

Morpho-Genetic Study and Detection of Boron Toxicity Tolerance of Wild Wheat Genotypes

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Abstract

The occurrence of boron toxicity becomes a major constraint in cereal production in the world, thus cause significant yield loss particularly in wheat, one of the most susceptible crops to boron toxicity. Development of tolerant variety is now of utmost importance since agronomic management of soil boron is becoming ineffective to mitigate the toxicity. Due to narrow genetic base of the existing wheat cultivars, genetic variation for this character was poorly reported. The present study was devoted to identify such variation by using two different genetic background of wheat including *Aegilops* and *Triticum*, which could be readily used in wheat breeding program. Morphological and genetic screening revealed that two species *Ae. longissima* and *Ae. sharonensis* expressed tolerance against boron toxicity. The molecular marker analysis such as unweighted pair group method and principal coordinate analysis confirmed these two species to be more tolerant to excessive boron with higher root length. Their derivatives genotypes TL-09 and TL-17 were found to explained considerable tolerance to boron. On other hand, among the SSR markers used, Xgwm192 was the most robust in identifying boron tolerance polymorphism. The diversity and variability observed in this work could open new avenue in developing B toxicity tolerant wheat variety.

Keywords: Boron-toxicity, Genetic diversity, SSR, *Aegilops*, *Triticum*, Root length, Cluster analysis

1. Introduction

Boron (B) is an essential micronutrient for plant growth and development. Crop production is hampered seriously under both conditions of deficient and excessive boron in the soil. Agronomic management of B toxicity is more of serious task than dealing with B deficiency (Sutton et al., 2007). Boron stress is one of the key limiting factors for crop production in

many areas of the world (Jefferies et al., 2000). Agronomic management of B toxic soils is a robust practice (Sutton et al., 2007) and also, detoxification of excess B from soils. Development of wheat cultivars that are tolerant to B is the only long-term viable approach to deal with B toxicity and crop production (Schnurbusch et al., 2010). Wheat is very susceptible to B toxicity and production is hampered in toxic environments. Due to the stock of different genes in an interbreeding population used in breeding programs, the genetic diversity among wheat cultivars has decreased (Wei et al., 2000, as cited in Li et al., 2006). Hence, Wheat improvement programs require the inclusion of diverse genetic background as a source of target genes. Landraces, weedy species and wild relatives of wheat are generally the principal source of these genes (Li et al., 2006). *Aegilops*, which is one of the most closely related genera of wheat, possesses tremendous genetic variability and could be the source of desirable B tolerant genes. Many other value-added genes of interest have been incorporated to cultivated wheat from *Aegilops* species (Schneider et al., 2008). In case of wheat, most of the earlier studies (Schnurbusch et al., 2008, Schnurbusch et al., 2007, Torun et al., 2006; Jefferies et al., 2000, Paull et al., 1992, 1991), for B toxicity tolerance, were conducted on bread wheat and durum wheat. The *Aegilops* genera are still neglected in B tolerance research, and the existence of such a large pool of genotypic variation is promising and can be used in wheat breeding programs for developing B-tolerant cultivars. So far only one report (Emon et al., 2012) is available which attempted to categorize the *Aegilops* species for B tolerance. Moreover, most of the previous studies (Konstantinos et al., 2010; Belkadi et al., 2011, Baghizadeh et al., 2011, Naghavi et al., 2009) that were devoted to identifying the genetic relationship and diversity of *Aegilops* species were based on RAPD markers. Previous studies were inappropriate in context of the number of species included and the type of molecular marker used for the diversity studies. On the other hand wild emmer wheat *Triticum dicoccoides* and rye (*Secale cereale*) are also important genetic stocks for improvement of cultivated wheat. Due to genetic similarities to cultivated wheat, the transfer of useful genes from these species is possible. Thus, the evaluation of genetic diversity of *Aegilops*, *Triticum* and rye could provide valuable information for genetic improvement of wheat. Therefore, in the present study 80 genotypes comprised of *Aegilops*, *Triticum* and rye species were subjected to a diversity study utilizing thirty-two SSR markers. The main objective of the study was to explore the genetic diversity and relationship of the species at the DNA level, and identify SSR markers linked to Boron toxicity tolerance.

2. Materials and Methods

2.1 Plant Materials

Seventy-nine genotypes from 12 wild species within the *Aegilops-Triticum* group were obtained from Dr. Moshe Feldman, Department of Plant Sciences, The Weizmann Institute of Science, Rehovot 76100, Israel. These genotypes were collected from several Mediterranean region sources (Table 1). One rye (*Secale cereale* 'Blanco') obtained from the USDA-Sears Collection; University of Missouri, USA was also included in this characterization.

2.2 Hydroponic screening

The hydroponic screening was done in a growth chamber with light set to 16 h day at 22 °C and 8 h night at 15 °C. High quality seeds were sterilized in a solution of 1.3% sodium hypochlorite (2.5% bleach solution) plus one drop of Tween 20 (surfactant) in distilled water

for 8 minutes and then thoroughly rinsed. Seeds were placed on filter paper containing 0.2% of Pipracil (Piperacillin Sodium antibiotic) solution in labeled petri dishes for germination. Petri plates were shifted to a refrigerator (4 °C) for adjusting seedling growth during germination. The hydroponic nutrient solution consisted of 0.4 mM CaCl₂; 0.65 mM KNO₃; 0.25 mM MgCl₂·6H₂O; 0.1 mM (NH₄)₂SO₄; and 0.04 mM NH₄NO₃ in dionized water (Yau, 2002). The nutrient solutions were supplemented with H₃BO₃ to make it at 3 mM and 10 mM of B concentrations. Four seedlings per accession with three replications were grown in 2 L of nutrient solution with 0, 3 and 10 mM B, respectively for 10 days. All genotypes were categorized as tolerant or susceptible based on their seedling root length.

Table 1. List of eighty *Aegilops-wheat* accessions collected from different country and used in SSR analysis of the present study

Name of The Species	*Genome	Country	Name of the accessions
<i>Aegilops speltoides</i>	S	Israel (7)	TS-100, TS-02, TS-41, TS-43, TS-47, TS-76, TS-01
		Unknown (1)	TS-117
		Turkey (1)	TS-118
		Syria (1)	TS-132
<i>Aegilops longissima</i>	S ¹	Israel (8)	TL-01, TL-02, TL-04, TL-05, TL-07, TL-09, TL-17, TL-21
		Jordan (1)	TL-24
<i>Aegilops sharonensis</i>	S ^{sh}	Israel (9)	TH-17, TH-02, TH-03, TH-04, TH-07, TH-10, TH-11, TH-15, TH-01
<i>Aegilops bicornis</i>	S ^b	Israel (2)	TB-04, TB-05
		Egypt (3)	TB-07, TB-10, TB-12
<i>Aegilops searsii</i>	S ^s	Israel (4)	TE-03, TE-09, TE-21, TE-27
		Syria (1)	TE-36
<i>Aegilops kotschy</i>	US	Israel (6)	TKK-01, TKK-03, TKK-06, Tkk-11, TKK-27, TKK-21
<i>Aegilops cylindrostachys</i>	US	Israel (4)	TKC-01, TKC-04, TKC-06, TKC-08
		Unknown (1)	TKE-02
<i>Aegilops euvariabilis</i>	US	Israel (9)	TKE-03, TKE-12, TKE-19, TKE-22, TKE-24, TKE-42, TKE-46, TKE-62, TKE-66,
		Unknown (1)	TO-01
<i>Aegilops geniculata syn. ovata</i>	UM	Unknown (1)	TO-01
		Israel (2)	TO-07, TO-13

		Lebanon (1)	TO-44
<i>Aegilops biuncialis</i>	UM	Israel (2)	TN-03, TN-13
<i>Aegilops triuncialis</i>	UC	Israel (3)	TW-03, TW-09, TW-11
			TTD-54, TTD-09, TTD-12,
			TTD-15, TTD-24,
<i>Triticum. dicoccoides</i>		Israel (10)	TTD-25, TTD-30, TTD-47, TTD-48,
			TTD-04
		Iran (1)	TTD-64
		Turkey (1)	TTD-68
<i>Secale cereale</i>		Brazil (1)	Blanco rye

*Slageren (1994).

2.3 DNA extraction

Fresh leaves from 13-days old seedlings were used for DNA extraction followed by CTAB mini-prep method (IRRI, 1997). The DNA samples were analyzed both qualitatively and quantitatively using a spectrophotometer and 0.8% agarose gel electrophoresis.

2.4 SSR marker genotyping

Thirty-four selected SSR primers (along with three Barley primers) were used for the study. The majority of the primer pairs were selected from chromosomes 7B and 7D of wheat and 2H, 3H, and 4H of barley, which are believed to be associated with the boron tolerance. Few primers were taken from wheat chromosomes, 5A, 5B, and 5D because recent study (Schnurbusch et al., 2008) identified some orthologous sequence of B tolerant genes on these chromosomes. The total PCR reaction volume was 13 µl, composed of 2.0 µl genomic DNA, 1.5 µl 10X PCR buffer (Tris with 15 mM MgCl₂, Conc. 10×), 0.75 µl dNTPs (Contains dCTP, dGTP, dTTP and dATP all in the conc. of 10 mM), 1.0 µl forward primer, 1.0 µl reverse primer, 0.5 µl Taq DNA polymerase (conc. 5 U/µl) and 8.25 µl sterile deionized water. Samples were subjected to the following thermal profile for amplification in a thermocycler: after the initial 7 min at 95 °C, SSR marker amplification comprised 10–15 touchdown cycles of 94 °C for 30 s, annealing for 30 s, decreasing the temperature by 0.5 °C per cycle until the specified annealing temperature was reached, and 72 °C for 30s. This was then followed by 25–35 cycles of amplification with the specified annealing temperature, and a final extension at 72 °C for 10 min. After amplification, the PCR tube was stored at 4 °C until electrophoresis. Visualization of amplification products was accomplished on a 3% agarose gel in 0.5 × TBE buffer. The agarose gels were stained with ethidium bromide solution for 20-25 min. The stained agarose gel was illuminated by UV-trans-illuminator and photographed for assessing the DNA profiles.

2.5 Statistical Analysis

The allele size at each microsatellite locus was measured in base pairs by using AlphaEaseFC

4.0 software (<http://www.alphainnotech.com/software.htm>: Alpha Innotech Inc.). The summary statistics including the number of alleles per locus, heterozygosity, gene diversity and polymorphism information content (PIC) values were calculated using Power Marker version 3.25 (Liu & Muse, 2005). Power Marker was also used to generate a distance matrix based on Nei (Nei, 1972) distance. The genetic distance matrices were then subjected to the neighbor-joining method (Saitou and Nei, 1987) of tree formation. MEGA 5.0 (Tamura et al., 2007) was used to produce graphical trees. External validation (bootstrapping) of tree wherever performed was done by the program Phylip ver. 3.69 (Felsenstein, 2009) with 1000 permutation and co-phenetic correlation coefficient was calculated by NTSYSpc program ver. 2.11 (Rohlf, 2005) for internal validation of the phylogenetic tree. Principal coordinate (PCoA) and analysis of molecular variance (AMOVA) were performed by the program GENALEX ver.6.0 (Peakall and Smouse, 2006). Where not mentioned, software CONVERT ver. 1.31 (Glaubitz, 2004) were used to convert the data in different format for the analysis of various software program.

2.6 Correlation of Root Length and SSR Data

Two dissimilarity distance matrixes were created with NTSYSpc software ver. 2.11 (Rohlf, 2005). The first matrix was produced from the standardized root length data using SIMINT procedure based on Average Taxonomic Distance (i.e. DIST coefficient in the procedure). Standardization of data was done by STAND procedure of NTSYS. The second matrix was generated with help of SSR data using Nei genetic distance (Nei, 1972). The allele frequency data from POWER MARKER was used to export the data in binary format (allele presence = “1” and allele absence = “0”) for analysis with NTSYS-PC. Correlation between root length and SSR data were done by the mantel two-way matrix correspondence test (Mantel, 1967) with the help of MXCOMP procedure of NTSYS-pc. The significance of the correlation between the matrices was tested using the normalized Mantel Z-statistics.

3. Results

3.1 Morphological Characterization

3.1.1 Root Length Variation

In the hydroponic screening, variation in root length of the genotypes of each species under different concentrations (0, 3 and 10 mM) is presented in the Figure 1. It was observed that many genotypes from *Ae. speltoides*, *Ae. longissima*, *Ae. sharonensis*, *Ae. searsii*, and *Ae. euvariabilis* exhibited good root length under the excess B concentrations. Whereas, *Ae. geniculata*, *Ae. biuncialis*, *Ae. triuncialis*, *T. dicoccoides* and *S. cereale* showed very poor root length with 0mM and 10 mM B concentration. But, surprisingly these species gave better root length under 3 mM B. Among the species evaluated *Ae. sharonensis*, *Ae. longissima* and *Ae. kotschyi* revealed better tolerance against excess B than the other species (Figure 1). *Ae. speltoides*, *Ae. euvariabilis* and *Ae. searsii* also experience tolerance performance against B toxicity. Due to the inconsistency, no reliable conclusion can be drawn for any of the genotypes from the species *Ae. kotschyi* and *Ae. bicornis*.

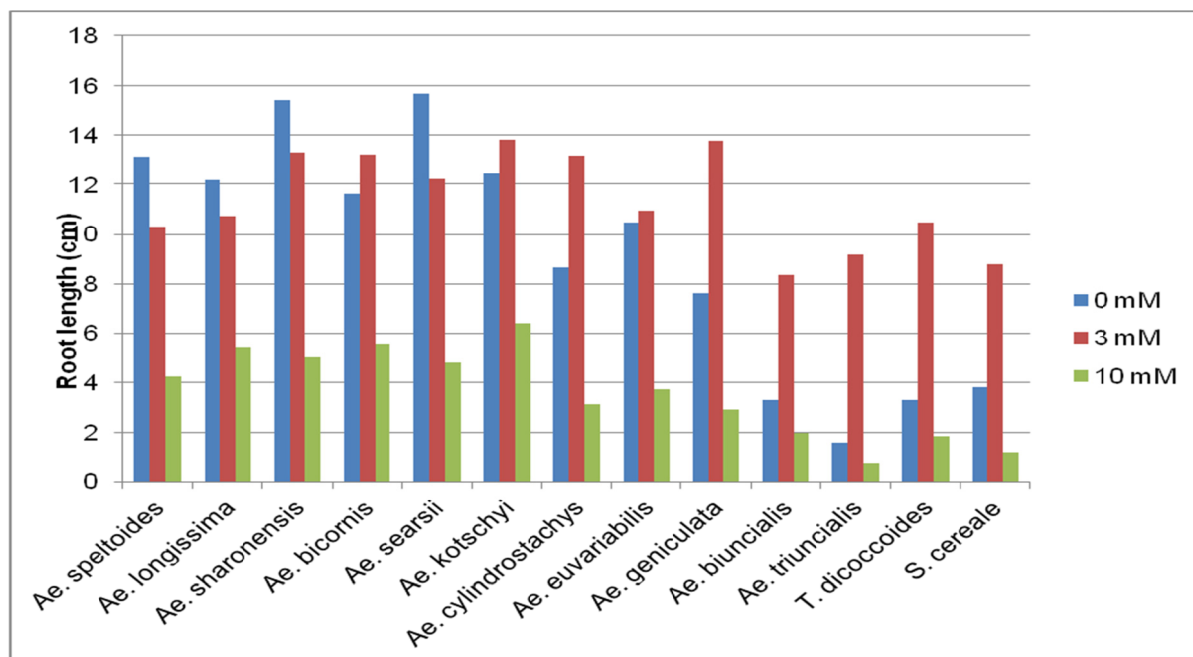


Figure 1. Tolerance of different species against different concentrations of Boron (mM = mili mole)

3.2 Genomic Characterization with SSR

3.2.1 Genetic Variation within the Species

In SSR based diversity analysis of eighty genotypes, a total of 265 alleles were identified utilizing thirty-four SSR makers. The number of alleles per locus ranged from 2 (Xgwm146 and Xgwm577) to 14 (Xgwm192) and average value of across all loci was 7.8. Marker WMC208 and WMC276 captured three alleles at each locus, which is also very close to the lowest value. The average heterozygosity over all the genotypes was 0.2, with the maximum heterozygosity (0.786) being found at locus Xgwm642 and the minimum (0.025) at EBmac0679. Several loci (BARC182, Bmag0603, WMC208, WMC276, WMC537, Xgwm146, Xgwm158, and Xgwm577) did not show significant heterozygosity in the eighty genotypes studied. Marker Xgwm192 revealed the highest (0.893) gene diversity value and loci Xgwm577 revealed the lowest value (0.077), with a mean gene diversity of 0.65 over all markers. The PIC values varied from a low of 0.074 (Xgwm577) to a high of 0.883 (Xgwm192) with a mean of 0.61. Eight markers viz. BARC123, Bmac0093, WMC208, WMC276, Xgwm46, Xgwm146, Xgwm344, and Xgwm635 produced a PIC value lower than 0.5. The results of all the diversity indices mentioned are presented in Table 2.

Table 2. Allelic variation of the 34 SSR loci in the 79 wild wheat and one rye accessions

Sl. No.	SSR marker	Chr. location	Repeat type	T (°C)	Allele no.	Gene diversity	Hetero-zygosity	PIC
1.	BARC10	4B, 5A, 7B	(GAA)13	52	8	0.628	0.181	0.583
2.	BARC32	5B, 7B	(ATT)10	52	11	0.855	0.144	0.842
3.	BARC123	6D, 7B	(CA)9	52	9	0.385	0.274	0.318
4.	BARC182	7B	(CT)15	58	5	0.723	0.000	0.679
5.	Bmac0093	2H	(AC)24	55	7	0.460	0.111	0.396
6.	Bmag0603	3H	(AG)24	55	6	0.698	0.000	0.660
7.	EBmac0679	4H	(AC)22	55	5	0.707	0.025	0.669
8.	WMC31	1B, 1D	(GT)19	61	7	0.734	0.130	0.688
9.	WMC112	2D	(CT)34	61	9	0.802	0.423	0.776
10.	WMC116	7A	(CT)12	61	8	0.723	0.098	0.674
11.	WMC208	5B	(GT)27	61	3	0.417	0.000	0.380
12.	WMC245	2B, 2D	(CA)10	61	10	0.819	0.242	0.795
13.	WMC276	7B	(CA)19	51	3	0.550	0.000	0.489
14.	WMC296	2A	(GT)28	61	6	0.712	0.303	0.667
15.	WMC331	4D	(CA)13	61	12	0.694	0.473	0.671
16.	WMC332	2B	(CT)12	61	12	0.818	0.220	0.797
17.	WMC357	5D	(GT)10	61	11	0.781	0.394	0.763
18.	WMC537	5B, 7B	(CT)10	51	6	0.550	0.000	0.513
19.	WMC581	7B	(GT)8	61	8	0.732	0.285	0.688
20.	Xgwm46	7B	(GA)2GC(GA)33	60	8	0.318	0.300	0.309
21.	Xgwm47	2A, 2B	(CT)7TT(CT)16	60	10	0.776	0.216	0.752
22.	Xgwm146	7B	(GA)5GG(GA)20	60	2	0.452	0.000	0.350
23.	Xgwm192	5D	(CT)46	60	14	0.893	0.160	0.883
24.	Xgwm193	6B	(CT)24imp(CA)8	60	8	0.661	0.493	0.621
25.	Xgwm264	1B, 3B	(CA)9A(CA)24	60	7	0.734	0.142	0.693
26.	Xgwm296	2D, 2A	(CT)28	55	10	0.799	0.546	0.775
27.	Xgwm344	7B	(GT)24	55	9	0.455	0.112	0.395
28.	Xgwm358	5D	(GA)18(G)2(GA)4	55	5	0.754	0.000	0.712
29.	Xgwm471	7A	(CA)34	60	10	0.726	0.268	0.695
30.	Xgwm493	3B	(CA)43imp	60	10	0.816	0.071	0.793

31.	Xgwm533.1	3B	(CT)18(CA)20	60	12	0.858	0.083	0.844
32.	Xgwm577	7B	(CA)14(TA)6	55	2	0.077	0.000	0.074
33.	Xgwm635	7A, 7D	(CA)10(GA)14	60	6	0.318	0.282	0.308
34.	Xgwm642	1D	(GT)14	60	6	0.737	0.786	0.707
Mean					7.794	0.650	0.199	0.617

Additionally, species-wise analysis of various diversity parameters like allele number, effective allele number, heterozygosity, gene diversity, PIC, shanon information index and percent of polymorphic loci (Table 3) revealed that genotypes from *Ae. euvariabilis* were the most diverse and those from *Ae. biuncialis* and *Ae. triuncialis* were least.

Table 3. Mean value of various diversity indices of different species

Species	AN	EAN	He	GD	PIC	SII	%P
<i>A. speltoides</i>	3.235	2.26	0.146	0.439	0.400	0.189	82.35%
<i>A. longissima</i>	2.852	2.05	0.194	0.417	0.370	0.733	82.35%
<i>A. sharonensis</i>	3.647	2.41	0.231	0.445	0.410	0.863	91.18%
<i>A. bicornis</i>	2.529	1.93	0.189	0.393	0.346	0.672	79.41%
<i>A. searsii</i>	2.558	2.03	0.221	0.402	0.352	0.689	85.29%
<i>A. kotschyi</i>	2.882	2.30	0.182	0.485	0.426	0.834	91.18%
<i>A. cylindrostachys</i>	2.323	1.95	0.220	0.398	0.344	0.651	79.41%
<i>A. euvariabilis</i>	3.735	2.38	0.267	0.515	0.469	0.962	97.06%
<i>A. geniculata</i>	2.352	1.90	0.237	0.407	0.347	0.662	85.29%
<i>A. biuncialis</i>	1.441	1.40	0.176	0.227	0.186	0.311	44.12%
<i>A. triuncialis</i>	1.823	1.62	0.225	0.276	0.230	0.455	64.71%
<i>T. dicoccoides</i>	3.000	1.84	0.148	0.376	0.337	0.688	82.35%
<i>Secale cereale</i>	0.735	0.73	0.041	0.352	0.345	0.041	5.88%

AN: Allele number; EAN: Effective allele number; He: heterozygosity; GD: genetic distance. PIC: polymorphism informayion content; SII: Shanon information index; % P: percent of polymorphic loci.

An UPGMA dendrogram generated from the root length of different *Aegilops-Triticum* species have shown two major group clusters. The group-I contained only *Ae. sharonensis* was clustered distinctly from the remaining species with 100% of the bootstrap value. This species was found to produce comparatively higher root length and good tolerance to B toxicity. On other hand, the group-II was consisted of the other 12 species which were either sensitive or moderately sensitive to the B toxicity and exhibit a relatively reduced root length.

For example the species *S. cereale* and *T. dicoccoides* that clustered together with very high (99%) bootstrap value were sensitive to B toxicity. Three groups were observed, but interestingly, the more tolerant species *Ae. speltoides*, *Ae. sharonensis* and *A. longissima* coordinated together in group-III while the sensitive and moderately sensitive clustered in group-I and II. The grouping obtained by PCoA analysis agreed with the cluster analysis of both SSR and morphological data. An UPGMA tree with high value of co-phenetic ($r = 0.71$) produced from the root length of the 80 genotypes. All these genotypes were partitioned into six clusters and the means root length of each cluster were calculated under different concentrations of B (Figure 2 & 3).

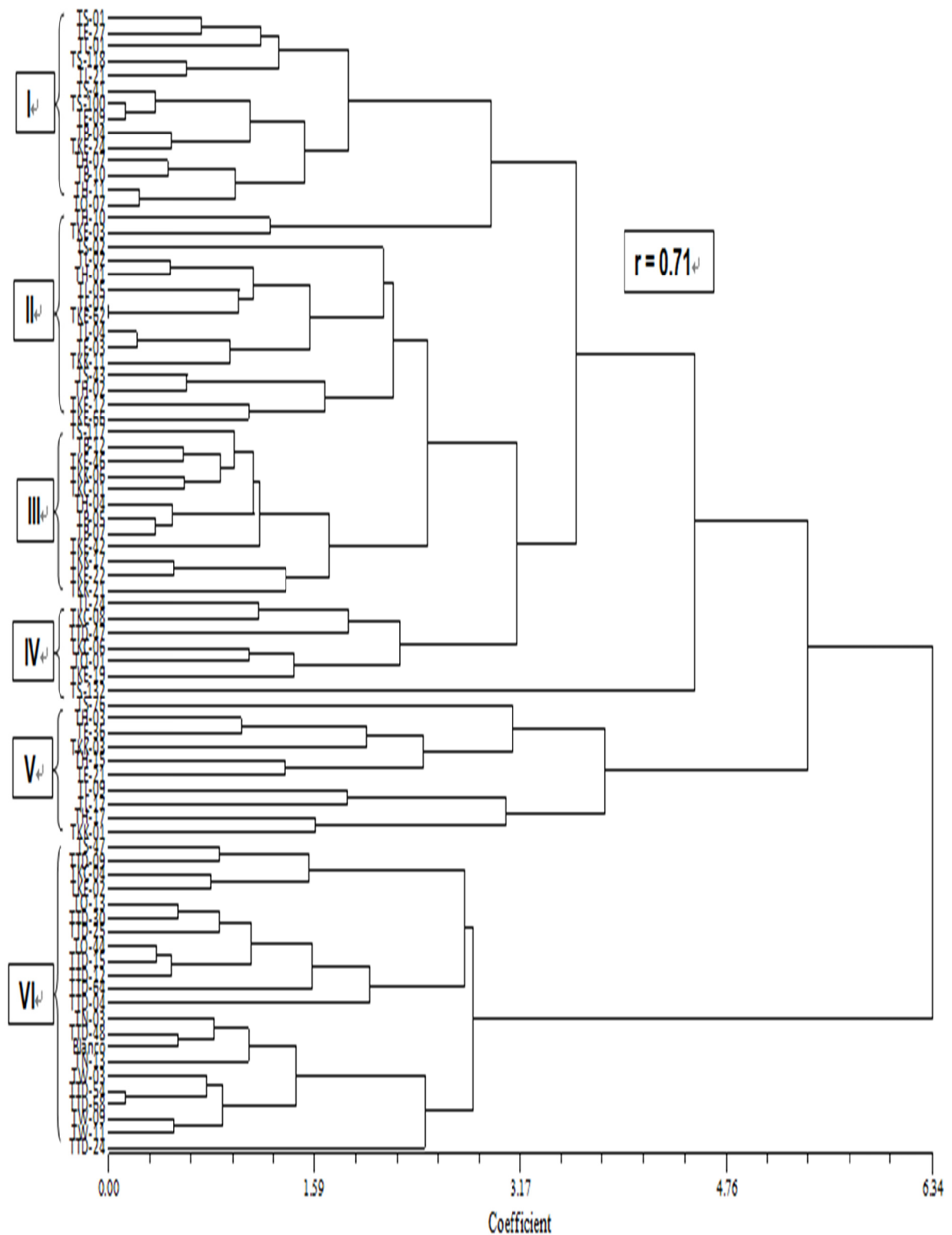


Figure 2. UPGMA Dendrogram of 80 genotypes obtained from the root length data. I, II, III, IV & VI are the different clusters

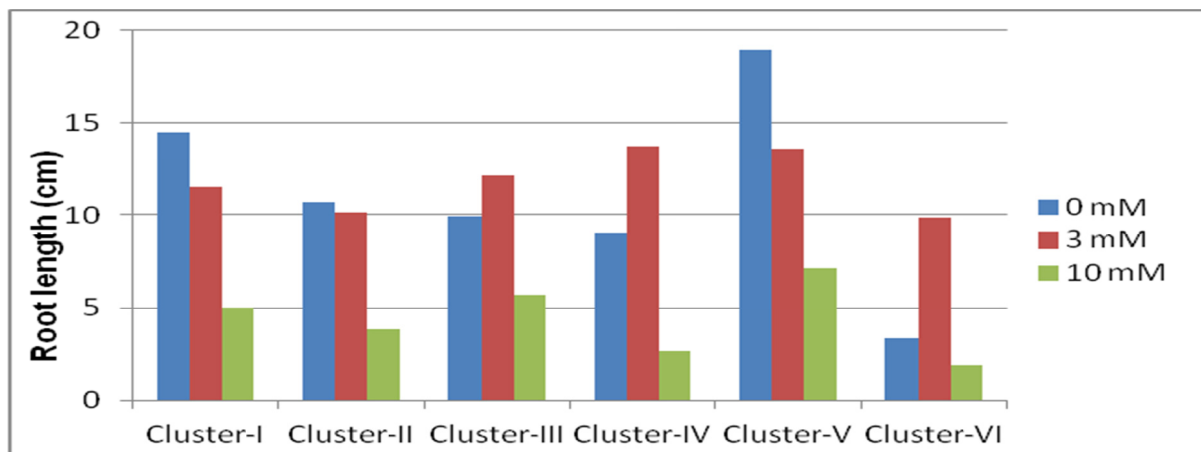


Figure 3. The mean of root length of genotypes obtained from different concentration of boron (mM= mili mole) in each cluster

Cluster-VI included the boron sensitive genotypes with lowest mean value of root length. In contrast, the genotypes grouped in cluster-V have explained a good tolerance to boron toxicity and produced higher root length. The genotypes in other clusters showed medium root length. The neighbor-joining (NJ) tree produced from SSR data based on the Nei genetic distance separated all the genotypes into two major groups. The first major group and second major group showed the similar results as UPGMA for cluster-VI and V, respectively. The efficiency of the NJ tree was assessed by calculating the correlation value (r) between the original distance matrix and the co-phenetic matrix obtained from the dendrogram. A high correlation value ($r=0.81$) between the matrixes indicated a good fit of the data set to the dendrogram (Figure 4).

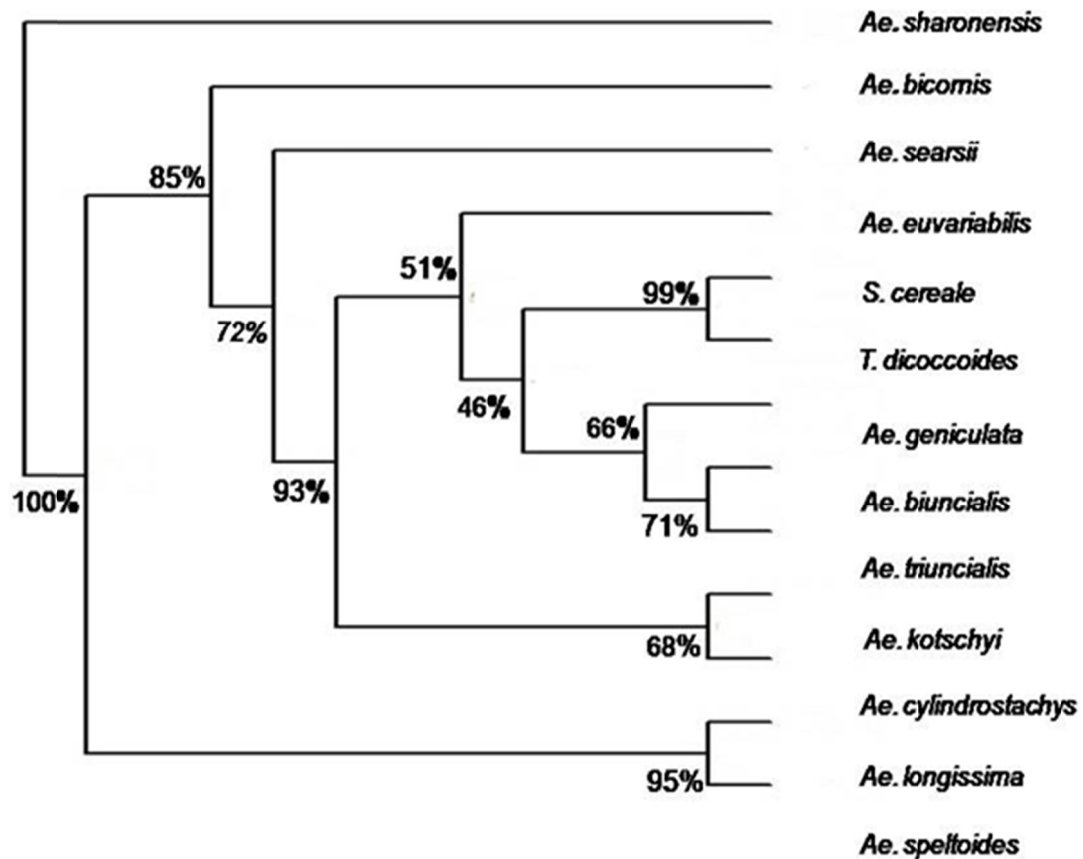


Figure 5. Cluster analysis based on SSR markers data of the *Aegilops-Triticum* complex.

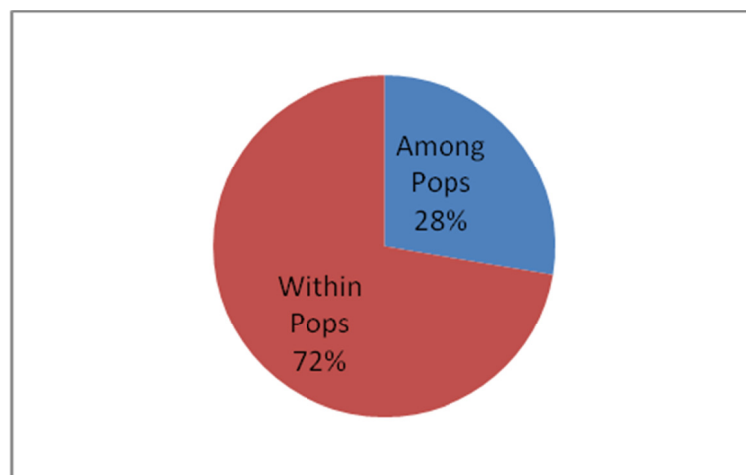


Figure 6. Genetic variation between *Ae. speltoides* and *A. longissima* populations (Pops). Blue color is the total variation from different species and Red color is the variation from different genotype within the species

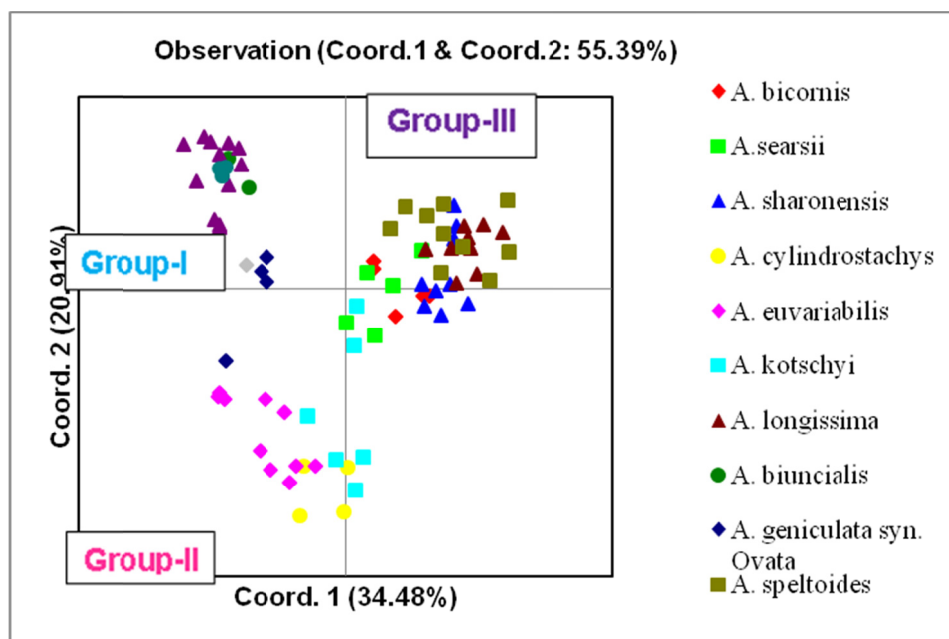


Figure 7. Result of the Principal Coordinate Analysis. Group I included *Ae. geniculata* syn. *ovata*, *Ae. biuncialis*, *Ae. triuncialis* and *T. dicoccoides* species with *S. cereale* (Blanco rye), group II contained *Ae. kotschy*, *Ae. cylindrostachys* and *Ae. euvariabilis* and group III composed of *Ae. speltoides*, *Ae. sharonensis*, *Ae. Bicornis*, and *Ae. Searsii*

Correlation between root length and SSR data: The correlation between SSR and root length data was assessed by Mantel matrix correspondence test. A positive significant correlation ($r=0.24$, $p=0.001$) was found but the value was low. Despite this low correlation, there were great similarities in separating the B sensitive genotypes from the B tolerant genotypes in the both dendrograms.

4. Discussion

Various *Aegilops* and *Triticum* species are very closely related to cultivated wheat. Historically, the utilization of these species for the improvement of wheat has achieved great success. In this study, a total of 80 genotypes including the cultivated wheat variety were grown under 3 treatments of B for evaluating their morpho-genetic characteristics and boron toxicity tolerance. Morphological screening of different species showed considerable variation for root length against B toxicity. *Ae. sharonensis* is likely to express a similar tolerance as *Aegilops longissima* (Figure 1). Moreover, in the dendrogram (Figure 2) generated from root length data, Cluster V (Figure 3) gave the highest mean root length and consisted mainly of the genotypes from *Ae. longissima* and *Ae. sharonensis*. The results found here agreed with the previous study (Emon et al., 2012), which identified these species as tolerant genotypes. In the cluster-V, two genotypes of *Ae. longissima* (TL-09 and TL-17) were grouped together (Figure 2), which was reported by Emon et al. (2012) as the most tolerant genotypes exhibiting very good root growth (10.3 and 10.9 cm, respectively) under 10mm B concentration. Moreover, variable response of some species (*Ae. geniculata*, *Ae. kotschy* and *Ae. euvariabilis*) to B toxicity was observed (Figure 2). Causes of this variation may be explained by various factors such as endosperm development and quality, seed

viability, seedling growth rate, and germination rate. Existence of such variation in seed between and within any *Aegilops* and *Triticum* species may be responsible for the non-uniform root growth observed in hydroponic screening (Figure 3). Therefore, sufficient care should be taken in hydroponic screening of any species. Regardless, this study suggests that some *Aegilops* species possesses significant tolerance to excess B and may be employed in wheat improvement programs for the development of B tolerant varieties.

In the DNA profiling of 80 genotypes by SSR markers, a total of 265 alleles were identified. The number of alleles per locus ranged from 2 (Xgwm146 & Xgwm577) to 14 (Xgwm192) and averaged 7.8 (Table 2). The results were very similar to other studies (Bandopadhyay et al., 2003; Sharma et al., 2002; Figliuolo & Perrino, 2004). Average allele number/locus found was larger than previously reported by Ehtemam et al. (2010). The *Aegilops-Triticum* complexes used in the present study were collected from the different countries, which may have contributed to the high level of allelic diversity observed. The mean PIC value was 0.61 with a range from 0.075 to 0.883 (Table 2). The PIC value was much larger than earlier reported (Thomas et al., 2010; Naghavi et al., 2009) but were quite comparable to Bandopadhyay et al. (2003).

Considerable variation was observed among the genotypes for heterozygosity, which ranged from 0.025 to 0.786 with a mean of 0.2 (Table 2). Moreover, the lowest heterozygosity (0.14) was found in the genotypes of species *Ae. speltoides* and *T. dicoccoides*. The results suggest that the sample analyzed for these species was very narrow-based and that a larger gene pool needs to be evaluated. These species are closely related to wheat and very important for wheat improvement. Analysis of molecular variances (AMOVA) identified that maximum variation (72%) existed among all genotypes of the species (Figure 6). Of the 34 markers, 26 (76%) produced PIC values greater than 0.5 (Table 2). Marker Xgwm192 was the most robust in detecting polymorphisms. Moreover, except for *Ae. biuncialis*, *Ae. triuncialis* and *S. cereale*, in the remaining genotypes more than 80% loci were polymorphic (Table 3). This very high discrimination power of the SSR markers revealed their efficiency in depicting the genomic relationship and diversity of these *Aegilops* and *Triticum* complexes.

Species-wise analysis of different diversity statistics showed that the genotypes from *Ae. euvariabilis* were the most diverse with 97.06% of the analyzed loci being polymorphic. *Aegilops biuncialis* and *Ae. triuncialis* genotypes were the least diverse species (Table 3). Moreover, in the UPGMA tree (Figure 4) the genetic relationship of the species studied, *Ae. sharonensis* was placed in a separate cluster from the rest of the species with 100% bootstrap confidence. Its genome is closely related to cultivated wheat and has been reported as a rich source of biotic and abiotic stress resistance/tolerance genes, but is still not widely used in wheat improvement programs (Olivera & Steffenson, 2009). Therefore, high tolerance to B toxicity and tremendous genetic variability observed in this species both by phenotypic and morphological data could help to draw the attention of wheat breeders for using this species in wheat improvement programs for B toxicity tolerance. In addition, it is evident from the dendrogram that all the susceptible genotypes were placed in the same cluster. Also within this cluster *T. dicoccoides* and *S. cereale* were grouped together with a 99% bootstrap value. *Aegilops geniculata*, *Ae. biuncialis* and *Ae. triuncialis* were grouped in a single cluster. These findings are quite similar to other studies (Thomas & Bebeli, 2010; Sallares & Brown, 2004;

Cenkci et al., 2008, Sasanuma et al., 2004). Furthermore, the close relation of *S. cereale* and *T. dicoccoides* may be the result of early introgression of organelle DNA of *Aegilops-Triticum* and *Secale* (Yamane & Kawahara, 2005). But, this surprising relationship needs to be elucidated with further rigorous studies including a combination of different types of SSR markers and DNA sequence data.

On the other hand, both the dendrograms (obtained from root length, Figure 2 and SSR data, Figure 4 separated all eighty genotypes into two main groups according to B tolerance. Almost all the sensitive genotypes were clustered in the same group. Most of the markers used were selected from wheat chromosomes 7B and 7D of the wheat, and barley (*Hordeum vulgare* L.) 2H, 3H and 4H, which were previously reported (Jefferies et al., 1999, Jefferies et al., 2000, Paull et al., 1991) as the harboring chromosome of boron toxicity tolerance loci. Some markers were also selected from wheat chromosomes 5A, 5B, and 5D because a recent study (Schnurbusch et al., 2008) identified B tolerant genes on these chromosomes. The present results identified tolerant and susceptible genotypes. However, we used only root length data for phenotypic screening of B toxicity tolerance. Although root length measurement provides quick screening other characters like grain yield, shoot B concentration, grain B content etc. should be considered in any screening experiment. Further extensive studies including an increased number of SSR markers from the B tolerant chromosomes and a larger genotype sample size of these species may yield additional sources of B tolerant genes.

5. Conclusion

Evaluating wheat germplasm pools for B tolerance at the seedling root growth stage, using hydroponics can be a useful technique, but careful selection of the seed within each accession is needed to prevent the vagaries of seed quality from influencing root growth in hydroponics. The present study established that several species including *Ae. sharonensis*, *Ae. kotschyi*, *Ae. bicornis*, *Ae. kotschyi*, *Ae. peregrina ssp. cylindrostachys*, *Ae. peregrina ssp. euvariabilis*, *Ae. geniculata syn. ovata*, and some *Ae. longissima* and *T. dicoccoides* contained accessions that were generally more B tolerant, and that there were major differences in B tolerance within the various *Aegilops/Triticum* species. Variation that occurs within wild cereal species involving differences in endosperm development and quality, seed viability, seedling growth rate, germination rate, etc. were all problems in getting uniform root growth under hydroponic growing conditions whether or not B was added to the hydroponics solution. Nevertheless, several wild *Triticeae* species expressed sufficient B tolerance to be considered as useful candidates for wheat improvement programs striving to produce B tolerant cultivars. The assessment of genetic diversity is an essential component in germplasm characterization and conservation. This study also identified several genotypes from the analyzed species, which are highly tolerant to B toxicity, and show considerable variation for B toxicity tolerance. The variability in the species analyzed could be readily employed in wheat improvement programs to develop B toxicity tolerant cultivars and genetic diversity at the DNA level could be used for designing effective breeding programs aiming to broaden the genetic bases of commercially grown varieties.

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