

Assessment of genetic diversity among wheat doubled haploid plants using TRAP markers and morpho-agronomic traits

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Abstract

The objectives of this study were to compare the agronomic performance of wheat doubled haploid (DH) lines derived via microspore culture against their corresponding parental lines under field conditions. We also estimated the genetic diversity using molecular markers and agronomic performance to find an association between molecular markers and agronomic traits. DH wheat lines derived from microspore cultures system of the four wheat populations (Irena × Ksu102, Lang × Ksu105, Klasic × Ksu105 and Yecora Rojo × Ksu106) were evaluated for agronomic traits under normal field conditions. Analysis of variance showed highly significant differences among the parental wheat genotypes and doubled haploid lines for all the agronomic traits in four studied populations. The results indicated that the DH16 (9.2 t ha⁻¹) was significantly different from their parents (Ksu105 and Klasic) for grain yield (7.5 and 4.1 t ha⁻¹ in parents, respectively). The DH4 and DH11 were also significantly different from the two parents; Ksu106 and Yecora Rojo for biological and grain yield. Forty different target region amplification polymorphism (TRAP) primer combinations were used to evaluate the variation among parental and doubled haploid lines. The dendrogram created based on morpho-agronomic traits differed from that based on TRAP markers. However, the combined analysis produced similar dendrogram to that produced using TRAP marker alone. Our results show that wheat doubled haploid production via microspore cultures can generate lines that have the potential for commercial production as well as lines that can be incorporated into a breeding program.

Keywords: Double haploid, Genetic diversity, TRAP markers, *Triticum aestivum* L.

Abbreviations: DH- doubled haploid; HMW- high-molecular-weight, TRAP- target region amplification polymorphism.

Introduction

The production of doubled haploid plants (DH) is valuable in plant biotechnology because it enables breeders to obtain homozygous lines or plants directly from hybrid individuals (Barakat et al., 2012). The main advantage of using doubled haploid breeding lines is reduction of time required to achieve homozygosity. True-breeding lines can be generated in one generation rather than several years of backcrossing or selfing. Because of its homozygosity and high purity, doubled haploid wheat can be used as a bread variety and also as a base in producing hybrids. Therefore, understanding the genetic diversity of doubled haploid wheat lines based on morphologic traits and DNA polymorphisms is important. This method is also beneficial for producers and consumers as high yielding cultivars with improved agronomic and quality traits can be developed more rapidly.

Estimation of genetic variation level among accessions is prerequisite for germplasm conservation and breeding programs. Assessment of genetic diversity based on phenotype has limitations, since most of the morphological characters of economic importance are dramatically influenced by environmental factors and plant developmental stage (Fufa et al., 2005). Despite these limitations, phenotypic characters have been successfully used for genetic

variation and cultivar development. In contrast, molecular markers based on DNA sequence polymorphisms are independent from environmental conditions and can be estimated using DNA from any growth stage (Tatikonda et al., 2009). Morphological markers reflect variation of expressed regions of genome while molecular markers indicate variation of all genome including expressed and non-expressed regions.

A rapid and efficient PCR-based target region amplification polymorphism (TRAP) technique was developed by Hu and Vick. (2003). TRAP uses bioinformatics tools and the EST database information to generate polymorphic markers around targeted putative candidate gene sequences. Thus, it should be useful in plant genomics research involved in genetic mapping and marker-trait association (Liu et al., 2005). Previously, TRAP also was successfully used to estimate the genetic diversity in genetic stocks of wheat (Xu et al., 2003; Al-Doss et al., 2011).

Molecular and morphological analyses are among the most used tools for the estimation of genetic distances within a group of genotypes. Molecular markers provide an excellent tool for obtaining genetic information. Their application (*Triticum aestivum* L.) has been increased in the assessment

of genetic diversity in wheat over the last few years (Chao et al., 2007; Jin et al., 2008; Wicker et al., 2009; Al-Doss et al., 2009, Barakat et al., 2010, Al-Doss et al., 2011). Molecular markers are useful complements 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 (Al-Doss et al., 2009, Barakat et al., 2010, Al-Doss et al., 2011). Molecular characterization of cultivars is also useful to evaluate potential genetic erosion, defined as a reduction of genetic diversity in time (Manifesto et al., 2001).

The objectives of the present study were to compare: (1) the agronomic performance of DH wheat lines derived through microspore culture against their corresponding parental lines under field conditions and (2) estimation of genetic diversity using molecular markers and agronomic performance to find association between these techniques. Further efforts to create and characterize DH lines of wheat with superior agronomic or quality characteristics with high-molecular-weight (HMW) glutenins are ongoing.

Results

Irena × Ksu102 population

Analysis of variance showed highly significant differences between the 49 wheat doubled haploid lines and their parents for all the agronomic traits (plant height, spike length, date of flowering, date of maturity, biological yield, grain yield and harvest index). Plant height ranged from 46.0 to 94.3 cm with an average of 76.8 cm. The tallest genotype was DH35 (94.3 cm), while the plant height for parents Irena and Ksu102 were 74.7 and 88.7 cm, respectively. Spike length was significantly different among the wheat genotypes. The DH29 had the shortest spike length (8.3 cm), while the DH33 had the tallest (15.7 cm). DH12 and DH14 were the earliest genotypes, while DH17 (108) was the latest. Days to flowering ranged from 61.7 to 108.0 days, with an average of 80.1 days. Maturity time was similar among the most of genotypes even the differences among genotypes were significant for this trait. Days to maturity ranged from 109.0 (DH14) to 141.0 (DH17) days with an average of 125.5 days. The DH43 (25.8 t ha⁻¹) and DH46 (22.8 t ha⁻¹) were significantly different from the parents (Ksu102 and Irena) for biological yield (18.5 and 6.8 t ha⁻¹, respectively). Grain yield varied from 1.03 to 7.38 t ha⁻¹ with an average of 4.15 t ha⁻¹. The most productive genotype was DH11 (7.38 t ha⁻¹) which was not significantly different from the parent; Ksu102 (6.63 t ha⁻¹), while the least productive was DH25 (1.03 t ha⁻¹). Harvest index ranged from 0.53 (Ksu102) to 0.05 (DH25), with an average of 0.31.

The clustering pattern of the wheat genotypes based on phenotypic data using Jaccard's coefficient method is depicted in Fig. 1A. The analysis assigned the genotypes into five clusters. Cluster 1 included four doubled haploid lines (DH10, DH16, DH23 and DH42) characterized by the shortest plant height, moderately in spike length and low biological yield. In the second cluster, two doubled haploid lines (DH19 and DH34) grouped together, which were shortest in spike length, low biological yield and early to flowering. The third cluster contains only DH49, which is characterized by the shortest plant height, low biological yield and grain yield. The fourth cluster comprised seven doubled haploid lines (DH12, DH14, DH20, DH21, DH24, DH28 and DH38) and one parent (Irena) characterized by the low biological yield and moderately grain yield. The last cluster comprised the rest of the genotypes.

Forty different TRAP primer combinations were used to amplify DNA segments from 51 wheat genotypes. The

number of amplification bands per primer varied between 0 to 28. A total of 242 bands were observed, with 6.1 bands per primer. 200 out of 242 bands (82.64 %) were polymorphic. An example of polymorphism with TRAP8 (Fixed primer; TGAGTCCAAACCGGAAT and Arbitrary primers; TTCTTCCTCCCGCTCATCCT) is shown in Fig. 5A. Cluster analysis using TRAP data grouped our 51 wheat genotypes into four main clusters with Jaccard's similarity coefficient ranging from 0.38 to 0.82 (Fig. 1B). The highest similarity was found between DH2 and DH3 (0.82) and the lowest was between DH3 and DH48 (0.38). The first cluster contained three doubled haploid lines (DH1, DH2 and DH3). The second cluster contained DH42, DH48 and Ksu102. The third cluster only included one wheat genotype (Irena). The fourth cluster contained the rest of the wheat genotypes.

The dendrogram generated by combined phenotypic traits and TRAP analysis is presented in Figure 1C. The combined analysis produced similar dendrogram to that produced by TRAP marker analysis. The genotypes were grouped in four clusters as revealed by earlier TRAP analysis alone. The similarity coefficient among the wheat genotypes varied from 0.25 to 0.81 with the highest being between DH2 and DH3 (0.81) and the lowest being between DH3 and DH48 (0.25) as revealed by earlier TRAP alone.

The comparison between agronomic traits and TRAP markers was carried out by Mantel correlation test (Mantel, 1967). The correlation between the two measurements was 0.01 and it was not significant, showing that they were not related. However, the correlation coefficient between agronomic traits and the combined matrix (agronomic traits and TRAP markers) was highly significant (0.21**, $p \leq 0.001$).

Lang × Ksu105 population

The analysis of variance indicated that there were statistically significant differences ($p \leq 0.01$) among the studied wheat genotypes for all the agronomic traits. Plant height ranged from 63.0 to 112.7 cm with an average of 83.6 cm. The tallest genotypes were DH 13 (112.7 cm) and DH14 (109.0 cm). The DH10 had the tallest spike length (17.0 cm). DH4, DH6 and DH8 were the earliest genotypes (65.7 days), while DH10 (102.0 days) was the latest. Days to maturity ranged from 113.0 (DH2, DH3, DH4 and DH8) to 141.0 (DH15) days with an average of 121.5 days. The DH5, DH7 and DH11 were significantly different from only the parent Ksu105 for both agronomic traits, biological and grain yield. However, the same doubled haploid lines were not significantly different from both parents; Ksu105 and Lang for harvest index.

Cluster analysis using phenotypic data grouped the 17 wheat genotypes into two main clusters with Jaccard's similarity coefficient ranging from 0.06 to 1.0 (Fig. 2A). Cluster 1 included one doubled haploid line (DH4) characterized by medium plant height and spike length, early flowering date and maturity as well as high grain yield and harvest index. The second cluster contained the rest of the wheat genotypes.

A total of 312 fragments were produced by the 40 different TRAP primer combinations. Of these 158 amplified fragments, 49.36% were not polymorphic, while 50.64% were polymorphic among the 17 wheat genotypes. The values of similarity coefficient obtained in TRAP analysis ranged from 0.99 to 1.0 among the studied wheat genotypes. An example of polymorphism with TRAP34 (Fixed primer; AGTAACCCACCGCCTCCTTC and arbitrary primer; CGGACAGTGGCGGAGTTA) is shown in Fig. 5B. The dendrograms obtained from TRAP markers was not

Table 1. The four crosses and the desired HMW-GS combinations.

Population	Genotype	Glu1A	Glu1B	Glu1D	Glu-1 quality score**
Irena × Ksu102	Irena	1 Ax1	Bx7+By8	Dx2+Dy12	10
	Ksu102	Ax2	Bx7+By8	Dx2+Dy12	8
Lang × Ksu105	Lang	Ax1	Bx7*+By8	Dx2+Dy12	11
	Ksu105	Ax1	Bx7+By8	Dx2+Dy12	8
Klasic × Ksu 105	Klasic	Ax1	Bx17+By18	Dx5+Dy10	10
	Ksu105	Ax1	Bx7+By8	Dx2+Dy12	8
Yecora Rojo × Ksu106	Yecora Rojo	Ax1	Bx17+By18	Dx5+Dy10	10
	Ksu106	Ax1	Bx17+By18	Dx2+Dy12	8

**Scored based on Pfluger gluten alleles scoring system (Gahazy et al., 2012).

completely consistent to cluster analysis using phenotypic data. Cluster analysis using TRAP data grouped the 17 wheat genotypes into two main clusters. Cluster I is comprised of the parent Lang, which stands alone and far from the other wheat genotypes. Cluster II is comprised from the parent Ksu105 and all of the doubled haploid lines (Fig. 2B).

UPGMA dendrogram obtained from the pooled data of TRAP and phenotypic data analyses is presented in the Fig. 2C. The 17 wheat genotypes are grouped into two major clusters. Cluster I consisted of the parent Lang and seven doubled haploid lines (numbers 2, 3, 5, 8, 9, 14 and 15). Cluster II is comprised of the parent Ksu105 and the rest of the doubled haploid lines.

The comparison between agronomic traits and TRAP markers was carried out by Mantel correlation test. The correlation between the two measurements was -0.20, which was not significant, showing that they are not related. However, the correlation coefficient between agronomic traits and the combined matrix (agronomic traits and TRAP markers) was highly significant (0.59**, $p \leq 0.001$).

Klasic × Ksu105 population

Analysis of variance showed highly significant differences between the 19 wheat doubled haploid lines and their parents for all agronomic traits. Plant height ranged from 48.3 to 105.7 cm with an average of 63.6 cm. The tallest genotypes were DH8 and DH9 (101.3 and 105.7 cm, respectively), while the shortest was DH2 (48.3 cm). Spike length ranged from 10.3 to 17.0 cm with an average of 13.3 cm. The parent Klasic had the shortest spike length (10.3 cm), while the DH9 had the tallest (17. cm). DH6 (62.3 days) was the earliest genotypes, while DH3 (102.7 days) was the latest. Days to flowering ranged from 62.3 to 102.7 days with an average of 82.1 days. Days to maturity ranged from 95.0 (DH 19) to 136.0 (DH 3) days with an average of 123.2 days. The DH8 (30.8 t ha⁻¹) and DH9 (29.3 t ha⁻¹) were significantly different from the parents (Ksu105 and Klasic) for biological yield (20.1 and 10.1 t ha⁻¹, respectively). The results also indicated that the DH16 (9.2 t ha⁻¹/ton/ha) was significantly different from the parents (Ksu105 and Klasic) for grain yield (7.5 and 4.1 t ha⁻¹, respectively). Harvest index ranged from 0.44 (DH14) to 0.18 (DH19) with an average of 0.31.

The clustering pattern of the wheat genotypes based on phenotypic data using Jaccard's coefficient method is depicted in Fig. 3A. The analysis assigned the genotypes into three clusters. Cluster 1 included three wheat genotypes (Klasic, DH17 and DH18) characterized by the shortest in spike length, early to flowering and maturity, and low biological and grain yield. In the second cluster, two doubled haploid lines (DH6 and DH10) grouped together which were moderately in spike length, early to flowering and maturity,

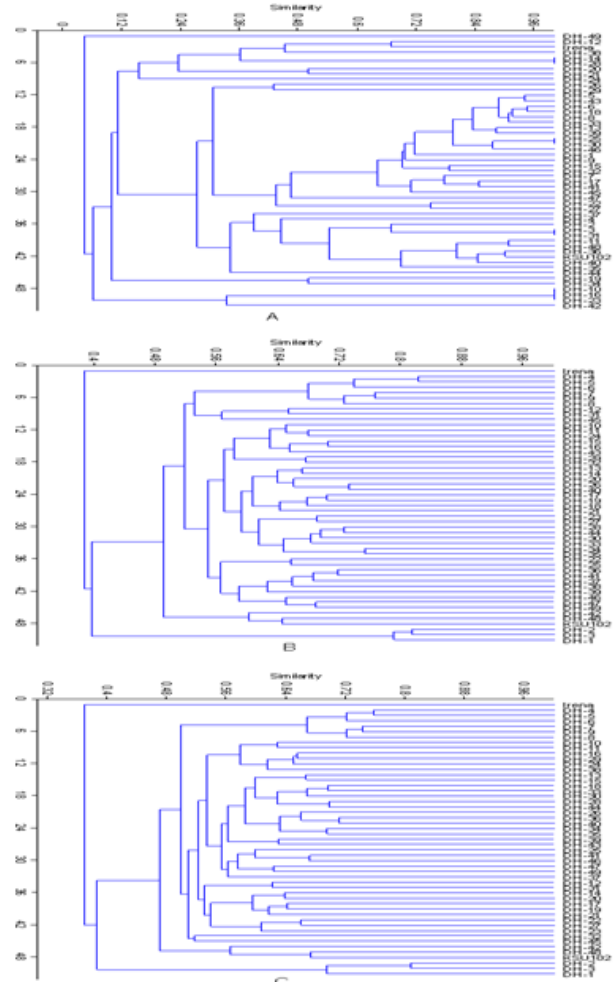


Fig 1. UPGMA dendrograms of cluster analysis of 51 wheat genotypes in Irena × Ksu102 population based on the similarity coefficients calculated using morphoagronomic traits (A), TRAP analysis (B) and morphoagronomic + TRAP data (C).

low biological yield and moderately in grain yield and harvest index. The last cluster comprised the rest of the genotypes.

The number of amplification bands per primer, derived from 40 different TRAP primer combinations, varied between 3 and 9. A total of 226 bands were observed, with 5.7 bands per primer. 50 out of 226 bands (22.12 %) were polymorphic. An example of polymorphism with TRAP38 (Fixed primer; AGTAACCCACCGCCTCCTTC and Arbitrary primers; CCCTCCACCAATCACAAT) is shown in Fig. 5C. Cluster

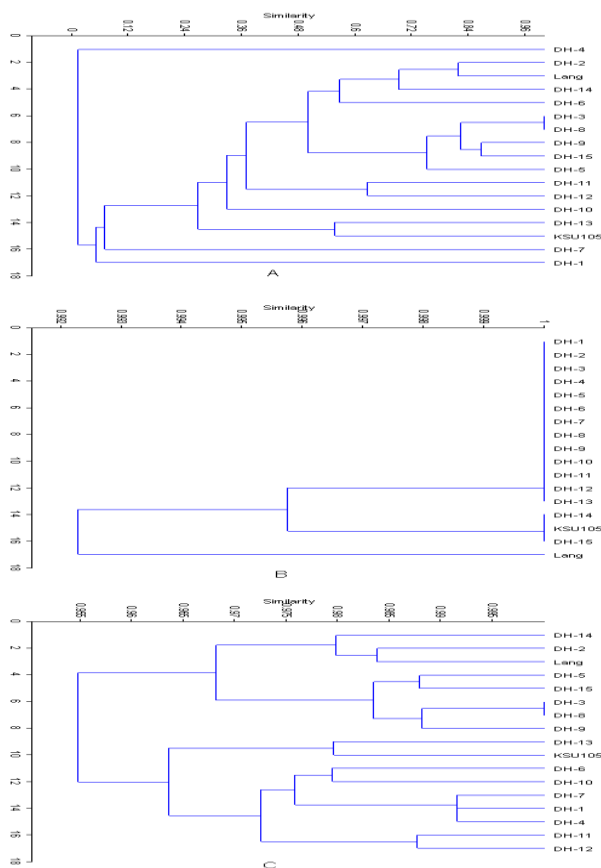


Fig 2. UPGMA dendrograms of cluster analysis of 17 wheat genotypes in Lang × Ksu105 population based on the similarity coefficients calculated using morphoagronomic traits (A), TRAP analysis (B) and morphoagronomic + TRAP data (C).

analysis using TRAP data grouped the 21 wheat genotypes into three main clusters with Jaccard's similarity coefficient ranging from 0.88 to 0.99 (Fig. 3B). The highest similarity was found between DH9 and DH16 (0.99) and the lowest was between Ksu105 and Klasic (0.88). The first cluster only included one wheat genotype (Ksu105). The second cluster contained two doubled haploid lines (DH5 and DH18). The third cluster contained the rest of the wheat genotypes.

The dendrogram generated by combined phenotypic traits and TRAP analysis is presented in Fig 3C. The combined analysis produced similar dendrogram to that produced using TRAP marker analysis; however, the DH18 was moved from Cluster II to Cluster III. The similarity coefficient among the wheat genotypes varied from 0.83 to 0.97 with the highest being between DH8 and DH9 (0.97) and the lowest being between DH5 and DH16 (0.83).

The comparison between agronomic traits and TRAP markers was carried out by Mantel correlation test. The correlation between the two measurements was 0.03, which was not significant, showing that they were not related. However, the correlation coefficient between agronomic traits and the combined matrix (agronomic traits and TRAP markers) was highly significant (0.48**, $p > 0.001$).

Ksu106 × Yecora Rojo population

The analysis of variance indicated that there were statistically significant differences ($p \leq 0.01$) among the wheat genotypes

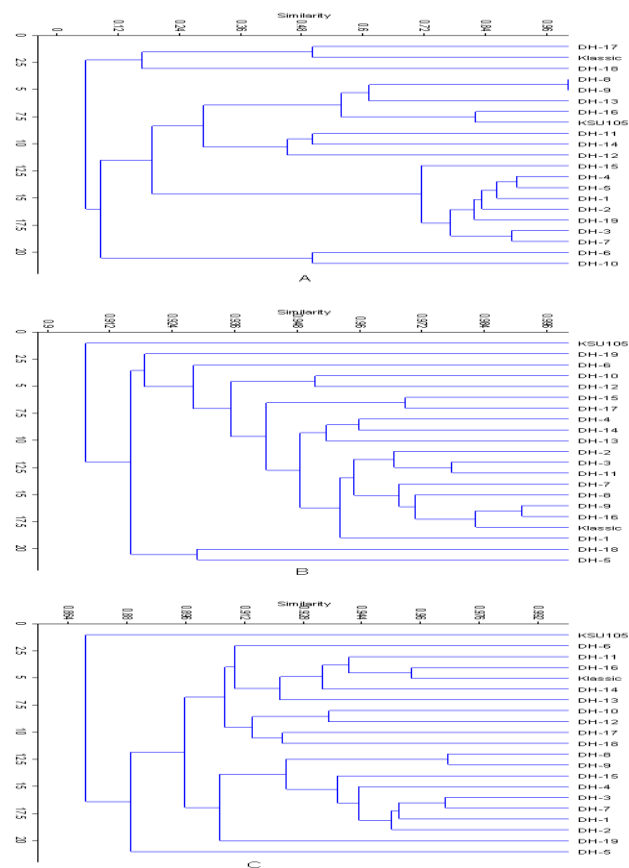


Fig 3. UPGMA dendrograms of cluster analysis of 19 wheat genotypes in Klasic × Ksu105 population based on the similarity coefficients calculated using morphoagronomic traits (A), TRAP analysis (B) and morphoagronomic + TRAP data (C).

for all the agronomic traits. Plant height ranged from 51.3 to 99.0 cm with an average of 74.0 cm. The tallest genotypes were DH8 (99.0 cm) and DH1 (94.3 cm). The DH1 and DH8 had the tallest spike length (18.1 and 18.0 cm, respectively). DH4 was the earliest genotypes (61.3 days), while DH2 (96.3 days) was the latest. Days to maturity ranged from 113.0 (DH3 and DH4) to 134.0 (DH8) days with an average of 121.8 days. The DH4 and DH11 were significantly different from the two parents Ksu106 and Yecora Rojo for both agronomic traits, biological and grain yield. However, the same double haploid lines were not significantly different from parents Ksu106 and Yecora Rojo for harvest index.

Cluster analysis using phenotypic data grouped the 14 wheat genotypes into three main clusters with Jaccard's similarity coefficient ranging from 0.06 to 0.88 (Fig. 4A). Cluster I included two doubled haploid lines (DH3 and DH9) characterized by short plant height, moderate spike length and flowering date, early maturity as well as low biological and grain yield. Cluster II included two wheat genotypes (DH5 and Yecora Rojo) characterized by short plant height and spike length and early in flowering date and maturity as well as low biological and grain yield. The third cluster contained the rest of the wheat genotypes.

A total of 276 fragments were produced by the 40 different TRAP primer combinations. Of these 144 amplified fragments, 52.17% were polymorphic, while 47.83% were not polymorphic among the 14 wheat genotypes. The values

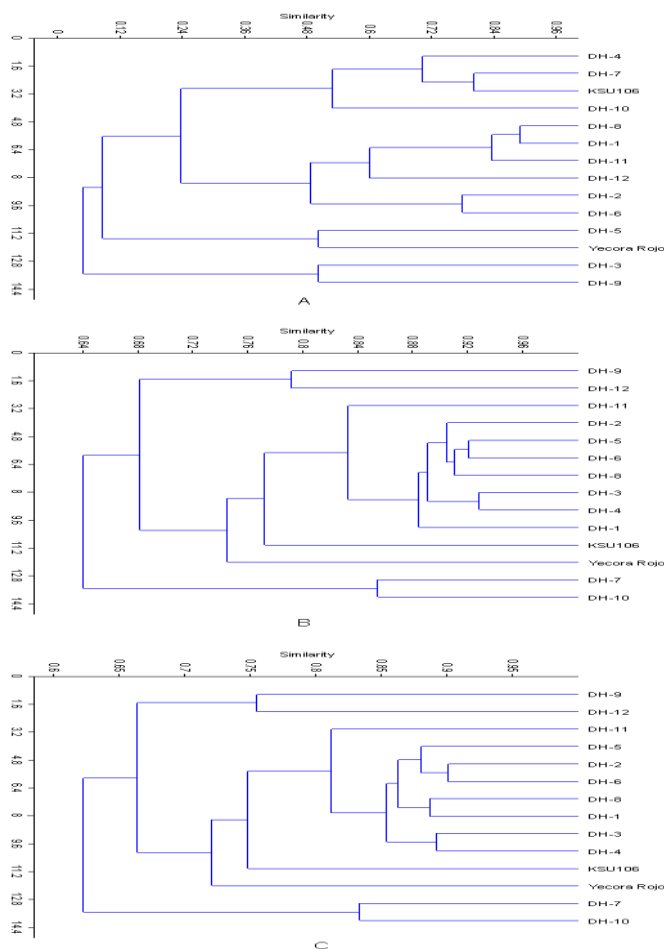


Fig 4. UPGMA dendrograms of cluster analysis of 14 wheat genotypes in Ksu106 × Yecora Rojo population based on the similarity coefficients calculated using morpho-agronomic traits (A), TRAP analysis (B) and morpho-agronomic + TRAP data (C).

of similarity coefficient obtained in TRAP analysis ranged from 0.46 to 0.92 among the studied wheat genotypes. An example of polymorphism with TRAP9 (Fixed primer; TGAGTCCAAACCGGAGC and arbitrary primers; TCACCCGCACCTTCTCC) is shown in Fig. 5D. The dendrograms obtained from TRAP markers were not completely consistent to cluster analysis of phenotypic data. Cluster analysis using TRAP data grouped the 14 wheat genotypes into three main clusters. Cluster I is comprised of two doubled haploid lines DH7 and DH10. Cluster II included two doubled haploid lines DH9 and DH12. The third cluster contained the rest of the wheat genotypes (Fig. 4B). UPGMA dendrogram obtained from the pooled data of TRAP and phenotypic data analyses is presented in the Fig. 4C. The 14 wheat genotypes were grouped into three major clusters as revealed by earlier TRAP alone. The highest similarity was found between DH2 and DH6 (0.90) and the lowest was between DH10 and DH12 (0.45).

The correlation between the two measurements was 0.13 and which was not significant, showing that they were not related. However, the correlation coefficient between agronomic traits and the combined matrix (agronomic traits and TRAP markers) was highly significant (0.23**, $p > 0.001$).

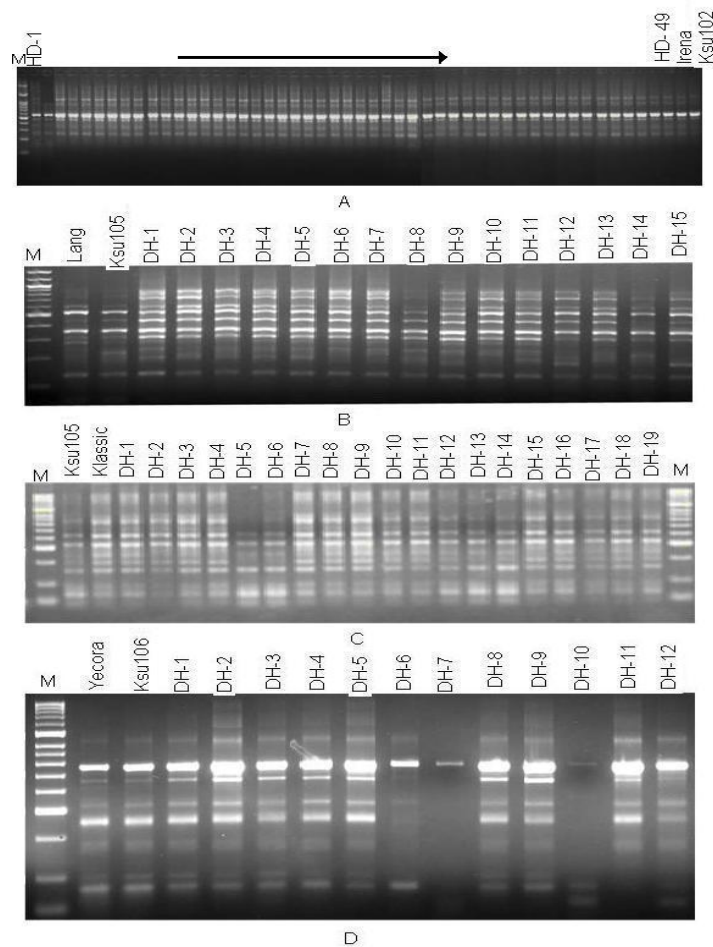


Fig 5. Examples of the TRAP fingerprinting produced by four TRAP primers in the four wheat populations: A- Irena × Ksu102 population with TRAP8; B- Lang X Ksu105 population with TRAP 34; C- Klasic X Ksu105 population with TRAP38; D - Ksu106 X Yecora Rojo population with TRAP9.

Discussion

Doubled haploid wheat can be used as a bread variety and also as a base in producing hybrids due to its homozygosity and high purity. Therefore, understanding the genetic diversity of doubled haploid wheat lines based on agronomic traits and DNA polymorphisms is important.

Genetic diversity, relatedness and structure of parental germplasm are important for breeders to design strategy in breeding programmes. Diversity analysis is important for deciphering genetic relationships including parentage and for the efficient management of germplasm and thereby, use in breeding of improved varieties (Al-Doss et al., 2011). In the present study, phenotypic diversity and TRAP markers were analyzed in four doubled haploid wheat line populations. The doubled haploid wheat lines were derived via microspore culture of F_1 crossing between four elite cultivars and three local Saudi lines to obtain different desired allelic combinations for HMW-GS in the A, B and D genomes.

Morpho-agronomic traits measured (7 traits) depicted significant differences among the parental wheat genotypes and doubled haploid lines in the four populations under investigation. Variation for most of the traits was observed. In the present investigation for the population Ksu105 × Klasic, the results indicated that the DH16 (9.2 t ha⁻¹) was

significantly different from the parents (Ksu105 and Klasic) for grain yield (7.5 and 4.1 t ha⁻¹, respectively). The results also indicated that the DH4 and DH11 were significantly different from the two parents Ksu106 and Yecora Rojo for both agronomic traits, biological and grain yield in the Ksu106 × Yecora Rojo. Agronomic and morphological traits are very important for grouping wheat genetic resources and also are essential and useful for plant breeders seeking to improve existing germplasm by introducing novel genetic variation for certain traits into the breeding populations (Lage et al., 2003; Pagnotta et al., 2005; Pagnotta et al., 2009; Al-Doss et al., 2011). Therefore, these characteristics have good potential to select and conserve genotypes.

Forty different TRAP primer combinations were tested for polymorphism among parental genotypes and doubled haploid lines in the four populations under investigation. Several primers had distinguishable banding patterns between parental genotypes and doubled haploid lines in each population. Our study using TRAP markers revealed a high level of genetic diversity among the wheat genotypes. The markers detected 6.0 polymorphic bands per primer with an average polymorphism of 51.9% across the four populations. Polymorphism between genotypes can arise through nucleotide changes that prevent amplification, 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). 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 = 28, 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. Liu et al. (2005) reported that TRAP markers were very efficient for rapid generation of markers scattered across the genome, which allows linkage groups to be joined and many gaps to be filled. TRAPs also showed the same ability as SSRs to assign linkage groups to chromosomes. Recently, TRAP marker technique has been used for analysis of genetic diversity among six genetically diverse durum wheat genotypes under heat stress (Al-Doss et al., 2011).

In the present study, the clustering based on morpho-agronomic traits did not match that of groupings derived through TRAP analysis. However, the combined analysis produced similar dendrogram to that produced using TRAP marker analysis. The main reason of mismatch between clustering based on molecular markers and quantitative traits may be due to the fact that most of the quantitative traits are controlled by a large number of genes (polygenes) and these traits are highly influenced by environment. Diversity analysis, based on morpho-agronomic traits alone, may not be completely reliable because the traits are limited in number and influenced by environment (Fufa et al., 2005). Despite these limitations, phenotypic characters have been successfully used for genetic variation and cultivar development. Molecular diversity evaluated by molecular markers is independent of environmental influence and can be estimated by DNA obtained from any growth stage (Tatikonda et al., 2009). Molecular characterization is now

the favored means to quantify variation within germplasm samples (Glaszmann et al., 2010). Molecular markers have clarified the structure of genetic diversity in a wide range of plant species. Molecular diversity studies evaluate all levels of genetic structure, ranging from relationships between species complex components to the origin of particular genotypes (Kilian et al., 2007).

The obtained TRAP clusters were not in accordance with the agronomic traits clusters. There was no correlation between variation measurements identified in wheat using TRAP markers and agronomic traits. Previously, low and non significant correlations (-0.23 and -0.25) were reported for AFLP and agronomic characters in wheat lines evaluated in Iran and Mexico, respectively (Esmailzadeh et al., 2005). Rana et al. (2005) reported a correlation of 0.04 between morphological traits and AFLP markers in cotton. Garcia et al. (2007) found no relationship (r = 0.03) between RAPD and morphological characters in perennial dalli grass. However, Autrique et al. (1996) calculated a moderate correlation (0.47) in their genetic diversity study in durum wheat using RFLP and agronomic traits, which was a result of using wider range of genotypes representing more than one ecotype. The low or no correspondence between variation measurements based on molecular markers and agronomic traits should not be considered as a limitation of these methods. The disparity of the two measurements indicates that germplasm classification and selection for crossing in plant breeding programs should not be relied on only one variation measurement. Semagn (2002) stated two reasons for the general lack of correlation between molecular and morphological variation: (1) molecular markers cover a large proportion of the genome, including coding and non-coding regions, and (2) molecular markers are less subjected to artificial selection compared with morphological markers. Correspondence between molecular and agronomic diversity might be improved by analyzing more morphological and DNA markers (Martinez et al., 2005).

Materials and methods

Population development and evaluation of doubled haploid lines

Four wheat populations were derived from crossing between four elite cultivars and three local Saudi lines to obtain different desired allelic combinations (Gahazy et al., 2012) for HMW-GS in the A, B and D genomes (Table 1). Crosses were made to obtain F₁ seeds during winter season 2009/2010 at the Experimental Research Station, King Saud University. F₁ plants were grown in the greenhouse. 49, 15, 19 and 12 doubled haploid (DH) wheat lines derived from microspore cultures of the four wheat populations (Irena × Ksu102, Lang × Ksu105, Klasic × Ksu 105 and Yecora Rojo × Ksu106, respectively) were obtained (Al-Doss et al., 2012). The experiment was laid out in a randomized complete block design (RCBD) with three replications for phenotypic evaluation at the College of Food and Agriculture Sciences, Experimental Research Station, at Dierab near Riyadh, King Saud University, during winter season 2010/2011 for preliminary evaluation for agronomic traits such as plant height, spike length, date of flowering and date of maturity, biological yield, grain yield and harvest index. The seeds were sown in plots 2 m long, with between- row distance of 20 cm and plant distance of 5 cm.

DNA extraction

Frozen young leaves (500 mg) of the doubled haploid lines were ground to a powder in a mortar with liquid nitrogen. The DNA extraction was done using CTAB method (Saghi-Marouf et al., 1984).

TRAP analysis

Forty different TRAP primer combinations (Hu and Vick, 2003) were tested for polymorphism among parental genotypes and doubled haploid lines. The PCR reaction mixture consisted of 20-50 ng genomic DNA, 1×PCR buffer, 2.0 mM MgCl₂, 100 μM of each dNTP, 0.1 μM primer and 1U *Taq* polymerase in a 25 μL volume. 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 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 transilluminator.

Statistical analysis

Analysis of variance appropriate to randomized complete block design (RCBD) was carried out using SAS. Least significant difference (LSD) test was used for the mean comparisons. Data from agronomic traits were standardized and used to estimate the distance matrix, according to Jaccard's coefficient (Jaccard, 1908).

TRAP data were scored for the presence (1), absence (0) or as a missing observation (Fernandez et al., 2002) and each band was regarded as a locus. Based on the similarity matrix, a dendrogram showing the genetic relationships between genotypes was constructed using the algorithm UPGMA (Unweighted Pair Group Method with Arithmetic Average) (Sokal and Michene, 1958) through the software NTSYS pc (Numerical Taxonomy and Multivariate Analysis System, version 1.80 (Applied Biostatistics Program; Rohlf, 1993). Comparison between the Jaccard distance matrix based on agronomic traits and genetic distance matrix obtained with molecular markers was performed for the wheat genotypes by calculating the correlation between the two data sets using the Mantel test (1967) in NTSYS-pc.

Conclusion

Our results indicate that not only we were able to generate DH lines that could be used in a crop improvement program, but also we developed DH lines that could be used directly as cultivars as these lines performed better than the parental line. Further efforts to create and characterize DH lines of wheat with superior agronomic or quality characteristics with high-molecular-weight (HMW) glutenins are ongoing during winter season 2011 /2012.

Acknowledgements

This research project (AR-29-208) is carried out under the Grant Program for Development Research. The program is administered by the King Abdul-Aziz City for Science and Technology (KACST).

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