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Original Contribution

THE IDENTIFICATION OF E^B CHROMOSOMES IN TRITIPYRUM PRIMARY LINES USING RANDOM AND SEMI RANDOM PRIMERS

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ABSTRACT

In order to detect the presence of E^b chromatin in intergeneric hybrids of durum wheat (2n=2x=28 AABB genoms) with *Thinopyrum bessarabicum* (2n=14 E^bE^b genome) genomic DNA of seven Tritipyrum primery lines, five wheat breeding cultivars, five Triticale primising lines and *Thinopyrum bessarabicum* were amplified using 30 random primers and 20 semi random primers. DNA amplified with primer OPF03 has shown the presence of Eb genome DNA fragment (1296 bp) in *Thinopyrum bessarabicum* and tritipyrum primary lines, but the absence of chines spring wheat, wheat breeding cultivars and triticale primising lines. Genomic DNA amplified with primer OPM 06 showed a DNA fragment (approximately 700bp) in *Thinopyrum bessarabicum* and tritipyrum primary lines with the exception of La(4B)4D/b and showed also a DNA fragment of approximately 800bp in *Thinopyrum bessarabicum* and tritipyrum primary lines with the exception La(4B)4D/b and Ka/b lines. Genomic DNA amplified with semi random primer of ET34, showed a DNA fragment (approximately 830bp) presence in, *Thinopyrum .bessarabicum* and triticale primising lines, but absence in Chinese spring wheat, wheat breeding cultivares and tritipyrum primary lines. Thus, OPM 06 and OPF03 bands could be used as a markers to detect the E^b chromosome in all the tritipyrum lines and materials carrying the E^b chromosome and also DNA fragment amplified to ET34 primer probability demonstrated the E^b genome is relatively closer to the R genome of secale These studies showed the usefulness of molecular markers in detecting E^b chromatin/ DNA fragments intrergeneric hybrids with durum wheat.

Key words: E^b chromosome, RAPD, semi random primer, *Thinopyrum bessarabicum*, tritipyrum, wheat, triticale

INTRODUCTION

Tritipyrum is on intergeneric hybrid between wheat durum (2n=4x=28 AABB) and wheatgrass *Thinopyrum bessarabicum* (2n=2x=14 E^bE^b) (1, 2, 3). Many wide species of the tribe *Triticeae* of grass family (Poaceae) are valuable sources for resistance to diseases (dwarf bunt and barley yellow dwarf virus), insect pests that can be used to improve the present cultivars of durum wheat (4). In the last four decades of 1936, the incorporation of alien genetic material from *Thinopyrum* into common wheat through wide crosses has increased the genetic diversity in wheat for improvement of disease resistance, drought and salinity tolerance and others traits(5). The most had notable salt tolerance wild species of wheat such as Thinopyrum *bessarabicum* which is a littoral diploid grass native to the Crimea,

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Ukraine. Primary hexaploid tritipyrum lines, amphiploid between *Triticum durum* and *Thinopyrum bessarabicum* can set seed in at last 250 mM Nacl (1, 2, 6).

The identification and characterization of alien chromatin or chromosome segments introgressed into wheat complement is important from the plant breeding standpoint (4). Molecular cytogenetic techniques such as in situ hybridization, DNA polymorphism by random amplified polymorphic DNA (RAPD) or restriction fragment length polymorphism (RFLP) or a combination of these methods have been employed to detect alien chromatin in intergeneric hybrid with wheat (4). The need for information about the target sequence would greatly limit the applicability of PCR as a mapping tool. An alternative strategy was developed based on the consensus sequences for the intron splice junction (ISJ) (7). The ISJ- based markers can be used for generating maps of the cereal genomes (7).

Two rye genome-specific RAPD markers (pSc10C and pSc20H) were detected in all of the tested materials that contained rye chromatin (8). RAPD markers to chromosome 4 of barley (*Hordeum Vulgare*) were detected in wheat (Chinese Spring) background (9). And RAPD markers specific for chromosome $5E^b$ for *Thinopyrum bessarabicum* were identified in wheat / alien recombinant (10). In earlier studies on development of molecular markers for common bunt resistance, a random amplified polymorphic DNA (RAPD) marker associated with common bunt resistance gene to race T_1 (*Tilletia Tritic*i) in spelt was identified (11). In studies in order to detection of DNA fragments in durum wheat hybrids with *Thinopyrum Junceiform* by RAPD markers, genomic DNA amplified with primer PR41 showed the presence of *Thinopyrum Junceiform* DNA fragments in the durum hybrids and BC_1 plants (4). And also RAPD markers associated with $2A_i$-2 chromosome of *Thinopyrum* genus in wheat background were identified (12).

The objective of this study was detected the presence of E^b chromatin in intergeneric hybrids tritipyrum and R chromatin in triticale lines using random and semi random primers based on PCR.

MATERIALS AND METHODS

2-1 plant materials

Tritipyrum primery lines including: Az/b, La/b, Ka/b, Ma/b, St/b, La(4B/4d)/b and Cr/b, *Triticale* primising lines including: 4116, 4115, 4103, 4108 and M_{45}, wheat breeding cultivars including: CS, St, D.H, *Hirmand* Sistan and *Hamoon* Sistan and wheatgrass *Thinopyrum bessarabicum*. Seeds of tritipyrum and triticale were collected from research farm of agriculture collage of *Kerman* Shahid Bahonar University. The experiment was performed in the Institute of agriculture biotechnology of *Zabol* University.

2-2 DNA extraction and PCR analysis

Total genomic DNA in leaves (bulk samples) were extracted using modification the method described by Chen and Ronald (13). The yield of DNA per gram of leaf tissue extracted was measured using Biophotometer (model Eppendorph). The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to 280 nm and electrophoreses on 1% agarose gel (14). PCR reactions were conducted using arbitrary 10-mer primers (Operan technology) and semi random primers of ET (exon targeting) and IT (Intron targeting) (7, 15, and 16). The names and sequences of the primers that gave clear, repetitive and polymorphism bands are listed in table 1. the reaction conditions were optimized and the mixtures (25 µl total volume) were composed of 50 ng DNA, 1X reaction PCR buffer, 1U tag DNA polymerase (Cinnagen company), 3mM $Mgcl_2$, 0/2 mM of each DNTPs and 0/4µM primer. Amplifications were carried out in Thermal cycler (model Corbett Research). Denaturation was executed at 93^{0c} for 150 s before the start of cycling. An amplification cycle was consisted of 60 s at 93^{oc}, 60 s at 36^{oc} for random primers, 60^{oc} for semi random 15-mer primers and 63^{oc} for semi

18-mer primers, and 120 s at 71^{oc}. A total of 42 cycles were performed and cycling ended with a final extension at 71^{oc} for 8 min. the amplification products were separated in 1/8% (w/v) agarose gel in 1X TBE buffer and visualized by staining with ethidium bromide 0/1% and ultraviolet light. Molecular weights were estimated using DNA marker (100-100bp) and Lambda DNA digested by ECOR I and HIND III enzyme.

2-3 Data analysis

RAPD and ISJ (Intron Splice Junction) amplified fragments, with the same mobility according to the molecular weight (bp), were scored manually for band presence (1) or absence (0). The primers that could amplify a specific band that was present in Thinopyrum *bessarabicum* and tritipyrum primary lines but absent in Chinese spring wheat, wheat breeding cultivars and triticale primising lines, were selected in order to detect E^b genome chromosomes in tritipyrum primary lines.

Table 1. *Primers of random and semi random analysis used in the study*

primer	sequenc (5`-3`)	temperture melting
semi random primers		
IT34	5`GCGGCATCAGGTAAG 3`	60
ET33	5` ACCTACCTGGCCGAT 3`	60
ET34	5` ACCTACCTGGGCGAG 3`	60
ET36	5` ACCTACCTGGGGCTC 3`	62
ET37	5` ACTTACCTGAGGCGCGAC	63
ET38	5` ACTTACCTGCTGGCCGGA 3	63
ET39	5` ACTTACCTGGCCAGCTGC 3	63
ET42	5` ACTTACCTGCCTACGCGG 3	63
random primers		
OPFo3	5` CCTGATCACC 3`	36
OPN 15	5` CAGCGACTGT 3`	36
OPN 06	5` GAGACGCACA 3`	36
OPC 05	5` GATGACCGCC 3`	36
OPM 04	5` GGCGGTTGTC 3`	36
OPN 12	5` CACAGACACC 3`	36
OPN 16	5` AAGCGACCTG 3`	36
OPM 06	5` CTGGGCAACT 3`	36
OPF 01	5` ACGGATCCTG 3`	36
OPC 11	5` AAAGCTGCGG 3`	36

RESULTS

Among the prescreened primers, 11 semi random and 8 random primers amplified polymorphic, repetitive DNA bands **(Table 1)**. Random primers produced a total of 123 bands, 119 bands (97/66) being polymorphic bands. The bands were characterized based on size and ranged from approximately 200-5000 bp. Genomic DNA amplified with primer OPF03 shown the presence of E^b genome DNA fragment (1296 bp) in *Thinopyrum bessarabicum* and tritipyrum primising lines but the absence of chines spring wheat , wheat breeding cultivars and triticale primising lines. Genomic DNA amplified with primer OPM 06 showed a DNA fragment (approximately 700bp) in *Thinopyrum bessarabicum* and tritipyrum primary lines with the exception La(4B)4D/b and showed also a DNA fragment of approximately 800bp in *Thinopyrum bessarabicum* and tritipyrum primary lines, with the exception La(4B)4D/b and Ka/b.**(Fig 1a)**. The result

has shown that OPM 06 and OPF03 primers bands could be used as markers to detect the E^b chromosome in all the tritipyrum lines and materials carrying the E^b chromosome. Genomic DNA amplified with semi random primer of ET34, showed a DNA fragment (approximately 830bp) presence in, *Thinopyrum bessarabicum* and triticale primising lines, but absence in Chinese spring wheat , wheat breeding cultivars and tritipyrum primary lines **(Fig. 1b)**.

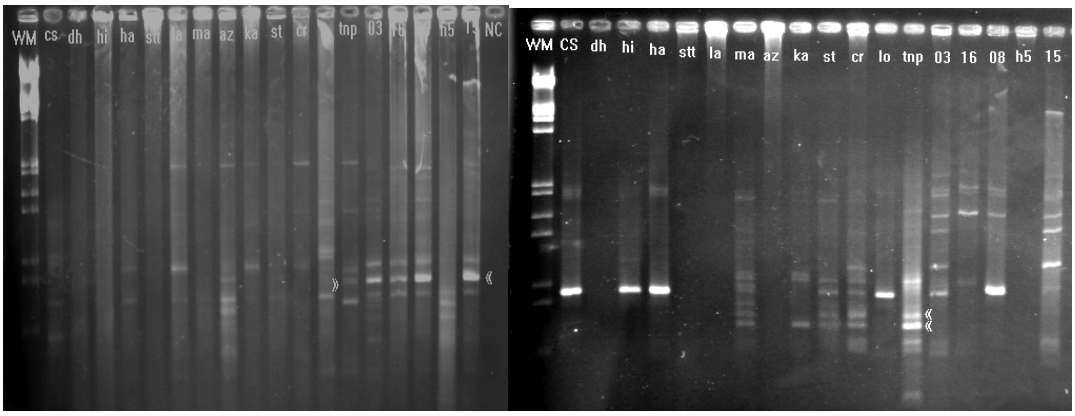

Fig. 1a and 1b *Genomic DNA amplified by ET34 and OPM 06 primers (right to left respectively)*

DISCUSSION

The most difficult and time-consuming process for constructing a set of addition lines can be alien chromosome identification (17). Molecular markers such as RFLP, SSR and radioisotope based AFLP are not easy or speedy methods for identification. The AFLP technique is an efficient DNA fingerprinting tool that generates a large number of molecular markers. However, most of the AFLP markers are difficult to convert to sequence- tagged-site (STS) markers (18). The RAPD and ISJ primers can be used by almost every laboratory and most RAPD markers can be easily converted to STS markers. Therefore, we investigated detection E^b chromosomes in tritipyrum primary lines, using RAPD and ISJ primers. In this study, 2 RAPD marker, including OPF03 and OPM06 were identified for each E^b chromosome in the tritipyrum primary lines. In a previous study 6 RAPD markers, associated with E^b genome were found in the *Cs*/*Thinopyrum bessarabicum* partial amphiploid and all disomic addition lines (17, 19). Primer $OPF03_{1296}$ previously reported by Zhang et al (10), this RAPD marker is dispersed along all seven chromosome of the E^b genomes. In this study presence of E^b genome- specific RAPD marker $OPF03_{1296}$ in the *Thinopyrum bessarabicum* and the tritipyrum primary lines and its absence in the Chinese spring wheat, wheat breeding cultivar and triticale primising lines, also demonstrated the transferred chromatin of E^b genome in tritipyrum primary lines. Primer OPM06 also amplified segment of E^b chromosome in the *Thinopyrum bessarabicum* and tritipyrum primary lines, but we do not known which where its site on E^b chromosomes. ET34 of semi random primers were amplified shared DNA fragment in the *Thinopyrum bessarabicum*, and triticale primising lines. This DNA fragment probability demonstrated the E^b genome is relatively closer to the R genome of secale. Shan e*et al* (18) reported that 22.3% of AFLP bands are barley chromosome-specific in a wheat background when only five dissomic addition lines were studied. This is much higher than the 10.9% reported by Zhang *et al* (19) for five E^b addition lines and indicates a more divergent relationship between the I genome of *Hordeum Vulgare* and ABD of common wheat than between the E^b genome of

Thinopyrum bessarabicum and ABD. In this study 4.06% of RAPD bands are *Thinopyrum bessarabicum* chromosome-specific in a wheat background when only 7 tritipyrum primary lines were studied, indicating useful of RAPD and ISJ primers in E^b chromosome identification, mapping and introgression.

This is the first report providing molecular markers such as RAPD and ISJ primers for successful in identification of E^b chromosome into tritipyrum primary line.

CONCLUSION

Our results show that RAPD and ISJ primers can be useful as molecular tools for detection E^b chromosome in tritipyrum primary lines. Future experiments will be conducted on the isolation and nucleotide sequencing of the specific bands of E^b genome. These molecular markers also will be used with in situ hybridization for physical identification of Eb chromosome.

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Notation: Cs=Chinse spring cultivar of wheat, dh= duble haploid cultivar of wheat, hi= Hirmand cultivar of wheat, ha= Hamun cultivar of wheat , stt= Stewart tetraploid cultivar of wheat , la= La/b of Tritipyrum line, ma= Ma/b of Tritipyrum line, az= Az/b of Tritipyrum, ka= Ka/b of Tritipyrum line, st= St/b of Tritipyrum line, cr= Cr/b of Tritipyrum lines, lo= La(4B/4D)/b of Tritipyrum line, tnp= *Thinopyrum bessarabicum*, 03= 4103 triticale line, 16= 4116 of triticale line, 08= 4108 of triticale line, h5=Mh5 of triticale line and 15= 4115 of triticale line