Effect of Callus Colour Variation and Texture in Different Colchicine Concentrations on the Induction of Rice Polyploids

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

I. 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 conducted to produce polyploid rice plants through the identification of effective Colchicines treatment duration, concentration and to identify the variations from polyploidy plantlets regeneration that may enhance abiotic stress tolerance, seed size, tillering and productivity.

II. 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 were 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 onto sterile filter papers. Surface sterilized seeds were introduced to test tubes (2 seed per tube) containing 3ml of MS basal medium with 2mgL^{-1} 2,4-D and 0.1 mgL^{-1} BAP for callus induction. MS medium was used with 30 gL^{-1} sucrose and solidified by Agar (8gL^{-1}). Medium was autoclaved for 21 minutes at 121°C after adjusting the pH to 5.8 (Dahanayake *et al*, 2012).Callus of 0.5cm^2 were introduced for different Colchicine concentrations(30 mgL^{-1},60 mgL^{-1},90 mgL^{-1} and 120 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 0.1mgL^{-1} IAA and 2mgL^{-1} BAP. Cultures were incubated at light intensity of 1000 µmolm^{2}sec^{-1} at 25±1°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).

III. RESULT AND DISCUSSION

Following experiments were only observed in Sullai and Suwadel rice cultivars effect of callus colour variation and texture in different colchicine concentrations after one month. In 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 mgL^{-1} were yellow colour. Treatment 120 mgL^{-1} in 24 hours and all the treatment of 78 hours exposed to colchicines were turned into brown colour after one month.

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

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).

CONCLUSION

Colchicine concentrations 30, 60, 90 and 120 mgL-1 in 12 hours and 30, 60 and 120 mgL-1 24 hours exposed callus have potential to survive. When, increasing Colchicine concentration and time duration were showed that regeneration ability of callus reduced in above cultivars.

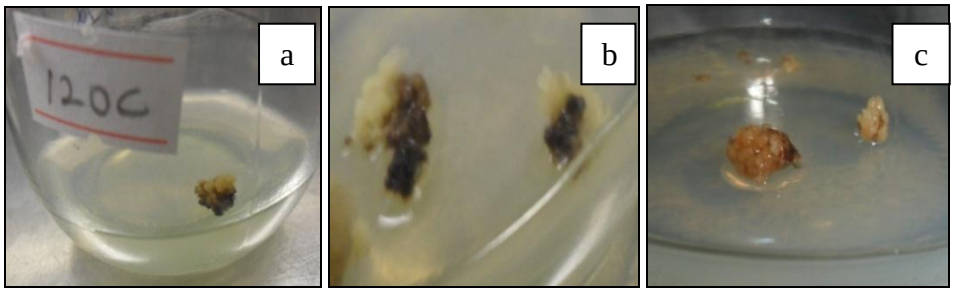

Figure 1. Browning of callus due to effect of colchicines on callus growth of Suwadel; a, 120mgL^{-1} 24hours; b, 60mgL^{-1} 78hours; c, 90mgL^{-1} 78hours.

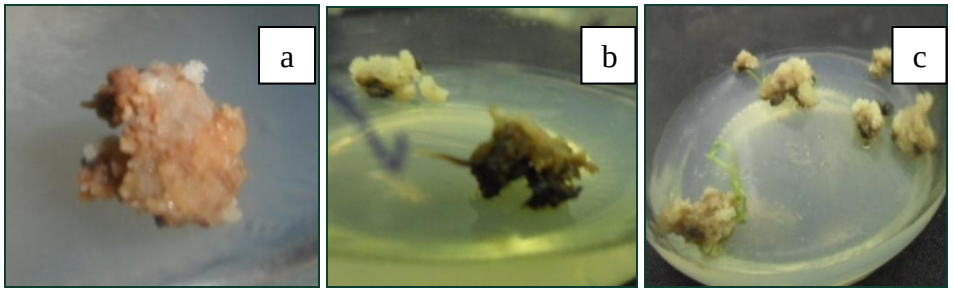

Figure 2.Browning of callus due to effect of colchicines on callus growth of Sulaai; a, 120mgL^{-1} 24hours; b, 120mgL^{-1} 78hours; c, 90mgL^{-1} 78 hours.

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

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

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

REFERENCES

Dahanayake N, Ranawake AL and Wanithunga U (2012) Potential of alternative cheap gelling agents for in vitro micro-propagation of Rice (*Oryza sativa* L.). International Symposium on Agriculture and Environment: 312-314.

Department of Agriculture (2006) [Online] Available: <http://www.agridept.gov.lk> [20 July 2014].

Dhahanayake N (2008) In vitro culture and chromosome doubling of purple coneflower (Echinacea purpurea L.). Ph.D. Thesis, Genetic Engineering Laboratory, Department of Biochemistry and Molecular Biology, College of Life Sciences, South China Agricultural University, Guangzhou 510642, P.R. China: 95-106.

Heong, KL, Chen YH, Johnson DE, Jahn GC, Hossain, M and Hamilton RS (2005) Debate over a GM rice trial in China. Letter in Science 310: 231-233

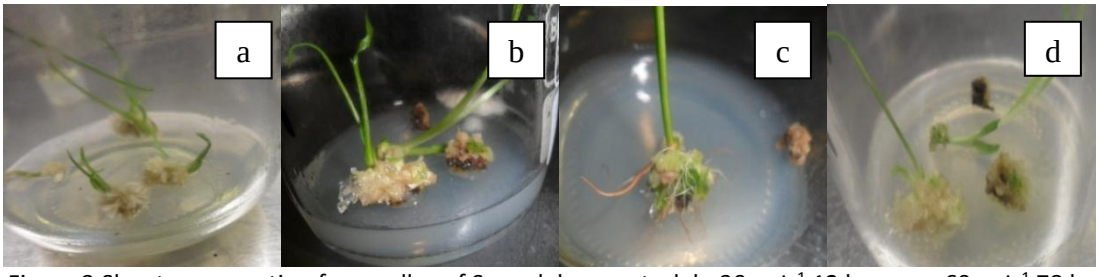

Figure 3.Shoot regeneration from callus of Suwadel; a, control; b, 30mgL^{-1} 12 hours; c, 60mgL^{-1} 78 hours; d, 90mgL^{-1} 12 hours.

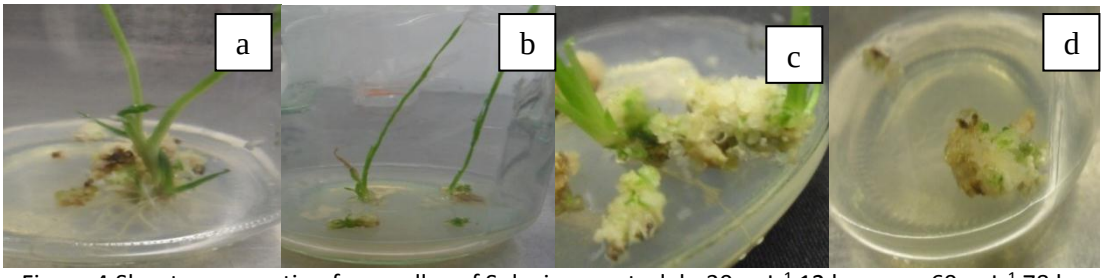

Figure 4.Shoot regeneration from callus of Sulaai; a, control; b, 30mgL^{-1} 12 hours; c, 60mgL^{-1} 78 hours; d, 90mgL^{-1} 12 hours

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