

RESEARCH PAPER

## Genetic variation among and within tritipyrum (*Thinopyrum bessarabicum* × *Triticum durum*) lines using PCR-based molecular markers

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### Abstract

**B. A. Siahisar, M. Allahdoo, and H. S. Hassani. 2011. Genetic variation among and within tritipyrum (*Thinopyrum bessarabicum* × *Triticum durum*) lines using PCR-based molecular markers. Cien. Inv. Agr. 38(1): 127-135.** Primary hexaploid tritipyrum lines are amphiploids between *Triticum durum* and *Thinopyrum bessarabicum*, which can set seed in at least 250 mM NaCl. Genetic variation within and among 14 lines of primary tritipyrum were investigated by using 2 random and 6 semi random primers that produced clear and reproducible DNA amplicons. Average values for Nei's gene diversity ( $h$ ) and Shannon indices ( $I$ ) were 0.115 and 0.178, respectively. These results indicated low variation within primary tritipyrum lines, probably related to unbiased chromosome and partial aneuploidy. Total genetic diversity ( $H_T$ ) and gene diversity within lines ( $H_S$ ) were 0.2114 and 0.1156, respectively. The coefficient of genetic differentiation ( $G_{ST}$ ) was equal to 0.4534. AMOVA showed highly significant ( $P < 0.001$ ) genetic differences among lines. From the total genetic diversity, 51.54% is attributable to 14 tritipyrum lines and 48.16% to differences within lines. Fixation index ( $F_{ST}$ ) was 0.4815. Genetic distance, calculated on the basis of allele frequencies among lines, ranged from 0.064 to 0.226 with an average of 0.1156. Grouping of populations based on UPGMA and Nei's gene diversity are indications of genetic similarity between some populations. Pop6 and populations located in group III had the highest genetic distance. These results could be used in breeding and cross programs to breed a new tritipyrum plant.

**Key words:** Aneuploidy, genetic variation, random primers, semi random primers, tritipyrum lines.

### Introduction

The introduction of alien genetic variation from the genus *Thinopyrum* through chromosome engineering into wheat is a valuable and proven technique for wheat improvement (Chen, 2005). Partial 8x amphiploids are lines that contain 42 (28-42) wheat and 14 (14-18) alien chromosomes. They are derived by back-

crossing wheat onto hybrids between wheat and either *Thinopyrum intermedium* (6X) or *Thinopyrum bessarabicum* (2X) (Fedak and Han, 2005). Triticale (2n=6X=42, AABBRR) the amphiploids between tetraploid wheat (AABB) and cultivated rye (2n=14, RR) is a successful example. Other examples are tritordeum (2n=6X=42, AABBH<sup>ch</sup>H<sup>ch</sup>), the amphiploids between tetraploid wheat (AABB) and *Hordeum chilense* (2n=14, H<sup>ch</sup>H<sup>ch</sup>) (Shahsevand Hassani *et al.*, 2003), Trigopiro (2n=8X=56, AABDDJJ), the amphiploids between *Triticum aestivum*

( $2n=6X=42$ , AABBDD) and *Thinopyrum ponticum* ( $2n=2X=14$ , JJ), tricepuro (AABBDDR), the amphiploids between hexaploid triticale (AABBRR) and trigopuro (AABBDDJJ) (Ferrari *et al.*, 2005), and tritipuro ( $2n=6X=42$ , AABBE<sup>b</sup>E<sup>b</sup>), another amphiploids between *Triticum durum* (AABB) and *Thinopyrum bessarabicum* ( $2n=2X=14$ , E<sup>b</sup>E<sup>b</sup>) (Shahsevand Hassani *et al.*, 2003). *Thinopyrum bessarabicum* is a wild, rhizomatous maritime sand couch grass distributed in Crima (Dewey, 1984). It is recognized for its high tolerance to salinity, being able to stand up in 350 mM of NaCl (Shahsevand Hassani *et al.*, 2003; King *et al.*, 1996). It also possesses resistance to Columbia root-knot nematode, *Melodogyne chitwodi*, and tolerance to scab disease caused by *Fusarium graminearum* (Zhang *et al.*, 2002). Primary hexaploid tritipuro lines, amphiploids between *Triticum durum* and *Thinopyrum bessarabicum*, have been produced which can set seed in at least 250 mM NaCl (King *et al.*, 1996). In an investigation of tritipuro line resistance to gall nematode and covered smut diseases, tritipuro lines of (Ma/b×Cr/b)F<sub>4</sub>, (Ka/b×Cr/b)F<sub>3</sub> and (Ka/b×Cr/b)F<sub>5</sub> were resistant to gall nematode t and (St/b×Cr/b)F<sub>4</sub> was susceptible. To covered smut, tritipuro lines of (St/b×Cr/b)F<sub>4</sub>, (Ka/b×Cr/b)F<sub>3</sub> and (Ma/b×Cr/b)F<sub>4</sub> were resistant and (Ka/b×Cr/b)F<sub>4</sub> was susceptible.

In recent years, marker systems based on hybridization techniques such as RFLP, have been replaced by faster and cheaper PCR-based methods. The RAPD system, which is useful for many crops, is not suitable for genetic analysis of large and complex genomes, such as wheat and triticale cereals (Gawel and Iwona, 2002). The main reason for this is the low rate of polymorphism detected by RAPD primers. Semi specific PCR and the use of primers with partial homology to sequences of intron-exon junctions (ET (exon targeted) and IT (intron targeted) primers) seem to be an alternative to RAPD and other tedious and expensive methods such as RFLP and AFLP (Gawel and Iwona, 2002). The former allow the generation of a great diversity of markers without any additional sequence information (Gawel and Iwona, 2002; Weining and Langridge, 1991). Introns have been identified

in most studied plant genes and the junctions to exons are highly conserved sequences. However, since the introns are generally subjected to only weak selective pressure by comparison to exons, they are usually highly variable in sequence and length (Weining and Langridge, 1991).

Molecular data has played an essential role in determining the genetic relationship among many plants and has also led to new genetic classifications that often disagree with traditional taxonomy (Jobst *et al.*, 1998). Wu *et al.* (2004) used RAPD and ISSR markers to analyze the genetic variation of *Oryza granulate* and revealed that there was a low level of genetic diversity within populations and a high genetic differentiation among populations. Shahsevand Hassani *et al.* (2003) used GISH technique to identify the number of E<sup>b</sup> chromosomes in tritipuro lines and F<sub>1</sub> hybrids (tritipuro×6X wheat) and showed a degree of instability in the form of aneuploidy, with a proportion of plants having lost a chromosome (41) and some having gained a chromosome (43).

In order to investigate less variable and homozygous tritipuro lines (relationship between lines, use of them in future breeding programs of hybridization with wheat and transfer of efficient genes of tritipuro to wheat), the genetic diversity among and within 14 primary and combined primary tritipuro lines, using random (RAPD) and semi random primers, was evaluated.

## Materials and methods

### Plant materials

Plant genotypes included primary tritipuro lines (Ne/b, St/b, Cr/b, Ka/b, Ma/b) and primary combined tritipuro lines {(Ma/b)(Cr/b)F<sub>3</sub>, (Ma/b)(Cr/b)F<sub>4</sub>, (St/b)(Cr/b)F<sub>3</sub>, (St/b)(Cr/b)F<sub>4</sub>, (Ka/b)(Cr/b)F<sub>2</sub>, (Ka/b)(Cr/b)F<sub>3</sub>, (Ka/b)(Cr/b)F<sub>4</sub>, (Ka/b)(Cr/b)F<sub>5</sub>, (Ka/b)(Cr/b)F<sub>6</sub>}. Plant material was prepared by Shahid Bahonar University of Kerman (SBUK), Iran.

### DNA extraction

Fifty seeds of each line were sown at 25 °C under ambient greenhouse conditions. At two weeks of age, 20 seedlings of each line were randomly selected for total DNA extraction, using Dellaporta *et al.* (1983) protocol with minor changes. For PCR analysis, the quality and quantity of DNA were assessed in a 1% agarose gel and a biophotometer (Biorad) according to Zidani *et al.* (2005). From 20 DNA sample results of 20 seedlings, 13 samples that had efficient quality and quantity were selected for PCR analysis.

### PCR analysis

After DNA amplification, each PCR reaction and electrophoresis were repeated at least twice. The 10 bp primers used for RAPD analysis were provided by Operon technology kits, F, C, M; and semi random primers were designed by Weining and Langridge (1991), Przetakiewicz *et al.* (2002), and Gawel and Iwona (2002). Two of 15 random primers (RAPD) and six of 12 semi random primers (3 ET and 3 IT primers) (Table 1) were selected on the basis of clear banding patterns. Reaction conditions were optimized for 25µl mixtures and were composed of 50 ng template DNA, 1X reaction PCR buffer, 1U *Taq* DNA polymerase (Cinnagen Company), 3mM MgCl<sub>2</sub>, 0.2 mM of each dNTP and 0.4 µM of each primer.

Amplifications were carried out in a Corbett Research Thermal Cycler as follows: initial strand separation in 93 °C for 150 seconds; an amplification cycle consisted of 60 seconds at 93 °C; 60 seconds at 36 °C for random primer, 60 °C for semi random 15-mer primer and 63°C for semi random 18-mer primers annealing; extension period of 120 seconds at 71 °C for all primers. A total of 42 cycles were performed and cycling ended with a final extension at 71 °C for 8 min. PCR products were separated on 1.8% agarose gels, which contained 1.8g agarose dissolved in 100 cc of TBE buffer (pH=8) and stained with 0.1% ethidium bromide. The size of the amplicons was estimated using Gene Ruler™ 100bp DNA ladder plus of Fermentas Company. Gel images were recorded and band sizes were quantified with a Gel Doc system model Vilber Lourmat.

### Data analysis

RAPD and ISJ bands of equal mobility (according to the molecular weight of 100-3,000 bp DNA ladder plus) were scored manually for presence (1) or absence (0), for each of the 13 seedlings. The following parameters were generated using the POPGEN 1.32 program (Yeh *et al.*, 1997) to describe intra and inter-line genetic variation: percentage of polymorphic *loci* (P), Nei's gene diversity (h), Shannon information index (i), number

**Table 1.** The nucleotide sequences of the eight selected primers, the numbers of bands scored and polymorphism percentage of primer.

	Primer	Primer sequence (5'-3')	AT <sup>1</sup>	NBR <sup>2</sup>	PP <sup>3</sup>
Semi random	ET32	5' ACTTACCTGGGCACG 3'	60	28	41.32
	ET33	5' ACCTACCTGGCCGAT 3'	60	30	35.41
	ET34	5' ACCTACCTGGGCGAC 3'	60	29	40.11
	IT32	5' GACTCGCCAGGTAAG 3'	60	33	55.21
	IT33	5' GATGCCCCAGGTAAG 3'	60	31	53.1
	IT34	5' GCGGCATCAGGTAAG 3'	60	29	47.81
Random	OPN 16	5' AAGCGACCTG 3'	36	26	22.10
	OPC11	5' AAAGCTGCGG 3'	36	29	25.14

<sup>1</sup>AT= annealing temperature; <sup>2</sup>NBR= number of recorded bands; <sup>3</sup>PP= polymorphism percentage; ET= Exon targeted; IT= Intron targeted.

of observed alleles ( $N_a$ ) and number of effective alleles ( $N_e$ ) (Nei, 1973; Lewontin, 1972). Genetic diversity among lines was investigated using Nei's unbiased genetic distances (GD) and genetic identities (GID) (Nei, 1978). Nei's unbiased genetic distances were calculated for all line pairs and used to construct a phylogenetic tree (UPGMA) (Nei, 1978). The genetic structure was further investigated using Nei's gene diversity statistics, including the total genetic diversity ( $H_T$ ), genetic diversity within lines ( $H_S$ ), and the relative magnitude of genetic differentiation among lines ( $G_{ST}=(H_T H_S)/H_P$ ) (McDermott and McDonald, 1993). An estimate of gene flow among lines ( $N_m$ ) was computed using the formula of  $N_m = (1 - G_{ST}) / 2G_{ST}$  (McDermott and McDonald, 1993). In order to describe variability among and within lines, the non-parametric Analysis of Molecular Variance (AMOVA) procedure was used as described in Excoffier *et al.* (1992), where the variation was partitioned within lines and among lines. This analysis was performed with the Arlequin program version 3.1 (Excoffier, 2007).

## Results

### *RAPD and ISJ primers variation*

The two selected random primers generated 55 clear, reproducible and fully polymorphic bands. The six selected semi random primers gave 180 unambiguous, 100% reproducible polymorphic bands that ranged between 300 and 3,000 bp. The number of bands varied from 26 (OPN16) to 33 (IT32) with an average of 29.37 bands per primer (Table 1).

### *Genetic diversity within lines*

In individual lines, the percentage of polymorphic loci ( $p$ ) ranged from 32.34% to 47.66%, with an average of 39.72% (Table 2). Nei's gene diversities ( $h$ ) varied from 0.096 to 0.131, with an average of 0.115 and Shannon's indices ( $I$ ) ranged from 0.146 to 0.205 with an

average of 0.178. Also, the values of the parameters  $h$  and  $I$  showed a similar trend. The mean of observed alleles ( $N_a$ ) ranged from 1.323 to 1.476, while the mean effective alleles ( $N_e$ ) varied from 1.152 to 1.216. The values of  $h$ ,  $I$ ,  $N_a$  and  $N_e$  were 0.207, 0.336, 2.000 and 1.310, respectively. Among 14 tritipyrum lines, St/b and (Ma/b) $\times$ (Cr/b) $F_4$  lines had the highest genetic variation and (Ka/b) $\times$ (Cr/b) $F_3$  and Ka/b lines had the lowest genetic variation (Table 2).

### *Line genetic structure*

Total genetic diversity ( $H_T$ ) and genetic diversity within all tritipyrum lines ( $H_S$ ) were 0.2114 and 0.1156, respectively, which shows considerable genetic differentiation among lines. Genetic differentiation coefficient ( $G_{ST}=0.4534$ ) indicated a high degree of genetic variation among tritipyrum lines and the estimated amount of gene flow was 0.6028.

### *Genetic relationships*

On the basis of allele frequencies for RAPD and ISJ markers, the genetic distance among all lines ranged from 0.064 to 0.239 with an average of 0.1156. Greatest genetic distance values were found between line 6 and 13. Lowest genetic distance values were found between line 9 and 10 (Table 3, Figure 1). Grouping of populations based on UPGMA and Nei's gene diversity indicated genetic similarity between some populations. In this grouping pop 1 in group I; pop2, pop3, pop12, pop4, pop11, pop7 and pop8 in group II; pop9, pop10, pop13 and pop14 in group III; pop5 in group IV and pop6 in group V were located. Pop6 with populations located in group III had the highest genetic distance. Pop6 had the lowest gene diversity and polymorphism, thus aneuploidy content in this line is lower than in other lines. Results regarding the high genetic distance could be used in breeding and cross programs to breed a new tritipyrum plant.

**Table 2.** Genetic variation parameters of tritipyrum lines based on random and semi random primers.

<i>Tritipyrum</i> lines <sup>1</sup>	Na <sup>1</sup>	Ne <sup>2</sup>	h <sup>3</sup>	I <sup>4</sup>	P (%) <sup>5</sup>
Cr/b	1.476	1.173	0.112	0.182	47.66
Ka/b	1.331	1.158	0.096	0.148	33.19
(Ka/b)(Cr/b)F <sub>2</sub>	1.357	1.186	0.111	0.169	35.74
(Ka/b)(Cr/b)F <sub>3</sub>	1.331	1.152	0.096	0.149	33.19
(Ka/b)(Cr/b)F <sub>4</sub>	1.408	1.208	0.126	0.194	40.85
(Ka/b)(Cr/b)F <sub>5</sub>	1.323	1.155	0.096	0.148	32.34
(Ka/b)(Cr/b)F <sub>6</sub>	1.387	1.187	0.115	0.178	38.72
Ma/b	1.387	1.196	0.119	0.184	38.73
(Ma/b)(Cr/b)F <sub>3</sub>	1.340	1.168	0.101	0.156	34.04
(Ma/b)(Cr/b)F <sub>4</sub>	1.476	1.208	0.130	0.205	47.66
Ne/b	1.404	1.208	0.126	0.192	40.43
St/b	1.455	1.216	0.131	0.201	45.53
(St/b)(Cr/b)F <sub>3</sub>	1.438	1.203	0.127	0.198	43.83
(St/b)(Cr/b)F <sub>4</sub>	1.442	1.212	0.128	0.199	44.26
Mean	1.396	1.187	0.115	0.178	39.72
Total	2.000	1.31	0.207	0.336	100

<sup>1</sup>Number of observed alleles; <sup>2</sup>Number of effective alleles; <sup>3</sup>Nei's gene diversity; <sup>4</sup>Shannon's information index; <sup>5</sup>Percentage of polymorphic loci.

### Analysis of Molecular Variance (AMOVA)

To assess the diversity among and within tritipyrum lines, an AMOVA was performed from the distance matrix. AMOVA showed highly significant ( $P \leq 0.001$ ) genetic differences among lines. From the total genetic diversity, 51.538% is attributable to variation of 14 tritipyrum lines and 48.16 % related to differences within lines (Table 3). Fixation index (FST) was 0.4815 (Table 4).

### Discussion

Although, for genetic variation within and among tritipyrum lines, plenty random primers were used, only 2 of them however, produced low polymorphic band (Table 1). In ET and IT semi random primers, the IT groups had higher polymorphism than ET (Table 1, Fig-

ure 2). These results agree with Weining and Langridge (1991) and Gawel and Iwona (2002) who showed that semi random primers revealed higher polymorphisms between wheat varieties, when compared to RAPD primers. This outcome suggests that semi random primers are more useful for evaluating genetic variation among cereals such as bread wheat, triticale and tritipyrum, than RAPD primers.

The h value (genetic diversity within tritipyrum lines) was not the same and varied as follows: St/b > (Ma/b)Cr/b > (St/b)(Cr/b)F<sub>4</sub> > (St/b)(Cr/b)F<sub>3</sub> > Ne/b > (Ka/b)(Cr/b)F<sub>4</sub> > Ma/b > (Ka/b)(Cr/b)F<sub>6</sub> > Cr/b > (Ka/b)(Cr/b)F<sub>2</sub> > (Ka/b)(Cr/b)F<sub>5</sub> > Ka/b > (Ka/b)(Cr/b)F<sub>3</sub>. In spite the equal sample size for all tritipyrum lines, the highest h values were found in St/b and the lowest were found in Ka/b and (Ka/b)(Cr/b)F<sub>3</sub> lines. Wu *et al.* (2004) showed that the mean genetic variation within a population of self-pollinated rice (*Oryza granulate*), using RAPD and ISSR, was 0.09 and 0.08, respec-

**Table 3.** Nei's genetic distances and genetic identities among lines of tritipyrum.

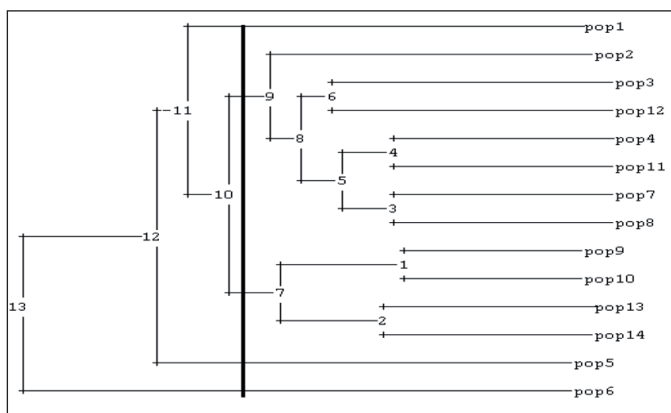
Lines	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	***	0.865	0.856	0.862	0.871	0.823	0.903	0.887	0.888	0.896	0.879	0.858	0.885	0.875
2	0.145	***	0.897	0.903	0.872	0.865	0.902	0.884	0.852	0.875	0.912	0.898	0.880	0.888
3	0.155	0.109	***	0.917	0.876	0.886	0.906	0.892	0.865	0.888	0.904	0.910	0.848	0.879
4	0.149	0.102	0.087	***	0.900	0.886	0.912	0.900	0.864	0.876	0.926	0.891	0.847	0.880
5	0.138	0.137	0.132	0.105	***	0.850	0.872	0.891	0.847	0.866	0.899	0.866	0.842	0.861
6	0.195	0.146	0.121	0.122	0.163	***	0.850	0.827	0.797	0.817	0.861	0.842	0.788	0.816
7	0.103	0.103	0.099	0.092	0.137	0.162	***	0.927	0.902	0.920	0.925	0.896	0.913	0.919
8	0.120	0.124	0.114	0.106	0.115	0.189	0.076	***	0.912	0.910	0.919	0.905	0.893	0.912
9	0.119	0.160	0.145	0.146	0.166	0.226	0.103	0.092	***	0.938	0.883	0.893	0.891	0.904
10	0.109	0.134	0.119	0.133	0.144	0.202	0.084	0.094	0.064	***	0.907	0.881	0.901	0.913
11	0.129	0.092	0.101	0.077	0.107	0.150	0.078	0.085	0.125	0.098	***	0.907	0.865	0.904
12	0.153	0.107	0.094	0.116	0.144	0.172	0.110	0.100	0.113	0.127	0.098	***	0.889	0.919
13	0.122	0.128	0.165	0.166	0.173	0.239	0.091	0.114	0.116	0.105	0.145	0.118	***	0.930
14	0.134	0.119	0.129	0.128	0.150	0.204	0.084	0.092	0.101	0.091	0.101	0.085	0.073	***

Above diagonal, genetic identities; below diagonal, genetic distance.

1=Cr/b, 2=Ka/b, 3=(Ka/b)(Cr/b)F<sub>2</sub>, 4=(Ka/b)(Cr/b)F<sub>3</sub>, 5=(Ka/b)(Cr/b)F<sub>4</sub>, 6=(Ka/b)(Cr/b)F<sub>5</sub>, 7=(Ka/b)(Cr/b)F<sub>6</sub>, 8= Ma/b, 9=(Ma/b)(Cr/b)F<sub>3</sub>, 10=(Ma/b)(Cr/b)F<sub>4</sub>, 11=Ne/b, 12=St/b, 13=(St/b)(Cr/b)F<sub>3</sub>, 14=(St/b)(Cr/b)F<sub>4</sub>.

**Table 4.** analysis of molecular variance (AMOVA) for tritipyrum lines.

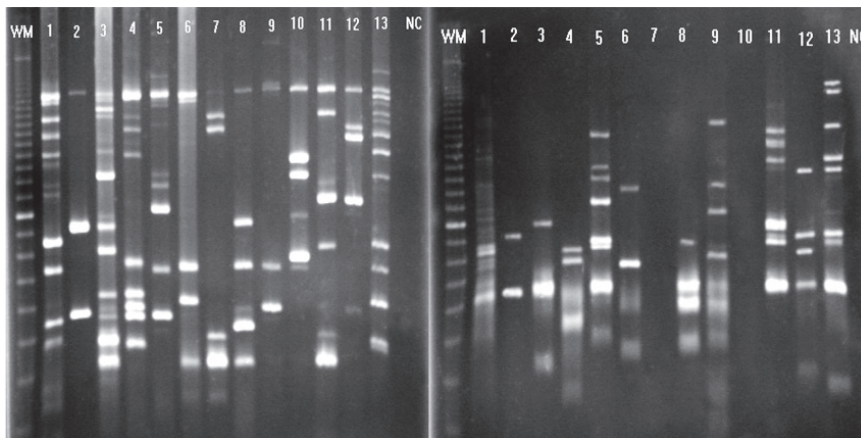
Source of variation	Df	Mean sum of squares	Variance components	Percentage of variation	P-value
Among lines	13	174.65	12.538	51.538	<0.001
Within lines	168	11.647	11.647	48.160	
Total	181	-	24.185		
Fixation index		FST=0.4815			



**Figure 1.** Dendrogram obtained using UPGMA, based Nei's genetic distance for 14 tritipyrum lines.

Pop1=Cr/b, Pop2=Ka/b, Pop3=(Ka/b)(Cr/b)F<sub>2</sub>, Pop4=(Ka/b)(Cr/b)F<sub>3</sub>, Pop5=(Ka/b)(Cr/b)F<sub>4</sub>, Pop6=(Ka/b)(Cr/b)F<sub>5</sub>, Pop7=(Ka/b)(Cr/b)F<sub>6</sub>, Pop8=Ma/b, Pop9=(Ma/b)(Cr/b)F<sub>3</sub>, Pop10=(Ma/b)(Cr/b)F<sub>4</sub>, Pop11= Ne/b, Pop12=St/b, Pop13=(St/b)(Cr/b)F<sub>3</sub>, Pop14= St/b)(Cr/b)F<sub>4</sub>.





**Figure 2.** The amplified DNA of 13 seedling of (St/b)(Cr/b) $F_3$  line using IT33 primer and (Ma/b)(Cr/b) $F_4$  line using ET32 primer, respectively (right to left). \*WM, weight marker; 1-13, number of tritipyrum seedlings studied in each primer; NC, negative control.

tively. Our results revealed a mean of Nei's gene diversity within tritipyrum lines of 0.115, which indicates low and non significant variation. This is probably due to very low partial aneuploidy in primary tritipyrum lines as was reported by Shahsevand Hassani *et al.* (2003).

The polymorphic bands within individual plants of all tritipyrum lines varied from 32.33 to 47.6%, with an average of 39.72%. Fixation index (FST) within these seedlings was 0.4815. This indicates a weak degree of heterogeneity and/or heterozygosity, which is in agreement with the findings of Tames *et al.* (2004) in 15-20 single seeds of triticale cultivars using SSR markers. In particular, the advanced breeding lines might be even more heterogeneous than the registered cultivars (Tams *et al.*, 2005).

Molecular data has played an essential role in determining the genetic relationship among many plants, and has led to new genetic classifications that often conflict with traditional taxonomy (Jobst *et al.*, 1998). Population variability is related to the method of reproduction. This has been shown in numerous species and is reflected by the positive association between the amount of outcrossing and genetic variation (Hamrick *et al.*, 1979). In general, autogamous species show greater differences among, rather than within, populations (Charlesworth

and Charlesworth, 1995). The genetic differentiation in this study ( $G_{ST}=0.453$ ) among tritipyrum lines was considerable and higher than the levels of differentiation detected among wheatgrass of *Thinopyrum junceum* based on isozymes (Nieto-Lopez *et al.*, 2003). Jensen *et al.* (1990) classified the *Thinopyrum junceum* complex as predominantly self-pollinating, while findings of Nieto-Lopez *et al.* (2003) led to new genetic classification (out crossing), and disagree with traditional taxonomy. The amount of genetic diversity present within lines of tritipyrum is low with a  $H_s$  value equaling 0.115, which is not surprising since tritipyrum is a highly self-crossing plant. These results were also reported for other self-crossing Poaceae family plants. For example, 59% RAPD and 64% ISSR was found among 14 populations of *Oryza granulate* (Wu *et al.*, 2004). Reports for outcrossing native plants indicate 92.6% eSSR variation within six populations of bluebunch wheatgrass and 7.4% among populations (Young and Thompson, 2006), 19% AFLP variation among three populations of *Festuca campestris* (Fu *et al.*, 2005), and 7% among six populations of *Schizachyrium scoparium* (Fu *et al.*, 2004). Genetic variation among 14 tritipyrum lines, using combined of RAPD and ISJ markers based on AMOVA was 51.538%, which was considerable and greater than outcrossing plants as bluebunch wheatgrass and *Schizachyrium scoparium*.

Analysis of molecular variance (AMOVA), Nei's gene diversity and grouping of populations based on UPGMA and Nei's gene diversity, suggested that the genetic variation among tritipyrum lines is more than genetic variation within tritipyrum lines. This difference is not comparable to out crossing plants such as *Thinopyrum junceum*. Intra line variation of tritipyrum is due to aneuploidy and chromosome imbalance during meiosis division. Fixa-

tion index (FST) intra tritipyrum lines was 0.4815, which could be due to heterogeneous or heterozygosity, which is attributed to earlier similar results of triticales lines by SSR markers. (St/b)(Cr/b) $F_5$  lines had the lowest gene diversity and thus the lowest aneuploidy. This line had the highest genetic distance with lines located in the group III, which could be considered in future crosses to artificially breed tritipyrum plants.

### Resumen

**B. A. Siahisar, M. Allahdoo y H. S. Hassani. 2011. Variación genética entre y dentro de las líneas de tritipyrum (*Thinopyrum bessarabicum* × *Triticum durum*), por marcadores moleculares basados en PCR. Cien. Inv. Agr. 38(1): 127-135.** Las líneas primarias hexaploides de tritipyrum son anfiploides entre *Triticum durum* y *Thinopyrum bessarabicum*, cuyas semillas pueden ser plantadas en, al menos, 250 mM de NaCl. Se investigó la variación genética dentro y entre las 14 líneas de tritipyrum primaria, por medio de 2 oligos aleatorios y 6 semi-aleatorios que producen amplicones de ADN claro y reproducible. Los valores medios de la diversidad génica de Nei (h) y los índices de Shannon (I) fueron 0,115 y 0,178, respectivamente. Estos resultados indican poca variación dentro de las líneas tritipyrum primaria, lo que probablemente esté relacionado con el cromosoma imparcial y aneuploidía parcial. La diversidad genética total (HT) y la diversidad genética dentro de las líneas (SA) fueron 0,2114 y 0,1156, respectivamente. El coeficiente de diferenciación genética (GST) fue 0,4534. El AMOVA mostró diferencias genéticas altamente significativa ( $P \leq 0,001$ ) entre las líneas. De la diversidad genética total, 51,54%, es atribuible a las 14 líneas tritipyrum y 48,16% a las diferencias dentro de las líneas. El índice de fijación (FST) fue 0,4815. La distancia genética calculada en base a las frecuencias alélicas entre las líneas, fluctuó entre 0,064 y 0,226, con una media de 0,1156. La agrupación de las poblaciones sobre la base de UPGMA y la diversidad genética de Nei, indican una similitud genética entre algunas poblaciones. La mayor distancia genética se produjo entre el grupo POP6 con las poblaciones ubicadas en el grupo III. De acuerdo con la alta distancia genética de esta línea con las líneas ubicadas en el grupo III, se podría utilizar estos resultados en programas de mejoramiento y cruzamiento, con el fin de mejoramiento de la nueva planta tritipyrum.

**Palabras clave:** Aneuploidía, variación genética, cebadores aleatorios, líneas tritipyrum.

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