Detection of wheat (*Triticum aestivum*) cultivars with contrasting performance under abiotic stresses

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Abstract: Drought tolerance is a complex trait of increasing importance. The present study aims at detecting molecular markers in eight bread wheat (*T. aestivum*) cultivars, based on ISSR and AFLP, differing in their performance under drought stress. Wheat is one of the most important cereal crops in the world. Therefore, the identification of molecular markers for drought tolerance is crucial for the future development of tolerant varieties through breeding. Across the two types of markers, a total of 109 cultivar-specific markers were recovered. Most markers were resulted for Sahel 1 cultivar indicating the genetic distance between it and the other wheat cultivars. AFLP analysis resulted in higher level of polymorphism indicating its efficacy in separating closely related germplasm. A number of 49 markers, across both types of markers, for drought tolerance were recovered. A few markers to link cultivars with possible share of a common ancestor were also recovered. The polymorphism information content (PIC) and average of heterozygosity (H_e) indicated no preference for any of ISSR or AFLP types of markers. The effective multiplex ratio (E), and the marker index (MI) indicated that AFLP revealed higher values. The results of ISSR data analysis indicated the suspicion in utilizing this type of marker in detecting genetic relatedness among bread wheat cultivars unless high number of markers is recovered. Some of the markers generated across both types of analyses can be utilized in breeding for drought stress tolerance in bread wheat via marker-assisted selection (MAS).

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1. Introduction

Wheat breeders have been able to alter wheat life cycle enabling cultivars to grow in diverse agro ecological regions in the world. These changes help crops avoid/tolerate stresses at critical development stages, thereby, improving their yield potential and water-use efficiency (William *et al*., 2007). Selection for drought tolerance through visible phenotypic drought tolerance traits is time consuming. The use of molecular markers to identify and locate genomic regions that control drought tolerance traits may accelerate selection (Monneveux *et al*., 2013). Future progress in breeding for yield potential and crop adaptation will be constrained by a number of factors; including available genetic variability for yield enhancing traits and the complexity of inheritance of economically important traits such as yield potential and drought tolerance (William *et al*., 2007). In this context, this paper explores the use of molecular markers in wheat improvement, with emphasis on using MAS as a tool that can help wheat breeders meet the challenge in the future.

Progress in plant breeding and cultivar identification mostly relies on morphological characteristics that require extensive observations of individuals (Wrigley *et al*., 1987). Factors, like the environment, multigenic and quantitative inheritance or partial and complete dominance virtually confound gene expression. Although protein and isozyme markers were used in many crops, major limitations are the lack of polymorphism among closely-related genotypes and the variation of protein content and type among different tissues and developmental stages under different environmental conditions (Beckmann and Soller, 1983). DNA-based genetic markers are recently integrated into several plant systems and expected to play a very important role in the future of plant breeding (marker assisted selection or MAS) and molecular genetics analysis.

Polymerase chain reaction (PCR) was initiated as a genetic assay based on selective DNA amplification (Saiki *et al*., 1988; Innis *et al*., 1990). Among the different types of PCR-based molecular markers, inter simple sequence repeats (ISSRs) were developed as an anonymous approach accessing variation in the numerous microsatellite regions dispersed throughout the genome (Zietkiewicz *et al*., 1994). ISSRs are based on the amplification of DNA regions between inversely oriented SSRs or microsatellites (Bussell *et al*., 2005). The ISSR markers are simple and reproducible. They require small amounts of DNA

and do not require information on DNA sequence. ISSR primers are designed from SSR motifs and can be undertaken for any plant species containing a sufficient number and distribution of SSR motifs in the genome (Buhulikar *et al*., 2004). Therefore, ISSRs are widely used in many respects such as the study of genetic diversity in barley (Brantestem *et al*., 2004) and cultivar identification in tobacco (Denduangboripant *et al*., 2010). Microsatellites are very short stretches of DNA that are "hypervariable", expressed as different variants within populations and among different species. They are characterized by mono-, di- or trinucleotide repeats that have 4-10 repeat unit side-by-side (Morgante and Olivieri, 1993). Amplified fragment length polymorphism (AFLP) utilizes fragments of DNA amplified using primers from restriction digested genomic DNA (Vos *et al*., 1995). AFLP provides the highest levels of resolution to allow delineation of complex genetic structures, to differentiate individuals in a population in gene flow experiments, and also to register plant varieties (Powell *et al*., 1996; Law *et al*., 1998; Barker *et al*., 1999; Aparajita & Rout, 2010; Misra *et al*., 2010).

The present study aims at exploring the usefulness of molecular markers, i.e., ISSR and AFLP, in characterizing the drought-tolerant versus drought-sensitive (*T. aestivum*) cultivars and in detecting possible cultivar-specific markers to be utilized in the future breeding for drought tolerance in wheat.

2. Materials and methods

Plant material

The study involved eight cultivars of wheat (*T. aestivum*) of Egyptian origin differ in their performance under drought stress to be compared on the molecular levels. Genotype- either positive (marker type 1) or negative (marker type 2) molecular markers were detected. Markers to detect possibly closely-related cultivars (marker type 3 for Sakha cultivars and marker type 4 for Gemiza cultivars) as well as cultivar-specific markers were also detected. Names, codes and performance under drought stress of these cultivars are shown in Table 1. Relatedness of these cultivars with no available pedigrees will be detected based on their molecular fingerprints. Seeds of each genotype were kindly provided by the Field Crops Research Institute (ARC, Egypt), where they were collected from plants in three locations (populations). Ten plants of different genotypes were selected in each location based on morphological homogeneity.

Genomic DNA extraction and purification

Extraction of total DNA was performed using the modified procedure of Gawel and Jarret (1991). The minimum number of plants to be bulked for each genotype to saturate polymorphisms within each cultivar was determined (data shown upon request). To remove RNA contamination, RNase A (10 mg/ml, Sigma, USA) was added to the DNA solution and incubated at 37°C for 30 min. Estimation of the DNA concentration in different samples was done by measuring optical density at 260 nm according to the following equation:

$$\text{Concentration (ug/ml)} = OD_{260} \text{ X 50x dilution factor}$$

PCR amplification was performed in a Perkin Elmer 2400 thermocycler (Germany), programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 37°C for 2 min, and an extension step at 72°C for 2 min, followed by extension cycle for 7 min at 72°C in the final cycle.

Table 1. Names and performance of the tested bread wheat cultivars.

Serial no.	Genotype name	Genotype
a	Misr 2	Tolerant
b	Gimeza 10	Tolerant
c	Sakha 93	Tolerant
d	Sakha 94	Tolerant
e	Sakha 95	Sensitive
f	Gimeza 9	Sensitive
g	Giza 168	Sensitive
h	Sahel 1	Sensitive

Inter simple sequence repeat (ISSR)

Thirty primers for ISSR were used in the study but only 17 were successful in generating reproducible and reliable amplicons for different genotypes. Names and sequences of the selected primers are shown in Table 2. PCR analysis was performed in 25 µl reaction and amplification (Perkin Elmer 2400 thermocycler, Germany) was programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 40°C for 2 min, and an extension step at 72°C for 2 min, followed by extension cycle for 7 min at 72°C in the final cycle.

Amplified fragment length polymorphism (AFLP)

AFLP analysis was performed using the AFLP Analysis System I (Invitrogen, cat. no. 10544-013) according to the manufacturer's protocol. Genomic DNA samples were digested with *Eco*RI and *Mse*I restriction enzymes in which *Eco*RI and *Mse*I adapters were ligated to the digested DNA fragments. Pre-amplification was carried out using *Eco*RI primer plus one extension base at the 3' position (A) and *Mse*I primer plus one extension base at the 3' position (C) to amplify fragments that contain complementary sequences. Six combinations of *Eco*RI primers plus three extension bases and *Mse*I primers plus three extension bases were used to selectively amplify the DNA fragments matching the primer-extension sequence, five of them succeeded to recover good quality polymorphic patterns. These five

combinations are: M-CCA/E-ACT, M-CAC/E-ACA, M-CAG/E-AAC, M-CTC/E-AAG and M-CAA/E-ACC.

Table 2. List of ISSR primers and their nucleotide sequences used in the present study.

No.	Name	Sequence	No.	Name	Sequence
1	814	$(CT)_8TG$	10	HB10	$(GA)_6CC$
2	844A	$(CT)_8AC$	11	HB11	$(GT)_6CC$
3	844B	$(CT)_8GC$	12	HB12	$(CAC)_3GC$
4	17898A	$(CA)_6AC$	13	HB13	$(GAG)_3GC$
5	17898B	$(CA)_6GT$	14	HB14	$(CTC)_3GC$
6	17899A	$(CA)_6AG$	15	HB15	$(GTG)_3GC$
7	17899B	$(CA)_6GG$	16	UCB-820	$(GT)_8C$
8	HB8	$(GA)_6GG$	17	UCB-827	$(AC)_8G$
9	HB9	$(GT)_6GG$			

Detection of PCR products

The products of ISSR were detected using electrophoresis on agarose gel (1.2% in 1x TBE buffer), stained with ethidium bromide (0.3 ug/ml), then visually examined with UV transilluminator and photographed using a CCD camera (UVP, UK). AFLP products were detected by capillary electrophoresis and virtual gels were prepared and analyzed. Fragments were separated and sized on an ABI 3500 DNA Sequencer (Applied Biosystems, Foster city, California). Using the program Genemapper 4.1 (Applied Biosystems), a genetic fingerprint was produced for each individual sample by scoring for the presence or absence of a standardized set of markers between 50 and 600 base pairs in size (Rogers, 2008).

Data analysis

The bands recovered by different techniques were considered reproducible and scorable only after observing and comparing them in three separate amplifications for each primer. Clear, unambiguous and reproducible bands recovered through different techniques were considered for scoring. Each band was considered a single locus. Data were scored as (1) for the presence and (0) for the absence of a given DNA band. Band size was estimated by comparing with 100-bp ladder (Bioron, Germany) using Gel Works 1D advanced gel documentation system (UVP, UK). The binary data matrices were entered into the TFPGA (Ver. 1.3) and analyzed using qualitative routine to generate similarity coefficient. Dissimilarity coefficients were used to construct a dendrogram using un-weighted pair group method with arithmetic average (UPGMA) and sequential hierarchical and nested clustering (SHAN) routine.

Matrix comparison

Similarity matrix produced by ISSR and AFLP were compared based on the genetic distance of the TFPGA, the normalized Mantel statistic (Mantel, 1967). The PIC (polymorphism information content) was calculated by applying the following formula given by Powell *et al*. (1996) and Smith *et al*. (1997):

$$PIC = 1 - \sum_{i=1}^{n} fi^2$$

Where, *fi* is the frequency of the i^{th} amplicon. The number of amplicons refers to the number of scored bands. The frequency of an amplicon was obtained by dividing the number of cultivars, where it was found, by the total number of cultivars. The PIC value provides an estimate of the discriminating power of a marker. Marker index (MI) was calculated for each primer as the product of PIC and the number of polymorphic bands.

Analysis of molecular variance (AMOVA)

Analysis of molecular variance (AMOVA) is a method of estimating population differentiation directly from molecular data and testing hypotheses about such a differentiation. A variety of molecular marker data (for example, ISSR or AFLP), direct sequence data, or phylogenetic trees may be analyzed using this method (Excoffier *et al*., 1992). AMOVA was performed using GENALEX 6 (genetic analysis in excel, Peakall and Smouse, 2006) in ISSR and AFLP to partition the total molecular variance between and within populations.

3. Results and Discussion

In this work, the two marker types, namely ISSR and AFLP were utilized to analyze eight cultivars of bread wheat (Table 1) differing in their origin and performance under drought stress. We estimated the optimal number of primers for ISSR or primer combinations for AFLP required in discriminating among genomic DNAs of different plant genotypes based on the reproducibility of data and level of polymorphism obtained by each type of molecular analysis (ex., ISSR, AFLP). The argument of the required value of genetic distance to classify correlated plants accessions as distinct cultivars have been raised (Cabrita *et al*., 2001; Papadopoulou *et al*., 2002). In the present study, 17 primers for ISSR and five combinations for AFLP with informative patterns were selected (samples of them are shown in Figures 2 & 3, respectively). Selection of ISSR primers and AFLP combinations was based on the number of amplicons recovered through PCR and the stability (or reproducibility) of the patterns. These ISSR

primers and AFLP combinations were used in the characterization of eight genotypes belonging to bread wheat. Less than 7% intra-plant polymorphism (within) was found across the two types of analyses for the plants of the same genotype (data provided upon request). As being dominant markers, pooling (bulk DNA) strategy in ISSR and AFLP analyses is thought to be ideal for saturating such an intra-plant polymorphism with no effects on the accuracy of the obtained results. Mengoni *et al*. (2000) indicated that 10% of intra-plant polymorphism, following the procedure of AMOVA (Excoffier *et al*., 1992), is statistically insignificant and acceptable.

Identification of molecular markers for different cultivars

ISSR is a relatively recent class of molecular markers as compared to random amplified polymorphic DNA (RAPD), which is based on inter tandem repeats of short DNA sequences. Such repeats were proven to be highly polymorphic even among closely-related genotypes due to the lack of functional constraints in these non-functioning DNA regions that was thought to result in the evolutionary changes in their DNA structures. Accordingly, a high level of polymorphism was generated utilizing the 17 ISSR primers. A total number of 1361 amplicons were obtained in which 911 of them were polymorphic (67%) and the rest were monomorphic (33%). The highest number of amplicons was generated for cv. Giza 168 (212 amplicons), while cv. Gemiza 9 generated the lowest (129 amplicons). The highest number of cultivar-specific markers (Tables 3 & 4) was scored for Sahel 1 (10 amplicons), while the lowest number was scored for Sakha 93 and Sakha 94 (2 amplicons).

Five combinations were used in the AFLP analysis and revealed a total of 1611 amplicons, 1081 of them were polymorphic (67%) among the different genotypes. The highest number of amplicons was generated for cv. Giza 168 (301 amplicons), while cv. Sahel 1 generated the lowest (163 amplicons). The highest number of cultivar-specific markers (Tables 3 & 4) was scored for Sahel 1 (17 amplicons), while the lowest was scored for Sakha 93 (3 amplicons). In conclusion, the five primer combinations of AFLP used in the present study allowed for a rate of distinction (67% polymorphism)similar to ISSRs (67% polymorphism).

The number of cultivar-specific markers scored across cultivars and type of marker was as high as 109 in which 67 of them were generated during AFLP analysis, while only 42 for ISSR (Tables 3 & 4). The highest number of cultivar-specific markers across types of markers was scored for cv. Sahel 1 (27 amplicons), while the lowest was scored for cv. Sakha 93 (5 amplicons) (Table 3).

Across the different genotypes (tolerant versus sensitive) and possible origins (within Sakha and Gemiza cultivars), a number of 194 markers were generated across both types of markers (Table 4). The highest number of markers (49) was scored for drought-tolerance (tolerant cultivars are Misr 2, Gemiza 10, Sakha 93 and Sakha 94), which are considered as positive markers for drought tolerance. While, the lowest number (10) of markers was scored for drought-sensitivity (sensitive cultivars are Sakha 95, Gemiza 9, Giza 168 and Sahel 1), which are considered as negative markers for drought tolerance. As for cultivars with possible linked origins, Sakha cultivars have shown 15 specific markers, while Gemiza cultivars showed only 11 specific markers.

Genetic relationships and cluster analysis

The genetic similarities among the eight cultivars of bread wheat, based on Nei's method (Nei's, 1978), within and across both markers are shown in Table 5 and Figure 3. The highest similarity indices resulted from ISSR, AFLP and across type of markers were 95%, 88% and 88%, respectively, while the lowest similarity indices were 85%, 76% and 77%, respectively. These results indicate that ISSR failed to detect high genetic distances among wheat cultivars, while AFLP succeeded to some extent to differentiate among them. Overall, the results of similarity indices and dendrograms across ISSR and AFLP data indicated accumulative information towards the complete separation of the drought-tolerant cultivars (a-d) from drought-sensitive cultivars (e-h). The data did not separate both groups of cultivars (Sakha and Gemiza groups) with possible similar ancestor. However, dengrograms generated from AFLP and across type of markers indicated that Sakha 93 and Sakha 94 are closely genetically related (88%). This indicates that they might be derived from one ancestor. The resulted dendrogram of AFLP data was the closest to that resulted across types of markers. In conclusion, it was obvious that ISSR data might have diluted the relationships among cultivars as similarity index and generated dendrograms indicated that cultivars Giza 168 and Gemiza 10 had the highest similarity percentage (95%, Table 5).

The partitioning of variation within and across bread wheat cultivars was studied with the analysis of the Dice's distance matrix by the analysis of molecular variance (AMOVA) approach. A hierarchical analysis of genetic diversity using a two-way nested AMOVA was performed. Results from AMOVA within and among population are shown in Table 6. Data indicated that 98% of the genetic variation is attributed to differences among populations, while only 2% of the genetic variation is attributed to differences within populations. The values of MS

indicated the high level of polymorphism among genotypes, hence, low level of experimental error. This reflects the homogeneity in leaf samples collected for the study as a perfect representative of the target genotypes. The polymorphism information content (PIC), average of heterozygosity (H_e), the effective multiplex ratio (E), and the marker index (MI) were computed for each assay based on experimental data (Table 7). The data for two types for PIC (0.32 and 0.32, respectively) and H_e (0.39 and 0.40, respectively) indicated no preference for any of ISSR or AFLP types of markers. Expectedly, AFLP revealed higher E and MI values (467 and 186.8, respectively) as compared to those for ISSR (19 and 7.41, respectively). The obtained results in the present investigation agreed with these of Powell *et al*. (1996) across both types of markers. Muzher (2005) also found that H_e of ISSR was more than AFLP. However, the results of ISSR data analysis indicated the suspicion in utilizing this type of marker in detecting genetic relatedness among bread wheat cultivars. It is possible to improve reliability on ISSR data if more primers were used in characterizing cultivars at the molecular levels.

It could be concluded that markers differ in their ability to differentiate individuals, the mechanism of detecting polymorphism, genome coverage, and the ease of application. They can be complementary to each other, as it is shown in the present study, depending on technical availability. Some of these markers can be linked to drought tolerance, hence, can be used in detecting possible relatedness among cultivars with unknown ancestors.

The allohexaploid nature of the wheat genome makes it the species with the largest genome among cereals. Wheat has 21 linkage groups and is generally known to possess low levels of polymorphism for marker systems such as restriction fragment length polymorphisms (RFLP) and simple sequence repeats or microsatellites (SSR). In contrast, rice with 12 linkage groups and maize with 10 linkage groups have well-saturated publicly available linkage maps. Although the International Triticeae Mapping Initiative (ITMI) population, the first publicly funded linkage map of bread wheat, currently has over 1,000 markers, significant gaps remain in some linkage groups (Somers *et al*., 2004; http://www.wheat. pw.usda.gov/GG2/ index.shtml). Moreover, genetic analysis in wheat is often complicated by interactions among the three genomes affecting the regulation of some important traits.

Molecular markers have been used extensively in cultivated species in trait characterization and are considered potentially valuable tools for crop improvement. A large number of traits in wheat have been genetically characterized using molecular markers (Hoisington *et al*., 2002). The availability of dense linkage maps with evenly distributed markers is important if genes associated with target traits are to be successfully characterized. Although a number of different marker systems are being used in genetic characterization of traits (Langridge *et al*., 2001; Hoisington *et al*., 2002), PCR-based markers such as ISSR and AFLP markers are two markers currently available in wheat that are amenable to large scale applications, a requirement in all molecular breeding procedures.

Plant growth and productivity are greatly affected by environmental stresses such as drought, high salinity, and low temperature (Zheng *et al*., 2010). Upon exposure to abiotic stress conditions, plants undergo a variety of changes from physiological adaptation to gene expression (Shinozaki and Yamaguchi-Shinozaki, 2007).

Drought is a major abiotic stress that adversely affects wheat production and quality in many regions of the world, the loss of which is the total for other natural disasters, with increasing global climate change making the situation more serious (Shao *et al*., 2005; Kirigwi *et al*., 2007).

Table 3. List of cultivar-specific markers of the eight bread wheat cultivars within and across the two marker types. The table indicates the type and number of markers along with their molecular weights (MW) in bp for different cultivars (a-h). Single red line represents drought-tolerant cultivars, while double blue line represents sensitive cultivars.

Marker type	Primer	Number (and MW in bp) of cultivar-specific markers							
		a[1]	b	c	d	e	f	g	h
ISSR	814	-	1 (630)	-	-	-	-	1 (430)	-
	844A	-	-	-	-	-	-	1 (2220)	1 (1340)
	844B	-	1 (2010)	-	-	-	-	-	-
	17898A	-	-	-	1 (1760)	-	-	-	2 (740, 1540)
	17898B	2 (490, 1030)	-	-	-	1 (2160)	-	-	-
	17899A	-	-	1 (1500)	-	-	1 (800)	-	-
	17899B	1 (550)	1 (990)	-	-	-	-	-	1 (970)
	HB8	1 (480)	-	-	-	-	-	-	-
	HB9	1 (950)	-	-	-	1 (2080)	-	-	-
	HB10	-	-	-	1 (620)	-	-	1 (570)	-
	HB11	-	-	-	-	1 (480)	1 (780)	-	1 (1440)
	HB12	-	1 (890)	-	-	-	-	-	2 (220, 500)
	HB13	1 (580)	-	-	-	-	-	2 (670, 2390)	-
	HB14	1 (1950)	-	-	-	-	-	-	1 (880)
	HB15	-	-	1 (2380)	-	-	1 (420)	-	-
	UCB-820	-	1 (720)	-	-	-	1 (700)	1 (1950)	2 (450, 1650)
	UCB-827	2 (1220, 1460)	-	-	-	-	-	1 (920)	-
	Total	**9**	**5**	**2**	**2**	**3**	**4**	**7**	**10 = 42**
AFLP	CCA/ACT	1 (1180)	4 (1080, 1060, 720, 620)	1 (1000)	2 (1130, 660)	3 (1160, 1100, 1030)	1 (600)	5 (1650, 1240, 800, 760, 740)	4 (1300, 1280, 1220, 700
	CAC/ACA	2 (930, 1020)	1 (520)	-	1 (630)	1 (740)	2 (360, 670)	2 (1040, 1390)	2 (1480, 2360)
	CAG/AAC	2 (500, 950)	-	1 (880)	1 (610)	-	-	3 (390, 700, 2030)	2 (750, 1490)
	CTC/AAG	1 (660)	1 (350)	1 (620)	-	1 (1420)	-	2 (990, 1240)	3 (680, 1010, 2160)
	CAA/ACC	4 (190, 540, 710 1680)	1 (630)	-	1 (960)	1 (1110)	2 (1230, 1600)	2 (440, 770)	6 (280, 480, 510 920, 2100, 2310)
	Total	**10**	**7**	**3**	**5**	**6**	**5**	**14**	**17 = 67**
Total		**19**	**12**	**5**	**7**	**9**	**9**	**21**	**27 = 109**

[1]See Table 1

Currently, drought study has been one of the main directions in global plant biology and biological breeding. Many advances in relation to this hot topic, including molecular mechanism of anti-drought and corresponding molecular breeding have taken place (Patnaikt and Khurana, 2001; Rellegrineschi *et al*., 2002; Chen and Gallie, 2004; Rampino *et al*., 2006; Zhao *et al*., 2008; Wei *et al*., 2009; Ashraf, 2010). Marker-assisted selection (MAS) provides a strategy for accelerating the process of wheat breeding (Wei *et al*., 2009) towards the development of drought-tolerant genotypes.

Table 4. Numbers of positive and negative markers for drought tolerance, markers for cultivars with linked origins as well as cultivar-specific markers of the eight bread wheat cultivars within and across the two marker types.

Marker type	Marker name	Marker[a] 1	2	3	4	5	**Total**
ISSR	814	1	-	-	-	2	3
	844A	2	-	1	-	2	5
	844B	1	1	-	1	1	4
	17898A	-	1	-	-	3	4
	17898B	2	-	1	-	3	6
	17899A	-	-	-	-	2	2
	17899B	-	1	1	-	3	5
	HB8	-	-	-	-	1	1
	HB9	2	-	-	1	2	5
	HB10	2	-	-	1	2	5
	HB11	-	1	-	-	3	4
	HB12	2	-	1	-	3	6
	HB13	1	-	-	-	3	4
	HB14	-	-	-	-	2	2
	HB15	1	-	-	-	2	3
	UCB-820	2	-	1	-	5	8
	UCB-827	-	-	-	1	3	4
	Total	**16**	**4**	**5**	**4**	**42**	**71**
AFLP	CCA/ACT	8	2	2	2	21	35
	CAC/ACA	12	1	-	1	11	25
	CAG/AAC	6	2	2	-	9	19
	CTC/AAG	4	-	3	2	9	18
	CAA/ACC	3	1	3	2	17	26
	Total	**33**	**6**	**10**	**7**	**67**	**123**
Total		**49**	**10**	**15**	**11**	**109**	**194**

[a]1: Tolerance positive marker , 2: Tolerance negative marker, 3: Sakha group marker , 4: Gemiza group marker, 5: cultivar-specific marker.

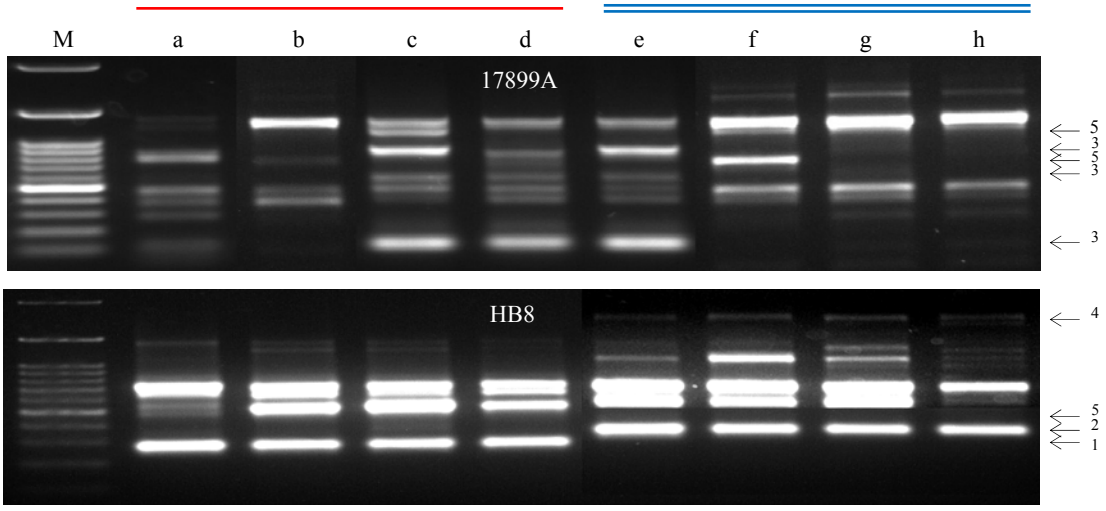

Figure 1. ISSR analysis with primers 17899A and HB8 of the eight bread wheat cultivars (a-h, see Table 1). M refers to DNA standard (100-bp ladder, Bioron). Primer 17899A indicated three markers for Sakha cultivars with 140, 650 and 900 bp and two cultivar-specific markers for Gemiza 9 (at 800 bp) and Sakha 93 (at 1500 bp). Primer HB8 indicated one positive (at 280 bp) and one negative (at 320 bp) markers for drought tolerance, one marker for Gemiza cultivars (at 2500 bp) and one cultivar-specific marker (at 480 bp) for Misr 2. Number 1-5 refer to different markers (See Table 4). M refers to DNA standard (100-bp ladder, Bioron). Single red line represents drought-tolerant cultivars, while double blue line represents drought-sensitive cultivars. Numbers 1-5 refer to different markers (See Table 4).

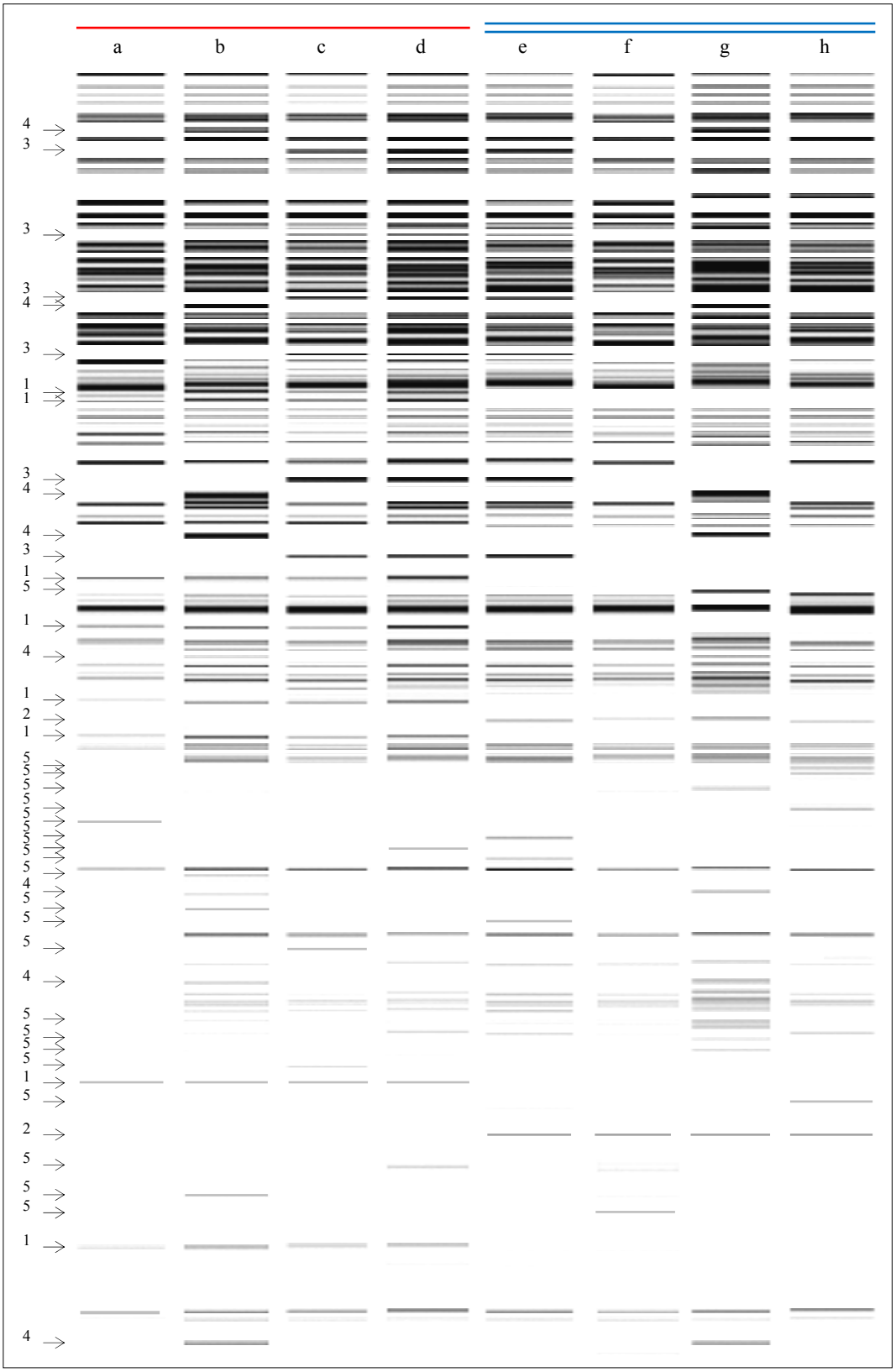

Figure 2. AFLP analysis with primer combination M-CCA/E-ACT of the eight cultivars (a-h, see Table 1) indicating 35 different markers (see Tables 3 & 4). Single red line represents drought-tolerant cultivars, while double blue line represents drought-sensitive cultivars. Numbers 1-5 refer to different markers (See Table 4).

Table 5. Similarity matrixes based on molecular data for the eight bread wheat cultivars (a-h, see Table 1). Single red line represents drought-tolerant cultivars, while double blue line represents drought-sensitive cultivars. Orange box indicates the highest values, while the green box indicates the lowest.

Marker type	**cultivar**							
	g	b	a	c	d	f	h	e
ISSR								
g	1.00							
b	0.95	1.00						
a	0.90	0.89	1.00					
c	0.89	0.91	0.89	1.00				
d	0.87	0.89	0.84	0.89	1.00			
f	0.88	0.87	0.88	0.90	0.89	1.00		
h	0.89	0.90	0.89	0.94	0.89	0.87	1.00	
e	0.92	0.90	0.88	0.87	0.85	0.93	0.87	1.00
AFLP								
g	1.00							
b	0.80	1.00						
a	0.80	0.80	1.00					
c	0.79	0.84	0.85	1.00				
d	0.80	0.82	0.82	0.88	1.00			
f	0.81	0.80	0.79	0.82	0.82	1.00		
h	0.77	0.76	0.80	0.82	0.81	0.77	1.00	
e	0.78	0.83	0.81	0.82	0.82	0.80	0.78	1.00
Overall								
g	1.00							
b	0.81	1.00						
a	0.81	0.83	1.00					
c	0.80	0.84	0.86	1.00				
d	0.80	0.83	0.82	0.88	1.00			
f	0.81	0.83	0.80	0.83	0.82	1.00		
h	0.77	0.77	0.81	0.83	0.82	0.78	1.00	
e	0.78	0.83	0.86	0.87	0.82	0.81	0.78	1.00

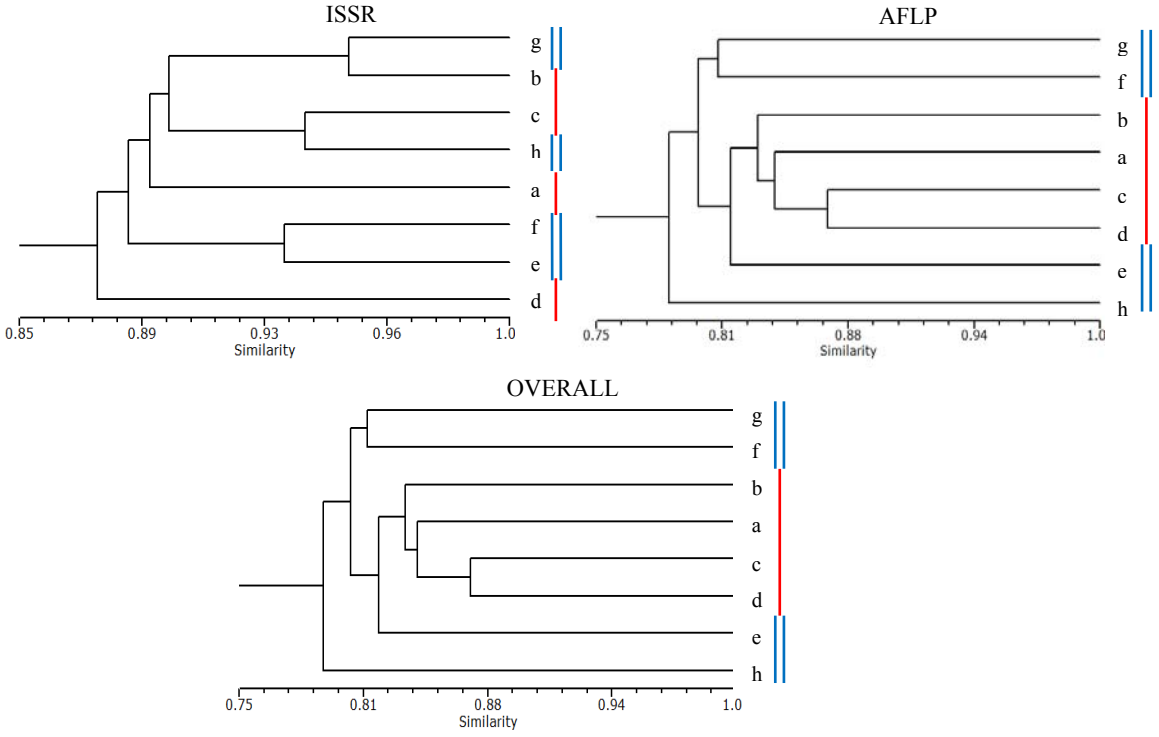

Figure 3. Dendrogram based on algorithm of unweighted pair group method with arithmetic averages among cultivars (a-h, see Table 1) within or across type of marker. Single red line represents drought-tolerant cultivars, while double blue line represents drought-sensitive cultivars.

Table 6. Analysis of molecular variance (AMOVA) of the different bread wheat cultivars.

Source	*df*[1]	*SS*[2]	*MS*[3]	*Variance (%)*
Among Pops	7	6.329	0.904	2
Within Pops	5656	1138.640	0.201	98
Total	5663	1144.969	1.105	

[1]*df* = Degrees of freedom, [2]*SS* = Sum of squares, [3]*MS* = Mean square

Table 7. Polymorphism information content (PIC), expected heterozygosity for polymorphic products (He), effective multiplex ratio (E) and the marker index (MI) of each marker type used across bread wheat cultivars.

Marker type	PIC	He	E	MI
ISSR	0.32	0.39	19	7.41
AFLP	0.32	0.40	467	186.8

Through marker-assisted breeding (MAB), it is now possible to examine the usefulness of thousands of genomic regions of a crop germplasm under water limited regimes, which was, in fact, previously not possible (Ashraf, 2010).

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References

Aparajita S, Rout GR (2010). Molecular analysis of *Albizia* species using AFLP markers for conservation strategies. J Genet 89:95-99.

Ashraf M (2010). Inducing drought tolerance in plants: recent advances. Biotech Adv 28:169-183.

Barker JHA, Matthes M, Arnold GM, Edwards KJ, Ahman I, Larsson S, Karp A (1999). Characterization of genetic diversity in potential biomass willows (*Salix* spp.) by RAPD and AFLP analyses. Genome 42:173-183.

Beckmann JS, Soller M (1983). Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. Theor Appl Genet 67:35-43.

Brantestem AK, Bothmer RV, Dayteg C, Rashal I, Tuvesson S, Weibull J (2004). Inter simple sequence repeat analysis of genetic diversity and relationship in cultivated barley of Nordic and Baltic origin. Hereditas 141:186-192.

Buhulikar RA, Stanculescu D, Preston CA, Baldwin IT (2004). ISSR and AFLP analyses of the temporal and spatial population structure of the post-fire annual *Nicotiana* attenuate in SW, Utah. BMC Ecol 4:1-13.

Bussell JD, Waycot M, Chappill JA (2005). Arbitrarily amplified DNA markers as characters for phylogenetic inference. Perspect Plant Ecol Evol. Syst 7:3-26.

Cabrita LF, Aksoy U, Hepaksoy S, Leitao JM (2001). Suitability of isozyme, RAPD and AFLP markers to assess genetic differences and relatedness among fig (*Ficus carica* L.) clones. Sci. Horticult. 87:261-73.

Chen Z, Gallie DR (2004). The ascorbic acid redox state controls guard cell signaling and stomatal movement. Plant Cell 16:1143-1162.

Denduangboripant J, Setaphan S, Suwanprasart W, Panha S (2010). Determination of local tobacco cultivars using ISSR molecular marker. Chiang Mai J Sci 37:293-303.

Excoffier L, Smouse PE, Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genet 131:479-491.

Gawel NJ, Jarret RL (1991). A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. Plant Mol Biol Rep 9:262-266.

Hoisington D, Bohorova N, Fennell NS, Khairallah M, Pellegrineschi A, Ribout JM (2002). Plant production and protection series. Available from http://www.fao.org (FAO document respository).

Innis MA, Gelfand DH, Snlnsky JJ, White TJ (1990). PCR protocols: a guide to methods and applications. Academic Press, New York.

Kirigwi FM, Van Ginkel M, Brown-Guedira G, Gill BS, Paulsen GM, Fritz AK (2007). Markers associated with a QTL for grain yield in wheat under drought. Mol Breed 20:401-413.

Langridge P, Lagudah ES, Holton TA, Appels R, Sharp PJ, Chalmers KJ (2001). Trends in genetic and genome analysis in wheat: a review. Aust J Agric Res 52:1043-1077.

Law JR, Donini P, Koebner RMD, Jones CR, Cooke RJ (1998). DNA profiling and plant variety registration III: The statistical assessment of distinctness in wheat using amplified fragment length polymorphisms. Euphytica 102:335-342.

Mantel NA (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209-220.

Mengoni A, Gori A, Bazzicalupo M (2000). Use of RAPD and microsatellite (SSR) variation to assess genetic relationships among populations of tetraploid alfalfa, *Medicago sativa*. Plant Breed 119:311-317.

Misra A, Shasany AK, Shukla AK, Darokar MP, Singh SC, Sundaresan V, Singh J, Bagchi GD, Jain SP, Saikia D, Khanuja SPS (2010). AFLP markers for identification of *Swertia* species (Gentianaceae). Genet Mol Res 9:1535-1544.

Monneveux P, Ramírez DA, Pino M-T (2013). Drought tolerance in potato (*S. tuberosum* L.). Can we learn from drought tolerance research in cereals ? Plant Sci (In press)

Morgante M, Olivieri AM (1993). PCR amplified microsatellites as markers in plant genetics. Plant J 3:175-182.

Muzher MB (2005). Application of biochemical and PCR based molecular markers to the characterization of Syrian Pears (*Pyrus syriaca* Boiss) genotypes. Intl Conf on: Promoting Community-driven Conservation and Sustainable Use of Dryland Agrobiodiversity, ICARDA, Syria, p18-21.

Nei M (1978). Estimation of average hetero-zygosity and genetic distance from a small number of individuals. Genet 89:583-590.

Papadopoulou K, Ehaliotis C, Tourna M, Kastanis P, Karydis I, Zervakis G (2002). Genetic relatedness among dioecious *Ficus carica* L. cultivars by random amplified polymorphic DNA analysis, and evaluation of agronomic and morphological characters. Genetica 114:183-194.

Patnaitk D, Khurana P (2001). Wheat biotech-nology: a mini-review. Elec J Biotech 4:74-102.

Peakall R, Smouse PE (2006). Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288-295.

Powell W, Morgante M, Andre C, Hanfey M, Vogel J, Tingey S, Rafalski A (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2:225-228.

Rampino P, Pataleo S, Gerardi C, Perotta C (2006). Drought stress responses in wheat: Physiological and molecular analysis of resistant and sensitive genotypes. Plant Cell Environ 29:2143-2152.

Rellegrineschi A, Ribaut JM, Trethowan R (2002). Looking beyond the details: a rise in system-oriented approaches in genetics and molecular biology. Curr Genet 41:1-10.

Rogers KB (2008). Using amplified fragment length polymorphisms to characterize purity of cutthroat trout in Colorado: Results from 2007. Colorado Division of Wildlife, Fort Collins.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-491.

Shao HB, Liang ZS, Shao MA, Sun Q (2005). Dynamic changes of anti-oxidative enzymes of10 wheat genotypes at soil water deficits. Colloids and Surfaces B: Biointerfaces 42:187-195.

Shinozaki K, Yamaguchi-Shinozaki K (2007). Gene networks involved in drought stress response and tolerance. J Exp Bot 58:221-227.

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell SE, Kresovich S, Ziegle J (1997). An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): Comparisons with data from AFLPs and pedigree. Theor Appl Genet 95:163-173.

Somers JD, Isaac P, Edwards K (2004). A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105-1114.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407-4414.

Wei B, Jing R, Wang Ch, Chen J, Mao X, Chang X, Jia J (2009). *Dreb1* genes in wheat (*Triticum aestivum* L.): development of functional markers and gene mapping based on SNPs. Mol Breed 23:13-22.

William HM, Trethowan R, Crosby-Galvan EM (2007). Wheat breeding assisted by markers: CIMMYT's experience. Euphytica 157:307-319.

Wrigley CW, Batey IL, Skerritt JH (1987). Complementing traditional methods of identifying cereal varieties with novel procedures. Seed Sci Tech 15:679-688.

Zietkiewicz E, Rafalski A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176-183.

Zhao CX, Guo LY, Cheruth AJ, Shao HB, Yang HB (2008). Prospective for applying molecular and genetic methodology to improve wheat cultivars in drought environments. C R Biologies 331:579-586.

Zheng J, Fu J, Gou M, Huai J, Liu Y, Jian M, Huang Q, Guo X, Dong Z, Wang H, Wang G (2010). Genome-wide transcriptome analysis of two maize inbred lines under drought stress. Plant Mol Biol 72:407-423.