INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–0158/2018/20–6–1397–1404
DOI: 10.17957/IJAB/15.0648
http://www.fspublishers.org

Full Length Article

Assessment of Biochemical Parameters and Genotoxicity of Rice (*Oryza sativa*) Variation Treated with Zinc Sulfate and Boric Acid

Roqaia Bibi[1†], Muhammad Adnan[1*†], Shaheen Begum[2], Azra Yasmin[2], Abdelaaty Abdelaziz Shahat[3,4], Mansour S. Al-Said[3], Riaz Ullah[3], Akash Tariq[5,6] and Nawab Ali[7]

[1]*Department of Botany, Kohat University of Science and Technology, Kohat-26000, Pakistan*
[2]*Department of Environmental Sciences, Fatima Jinnah Women University, The Mall Rawalpindi-46000, Pakistan*
[3]*Department of Pharmacognosy and Medicinal Aromatic, and Poisonous Plants Research Center, College of Pharmacy King Saud University, Riyadh 11451, Saudi Arabia*
[4]*Phytochemistry Department, National Research Centre, 33 El Bohouth st., Dokki, P.O. Box 12622, Giza, Egypt*
[5]*Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu-610041, Sichuan, China*
[6]*University of Chinese Academy of Sciences, Beijing, China*
[7]*Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat-26000, Pakistan*
[*]For correspondence: ghurzang@hotmail.com
[†]Equally contributing principal authors

Abstract

Zinc sulfate and boric corrosive are transcendently utilized as manufactured composts for rice cropping system in Pakistan. The present research work was conducted to investigate the adverse effects of these fertilizers at 150 and 350 mg/L concentrations in rice (*Oryza sativa* L.). The biochemical and molecular parameters of NIAB-IR9 and KSK-282 varieties of rice were studied being treated with the selected fertilizers' concentrations. Both fertilizers showed positive effects on the biochemical parameters (total soluble sugars, chlorophyll a and b, total carotenoid) of rice seedlings as compared to controls. However, both fertilizers' concentrations with exception of zinc sulfate (150 mg/L) caused more cell membrane injuries and genotoxic effects in to rice seedlings. Zinc sulfate at 150 mg/L significantly decreased the cell membrane injury in the shoots (1.07 µs/cm) and roots (2.0 µs/cm) of KSK-282 as compared to the shoots (1.9 µs/cm) and roots (3.7 µs/cm) of control, respectively. Similarly, zinc sulfate at its lower concentration remained non-toxic for the genome of rice seedlings. Therefore, it can be concluded that zinc sulfate at 150 mg/L can be the suitable fertilizer's concentration to both rice varieties subjected to further field trials.

Keywords: Toxicity; Cell membrane injury; Chlorophyll content; Soluble sugars; RAPD Primers

Introduction

Zinc sulfate and boric acid are important synthetic fertilizers being used as good sources of zinc and boron for the rice cropping system in Pakistan (Abbas *et al*., 2013). Pakistani soils are dominantly carbonated rich, with high pH, low organic matter and deficient in several micronutrients (Parveen *et al*., 2011; Zia *et al*., 2012). According to studies, the deficiency of zinc and boron elements is dominant in rice growing regions of Pakistan due to the soil nature (Rashid *et al*., 2008; Bhutto *et al*., 2013). Therefore, both of these are supplied adequately to soil for rice cultivation across the country. Since zinc is an important trace element, its deficiency leads to some metabolic dysfunctions in plants (Alloway, 2008), which would not only affects the plants but also the humans (Cakmak, 2008; Hussain *et al*., 2012). Likely, boron has also been recognized as a vital micronutrient for all plants (Tariq and Mott, 2007). Its deficiency causes biochemical and physiological damages in plants as it is involved in the maintenance of cell walls and some metabolic activities (Brown *et al*., 2002).

Conclusively, zinc and boron act as micronutrients when provided in lower quantities can positively regulate the metabolic activities of a plant. However, these may become toxic at higher concentrations and may cause biochemical and molecular damages in crops. It has been proved that zinc sulfate and boric acid at 150 ppm and 350 ppm caused molecular damages (genotoxicity) in the bean seedling at Turkey (Cenkci *et al*., 2009). Genotoxicity assessment deals with changes in the hereditary material of organisms (Masood *et al*., 2012) and RAPD-PCR analysis is one of the most popular and efficient technique used for its

To cite this paper: Bibi, R., M. Adnan, S. Begum, A. Yasmin, A.A. Shahat, M.S. Al-Said, R. Ullah, A. Tariq and N. Ali, 2018. Assessment of biochemical parameters and genotoxicity of rice (*Oryza sativa*) variation treated with zinc sulfate and boric acid. *Int. J. Agric. Biol*., 20: 1397–1404

determination (Zheng, 2003). According to a study, boron being the essential nutrient for plants caused genotoxicity in bean and wheat at different concentrations by using RAPD analysis (Kekec *et al*., 2010). Likely in Iran, DNA changes were observed in maize seedling at 40 mg/L and 80 mg/L of cadmium (Shahrtash *et al*., 2010).

Despite of the above facts and figures, scarce studies have been done on the assessment of genotoxicity and biochemical parameters of rice across the world. In addition, the genotoxicity of rice induce by the application of fertilizers (zinc sulfate and boric acid) is unchecked at global level. Hence, this study has been planned with the objective to investigate the biochemical and molecular damages in rice varieties due to the application of these two chemical fertilizers. Thus, it was hypothesized that zinc sulfate and boric acid may cause biochemical damages and genotoxicity in rice if used in higher concentrations.

Materials and Methods

Seeds Selection, Sterilization, and Germination

Two rice varieties (KSK-282 and NAIB–IR9) were obtained from National Agriculture Research Center (NARC) Islamabad in April 2015. Seeds of both rice varieties were subjected to viability test and sterilization. Afterwards, the seeds were germinated in a glass jar having two-layered channel paper (Whatman No.1) for 3 days in dark condition at 37–39°C. Then, 10 germinated seeds were carefully chosen for each sterilized Petri plate (with doubled layered filter paper) and were transferred to them. Three replicates were selected for each treatment. The prepared Petri plates with seedlings were transferred to light. Growth chamber was used for the supply of light in the Department of Botany, KUST, where the temperature was kept 37–39°C and relative humidity was 60%. The seedlings were treated separately with zinc sulfate and boric acid at concentrations (0, 150 mg/L and 350 mg/L). After 10 days of treatment, the seedlings were used for different biochemical tests and assessment of genotoxicity (Cenkci *et al*., 2009).

Biochemical Parameters

Determination of cell membrane injury: For cell injury analysis, 20 pieces of 1 cm of fresh leaves from each replica were taken and were placed in 20 mL of sterile distilled water and were incubated at 10°C for 24 h. After this, samples were kept at room temperature for 10–15 min. Then the electric conductivity "EC-I" was examined through the Conductivity Meter (BMC EC meter-400 L). After that these samples were autoclaved, kept at room temperature for 10–15 min and the electric conductivity "EC-II" was examined again (Jamil *et al*., 2009). Following formula was used for the determination of the cell membrane injury.

EC-I/EC-II * 100

Count of Total Soluble Sugars

Soluble sugars were determined following the protocol of (Dey, 1990). Fifty milligrams of fresh leaves were homogenized and extracted with 3 mL of 90% hot ethanol at 60–70°C temperature. The extract was poured into a 25 mL flask and the remaining residue was then extracted again. Twenty five milliliter ethanol was added and volume was raised. One milliliter aliquot was added with 1 mL of 5% phenol and Sulphuric acid (5 mL, analytical grade). After cooling, the mixture was used in BMSUV1900 spectrophotometer and the absorbance was noted at 485 nm wave length. Finally, a standard curve for glucose solution was prepared and the concentration of soluble sugars (mg/g fresh weight) was calculated with the help of it.

Calculation of Chlorophylls and Total Carotenoids

Twenty-five milligram MgO was added to 25 mg of the dried plant sample. Methanol (5 mL) was added and shook for 2 h. The turbid pigments extract was then centrifuged for 5 min at 4000 rpm. The supernatant was used in a BMSUV-1900 spectrophotometer where absorbance readings were noted at 666 nm, 653 nm, and 470 nm wavelengths. Finally, following formula was used for the calculation of chlorophyll (*a* and *b*) and total carotenoid (Lichtenthaler and Wellburn, 1983).

$$C_a = 15.65\ A_{666} - 7.340\ A_{653}$$
$$C_b = 27.05\ A_{653} - 11.21\ A_{666}$$
$$C_{x+c} = (1000\ A_{470} - 2.860\ C_a - 129.2\ C_b)/245$$

Data Analysis

Microsoft Word Excel was used for the calculation of mean and standard deviation of each and every parameter. The difference between control and the treatments were considered to be significant by Paired-sample *t*-Test in SPSS version 16.0 (SPSS, 2007). Data was considered significant if two-tailed significance value was ≤ 0.05.

Genotoxicity Assessment

DNA isolation: Plant leaves (200 mg) were ground to obtain a paste by using 500 µL of CTAB buffer. The mixture of CTAB/plant extract was shifted to the microfuge tube and incubated for about 15 min at 55°C. After that, the mixture was spin at 12000 g for 5 min in order to spin down cell debris. The supernatant was transferred to another clean microfuge tube and mixed with 250 µL of Chloroform: Isoamyl Alcohol (24:1). Then, the mixture was spin for a min at 13000 rpm. Again the upper aqueous phase (contains the DNA) was transferred to a clean microfuge tube and 50 µL of 7.5 M Ammonium acetate was added. Finally, 500 µL of ice cold absolute ethanol was supplied and left at -20°C for 1 h to precipitate the DNA (Doyle and Doyle, 1987).

RAPD-PCR Amplification

RAPD-PCR amplification was done in order to check polymorphism, readability, and reproducibility. A total of 12 random primers from Operon Technologies Inc. (Alameda, California, USA) were used for the amplification. Selected primers and their sequences are shown in the Table 1. The protocol of Williams *et al*. (1990) was used for RAPD analysis with some modification. Amplification reactions were done in a volume of 20 µL having 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM each deoxynucleotide triphosphate (dNTP), 0.4 µM of the primer (Operon Technologies Inc.), 1 unit DNA polymerase (Ampli Taq Gold) and 20 ng of DNA template. Gene Amp PCR System 9700 (PE Applied Biosystems, USA) was used for amplification process. For initial denaturation, the cycling of PCR was programmed as 1 cycle of 5 min at 94ºC. This was followed by 45 cycles of 1 min at 94ºC for denaturation. Then, a cycle of 1 min at 36ºC for annealing and another of 2 min at 72ºC for primer extension. Finally, for the final extension 1 cycle of 7 min at 72ºC was programmed. The soaking was done at 4ºC.

Agarose-gel Electrophoresis of RAPD Products

The RAPD-PCR products were checked on 1.5% agarose-gel. For size measurement, 1 kb plus the molecular marker was used. After electrophoresis, the gels were documented using a Fluor Chem FC2 Imaging System (Alpha Innotech Corporation, USA).

RAPD Data Analysis

The genomic changes i.e., appearance and/or the disappearance of bands of treated samples as compared to control by using RAPD primers were observed in the RAPD profiles and tabulated. The percentage of polymorphism of amplified DNA was calculated using the following equation:

$$P = [(a + b)/c] \cdot 100$$

Where, "a" shows the number of newly appeared bands, "b" shows the number of bands that disappeared and "c" is for the total number of bands for each primer used.

Results

Biochemical Parameters

In a laboratory experiment, two rice cultivars (KSK-282 and NIAB-IR9) were treated with zinc sulfate and boric acid fertilizers. Both cultivars showed different biochemical responses to the fertilizers. These observed biochemical parameters include cell membrane injury, the amount of total soluble sugars, the contents of chlorophyll a and b, and total carotenoid contents in both varieties.

Zinc sulfate at 150 mg/L significantly decreased the cell membrane injury in the shoots (1.07 µs/cm) and roots (2.0 µs/cm) of KSK-282 as compared to the shoots (1.9 µs/cm) and roots (3.7 µs/cm) of control, respectively. Similarly, a significant decrease of the cell membrane injury in the roots (1.37 µs/cm) and shoots (1.34 µs/cm) of NIAB-IR9 was also found compared to the roots (2.5 µs/cm) and shoots of (3.4 µs/cm) control, respectively. However, the remaining concentrations of both fertilizers significantly increased the injuries in the seedlings of both varieties Fig. 1a.

Total soluble sugars in both rice verities were observed at different concentrations in response to the application of two fertilizers Fig. 1b. In total, the quantity of sugar in shoots was greater than the roots across the treatments. For example, in the KSK-282 seedlings treated with zinc sulfate 150 mg/L, the shoots showed sugar contents of 11.2 mg/g (fw) while the roots showed only 3.2 mg/g (fw). Further, in comparison to control, the amount of sugar was significantly increased in both rice varieties in all treatments except Niab-IR9 treated with zinc sulfate 350 mg/L. Overall, total soluble sugar was significantly increased in Niab-IR9 (shoots: 16.8 mg/g and roots: 5.1 mg/g) treated with 150 mg/L of boric acid in comparison to control (shoots: 9.3 mg/g and roots: 3.5 mg/g).

The chlorophyll contents (*a* and *b*) of Niab-IR9 were observed higher than KSK-282. However, in general, both rice varieties have significantly higher chlorophyll contents in comparison to control. Individually in comparison to control, significantly highest chlorophyll *a* contents were found in Niab-IR9 (0.02 mg/g) treated with zinc sulfate 350 mg/L followed by Niab-IR9 (0.017 mg/g) treated with zinc sulfate 150 mg/L. Similar results were also found for chlorophyll *b* Fig. 1c.

Overall, the total carotenoid contents were observed higher in KSK-282 in comparison to Niab-IR9. In comparison to control (10.99 mg/g), KSK-282 seedlings being treated by boric acid 350 mg/L have the highest concentration of carotenoids (25.79 mg/g) followed by the carotenoids (21.68 mg/g) of KSK-282 being treated by zinc sulfate 350 mg/L. Moreover, there was no significant difference as compared to control was observed in the carotenoid contents of KSK-282 and Niab-IR9 being treated by zinc sulfate 150 mg/L Fig. 1d.

Genotoxicity Assessment

RAPD analysis was carried out on the genome extracted from rice leaves for the determination of genotoxic effects of chemical fertilizers. In total 12 random primers were used in the analysis, of which only five primers (OPC-13; OPM-14; OPA-10; OPA-11 and OPB-12) resulted in genomic DNA amplification. OPC-13 and OPM-14 showed polymorphism in fertilizers' induced rice varieties as compared to control. Moreover, OPC-13 showed more polymorphism (%) than OPM-14 in both rice varieties.

Table 1: The RAPD Primers Used for Analysis. In all cases, the annealing temperature was 36ºC/Sec

Serial #	Primer name	Sequence (5' to 3')
1	OPA-01	CAGGCCCTTC
2	OPA-02	TGCCGAGCTG
3	OPA-10	GTGATCGCAG
4	OPA-11	CAATCGCCGT
5	OPA-20	GTTGCGATCC
6	OPB-08	GTCCACACGG
7	OPB-12	CCTTGACGCA
8	OPB-18	CCACAGCAGT
9	OPC-05	CCACAGCAGT
10	OPC-07	CCACAGCAGT
11	OPC-13	AAGCCTCGTC
12	OPM-14	AGGGTCGTTC

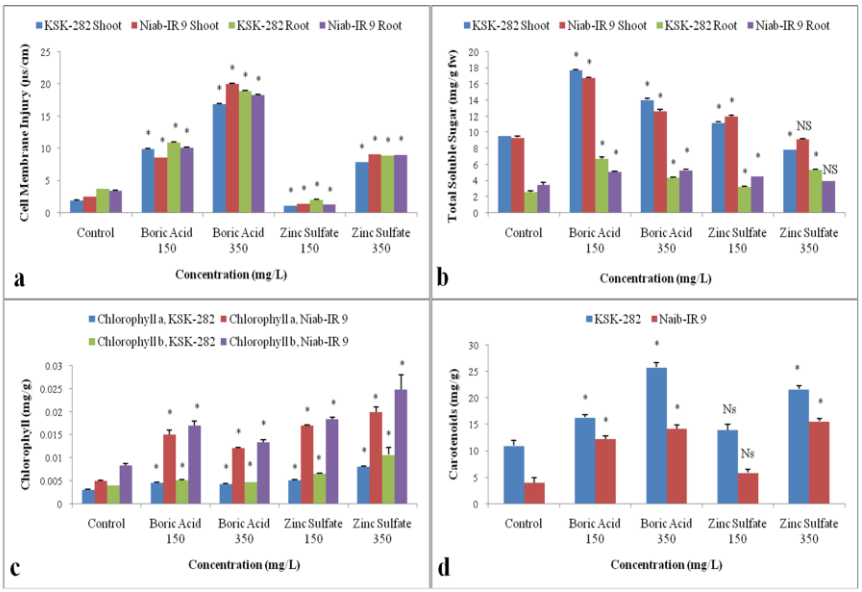

Fig. 1: Effects of boric acid and zinc sulfate (control, 150 and 350 mg/L) on KSK-282 and NAIB IR 9 varieties of rice. Each bar represents the mean value of three independent replicates and the error bars show standard error. An asterisk (*) shows the significant difference as compared to the control

By using OPC-13; 50% and 83.3% polymorphism were observed in KSK-282at 150 mg/L and 350 mg/L of boric acid, respectively. Similarly, in NIAB-IR9, 100%, and 60% polymorphisms were observed at 150 mg/L and 350 mg/L of boric acid, respectively. Although no polymorphism was noticed by using the same primers (OPC-13 and OPM-14) at 150 mg/L of zinc sulfate, yet it was observed at 350 mg/L (Table 2). Total DNA bands, the molecular sizes of disappeared bands, and newly appeared bands are shown in Table 3. The bands were 14 (control) and 57 (treatments) in KSK-282while 17 (control) and 65 (treatments) in NIAB-IR 9. OPA-10 and OPB-12 primers produced same RAPD profiles for both varieties of rice. The number of bands was higher in NIAB-IR9 as compared to KSK-282 genome by using RAPD primer OPA-11 Fig. 2. Furthermore, the RAPD profiles showed some genomic changes in seedlings treated with the selected fertilizers at 150 and 350 mg/L as compared to control, for OPC-13 Fig. 3a and b and OPM-14 Fig. 3c.

Discussion

Zinc sulfate and boric acid are commonly used fertilizers in rice cropping system across Pakistan. The present study reveals that zinc sulfate at 150 mg/L decreased cell membrane injuries in both rice varieties. Earlier research studies have proven that zinc is involved in the maintenance of biological membranes (Sadeghzadeh and Rengel, 2011; Mousavi *et al*., 2013). It might be due to the fact that it is an important part of Cu/Zn-superoxide dismutase (SOD), which detoxify the reactive oxygen species (ROS) (Jing *et al*., 2015). These ROS are very harmful to cell membrane lipids and SOD converts them into ordinary oxygen molecules. Thus, protects the cell membrane from injury (Cakmak, 2000; Apel and Hirt, 2004). However, the higher concentration of this fertilizer caused damage to rice by affecting its membrane. Thus, 150 mg/L might be the optimum or near to optimum concentration for the crop.

Table 2: Polymorphism (P) % of amplified RAPD primers

Serial #	Primer name	(P) % in KSK-2282				(P) % in NIAB-IR 9			
		Boric acid 150 mg/L	Boric acid 350 mg/L	Zinc sulfate 150 mg/L	Zinc sulfate 350 mg/L	Boric acid 150 mg/L	Boric acid 350 mg/L	Zinc sulfate 150 mg/L	Zinc sulfate 350 mg/L
1	OPA-10	0	0	0	0	0	0	0	0
2	OPA-11	0	0	0	0	0	0	0	0
3	OPB-12	0	0	0	0	0	0	0	0
4	OPC-13	50	83.3	0	66.6	100	60	0	33.3
5	OPM-14	33.3	50	0	50	33.3	20	0	33.3

Table 3: The number of bands in control and molecular sizes (base pair, bp) of disappearance (-) and/or appearance (+) of DNA bands for RAPD primers in the leaves of rice seedlings treated with boric acid and zinc sulfate

Primers	Rice variety	Control	Appearance/disappearance of bands	Boric acid 150 mg/L	Boric acid 350 mg/L	Zinc sulfate 150 mg/L	Zinc sulfate 350 mg/L
OPC-13	KSK-282	3	_	500	776	0	776
			+	0	325; 582; 673; 842	0	670
OPC-13	NIAB-IR 9	4	_	375; 470	740	0	375
			+	0	610; 910	0	0
OPM-14	KSK-282	4	_	650	540	0	290
			+	0	710	0	400
OPM-14	NIAB-IR 9	4	_	650	0	0	290
			+	0	350	0	0
OPA-10	KSK-282	3	_	0	0	0	0
			+	0	0	0	0
OPA-10	NIAB-IR 9	3	_	0	0	0	0
			+	0	0	0	0
OPA-11	KSK-282	3	_	0	0	0	0
			+	0	0	0	0
OPA-11	NIAB-IR 9	5	_	0	0	0	0
			+	0	0	0	0
OPB-12	KSK-282	1	_	0	0	0	0
			+	0	0	0	0
OPB-12	NIAB-IR 9	1	_	0	0	0	0
			+	0	0	0	0
Total	KSK-282	14		2 (-); 0 (+)	2 (-); 5 (+)	0 (-); 0 (+)	2 (-); 2 (+)
	NIAB-IR 9	17		3 (-); 0 (+)	1 (-); 3 (+)	0 (-); 0(+)	2 (-); 0 (+)

Unlike zinc sulfate in our study, boric acid damaged the cell membrane at both 150 mg/L and 350 mg/L concentrations. This might be due to the trans-membrane movement of boric acid and its ability to partition the lipid bilayer (Dordas and Brown, 2000).

Our study has proven that zinc sulfate and boric acid have increased the total soluble sugars in both rice varieties. The quantity of sugar in shoots was greater than the roots across all treatments of both rice varieties. Our findings are in line with the study of Verma and Dubey (2001), in which the application of cadmium has increased the amount of sugar more in shoots than in roots. Similarly in another study, the application of NaCl to wheat seedlings showed a higher level of sugar contents in the aerial parts as compared to roots (Kerepesi and Galiba, 2000). The present findings further showed that in comparison to control the amount of sugar was significantly increased in both rice varieties in all treatments except Niab-IR9 treated with zinc sulfate 350 mg/L. The accumulation of sugar in zinc and boron treating plants might be due the nature of both elements, in order to prepare the plant to tolerate the toxicity caused due to increased elemental concentration (Reda *et al.*, 2014). Additionally, another study showed that wheat plants accumulated sugars in their subcellular compartments to maintain growth under a stressful condition of boron toxicity (Masood *et al.*, 2016). Overall, the results demonstrate that total soluble sugar was found highest in both rice varieties treated with 150 mg/L of boric acid. Thus, boric acid might be the best fertilizer for increasing the sugar content of rice at this specific concentration.

The results presented herein demonstrate that the rice varieties treated with the tested fertilizers have significantly higher chlorophyll contents in comparison to control plants. Previous studies have shown that the application of boron and zinc significantly increases the chlorophyll content, growth and yield of rice crops. According to Patil *et al.* (2008), elements like zinc and boron are considered as essential micronutrients for improving the chlorophyll content of plants. This in turn enhances the photosynthetic and metabolic activities in the plant cell (Hatwar *et al.*, 2003). Individually, in comparison to control, significantly highest chlorophyll *a* contents were found in Niab-IR9 treated with zinc sulfate. Similar results were also found for chlorophyll *b*. The increase in chlorophyll content due to the

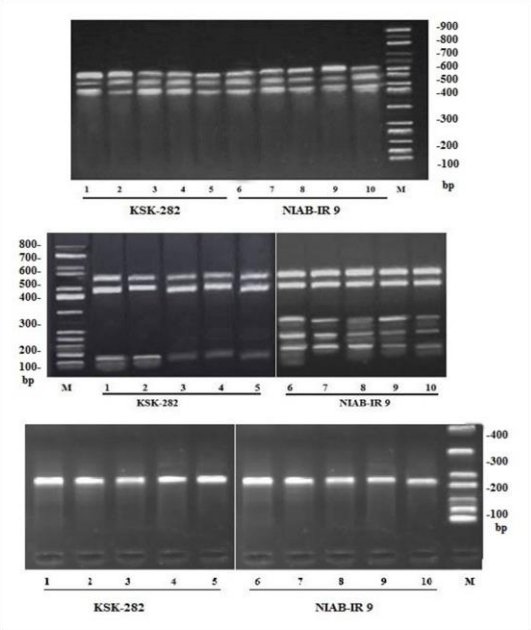

Fig. 2: RAPD profiles of genomic DNA from leaves of rice seedlings exposed to untreated control (1, 6), boric acid 150 mg/L (2, 7), boric acid 350 mg/L (3, 8), zinc sulfate 150 mg/L (4, 9),and zinc sulfate 350 mg/L (5, 10), using primers OPA-10 (a), OPA-11 (b, c) and OPB-12 (d, e).M: DNA molecular size marker 1000 bp (100 bp DNA ladder)

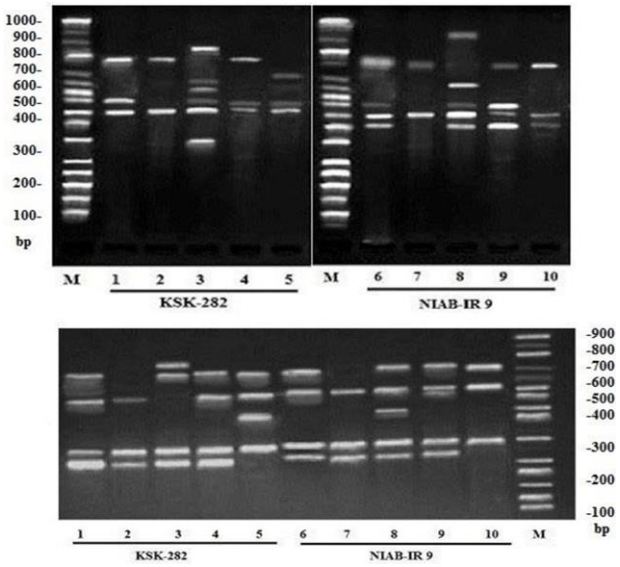

Fig. 3: RAPD profiles of genomic DNA from leaves of rice seedlings exposed to untreated control (1, 6), boric acid 150 mg/L (2, 7), boric acid 350 mg/L (3, 8), zinc sulfate 150 mg/L (4, 9) and zinc sulfate 350 mg/L (5, 10), using primers OPC-13 (a, b) and OPM-14 (c).M: DNA molecular size marker 1000 bp (100 bp DNA ladder)

zinc sulfate might be due to the fact that with a proper supply of zinc the formation of Cu/ Zn-superoxide dismutase enzyme (SOD) increases, which protects the photosynthetic pigments from ROS (Sharma *et al*., 2012).

Total carotenoids contents were observed higher in KSK-282 in comparison to Niab-IR9. In comparison to control, both fertilizers at 350 mg/L showed the highest concentration of the carotenoids in KSK-282 seedlings. Our results are in line with the findings of Kalefetoglu and Ekmekci (2009), Nahed *et al*. (2007) who reported a high level of carotenoids in their plants treated with zinc as compared to control. Similarly, according to Ilyas *et al*. (2015), foliar application of Zn, Cu, and B significantly increased the compound in citrus plants. As it has been investigated that plants with the high level of carotenoids are more tolerant to stress (Khan *et al*., 2016) therefore, plants accumulate more carotenoids for future adverse situations. Secondly, the increase might be due to the enhancement of secondary metabolites by the action of micronutrients (Shitole and Dhumal, 2012).

In the present case, the genotoxicity of chemical fertilizers with RAPD technique was monitored for the first time in Pakistan. It has been proved that this technique is mostly used to find out various types of damages and mutations in the plant's genome (Enan, 2007; Liu *et al*., 2007). In this study, RAPD profile was used for the observation of the modified genomic DNA of rice seedlings. The DNA changes including the disappearance of normal bands and appearance of new bands in rice seedlings due to the action of zinc sulfate and boric acid was compared to untreated control. RAPD primer OPC-13 and OPM-14 showed the disappearance and/ or appearance of bands in all treatments, except zinc sulfate at 150 mg/L. The loss of the band (disappearance) may be due to DNA strand breaks, modification of bases, oxidation of bases, point mutations and/or the complex rearrangement of chromosomes produced through the applied genotoxins (Atienzar *et al*., 2002; Wolf *et al*., 2004; Atienzar and Jha, 2006). The appearance of new bands in RAPD profiles may expose changes in the genome, which might be due to mutations, deletions, and/or homologous recombination (Atienzar *et al*., 1999). Our results support the previous findings (Cenkci *et al*., 2009), where zinc was found to be dose-dependent, similarly in the present study zinc sulfate at 150 mg/L showed no polymorphism in the DNA of the rice seedling, although at the higher dose the polymorphism was noticed.

Conclusion

Zinc sulfate and boric acid are good sources of zinc and boron elements, respectively, but the higher concentration are injurious for different metabolic activities of rice plants. The mechanism of action of the two elements was variable. Total soluble sugars, chlorophyll a and b, and total

carotenoids of fertilizers' induced rice varieties showed increased values in comparison to control treatments. However, both low and high concentrations of boric acid damaged the cell membrane. Zinc sulfate at lower concentration has decreased the cell membrane injury in comparison to control treatments of rice varieties. Similarly, genotoxic effects revealed the less toxic behavior of zinc sulfate as compared to boric acid, especially at 150 mg/L. Thus, it can be concluded that among all treatments only zinc sulfate at 150 mg/L was found to be a suitable fertilizer in comparison to boric acid. Detailed biochemical and molecular level studies are required for a deep insight of fertilizers' application onto the mechanism of action in rice varieties. Moreover, field experiments are also recommended to draw general conclusions on the use of these fertilizers in rice fields. Moreover, the effects of zinc and boron treated rice crops should also be tested for human health.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no RGP-262. Authors acknowledge Kohat University of Science and Technology (KUST) for financial, technical and intellectual support. This study is part of Roqaia Bibi's M.Phil research thesis.

References

Abbas, M., T.M. Zahida, R. Uddin, I. Sajjid, A. Akhlaq, K. Moheyuddin, J. Salahuddin, A.H. Mari and R.N. Panhwar, 2013. Effect of zinc and boron fertilizers application on some physicochemical attributes of five rice varieties grown in agro-ecosystem of Sindh, Pakistan. *Amer.-Eur. J. Agric. Environ. Sci*., 13: 433–439

Alloway, J.B., 2008. *Zinc in Soils and Crop Nutrition*. International Zinc Association and International Fertilizer Industry Association, Belgium, and France

Apel, K. and H. Hirt, 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol*., 55: 373–399

Atienzar, F.A. and A.N. Jha, 2006. The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mutat. Res*., 613: 76–102

Atienzar, F.A., P. Venier, A.N. Jha and M.H. Depledge, 2002. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. *Mutat. Res*., 521: 151–163

Atienzar, F.A., M. Conradi, A.J. Evenden, A.N. Jha and M.H. Depledge, 1999. Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* exposed to benzo[a] pyrene. *Environ. Toxicol. Chem*., 18: 2275–2282

Bhutto, M., Z.T. Maqsood, S. Arif, Riazuddin, S. Iqbal, Q. Mahmood, A. Akhlaq, R. Bhutto, K. Moheyuddin, A.H. Mari, R.N. Panhwar and J. Salahuddin, 2013. Effect of zinc and boron fertilizer application on the uptake of some micronutrients into the grain of rice varieties. *Amer.-Eur. J. Agric. Environ. Sci*., 13: 1034–1042

Brown, P.H., N. Bellaloui, M.A. Wimmer, E.S. Bassil, J. Ruiz, H. Hu, H. Pfeffer, F. Dannel and V. Römheld, 2002. Boron in plant biology. *Plant Biol*., 4: 203–223

Cakmak, I., 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification. *Plant Soil*, 3: 1–17

Cakmak, I., 2000. Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol.*, 146: 185–205

Cenkci, S., M. Yıldız, I.H. Cigerci, M. Konuk and A. Bozdag, 2009. Toxic chemicals-induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean *Phaseolus vulgaris* L. seedlings. *Chemistry*, 76: 900–906

Dey, P.M., 1990. Oligosaccharides. *In*: *Methods in plant biochemistry Carbohydrates*, pp: 189–218. Dey, P.M. (ed.). Academic Press, London

Dordas, C. and P.J. Brown, 2000. The permeability of boric acid across lipid bilayer and factors affecting it. *Membrane Biol.*, 175: 95

Doyle, J.J. and J.L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry*, 19: 11–15

Enan, M.R., 2007. Assessment of genotoxic activity of para-nitrophenol in the higher plant using arbitrarily primed-polymerase chain reaction AP-PCR. *Amer. J. Biochem. Biotechnol*., 3: 103–109

Hatwar, G.P., S.V. Gondane and S.M. Urkude, 2003. Effect of micronutrients on growth and yield of chilli. *Soil Crop*, 13: 123–125

Hussain, S., M.A. Maqsood, Z. Rengel and T. Aziz, 2012. Biofortification and estimated human bioavailability of zinc in wheat grains as influenced by methods of zinc application. *Plant Soil*, 4: 223–237

Ilyas, A., M.Y. Ashraf, M. Hussain, M. Ashraf, R. Ahmed and A. Kamal, 2015. Effect of micronutrients (Zn, Cu andBo) on photosynthetic and fruit yield attributes of citrus *Reticulate blanco* var. kinnow. *Pak. J. Bot*., 47: 1241–1247

Jamil, M., M. Ashraf, S. Rehman and E.S. Rha, 2009. Salinity and water stress. *In: Cell membrane stability (CMS): A simple technique to check salt stress alleviation through seed priming with GA3 in canola*, pp: 117–127. Springer, Netherlands

Jing, X., P. Hou, Y. Lu, S. Deng, N. Li, R. Zhao, J. Sun, Y. Wang, Y. Han, T. Lang, M. Ding, X. Shen and S. Chen, 2015. Overexpression of copper/zinc superoxide dismutase from mangrove *Kandelia candel* in tobacco enhances salinity tolerance by the reduction of reactive oxygen species in chloroplast. *Front. Plant Sci.*, 5: 23

Kalefetoglu, M.T. and Y. Ekmekci, 2009. Alterations in photochemical and physiological activities of chick pea (*Cicer arietinum* L.) cultivars under drought stress. *J. Agron. Crop Sci*., 195: 335–346

Kekec, G., M.S. Sakcali and I. Uzonur, 2010. Assessment of genotoxic effects of boron on wheat *Triticum aestivum* L. and bean *Phaseolus vulgaris* L. by using RAPD analysis. *Bull. Environ. Contam. Toxicol*., 84: 759–764

Kerepesi, I. and G. Galiba, 2000. Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat seedlings. *Crop Sci.*, 40: 482–487

Khan, R., S. Gul, M. Hamayun, M. Shah, A. Sayyed, Ismail, H.A. Begum and H. Gul, 2016. Effect of Foliar Application of Zinc and Manganese on Growth and Some Biochemical Constituents of *Brassica Junceae* Grown under Water Stress. *Amer. Eur. J. Agric. Envirn. Sci*., 16: 984–997

Lichtenthaler, H.K. and A.R. Wellburn, 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochem. Soc. Trans*., 11: 591–592

Liu, W., Y.S. Yang, Q. Zhou, L. Xiea, P. Lia and T. Sun, 2007. Impact assessment of cadmium contamination on rice *Oryza sativa* L. seedlings at molecular and population levels using multiple biomarkers. *Chemistry*, 67: 1155–1163

Masood, F., R. Anjum, M. Ahmad and A. Malik, 2012. Methods for genotoxicity testing of environmental pollutants *In: Environmental protection strategies for sustainable development strategies for sustainability*, pp: 229–260. Malik, A. and E. Grohmann, (Eds.).. Springer, Netherlands

Masood, S., I. Muhammad, J.C. Hassan and M.F.S. Munis, 2016. Subcellular Compartmentation of Sugars in Wheat Leaves under the Influence of Salinity and Boron Toxicity. *J. Plant Nutr*., 39: 2100–2105

Mousavi, S.R., M. Galavi and M. Razaei, 2013. Zinc (Zn) importance for crop production - A review. *Int. J. Agron. Plant Prod*., 4: 64–68

Nahed, G., A. Aziz, K. Balbaa and K. Laila, 2007. Influence of tyrosine and zinc on growth, flowering and chemical constituents of *Salvia farinacea* plants. *J. Appl. Sci. Res*., 3: 1479–1489

Parveen, R., Y. Jamil, M. Ashraf, Q. Ali, M. Iqbal and M.R. Ahmad, 2011. He-Ne laser-induced improvement in biochemical, physiological, growth and yield characteristics in sunflower *Helianthus annuus* L. *Photochem. Photobiol*., 87: 1453–1463

Patil, B.C., R.M. Hosamani and P.S. Ajjappalavara, 2008. Effect of Foliar application of micronutrients on growth and yield components of tomato *Lycopersicon esculentum* Mill. *Karnataka J. Agric. Sci*., 21: 428–430

Rashid, A., M. Yasin, M.A. Ali, Z. Ahmad and R. Ullah, 2008. Boron deficiency in rice in Pakistan. *In*: *A Serious Constraint to Productivity and Grain Quality*, pp: 213–219. Ashraf, M., M. Ozturk and H.R. Athar, (Eds.). Salinity and Water Stress. Springer, Germany

Reda, F., M.T. Abdelhamid and S.R. El-Lethy, 2014. The Role of Zn and B for Improving *Viciafaba* L. Tolerance to Salinity Stress. *Middle East J*., 3: 707–714

Sadeghzadeh, B. and Z. Rengel, 2011. Zinc in soils and crop nutrition. *In*: *The Molecular Basis of Nutrient Use Efficiency in Crops*, pp: 335–376. Hawkesford, M.J. and P. Barraclough, (Eds.). Wiley-Blackwell, Oxford, UK

Shahrtash, M., S. Mohsenzadeh and H. Mohabatkar, 2010. Cadmium-induced genotoxicity detected by the random amplification of polymorphism DNA in the maize seedling roots. *J. Cell Mol. Res*., 2: 42–48

Sharma, P., A.B. Jha, R.S. Dubey and M. Pessarakli, 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot*., 2012: 1–26

Shitole, S.M. and K.N. Dhumal, 2012. Influence of foliar applications of micronutrients on photosynthetic pigments and organic constituents of medicinal plant *Cassia angustifolia* Vahl. *Ann. Biol. Res*., 3: 520–526

SPSS, Inc., 2007. *SPSS Version 16.0 for Windows*. SPSS, Chicago, Illinois, USA

Tariq, M. and C.J.B. Mott, 2007. The significance of boron in plant nutrition and environment-A review. *J. Agron*., 6: 1–10

Verma, S. and R.S. Dubey, 2001. Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. *Biol. Plant*., 44: 117–123

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acid Res*., 18: 6531–6535

Wolf, H.D., R. Blust and T. Backeljau, 2004. The use of RAPD in ecotoxicology. *Mutat. Res*., 566: 249–262

Zheng, J.S., 2003. Lab. PCR technique and its practical methods. *Mol. Plant Breed*., 1: 381–394

Zia, H., Y. Jamil and S. Irum, 2012. Enhancement in the germination, seedling growth and yield of radish (*Raphanus sativus*) using seed pre-sowing magnetic field treatment. *Pol. J. Environ. Stud*., 21: 369–374

(Received 11 February 2017; Accepted 24 February 2018)