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RAPD AND SSR BASED GENETIC DIVERSITY ANALYSIS OF ELITE-2 SET OF SYNTHETIC HEXAPLOID WHEATS

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Abstract

Background: Synthetic hexaploid wheats are artificially reconstituted hexaploid wheats that possess high genetic variation which could be utilized for the development of new improved wheat varieties. One such group of synthetic wheats is called the Elite-II set of synthetic wheats that are derived from crossing durum wheat with different *Aegilops tauschii* wheats.
Materials and Methods: In the current study genetic diversity was investigated among 18 Elite-II synthetic hexaploid wheat lines at DNA level. Two types of molecular markers i.e. RAPD and SSR were used for this purpose.
Results: Both types of markers proved useful in estimating the overall genetic diversity among these lines. Based on RAPD data range of genetic distances in these lines was from 0 to 100 percent. Seven D- genome specific SSRs were also used to get further estimation of the genetic diversity contributed by *Aegilops tauschii* parent. On the basis of results obtained it is inferred that the *Aegilops tauschi* accessions used in the production of these synthetic lines were genetically different and they contributed to the enhancement of genetic variation in the synthetic lines. These results could be helpful for future genome mapping programs.
Conclusion: The overall extensive genitive diversity indicates that these lines are good candidates for development of improved wheat varieties by crossing with cultivated wheat varieties.

Key words: Elite-II, molecular markers, RAPD, SSR, synthetic hexaploid wheat

Introduction

Bread wheat is one of the most important crops of the world. Its importance will increase further as the world population is increasing very rapidly. Bread wheat is an amphidiploid (2n= 6x= 42, AABBDD) with three genomes (ABD) and it is believed that it originated as a result of spontaneous hybridization between a tetraploid wheat *Triticum Trugidum* (2n= 4x= 28, AABB) and a diploid wheat *Aegilops tauschii* (2n=2x=14, DD) (Yang et al., 2009). However, it is thought that this evolution of bread wheat involved very few members of the two species. Consequently, the gene pool of hexaploid wheat is much narrowed as compared to its progenitors. This has further been confirmed by molecular genetic analysis of bread wheat and its evolutionary parents (Nazem and Arzani, 2013).

The narrow gene pool of the cultivated wheat has been utilized in the development of improved cultivars over the years for many traits. However, today`s bread wheat is faced with an increasing pressure from biotic and abiotic stresses. The existing genetic diversity has exhausted to bring further improvements in bread wheat. On the other hand, its progenitors have been growing in the wild and have accumulated many useful genes during evolution to better withstand the biotic and abiotic stresses (Tariq-Khan et al. 2012). Hence for sustainable agriculture, these wild relatives of wheat have to be utilized to bring more genetic variation in cultivated wheat (Talbert et al., 1998). Of these wild relatives, *Aegilops tauschii* (DD) is especially important (Mujeeb-Kazi, 2006).

One important solution to increase genetic diversity in common wheat is the production of synthetic hexaploid wheat. Synthetic hexaploid wheat is produced artificially through a method analogous to the evolutionary development of hexaploid wheat. Synthetic hexaploid wheat is a hybrid between tetraploid wheat e.g. durum wheat (triticum turgidum 2n= 4x = 28, AABB) with diploid wheat *Aegilops tauschii* (2n= 2x = 14, DD) (Mujeeb-Kazi, 2006). The resulting hybrid is treated with colchicines to make hexaploid wheat (amphidiploid 2n = 6x = 42, AABBDD) by chromosomes doubling. Till date, more than thousand synthetic hexaploid wheat lines have been produced in CIMMYT (International Maize and Wheat improvement Centre, Mexico) which are being utilized for wheat improvement throughout the world (Mujeeb-Kazi et al., 1996). A number of desirable traits have been incorporated into cultivated wheat from wild relatives (Khan and Khan, 2010). Synthetic hexaploid wheat have been found to have greater genetic diversity and desirable traits (Dreisigacker et al., 2008; Tang et al., 2009), resistance to pests and diseases (Arraiano et al., 2001; Lage et al. 2003) and more adaptability to abiotic stresses (Imtiaz et al., 2008; Trethowan et al., 2008).

Estimation of genetic diversity is an important tool that provides means for development and improvement of plant varieties. A high genetic diversity means that there are more chances of selecting and evolving better varieties. Genetic diversity can be estimated using pedigree analysis, morphological, physiological and cytogenetic characters or molecular markers (Habash et al., 2009). The conventional methods of diversity estimation are not stable and are affected by environmental conditions (Marmar et al., 2013). However, molecular markers methods are not affected by environment and are more reliable. The most common types of molecular markers include restriction fragment length polymorphism (Kim and Ward, 2000), random amplified polymorphic DNA (McDonald, 1995), amplified fragment length polymorphism (Barrett and Kidwell, 1998), and simple sequence repeats (Masmoudi et al., 2006). Simple sequence repeats (SSR) are repeating DNA sequences of 2-6 base pairs (Varshney et al., 2005). They have high reproducibility, are multiallelic, chromosome specific, co-dominant and relatively abundant (Roeder, 1998). They have been utilized for genetic diversity studies in wheat (Röder et al., 2004), gene localization (Röder et al., 2004) and determination of quantitative trait loci (Ganal et al., 2007). RAPDs are another group of simple, cheaper, and more time effective molecular markers (Williams et al., 1990).

Among the various groups of synthetic wheat created in CIMMYT, one is called the Elite-II set of synthetic hexaploid wheat which carries resistance to a number of biotic stresses (Mujeeb-Kazi and Delgado, 2001). The Elite-II set consists of 33 lines. Current studies were aimed at estimation of genetic diversity in 18 of the Elite-II synthetic hexaploid wheat lines using RAPD and SSR markers. The data generated will be utilized in breeding programmes and marker assisted selection to help in the development of better yielding and resistant wheat varieties.

Materials and methods

Genomic DNA isolation

The 18 synthetic Elite-II synthetic lines (line no. 1, 2, 7, 8, 10, 12, 14, 15, 16, 18, 19, 20, 21, 22, 23, 25, 26, 31) (Mujeeb-Kazi and Delgado, 2001) were grown in greenhouse for collection of leaf material. Total genomic DNA was isolated from young seedling using a mini

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scale DNA isolation protocol of Weining and Langridge (1991) with slight modifications. To remove RNA contamination, the isolated DNA was treated with RNAase A at 37°C for 1 hour. DNA was quantified with spectrophotometer and about 50 ng DNA was used for PCR reaction.

Polymerase chain reaction

For RAPD analysis, two RAPD primers GLA07 and GLA09 (Table 1, Genelink, USA) were used. For SSR analysis seven D-genome specific SSR primers (DMS-46, DMS-61, DMS-67, DMS-84, GDM-46, GDM-61, GDM-67 and GDM-84, Table 2) were used. PCR reactions were carried out according to standard procedures. A typical reaction volume consisted of 25 µl. For RAPD-PCR, the reaction profile consisted of an initial denaturation step of 5 min at 95°C, followed by a 1 min denaturation step at 95°C, annealing for 1 min at 37°C and extension for 2 min at 72°C. A total of 35 cycles were performed. A 7 min extra extension step was used after the last cycle in order to allow completion of incomplete reactions. For SSR Analysis, primers specific annealing temperatures were used. The rest of PCR profile was similar to RAPD- PCR.

Table-1: RAPD primers used in present study.

RAPD primer	Sequence (5´-3´)	Size (bp)	Molecular Weight	% GC
GL DecamerA-07	GAAACGGGTG	10	3117.04	60
GL DecamerA-09	GGGTAACGCC	10	3053.01	70

Table-2: SSR (DMS) primers (Dr Manilal, CIMMYT, Mexico/ Dr. Mujeeb Kazi, personal communication and SSR (GDM) primers (Pestsova et al., 2000) used in SSR analysis**.**

Primer	Chromosome	Melting temperature (C°)	Repeat
DMS 46	7D	60	(CA)11
DMS 61	4D	60	(GT)12
DMS 67	7D	60	(GT)20
DMS 84	7D	60	(GT)4GCC(GT)15
gdm46	7D	60	(CA)11
gdm61	4D	60	(GT)12
gdm86	7D	60	(CT)17

Gel electrophoresis

Standard procedures (Sambrook et al., 1989) were followed for gel electrophoresis of PCR amplified products. The PCR products were separated using a 2% agarose/TBE gel. The separated PCR products were then visualized under UV light and the gels were photographed.

Statistical Analysis

For diversity analysis, all scorable bands were scored as present=1/absent=0. The bivariate 1-0 data were used to estimate dissimilarity on the basis of polymorphic loci following Nei *and* Li (1979) by the formula, $D_{xy} = 1- N_{xy} / (N_x + N_y - N_{xy})$, where D_{xy} is the distance (dissimilarity) between two genotypes X and Y, N_{xy} is the number of common bands (alleles) between two genotypes X and Y, N_x is the total number of bands (alleles) present in genotype X and N_y is the number of bands present in genotype Y. The 1-0 bivariate data matrix based on the data of two RAPD and seven SSR primers were used to construct a dendrogram using statistical software "PopGene32" (http://www.ualberta.ca./~fyeh/fyeh).

Results

RAPD analysis

Two RAPD primers (GLA-07 and GLA-09) were used to estimate genetic diversity in the material. Size range of scorable bands (alleles) was from 100-9000 bp (an example of PCR amplification using RAPD is presented in Figure 1). The two RAPD primers revealed high degree of polymorphism between the synthetic lines. Range of mean genetic distances estimated in these lines was 0 to 100%. Maximum mean genetic distance (100%) was observed between synthetic line 19 and synthetic line 1, synthetic line 19 and synthetic line 10, and synthetic line 19 and synthetic line 26.

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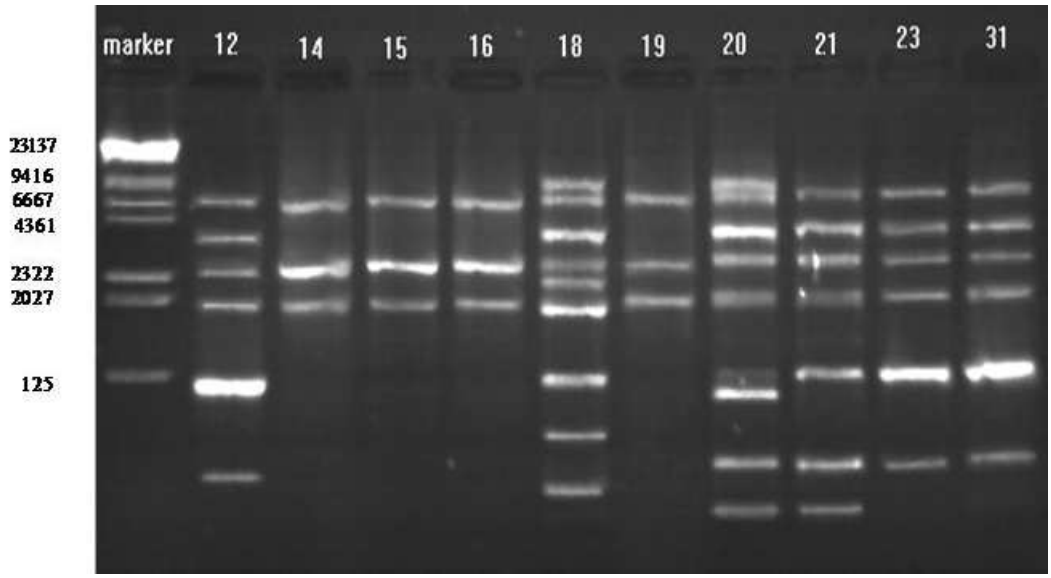

Figure-1: PCR profile of synthetic hexaploid lines, using GL DecamerA-09. Left to right; molecular size λ Hind3/EcoR1 marker (size of fragments are indicated in bp on left), synthetic line no. 12, synthetic line no. 14, synthetic line no. 15, synthetic line no. 16, synthetic line no. 18, synthetic line no. 19, synthetic line no. 20, synthetic line no. 21, synthetic line no. 23 and synthetic line no. 31

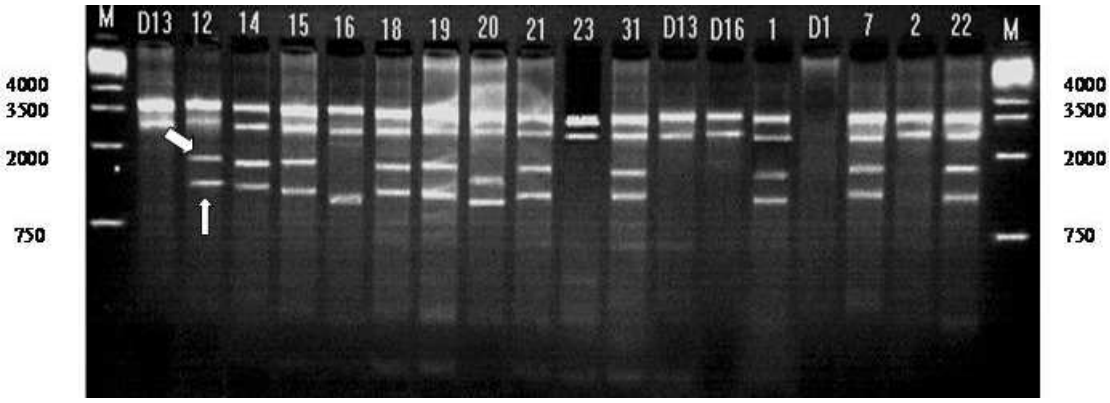

Figure-2: PCR profile of durum parents and their respective derivative synthetic lines using GDM 46 primer. Left to right: molecular size 1 kb ladder DNA (size of fragments are indicated in bp), durum parent D13 and its derivatives; synthetic line no. 12, synthetic line no. 14, synthetic line no. 15, synthetic line no. 16, synthetic line no. 18, synthetic line no. 19, synthetic line no. 20, synthetic line no. 21, synthetic line no. 23, synthetic line no. 31, durum parent D13, durum parent D16 and its derivatives; synthetic line no. 1, synthetic line no. 7, durum parent D1 and its derivatives; synthetic line no. 2, synthetic line no. 22, molecular size 1 kb ladder DNA (size of fragments are indicated in bp). Arrows indicate the polymorphic bands inferred to be contributed by the *Aegilops tauschii* accessions used in these crosses.

Statistical analysis

The dissimilarity coefficient matrix of 18 synthetic lines based on the data of two RAPD and seven SSR primers was used to construct a dendrogram (Figure 3). The genotypes were clustered into one major cluster (A) and three small clusters (B, C and D) while one genotype (E) did not fall into any group. Genotypes in clusters A, B, C, D and E were progeny of durum parents Cerceta, D67.2/P66.270, Sora, Croc-1 and LCK 59.61, respectively. Hence the dendrogram indicates the common ancestry (durum parent) of the synthetic lines along with the diversity contributed by D-genome. As none of the lines showed 100% similarity, it is further inferred that the *Aegilops tauschii* accessions used in the development of these synthetic lines were genetically diverse.

Discussion

In the present study, RAPD analysis was found to be an effective DNA marker system for estimation of genetic diversity among synthetic hexaploid wheats. Two RAPD primers (GLA-07 and GLA-09) used in the study revealed high degree of polymorphism between the synthetic lines. Range of mean genetic distances estimated in these lines was 0 to 100%. Devos and Gale (1992) detected only a few polymorphisms in hexaploid wheat, attributing this to the large portion of repetitive DNA in the common wheat genome. On the other hand the level of RAPD polymorphism in wild wheat has been found to be higher than that in common bread wheat. For example, Castagna et al. (1997) studied genetic diversity in 49 *Triticum urartu* lines. They were able get a very high genetic polymorphism (96%). In the present study, a high diversity (up to 100 percent among some genotypes) is a clear indication of the role the synthetic hexaploid wheat can contribute towards broadening the genetic base of wheat breeding populations. These studies indicate that synthetic lines are genetically diverse and possess a high

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amount of polymorphism. Hence, RAPD technique can be utilized as DNA fingerprint technique for variability identification in hexaploid wheat. The information would be helpful for future genome mapping programs.

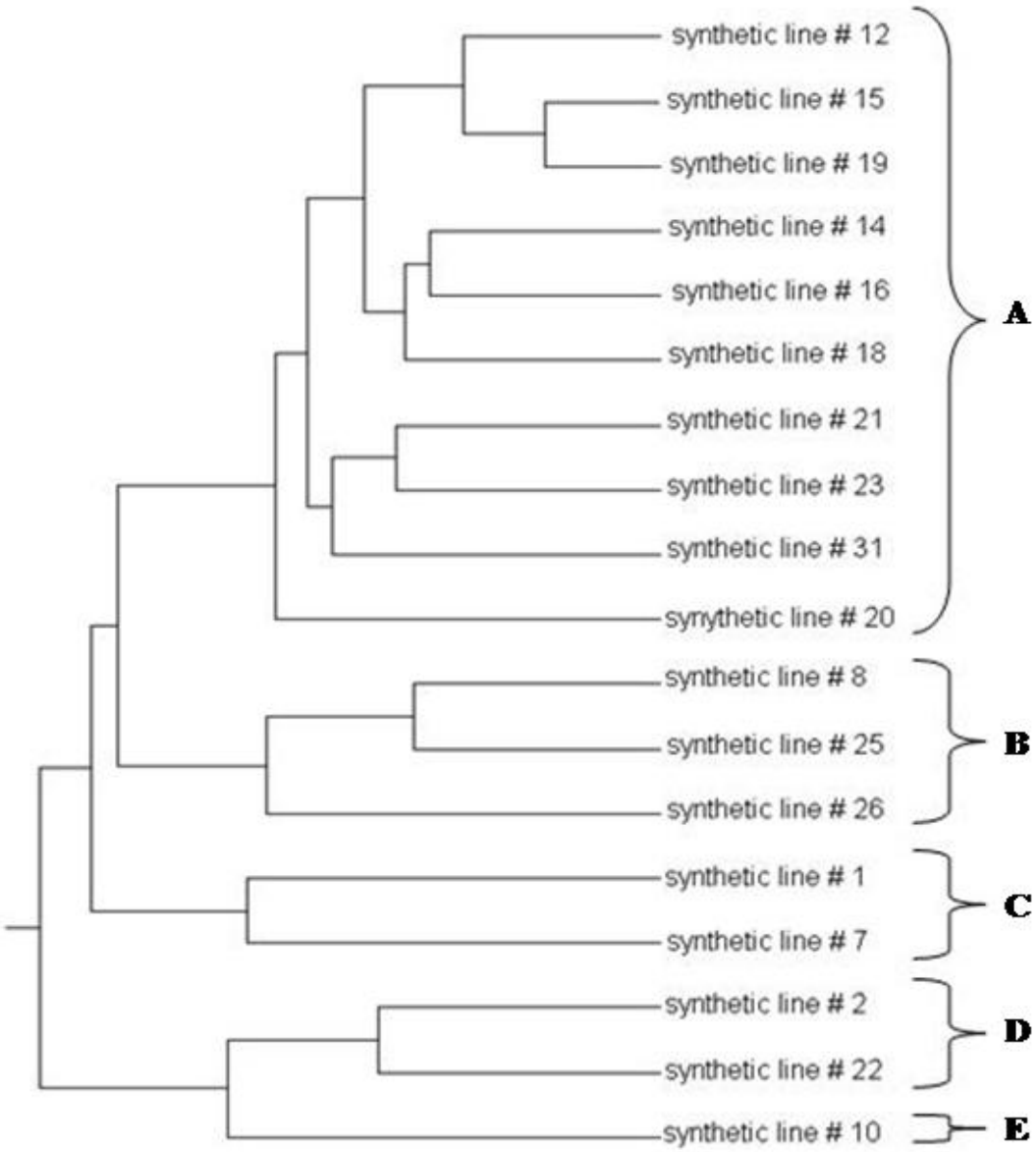

Figure 3: Dendrogram of eighteen synthetic hexaploid wheat lines constructed by using bivariate 1-0 data matrix of two RAPD and seven SSR primers

Although few D-genome specific alleles were found common among different synthetic lines, the overall result of the seven D-genome specific primers revealed that none of these synthetic lines share 100 % similarity for their D-genome parents. Hence it is inferred that the *A. tauschii* accessions used in crosses were genetically different. However, because of the unavailability of seeds/DNA of *A. tauschii* accessions, these results could not be confirmed. Present findings were in agreement with earlier reports of Röder et al. (1998), Peng et al. (1999), Korzun et al. (1998), Huang et al. (2000), Fahima et al. (2002) who also reported high level of polymorphism detected using SSR primers. It is clear from the current studies that more molecular analyses are necessary to find a better and more detailed structure of D-genome in general and *Triticum aestivum* in particular.

This novel diversity residing in synthetic hexaploid wheat is anticipated to add to the durability status and give sustainable outputs (Mujeeb-Kazi and Rajaram, 2002). The integration of molecular output data will enhance breeding efficiency which could also be helpful in PCR based marker assisted selection. In view of the massive information about the high genetic diversity residing in synthetic hexaploid wheats, it is suggested that molecular marker based breeding projects should be initiated to utilize these synthetics to produce distinct cultivars/genotypes by crossing them to different cultivated *T. aestivum* varieties that will maintain the steady genetic improvement.

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