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Comparative biochemical and RAPD analysis in two varieties of rice (*Oryza sativa*) under arsenic stress by using various biomarkers

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ABSTRACT

Multiple biomarker systems have been frequently used to measure the genotoxic effects of environmental pollutants (including heavy metals) on living organisms. In this study, we used leaves of hydroponically grown 14 days old seedlings of rice (*Oryza sativa*) varieties (PB1 and IR64) treated with 50, 150 and 300 μM arsenite (As(III)) for 24 and 96 h duration. Reduction in seed germination, root–shoot length, chlorophyll and protein were observed with increasing As(III) concentration and duration in both varieties, being more in IR64. Increase/decrease of antioxidant enzymes and stress related parameters showed much changes at higher concentration for 24 and 96 h duration in both varieties. Eleven primers were found in RAPD analysis to produce polymorphic band pattern and produced a total of 51 (control), 79 (treated) and 42 (control) and 29 (treated) bands in PB1 and IR64 varieties, respectively. These results indicated that genomic template stability (GTS, changes in RAPD profile) was significantly affected at all tested As(III) concentration, when compared with other parameters. Differential response was observed in both varieties with PB1 being more tolerant. We concluded that DNA polymorphism detected by RAPD analysis in conjunction with other biochemical parameters could be a powerful eco-toxicological tool in bio-monitoring arsenic pollution.

1. Introduction

Heavy metal arsenic (As) is bioactive and potentially toxic to plants, animals and humans. It is a non-essential metal and the exploitation of As-containing groundwater in large areas of Asia causes problem for millions of people via consumption of As-contaminated drinking water and food [1]. Arsenic is present in the environment in both organic and inorganic form. Inorganic arsenate (V) and arsenite (III) are the main soluble As species found both in soil and water. As(V) predominates under aerobic conditions and considered as an analogue of phosphate, enters the plant through phosphate transporters and acts as an uncoupler of oxidative phosphorylation. On the other hand As(III) is the dominant form under anaerobic conditions, which mainly reacts with –SH groups and is an effective inhibitor of enzymes requiring free sulfhydryl group [1,2]. Arsenic exposure interrupts several physiological and biochemical processes in plants [1]. Heavy metal induced DNA damage lies in the fact that overproduction of ROS could induce genotoxicity events [3]. Oxidative DNA damages can produce a multiplicity of modifications in DNA including base and sugar lesions, strand breaks, DNA–protein crosslinks and base-free sites [4]. Beside direct oxidation, DNA bases may get indirectly damaged through reaction with reactive products generated by ROS attack to lipids. Lipid peroxidation-induced aldehydes are mutagenic molecules and create MDA–guanines adducts [4,5]. There is a significant evidence that inorganic As exposure results in the generation of reactive oxygen species (ROS) through the conversion of arsenate to arsenite, which leads to the synthesis of enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione, etc. [6,7]. ROS generated as a result of oxidative stress has been widely implicated in damage to cell membrane and DNA [8].

Developing an understanding of the mechanisms of heavy metal tolerance in plants at a biochemical and molecular level is the focus of today's ongoing research efforts. Toxicant induced population genetic effects may arise from the direct action of the toxicant at the DNA level (mutagenic effects) [9] or may indirectly result from population mediated process that are related to the toxicant exposure [10]. Initially protein markers (i.e. allozymes) were used to infer the population genetic effects of toxicant exposure [11], but currently a wide variety of DNA markers/techniques are available. These techniques can be applied to infer all routes through which toxicants may affect the genetic structure of exposed plants/organisms. Mutagenic activity of chemicals has already been analyzed for different plants *Allium cepa* [12], *H. vulgare* [13], *Glycine max* [14] and *Vicia faba* [15].

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The recent advances in molecular biology have led to the development of several PCR-based techniques, which can be used for DNA analysis in the field of genotoxicology [16]. The random amplified polymorphic DNA (RAPD) method is a PCR-based technique that amplifies random DNA fragments with the use of single short primers of arbitrary nucleotide sequence under low annealing conditions. This technique is a simple, fast and capable of detecting not only point mutations but also temporary alteration of DNA that may not finally manifest themselves as mutation in future and allow detection of low doses of pollutants. Until now it has been widely used in genotoxicity studies to evaluate, detect and identify changes in DNA, caused due to environmental stressors [9,17,18]. The changes in RAPD profile can be evaluated as alteration in genomic stability test (GTS, a qualitative measure of genotoxic effect) [11]. The changes in parameters such as root–shoot length, chlorophyll and protein contents support GTS [16–18].

While As contamination in drinking water has attracted much attention, plant-based foods are also an important source of As. Excessive accumulation of As, particularly inorganic As in rice (*Oryza sativa*) poses a potential health risk to populations with high rice consumption. Previous studies showed that rice is much more efficient in As accumulation than other cereals such as wheat and barley [19]. Since rice is a staple food for many countries, therefore, biomarkers are necessary for detection of high concentration of heavy metals in this crop, particularly where it is growing and to keep human health free from hazardous material.

The present study was designed to see arsenite (As(III)) induced changes in two varieties of rice (PB1 and IR64) using RAPD assay. Changes in RAPD profiles were compared to population (root–shoot length), physiological (chlorophyll, protein) and biochemical parameters (SOD, CAT, Proline and MDA content). Results obtained may suggest that molecular, physiological and biochemical assays could be used together as reliable and powerful biomarkers to determine genotoxic effects of heavy metals in ecotoxicology.

2. Materials and methods

2.1. Plant material and treatment conditions

Seeds of two varieties of rice (viz. IR64 and PB1) were obtained from IARI, Pusa, New Delhi, India. Seeds were surface sterilized in 3% H_2O_2 and washed with distilled water prior to germination on a moist cotton bed and watered with 10% Hoagland nutrient medium with and without arsenic (As(III); prepared using salt $NaAsO_2$) and kept in dark for 2 days. The seedlings were transferred to light (a 16 h photoperiod) with a day/night temperature of 25 °C ± 2 °C. Fourteen days old plants were treated with different concentrations of arsenic (50, 150 and 300 μM) for 24 and 96 h duration. After harvesting each plant was separated into leaf and roots, washed thoroughly with distilled water, frozen in liquid nitrogen and kept in −80 °C for biochemical and molecular analysis. Plants treated without metals served as controls. Each experiment was carried out in triplicates and each replicate contained equal number of seedlings of equal size.

2.2. Isolation and quantification of total chlorophyll and total protein

Total chlorophyll was isolated according to Arnon [20]. Total protein was estimated following Bradford [21] method, with bovine serum albumin (BSA) as a standard.

Table 1
Sequences of 11 primers used in the study.

S. no.	Primer name	Sequence (5′-3′)
1	OPG-08	TCACGTCCAC
2	OPG-09	CTGACGTCAC
3	OPG-10	AGGGCCGTCT
4	OPG-11	TGCCCGTCGT
5	OPG-12	CAGCTCACGA
6	OPG-14	GGATGAGACC
7	OPG-15	ACTGGGACTC
8	OPG-16	AGCGTCCTCC
9	OPG-17	ACGACCGACA
10	OPG-18	GGCTCATGTG
11	OPG-19	GTCAGGGCAA

2.3. Enzymatic cellular antioxidants

Frozen plant leaves (0.4–0.8 g) were homogenized in ice cold extraction buffer (pH 7.5) containing 50 mM HEPES, 0.4 mM EDTA, 5 mM $MgCl_2$, 10% glycerol, 1% PVP, 2 mM DTT and 1 mM phenyl methyl sulfonyl fluoride. The homogenate was centrifuged (14,000 × *g*) at 4 °C for 20 min. The supernatant was used for enzyme activity. Superoxide dismutase activity (SOD, EC 1.15.1.1) measured according to Dhindsa et al. [22]. Catalase activity (CAT, EC 1.11.1.6) determined by monitoring the disappearance of H_2O_2 by the method of Aebi [23]. Activity of all the enzymes is expressed in percent of control.

2.4. Stress related parameters

Malondialdehyde (MDA) content was estimated following Heath and Packer [24] by reaction with thiobarbituric acid (TBA). The amount of MDA was calculated from the difference in absorbance at 532 and 600 nm using an extinction coefficient of 155 mM^{-1} cm^{-1}. Level of proline was measured following Bates [25]. Plant leaves (0.5 g) were crushed in 3% sulfosalicylic acid and centrifuged at 4000 × *g* for 10 min. To 2 ml of supernatant, 2 ml of ninhydrin was added with 2 ml acetic acid and incubated at boiling temperature for 1 h. The mixture was extracted with toluene, and proline was quantified spectrophotometrically at 520 nm from the organic phase.

2.5. DNA extraction, and RAPD procedure

Genomic DNA isolation from arsenite treated leaves and RAPD procedure were according to Gupta and Sarin [9]. Twenty decamers oligos from Kit G and Ten from Kit B (Operon Technologies, Alameda, USA) were used as primers (Table 1). Data (fragment sizes of all the amplification products, estimated from the gel by comparison with marker) were scored as presence and absence of bands. Polymorphism observed in profiles included disappearance or appearance of bands in comparison to controls.

2.6. Estimation of genomic template stability (GTS)

Changes in the RAPD profiles were expressed as GTS, a qualitative measure showing the obvious changes in the number of RAPD profiles. The GTS was calculated by the formula:

$$\mathrm{GTS} = \left(1 - \frac{a}{n}\right) \times 100$$

where *a* is the average number of changes in DNA profiles and *n* is the number of bands selected in control DNA profiles. Polymorphism in RAPD profiles included disappearance of a normal band and appearance of a new PCR band in comparison to control RAPD profiles. The average was calculated for each test group exposed to a different As treatments. In order to compare the sensitivity of

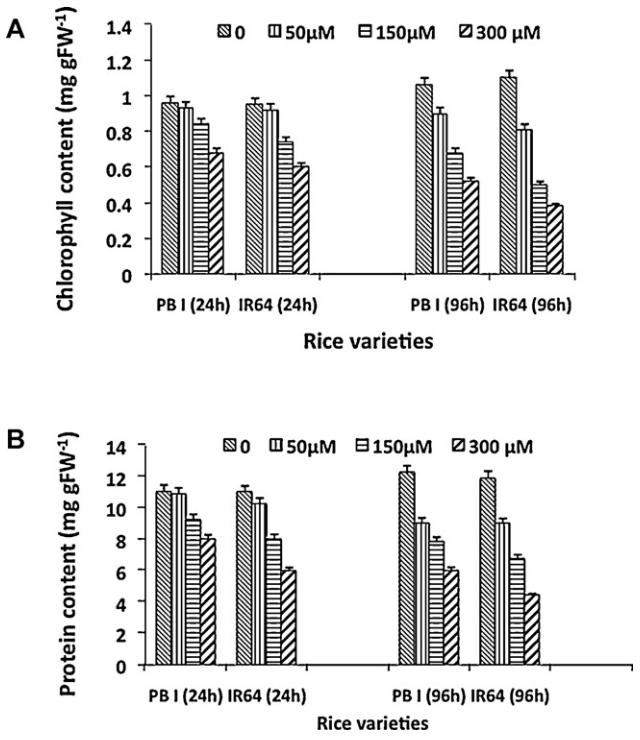

Fig. 1. Effect of increasing As(III) concentration on chlorophyll (A) and protein (B) content in leaves of 14 days old seedlings of PB1 and IR64 rice varieties after 24 and 96 h duration. The error bars indicate ± SE (n = 3).

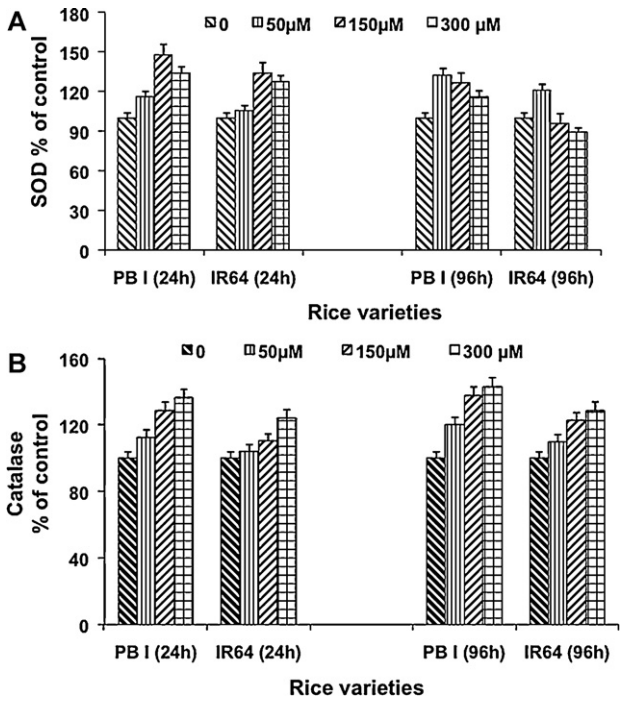

Fig. 2. Effect on the activities of SOD (A) and CAT (B) in leaves of 14 days old seedlings of PB1 and IR64 rice varieties at the given As(III) concentrations after 24 and 96 h duration. The error bars indicate ± SE (n = 3).

each parameter (chlorophyll, protein, SOD, CAT, MDA, proline and GTS), changes in these values were calculated as a percentage of their control value (set to 100%).

3. Results

3.1. Effect of As(III) on seed germination, root–shoot length, chlorophyll and protein content

Percentage seed germination and root–shoot length (cm) in both the varieties of rice (PB1 and IR64) are shown in Table 2 after 7 day of As(III) exposure. Germination of seeds inhibited as the concentration increases in both the varieties, however, inhibition was more in IR64.

Root and shoot length were also substantially decreased with increasing As(III) concentration after 7 day of exposure, as compared to their control. The inhibition rates of the metal ion (50, 150 and 300 μM) on root and shoot length of PB1 and IR64 increased with increasing concentration (Table 2).

Changes in chlorophyll and protein content in the leaves of both rice varieties are presented in Fig. 1A and B. When 14 days old rice seedlings were treated with different As(III) concentration for 24 and 96 h, both chlorophyll and protein content significantly decreased at higher concentration (300 μM) and duration (96 h), however, at lower concentration (50 μM) not much changes were observed. Further, inhibition in protein content was more in IR64 as compared to PB1 variety.

3.2. As(III) induced changes in SOD, CAT, MDA and proline content

Comparing the activities of two enzymatic antioxidants SOD and CAT (Fig. 2A and B), it is evident that As(III) induced a strong antioxidative response in the leaves of both varieties. The trend shown by the plants was increase of enzyme level at all the concentration and duration. However, at 300 μM for 96 h, there was a decrease in SOD activity as compared to the control in IR64 variety. Superoxide dismutase plays an important role in antioxidative defense system. The effect of As(III) on SOD activity is shown in Fig. 2A. The increase in SOD activity of PB1 and IR64 variety was 115.76% and 105.43% compared to control at 50 μM after 24 h, respectively. However, SOD activity showed 116.26% in PB1 and 89.06% in IR64 variety at 300 μM after 96 h exposure. Comparing the activities of both the varieties PB1 always maintained higher SOD activity than IR64.

Catalase is H_2O_2 capturing enzymes. Leaves of both the varieties showed the differential response in CAT activity with increasing concentration by 136.19% and 124.45% of control, in PB1 and IR64 variety respectively at 300 μM after 24 h exposure, though, the increase was maximum 148.18% in PB1 at all concentrations after 96 h duration (Fig. 2B). Furthermore, activity of CAT was relatively higher in PB1 variety as compared to IR64.

The results so far indicated that PB1 variety is more tolerant to As(III) stress as compared to IR64. In order to examine further tolerance, we investigated proline and malondialdehyde content as stress related parameter. A major effect of heavy metal toxicity is the chemical modification of membrane lipids triggered by the oxidative stress. We investigated a biomarker indicative to lipid peroxidation (LPO) status of the cells and measured MDA content that is the major product of LPO (Fig. 3A). At 96 h, the MDA content increased 152.31%, 219.43% and 141.00% in leaves treated with 50, 150 and 300 μM, respectively in IR64 variety. Furthermore, MDA content was less in PB1 variety as compared to IR64 variety, maximum being at 150 μM after 96 h (199.33%).

Fig. 3B depicts the effect of As(III) on proline content in both the varieties. Proline levels increased in both varieties as compared to their control. PB1 showed 270.44% and IR64 170.00% increase, respectively at 150 μM after 96 h exposure. IR64 maintained low level of proline content as compared to PB1 variety.

Table 2
Effect of different concentrations of As(III) on percentage seed germination, shoot and root length in PB1 and IR64 rice varieties after 7 days of treatment ($n = 3$).

AsIII conc.	PB1			IR64		
	Seed germination (%)	Shoot length (cm)	Root length (cm)	Seed germination (%)	Shoot length (cm)	Root length (cm)
0	100	1.40	1.56	100	1.09	1.19
50 μM	92	1.17	0.88	85	0.78	0.59
150 μM	86	0.84	0.41	59	0.63	0.37
300 μM	52	0.54	0.32	26	0.18	0.12

3.3. Effect of As(III) on RAPD profile

To see the genetic effect of As(III) contamination RAPD analysis was performed on DNA extracted from leaves of both the varieties at 150 and 300 μM As(III) for 24 and 96 h duration. Twenty 10-mer oligonucleotide primers were screened for RAPD analysis and only eleven gave clear and reproducible bands. Table 3 and Fig. 4 present a summary of all RAPD profile modifications and products of selected eleven primers. Total number of bands produced in control plants of PB1 and IR64 was 51 and 42, respectively. RAPD profiles in treated plants of PB1 and IR64 showed substantial differences between control and treated plants. Apparent changes were observed in both varieties, like appearances of some new bands or disappearance of bands as compared to their control. Total number of bands were more in PB1 variety (51 in control, 79 in treated) as compared to IR64 (42 in control, 29 in treated) (Fig. 4). The number of disappearing RAPD bands for primers 10, 12, 16 18, 19 were more in 300 μM As(III) treated leaves in both varieties as compared to their control after 96 h exposure, maximum being in PB1 variety. On the other hand, 26 and 5 extra bands appeared at 150 and 300 μM in PB1 and IR64 variety, respectively after 24 and 96 h duration with all 11 primers. Polymorphisms were due to the disappearance or appearance of amplified bands in the treated profiles in comparison to control profiles.

3.4. Comparison of RAPD profiles (GTS, %), root–shoot growth, chlorophyll, protein, SOD, and MDA content

The genomic template stability (GTS, %) values, a qualitative measure shows changes in RAPD profile was calculated for each 11 primers in both the varieties and presented in Table 3. GTS values decreased with increasing concentration and duration. However, value of GTS was more in PB1 as compared to IR64 variety as compared to their control. To compare the sensitivity of RAPD profiles, root–shoot growth, chlorophyll, protein, SOD and MDA content were calculated as a percentage of their control value (set to 100%, Table 4) at 150 and 300 μM after 96 h exposure. The results showed that root–shoot growth, chlorophyll, protein and GTS were reduced with increasing As(III) concentration. However, SOD and MDA level increased after the exposure of As(III) which are known as stress related markers. This effect may be due to multiple changes in RAPD profile which tend to counter balance each other through the appearance/disappearance of bands.

4. Discussion

Heavy metal toxicity causes various damages in growth and developmental process. Analysis of plant height, chlorophyll, protein, lipid peroxidation and antioxidative enzymes that give

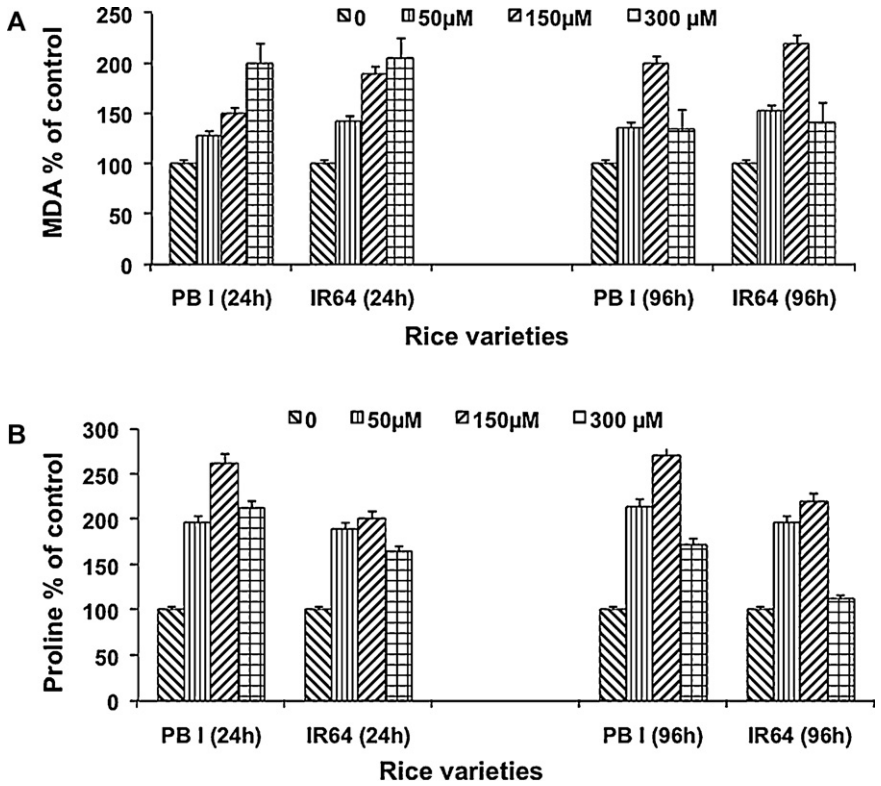

Fig. 3. Effect of different As(III) concentration on MDA (A) and proline (B) content in leaves of 14 days old seedlings of PB1 and IR64 rice varieties after 24 and 96 h duration. The error bars indicate ± SE ($n = 3$).

Table 3
Changes of total bands and genomic template stability (GTS, %) in control and As(III) treated leaves of PB1 and IR64 rice varieties for 24 and 96 h duration. Genomic template stability for each primer was calculated by the formula GTS (%) = $(1 - a/n) \times 100$, where a is the average number of changes in DNA profiles and *n* the number of bands selected in control DNA profiles.

Primers	Control	PB1				Control	IR64			
		150 μM a/b		300 μM a/b			150 μM a/b		300 μM a/b	
		24 h	96 h	24 h	96 h		24 h	96 h	24 h	96 h
OPG-08	3	0/0 (100)	1/0(66)	0/0(100)	1/0(66)	2	0/0(100)	0/1(50)	0/0(100)	0/0(100)
OPG-09	4	2/0 (50)	1/1(50)	1/2(25)	1/1(50)	4	0/0(100)	0/1(75)	0/0(100)	0/0(100)
OPG-10	5	0/0 (100)	0/3(40)	0/0(100)	0/3(40)	4	1/0(75)	0/2(75)	2/0(50)	¼(25)
OPG-11	3	1/0 (66)	0/2(33)	0/1 (66)	0/2(33)	3	0/0(100)	0/0(100)	0/0(100)	0/0(100)
OPG-12	5	0/1 (80)	0/3(40)	0/0(100)	0/3(40)	4	0/0(100)	0/0(100)	0/0(100)	1/1(50)
OPG-14	6	0/0 (100)	½(50)	0/1(83)	1/2(50)	5	0/0(100)	0/1(80)	0/1(80)	0/1(80)
OPG-15	5	0/0 (100)	1/3(20)	0/0(100)	1/2(40)	3	0/0(100)	0/0(100)	0/1(66)	0/1(66)
OPG-16	7	1/0 (85)	0/3((57)	1/1(71)	0/3(57)	6	0/1(83)	0/1(83)	0/1(83)	0/1(83)
OPG-17	4	3/0 (25)	3/1(25)	3/0(25)	3/1(25)	4	0/0(100)	1/0(75)	0/0(100)	1/0(75)
OPG-18	4	0/1 (75)	0/3(25)	0/1(75)	0/3(25)	3	0/1(66)	0/1(66)	0/1(66)	0/1(66)
OPG-19	5	0/0 (100)	0/1(80)	0/0(100)	0/3(40)	4	0/0(100)	0/0(100)	0/0(100)	0/0(100)
Total average (GTS)	51 (100)	9 (80)	29 (44.1)	11 (76.8)	30 (42.3)	42 (100)	3 (93.1)	8 (82.1)	6 (85.9)	12 (76.8)

a, appearance of each band; b, disappearance of normal bands.
GTS(%) for each primer is given in parantheses.

information at the population level were presented in many studies but only a few studies are reported on DNA marker techniques [9,17]. The measure of parameters at the population level helps to interpret the data at molecular level [26]. Most of the work regarding DNA damage under heavy metal stress was focused on chromosome aberration, comet assay and micronucleus assay [3,27] but they analyze only total nuclear DNA. PCR-based molecular markers such as RAPD and AFLP are more sensitive as they give evidence about DNA mutation. Among all the marker techniques RAPD profiles are widely used to determine the level of genotoxicity in organisms and can be useful for preliminary assessment of toxic effects to populations [9,10,16–18]. Although, regarding reliability and reproducibility of RAPD technique, questions has been raised, but after proper optimization it is a reliable, sensitive and reproducible assay to detect DNA damage or mutations in a short period of time with relatively low cost and can be applied to genotoxicity and carcinogenesis studies [28].

We here report the effect of As(III) in two varieties of rice and its comparison using various parameters. The effect of As(III) was dependent on its concentration and duration. In both the varieties (PB1 and IR64), effect on growth parameters like seed germination and root–shoot length decreased with increasing As(III) concentration. Similar effect on growth parameters was observed with increasing arsenic concentration in wheat seedlings [29].

Our results indicate that As exposure had greater effect on chlorophyll content. IR64 variety showed more decrease in cholorophyll content at higher concentrations and duration. Earlier report by Seth et al. [30] showed that arsenic species can have a hormesis effect on photosynthetic pigments, which is a stimulatory effect induced by low doses of toxic substances. The decrease in chlorophyll content may also be due to the peroxidative breakdown of pigments and chloroplast membrane lipids by ROS [31], impaired uptake of nutrients [32] or chlorophyll degradation through an increase in chlorophyllase activity [33]. Protein contents also showed decrease at higher concentrations and duration in both the varieties may be due to the adverse effects of ROS which is very much in line with the earlier findings [30]. IR64 variety which is considered as sensitive variety for As(III), showed less protein content as compared to PB1 .

Antioxidant enzymes (SOD, CAT) are considered to be an important defense system of plants against oxidative stress caused by metals. SOD plays an important role in dismutation of superoxide anion converting to H_2O_2 which acts as substrate for catalase. A bell-shaped concentration–response trend was observed, where the activity of SOD increased in both varieties at all the tested concentration and duration as compared to their control, however, it decreased during a prolonged exposure (300 μM, 96 h) and it was more in sensitive variety (IR64) as compared to tolerant variety (PB1). A similar case was observed in a previous study of the antioxidant responses to arsenic exposure in plants [34]. Higher SOD activity in tolerant varieties indicated higher O^{2-} scavenging activity under stress and lesser amount of activity in sensitive variety is probably due to the enhanced level of H_2O_2 produced in different cellular compartments [7]. The accumulation of H_2O_2 is prevented in the cell by CAT, which increased at all the given concentration and duration, although IR64 variety showed lesser amount of CAT as compared to PB1. The function of CAT is to degrade H_2O_2, which is a potential source of highly reactive hydroxide radical and singlet oxygen. This singlet ion can initiate lipid and organic peroxidation. Thus, greater CAT activity generally indicates greater stress for a given plant. Similar studies have been reported for other plants under heavy metal stress [7,35]. Further, increase/decrease of CAT activity could be due to an increase in the amount of CAT substrate or inactivation by peroxisomal protease [36].

The level of MDA in the tissue is considered a measure of lipid peroxidation status which determines the oxidative stress level of the plant cell. Lipid peroxidation is linked to the production of free chain radical reaction that causes degeneration of cell membranes. Effect of As(III) on plant cell membrane leads to the inhibition of

Table 4
Comparison of chlorophyll, protein, SOD, CAT, MDA, proline and RAPD profile (GTS) in PB1 and IR64 rice varieties exposed to 150 and 300 μM As(III) concentration for 96 h duration. The control was accepted as 100%.

AsIII μM	Chlorophyll		Protein		SOD		CAT		MDA		Proline		RAPD Profile (GTS)	
	PB1	IR64	PB1	IR64	PB1	IR64	PB1	IR64	PB1	IR64	PB1	IR64	PB1	IR64
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
150	64.15	45.4	64.1	49.0	126.3	95.5	137.6	122.6	199.3	210.2	270.4	220	44.1	82.1
300	49.0	34.5	56.8	36.8	116.2	89.06	143	128.8	134	141	172.2	112	42.3	76.8

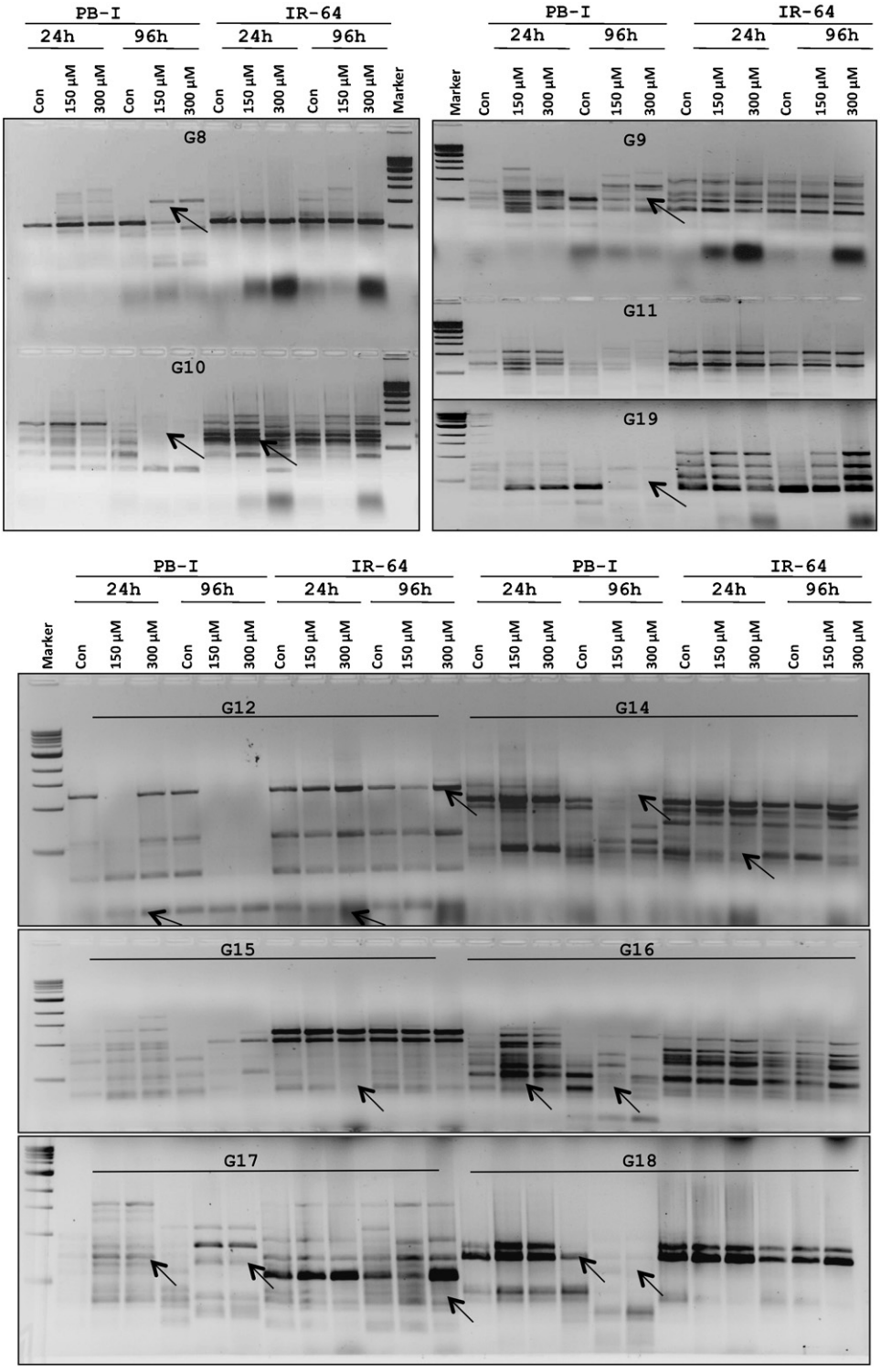

Fig. 4. RAPD profiles from leaves of PB1 and IR64 rice varieties exposed to 150 and 300 μM As(III) concentration for 24 and 96 h duration. M – marker (100 base pair ladder); Con – control.

cellular function and death [37]. The oxidative stress produced by arsenic is evident from an enhancement in lipid peroxidation, which may damage chloroplasts, decrease plant biomass, inhibit chlorophyll synthesis, and protein level [32]. MDA levels were found to be increasing in both the varieties studied. Although, it was less in PB1 (tolerant) variety as compared to IR64 (sensitive) variety. Similar report has been showed in pea genotypes under Cd stress indicating that the tolerant genotype had a high ability to cope up with metal stress or arsenic species may modify the activities of the enzyme lipoxygenase [38,39], which could result in altered membrane permeability and, consequently, increased ion leakage. Similarly, increase was observed in proline content in both varieties, being more in PB1 variety. Proline is known to function as radical scavenger and cellular redox potential buffer [8]. Hence, the capacity of proline to quench ROS was available to a lower extent for IR64 (sensitive) variety, whereas, in PB1 (tolerant) variety,

proline accumulation would have acted as a supporting molecule to assist the enzyme mediated dismutation of reactive oxygen species.

Changes in RAPD patterns under As(III) stress in both varieties comprised a loss and/gain of bands as compared to their control RAPD pattern. Previous studies under heavy metal stress have shown that metals can induce a range of DNA damage such as single- and double strand breaks, modified bases, point and deletion mutations and DNA–protein cross links. The disappearance of normal RAPD bands may be related to the events such as DNA damage, point mutations or complex chromosomal rearrangements induced by genotoxins [11,40]. Presence of new RAPD products reveal a change in some oligonucleotide priming sites due to mutations (new annealing events), large deletions (bringing to pre-existing annealing site closer), and/or homologous recombination (just exposing two sequences that match the sequences of primer) [11].

Our results indicate, that the frequency of band loss was increased with increasing concentration and duration in both the varieties. Both loss and gain of RAPD bands were more in PB1, the tolerant variety of the two. The changes observed in RAPD profiles could be regarded as modifications in genomic template stability (GTS) and this could be directly compared to the alterations in physiological and biochemical parameters [17,18]. The changes in all parameters (except for stress related parameters) there was an inverse relationship with As(III) concentration. An apparent inhibition in chlorophyll and protein content correlated well with the changes in GTS (RAPD profiles), which was more apparent in IR64, although, GTS was less in this variety as compared to PB1 (tolerant). GTS is related to the level of DNA damage, the efficiency of DNA repair and replication. The reason for high change in GTS in tolerant variety may be due to the efficiency of molecular machinery to adapt itself in response to As(III) stress is higher which resulted in more changes in the DNA profile. Though this needs to be investigated further and to be correlated with grain yield.

The present study showed the inhibition in biochemical parameters along with the increase in enzyme activity, which are earlier, reported as an indicator of metal stress. Little is known about the marker based selection of crops under arsenic stress, mainly through the use of molecular markers. This is the first report on As-induced RAPD profiling in rice (major crop suffering As stress) along with other biochemical markers. Our results suggest that molecular, physiological and biochemical assay could be used together as reliable and powerful biomarkers to detect genotoxic effect of heavy metals in different varieties of plants, which will help for accumulating more information to understand the effect of contaminants on organism in ecotoxicology.

Acknowledgments

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