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Effect of nickel on regeneration in *Jatropha curcas* L. and assessment of genotoxicity using RAPD markers

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Abstract The aim of the present study was to determine the effect of nickel on shoot regeneration in tissue culture as well as to identify polymorphisms induced in leaf explants exposed to nickel through random amplified polymorphic DNA (RAPD). In vitro leaf explants of *Jatropha curcas* were grown in nickel amended Murashige and Skoog (MS) medium at four different concentrations (0, 0.01, 0.1, 1 mM) for 3 weeks. Percent regeneration, number of shoots produced and genotoxic effects were evaluated by RAPD using leaf explants obtained from the first three treatments following 5 weeks of their subsequent subculture in metal free MS medium. Percent regeneration decreased with increase in addition of nickel to the medium up to 14 days from 42.31% in control to zero in 1.0 mM. The number of shoot buds scored after 5 weeks was higher in control as compared to all other treatments except in one of the metal free subculture medium wherein the shoot number was higher in 0.01 mM treatment (mean = 7.80) than control (mean = 7.60). RAPD analysis produced only 5 polymorphic bands (3.225%) out of a total of 155 bands from 18 selected primers. Only three primers OPK-19, OPP-2, OPN-08 produced polymorphic bands. The dendrogram showed three groups A, B, and C. Group A samples showed 100% genetic similarity within them. Samples between groups B and C were more genetically distant from each other as compared to samples between groups A and B as well as groups A and C. Cluster analysis based on RAPD data correlated with treatments.

Keywords Regeneration · Nickel · Genotoxicity · Molecular markers · *Jatropha curcas*

Abbreviations

MS	Murashige and Skoog medium
TDZ	Thidiazuron
BAP	6-Benzylaminopurine
IAA	Indole 3-acetic acid
RAPD	Random amplified polymorphic DNA
AFLP	Amplification fragment length polymorphism
SSR	Simple sequence repeat
AP-PCR	Arbitrarily primed PCR
JSC	Jaccard's similarity coefficients
CTAB	Cetyl trimethyl ammonium bromide

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Introduction

Nickel is considered an essential element in plants (at least for legumes) primarily because of its function as an irreplaceable component of urease (Gerendas et al. 1999). Among the endemic serpentine flora, ca. 75% of the plants have shown to hyperaccumulate this metal (Reeves and Baker 2000) without any toxic effects. This phenomenon is widely implicated as a defence mechanism protecting the plants against herbivores (Boyd 2004, 2007; Palomino et al. 2007). Increased anthropogenic activities such as vehicular traffic, burning of fossil fuels, soil fertilization, use of pesticides, mining and metallurgical activities, and the disposal of sludge have resulted in widespread contamination of this metal across different parts of the globe (Chen et al. 2009). Nickel, a non redox heavy metal, induces oxidative stress indirectly by acting on functional groups of proteins thereby producing toxic symptoms in sensitive, moderately tolerant and even in hyperaccumulating plants, at various exposure levels (Sharma and Dietz 2009).

The role of nickel as a potent carcinogen and as a mutagen has been clearly established in animal and human cell lines where it is known to inhibit DNA repair enzymes (Hartwig et al. 1994; Lynn et al. 1997; Kim et al. 2002; Lu et al. 2005). Not only genotoxins shorten the life expectancy of the organisms but they can also result in alterations in population dynamics having an effect on intra and inter species diversity with adverse ecological consequences (Atienzar et al. 2001). Reactive oxygen species (ROS) generated as a result of oxidative stress has been widely implicated in damage to cell membrane and DNA (Sharma and Dietz 2009). However, the available literature does not clearly demonstrate nickel induced changes in DNA sequence and chromosomal aberrations in plants. Molecular markers, such as random amplified polymorphic DNA (RAPD), arbitrarily primed PCR (AP-PCR), amplification fragment length polymorphism (AFLP), simple sequence repeat (SSR) have been widely used in genotoxicity studies to evaluate, detect and identify changes in the sequence of DNA caused by various environmental pollutants and mutagens (Atienzar et al. 2002; Rancelis et al. 2006; Monteiro et al. 2007; Lu et al. 2007; Enan 2007; Swaileh et al. 2008; Liu et al. 2009; Cenkci et al. 2009). Among these multilocus markers, RAPD is a simple, fast, reliable technique than classic genotoxic analysis tools, as it is capable of detecting not only point mutations but also temporary alteration of DNA that may not finally manifest themselves as mutation in future (Labra et al. 2003) and allow detection of low doses of pollutants (Liu et al. 2009).

Further nickel is a component of Heller's medium (Heller 1953) and has been recommended to be included as a normal constituent of MS medium (Witte et al. 2002). Although a wealth of information is available in the literature about the effect of nickel on nutrient imbalances, inhibition of growth and yield, disruption of photosynthesis and oxidative stress but literature highlighting the effect of higher doses of nickel on plant regeneration in tissue culture is very limited. *Jatropha curcas* has ability to grow on marginal wastelands thus enabling not only in vegetating those lands but also serving as a source of obtaining monetary benefits for the communities and small farmers of those areas. Recently there have been several reports suggesting the use of *J. curcas* in phytoremediation of polluted soils including metal contaminated sites and those fertilized with sewage sludge (Kumar et al. 2008; Mangkoedihardjo and Surahmaida 2008). These sites are known to contain high concentration of heavy metals of which nickel is one of the contaminants. Therefore the present investigation was carried out to study the effect of nickel on shoot number, percent regeneration and determine by RAPD technique as to whether nickel induces changes in the DNA sequence of *J. curcas* which has been identified as a potential biofuel crop in India, under the programme of National Mission on Biofuels (http://www.planningcommission.nic.in).

Materials and methods

Culture and growth conditions

Leaves collected from cultures of *J. curcas* established in vitro served as an explant source. The explants were obtained from 6-week old nodal cultures maintained on MS medium (Murashige and Skoog 1962) supplemented with vitamins, sucrose 30 g l^{-1}, 0.5 mg l^{-1} 6-benzylaminopurine (BAP) and 0.5 mg l^{-1} indole 3-acetic acid (IAA) and solidified with 7 g l^{-1} agar at pH 5.7 set prior to autoclaving at 121°C for 15 min. Care was taken to

ensure that neither old senescent nor young leaves were used for the experiment. The explants thus obtained were exposed to 0, 0.01, 0.1, 1 mM concentration of nickel following filter sterilization though 0.22 μm Millex-GV syringe filters (Millipore). Nickel was supplemented as nickel chloride hexahydrate (Sigma) at around 45°C to autoclaved MS medium (MST) amended with vitamins, sucrose 30 g l^{-1}, 0.5 mg l^{-1} thidiazuron (TDZ) and solidified with 7 g l^{-1} agar at pH 5.7 (Sharma 2008). Each concentration of nickel used was considered as a treatment. The experiment was completely randomized and carried out with a minimum of at least 20 replications per treatment and was repeated twice. A single in vitro leaf explant in 38 × 200 mm culture tube containing 40 ml of MST medium served as a single replication. The number of explants regenerating in each of the treatment was scored after 7, 14 and 21 days and percent regeneration was calculated. All cultures were maintained at 26 ± 2°C under 12 h light/12 h dark photoperiod in a culture room through out the experiment.

At least five explants obtained from each treatment except 1 mM were subsequently subcultured after 3rd week in nickel free MS medium (proliferation medium) supplemented with vitamins, sucrose 30 g l^{-1} and 4 different combinations of BAP and IAA which was solidified with 7 g l^{-1} agar at pH 5.7. After 5 weeks of subculture in proliferation medium, the number of shoots/shoot buds was scored in each case and the data recorded. From one of the combinations used in subculture viz., 0.5 mg l^{-1} BAP and 1.0 mg l^{-1} IAA, a total of nine tissue samples (leaves) were collected (3 sample repeats from each treatment of nickel viz., T_1R_1,T_1R_2,T_1R_3 of control, T_2R_1, T_2R_2, T_2R_3 of 0.01 mM and T_3R_1,T_3R_2,T_3R_3 of 0.1 mM) and used for RAPD analysis.

DNA extraction and RAPD analysis

DNA was extracted from each of the samples by following CTAB method described by Sudheer et al. (2009a, b). Leaves (100 mg) from each of the nine samples were ground using liquid nitrogen. To the ground samples 0.5 ml of extraction buffer (2% CTAB, 100 mM Tris–HCl, 3.5 M NaCl, 20 mM EDTA, 0.2 M β-Mercaptoethanol, 2% PVP, pH 8.0) was added and incubated at 65°C for 90 min. The above samples were extracted with equal volume of chloroform: isoamyl alcohol (24:1) and each supernatant was transferred into a new tube. The samples were treated with RNase and extracted with Tris saturated phenol. The supernatant after extraction with Tris saturated phenol was taken and extracted further with chloroform: isoamyl alcohol (24:1) twice, and precipitated with 80% of ethanol. The pellet was washed with 70% ethanol, air dried and dissolved in 100 μl of Milli Q water.

The polymerase chain reaction (PCR) was carried out using methodology developed by Williams et al. (1990). A total of 18 selected decamer arbitrary RAPD primers (Operon technologies Inc, USA; IDT, USA) produced good amplification profiles and later on used for RAPD analysis. PCR amplification was carried out on a Thermal cycler (Master Cycle Epgradient S, Eppendorf, Germany) with 15 μl reaction mixture containing 25 ng template DNA, 20 pmol each primer, 1U Taq polymerase, 1X Taq buffer [10 mM Tris–HCl (pH 9.0),150 mM KCl, 0.1% Triton X 100], 0.12 mM each dNTPs and 1.8 mM $MgCl_2$ (Biogene, USA). The amplification reaction was carried out using the program of initial denaturation at 94°C for 3 min followed by 42 cycles of denaturation at 94°C for 30 s, annealing at 30°C for 30 s, extension at 72°C for 2 min and a final extension at 72°C for 5 min. The amplification products were electrophoresed at 80 V in 1.5% agarose gel in 1× TBE buffer for 90 min. The gel was stained with ethidium bromide and documented using gel documentation system (Syngene, UK). The size of each RAPD band was determined by using Gene Tool Analysis Software (Syngene, UK).

Data analysis

Statistical analysis of treatments was carried out using SPSS software version 10 (SPSS Inc., Chicago). RAPD data (binary matrix) was fed into and the dendrogram constructed using NTSYS version 2.20 software package. Each profile of RAPD was analyzed by scoring presence of band as (1) and absence of band as (0) and the scores were entered into a binary matrix. Faint and unclear band was not considered for further analysis. Genetic similarity based on Jaccard's coefficient were calculated among all possible pairs of the nine samples using SIMQUAL module and arranged in a similarity matrix. A dendrogram was constructed by using UPGMA (Unweighed Pair Group Method with Arithmetic Average) (Sokal and Sneath 1963)

option of SHAN module. The percentage of polymorphism (PP) was calculated by using the formula P = total number of polymorphic bands/total number of bands multiplied with 100.

Results

Effects of nickel on shoot regeneration from leaf explant

Percent regeneration of leaf explants exposed to nickel was higher in control (0 mM Nickel) as compared to other treatments up to 14 days in MST medium. But at 21 days of exposure, percent regeneration in 0.01 mM treatment was higher (63.83%) as compared to 53.85% in control (Table 1). Explants remained mostly green and healthy throughout the experiment (Fig. 1) in all the treatments, except 1 mM, where cytotoxic symptoms were manifested in 25% of the explants within 7 days, followed by complete necrosis on 14th day. Hence during the second repetition of the experiment 1 mM treatment was excluded from the experiment. Regeneration of explants in 0.1 mM treatment was very low at 8% after 14 days and remained the same even after 21 days of exposure. The total number of shoots produced in control was higher as compared to other treatments when subcultured in all but one of the combinations of nickel free proliferation medium supplemented with 0.5 mg l^{-1} BAP and 0.5 mg l^{-1} IAA. In this combination, the number of shoots in 0.01 mM nickel treated leaves (mean shoots = 7.80) was higher than that of the control (mean shoots = 7.60). Of the four different combinations used, robust growth was visualized in the proliferation medium with hormonal combination 0.5 mg l^{-1} BAP and 1.0 mg l^{-1} IAA which also induced relatively higher number of shoots/buds in most of the treatments as compared to other combinations, with 0.1 mM nickel exposure also producing mean of 5.60 shoots/buds (Table 2). Analysis of Variance (ANOVA) for the number of shoots in different combinations of BAP and IAA proliferation medium showed that only treatment was statistically significant ($p < 0.0005$) while other parameters viz., hormonal combination and treatment × hormonal combination were insignificant. Hence explants for subsequent RAPD analysis were chosen from this combination. Further post hoc multiple comparisons using least significant difference (LSD) has revealed that the treatment means of control and 0.01 mM treatment were insignificant with respect to each other while these two treatments were significant with that of 0.1 mM treatment mean (Tables 2, 3).

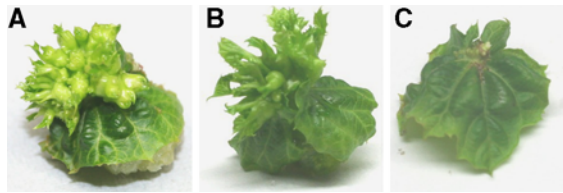

Fig. 1 Regeneration of shoots buds from leaf explants in nickel amended MS medium containing 0.5 mg l^{-1} thidiazuron **A** control with no nickel; **B** 0.01 mM nickel; **C** 0.1 mM nickel

Evaluation of genotoxicity with RAPD markers

A total of 155 amplicons from 18 primers were produced in PCR amplification reaction. The amplicons ranged from 273 to 3,095 bp in size and 5 bands (3.225%) were polymorphic out of total 155 bands generated. The number of bands scored in each primer varied from 4 (primer OPP-10) to 15 (OPJ-20).The number and percentage of monomorphic bands were 150 and 96.774 respectively. Only three primers OPK-19 (20%), OPP-2 (16.66%) and OPN-08 (16.66%) produced polymorphic bands across all the samples studied. Maximum polymorphism of 20% was shown by the primer OPK-19 where a total

Table 1 Percent regeneration of leaf explants obtained following exposure to various concentrations of nickel at different time intervals in MS medium containing 0.5 mg l^{-1} thidiazuron

Treatments (mM)	Percent regeneration			Total number of explants used
	7 days	14 days	21 days	
Control	17.31	42.31	53.85	52
0.01	14.89	38.30	63.83	47
0.1	2	8	8	50
1.0	0	0	0	24

Table 2 Total number of shoots produced in different combinations of BAP and IAA of nickel treated leaf explants scored after 5 weeks of culture in MS medium

Hormonal combinations (mg l^{-1})		Treatments		
BA	IAA	Control	0.01 mM	0.1 mM
0.5	0.1	13.00 ± 2.30[a]	7.00 ± 1.79[a]	2.20 ± 0.58[b]
0.5	0.2	10.60 ± 4.08[a]	9.20 ± 2.01[a]	2.00 ± 1.05[b]
0.5	0.5	7.60 ± 2.25[a]	7.80 ± 2.13[a]	1.40 ± 0.87[b]
0.5	1.0	11.00 ± 2.74[a]	7.20 ± 1.74[a]	5.60 ± 2.77[b]

Values represented are mean ± SE of 5 replicates

Means followed by the same letter in a row are not significantly different at $P < 0.05$ by post hoc comparison using least significant difference (LSD)

Table 3 ANOVA summary for the number of the shoots produced in different combinations of BAP and IAA subsequently following nickel exposure at different concentrations

Source of variation	Mean square	*df*	*F*	*p* value
Treatment	308.750	2	12.517	<0.0005
Hormonal combination	15.261	3	0.619	0.606
Treatment × hormonal combination	16.261	6	0.659	0.683
Error	24.667	48		

of 5 bands were produced (Table 4). On an average 8.61 bands were generated per primer used in this study. Jaccard's similarity coefficients (JSC) varied from 1.00 to 0.974 across all the samples. In the dendrogram, the samples diverged into three groups viz. A, B, C. Group A contained 4 samples of T_1R_1,T_1R_2,T_1R_3 and T_2R_1, while group B included T_2R_2, T_2R_3 and T_3R_1,T_3R_2,T_3R_3 were in Group C (Fig. 2). Only three polymorphic bands were shared by group C samples, only two bands were shared by group B samples. Group A samples showed 100% genetic similarity within them. Group C samples showed JSC ranging from 0.974 to 0.993 respectively. Lowest similarity matrix value of 0.974 was observed between T_2R_3/T_3R_1 and T_2R_3/T_3R_2 (Table 5). Maximum number of two polymorphic bands was produced by each of the primers OPP-02 and OPN-08. Fifteen primers, OPP-15, OPQ-15, OPR-08, OPO-05, OPJ-09, OPJ-20, OPP-10, OPL-02, OPN-12, OPQ-11, OPN-10, OPQ-18, OPN-05, OPQ-12 and OPN-04 out of total eighteen primers produced no polymorphism across all the samples (Table 4). Within groups B and C, JSC ranged from 1.000 to 0.993 and 0.993 to 0.987 respectively. Between groups A and B, JSC ranged from 0.993 to 0.987 and between groups A and C the similarity matrix values ranged from 0.993 to 0.987, while between groups B and C, similarity matrix values ranged from 0.987 to 0.974 indicating samples between groups B and C are more genetically distant from each other as compared to samples between groups A and B as well as groups A and C (Table 5).

Discussion

Effects of nickel on shoot regeneration from leaf explant

In this study explants exposed to 0.01 mM nickel treatment showed a higher percent regeneration than control after 21 days. This is suggestive of the fact that lower concentrations of nickel (≤0.01 mM) may be required for growth and regeneration. Similar observations have been made in callus cultures of *J. curcas* treated with Nickel (Anand et al. 2008) and in sugarcane callus stressed with Cadmium (Fornazier et al. 2002). In *Capsicum annuum*, Joshi and Kothari (2007) reported an increase in the frequency of response and number of shoot buds at higher levels of

Table 4 List of primers, sequences of primers, number of monomorphic and polymorphic bands, number and length of amplicons, percentage of polymorphism

Primer	Sequence of primer (5′–3′)	MB[a]	PB[b]	Total	Length of amplicons (bp)	PP[c]
OPJ-09	TGAGCCTCAC	10	0	10	575–2,889	0
OPJ-20	AAGCGGCCTC	15	0	15	369–1,900	0
OPK-19	CACAGGCGGA	4	1	5	354–1,746	20
OPL-02	TGGGCGTCAA	9	0	9	682–2,303	0
OPN-12	CACAGACACC	6	0	6	325–2,158	0
OPN-10	ACAACTGGGG	8	0	8	482–2,067	0
OPN-05	ACTGAACGCC	8	0	8	326–1,214	0
OPN-04	GACCGACCCA	9	0	9	372–2,462	0
OPN-08	ACCTCAGCTC	10	2	12	373–2,354	16.66
OPO-05	CCCAGTCACT	10	0	10	670–2,260	0
OPP-02	TCGGCACGCA	10	2	12	486–3,095	16.66
OPP-15	GGAAGCCACC	11	0	11	326–1,291	0
OPP-10	TCCCGCCTAC	4	0	4	609–1,845	0
OPQ-15	GGGTAACGTG	6	0	6	273–1,000	0
OPQ-11	TCTCCGCAAC	6	0	6	634–1,981	0
OPQ-18	AGGCTGGGTG	5	0	5	422–1,526	0
OPQ-12	AGTAGGGCAC	10	0	10	678–2,078	0
OPR-08	CCCGTTGCCT	10	0	10	400–1,395	0
Total		150	5	155		

[a] Number of monomorphic bands

[b] Number of polymorphic bands

[c] Percent of polymorphism

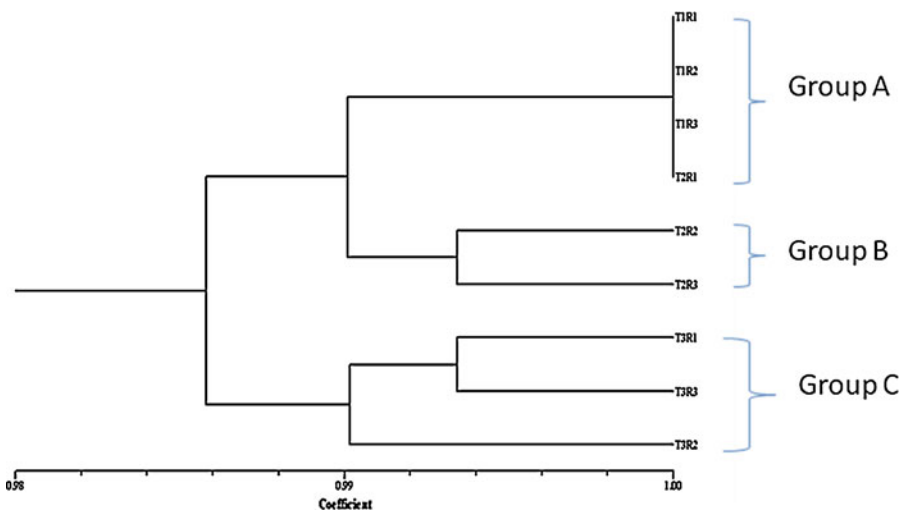

Fig. 2 Dendrogram of 9 samples based on Jaccard's similarity coefficient

Copper and Silver than at lower levels. No regeneration of the explants was observed in 1 mM treatment indicative of the fact that exposure at this dosage is lethal and cytotoxic to the explants. The toxicity observed in 1 mM treatment might be due to oxidative stress induced ROS with concomitant

Table 5 Matrix of pairwise Jaccard's similarity coefficient of 9 samples

	T_1R_1	T_1R_2	T_1R_3	T_2R_1	T_2R_2	T_2R_3	T_3R_1	T_3R_2	T_3R_3
T_1R_1	1.000								
T_1R_2	1.000	1.000							
T_1R_3	1.000	1.000	1.000						
T_2R_1	1.000	1.000	1.000	1.000					
T_2R_2	0.993	0.993	0.993	0.993	1.000				
T_2R_3	0.987	0.987	0.987	0.987	0.993	1.000			
T_3R_1	0.987	0.987	0.987	0.987	0.980	0.974	1.000		
T_3R_2	0.987	0.987	0.987	0.987	0.980	0.974	0.987	1.000	
T_3R_3	0.993	0.993	0.993	0.993	0.987	0.980	0.993	0.993	1.000

damage to DNA, proteins and lipids (Boominathan and Doran 2002; Hao et al. 2006; Sharma and Dietz 2009). The frequency of regeneration observed in 0.1 mM was almost constant throughout the 3rd week from 14 to 21 days of exposure indicative of the fact that the threshold level/tolerance level may be at this dosage. Moreover, explants from 0.1 mM nickel treatment that failed to regenerate initially also produced shoots/buds when transferred to nickel free proliferation medium. This is suggestive of the fact that nickel has some inhibitory effect upon regeneration in *J. curcas*. A similar result was reported in shoot cultures of *Allysum markgrafii* where an arrest of shoot growth was observed at concentrations >10 mM nickel followed by its subsequent proliferation upon transfer to nickel free medium (Vinterhalter and Vinterhalter 2005).

Evaluation of genotoxicity with RAPD markers

In this study, there was only appearance of five polymorphic bands without disappearance of any amplified bands in relation to control. New PCR amplicon may express changes in some oligonucleotide priming sites due to mutation, large deletions and homologous recombinations (Atienzar et al. 1999). New amplified fragments are generated as a new site of genomic sequence becomes accessible to primer due to alteration at the structural level of DNA (Pietrasan et al. 2000; Enan 2006; Swaileh et al. 2008). A single point mutation within binding site can be responsible for changes in RAPD banding pattern (Williams et al. (1990)). Out of 9 regenerants (3 control replicates and 6 nickel treated regenerants) used for RAPD analysis, 5 plantlets (out of 6 nickel treated regenerants) showed variation in amplification pattern in relation to control plants corresponding to a genetic variation frequency of 55.55%. Control regenerants showed no genetic variation (DNA polymorphism) within them. Further a higher level of genetic variation frequency thus generated may be due to nickel induced mutagenic effect but not as a result of tissue culture induced DNA polymorphism. Low level of overall DNA polymorphism (3.125%) and higher level of similarity coefficient values (1.00–0.974) shows that nickel might be a weak mutagen. The level of tissue culture induced DNA polymorphism may be very less (Munthali et al. 1996; Al-Zahim et al. 1999; Gesteira et al. 2002) to zero (Devaux et al. 1993; Isabel et al. 1993; Valles et al. 1993).

In our study, the percentage of polymorphic bands (PPB) within samples derived from leaf explants treated with 0.01 and 0.1 mM concentrations of nickel are 11.11 and 16.66 respectively as compared to control. This study also showed there was a within treatment genetic variation within each three sample of a treatment based on presence or absence of particular novel band (s) produced by a single primer. For instance, primer OPK-19 produced a polymorphic band of 1,746 bp in T_2R_2 and T_2R_3 replicates but not in T_2R_1 (Fig. 3). All these three replicates were derived from leaf explants treated with 0.01 mM concentration of nickel. In this research work, we found out that same novel band (s) appeared in one/two or all the three regenerants derived from leaf explants treated with same concentration of nickel as compared to control, but were not found in regenerants treated with different concentrations of nickel as compared to control. For

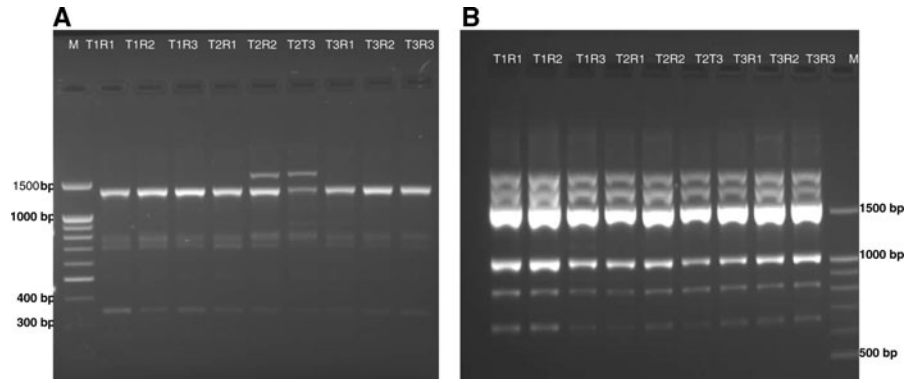

Fig. 3 **a** RAPD profile with primer OPK 19 showing polymorphic bands in T_2R_2 and T_2R_3, **b** RAPD profile of primer OPQ11 showing monomorphic bands. Lane M represents markers

example, primer OPN-08 produced one novel band of 822 bp in T_3R_1, T_3R_2, and T_3R_3 replicates derived from different leaf explants treated with 0.1 mM nickel, this primer also produced another novel band of 445 bp in only T_3R_2 sample without producing this band in other two samples T_3R_1 and T_3R_3. The two aforesaid bands were totally absent in samples derived from leaf explants treated with 0.01 mM nickel and control. Similar results also obtained in Chinese narcissus (Lu et al. 2007).This finding suggests that there might be some specific regions in *J. curcas* genome that are sensitive to mutation induced by nickel treatment in vitro culture condition. Our work suggests that 0.01 mM nickel induces genotoxicity to a lesser extent to the sample of group B without any cytotoxic and inhibitory effect on leaf regeneration. But 0.1 mM nickel induces genotoxicity and shows toxic symptoms correlating with its inhibitory effect on regeneration. Appearance of new bands occurred with increased concentration of nickel indicating that the effect of nickel on DNA is dose specific. Similar results were obtained in bean seedlings (Cenkci et al. 2009).

Conclusion

Our data suggests that lower concentration of nickel ($\leq$0.01 mM) may stimulate growth and regeneration but higher concentrations adversely affects leaf regeneration of *J. curcas* in tissue culture and is responsible for inducing polymorphism through point mutations and subsequent changes in DNA sequences. This work thus provides clear evidence of nickel induced alterations in the sequence of DNA in plants. Genotoxicity was effectively evaluated by RAPD analysis. Furthermore in this study, RAPD analysis supports the data obtained from inhibitory effects of nickel on leaf regeneration. But further research is required to ascertain the bands reflecting stable alteration in DNA is allelic or due to somatic mutation.

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