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Assessment of Genotoxic Effects of Boron on Wheat (*Triticum aestivum* L.) and Bean (*Phaseolus vulgaris* L.) by Using RAPD Analysis

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Abstract In boron-rich soils of Turkey, boron tolerant wheat (*Triticum aestivum* L.) and sensitive bean (*Phaseolus vulgaris* L.) are most widely cultivated crops. In this study they have been studied to elucidate the probable genotoxic effects of boron by using RAPD analysis. During the study, root and stem lengths have been measured and inhibitory rates (%) of root growth have been found to be significant, starting from 10 (13%) and 5 ppm (19%) for wheat and bean, respectively, which is in strong correlation with the root DNA alterations; RAPD variations starting from 100 ppm for wheat and 25 ppm for bean. The preliminary findings encourage the use of these tools in investigation of genotoxic effects of boron on wheat, bean and the other crops.

Keywords RAPD-PCR · Genotoxicity · Boron · Wheat · Bean

In recent years, there has been a drastic increase in the use of industrial Boron (B). This circumstance encourages excessive mining of B. One of the largest world B reserves is found in western parts of Turkey; in Emet, Bigadiç, Kırka and Mustafakemalpaşa Districts (72% of the world boron reserves). In addition to industrial use and water desalination processes for healthy irrigation, mining processes give way to a dramatic increase in the accumulation of B in agricultural soils (Woods 1994; Parks and Edwards 2005).

The sources of B in agricultural areas of Turkey are informal mining processes. Bigadiç mining area is a good example for this situation. The wastewater of the mining processes, which flows to Simav River influences a large area of Balıkesir plain and its environment, about 40.000 ha of agricultural area(Şener and Özkara 1989).

Boron plays important roles in many plant metabolic pathways (Lovatt and Dugger 1984; Lou et al. 2001) as an essential micronutrient (Marschner 1995), however, excessive amount of B creates some disorders in crop productivities (Hale and Orcutt 1987; Nable et al. 1997; Cervilla et al. 2007). Boron-induced symptoms are reduced vigor, slow development and growth of plants (Cervilla et al. 2007), cellular disorders and DNA damage as well. Some methods to assess genotoxicity on plants are comet, micronucleus or chromosome aberration assay (Steinkellner et al. 1999; Angelis et al. 2000; Reinecke and Reinecke 2004; Liu et al. 2005; Liu et al. 2007; Osman et al. 2008). For genotoxicity monitoring, it is important to use sensitive but non-specific assays that indicate a wide range of DNA damage types. RAPD-PCR is a widely applicable technique developed by Williams et al. (1990); Welsh and McClelland (1990), that is capable of detecting variations in intensity as well as gain or loss of DNA bands following toxicant exposures that might be an indicator for DNA changes (Uzonur et al. 2004; Rong and Yin 2004; Liu et al. 2005; Atienzar and Jha 2006).

In the present study, we screened genome-wide DNA alterations in *Triticum aestivum* L. and *Phaseolus vulgaris* L. root cells exposed to various concentrations of B by using the random amplified polymorphic DNA (RAPD) method to investigate (1) Boron-induced genomic instability and (2) the correlation of RAPD profile changes with root growth inhibition factors in wheat and bean.

Materials and Methods

Experiments were designed with starting germination tests and followed by RAPD-PCR analysis of wheat (*Triticum*

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aestivum L.) and bean (*Phaseolus vulgaris* L.) exposed to 0, 5, 10, 25, 50, 100, 125, 150 ppm B. Seeds were surface-sterilized with 1% sodium hypoclorite and placed in petri dishes containing two layers of Whatman No 1 filter paper with test solutions and a control solution (distilled water). Boric acid (H_3BO_3) stock solution (150 ppm) was prepared and diluted to 0 (control), 5, 10, 25, 50, 100, 125 ppm concentrations with distilled water. Incubation was done in a climatic conditioner at 23°C in the dark for 7 days. After 7 days of incubation, root and stem lengths of wheat and bean were measured and inhibitory rates (%) of root growth for both were calculated using the following formula:

$$\mathrm{IR} = \left(1 - \frac{x}{y}\right) \times 100$$

x The average root lengths of control plants. y The average root lengths of treated plants.

DNA isolation was carried out using the Dneasy Plant DNA Extraction Mini Kit (Qiagen) according to the supplier's instructions. DNA concentrations and sizes were estimated by comparing them with a standard sample (GeneRuler^{TM} 100 bp DNA Ladder, ready-to-use, MBI Fermentas) in a 2% agarose gel (fragment, (bp) and DNA quantity in band, (ng) were given, respectively, for 10 bands in descending order of the fragment sizes 1: 1,031–169, 2: 900–147, 3: 800–131, 4: 700–115, 5: 600–98, 6: 500–164, 7: 400–65, 8: 300–49, 9: 200–33, and 10: 100–16. RAPD amplification was done in a 25 μL PCR mix, containing 1× PCR buffer $(NH_4)_2SO_4$, 0.2 mM from each dNTP (2 mM dNTP mix), 25 pmol of primer OPA-08 5′CCACAGCAGT 3′ from QIAGEN Operon RAPD® 10 mer Kits, 20–200 ng of genomic DNA, and 0.5 units of Taq DNA polymerase, and filled up with sterile deionized water to the final volume. PCR chemicals were obtained from MBI Fermentas, except when otherwise stated.

Tubes containing all reaction components, except template DNA, were included as controls for each reaction. Amplifications were performed in a Techne Endurance TC-512 Gradient Thermal Cycler, programmed for 3 min at 95°C (initial denaturation of template DNA) followed by 45 cycles of 1 min at 94°C (denaturation), 1 min at 37°C (primer annealing temperature), 2 min at 72°C (elongation), and 5 min at 72°C (final extension step).

Amplification products were analyzed by 2% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV-light. Results were documented with GelDoc 2000 (BioRAD). To confirm intraindividual variation in RAPD profiles for each DNA sample three replicates (i.e. n$x = 3x$) were prepared. Clearly observed bands were scored and used to create the genetic profiles of each plant sample. The genomic instability was determined and DNA variations were observed according to increase or decrease in band intensities, loss and gain of bands.

Three replicates were set up for each treatment and experiments. The values of experimental results were statistically investigated using *t*-tests.

Results and Discussion

After 7 days of varying amounts of B, at 10 ppm inhibitory rate of root growth of bean was calculated as 7% and the other concentrations decreased gradually from (−) 19 to (−) 86 ($p < 0.001$; Fig. 1). The inhibitory rate of root growth of wheat was calculated as 13% at 5 ppm B and the other concentrations decreased gradually from (−) 13% to (−) 87% ($p < 0.001$; Fig. 2).

The effects of varying B concentrations on the root cells' DNA were analyzed by comparing their RAPD-PCR profiles. The RAPD-PCR analysis was performed with DNA extracted from roots of each group of plants treated with different B concentrations (0, 5, 10, 25, 50, 100, 125, 150 ppm) for 7 days.

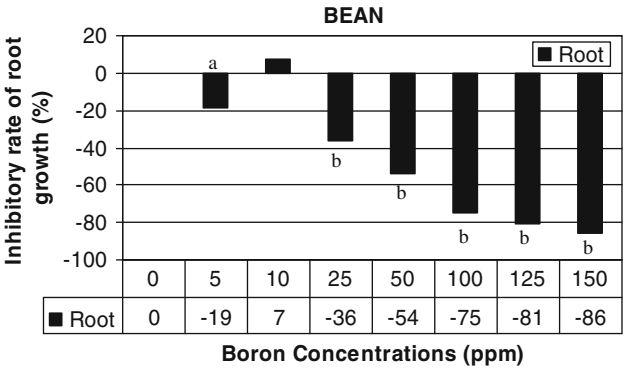

Fig. 1 Root growth inhibition rates in bean seedlings exposed to different B concentrations. [a]$p < 0.05$, [b]$p < 0.001$

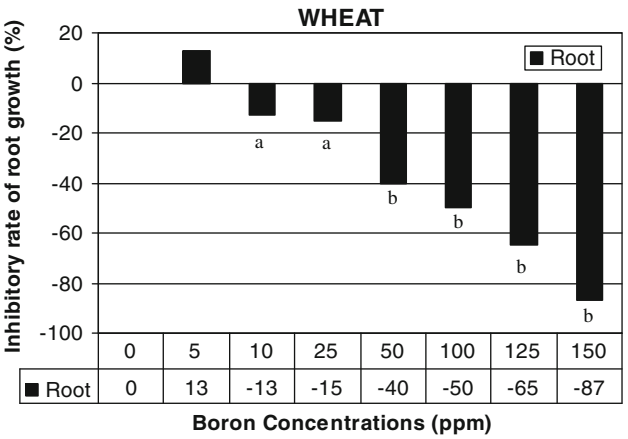

Fig. 2 Root growth inhibition rates in wheat seedlings exposed to different B concentrations. [a]$p < 0.05$, [b]$p < 0.001$

RAPD profiles of B treated and the control plants were compared on the basis of specific RAPD band alterations: Increase and decrease in band intensities and the loss and the gain of bands. In bean, the molecular size of the bands obtained with OPA08 range from 210 to 1,559 bp and decreasing of band intensities were especially at 125 and 150 ppm boron exposure. Furthermore intensity of the RAPD band with 964 bp in molecular size substantially varied with increase of boron concentration (Tables 1, 2). Whereas intensity of RAPD band with ~392 bp substantially decreased with increase of boron concentration. At 10, 25, 50, 100 ppm 210 bp normal RAPD band was lost whereas at 125 ppm there are two missing bands of sizes about 210 and 254 bp and at 150 ppm B only 392 bp and disappeared. Extra bands of molecular sizes between ~1,559, 1,177 and 700 bp appeared at 25, 50, 150 ppm B (Fig. 3).

In wheat, the molecular size of the bands obtained with OPA08 range from 254 to 954 bp and decreasing of band intensities were especially at 100 and 150 ppm B exposure. Furthermore intensity of the RAPD band with 943 bp in molecular size substantially varied with increase of boron concentration (Tables 3, 4). Whereas intensity of RAPD band with ~254 bp substantially decreased with increase of boron concentration. At 50 ppm 344 bp and at 100 ppm 954 bp bands appeared. In wheat there are no missing bands (Fig. 4).

Boron is an essential element for higher plants. Many studies show that certain B concentrations are necessary for plants' biochemical, physiological and morphological development. It is also confirmed in our work that B is an essential requirement for bean and wheat with the findings in root growth rate and genomic stability increase at 10 ppm B concentration. Similar findings were reported by Kocacaliskan and Olcer (2006) and Konuk et al. (2007). However, in amounts greater than 10 ppm, it may be acting as a toxic agent.

Boron toxicity may limit crop productivity in different regions of the world where B is found in agriculturally important fields. More than 50% of the world B reserves are in Turkey's agronomically most important areas (Kalafatoglu and Ors 2000). Boron toxicity has been evaluated for its genetic and epigenetic aspects in some recent studies. Boron could induce physiologic and metabolic problems (Papadakis et al. 2004) due to its genotoxicity. Badr and Ibrahim (1987) and Konuk et al. (2007) have investigated the genotoxicity of B with reference to the mitotic index and reported mitotic abnormalities in some plant species. Konuk et al. (2007) observed that B inhibits mitosis in *Allium cepa* L. at doses of 100 ppm and above. Karabal et al. (2003) and Cervilla et al. (2007) have found that B causes oxidative damage, but its genotoxic effect is still unclear.

RAPD-PCR based assays are important as a genome-wide DNA variation screening strategy. Toxicant-induced genotoxic effects, DNA variation, DNA damage, genetic instability and mutagenic effects have been evaluated with RAPD analysis successfully by previous work. RAPD assay has proved useful to detect genomic instability

Table 1 RAPD profile alterations in number and intensities of bands (*a*, *b*, *c*, and *d*) as detected with primer OPA-08 in B exposed bean seedlings in comparison to the non-exposed control seedlings

Boron concentrations (ppm)		Bean			
	Total bands	*a*	*b*	*c*	*d*
Control	6				
5 ppm	8	2	–	2	2
10 ppm	6	1	1	1	2
25 ppm	8	3	1	2	1
50 ppm	6	2	1	2	–
100 ppm	8	3	1	–	3
125 ppm	5	1	2	3	1
150 ppm	6	2	1	3	–

a Indicates appearance of new bands, *b* disappearance of normal bands, *c* decrease in band intensities and *d* increase in band intensities

Table 2 Molecular sizes (bp) of appearing, disappearing bands, and bands with changes in intensities

Primer: OPA08				
Boron concentrations (ppm)	Bean			
	a	*b*	*c*	*d*
5 ppm	814; 692	–	964; 358	327; 254
10 ppm	700	210	254	327; 964
25 ppm	1,559; 1,177; 254	210	392; 254	330
50 ppm	700; 344	210	392; 254	–
100 ppm	1,470; 1,100; 700	210	–	964; 392; 327
125 ppm	700	210; 254	392; 327; 254	964
150 ppm	1,470; 700	392	344; 327; 210	

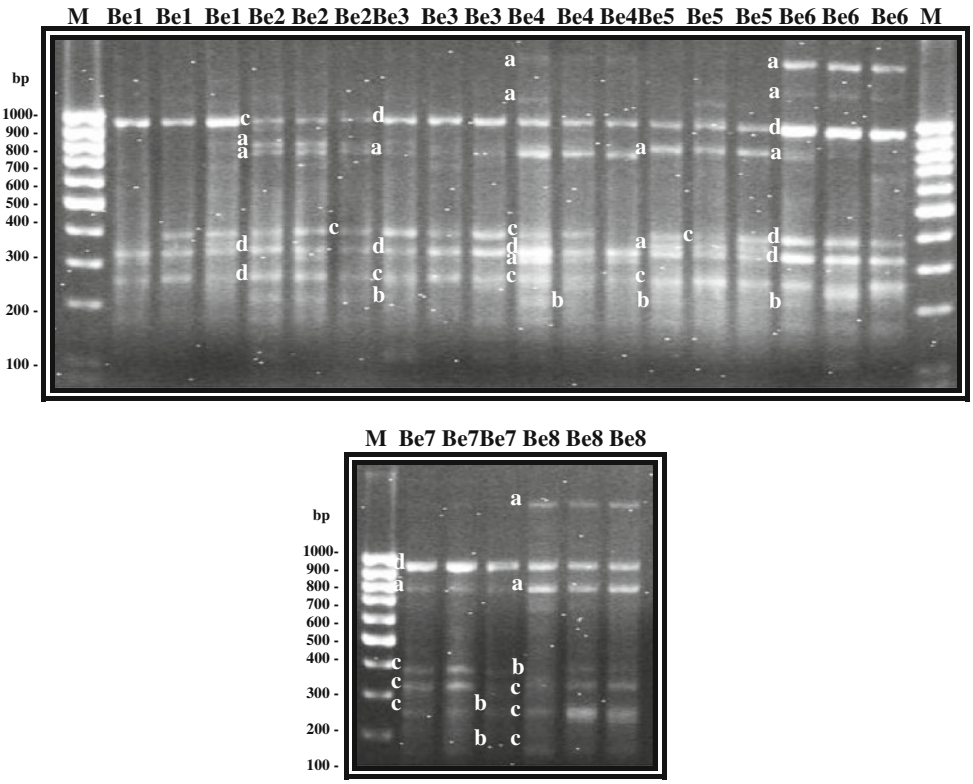

Fig. 3 RAPD profiles of genomic DNA from root-tips of bean seedlings exposed to different B concentrations (*Be1* control, *Be2* 5 ppm, *Be3* 10 ppm, *Be4* 25 ppm, *Be5* 50 ppm, *Be6* 100 ppm, *Be7* 125 ppm, *Be8* 150 ppm). **a** indicates appearance of new bands, **b** disappearance of normal bands, **c** decrease in band intensities, **d** increase in band intensities

Table 3 RAPD profile alterations in number and intensities of bands (*a*, *b*, *c*, and *d*) as detected with primer OPA-08 in B exposed wheat seedlings in comparison to the non-exposed control seedlings

Primer: OPA08					
Boron concentrations (ppm)		Wheat			
	Total bands	*a*	*b*	*c*	*d*
Control	4				
5 ppm	4	–	–	–	–
10 ppm	4	–	–	–	–
25 ppm	5	1	–	–	–
50 ppm	5	1	–	1	–
100 ppm	4	–	–	2	–
125 ppm	4	–	–	1	–
150 ppm	4	–	–	2	–

a Indicates appearance of new bands, *b* disappearance of normal bands, *c* decrease in band intensities and *d* increase in band intensities

Table 4 Molecular sizes (bp) of appearing, disappearing bands, and bands of wheat with changes in intensities

Primer: OPA08				
Boron concentrations (ppm)	Wheat			
	a	*b*	*c*	*d*
5 ppm	–	–	–	–
10 ppm	–	–	–	–
25 ppm	–	–	–	–
50 ppm	344	–	254	–
100 ppm	954	–	805; 254	–
125 ppm	–	–	254	–
150 ppm	–	–	805; 254	–

manifested such as point mutations, genetic and chromosomal rearrangements, deletion and insertions (Liu et al. 2007).

RAPD is likely to detect genomic instability as the newly growing and developing cells will produce a clone of dividing daughter cells. Thus the proportion of cells presenting the same genomic instability is high and easy to detect. In the field of genetic toxicology most RAPD studies describe changes such as differences in band intensity as well as a gain/loss of RAPD bands, defined as diagnostic RAPD.

According to disappearance of normal RAPD bands as a genomic instability parameter, B tolerance of plants can be classified as "B sensitive"; for the ones which have many missing normal bands at all concentrations, "B semi tolerant"; for the ones which have missing bands at only high concentrations of B (100, 125, 150 ppm) and "B tolerant"; when no missing bands have been observed at any

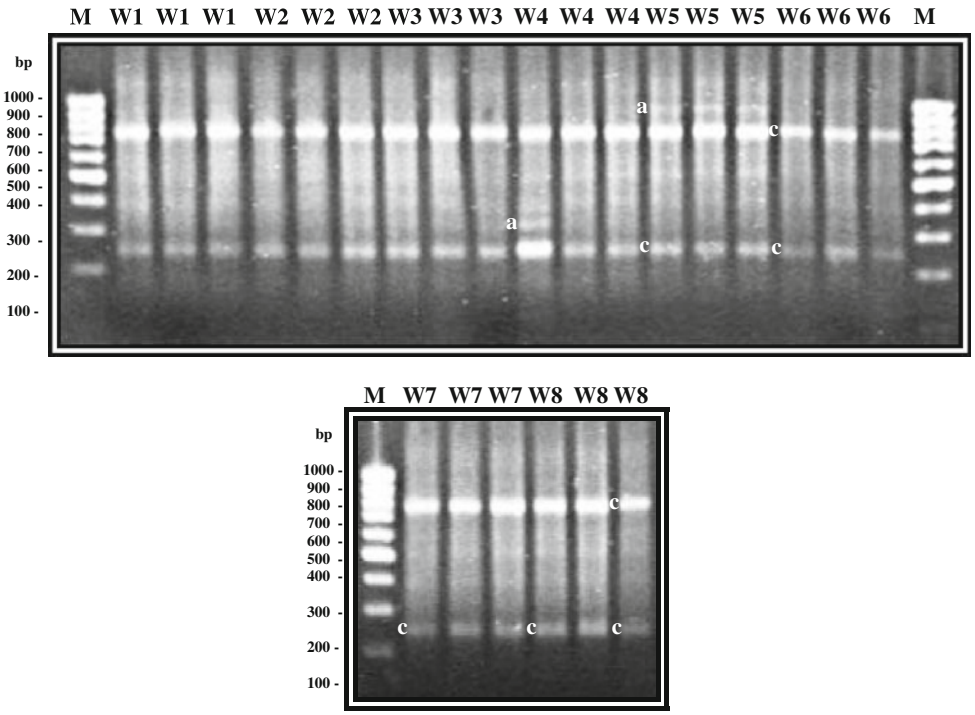

Fig. 4 RAPD profiles of genomic DNA from root-tips of wheat seedlings exposed to different B concentrations (*W1* Control, *W2* 5 ppm, *W3* 10 ppm, *W4* 25 ppm, *W5* 50 ppm, *W6* 100 ppm, *W7* 125 ppm, *W8* 150 ppm). **a** Indicates appearance of new bands, **b** disappearance of normal bands, **c** decrease in band intensities, **d** increase in band intensities

concentrations. So bean is confirmed to be a B sensitive, and wheat is a B tolerant species by both evaluation parameters.

In conclusion RAPD-PCR method can be used as an investigational tool for Boron-induced genomic alterations. Furthermore, the present results suggest that RAPD-PCR fingerprinting together with physiological parameters can be a powerful strategy for assessing levels of Boron exposure. OPA-08 primer was informative for detecting Boron-induced specific genomic alterations, but the nature and amount of DNA impact in RAPD band can only be understood by sequencing or probing (Atienzar and Jha 2006). Genomic targets of Boron exposure should further be assessed with systematic sequencing to make RAPD-PCR assay a quantification method rather than a qualification method.

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