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DNA INTEGRITY OF ONION (*ALLIUM CEPA* L.) ROOT CELLS EXPOSED TO BALLAST WATER

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ABSTRACT: Contaminated ballast water can pollute fresh waters. Regular sampling followed up by detailed analysis appears to ensure the proper monitoring that shipping activities do not encourage its indiscriminate discharge on the environment. In this study, the random amplified polymorphic DNA (RAPD) assay was used to assess the level of DNA damage in *Allium cepa* L. roots exposed to ballast water at different concentrations [0.5, 1, 5 and 10% (v/v)]. Compared to the control (tap water), the DNA obtained from the onion roots exposed to the wastewater caused greater changes in the RAPD patterns. This was discernible with appearance/disappearance of bands in the treated plants. A total of 116 RAPD bands were obtained using five oligonucleotide primers and 61 (52.5%) of these showed polymorphism. Onion bulbs exposed to the ballast water caused 17 new bands to appear and 18 to disappear; the loss and gain of bands decreasing with the raise of wastewater concentration. The genetic distances shown on the dendrogram revealed that genotoxicity of the wastewater was concentration-dependent. The data obtained from the RAPD fingerprinting imply that proper treatment should be given to ballast water to prevent, minimize, and ultimately eliminate the risks associated with its discharge into the environment as the wastewater is capable of inducing genotoxic effects.

Keywords: ballast water, rapd, dna damage, allium cepa, seagoing vessels

INTRODUCTION

To ensure buoyancy, stability and maneuverability, oceangoing ships need ballast water. Based on estimation that the world seaborne trade in 2013 amounted to 9.35 billion tons of cargo, the global ballast water discharge in 2013 are estimated to about 3.1 billion tons. Environmental pollution with ballast water is a worldwide problem. Over the past decades, the environmental impact of ballast water has received attention in the international scene. Control of discharge of ballast water is thus an international issue, handled by the International Maritime Organisation (IMO). In order to address this challenge, the IMO has adopted the international convention for the control and management of ships ballast water and sediments. This convention requires that ballast water quality shall meet strict standards regarding number of viable organisms and residual toxicity at the time of discharge (Delacroix et al., 2013).

Due to the global impacts of ballast water, the Ballast Water Management Convention (BWM convention) provided a set of management regulations through which the marinetime industry can be regulated. One of such regulations is the ballast water exchange standard (Regulation D-1) which requires ships to exchange a minimum of 95% ballast water volume at least 50 nautical miles from the nearest shore and in waters of 200 m deep or more (Werschkun 2014).

In Nigeria, cargo throughput in the national ports in the first quarter of 2014 stood at 19,659,946 million metric tonnes, an increase of 14 per cent over 17,245,923 metric tonnes achieved in 2013 (NPA, 2014). A total of 1,327 oceans going vessels with a total Gross Registered Tonnage (GRT) of 33,940,386 called at Nigerian Ports compared with 1,172 vessels with a GRT of 28,830,386 in 2013 (NPA, 2014). Although Nigeria joined the 1976 Convention and 2010 Protocol for the implementation and enforcement of the Ballast Water Management requirements, the regulatory bodies charged with responsibility for the management of the marine environment are yet to enforce full implementation of the Convention and other important international agreements and programs, including the London Protocol to protect the marine environment (IMO/NIMASA, 2013).

Higher plants provide a useful genetic system for screening and monitoring environmental pollutants *in situ* and *ex situ* with numerous advantages (Nilan, 1978; Grant, 1994; Maluszynska and Juchimiuk, 2005; Enan, 2009; Barbério, 2013; Firbas and Amon, 2013). Their genotoxicity assays offer attractive alternative to mammalian and non-mammalian animal assays in mutagen screening programmes (Grant, 1994). They are good indicators of cytogenetic and mutagenic effects (Constantin and Owens, 1982). Results obtained from an earlier study on the genotoxicity of ballast water discharged from a merchant ship berthed in a Nigerian Port revealed that the wastewater contained substances capable of inducing somatic mutation in plant cells (Olorunfemi *et al*., 2012).

Recent advances in molecular biology have led to the development of a number of selective and sensitive assays for DNA analysis in eco-genotoxicology. Among the DNA based techniques, RAPD is used to evaluate the variation at the DNA base pairs level. RAPD is a reliable, sensitive and reproducible assay

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and has the potential to detect a wide range of DNA damage, as well as mutations caused by heavy metal stress and therefore, it can be applied to study genotoxicity (Atienzar *et al*., 2004). The random amplified polymorphic DNA (RAPD) technique has been used to detect the differences in DNA fingerprints generated between onion plant cells exposed to genotoxic agents and control in the recent past (Baeshin *et al*., 2009; Quari, 2010; Ozakca and Silah, 2013; Hassan and Yassein, 2014). We are not aware of any documented information on the phyto genotoxicity evaluation of ballast water using molecular markers, therefore, this study was undertaken to determine whether or not the RAPD assay could detect DNA damage in onion roots exposed to ballast water obtained from a seagoing vessel berthed in Lagos Port Complex in Nigeria.

MATERIALS AND METHODS

Collection of water samples

The ballast water used for the study was collected in April 2014 from "M/T Azuryth" (3922 gross tonnage, IMO number 8111518, built Appledore 1982, Flag: Vanuata), a seagoing vessel used for transportation of crude oil along the Atlantic Ocean. It originated from Port Vila, and was berthed on high Sea at Lagos Port Complex (port of Lagos) located at the Apapa area of Lagos, South West Nigeria.

Plant Material and Treatment

The purple variety of average sized onion bulbs, *Allium cepa* L. (about 30 g, 15-22 mm diameter) were purchased from a local market in Benin City, Nigeria (6°15′N, 5°25′E) and sun-dried for two weeks. The dried roots present at the base of the onion bulbs were carefully shaved off with a sharp razor blade to expose the fresh meristematic tissues. The bulbs were then placed in freshly prepared distilled water to protect the primordial cells from drying up. Thereafter, the bulbs were removed from the distilled water and placed on a blotting paper to remove excess water. Seven onion bulbs were utilized for each water sample and the control (tap water). The base of each of the bulbs was suspended on the water sample inside 100 ml beakers in the dark for 7 days. Test samples were changed daily. At the end of the exposure period, the roots with the best growth for every single onion tested were removed with a forceps and utilized for chromosomal DNA extraction and RAPD analysis.

DNA Extraction

After one week of growth, approximately 2.5-3.5 cm of the onion roots were collected, ground in liquid nitrogen, and total genomic DNA was isolated by a CTAB method based on that of Padmalatha and Prasad (2006) method with minor modifications (Qari, 2010). Purity of DNA was determined by measuring its optical density in spectrophotometer at 260 nm/280 nm ratios and the quality of DNA samples was checked by loading them on 0.8% agarose gel and observing it on UV illuminator.

RAPD Fingerprinting

The conditions of DNA amplification followed the procedure of Williams *et al*. (1990) with some modifications (Qari, 2010). Random Amplified Polymorphic DNA (RAPD) was performed using primers OPH02 (5'-TCGGACGTGA-3'), OPT04 (5'-CACAGAGGGA-3'), OPT05 (5'-GGGTTTGGCA-3'), OPT17 (5'-CCAACGTCGT-3') and OPT19 (5'-GTCCGTATGG-3') (Operon technologies Inc., Alameda, California, USA) for each amplification. Each reaction (25 µL) consisted of 1 mM of $MgCl_2$, 4 mM each of dATP, dCTP, dGTP, dTTP (Boehringer-Manheim, Germany), 400 nM primer, 1.0 U of Taq DNA polymerase (Appligene-Oncor, France), reaction buffer (1 mM $MgCI_2$, 20 mM Tris HCl pH 8.0, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol, and 50% glycerol). The reaction mixture was overlaid with a drop of mineral oil and incubated in a thermal cycler (thermal cycler 480, Perkin Elmer-Cetus, USA) programmed as follows: 48 cycles of 1 mm denaturation at 94°C, 1 mm annealing at 37°C and 1.5 mm extension at 72°C, followed by final extension at 72°C, followed by a cooling at 4°C. Tubes containing all reaction products except template DNA were used as negative control.

PCR reaction products were mixed with one-sixth volume of gel loading buffer (analytical grade water containing 36% glycerol, 0.05% bromophenol, 30 mM EDTA and 0.05% xylene cyanol), and then separated by electrophoresis in a 2.4% agarose gel, using a Tris-borate-EDTA (TBE) system (0.5 × TBE = 45 mM Tris-base, 45 mM boric acid, and 1 mM EDTA). Agarose gel dimensions were 12×6×0.5 cm^3. For comparison, DNA molecular size marker (1 kb) was used for each agarose gel.

Statistical Analysis

The data were expressed as mean ± SD. The differences between mean values and the controls were statistically investigated using student t-tests. Genomic template stability (%) was calculated as 100 − (100 **a/n**), where **a** was RAPD polymorphic profiles detected in each treated sample while **n** was the total number of bands in the control. Polymorphism observed in RAPD profiles include disappearance of a normal band and appearance of a new band in comparison with control RAPD profiles (Wiiliams *et al*., 1990; Atienzar *et al*., 1999). The average was then calculated for each experimental group exposed to different concentrations of ballast water samples.

RESULTS AND DISCUSSION

The range of the purity of DNA extracted from root tips of onion bulbs cultivated in the ballast water samples and control was in the range of 1.71 – 2.04. Result of the RAPD profile of nucleotide sequence of the five primers used is presented in Table 1. The RAPD profile obtained with the five oligonucleotide primers used produced bands between 110 and 1125 bp in length (Plates 1-5). In all, 116 bands were scored, 61 (52.5%) were polymorphic. The number of fragments yielded by onion bulbs increased with increase in concentration. Altogether, 17 new bands were formed while 18 were lost (Table 2).

Several investigators have used the DNA-RAPD fingerprinting as a biomarker assay to detect DNA

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damage and mutational events in cells of *Allium cepa* (Qari, 2010; Ozakca and Silah, 2013; Hassan and Yassein, 2014). Ballast water has been implicated in the induction of chromosome aberration in *Allium cepa* root tips exposed to the wastewater (Olorunfemi *et al*., 2012). It is possible that the detected DNA polymorphism could lead to genotoxic effects in the case of treated plants most probably due to chemical substances present in waste water. It is also possible that the observed DNA polymorphism/damage could affect mitosis suggesting a possible genotoxic effect for the analyzed wastewater.

In this study, DNA damage/polymorphism was evident in RAPD profiles via appearance or disappearance of bands in the wastewater compared with the control. Disappearing bands are likely due to changes in oligonucleotide priming sites, originated from rearrangements and less likely from point mutations and DNA damage in the primer binding sites (Liu *et al*., 2009). The RAPD fingerprinting method can be applied to increase our understanding of the nature of wastewaters (Raj *et al*., 2014) and to a wide range of bioindicator organisms and may become a universal methodology for the identification of target genes for specific genotoxic agents (Al-Qurainy *et al*., 2010).

Table 1

Nucleotide sequence of five primers used showing the total bands and the polymorphism in percentage as calculated from control and ballast water samples

RAPD Primer	Sequence (5'---3')	G=C Content (%)	Total Bands	Polymorphism (%)
OPH02	TCGGACGTGA	60	11	54.54
OPT04	CACAGAGGGA	60	32	68.75
OPT05	GGGTTTGGCA	60	15	0.00
OPT17	CCAACGTCGT	60	29	48.28
OPT19	GTCCGTATGG	60	29	65.52
		Total	116	

Table 2

Changes in RAPD profiles scored compared with control

Treatments (%)	RAPD Band Gain (+)	RAPD Band Loss (-)
0.50	7	7
1.00	5	7
5.00	4	2
10.00	1	2
Total	17	18

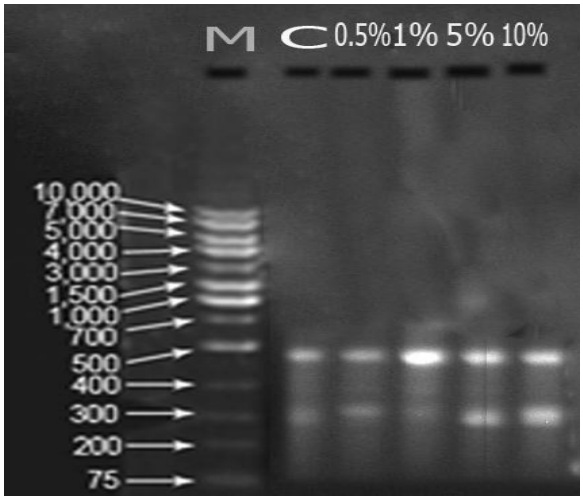

Plate 1: Profiles RAPD of genomic DNA from root cells of *Allium cepa* grown in the ballast water samples using OPH02 primer.

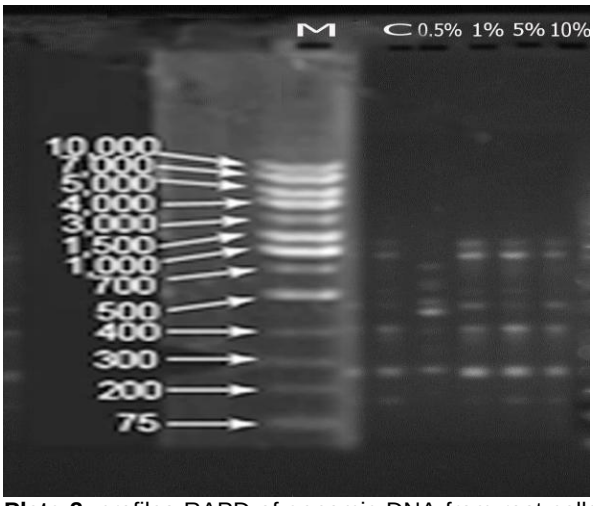

Plate 2: profiles RAPD of genomic DNA from root cells of *Allium cepa* grown in the ballast water samples using OPT04 primer.

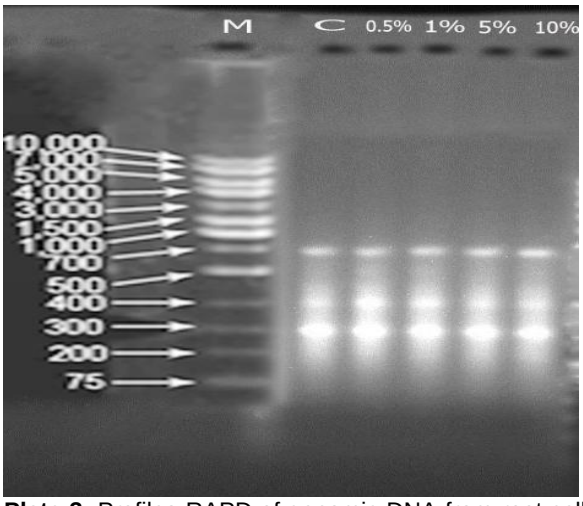

Plate 3: Profiles RAPD of genomic DNA from root cells of *Allium cepa* grown in the ballast water samples using OPT05 primer.

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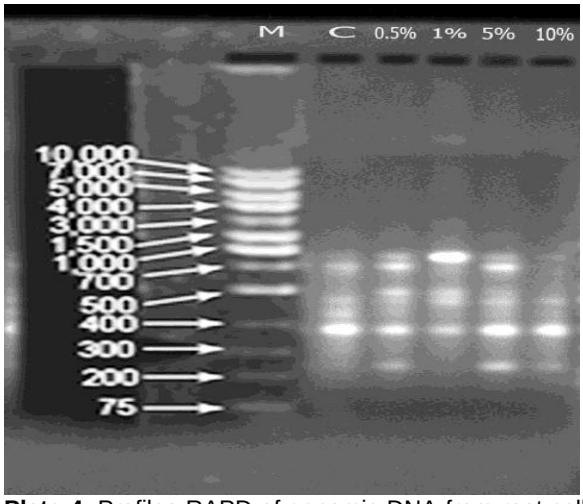

Plate 4: Profiles RAPD of genomic DNA from root cells of *Allium cepa* grown in the ballast water samples using OPT17 primer.

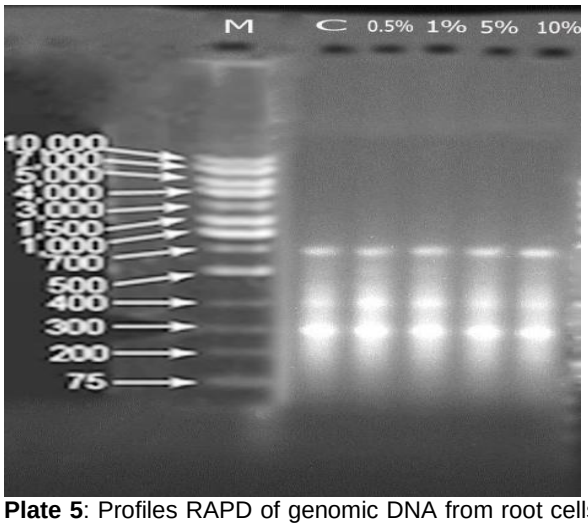

Plate 5: Profiles RAPD of genomic DNA from root cells of *Allium cepa* grown in the ballast water samples using OPT19 primer.

The Squared Euclidean distance method was used to construct dissimilarity values to estimate the level of DNA polymorphism among control and test samples. The Squared Euclidean distance between the control plants and those exposed to the various concentrations of ballast water is shown in Fig. 1. The Squared Euclidean distance between the control and 0.5% ballast water was 1, while a Squared Euclidean distance of 8 existed between control and onion bulbs exposed to 1% sample. However, the higher concentrations of the wastewater (5 and 10%) were separated at a longer (16 and 25) rescale distances respectively.

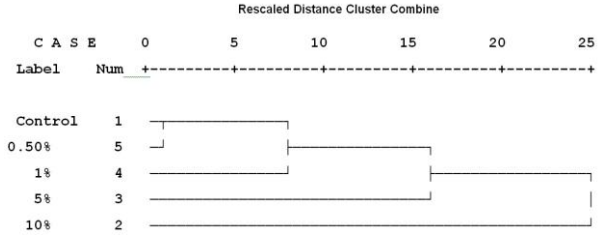

Fig.1 Dendrogram representing genetic distances among five concentrations of ballast water treated *Allium cepa* by UPGMA method based on RAPD analysis

DNA damage induced by ballast water was reflected in changes in RAPD profiles through changes in band intensity, disappearance of bands and appearance of new bands in the exposed plant. The disappearance of bands may be due to the formation of pyrimidine dimers, single and double strand breaks, modified bases, basic sites, oxidized bases, bulky adducts and DNA-protein cross-linked, point mutation and complex chromosomal rearrangement induced by genotoxins (Wolf *et al*, 2004). Any of these events can act to block or reduce (by-pass event) the polymerization of DNA in PCR reaction. The highest number of disappeared bands was observed at concentrations 5% and 10% of ballast water. This suggests that ballast water at these concentrations is capable of inducing DNA damage that will result in band loss. The appearance of new PCR products may reveal a change in some oligonucletide priming due to mutations and juxtaposing two sequences that match the sequence of the primers (Atienzar *et al*., 1999). According to Atienzar and Jha (2006), mutation can only be responsible for the appearance of new bands if they occur at the same locus in a sufficient number of cells. A minimum of 10% of mutation may be required to get new PCR products to be visible in agarose gel. The appearance and disappearance of bands in this study could be attributed to changes or mutation of DNA of the test plant induced by the wastewater.

CONCLUSION

To the best of our knowledge, there is no documented report on RAPD fingerprinting of *Allium cepa* DNA polymorphism/damage induced by ballast water. The findings in the present study has improved our understanding of the genotoxic effects of ballast water. In conclusion, the data presented here shows that ballast water induced DNA polymorphism/damage in *Allium cepa* root cells and that indiscriminate disposal of the wastewater in the aquatic ecosystem could pose health hazards to the environment and plant life. Serious measures should be put in place to regulate ballast water disposal.

REFERENCES

Al-Qurainy F, Alameri AA, Khan S, RAPD profile for the assessment of genotoxicity on a medicinal plant; *Eruca sativa*. Journal of Medicinal Plants Research, 4(7): 579-586, 2010.

Atienzar FA, Cordi B, Evenden AJ, 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. Environmental Toxicology and Chemistry, 18: 2275-2282, 1999.

Atienzar FA, Jha AN, The Random Amplified Polymorphic DNA (RAPD) assay to determine DNA alterations, repair and transgene rational

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effects in B(α)P exposed *Daphnia magna*. Mutation Research, 552: 125–140, 2004.

Atienzar FA, Jha AN, The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. Mutation Research, 613(2-3): 76-102, 2006.

Baeshin NA, Sabir JSM, Qari SH, Cytogenetic and molecular evaluations of genetic effects of leaf extract of *Rhazya stricta* (Decne) on *Allium cepa* root tip meristems. Egyptian Journal of Genetics and Cytology, 38: 73-83, 2009.

Barbério A, Bioassays With Plants in the Monitoring of Water Quality. In: Elshorbagy W, Chowdhury RK editors. Water Treatment. Rijeka, Croatia: InTech, pp. 317-334, 2013. Available at http://dx.doi.org/10.5772/2883. [accessed 09 October 2014]

Constantin MJ, Owens ET, Introduction and perspectives of plant genetic and cytogenetic assays, A Report of the US Environmental Protection Agency Gene-Tox Program. Mutation Research, 99:1-12, 1982.

Delacroix S, Vogelsang C, Tobiesen A, Liltved H, Disinfection by-products and ecotoxicity of ballast water after oxidative treatment - Results and experiences from seven years of full-scale testing of Ballast Water Management Systems. Marine Pollution Bulletin, 73(1):24-36, 2013.

Enan MR, Genotoxicity of the herbicide 2, 4-dichlorophenoxyacetic acid (2,4-D): Higher plants as monitoring systems. American-Eurasian Journal of Sustainable Agriculture, 3(3): 452-459, 2009.

Firbas P, Amon T, *Allium* chromosome aberration test for evaluation effect of cleaning municipal water with Constructed Wetland (CW) in Sveti Tomaž, Slovenia. Journal of Bioremediation and Biodegradation, 4:189-193, 2013

Grant WF, The present status of higher plant bioassays for the detection of environmental mutagens. Mutation Research, 310: 175-185, 1994.

Hassan GM, Yassein AA, Cytogenotoxicity evaluation of water contaminated with some textile azo dyes using RAPD markers and chromosomal aberrations of onion (*Allium cepa*) root cells. Egyptian Journal of Genetics and Cytology, 43:39-57, 2014.

International Maritime Organisation/Nigeria Maritime Administration and Safety (IMO/NIMASA), Report of the National Workshop on the London Protocol in Nigeria, Lagos, 26-28 February 2013, 42p.

Liu W, Yang YS, Li PJ, Zhou QX, Xie LJ, Han YP, Risk assessment of cadmium-contaminated soil on plant DNA damage using RAPD and physiological indices. Journal of Hazardous Materials, 161:878-883, 2009.

Maluszynska J, Juchimiuk J, Plant Genotoxicity. Arh Hig Rada Toksikol, 56:177-184, 2005

Nigerian Ports Authority (NPA), Nigerian Ports Authority Press Releases, 26/28 Marina, Lagos. Available online: http://www.nigerianports.org/news.aspx?id=375 (accessed on 11 November, 2014).

Nilan RH. Potential of plant genetic systems for monitoring and screening mutagens. Environmental Health Perspectives, 27:181–196, 1978.

Olorufemi DI, Duru EC, Okieimen FE, Induction of chromosome aberrations in *Allium cepa* L. root tips on exposure to ballast water. Caryologia, 65:147–151, 2012.

Ozakca DU, Silah H, Genotoxicity effects of flusilazole on the somatic cells of *Allium cepa*. Pesticide Biochemistry and Physiology, 107: 38-43, 2013.

Padmalatha K, Prasad MNV, Optimization of DNA isolation and PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India. African Journal of Biotechnology, 5(3): 230-234, 2006.

Qari SHM, DNA-RAPD fingerprinting and cytogenetic screening of genotoxic and antigenotoxic effects of aqueous extracts of *Costus speciosus* (Koen). Journal of King Abdulaziz University: Science, 22: 133-152, 2010.

Raj A, Kumar S, Haq I, Kumar M, Detection of tannery effluents induced DNA damage in mung bean by use of Random Amplified Polymorphic DNA markers. International Scholarly Research Notices Biotechnology, Article ID 727623, 1-8, 2014.

Werschkun B, Banerji S, Hofer T, Emerging risk from Ballast Water Treatment: The run-up to the International Ballast Water Management Convention. Journal of Chemosphere, 112: 256-266, 2014.

Williams J, Kubelik AR, Livak KJ, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research, 18: 6531-6535, 1990.

Wolf HD, Blust R, Backeljau T, The use of RAPD in ecotoxicology. Mutation Research, 566(3): 249-262, 2004

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