

Effect of Colchicine on some Morphological and Anatomical Characteristics of Homnil Rice Seedling (*Oryza sativa* L.), Landrace Rice of Thailand

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บทคัดย่อ

ศึกษาผลของโคลชิซินต่ออัตราการรอดชีวิต ลักษณะทางสัณฐานวิทยาและกายวิภาคศาสตร์บางประการของข้าวหอมนิล (*Oryza sativa* L.) โดยพอกฆ่าเชื้อเมล็ดข้าวด้วยสารละลายโซเดียมไฮโปคลอไรท์ (Clorox) แล้วแช่เมล็ดข้าวในสารละลายโคลชิซินที่ความเข้มข้น 0 0.01 0.02 0.03 และ 0.04% ในที่มืดเป็นเวลา 24 และ 48 ชั่วโมง เพาะเลี้ยงในอาหารสูตร Murashige & Skoog (MS, 1962) เป็นเวลา 6 สัปดาห์ พบว่าข้าวหอมนิลที่แช่ในสารละลายโคลชิซินเป็นเวลา 24 ชั่วโมง ที่ความเข้มข้น 0.01 และ 0.02 % มีอัตราการรอดชีวิตสูงสุดเท่ากันคือ 66.67% ในขณะที่การแช่ในสารละลายโคลชิซิน เวลา 48 ชั่วโมง พบว่า ต้นข้าวมีอัตราการรอดชีวิตสูงสุดเมื่อแช่ในสารละลายโคลชิซินที่ความเข้มข้น 0.02% ซึ่งมีอัตราการรอดชีวิตเท่ากับ 52.38% การศึกษาลักษณะสัณฐานวิทยา พบว่า ต้นข้าวที่ผ่านการแช่ในสารละลายโคลชิซินมีลักษณะสัณฐานวิทยาเหมือนกับต้นที่ไม่ผ่านการแช่ในสารละลายโคลชิซิน แต่มีจำนวนใบ ความกว้างและความยาวของใบ มากกว่าต้นข้าวที่ไม่ผ่านการแช่ในสารละลายโคลชิซิน สำหรับปากใบของต้นที่ผ่านการแช่ในสารละลายโคลชิซินมีขนาดที่ใหญ่กว่าต้นที่ไม่ผ่านการแช่ในสารละลายโคลชิซิน ผลการศึกษาครั้งนี้สามารถเป็นข้อมูลพื้นฐานสำหรับการปรับปรุงพันธุ์ข้าวในอนาคตได้

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Abstract

Effects of colchicine on survival rate, morphological and anatomical characteristic of Homnil rice (*Oryza sativa* L.) were investigated. Dehusked seed were sterilized in sodium hypochlorite (clorox) and soaked in various concentrations of colchicine (0, 0.01%, 0.02%, 0.03% and 0.04%) in dark incubation for 24 and 48 hours. The treated seeds were then transferred to Murashige & Skoog (MS, 1962) medium for 6 weeks. The results showed that the treated seed for 24 hours at 0.01% and 0.02% colchicine showed the highest survival rate (66.67%). The highest survival rate of 48 hours treatment was found in 0.02% colchicine treatment (52.38%). Morphological studies showed that there was no difference between control and treated plantlet but some characters of treated plant were higher than control in term of average number of leaf, height and width of leaf and guard cells size. The results of this study are also useful as basic data for Homnil rice improvement in the future.

Keywords: colchicine, stomata, Homnil rice (*Oryza sativa* L.)

1. Introduction

Rice is the staple food of around 30-40% of the world population. It is the world most important food crop after wheat and maize. Over 90% of rice is produced and consumed in Asia. *Indica* type rice feeds more than two billion people, predominantly in developing countries. Global population is expected to reach around 10 billion by 2050 (Bano, Jabeen, Rahim & Ilahi, 2005). In the past, Thailand has many varieties of rice, both traditional varieties and improved varieties. For landrace rice or native rice, they

have dominant feature such as suitable for their natural habitat or tolerance to drought and salt that appropriate to the landraces climate. In addition, some landraces variety showed outstanding character such as disease resistance or insect resistance. Moreover, some landraces or traditional varieties have high nutritional value or medicinal value than general distribution rice of Thailand. However, many landraces rice varieties in Thailand are risk in extinction. This study is the first report on colchicine treatment of Homnil rice, landraces rice

of Thailand which could lead to promoted landrace rice valuable and improvement in the future. Moreover, preservation of rice genetic conservation and processing of landraces rice should be more investigated.

2. Material and Methods

Dehusked seed (Figure 1) were sterilized in sodium hypochlorite (clorox) prior to wash in sterile stillled water for 3 times and soaked in various concentrations of colchicine (0, 0.01%, 0.02%, 0.03% and 0.04%) in dark incubation for 24 and 48 hours. After washed in sterile stillled water for 3 times, the treated seed were then transferred to MS (1962) medium for 6 weeks. Some morphological and anatomical characteristics of Homnil rice (*O. sativa* L.) in term of survival percentage, leaf number, leaf length, root number, plant number per clump, clump height and guard cell size were investigated.

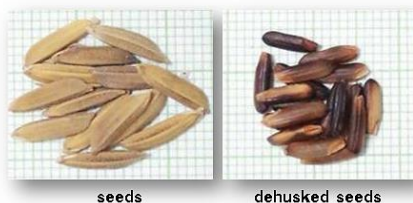


Figure 1 Seeds and dehusked seeds of Homnil rice (*Oryza sativa* L.)

3. Results and discussion

Effects of colchicine on survival percentage, morphology and anatomy of rice seedling were investigated. After *in vitro* cultured for 6 weeks, the results showed that the highest survival percentage of 24 h treatment was recorded when treated at 0.01 and 0.02% colchicine, survival percentage was 66.67% while the lowest survival percentage was 42.86% when treated at 0.04% colchicine. For 48 h treatment, the highest survival percentage was 52.38% when treated at 0.02% colchicine and other treatments (0.01 0.03 and 0.04% colchicine) showed lowest survival percentage, the average survival rate was equal to 14.29% (Table 1). Colchicine treatment was widely used in the crop improvement, but suitable concentration and incubation time should be proved because colchicine has highly toxic to plants. High toxicity would present when duration of incubation was increased (Escandon, Hagiwara & Alderete, 2006; Takamura and Miyajima, 1996).

Morphological study of treated plants show the highest average leaf number of 24 h and 48 h treatments was 13.33 and 14.00 when treated at 0.03% and 0.02%

colchicine, respectively (Table 1). Although leaf number of treated plants was increased but leaf length was decreased in all treatment when comparison with control. However, average root length and number of stem per clump of all treated plants are also difference from control. Table 2 presents an average root length, stem number and height of treated plants after cultured for 6 weeks. However, after planted in *ex vitro*, growth rate of treated plants are also slow when comparison with control but size of stems and leaf larger than control (data not shown). The result of this study was similar to other reports such as in palm oil (Madon, Clyde, Hashim, Mohd, Mat & Saratha, 2005), *Cucumber* (Walters & Wehner, 2002) and *Citrus* callus (Wu and Mooney, 2002). Colchicine may cause toxicity or delay growth of explants. However, more experiments in term of chromosome number, DNA content using flow cytometry, chlorophyll content and more morphological characteristics of treated plants after propagation in the field should be investigated in further study.

Guard cells of stomata were measured after *in vitro* culture for 6 weeks. Guard cells size of all treated plants showed larger size than control (Table 3). Guard

cells size was increased when treated with suitable colchicine concentrations. The highest guard cell width of all treatment was observed in 0.01% colchicine after treated for 48 h (2.75 μm) while the highest guard cell length was 3.63 μm after treated with 0.03% colchicine for 48 h. Almost of treated plant showed significant difference when comparison with control. The result of this study was similar to many reports. (Gu, Yang, Meng & Zhang, 2005; Rose, Kubba & Tobutt, 2000; Sari, Abak & Pitrat, 1999; Stanys, Weckman, Staniene & Duchovskis, 2006). However, Stomatal Index (SI) of treated plant should be done in the future. In conclusion, this study is the first report on colchicine treatment of Homnil rice seedling. Almost treated plants both 24 and 48 h showed significant difference characters when comparison with control. The results of this report may provide a basic data for Homnil rice improvement in the future.

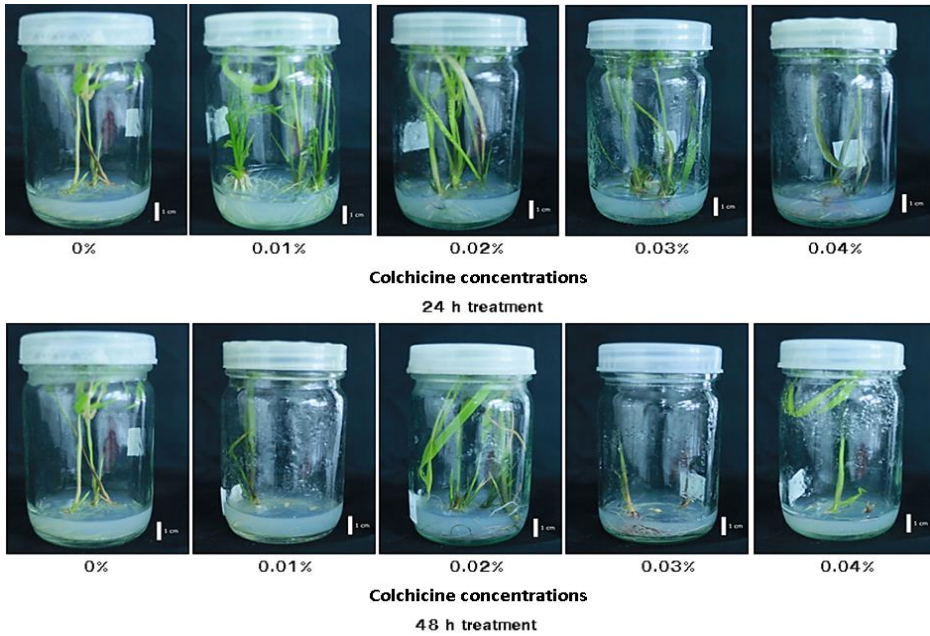


Figure 2 Plantlets of treated rice in all experiments after *in vitro* culture for 6 weeks.

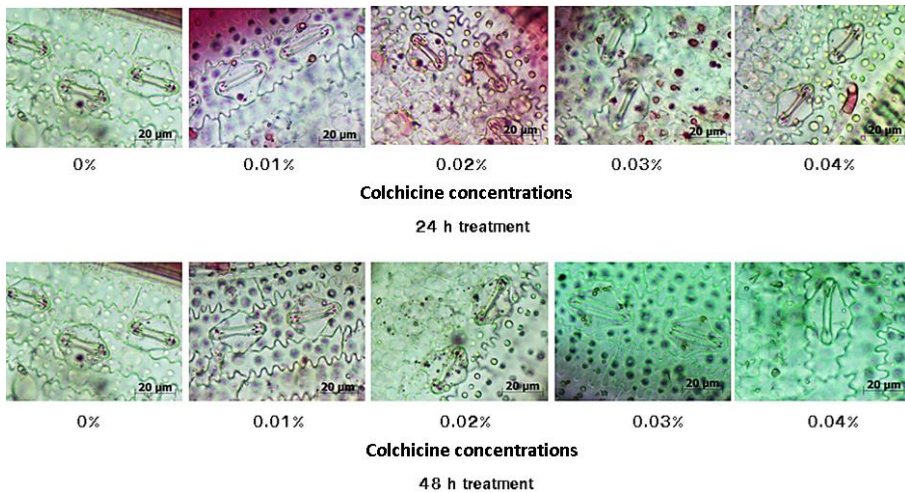


Figure 3 Stomata of treated plants observed from mature leaf after propagation for 10 weeks.

Table 1.

Survival percentage, leaf number and leaf length of treated plants after cultured for 6 weeks.

Colchicine concentration s (%)	Survival percentage (mean±SE)		Leaf number (mean±SE)		Leaf length (cm) (mean±SE)	
	24 h	48 h	24 h	48 h	24 h	48 h
	0	85.71±6.73 ^a	85.71±6.73 ^a	5.67±0.33 ^a	5.67±0.33 ^a	15.67±4.04 ^a
0.01	66.67±10.29 ^b	14.29±6.73 ^b	10.00±2.51 ^b	6.67±3.53 ^a	12.44±3.91 ^{ab}	10.50±1.83 ^b
0.02	66.67±10.29 ^b	52.38±14.29 ^c	10.33±0.33 ^b	14.00±5.58 ^b	12.44±1.98 ^{ab}	10.22±1.64 ^b
0.03	47.62±17.60 ^c	14.29±6.73 ^b	13.33±2.40 ^b	4.33±2.96 ^a	12.89±1.97 ^{ab}	6.83±5.50 ^c
0.04	42.86±9.52 ^c	14.29±6.73 ^b	12.00±2.64 ^b	4.67±2.40 ^a	10.11±1.93 ^b	13.33±1.33 ^{ab}

Means within columns followed by the same letter are not different at the 5% level of significance, (LSD test).

Table 2.

Root length, plant number and height of treated plants after cultured for 6 weeks.

Colchicine concentrations (%)	Root length (cm) (mean±SE)		plant number per clump (mean±SE)		clump height (cm) (mean±SE)	
	24 h	48 h	24 h	48 h	24 h	48 h
	0	4.50±1.75 ^{ab}	4.50±1.75 ^a	1.00±0.00 ^a	1.00±0.00 ^a	8.83±3.33 ^a
0.01	7.00±2.22 ^a	6.50±0.83 ^a	1.33±0.33 ^a	3.00±0.00 ^{ab}	9.00±0.58 ^a	7.33±0.00 ^a
0.02	3.11±1.31 ^{ab}	11.22±5.44 ^b	3.33±1.20 ^b	4.33±0.33 ^b	7.83±1.34 ^a	7.33±0.51 ^a
0.03	1.11±0.25 ^b	6.83±2.83 ^a	7.67±4.70 ^c	2.50±1.50 ^{ab}	4.33±1.00 ^b	4.50±1.50 ^b
0.04	4.50±1.60 ^{ab}	9.67±6.33 ^{ab}	5.67±1.76 ^{bc}	3.00±1.00 ^{ab}	6.50±2.59 ^{ab}	5.12±0.88 ^{ab}

Means within columns followed by the same letter are not different at the 5% level of significance, (LSD test).

Table 3.Guard cells size of 24 h and 48 h treatments after *in vitro* culture for 6 weeks.

colchicine (%)	Guard cells size (mean±SE)			
	24 h treatment		48 h treatment	
	Width (µm)	Length (µm)	Width (µm)	Length (µm)
0	2.05±0.09 ^a	2.95±0.06 ^a	2.05±0.08 ^a	2.95±0.06 ^a
0.01	2.25±0.06 ^b	3.20±0.07 ^b	2.75±0.08 ^b	3.25±0.10 ^b
0.02	2.43±0.08 ^b	3.05±0.07 ^{ab}	2.63±0.04 ^b	3.35±0.08 ^b
0.03	2.35±0.04 ^b	3.00±0.07 ^{ab}	2.55±0.07 ^b	3.63±0.09 ^c
0.04	2.28±0.07 ^b	2.95±0.07 ^a	2.65±0.06 ^b	3.23±0.04 ^b

Means within columns followed by the same letter are not different at the 5% level of significance, (LSD test).

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