

# Chromosome Observation of Rice Root Tip and Effect of Callus Colour Variation and Texture in Different Colchicine Concentrations on the Induction of Rice Polyploids

PCD Perera\*, Nilanthi Dahanayake

Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, Sri Lanka

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**Abstract** The abiotic stresses course major environmental factors that determine the most serious yield reduction in rice (*Oryza sativa* L.). The abiotic stresses tolerant ability can be improved by changing typical chromosome number of plants. The objectives of the studies were to evaluate the performance of callus of rice cultivars (Suwadel and Sulaai) for different Colchicine concentrations to produce polyploidy for enhance tolerant characteristics to drought and salinity stresses. Surface sterilized seeds were introduced to Murashige and Skoog (MS) basal medium with hormone  $2\text{mgL}^{-1}$  2,4-D (2, 4-dichlorophenoxyacetic acid) and  $0.1\text{mgL}^{-1}$  BAP (6- benzylamino purine) for callus induction. Callus of  $0.5\text{cm}^2$  from rice cultivars introduced to different Colchicine concentrations (0, 30, 60, 90 and  $120\text{mgL}^{-1}$ ) and different time durations (12, 24 and 78 hours). Treated callus were introduced to shoot regeneration on MS medium with  $0.1\text{mgL}^{-1}$  IAA (Indole acetic acid) and  $2\text{mgL}^{-1}$  BAP. Colour, texture and regeneration ability of callus were recorded after one month. Completely Randomized Design (CRD) with five replicates was used for study. Statistical analysis was performed with Duncan's multiple range tests using SAS software (version 9.1.3). Results showed that callus treated from Colchicine 30, 60, 90 and  $120\text{mgL}^{-1}$  in 12 hours and, 30, 60, 90  $\text{mgL}^{-1}$  in 24 hours have potential to survive. Increasing Colchicine concentration and time duration showed that regeneration ability of callus reduced in selected rice varieties.

**Keywords** Colchicine, *Oryza Sativa* L., Abiotic Stresses, Regeneration Ability

## 1. Introduction

Rice (*Oryza sativa* L.) is the primary source of calories for people more than half of the world's population mainly in

Asia (Heong *et al*, 2005). Rice is the single most important crop occupying 34 percent (0.77 /million ha) of the total cultivated area in Sri Lanka. About 1.8 million farm families are engaged in paddy cultivation island-wide (Department of Agriculture, 2006). Rice is a cereal monocotyledonous plant in family Poaceae with a genome consisting of 430Mb across 12 ( $2n=24$ ) chromosomes. Genetic engineering offers an alternative approach for developing tolerant crops. Plants that have more than the normal two sets of chromosomes are termed "polyploidy" in general although specific names are given to the certain chromosome numbers (e.g. tetraploid or  $4N$  plants have four sets of chromosomes). Polyploid plants are generated in an effort to create new plants that have new characteristics. Colchicine is a toxic natural product (allelopathic compound) and secondary metabolite, originally extracted from plants of the genus *Colchicum*. It uses for inducing polyploidy in plant cells during cellular division by inhibiting chromosome segregation during meiosis (prevents the spindle formation). This study was done to produce polyploid paddy plants to enhance characteristics (Abiotic stresses, large seed, improve tillering, to increase productivity, ect.) to identify the effective treatment duration, the effective concentration of colchicine and the variations from polyploidy plantlets regeneration.

## 2. Methodology

The experiments were conducted at the Department of Agricultural Biology research laboratory, Faculty of Agriculture, University of Ruhuna, Sri Lanka. Seeds of traditional rice cultivars; Sulaai and Suwadel were used for the experiment. First, seeds were dehusked using forceps and seeds rinsed with soap washed with distilled water. Then seeds were sterilized once with 70% (v/v) ethanol for 3 min. The seeds were soaked in 20% (v/v) Chlorox solution (Sodium hypochlorite) for 20 min followed by rinsing in

sterile distilled water for 3 times. They were dried on sterile filter papers. Surface sterilized seeds were introduced to test tubes (2 seed per tube) containing 3ml of MS basal medium with  $2\text{mgL}^{-1}$  2,4-D and  $0.1\text{mgL}^{-1}$  BAP for callus induction. MS medium was used with  $30\text{gL}^{-1}$  sucrose and solidified by Agar ( $8\text{gL}^{-1}$ ). Medium was autoclaved for 21 minutes at  $121^\circ\text{C}$  after adjusting the pH to 5.8 (Dahanayake *et al*, 2012). Callus of  $0.5\text{cm}^2$  were introduced for different Colchicine concentrations ( $30\text{mgL}^{-1}$ ,  $60\text{mgL}^{-1}$ ,  $90\text{mgL}^{-1}$  and  $120\text{mgL}^{-1}$ ) and kept them in different duration hours (0h, 24h, 48h and 72h). Treated callus were introduced to shoot regeneration on MS medium with  $0.1\text{mgL}^{-1}$  IAA and  $2\text{mgL}^{-1}$  BAP. Cultures were incubated at light intensity of  $1000\ \mu\text{mol/m}^2/\text{sec}$  at  $25\pm 1^\circ\text{C}$  and 70-80% relative humidity with a 16/8 hrs light/dark photoperiod. All experiments reported here were done Completely Randomized Design (CRD) with five replicates. Data were collected after one month of Colchicine treatment. Callus colour were given numbers 1-3 according to sequential of yellow, Greenish yellow and brown and texture were ranked according to numbers 1-2 sequential of compact or soft and were taken the mean value of given marks in all kind of tested specimens. Also, number of dates for shoot initiation and number of emerging buds per callus were observed. Statistical analysis was carried out using Duncan's multiple range test of SAS software (version 9.1.3).

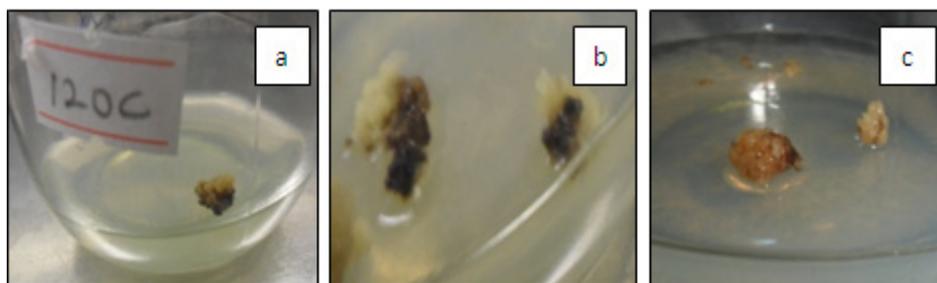
**Chromosome observation:** Immature root tips of rice (about 0.5cm) were taken and dipped in 0.01% colchicines for 2 hours. After that, it was washed with distilled water and kept in 18 hours in Ethanol(95%) and Acetic acid combination; 3:1 (V/V) respectively. Again root tips were washed with distilled water and put into 1N HCl solution. It was kept in water bath three minutes at  $65^\circ\text{C}$  temperature. After hydrolysis, root tips were rinsed with distilled water for 10 min and cut to obtain shorter root tips about 1.5 mm.

These prepared root tips were then placed on slid glass, stained with one drop of carbol fuchsin solution for 1-2 minutes, squashed under cover glass and cell samples of the root tips were observed for chromosomes under a microscope (Leica DLMB2), and photos were taken with the associated apparatus. A plant with all the root tip cells showing 24 chromosomes was determined as diploid, a plant with some cells showing 24 and the other cells showing 48 chromosomes was determined as chimera, and a plant with all the cells showing 48 chromosomes was determined as tetraploid.

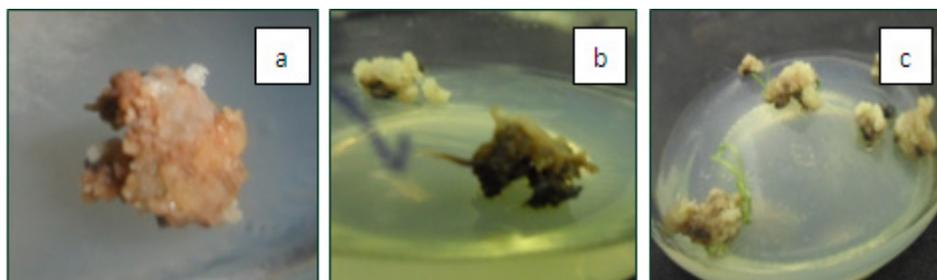
For preparation of staining solution (Carbol fuchsin solution), solution A was prepared by dissolving 0.3 g of basic fuchsin in 10 ml of 70% ethanol and adding 90 ml of 5% phenol, which should be used in 2 weeks. Solution B was made by mixing 55 ml of solution A with 6 ml of glacial acetic acid and 6 ml of 37% formaldehyde, which can keep for a long time. The staining solution was prepared by mixing 10 ml of solution B with 90 ml of 45% glacial acid and adding 1.8 g of sorbitol (Li and Zhang, 1991). A few drops of glycerin were added to enhance the staining capacity and prolong the time of preservation and observation.

### 3. Result and Discussion

Experiments were only observed in Sullai and Suwadel rice cultivars effect of callus colour variation and texture in different colchicine concentrations after one month. Both Sulaai and Suwadel were showed that control treatment and all the colchicine treatments of 12 hours exposed callus and 24 hours exposed callus in 30, 60 and  $90\text{mgL}^{-1}$  were yellow colour. Treatment  $120\text{mgL}^{-1}$  in 24 hours and all the treatment of 78 hours exposed to colchicines were turned into brown colour after one month.



**Figure 1.** Growing of callus due to effect of colchicines on callus growth of Suwadel; a,  $120\text{mgL}^{-1}$  24hours; b,  $60\text{mgL}^{-1}$  78hours; c,  $90\text{mgL}^{-1}$  78hours



**Figure 2.** Browning of callus due to effect of colchicines on callus growth of Sulaai; a,  $120\text{mgL}^{-1}$  24hours; b,  $120\text{mgL}^{-1}$  78hours; c,  $90\text{mgL}^{-1}$  78 hours

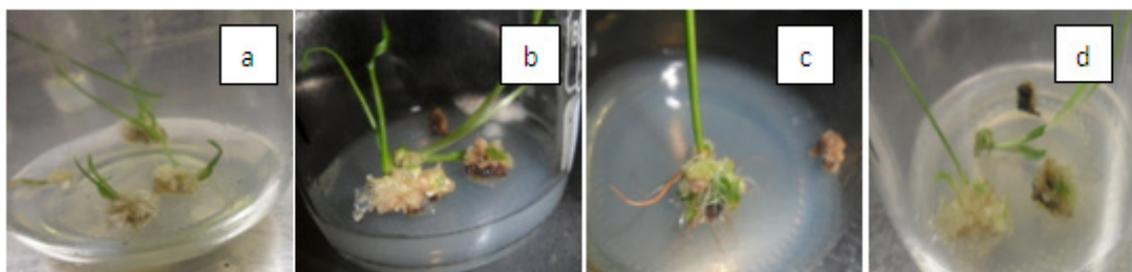
After one month, when observed callus texture in all different colchicine concentrations were compact.

**Table 1.** Number of days to regenerate plantlets and number of buds from callus

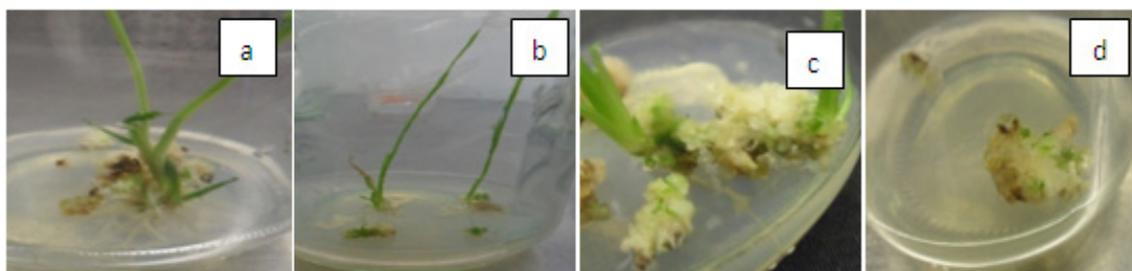
Hour	Colchicine concentrate (mgL <sup>-1</sup> )	Average No. of buds		No. of days for shoot initiation
		Suwadel (after month)	Sulaai (after month)	
	Control	6 <sup>a</sup>	8 <sup>a</sup>	22 <sup>d</sup>
12	30	5 <sup>a</sup>	6 <sup>a</sup>	31 <sup>c</sup>
	60	4 <sup>b</sup>	5 <sup>b</sup>	35 <sup>b</sup>
	90	1 <sup>c</sup>	2 <sup>c</sup>	36 <sup>b</sup>
	120	0 <sup>d</sup>	0 <sup>d</sup>	-
24	30	3 <sup>a</sup>	4 <sup>a</sup>	32 <sup>c</sup>
	60	3 <sup>a</sup>	3 <sup>b</sup>	40 <sup>a</sup>
	90	1 <sup>b</sup>	1 <sup>c</sup>	42 <sup>a</sup>
	120	0 <sup>c</sup>	0 <sup>d</sup>	-

Same letter are not significantly different as determined by Duncan's multiple range test ( $P=0.05$ ).

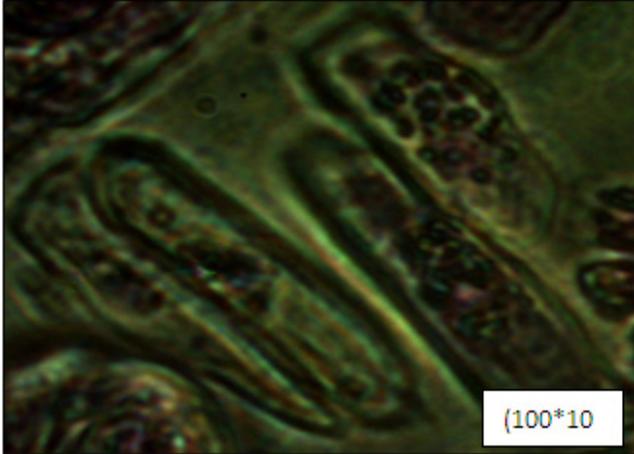
The regeneration rates in the colchicine treatments were lower than those of the control, especially at higher concentrations and longer durations. The first visible effect was shoot regeneration ability and growth of buds delayed significantly on colchicine containing medium compared with non-treated regeneration medium, especially higher concentrations and longer durations inhibited more heavily (Table 1). Higher concentration of colchicine was taken long period to regeneration (Dhahanayake, 2008).



**Figure 3.** Shoot regeneration from callus of Suwadel; a, control; b, 30mgL<sup>-1</sup> 12 hours; c, 60mgL<sup>-1</sup> 78 hours; d, 90mgL<sup>-1</sup> 12 hours.



**Figure 4.** Shoot regeneration from callus of Sulaai; a, control; b, 30mgL<sup>-1</sup> 12 hours; c, 60mgL<sup>-1</sup> 78 hours; d, 90mgL<sup>-1</sup> 12 hours.



**Figure 5.** Diploid chromosomes in rice cell ( $2n = 24$ ) in transition to metaphase under light microscope.

#### 4. Conclusions

Colchicine concentrations 30, 60, 90 and 120 mgL<sup>-1</sup> in 12 hours and 30, 60 and 120 mgL<sup>-1</sup> 24 hours exposed callus have potential to survive. When, increasing Colchicine

concentration and time duration were showed that regeneration ability of callus reduced.

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