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*Full Length Research Paper*

# *In vitro* mutagenesis in *Rosa hybrida* using oryzalin as a mutagen and screening of mutants by randomly amplified polymorphic DNA (RAPD) marker

**S. K. Senapati[1]* and G. R. Rout[2]**

[1]MITS School of Biotechnology, Infocity, Bhubaneswar, Orissa, 751015, India.
[2]Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India.

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**Apical and axillary meristems of *Rosa hybrida* Cv. First red were pretreated with various concentrations (0, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 µM) of oryzalin ($C_{12}H_{18}N_4O_6$) to induce variation *in vitro*. The present results indicate that fifty percent survivability ($LD_{50}$) was obtained in 20 µm oryzalin. Both the treated and untreated meristems were cultured in Murashige and Skoog (MS) basal medium supplemented with 0.5 mg/l benzylaminopurine (BAP), 0.01 mg/l indole acetic acid (IAA), 25 mg/l adenine sulphate (Ads) and 20 µm oryzalin. The elongated shoots were rooted in the half strength MS basal medium supplemented with 0.25 mg/l indole-3-butyric acid (IBA) and about 60% rooted plants survived in the green house. A total of 28 mutants were obtained and evaluated by randomly amplified polymorphic DNA (RAPD) markers using control as untreated meristems. Out of the twenty-eight mutants, eight mutants, which deviate from the DNA banding pattern when compared with the control plants, were obtained. This result showed the efficiency of oryzalin to induce *in vitro* variability in hybrid rose and detect variation through molecular markers. This investigation will give a better understanding on the rose breeding program.**

**Key words**: Hybrid rose, *in vitro*, oryzalin, mutation.

## INTRODUCTION

The genus Rosa includes over 100 species, among which about 20% were developed on the basis of spontaneously derived sports (Krusman). This relatively high percentage may provide the opportunity of involving the mutation induction technique in rose breeding programs. In the past few decades, breeders have faced a lot of problems in rose improvement due to low sexual reproduction and poor germination of seed because of embryo abortion. Rose improvement could be possible by using physical and chemical mutagens, although chemical mutagen is not widely used due to their low penetration into plant tissue. In order to avoid this problem, we used the *in vitro* system, in which chemical mutagens are used to evolve new variants. The number of commercially propagated mutant varieties is however very small. The reason is that it takes too much time from the mutagenic treatments up to the detection of the mutants and their large scale propagation (Broertjes and Van Harten, 1978). Since the *in vitro* propagation of rose is a very well known method, *in vitro* mutagenesis of rose, which is a combination of *in vitro* culture and mutation induction, provides the opportunity to increase variability of economically important cultivars. A mutation is an event that results in a change in the hereditary material, and so, a distinction is made between genes and chromosome mutation. Gene mutation is the localized changes that typically arise from errors in DNA replication, but chromo-

*Corresponding author. E-mail: sunil_k_senapati@yahoo.com. Fax: 0674-2741584.

**Abbreviations: IAA,** Indole-3-acetic acid; **NAA,** naphthaleneacetic acid; BAP, benzylaminopurine; Ads, adenine sulphate; **BA,** benzyl adenine; **Kn,** kinetin; **CTAB,** cetyltrimethylammonium bromide; **EDTA,** ethylenediaminetetraacetic acid; **PCR,** polymerase chain reaction; **RAPD,** randomly amplified polymorphic DNA.

somal mutation is the larger scale changes that involve many genes. Plant propagation by tissue culture usually aims at the possible highest multiplication rate without direct vitrification and mutation from nodal segments, lateral and auxiliary buds. In order to obtain high multiplication rates, a relatively large amount of cytokinins are used in the multiplication media. However, in micro propagation of some plants, the multiplication rates decrease with an increase of the concentration of cytokinins in the medium (Corchete et al., 1997). Though multiplication is our requirement, its rooting and successful establishment is our target. In order to obtain good rooting, generally, auxins of different types are used either singly or in combination in the ½ Murashige and Skoog (MS) medium.

The application of tissue culture techniques for the regulation and commercial propagation of rose is recently developed. Many works have been done on roses since 1970, when Elliott (the first man) reported shoot multiplication and rooting of rose on different concentrations of hormones for the initiation of callus. Its maintenance and regeneration of shoot and roots from the callus was gotten directly from nodal segments, lateral and axillary buds and shoot tips (Vijya and Satyanarayan, 1991; Ali, 1993; Chu, 1993; Syamal and Singh, 1994; Jahan, 1997; Dobres, 1998; Kintzios, 1999; Rout, 1999; Dobois, 2000; Soomro, 2001; Senapati and Rout 2008). In the present investi-gation, the experiments were conducted to test a number of new concentrations of growth hormones and their combination which will provide a comparative account of the *in vitro* responses of three cultivars of *Rosa hybrida* with respect to bud break, bud proliferation, shoot multiplication and rooting. Also, their establishment in green house was subsequently transferred to the field. The objective of the present investigation is to induce mutation in apical and axillary meristems of *R. hybrida* cv First red by using oryzalin ($C_{12}H_{18}N_4O_6$) as the chemical mutagen.

## MATERIALS AND METHODS

### Initiation of nodal culture

Internodal segments (8 to 10 cm long) of *R. hybrida* cv. First Red were collected from the Rose garden of Regional Plant Resource Center, Bhubaneswar. They were recut into 3 to 4 cm length and washed thoroughly with 2% (v/v) 'Teepol' (Qualigen, Bumbai, India) for about 15 min with constant shaking, and were then rinsed with water from a running tap. Further, its surface was sterilized with 0.1% (w/v) mercuric chloride solution for 30 min with constant shaking followed by rinsing with sterile distilled water thrice. The internodal segments were further cut into 0.5 to 1.0 cm length with a single node and were used as explants source. The nodal explants were cultured on a semi-solid MS basal medium supplemented with different concentrations of benzyl adenine (BA) (0, 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l), kinetin (Kn) (0, 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l), indole-3-acetic acid (IAA) or naphthaleneacetic acid (NAA) (0, 0.1, 0.25 and 0.5 mg/l) alone or in combination for shoot multiplication. Agar-agar (7 g/l) was added to the medium as a gelling agent after adjusting the pH to 5.7 to 5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving. Routinely, 20 ml of the molten medium was dispersed into the culture tube (25 x 15 mm) plugged with non-absorbent cotton wrapped in one layer of cheesecloth and autoclaved at 121°C or 1.06 kg/cm$^2$ for 15 min. Consequently, the explants were inoculated aseptically. The cultures were maintained at 25 ± 2°C under 16 h photoperiod in cool white fluorescent lamps (Philips, Mumbai, India) with 3.0 Klux, and also in a fresh medium (MS + 2.0 mg/l BA + 50 mg/l Ads + 0.25 mg/l IAA + 3% sucrose) at 4 weeks interval.

### Induction of mutation

After a successful development of the multiple shoots, the epical and axilary meristems were isolated aseptically and treated with different concentration (0, 5, 10, 15, 20, 30 and 40 µm) of oryzalin for different time periods (1, 3, 6, 12, 18 and 24 h) with constant shaking at 100 rpm in a rotary shaker. After pretreatment, both treated and control apical axilary meristems were removed and shocked in the blotting paper aseptically. Subsequently, the meristems were transferred to the glass petriplate (Figures 1A and B) containing the basal MS medium (Murashige and Skoog, 1662) supplemented with 0.5 mg/l 6-benzylaminopurine (BAP), 0.01 mg/l IAA, 25 mg/l adenine sulphate (Ads) and oryzalin of the same concentration in which the explants were pretreated and incubated under 16 h. As such, the photoperiod was provided by cool and fluorescent light (3000 lux). After two weeks, the treated explants were transferred to the test tube containing MS basal medium supplemented with 0.5 mg/l BAP, 0.01 mg/l IAA and 25 mg/l Ads without oryzalin. After another 4 weeks, the explants were transferred to the multiplication medium, that is, the MS medium supplemented with 2 mg/l BAP, 0.25 mg/l IAA and 50 mg/l Ads (Figure 1D). After a successful multiplication, shoots from the shoot clumps were separated and inoculated into a semi-solid half-strength MS medium supple-mented with 2% (w/v) sucrose and different concentrations of IBA, IAA and NAA (0.1, 0.25 and 0.5 mg/l) alone or in combination. Thus, one excised shoot was placed in each tube (25 x 150 mm) having 15 ml of the culture media. All the cultures were incubated at 25 ± 2°C under 16 h photoperiod with 3.0 Klux intensity. Rooted micropropagules were thoroughly washed to remove the adhering gel and were planted in an earthen pot containing 100% sand for 2 weeks in the green house. After developing the good root systems, it was subsequently transferred to 6" earthen pots containing sterile mixture of sand, soil and cow-dung manure in the ratio of 1: 1: 1 (v/v). The pots were kept in the green house for acclimatization and the experiment was repeated at least three times. The data pertaining to mean percentage of cultures showing response, number of shoots per culture, mean percentage of rooting and number of roots per shoot, were statistically analyzed by the Post-Hoc Multiple Comparison test (Marascuilo and McSweeney, 1977). Between the treatments, the average figures followed by the same letters were not significantly different at $P < 0.05$ levels.

### DNA isolation and quantification

In the preliminary identification of mutants, the mutant leaf material was collected from 28 treated explants of *R. hybrida cv.* First red for DNA isolation. DNA was extracted from young leaves of 2 months old *in vitro* raised shoots using N-cetyl-N, N, N-trimethyl ammonium bromide (cetyltrimethylammonium bromide (CTAB)) method described by with some modification. 0.5 g of the leaf sample were collected aseptically and then ground in liquid nitrogen. 1 ml pre heated extraction buffer (2% CTAB w/v 0.2% β-mercaptoethanol v/v, 100 mM Tris HCl pH = 8.0, 2 mM EDTA, 1.4 M NaCl) was then added per 0.5 g of the fresh material. Then it was incubated for 2 h

at 65°C with moderate shaking.

Extraction with chloroforom:isoamylalcohol (24:1) was done once and the DNA was precipitated with a two-third cold isopropanol. As such, the DNA pellet was resuspended in 100 µl of the TE buffer (10mM Tris-HCl pH-8.0 and 0.1 mM EDTA pH-8.0). Subsequently, the crude DNA was purified and the first RNase-A was added (10 ug/ml), followed by an incubation at 37°C for 1 h. Final extraction was done with Phenol:chloroform:isoamyl (25:24:1) followed by chloroform:isoamylalcohol (24:1). The DNA was then precipitated with a one-tenth volume of sodium acetate and with chilled ethanol for 2.5 times. Afterward, the DNA pellet was resuspended in 20 µl of Tris-ethylenediaminetetraacetic acid (EDTA) buffer. In order to know the quality and quantity of the isolated genomic, DNA was examined by gel electrophoresis in 1% agarose in Tris acetate-EDTA (TAE) buffer pre-stained with ethidium bromide (0.5 ug/ml). Electrophoresis was done at 50 V for 1 h and the DNA was visualized under U.V transilluminator. Nonetheless, the concentration of DNA was estimated by comparing it with an uncut lambda DNA marker.

**Polymerase chain reaction (PCR) amplification for randomly amplified polymorphic DNA (RAPD) assay**

Forty decamer primers corresponding to kit A, C, D and N from operon technologies (Alameda, California) for RAPD were initially screened to determine the suitability of each primer for the study. A total of 8 out of the forty RAPD primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products of treated explants. A reaction mixture of 25 µl for RAPD assay contained 20 ng template DNA, 100 mM for each dNTPs, 15 ng of decanucleotide primers (M/S Operon Technology, Inc., Almeda, CA 94501, USA), 1.5 mM MgCl, 1x Taq buffer (10 mm Tris-HCl, pH 9.0, 50 mM KCl and 0.01% gelatin) and 0.5 U Taq DNA polymerase (M/S Bangalore Genei, India). DNA amplification was performed in a PTC 100 thermal cycler (MJ research, USA) programmed as follows: preliminary activation at 94°C for 2 min followed by 40 cycles at 94°C for 20 s, 38°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 5 min. Before amplification, PCR reaction was optimized for a concentration of $MgCl_2$, template DNA, primer and also for a number of cycles. Amplification products were visualized in 1% agarose gel electrophoresis in 1X TAE (Tris acetate-EDTA) at 60 V for 3 h and pre-strained with ethidium bromide. A size marker, which is a 1.0-kb plus ladder (M/S Bangalore Genei, India), was run in each gel, while the gel photographs were scanned through Gel Documentation System (Gel Doc. 2000, BioRad, USA) and the amplification products size was evaluated using the software Quantity One (BioRad, USA). The analysis used for each primer was repeated twice to establish reproducibility of the banding pattern.

## RESULTS AND DISCUSSION

### Micro propagation of the control plant

Among the three cytokinins tested, the medium containing BA plus Ads showed a higher rate of bud break that subsequently developed into multiple shoots as compared to Kn or Kn + Ads. Bud break was achieved at about 85 to 90% within 7 to 8 days of inoculation in the MS medium supplemented with 0.5 mg/l BA + 0.01 mg/l IAA +25 mg/l Ads. Feeble callusing was observed at the base of the explants because of the presence of endogenous auxin levels in the explants. The maximum rate of shoot multiplication was achieved on the nutrient medium containing 2 mg/l BA, 50 mg/l Ads and 0.25 mg/l IAA (Figure 1C). The rate of multiplication was 4.84 in the medium supplemented with 2 mg/l BA + 0.25 mg/l IAA + 50 mg/l Ads after four weeks of subculture (Table 1). The rate of shoot multiplication depends on the period of subculture and type of nutrient medium. While the high frequency of shoot multiplication was obtained during the 6th to 7th subculture, it declined in subsequent subculture. Maximum percentage of rooting was 62.8% in the nutrient medium having 0.25 mg/l IBA within 21 days of culture (Table 2 and Figures 1E and F). Little friable callus was formed at the cut end of the micro shoot when it was transferred to the medium containing a higher concentration of IBA (0.5 mg/l), because of the imbalance of IBA concentration and the physiological status of microshoots.

### Effect of oryzalin

A decrease in the survival of nodal cultures was observed as the concentration of oryzalin in the medium increased. It was also observed that with the increasing pre-treatment time period, the percentage of survival decreases, and at 20µM oryzalin for 24 h, we got the fifty percent survivality (Figure 2 and Tables 3 and 4). After getting the 50% survivability, the treated shots were transferred to the different medium for multiplication and rooting, followed by acclimatization. However, it showed a+ significant difference than that of the control (Table 5).

### Screening of mutants by RAPD markers

Mutations resulting in polymorphisms are those occurring on primer binding sites, leading to an increase or decrease in the total number of primer binding sites, and consequently in the number of amplified fragments. In this study, forty primers were assayed, out of which 8 primers revealed a total of 72 PCR fragments over the entire nodal culture tissues that were used. Among those fragments, 55 were monomorphic across all treatments (including control) and thus, generated electrophoretic profiles. However, primers OPN-8, OPN-2 and OPN-7 generated polymorphic fragments, which were also confirmed in a repeated experiment. The polymorphism generated by the primer OPN-2 produced polymorphic fragments of ~800 bp in size (Figure 3A). Similarly, primer OPN-7 generated polymorphic fragments of ~900 and ~2100 bp in size (Figure 3B). Also, gel electrophoresis revealed some missing bands in different regions. Changes in DNA, caused by mutagens, result in a genetic variation detected by RAPD analysis (Teparkum and Veilleux, 1998; Lanteri, 2001; Hofman et al., 2004). However, it has to be noted that direct-acting alkylating agents, such as EMS, primarily cause point

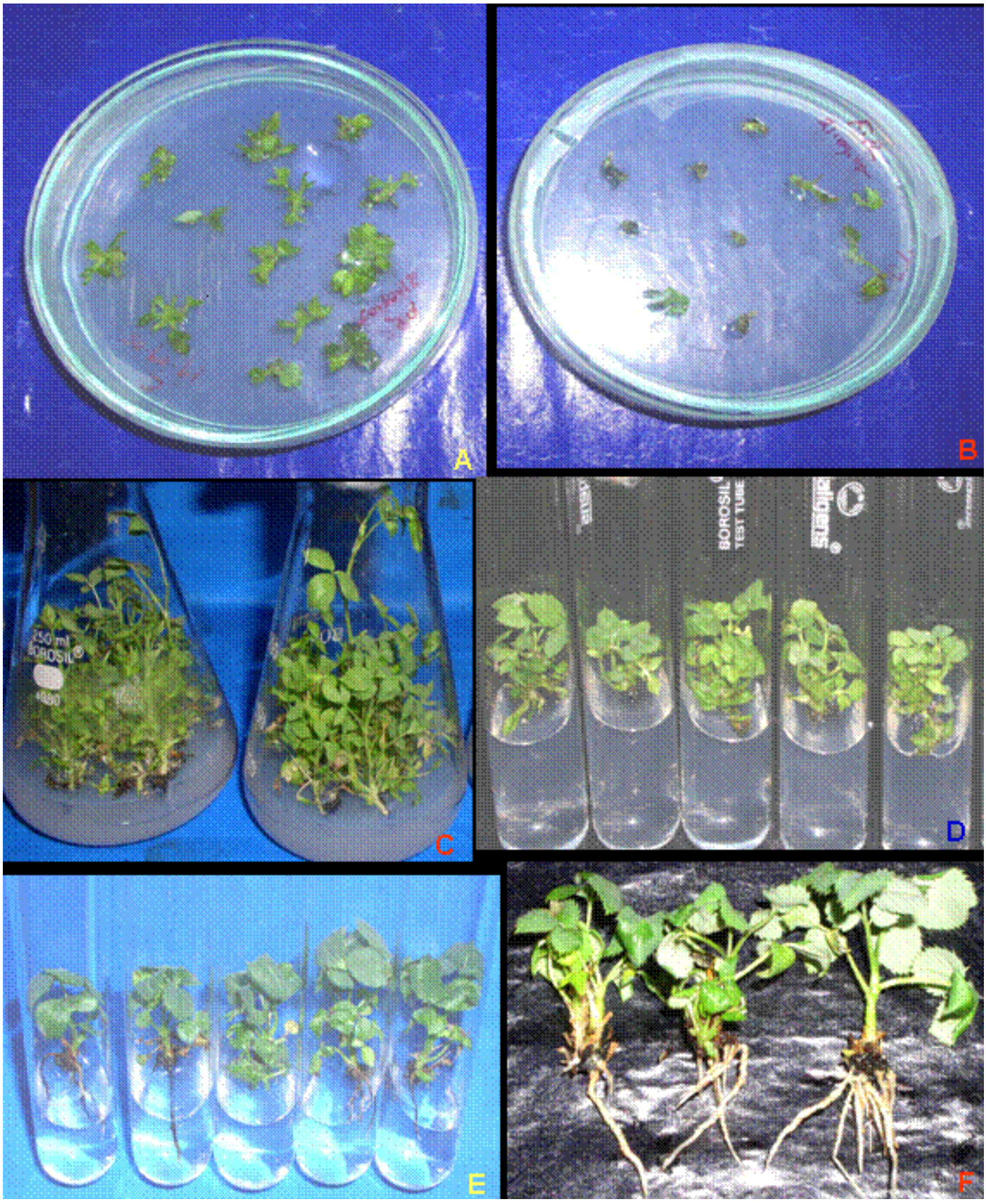


**Figure 1.** A, Control shoots in the control medium; B, treated shoots in the treated medium; C, Multiplication of the control shoots; D, multiplication of the treated shoots; E and F, rooting of the micro shoots.

**Table 1.** Effect of cytokinins and auxins on shoot multiplication of *R. hybrida* cv. First Red after 4 weeks of subculture.

| MS + Growth regulators (mg/l) | | | | | Percent of multiple shoots (Mean ± S.E)* | Av. number of shoots per culture (Mean± S.E)* |
|---|---|---|---|---|---|---|
| BA | Kn | Ads | NAA | IAA | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0 | 0 | 0 | 0 | 32.6 ± 1.1 $^{d}$ | 2.14 ± 0.8$^{c}$ |
| 1.0 | 0 | 0 | 0 | 0 | 36.8 ± 0.9 $^{f}$ | 2.86 ± 0.6$^{d}$ |
| 1.5 | 0 | 0 | 0 | 0 | 46.2 ± 1.2 $^{g}$ | 3.10 ± 0.5$^{e}$ |
| 2.0 | 0 | 0 | 0 | 0 | 50.8 ± 1.3 $^{h}$ | 3.34 ± 0.7$^{f}$ |
| 2.5 | 0 | 0 | 0 | 0 | 52.6 ± 1.2 $^{i}$ | 3.67 ± 0.8$^{g}$ |
| 0 | 1.0 | 0 | 0 | 0 | 20.6 ± 1.0 $^{a}$ | 1.56 ± 0.4$^{a}$ |
| 0 | 1.5 | 0 | 0 | 0 | 28.4 ± 0.6 $^{b}$ | 1.88 ± 0.6$^{b}$ |
| 0 | 2.0 | 0 | 0 | 0 | 30.6 ± 0.8 $^{c}$ | 2.12 ± 0.7$^{c}$ |
| 0 | 0 | 25 | 0 | 0 | 32.6 ± 1.0 $^{d}$ | 1.87 ± 0.5$^{b}$ |
| 0 | 0 | 50 | 0 | 0 | 34.8 ± 0.9 $^{e}$ | 2.10 ± 0.4$^{c}$ |
| 2.0 | 0 | 0 | 0.10 | 0 | 62.6 ± 0.8 $^{l}$ | 4.12 ± 0.8$^{i}$ |
| 2.0 | 0 | 0 | 0.25 | 0 | 63.2 ± 1.2 $^{l}$ | 4.26 ± 0.6$^{i}$ |
| 1.5 | 0 | 0 | 0 | 0.10 | 65.6 ± 0.8 $^{m}$ | 4.10 ± 0.7$^{i}$ |
| 2.0 | 0 | 0 | 0 | 0.25 | 78.8 ± 1.0 $^{p}$ | 3.84 ± 0.8$^{h}$ |
| 2.0 | 0 | 0 | 0 | 0.50 | 70.2 ± 1.0 $^{n+}$ | 3.92 ± 0.6 $^{h}$ |
| 2.0 | 0 | 25.0 | 0 | 0.25 | 76.2 ± 0.8 $^{o}$ | 4.11 ± 0.8$^{i}$ |
| 2.0 | 0 | 50.0 | 0 | 0.25 | 80.4 ± 1.2 $^{q}$ | 4.84 ± 0.5$^{i}$ |
| 0 | 2.0 | 0 | 0.10 | 0 | 54.6 ± 0.8 $^{j}$ | 3.22 ± 0.6$^{d}$ |
| 0 | 2.0 | 0 | 0.25 | 0 | 58.8 ± 1.0 $^{k}$ | 3.42 ± 0.8$^{f}$ |
| 0 | 2.0 | 25.0 | 0.25 | 0 | 64.4 ± 1.2 $^{m}$ | 3.62 ± 0.5$^{g}$ |
| 0 | 2.0 | 50.0 | 0.25 | 0 | 62.0 ± 0.8 $^{l+}$ | 3.54 ± 0.6$^{g}$ |

*20 Replicates/treatment repeated thrice; a to q: Mean having same letter are not significantly different by Post-Hoc multiple comparison test at $P < 0.05$ level.

**Table 2.** Effect of auxins on root induction from microshoots of *R. hybrida* cv. First Red after 3 weeks of culture.

| ½ MS + Growth regulators (mg/l) + 2% sucrose (w/v) | | | Percent of shoot rooted (Mean ± S.E)* | Av. No. of roots/shoot (Mean ±S.E)* |
|---|---|---|---|---|
| IAA | IBA | NAA | | |
| 0 | 0 | 0 | 0 | 0 |
| 0.10 | 0 | 0 | 0 | 0 |
| 0.25 | 0 | 0 | 24.0 ± 0.5 | 2.12 ± 0.4 |
| 0.50 | 0 | 0 | 32.7 ± 0.6 $^{+}$ | 3.14 ± 0.6 $^{+}$ |
| 0 | 0.10 | 0 | 42.0 ± 0.7 | 3.11 ± 0.5 |
| 0 | 0.25 | 0 | 62.8 ± 1.2 | 5.23 ± 0.6 |
| 0 | 0.50 | 0 | 54.0 ± 0.7$^{+}$ | 4.48 ± 0.7 $^{+}$ |
| 0 | 0 | 0.10 | 35.3 ± 0.6 | 3.82 ± 0.6 |
| 0 | 0 | 0.25 | 40.7 ± 0.6 | 3.68 ± 0.5 |
| 0 | 0 | 0.50 | 41.3 ± 0.5 $^{+}$ | 3.0 ± 0.7 $^{+}$ |
| 0.10 | 0.25 | 0 | 52.8 ± 0.7 | 4.23 ± 0.6 |
| 0.25 | 0.10 | 0 | 50.6 ± 0.8 $^{+}$ | 4.06 ± 0.3 $^{+}$ |
| 0.10 | 0 | 0.50 | 46.2 ± 0.5 $^{+}$ | 3.27 ± 0.5 $^{+}$ |

*50 Microshoots per treatment repeated thrice; $^{+}$callusing at the basal cut end.

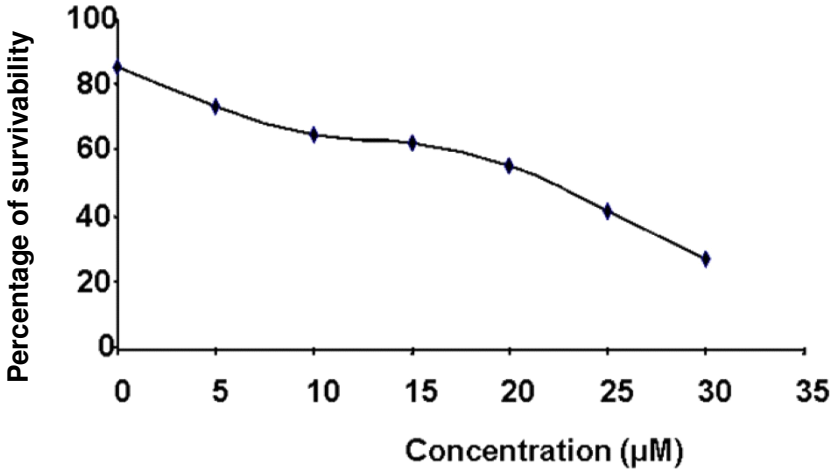


**Figure 2.** Survival percentage of meristems treated with different concentration of oryzalin.

**Table 3.** Percentage of survivability in different concentrations at a constant time period after 2 weeks of transfer to the solids medium.

| Concentration (µM) | Treatment time period (in hours) in the liquid medium | Percentage of survivability |
|---|---|---|
| 0 | 24 | 85.30 |
| 5 | 24 | 73.50 |
| 10 | 24 | 64.50 |
| 15 | 24 | 62.50 |
| 20 | 24 | 55.33 |
| 25 | 24 | 41.39 |
| 30 | 24 | 26.38 |

**Table 4.** Percentage of survivability at different time periods at constant concentration after 2 weeks of transfer to the solid medium.

| Treatment time period (in hours) in liquid medium | Concentration (µM) | Percentage of survivability |
|---|---|---|
| 0 | 20 | 98.60 |
| 6 | 20 | 76.80 |
| 18 | 20 | 63.11 |
| 24 | 20 | 55.33 |
| 36 | 20 | 37.30 |

mutations which are the result of a single base pair deletion, addition or substitution (Schy, 1989). Polymorphic amplification products which represent one allele per locus can result from changes in either the sequence of the primer binding site, such as point mutations, or from changes altering the size or preventing successful amplification of a target DNA such as insertions, deletions and inversions (Rani, 1995). In the present study, eight RAPD primers have revealed an average of 23.6% polymorphisms among nodal cultures, which are most likely due to treatment with the chemical mutagen oryzalin (Table 6). These polymorphisms indicate the presence of genetic differences in nodal explants of *R. hybrida* cv. First red treated with various

**Table 5**. A comparative analysis between the control and treated plants after 4 weeks of sub-culture.

| Characters | Control plant | Treated plant |
|---|---|---|
| Percentage of shoot multiplication | 80.4% | 76.4% |
| Average no. of multiple shoots per plant | 4.84 ± 0.5 | 4.56 ± 0.28 |
| Percentage of shoots rooted | 62.8% | 72.8% |
| Average no. of roots/shoot | 5.23 ± 0.6 | 5.86 ± 1.2 |
| Percentage of survivability after acclimatization | 60% | 70% |

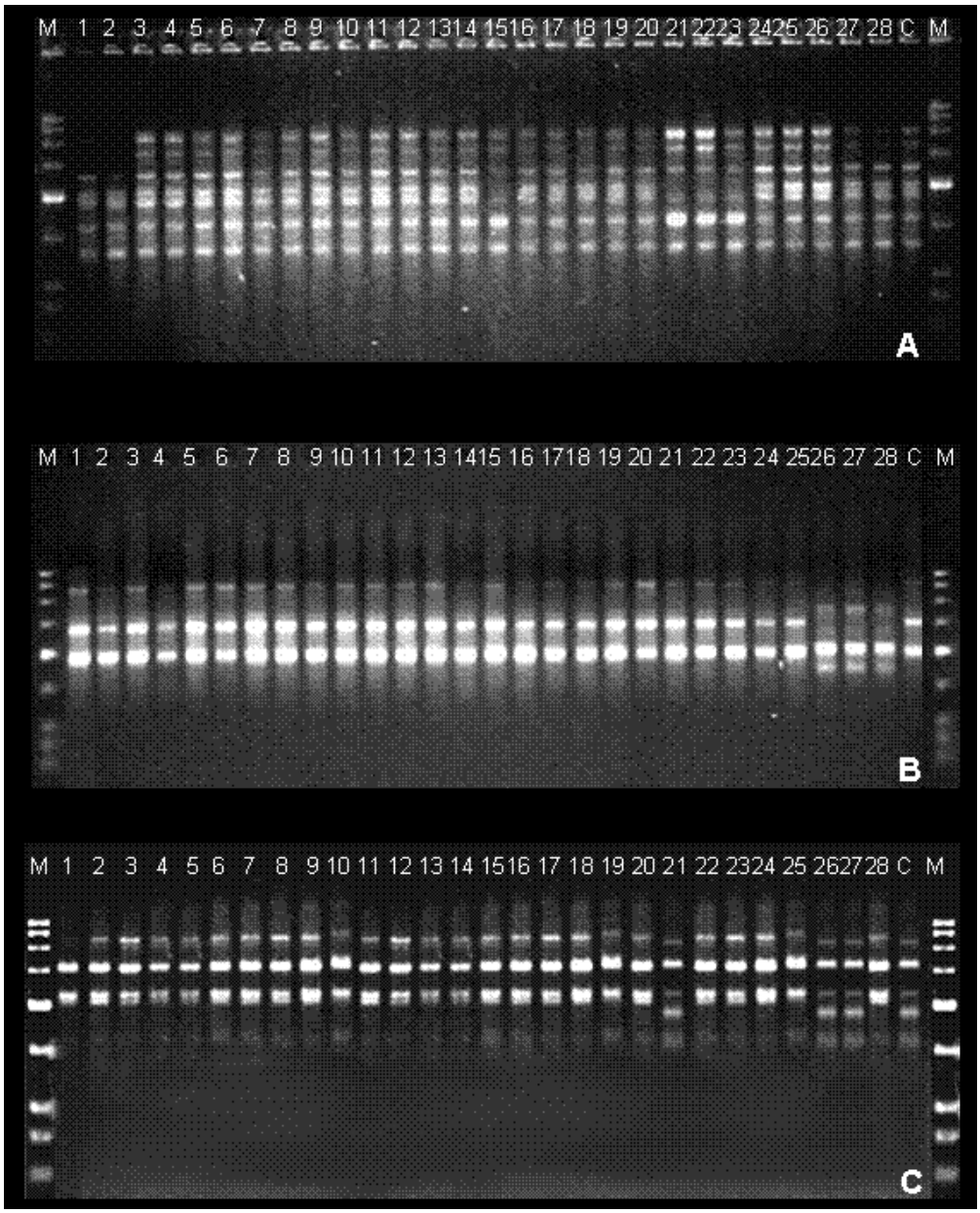


**Figure 3**. RAPD banding pattern with primers. A, OPN-08; B, OPN-02 and C, OPN-07 of the treated microshoots compared with the untreated microshoots of *R. hybrida* cv First red.

concentrations of oryzalin. It is possible that these mutations have occurred in different loci, although it is not yet known whether these mutations have resulted in alterations of agronomical useful traits. RAPD polymor-

**Table 6.** Details of RAPD primers used for primer screening of oryzalin treated plants.

| Primer | Primer sequence | Total no. of bands | Monomorp-hic bands | Polymorphic bands | Percentage of polymorphism | Band range In kbP |
|---|---|---|---|---|---|---|
| OPN-02 | 5-'ACCAGGGGCA-3' | 07 | 04 | 03 | 42.8 | 0.80-2.8 |
| OPN-07 | 5'-CAGCCCAGAG-3' | 07 | 04 | 03 | 42.8 | 0.70-2.3 |
| OPN-08 | 5'-ACCTCAGCTC-3' | 09 | 07 | 02 | 28.5 | 0.40-2.1 |
| OPA-02 | 5'-TGCCGAGCTG-3' | 07 | 05 | 02 | 28.5 | 0.40-1.9 |
| OPA-04 | 5'-AATCGGGCTG-3' | 13 | 12 | 01 | 07.6 | 0.32-2.0 |
| OPD-03 | 5'-GTCGCCGTCA-3' | 08 | 06 | 02 | 25.0 | 0.30-1.7 |
| OPD-08 | 5'-GTGTGCCCCA-3' | 10 | 09 | 01 | 10.0 | 0.35-1.9 |
| OPD-20 | 5'-ACCCGGTCAC- 3' | 11 | 08 | 03 | 27.2 | 0.50-2.1 |
| Total | | 72 | 55 | 17 | 23.61 | 0.30-2.8 |

phisms observed in this study are likely due to alterations in the number of primer binding sites following mutagenesis. Al-Zhaim et al. (1999) have reported that although RAPDs are capable of detecting polymorphism in somaclonal variants of garlic derived from *in vitro*-grown embryogenic callus cultures, no association has been found between the frequency of the cytological changes and the frequency of RAPD polymorphisms observed. Similarly, Zucchi, (2002) have detected polymorphisms among somaclonal variants of sugarcane plants derived from rhizome and meristem cultures, but these polymorphisms have not been correlated with a number of subcultures. In this study, 17 polymorphisms were detected out of the 72 fragments assayed, and it indicated a high level of mutation due to chemical mutagenesis as RAPD markers tend to underestimate the level of polymorphisms observed.

Thus, RAPD markers are useful in detecting polymorphisms in nodal cultures of *R. hybrida* cv. First red subjected to chemical mutagenesis as they provide sufficient numbers of DNA fragments for conducting assays. This approach can then be used to rapidly screen large numbers of cultures in the efforts of detecting mutants with desirable agronomic.

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