Journal of Agroecology and Natural Resource Management
Print ISSN: 2394-0786, Online ISSN: 2394-0794, Volume 2, Number 3; April-June 2015 pp. 203-204

http://www.krishisanskriti.org/janrm.html

Silicon Mediates Genotoxic Alterations in *Brassica juncea* Roots under Arsenic Stress

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Abstract—*Arsenic (As), a heavy metal is toxic to both plants and animals and severe contamination of As occurs in crop plants mainly rice which is an essential diet for millions of people. In this study, roots of hydroponically grown 14 days old seedling of Brassica juncea (var. Varuna) were used to observe the genotoxic effect of As (150 µM) in presence of Silicon (Si)- (1.5 mM), after 96 hours of exposure of these metals either single or in combination and at the same time those without metals serve as control. RAPD (Random Amplification of Polymorphic DNA), a molecular marker assay is used to study the alteration in DNA profile under As stress and these altered DNA profile become visible in the form of appearance of new bands and loss of existing bands as compared to control. For the RAPD analysis, 20 primers were used and out of which only 7 primers produce polymorphic bands. In roots of B.juncea, a total number of 25 and 52 polymorphic bands present in control and treated seedlings respectively and intensity of bands was more in the range of 500 bp to 1.5 KB. Total number of bands were 23 and 15 in As and As+Si respectively. Genomic template stability (%GTS, changes in RAPD profile) and polymorphism ratio of the primers was 8%, 92% respectively under As stress whereas 40 %, 60% respectively in As+Si. These results indicated that RAPD assay could be used as an ecotoxicological tool for the detection of heavy metal contamination at molecular level and supplementation of Si along with As mitigate the genotoxic effect of As.*

1. INTRODUCTION

Heavy metals contaminations have negative effect on human health as well as on plants and are considered major environmental problems all over the world. Arsenic (As), an important environmental contaminant raise a potential health risk to people consuming excess ofAs contaminated rice [1]. As toxicity cause changes at morphological, physiological and molecular level in plants including reduction in shoot and root length, chlorosis in leaves and necrosis in aerial plant parts. Toxicity of As is mainly due to oxidative stress induced by it. Silicon (Si), second most abundant element in soils taken up via the same pathway as As due to physiochemical similarities between them. Addition of Si fertilizer to the soil may be an effective way to reduce the accumulation of As in rice grown in soil contaminated with As. It has been reported that external Si supplementation of 1.873 mM concentration decreases the total As uptake and As content in roots and significantly increases the As translocation from roots to shoots [2]. RAPD (Random Amplified Polymorphic DNA) is a DNA molecular marker, used to assess the genotoxicity and to evaluate the mutagenic effects of metals in plants. RAPD, a PCR based technique is simple and fast capable of detecting point mutations as well as temporary alteration of DNA that may not finally manifest themselves as mutation in future [3]. Alternation in genomic stability test (GTS%, a qualitative measure of genotoxic effect) is a measure to evaluate the changes in RAPD profile caused due to exposure of As [4]. In the present study, roots of *B. juncea* (variety Varuna) are used to observe the genotoxic effect of As in presence of Si. *B. juncea* is used as model plant as it is known as hyperaccumulator of several heavy metals i.e. able to tolerate the range of metals that are toxic to other plants.

2. METHODOLOGY

Seeds of *Brassica juncea* (var. Varuna) were obtained from IARI, Pusa, New Delhi. Seeds were surface sterilized in 70% ethanol, washed with distilled water and then soaked in double distilled water for overnight. These soaked seeds were transferred to plastic pots under hydroponic condition with 5% Hoagland medium. These pots were kept in dark for 2 days and then transferred to the growth chamber (14 hours photoperiod) with day/night temperature of 25±2°C and 70% relative humidity. In 14 days old seedlings three different treatments were given for 96 hours (i) As-150 µM (ii) Si-1.5 mM (iii) As and Si in combination, and those without metal stress served as control. Arsenic was added in the form of $NaAsO_2$ and Si as silicic acid. In Si solution the pH was adjusted close to 6.5 using diluted HCl or NaOH. The nutrient solutions were changed every 2 days to prevent the depletion of nutrients. Each treatment was repeated in triplicate and contained equal sized seedlings. After harvesting, roots and leaves were separated and roots were frozen in liquid nitrogen, kept at −80°C for RAPD procedures. For the RAPD analysis, 20 primers were used out of which 7 primer produce polymorphic bands. Genomic template stability (GTS) values were calculated according to the results of RAPD analysis. The GTS was calculated as GTS = (1-a/n)x100, Where a = RAPD polymorphic profiles in each sample., n = Total no. of bands in the control. Changes in the RAPD pattern were expressed as a decrease in GTS. Polymorphism ratio of the primers were calculated as, (polymorphism ratio=polymorphic

bands/Total no. of bands x100) under various treatments, where polymorphic bands are no. of extra bands appear or disappear in comparison to control and total no. of bands include both polymorphic bands plus bands in controls. A dendrogram was constructed by the between-groups linkage method using the Numerical Taxonomy and Multivariate Analysis System (NTSYSps) program with the SAHN module.

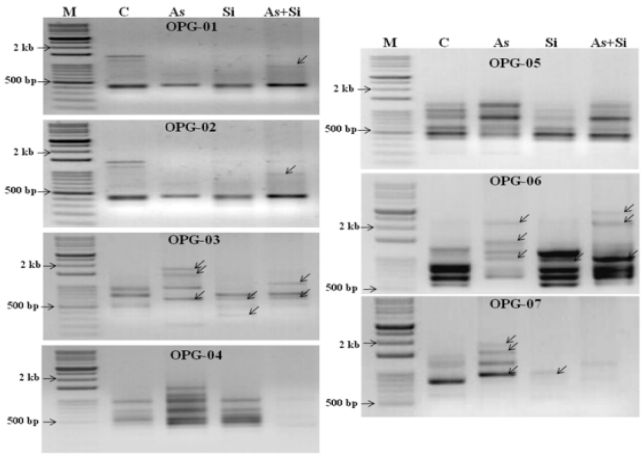

Fig. 1: RAPD profiles from roots of 14-d-old mustard seedlings treated using primers OPG-01–0PG-07. M: marker-1 kb plus DNA ladder. Changes in band intensity, appearance and disappearance of bands in control and treated samples are indicated by arrows.

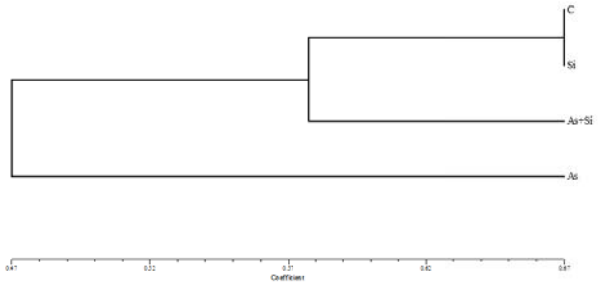

Fig. 2: Dendrogram represent two main groups with each one subdivided into two clusters. Control showed higher genetic similarity with Si and lowest with the As treated seedlings.

Table 1: Changes in total bands and genomic template stability (GTS %) in control and treated roots for each primer: GTS% value was only 8% in As, whereas it was 40% in combination of As and Si when control is considered as 100%.

Primers	C a/b	As a/b	Si a/b	As+Si a/b
OPG-01	4	0/2 (50)	0/2 (50)	1/2 (25)
OPG-02	3	0/2 (34)	0/1 (67)	1/1 (34)
OPG-03	3	3/2 (2.75)	2/1 (6.25)	2/2 (3.2)
OPG-04	3	3/0 (6.25)	2/0 (34)	0/0 (100)
OPG-05	5	0/1 (80)	0/1 (80)	0/0 (100)
OPG-06	5	3/3 (3.2)	1/1 (60)	3/2 (6.25)
OPG-07	2	3/1 (2.75)	1/2 (3.2)	0/1 (50)
Total average GTS(%)	25 (100)	23 (8)	14 (44)	15 (40)

Table 2: Polymorphism ratio of the primers under various treatments. Polymorphism ratio of the primers under various treatments was 92% in case of As,whereas it was 60% in As+Si treatment.

Treatments	Polymorphism Ratio
As	92%
Si	56%
As+Si	60%

3. CONCLUSION

GTS% value was only 8% in As, whereas, it was 40% in combination of As and Si when control is considered as 100%. Polymorphism ratio of the primers under various treatments was 92% in case of As, whereas, it was 60% in As+Si. These result showed that RAPD can be used as suitable biomarker for genotoxic alteration analysis under heavy metals stress and Si helps in mitigating the genotoxic effect caused by As.

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