

GENETIC CHARACTERIZATION OF OCTOPLOID (AABBDDRR) AND HEXAPLOID (AABBRR) TRITICALES

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Abstract

The genetic variability analysis of octoploid and hexaploid triticales, evidenced by the RAPD-PCR method has allowed the triticales' (hybrids) differentiation in terms of their ploidy levels corresponding to their primary or secondary nature. The molecular genetic distances derived from Dice's similarity coefficients are based on 84 RAPD markers of which 40 bands are polymorphic (47.61%). The analysis of the matrix shows that the Sørensen–Dice's indices of similarity vary between 1 and 0 with an average of 0.52. The dendrogram shows a segregation of three groups: The first group is consists of triticales 8x ([Mahon-demias/ Merced], [KVZ/alb/RC9] and [Mahon-demias/RC9]). The second group is constituted of triticales 6x (LAMB 2 and CHREA) and the third group encloses only FAHAD 6x triticales. It clearly exposed an intraspecific variability ($i = 0; i = 0.81; i = 1$) and also, the presence an intervarietal polymorphism in the hexaploid group ($i=0, i=0,12, i=1$). The latter produced a high number of specific RAPDs (16) whereas the octoploid triticales amplified a lower number (2). This result confirmed that the specific markers (RAPD) are conversely proportional to the ploidy levels. The triticales (FAHAD5) could be a choice material and could be used as a gene donor for the accession of hybrid triticales. Despite the genomic kinship, the important genetic distance between octoploids (AABBDDRR) ($D < 10$) and hexaploids (AABBRR) ($D > 15$) was revealed in this study. This is explained by a genetic differentiation between to the ploidy level.

Keywords: *X-Triticosecale* Wittmack (8x, 6x), RAPD markers, genetic distance, intraspecific polymorphism, genomic parent

Introduction

Triticale (x -*Triticosecale* Wittmack.) is a synthetic species resulting from the hybridization of female wheat parent (*Triticum* sp) and male rye parent (*Cereale secale* L.). Some of its characteristics are: (i) complex genome due to its high ploidy levels, (ii) the large size of its genome (Ma and Gustafson 2006; Bento et al. 2011), (iii) and the distant relation between the two parental genomes contained in a polyploid nucleus. These characteristics make the triticale very invaluable species for the genetic analysis of genomic changes. Furthermore, the specie represents a gain in terms of biodiversity and economics due to its many genes with high potential (Ammar et al. 2004; Oetler 2005). Several researchers has confirmed that triticales improve generally the diet of humans and animals (Benbelkacem 2006) and that are highly adaptable (Bensalem 1982; Francois 1988; Royo 1995).

The octoploid triticales (8x) which are primary, are produced either by direct amphiploidization of “*Triticum aestivum* x *Secale cereale* L.” hybrids or by interbreeding between two triticales of the same species. These triticales represent a sort of a “bridge” between the parent species and the hexaploids. However, hexaploid triticales (6x) are primary, resulting from crossbreeding wheat and rye, or secondary ensuing from crossbreeding of two primary triticales. These triticales (6x) are the most cultivated and widely commercialized crop on a global scale.

RAPD markers have been used for long to trace the hybrid origins of several species (Nevo et al. 1994; Vaillancourt et al. 2007). It is used not only to differentiate the genotypes of different cultivars of a species of economic interest (Eagles 2001; Ma et al. 2004). But it is also predicted that it will be of a great importance in improving the agronomic value of crops and the selection programs in cereals (Shaked 2001; Dekkers 2002). A lot of research has demonstrated the effectiveness of RAPD markers to identify, characterize, and evaluate the genetic diversity of hexaploid triticales (Terzi 1997; Gupta et al. 2001; Rafalski 2002; Bednarek et al. 2003). However, this method has ever hardly been applied to the study of octoploid triticales.

This paper is part of a genetic variability study of octoploid triticales (AABBDDRR) and hexaploid triticales (AABBRR). RAPD-PCR molecular technique was used with the following objectives:

- Polymorphism analysis of the genomic DNA.
- Identification and characterization of each hybrid as well as highlighting the ratio of specific markers (alleles) to the ploidy level.
- Assessment of genetic distances and the highlighting of the phylogenetic relations between the triticales.

Material and method

Plant material and its origin: Three primary octoploid triticales ($2n=8x=56$) and three secondary hexaploid triticales ($2n=6x=42$) were used for the study (table 1).

-The octoploid triticales (local lines), which resulting from the amphiploidisation between hybrids (*Triticum aestivum* L. x *Secale cereale* L.), were provided by Batna's Agronomy Institute (Algeria).

- The hexaploid triticales (varieties), which resulting from the cross breeding of two different primary triticales, were selected in Algeria and originally cultivated in the International Maize and Wheat Improvement Center (CIMMYT), except CHREA that is a local variety.

Characteristics

The octoploid triticales, lines ([Mahon_demias/RC9], [Kvz/alb/RC9]) have given a good technological quality and a higher performance. But, [Mahon_demias/Merced] line was characterized by a higher performance and an adaptation to unfavorable environmental conditions. This line was sensible to cryptogamic diseases.

The hexaploid triticales, varieties (CHREA, LAMB2) were characterized by a higher seed yield and good technological quality. FAHAD5 variety had a higher performance and an adaptation to unfavorable environmental conditions. This variety was resistant to many cryptogamic diseases.

Table1. Triticale lines (8x) and varieties (6x) included in the analysis of RAPD polymorphism.

Species (ploidy levels)	Hybrids (lines/varieties)	code	Pedigree	G
Octoploid triticale (8x) AABBDDRR	Mahondemias/Merced	Mah/Md	Mah/Mer	F5
	Kvz/alb/RC9	Kv/RC9	Kv/RC9	F5
	Mahondemias/RC9	Mah/RC9	Mah/RC9	F5
Hexaploid triticale (6x) AABBRR	FAHAD 5	FA5	(CTM18931-0Y-3M- y1M- 2B-OY).	F6
	LAMB 2	LA2	(CTSS95Y00296S-M-Y- 0B-0Y-0B-B-Y).	F6
	CHREA	CH	(CTSS95Y00296S-0M-Y- 0B-0Y-0B-1B- Y)	F6

RAPD-PCR technic

RAPD PCR was applied to the samples as described by Williams et al. (1990). The polymorphism of genomic DNA requires the use of many primers, albeit six short sequence primers – from different kits - are kept at

the end (15pb) (OPO-07, OPO-05, OPC-15, OPO-16, OPC-05, and OPO-10) (table 2). The technique comprises the following steps:

a-The extraction of genomic DNA: it is performed according to the method described by Dellaporta et al. (1983) on young leaves (500 mg) of each hybrid, using the SDS method.

b-RAPD analysis with a primer: the amplification was carried out in a Thermal cycler (Perkin-Elmer 2400 modal) programmed to perform: first, a cycle of incubation at 94°C for 4 minutes, followed by 40 cycles of 94°C for 45 seconds each (denaturation of the DNA), then 36°C for 1 minute and 72°C for 2 minutes (polymerization), last but not least the final extension (or elongation of strands) at 72° C for 12 minutes. The reaction mixtures containing 25 pi (Polymerase Chain Reaction) is composed of 20 ng of DNA template, 0.2 µM dNTP, 0.2 µM of primer, 0.25 µ of Taq polymerase and 10x PCR reaction buffers. The mix was diluted with distilled water to 25 pi. Electrophoresis was used to separate the samples of amplified DNA on agarose gel at 80 Voltas.

Table2. Primers' codes, sequences, and percentage in content (C + G)

Code Primer	Sequences 5' → 3'	% (C + G)
OPO- 07	CAGCACTGAC	60 %
OPO- 10	TCAGAGCGCC	70 %
OPO- 05	CCCAGTCACT	60 %
OPO- 16	TCGGCGGTTC	70 %
OPC- 05	CATGACCGCC	70 %
OPC-15	GACGGATCAG	60 %

c- Statistical analysis: Unweighted Pair Group Method with Arithmetic Mean was used to estimate the genetic relations based on the Sørensen–Dice indices. The calculations were carried out by the NTSYS-PC software (Rohlf 1990).

Results

The selected primers were used for the identification and genetic characterization of the triticales. The presence of a marker (or a band) is an indicator of RAPD phenotype. The amplified DNA fragments of each marker are registered as (1) if present and (0) if absent. Each presence-absence couple is therefore deemed a locus (allele).

The primers that produced a high number of amplified DNA fragments are represented in figure 1. In this study, the analysis of primers revealed that the number of bands varied between 12 bands (primers OPC-05 and OPC-15) and 18 bands (primer OPJ-07) (table 3). In fact, the total number of RAPD markers was 84 of which 44 bands were monomorphic (52.38%) and 40 bands were polymorphic (47.61%) (table3). The latter's

size vary between 1 bp -1626 bp. Their precise position on the gel was determined in relation to the molecular weight of the standard marker Gene Ruler (50 bp DNA Ladder) (fig 1, fig 2).

The primer OPO-16 yielded a high level of amplified DNA fragments variability wherein polymorphic bands (10 bands, 76.92%), followed by three primers: OPO-10 (4 bands, 53.33%), OPO-07 (9 bands, 50%), OPO-05 (6 bands, 42.85%) (fig 1, table 3). Meanwhile, the primers OPC-05 ad OPC-15 amplified a lower number of polymorphic bands in the following order: 4 bands, (33.33%) and 3 bands (25%) (fig 2, table 3).

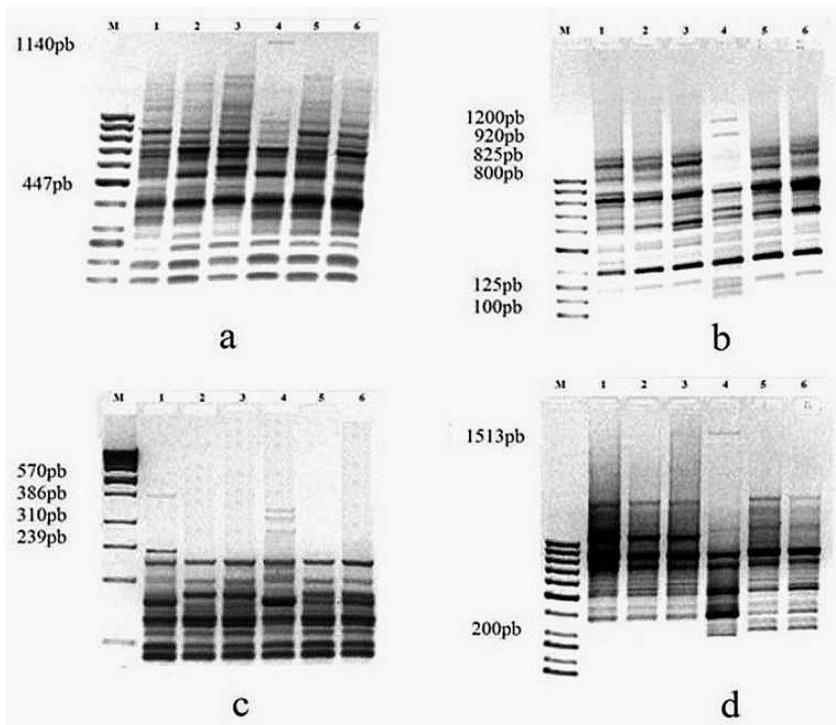


Figure1. RAPD profiles of octoploid and hexaploid triticales obtained with four primers: (a) OPO- 07, (b) OPO- 10, (c) OPO- 05 and (d) OPO- 16. Lane (M): DNA size marker molecular (50 bp DNA Ladder). Lane (1): Mahon-demias/Merced, Lane (2): KVZ/alb/RC9, Lane (3): Mahon-demias /RC9, Lane (4): FAHAD5 (majority of specific markers), Lane (5): LAMB2, Lane (6): CHREA.

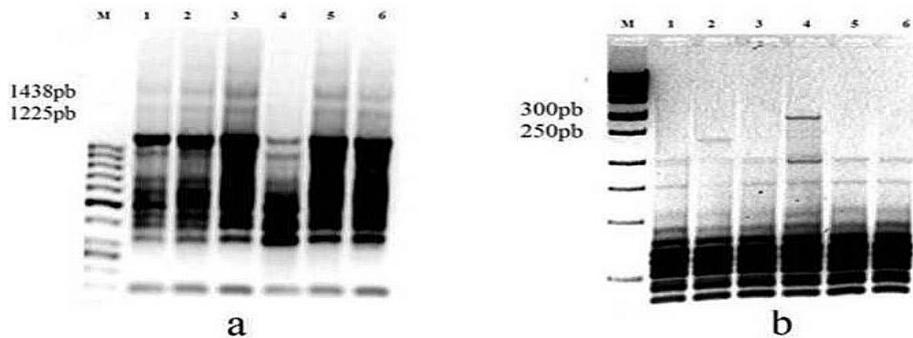


Figure2. RAPD profiles of octoploid and hexaploid triticales obtained with two primers: (a) OPC- 05, (b) OPC- 15 Lane (M): DNA size marker molecular (50 bp DNA Ladder). Lane (1): Mahon-demias/Merced, Lane (2): KVZ/alb/RC9, Lane (3): Mahon-demias/RC9, Lane (4): FAHAD5, Lane (5): LAMB2, Lane (6): CHREA.

Table3. Types of amplified DNA and detected polymorphism percentage in six primers in triticales (8x) and (6x).

Primers	Monomorphic Bands	Polymorphic Bands (loci)		Bands total	% of polymorphism
		unique bands (specific markers)	non-unique bands		
OPO- 07	9	4	5	18	50 %
OPO- 10	7	4	4	15	53,33%
OPO-05	8	6	0	14	42 ,85%
OPO-16	3	2	8	13	76,92 %
OPC- 15	9	2	1	12	25 %
OPC- 05	8	2	2	12	33,33%
Total	44	40		84	

The majority of specific bands (alleles) is observed in FAHAD5 variety (100 bp, 125bp, 200bp, 239 bp, 300 bp , 310 bp, 447 bp, 570 bp, 800 bp, 825 bp, 920 bp, 1200 bp, 1140 bp, 1225 bp, 1438 bp, 1513 bp) (fig 1). There is also another specific band detected in the hybrid Mahon-demias /Merced (386 bp) (fig 1) and another in the hybrid KVZ/alb/ RC9 (250 bp) (fig 2). These bands (or specific markers) can be used for the identification and differentiation of the studied hybrids.

Analysis of genetic distance

The matrix of genetic Dissimilarity between different triticales can be substantiated by the visualization of RAPD profiles on the Agarose gel. The analysis of this matrix shows that the Sørensen–Dice’s indices of similarity vary between 1 and 0 with an average of 0.52 (table 4). This interval expresses an important intra-specific genetic variability. The most important

genetic affinity was characterized by two varieties LAMB2 and CHREA

	Mah/Mer	Kv/RC9	Mah/RC9	FA5	LA 2	CH
Mah/Mer	1					
Kv/RC9	0,79	1				
Mah/RC9	0,64	0,81	1			
FA5	0,19	0,12	0,00	1		
LA 2	0,47	0,52	0,42	0,18	1	
CH	0,47	0,64	0,61	0,29	1,00	1

($i=1$). The Mahon-demias/RC9 hybrid, Kvz/alb/RC9, and Mahon-demias/Merced are equally close to each other ($i=0.81$; $i=0.79$) whereas FAHAD variety is clearly distinct ($i=0$) (table 4).

Table4. Similitude matrix based on Dice's indices

Discussion

A lot of research has been conducted on the octoploid (Ma and Gustafson 2006; Hammouda and khalfallah 2008) and hexaploid triticales using morphological (Gupta 1986; Benbelkacem and Zeghida 1994; Ammar et al. 2004), cytogenetic (Bernard et al. 1992; Feldeman and Levy 2005; Hammouda and khalfallah 2010) and biochemical markers (Amiour et al. 2001; Lukaszewskii 2006; Bellil et al. 2010; Bento et al. 2010; Lukaszewskii 2011). But, in this study concerns itself with molecular polymorphism of two types of triticales has been studied using RAPD markers.

Monica et al. (2006) showed that, the number and the size of the amplified DNA fragments of hybrids (triticales x wheat) were differed by different primers used. Notably, in our study, OPC-15 and OPC-05 primers had yielded the same results as the amplified DNA fragments (fig 2).

The genetic divergences between octoploid and hexaploid triticales were estimated by POPGENE freeware (Nei 1978). These distances have been presented in figure 3.

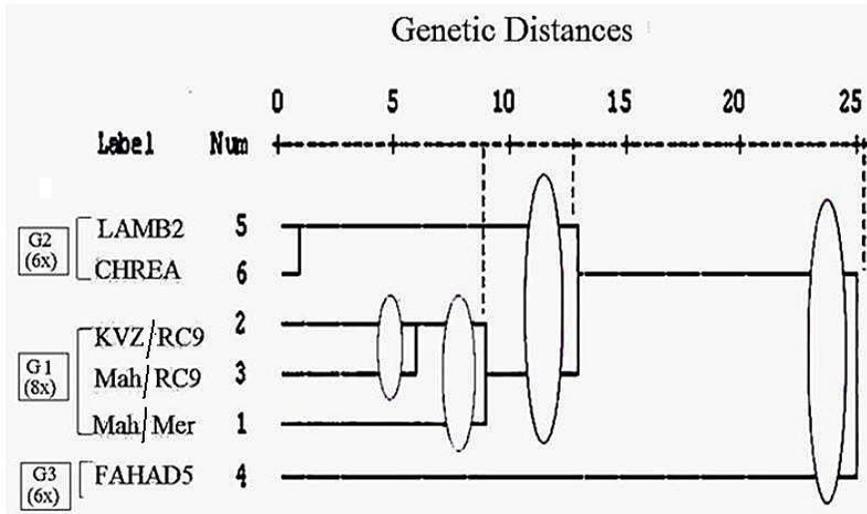


Figure3. UPGMA dendrogram of genetic distances determined from 84 RAPD markers on three octoploid triticales and three hexaploid triticales, demonstrating the phylogenetic relationships. G1: Lines triticales (8x) (Kvz/alb/RC9, Mahon-Demias/RC9, Mahon-Demias/Merced), G2: varieties triticales (6x) (LAMB2, CHREA), G3: variety triticales (6x) (FAHAD5).

From the analysis of 84 RAPD markers used, substantial genetic distances showed segregation of varieties and in-between triticales lines. These distances varied from 0-25.

The three lines or hybrids ([Mahon-demias/RC9],[Kvz/alb/RC9],[Mahon-demias/Merced]) segregated at $D < 10$ distances. The latter are low in differentiation between [Mahon-demias/Merced] and the two others. The LAMB 2 and CHREA varieties similarly segregated at almost identical distances. Therefore, they show a genetic affinity; however, FAHAD5 variety showed a greater genetic distance ($D=25$) and is consequently quite removed from the other tested varieties. FAHAD5 triticales represents a strong heterogeneity in comparison to all the studied material.

The dendrogram of genetic dissimilarity shows a segregation of three groups. The first group is made up of three triticales 8x ([Mahon-demias/Merced],[Kvz/alb/RC9],[Mahon-demias/RC9]). The second group is constituted of two triticales 6x (LAMB 2 and CHREA) and the third group encloses only FAHAD 6x triticales (fig 3):

FAHAD5 (triticales 6x) is characterized by the presence of the majority of specific markers (16 loci), a genetic distance ($D=25$) higher than all the varieties and lines we dealt with so far. It also shows low levels of heterochromatin (highly repeated sequence of DNA) detected by cytogenetic markers (Hammouda and Khalfallah 2010). In agronomy, this triticales

doesn't only have a high performance but it also has a high adaptability to a lot of harsh environmental conditions. Furthermore, it is resistant to many foliar diseases which means it could be a choice material and could be used as a gene donor for the accession of hybrid triticales.

The investigation works on hybrids conducted by authors (Gradzielewska et al. 2009) *Aegilops juvenalis* Thell (DD) and *xTriticosecale* Wittmack (AABBRR), using RAPD markers show four specific bands. These markers helped identifying *Argilops juvenalis*' DNA in the hybrid triticale. The same authors (Gradzielewska et al. 2010) worked on hybrids that resulted from the cross-breeding of *x-Triticosecale* Wittmack (ABR) and *Aegilops.crassa* Boiss (DM). They obtained 14 specific RAPDs which allowed the identification of genetic material of the uncultivated parent. The results of present study confirmed the genetic affinity between the two groups ($i=0.84$) of the studied hybrids. Nevertheless, our study case showed 18 specific RAPDs that were amplified in *x-Triticosecale* Wittmack, including the two groups (octoploid and hexaploid). On one hand, It clearly exposed an intra-specific polymorphism ($i = 0$; $i = 0.81$; $i = 1$). On the other hand, it is noteworthy that there was also an inter-varietal diversity in the hexaploid group.

The authors (Bolaric et al. 2005) demonstrated that in *Lolium sp*, RAPD markers presented a more elevated intra cultivar genetic variation (66%) than between cultivars (33%). Other researchers (Wilson et al. 2001; Amirouche and Misset, 2003) working on other *Poaceae*, such as *Elymus glaucus* and *Hordeum murinum* L., found a differentiation coefficient. They also declared that genetic variability (genetic differentiation) is frequently more important in *Poaceae* than other plant families.

After considering the obtained results, we could ascertain that the RAPD method is a reliable one because it allowed the triticales' (hybrids) differentiation in terms of their ploidy levels that relates to their primary or secondary nature. Although we noticed that the hexaploid triticales (6x) produced a high number of specific RAPDs (16) whereas the octoploid triticales (8x) amplified a low number (2). This finding confirmed that the specific markers (RAPD) are conversely proportional to the ploidy levels. Other authors (Bento et al. 2011) demonstrated that 30% of the polymorphic bands were observed in octoploid triticales and 40% were observed in hexaploid triticales. In other words (Ma and Gustafson 2006; Gradzielewska et al. 2012) the effects of ploidy levels play a significant role in the genomic changes of *x-Triticosecale* Wittmack during its formation. There were other works signaling that the effects of ploidy levels were weak in other allopolyploid species. The sole exception to that was *Triticum sp* complex (4x, 6x, 8x) which reflects no difference in genomic variations between different levels of ploidy (Ozkan et al. 2001).

Phylogenetic relations

Additionally, the phylogenetic relations between octoploid and hexaploid triticales by our interpretations are illustrated in figure3. According to their genomic constitution; octoploid and hexaploid triticales exhibit three common genomes (AA, BB, RR) and a supplementary genome (DD) present only in octoploids. The genome A donor is *Triticum monococcum* L., the genome B donor is *Aegilops spletoides*, the genome D donor is *Aegilops squarrosa* (Coss) (Syn.*Triticum tauschii*) and the genome R donor is *Secale cereale* L. Despite this genomic kinship, an important genetic distance between octoploids ($D < 10$) and hexaploids ($D > 15$) was revealed in our study. This is explained by a genetic differentiation between to both levels of ploidy.

Conclusion

The obtained results by RAPD markers analysis allowed precise identification of studied triticales and evaluation of genetic diversity across all the genome. A Intraspecific variability and a other intervarietal are observed. Despite this genomic kinship, the important genetic distance between octoploids ($D < 10$) and hexaploids ($D > 15$) was revealed in our study. This result suggests that the genetic differentiation is due to octoploid triticales are different to hexaploid triticales

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