishable among these landraces, they suggested that SRAP technology is useful for genetic diversity and relationship analyses, marker assisted selection and genetic map construction in durum wheat. On the other hand, Elshafei *et al.* (2013) used SRAP markers to identify new markers linked to chlorophyll concentration, flag leaf senescence and cell membrane stability in wheat under water stress conditions; they reported that SRAP generated successfully several QTLs linked to these traits. In addition, Moustafa *et al.* (2014) reported that, TRAP and SRAP markers, combined with bulked segregant analysis, could be used to identify molecular markers linked to six agronomic traits; (days to heading, plant height, spike number/m^2, kernel number/spike, 1000-kernel weight and grain yield), as indicators for drought tolerance genes in wheat. Recently, El-Rawy & Youssef (2014) reported the efficiency of SRAP in discriminating wheat genotypes under drought conditions at seedling stage using polyethylene glycol. They found that SRAP generated up to 85.71% polymorphism among the tested lines as well as SRAP showed its effectiveness by gathering all lines which have a high DSI in one sub-cluster and generated several unique and specific bands for high-DSI-lines and other for low-DSI-lines, suggesting that these bands could be used for further work as SRAP markers associated with drought tolerance in wheat.

Our results in generating unique bands specific for certain traits evaluated under drought stress were in accordance with previous studies. In this regards, the primer combination used in our study (4- ACC/TGC) which generated 10 specific bands specific for low-yield (2 bands), low-WUE (4 bands), high-RWC (3 bands) and low-AO (one band), has been reported to generate QTL specific for flag leaf senescence (Elshafei *et al.,* 2013) and to generate 3 bands specific for lines with high drought susceptibility index (DSI) and one band for low-DSI-lines (El-Rawy & Youssef, 2014). Moreover, the primer combination 9-AAG/GAC which generated 5 bands specific for high-RWC lines in our study has been reported as well to generate QTL specific for flag leaf senescence (Elshafei *et al.,* 2013).

Conclusion

Drought caused significant reduction in all evaluated agronomic traits. Comparing the two bulks of the highest and lowest performance lines in some agronomic traits evaluated under drought stress molecularly was sufficient to expose the difference between the tolerant and susceptible lines. SRAP showed its effectiveness in discriminating the tested genotypes by generating several unique and specific bands for high and/or low-performance in some agronomic traits evaluated under water stress. These bands could be identified as markers associated with drought tolerance in wheat. Additionally, evaluation of WUE in this study showed its efficiency in differentiating among the tested lines and was in agreement with SRAP analysis which showed the maximum number of specific markers when the high- and low-WUE bulks were compared unlike the other traits.

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واسمات الـ SRAP المرتبطة مع كفاءة استخدام الماء وبعض الصفات المحصولية فى قمح الخبز تحت معدلات رى مختلفة

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تم تقييم 50 سلالة من قمح الخبز للتحمل للجفاف و مقارنتهم بستة أصناف محلية تحت ثلاث معدلات من الرى (الرى بدون اجهاد = 0, 8 من النتح بخر , جفاف متوسط = 0,6 من انتح بخر و جفاف قاسى = 0,4 من النتح بخر). تم تقييم 8 صفات محصولية وهم عدد الأيام حتى طرد السنابل , عدد السنابل / النبات, وزن الـ 100 حبة , محتوى الماء النسبى , تركيز الكلوروفيل, محصول النبات الفردى , معامل الحصاد و كفاءة استخدام الماء فى كل من الرى العادى وظروف الأجهاد. و قد اظهر تحليل التباين فروق معنوية جدا بين السلالات المدروسة. وكانت صفة تركيز الكلوروفيل اكثر الصفات تأثرا بالجفاف تتبعها صفة محصول النبات الفردى و كفاءة استخدام الماء بينما كان معامل الحصاد اقل الصفات تأثرا نتيجة للجفاف. أظهرت 5 سلالات (س 1, س 5, س 11, س 41, س 42) تحملا للجفاف عن طريق الاداء العالى فى صفة محصول الحبوب للنبات الفردى تحت كل ظروف الرى. تم استخدام تقنية الـ SRAP لتحديد واسمات جزيئية مرتبطة بصفة تحمل الجفاف فى القمح. أوضحت النتائج قدرة الـ SRAP العالية فى التفرقة بين مجاميع السلالات عالية ومنخفضة الأداء فى بعض الصفات المحصولية التى تم دراستها. فضلاً عن ذلك، تم تخليق العديد من الحزم الفريدة والخاصة بالسلالات عالية أو منخفضة الأداء مما أوضح فعالية هذا الواسم الجزيئي فى تعريف التراكيب الوراثية وتحديد التنوع الوراثي في القمح. أوضح تقييم صفة كفاءة استخدام الماء القدرة العالية فى التمييز بين السلالات وكان ذلك متوافقاً مع نتائج تحليل الـ SRAP حيث تكَون أعلى عدد من الحزم عندما تم مقارنة مجاميع السلالات عالية ومنخفضة الأداء لصفة كفاءة استخدام الماء. ويمكن بصورة أولية استخدام هذه الحزم المخلقة كواسمات جزيئية لإنتخاب القمح تحت ظروف الجفاف.