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Full Length Research Paper

Grain yield stability and molecular characterization of durum wheat genotypes under heat stress conditions

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The objectives of the present study are to determine the performance of six promising durum wheat genotypes for yield stability under heat stress conditions and to compare the application and utility of SRAP (Sequence-Related Amplified Polymorphism) and TRAP (Target Region Amplified Polymorphism) marker techniques, for analysis of genetic diversity among durum wheat genotypes under heat stress. Field experiments were conducted for four sowing dates, over two seasons, to expose genotypes to different levels of heat stress during the grain-filling period. Grain yield and yield attributes during the grain filling period were investigated. Results indicated that significant variations were observed among different durum wheat genotypes in respect of all yield attributes. The effect of sowing date on the relative grain yield of durum genotypes was of greater magnitude than the effect of year. On the other hand, under the fourth sowing date (20th January), where heat stress was imposed, line KSUDW104 was the best performing line (3.26 ton/ha) out yielding Benysowef (2.21 ton/ha) by 47.5% and Kronos (2.41 ton/ha) by 35.3%. This line should be recognized as heat tolerant germplasm. The regression coefficients were significant for the six durum genotypes which indicated that they were highly responsive to the change in the average productivity of the growing season. SRAP and TRAP markers, were assayed to determine the genetic diversity of 6 durum wheat genotypes. In SRAP analysis, 45 out of 128 bands (35.16%) were polymorphic while in TRAP analysis, 22 out of 55 bands (40.0%) were polymorphic. The dendrogram based on SRAP markers differed from that based on TRAP markers.

Key words: Durum wheat**,** genetic diversity, heat tolerance, SRAP markers, TRAP markers, yields stability.

INTRODUCTION

Durum wheat currently represents 8-10% of the wheat grown and produced worldwide (FAO STAT data, 2006). However, it is concentrated in relatively small geo-graphical areas where it often plays a major role in the food security of urban populations and in the livelihood and nutrition of urban communities. More than 80% of the spring durum cultivars released in the developing world, covering more than 50% of the area planted with this crop, are semi-dwarf types, either from CIMMYT (International Maize and Wheat Improvement Center) crosses or from crosses involving at least one CIMMYT parent (Lantican et al., 2005).

The productivity of durum wheat is often limited by an array of abiotic stresses that affect a successful growth and a complete grain filling. Heat stress, due to increased temperature, is an agricultural problem in many areas in the world (Wahid et al., 2007).

Post-anthesis high tempe-rature stress in wheat is a major cause of yield reduction in some regions in Saudi Arabia as well as in many wheat-growing regions of the world. Some attempts to develop heat-tolerant genotypes via conventional plant breeding protocols have been successful (Ehlers and Hall, 1998; Camejo et al., 2005) and via molecular breeding which provided additional tools to develop crops with improved heat tolerance(Al-Doss et al., 2009).

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Table 1. List of genotypes.

No.	Name	Pedigree	Origin
1	KSUDW 101	L14\Benyswef-7-17-1	Plant Production Department
2	KSUDW 102	L18\Benyswef- 3-22-2	Plant Production Department
3	KSUDW 103	Stork\Benyswef-34-2-3	Plant Production Department
4	KSUDW 104	Sham1\Benyswef-57-9-5	Plant Production Department
5	Benysowef	Cultivar	Egypt
6	Kronos	Cultivar	USA

Grain yield stability is one of the most important goals of agriculture research, especially in the sub-tropical environment. The ideal wheat genotype should be high yielding under any environmental conditions, but as genetic effects are not independent of environmental effects, most genotypes do not perform satisfactorily in all environments (Carvalho et al., 1983). When interaction between genotype and environment occur, the relative ranking of cultivars for yield often differs when genotypes are compared over a series of environments and/or years.

This poses a serious problem for selecting geno-types significantly superior in grain yield (Stafford, 1982). Various statistical techniques have been developed to identify systematic variation in individual genotypic responses. Among these, Eberhart and Russell (1966) model has been widely used in studies of adaptability and stability of plant materials (Espitia-Rangel et al., 1999; Rharrabti et al., 2003).

Therefore, the choice of an adequate model to measure the stability of different genotypes is a question to be resolved by researchers. According to Crossa et al. (1988), the selection of superior genotypes in a plant-breeding program is based mainly on their yield potential and stable performance over a range of environmental conditions. Molecular markers provide an excellent tool for obtaining genetic information and their use in the assessment of genetic diversity in wheat has increased in the last few years (Manifesto et al., 2001; Roy et al., 2004).

Molecular markers are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification, early in plant development (Barakat et al., 2010). Recently, the new types of molecular markers, sequence-related amplified polymorphism (SRAP) and target region amplification polymorphism (TRAP), were developed and used in genetic mapping (Li and Quiros, 2001; Hu and Vick, 2003; Liu et al., 2005; Wang et al., 2005).

The objectives of the study are to (1) determine the potential of promising durum wheat genotypes for yield stability under heat stress conditions and (2) compare the application and utility of SRAP and TRAP marker techniques, for analysis of genetic diversity among durum wheat genotypes under heat stress.

MATERIALS AND METHODS

Field trails and traits evaluation:

Six genetically diverse durum wheat genotypes were used in this study. These included the two check cultivars as well as four advanced lines (F_9) (Table 1), selected from the wheat breeding program at the Plant Production Department, College of Food and Agriculture Sciences, King Saud University, Saudi Arabia. They were evaluated phenotypically for heat tolerance under four sowing dates (20th of October, November, December and January), over two season (2005 / 2006 and 2006 / 2007) to expose genotypes to different levels of heat stress during the grain-filling period. The first two dates represent the normal conditions while the other two dates are considered stress conditions in Saudi Arabia. The seeding rates were 160 kg/ ha. The fertilizers were applied at the rate of 120 kg N and 80 kg P_2O_5 per hectare. The cultural practices were carried out according to the recommended practices followed in Riyadh area.

Ten agronomic traits were scored for the durum wheat genotypes. These are heading date(DH), maturity date(DM), grain filling period(FP), plant height(PH), grain yield(GY), harvest index(HI), spike number per m^2(NS/m^2), kernels per spike(NG/S), 1000- kernel weight grain (KW) and number of tillers (NT/m^2).Grain yield was determined from the central rows and converted to grain yield per hectare. Spike number was determined by counting the number of grain-bearing tillers in an area of 50×50 cm and converted to number per m^2. Kernels per spike was determined in spikes of 10 random tillers which were hand threshed and number of kernels were counted and recorded as the average number of kernels per spike. Filling period was calculated by subtracting the number of days to heading from the number of days to maturity.

Statistical analysis

The layout of the experiment was a split-plot design with four replications. The four sowing date were assigned to the main plots while the six durum genotypes were allocated to the sub plots. Each sub plot consisted of 4 rows, 2 m long and 20 cm wide.

Data from the growing seasons were statistically analyzed using the ANOVA procedure for split-plot design, combined over the two years using the SAS program (1992). Each combination of year and sowing date was considered as one distinct environment. Genotype means for the eight environments were subjected to stability index analysis, as given by Eberhart and Russell(1966). Stability parameters were estimated using regression analysis of genotype means over an environmental index, estimated as the mean of all genotypes at a specific environment, minus the grand mean. By regressing the mean yield of each genotype upon the environmental index, a regression coefficient (b) and deviations from regression mean squares (S^2d) were obtained as parameters for evaluating the stability of yield over different environments. Correlation coefficients between the different characters were calculated using the individual plot values.

Table 2. Significance of the combined analysis of variance for the effect of seasons, sowing dates and genotypes on yield and agronomic traits of durum wheat.

SOV	GY	KW	NG/S	NS/m^2	HI	NT/m^2	PH	DH	DM	FP
Years(Y)	**	n.s	**	**	n.s	**	**	**	**	*
Sowing Dates(D)	**	**	**	**	**	**	**	**	**	**
Y X D	**	*	**	**	**	*	n.s	n.s	**	n.s
Genotypes (G)	**	**	**	**	**	**	**	**	**	**
GXY	*	n.s	n.s	**	*	**	n.s	n.s	**	n.s
GXD	**	n.s	**	**	**	**	**	**	**	**
GXDXY	n.s	**	n.s	**	**	**	n.s	n.s	n.s	n.s

n.s ,*,** indicate not significant and significant at 0.05 and 0.01 levels of probability, respectively; GY=grain yield (ton /h); KW=1000-kernel weight (gm); NG/S=No. of grains /spike; NS/m^2= No. of spikes/m2;HI= Harvest index (%);NT/m^2=No. of Tiller /m^2 ;PH=plant height (cm);DH=days to 50% heading; DM=days to 75% maturity; FP=filling period.

Molecular characterization

DNA extraction

Frozen young leaves (500 mg) were ground to powder in a mortar centrifuged for 10 min at 3200 rpm. The supernatants were pipetted off into new tubes and 6 ml isopropanol was added. After 60 min, the tubes were centrifuged for 10 min and the obtained pellets were put in sterile Eppendorf tubes, containing 400 µl of TE buffer of a pH 8.0 (10 mM Tris-HCl, pH 8.0 + 1.0 mM EDTA, pH 8.0). The DNA's from genotypes were then extracted and stored at -20°C until use.

SRAP and TRAP analysis

A total of 19 primers (Table 8) were used in SRAP analysis, and 9 primers were used in TRAP analysis (Table 9), from Pharmacia Biotech. (Amersham Pharmacia Biotech UK Limited, England HP79 NA). PCR amplification for SRAP and TRAP was carried out in a 20 *l* reaction mixture containing 1 x buffer, 1.5 mmol l^{-1}MgCl2, 0.1 mmol l^{-1} dNTPs, 500 nmol l^{-1} primer, 1U Taq polymerase, and 50–60 ng template DNA. After 5 min at 94°C , 5 cycles were performed with 1 min at 94°C, 1 min at 35°C, 1 min 40 s at 72°C, then 35 cycles the same as the previous, except for the annealing temperature at 50°C and a final 7 min at 72°C. Amplification products were electrophoretically resolved on 1.5% agarose gels containing 0.1 µg/ml ethidium bromides, and photographed on a UV trans-illuminator.

Data handling and cluster analysis

Data were scored for computer analysis on the basis of the presence of the amplified products for each primer. If a product is present in a cultivar, it will be designated as "1", if absent, it will be designated as "0", after excluding the unreproducible bands. Pair-wise comparisons of cultivars, based on the presence or absence of unique and shared polymorphic products, was used to determine similarity coefficients, according to Jaccard (1908). The similarity coefficients was used to construct dendograms, using the unweighted Pair Group Method with Arithmetic Averages (UPGMA), employing the SAHN (Sequential, Agglomerative, Hierarchical and Nested clustering) from the NTSYS– PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics) program (Rohlf, 1993).

RESULTS AND DISCUSSION

Field evaluation

Yield and other characters

Significant differences were found between the seasons for all traits except 1000-grain weight and harvest index. Sowing date was also significant for all traits and their interaction with seasons were also significant for all traits except heading date, grain filling period and plant height (Table 2). Significant variation was observed among different durum wheat genotypes in respect of all yield attributes. The interaction between sowing dates and genotypes was also significant for all traits except 1000-kernel weight (Table 2) .The effect of sowing date on the relative grain yield of durum genotypes was of greater magnitude than the effect of year.

Means of the sowing dates averaged over six durum wheat genotypes, for the agronomic traits under study are presented in (Table 3). All traits varied considerably among sowing dates for yields and other characters in the sowing date (November 20th) being superior to the other sowing dates .This superiority could have been due to the moderate temperatures and was the coolest sowing date especially during the critical stage of grain filling. This was reflected on the most agronomic traits (Table 4). The differences between sowing dates in grain yield were mainly due to No. of grains /spike, No. of spikes/m^2 and No. of Tiller /m^2 and these are temperature-dependent which means that cool temperature will extend the periods of vegetative growth and longer for the flowering date and consequently would be expected to give high grain yield. The reduction in grain yield and other associated traits in the other sowing dates could have been due to higher temperature in March through May. This was brought about by shorter periods of heading date, days to 75% maturity and filling period as a result of higher temperatures (Table 3).

The differences among the six durum genotypes were highly significant for the ten agronomic traits (Table 2).

Table 3. Sowing date means for the agronomic traits averaged over genotypes and years.

Sowing date	GY	KW	KN/S	SN/m^2	HI	TN/m^2	PH	DH	DM	FP
October 20th	4.96b	62.22a	40.75d	601.7b	37.02a	640.21b	79.01b	71.38b	144.69a	73.29a
November 20th	6.05a	53.06b	50.42b	809.4a	37.82a	840.00a	84.67a	78.33a	124.69b	46.35b
December 20th	6.26a	46.40c	53.72a	783.1a	38.89a	822.50a	76.95b	72.38b	115.83c	4344b
January 20th	2.66c	30.70d	45.56c	534.2c	28.28b	568.96c	65.64c	62.68c	96.85d	34.15c
LSD $_{0.05}$	0.61	2.70	2.43	30.8	2.31	33.19	2.027	3.46	1.06	3.51

[a] Means followed by same letter(s) are not significantly different according to LSD at 0.05 level of probability. GY=grain yield (ton /h); KW=1000-kernel weight (gm); KN/S=No. of grains /spike; SN/m^2= No. of spikes/m2; HI= Harvest index (%);TN/m^2=No. of Tiller /m^2; PH=plant height (cm); DH=days to 50% heading; DM=days to 75% maturity; FP=filling period.

Table 4. Means of agronomic traits for six wheat genotypes over eight environments.

Genotype	GY	KW	KN/S	SN/m^2	HI	TN/m^2	PH	DH	DM	FP
KSUDW 101	4.98b	49.64b	49.8ab	708.8ab	34.3bc	751.9a	78.4a	72.13b	121.3a	49.2bc
KSUDW 102	5.22ab	47.54c	47.9bc	697.2ab	36.3a	731.7ab	77.1ab	72.50b	120.7bc	48.2cd
KSUDW 103	4.53c	43.41d	47.3c	715.3a	34.1c	756.4a	76.1b	69.63c	120.3c	50.6ab
KSUDW 104	5.53a	47.54bc	51.1a	687.5b	36.7a	717.5bc	78.3a	71.63bc	120.4c	48.8bc
Benysowef	5.27ab	48.48bc	46.7c	659.4c	35.8ab	693.1c	77.6ab	74.81a	121.1ab	46.3d
Kronos	4.38c	52.03a	42.8d	624.4d	35.9a	657.8d	72.0d	66.53d	119.3d	52.7a
LSD 0.05	0.37	2.13	2.1	27.4	1.6	28.4	1.7	2.1	0.6	2.1

[a] Means followed by same letter(s) are not significantly different, according to LSD at 0.05 level of probability. GY=grain yield (ton /h); KW=1000-kernel weight (gm); KN/S=No. of grains /spike; SN/m^2= No. of spikes/m2; HI= Harvest index (%); TN/m^2=No. of Tiller /m^2; PH=plant height (cm); DH=days to 50% heading; DM=days to 75% maturity; FP=filling period.

Table 5. Combined analysis of variance of grain yield for durum genotypes according to Eberhart and Russell (1966).

Source of variation	D.F.	M.S.
Environment (E)	7	80.8**
Genotypes (G)	5	6.4**
E x G	35	1.5**
Environment(E)+G x E	42	14.68**
Environment (Linear)	1	565.3**
G x E (Linear)	5	8.72**
Pooled deviation	36	0.214
Pooled error	120	0.548

Also, highly significant interactions between sowing dates and durum genotypes were found except 1000-kernel weight, indicating that some genotypes ranked differently during growing seasons for the nine traits. The KSUDW 103 and Kronos had the lowest values of the most traits. However, Kronos cultivar had the maximum values for 1000-grain weight and grain filling period (Table 4).

On the other hand, under the fourth sowing date (20th Jan.), where heat stress was revealed, line KSUDW104 was the best performing line (3.26 ton/ha) out yielding Benysowef (2.21 ton/ha) by 47.5% and Kronos (2.41 ton/ha) by 35.3% (Table 6). This line should be recognized as heat tolerant germplasm. These results are in general agreement with those reported by several investigators (Purchase et al., 2000; Cooper et al, 2001; Asif et al., 2003; Matus-Cadiz et al., 2003; Amin et al., 2005; Okuyama et al., 2005), whose results indicated that there are genotypic variations for heat tolerance and demonstrated that the reaction to heat stress varied from one genotype to another.

Yield stability

The combined analysis of variance of grain yield is given in Table 5. The stability analysis suggested by Eberhart and Russell (1966) showed significance for environments (E), genotypes (G), environments (linear) and genotype by environment (G x E) linear interaction. The mean squares of environments were of greater magnitude than genotypes and the G x E interaction. The significant G x E (linear) interactions in all combined analyses indicated that the linear grain yield response of genotypes was not the same at various environments. The pooled deviations were also highly significant when tested against the pooled experimental error (Table 5). These results are in agreement with those found by several investigators (Patil et al., 1992; Pecettil and Annicchiarico, 1993;

Table 6. Mean grain yield for six durum genotypes under four sowing dates and their stability statistics.

Genotypes	Sowing date				Overall mean	Stability statistics b	
	Oct. 20th	Nov. 20th	Dec. 20th	Jan.20th		S^2d	C.V.
KSUDW101	4.69bc	6.01b	6.56a	2.68ab	4.99b	1.08**	0.20
KSUDW102	5.53ab	5.91b	6.65a	2.78ab	5.22ab	1.09**	0.21
KSUDW103	4.00c	5.91b	5.59b	2.60ab	4.53c	0.81**	0.25
KSUDW104	5.13b	7.10a	6.61a	3.26a	5.53a	1.03**	0.24
Benysowef	6.34a	5.74b	6.78a	2.21b	5.27ab	1.11**	0.84
Kronous	4.06c	4.06c	5.39b	2.41b	4.38c	0.88**	0.08

Means followed by same letter (s) are not significantly different, according to LSD at 0.05 level of probability.

Table 7. Phenotypic correlation coefficients between the nine characters under study.

Traits	GY	KW	KN/S	SN/m^2	HI	TN/m^2	PH	DH	DM
KW	0.47**	-							
KN/S	0.53**	-0.15*	-						
SN/m^2	0.61**	0.14ns	0.65**	-					
HI	0.67**	0.55**	0.18**	0.19**	-				
TN/m^2	0.59**	0.14ns	0.56**	0.99**	0.19**	-			
PH	0.69**	0.52**	0.41**	0.67**	0.36**	0.68**	-		
DH	0.65**	0.37**	0.40**	0.58**	0.38**	0.56**	0.74**	-	
DM	0.43**	0.87**	-0.17*	0.18**	0.47**	0.18**	0.57**	0.43**	-
FP	12 ns	0.76**	-0.41**	-0.11ns	0.31**	-0.10ns	0.23**	-.59**	0.89**

n.s,*,** indicate not significant and significant at 0.05 and 0.01 levels of probability, respectively; GY=grain yield (ton /h); KW=1000-kernel weight (gm); KN/S=No. of grains /spike; SN/m^2= No. of spikes/m2; HI= Harvest index (%);TN/m^2=No. of Tiller /m^2 ;PH=plant height (cm);DH=days to 50% heading; DM=days to 75% maturity; FP=filling period

Pecettil et al., 1994; Boggini et al., 1997; Ozkan et al., 1998).

The values of grain yield, regression coefficient (b) and regression deviation (S^2d) of the six durum genotypes are given in (Table 6). These parameters were studied separately for each genotype in groups of environments. According to the Eberhart and Russell (1966) model, a stable cultivar is one with a high mean yield, unit regression coefficient (b=1) and deviation from regression as small as possible ($S^2d = 0$).The regression coefficients were significant for the six durum genotypes which indicated that they were highly responsive to the change in the average productivity of the growing season. These results are in agreement with those found by several investigators (Kakar et al., 2003; Amin et al., 2005; Akcura et al., 2005; Okuyama et al., 2005). In the analysis of 8 environments, the genotype KSUDW 104 was the most productive and stable genotype in all groups of environments, followed by KSUDW101 and KSUDW102 genotypes. Kronos is the recommended variety for Saudi Arabia; however, it was the lowest yielding variety, as an average of the eight environments. This variety was the earliest among the tested genotypes (Table 4) due to short vegetative growth period and consequently reduced filling rate. Therefore, the KSUDW104 would be recommended as promising variety and need further testing in the Central Region of Saudi Arabia.

The relationships between grain yield and other agronomic traits for the eight environments were expressed in terms of pooled phenotypic correlation coefficients (Table 7). A highly significant correlation was found between GY and each of SN/m^2 (0.61**), HI (0.67**), PH (0.69**) and DH (0.65**), whereas, an insignificant correlation was found with FP (Table 7). Moreover, the highest significant values for correlations were found between TN/m^2 and SN/m^2 (0.99**), FP and DM (0.89**) and DM and KW (0.87**).

Molecular characterization

Identification and evaluation of SRAP and TRAP markers for diversity estimates

Nineteen primers were screened for their ability to amplify the genomic DNA from 6 durum wheat genotypes. The number of amplified DNA fragments ranged from 0.0 to 17.0 depending on the primer and the DNA sample, with a mean value of 6.7 bands per primer (Table 8). In the present investigation, the size of fragments ranged from 100 to 1300 bp. A total of 128 fragments were produced by the 19 primers. Of these 128 amplified fragments, 65.0% were not polymorphic while 35.0% were

Table 8. Number of amplifications and polymorphic products, using nineteen SRAP primers in durum wheat cultivars.

Primer number	Nucleotide sequence (5` - 3`)		No. of Amplifications a	No. of polymorphics b	Polymorphism b/a (%)
	Forward primers	Reverse primers			
1	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTCTG	6	1	16.66
2	TGAGTCCAAACCGGTCC	GACTGCGTACGAATTGTC	8	1	12.5
3	TGAGTCCAAACCGGTCA	GACTGCGTACGAATTAAT	5	1	20.0
4	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTCGA	4	3	75.o
5	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTCAG	11	5	45.45
6	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTTGA	11	5	45.45
7	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTGCA	0	0	0.0
8	TGAGTCCAAACCGGTTG	GACTGCGTACGAATTGGT	8	2	25.0
9	TGAGTCCAAACCGGTCA	GACTGCGTACGAATTCGA	7	2	28.57
10	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTCAA	17	10	58.82
11	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTTGC	2	1	50.0
12	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTGGT	14	7	50.0
13	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTGAC	5	2	40.0
14	TGAGTCCAAACCGGACC	GACTGCGTACGAATTTAG	6	0	0.0
15	TGAGTCCAAACCGGACC	GACTGCGTACGAATTCAG	5	1	20.0
16	TGAGTCCAAACCGGACC	GACTGCGTACGAATTAGC	5	1	20.0
17	TGAGTCCAAACCGGACC	GACTGCGTACGAATTTAG	5	1	0.0
18	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTTGA	4	1	25.0
19	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTTCG	5	1	40.0

Table 9. Number of amplifications and polymorphic products, using nine TRAP primers in durum wheat cultivars

Primer number	Nucleotide sequence (5` - 3`)		No. of amplification a	No. of polymorphics b	Polymorphism b/a (%)
	Fixed primers	Arbitrary primers			
1	TGAGTCCAAACCGGT	TCACCCGCACCTTCTCC	5	0	0.0
2	TGAGTCCAAACCGGC	CGGACAGTGGCGGAGTTA	6	2	33.33
3	TGAGTCCAAACCGGC	GGCGAACTCCGACATCTT	5	4	80.0
4	TGAGTCCAAACCGGC	GAGGAAGACGACGAGGT	10	8	80.0
5	TGAGTCCAAACCGGA	TTCTTCCTCCCGCTCATT	7	3	42.85
6	TGAGTCCAAACCGGT	CCCTCCACCAATCACAAT	6	1	16.66
7	AGTAACCCACCGCTTC	TCCTACAAACATTGCCTT	3	0	0.0
8	TGCCGCTTCCAACAAA	TCACCCGCACCTTCTTCC	8	3	37.5

polymorphic among the 6 durum wheat genotypes. Primer SRAP-4 generated the greatest polymorphism (75.0%), while the lowest level of polymorphism (0.0%) was obtained by primers SRAP-14 and SRAP-17. Out of the 19 primers, 4 revealed more than 50% polymorphism (Table 8). Figure 1 shows the amplification profiles, generated by primer SRAP-5 across the 6 durum wheat genotypes, all of which had distinguishable banding patterns. Polymorphism between genotypes can arise through nucleotide changes that prevent amplification by introducing a mismatch at one priming site; deletion of a priming site; insertions that render priming sites too distant to support amplification and insertions or deletions that change the size of the amplified product (Williams et al., 1990). SRAP is a PCR-based DNA marker system that generates multiple fragments in a single PCR reaction (Li and Quiros, 2001). SRAPs amplify several reproducible and polymorphic loci and alleles and they may amplify functional genes since they are in related sequence. SRAP markers possess multi-loci and multi-allelic features, which make them potentially more efficient for genetic diversity analysis, gene mapping and finger-printing genotypes. However, SRAP markers may not be randomly distributed across the genome (Li and Quiros, 2001).

Nine TRAP primers were used to amplify DNA segments from 6 durum wheat genotypes. The number of amplified bands per primer varied between 3 and 10 (Table 9). A total of 55 bands were observed, with 6.11 bands per primer. 22 out of the 55 bands (40%) were

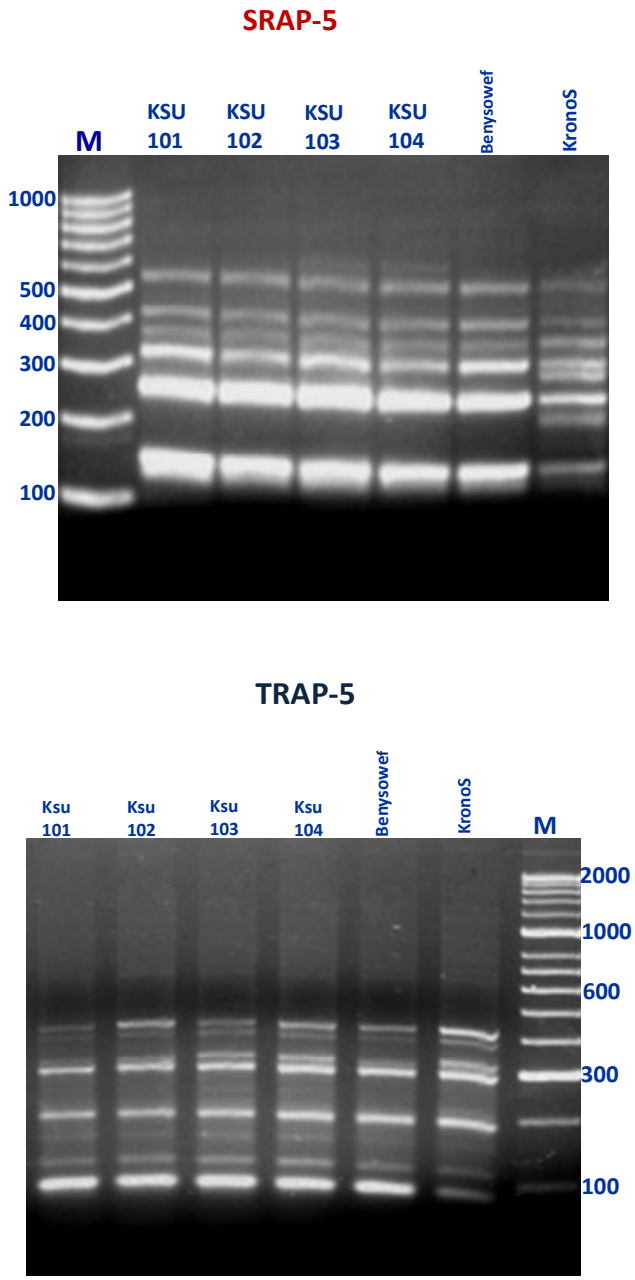

Figure 1. Polymorphism revealed using primer SRAP-5 and primer TRAP-5 to amplify genomic DNA purified from durum wheat genotypes.

polymorphs. An example of polymorphism is shown in Figure 1 which shows the amplification profiles, generated by primer TRAP-5 across the 6 durum wheat genotypes, all of which had distinguishable banding patterns. Previously, Hu and Vick (2003) developed a new marker technique known as target region amplified polymorphism (TRAP), which is a rapid and efficient PCR-based technique that employs two 18-mer primers. One ‘‘fixed’’ primer is designed from a known expressed sequence tag (EST) , while the other primer is arbitrary with either an AT- or GC- rich core to anneal with an intron or exon , respectively. Xu et al. (2003) used TRAPs to characterize genetic stocks of tetraploid wheat (*Triticum turgidum* L., 2n =4x =2 8, AABB genomes) and found that a large number of chromosome- specific markers could be generated with this technique. The results indicated that TRAPs might be suitable for rapidly mapping the wheat genome .Recently, Liu et al. (2005) reported that TRAP markers were very efficient for rapidly generating a large number of markers scattered across the genome, which allowed linkage groups to be joined and many gaps to be filled. TRAPs also showed

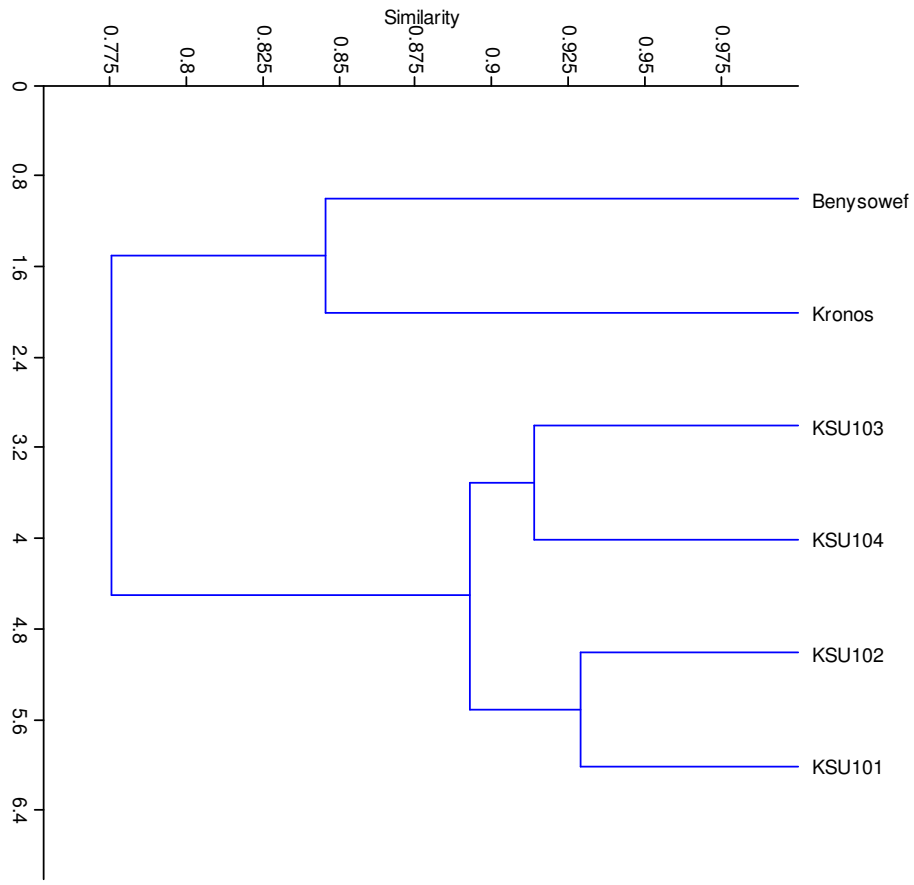

Figure 2. Dendrogram based on Jaccard similarity coefficient of 6 durum wheat genotypes, generated using SRAP markers.

the same ability as SSRs to assign linkage groups to chromosomes.

Genetic diversity of molecular markers

The relationships among durum wheat genotypes were estimated by a UPGMA cluster analysis of genetic similarity matrices. The composition of clusters obtained using SRAP markers alone (Figure 2) and TRAP markers alone (Figure 3), revealed similar groupings in some cases.

Cluster analysis using SRAP data, grouped the 6 durum wheat genotypes into two main clusters with Jaccard's similarity coefficient ranging from 0.71 to 0.93 (Figure 2). The highest similarity was found between 'KSUDW102' and 'KSUDW101' (0.93) and the lowest was between 'KSUDW103' and 'Kronos' (0.71). The first cluster included the commercial cultivars 'Benysowef' and Kronos, which are heat sensitive while the second cluster included the new durum wheat genotypes 'KSUDW101,KSUDW102,KSUDW103 and KSUDW104', which are more closely related with each other .These wheat genotypes had one parent in common (Benysowef) (Table 1) and subsequently, they clustered together. Previously, SRAP markers have been used to detect the genetic diversity of some accessions of *Cucurbita maxima* from Spain (Ferriol, 2003). SRAP markers were employed to examine their potential for genetic diversity analyses in hard red winter wheat (Fufa et al., 2005). The potential of the sequence-related amplified polymorphism (SRAP) technique, which preferentially amplifies gene-rich regions, was evaluated to assess the genetic relationships among members of the *Saccharum* species (Suman et al., 2008). Recently, Wang et al. (2009) reported that SRAP is a new molecular marker which could provide high polymorphism and plentiful in formation. It is simple and has not the species-specific character. It had been widely used for genetic diversity, comparing genome analysis and map construction.

The dendrogram generated from TRAP data clearly indicated two main clusters (Figure 3). The Jaccard similarity coefficient ranged from 0.46 to 0.84. Maximum similarity was found between 'KSUDW101' and 'KSUDW102'. The first cluster included the adapted commercial cultivar Kronos alone. The second cluster included the new durum wheat genotypes 'KSUDW101,

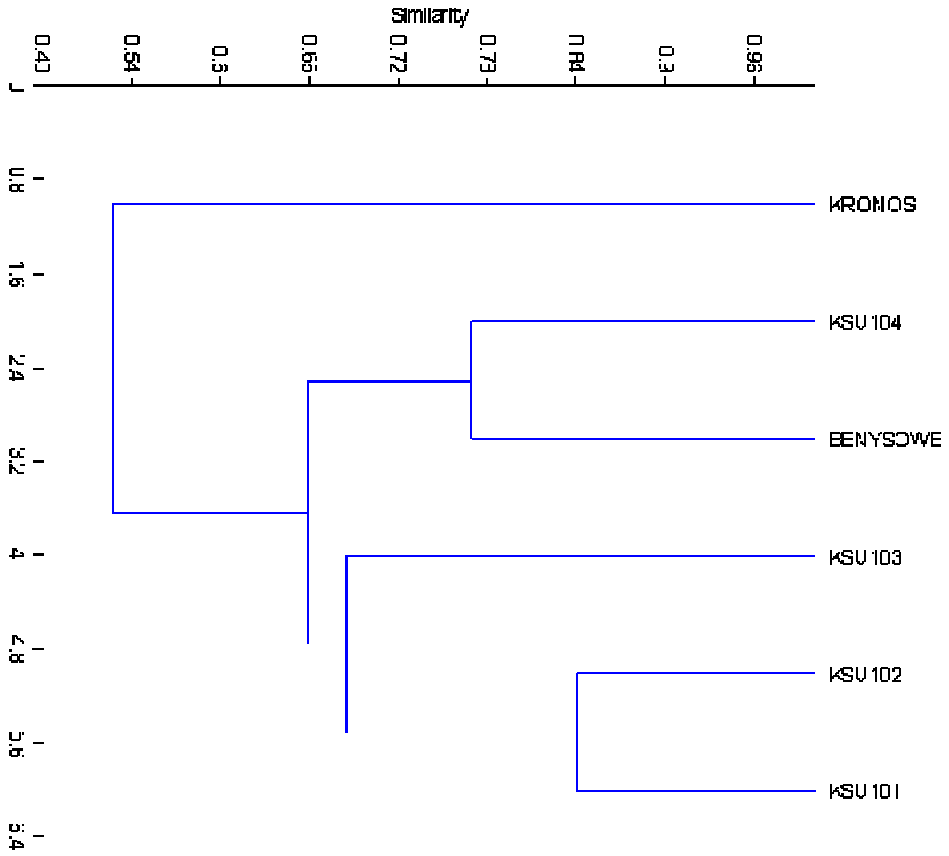

Figure 3. Dendrogram based on Jaccard similarity coefficient of 6 durum wheat genotypes, generated using TRAP markers.

KSUDW102, KSUDW103 and KSU101, which are more closely related with each other as well as the commercial cultivar, Benysowef.

These new durum wheat genotypes had one parent in common (Benysowef) (Table 1) and subsequently, they clustered together. Previously, TRAP was successfully used to estimate the genetic diversity in genetic stocks of tetraploid wheat (*T. turgidum* L., 2n =4x =2 8, AABB genomes) (Xu et al., 2003).

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