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PHYSIOLOGICAL AND ANATOMICAL CHANGES IN THAI RICE LANDRANCE (*ORYZA SATIVA* L.) CV PAKAUMPUEL AFTER COLCHICINE TREATMENT

WORASITIKULYA TARATIMA[1*], PRADUB REANPRAYOON[2], SAYAM RASO[2], MALLIKA CHANTARANGSEE[3] AND PITAKPONG MANEERATTANARUNGROJ[4]

[1]Salt-tolerant Rice Research Group, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Faculty of Science and Technology, Surindra Rajabhat University, Surin 32000, Thailand
[3]Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus, Nong Khai 43000, Thailand
[4]Faculty of Veterinary Science, Khon Kaen University, Khon Kaen 40002, Thailand
**Corresponding author's email: worasitikulya@gmail.com*

Abstract

Physiological and anatomical characteristics of the Thai rice landrace cv Pakaumpuel were investigated. Dehusked seeds were soaked in various concentrations of colchicine for 24 and 48 h in the dark, prior to washing in distilled water three times before germination. Two weeks' old seedlings were cultured up to 16 weeks with transfer into bigger pots and physiological and anatomical parameters were recorded. The abaxial epidermis of mature leaves was examined by peeling technique, while leaf blade anatomy was investigated by transverse free hand section using 2% (w/v) Safranin as staining agent. Thirty-one anatomical characteristics were recorded. Results showed that colchicine treatment increased all growth parameters with the exception of tiller number. Eighteen anatomical characteristics showed significant differences after treatments including guard cell length, midrib thickness, and vertical and horizontal length of midrib and lamina vascular bundles. Effects of colchicine on growth and anatomical characteristics provide confirmatory evidence for polyploidy. This is the first report on Thai rice landrace anatomy. Basic data may be useful for product improvements in other rice cultivations.

Key words: Thai rice landraces, Leaf anatomy, Growth, Colchicine.

Introduction

Rice represents the most important staple food for 30-40% of the global population with over 90% grown and consumed in Asia (Khush, 1997; Bano *et al*., 2005). Global demand for rice is increasing in line with world population (Mahathanaseth, 2014). Breeding using rice landrace germplasm as a genetic donor offers an alternative choice to amend the production yield of local or native varieties/cultivars. Rice landraces evolved from wild progenitors and still retain high genetic diversity (Ray *et al*., 2013). These landraces can be identified using morphological characteristics and many have been named by local farmers. Genetic structures of rice landraces are heterogeneous; they show variable phenology with the ability to grow in both biotic and abiotic stress environments, providing valuable data for crop improvement (Dwivedi *et al*., 2016). Thailand is a global center for rice diversity and Thai rice landraces are necessary and valuable resources for rice breeding programs (Rerkasem & Rerkasem, 2002).

Pakaumpuel rice is a landrace grown in Surin and neighboring Thai provinces bordering Cambodia. This landrace provides greater vitamin E, lutein and iron than Khao Dok Mali (KDML 105) rice cultivars at 0.8, 0.4 and 1.1-fold, respectively (Maneerattanarungroj *et al*., 2011). Pakaumpuel rice seeds are small in comparison with other commercial Thai rice cultivars and yield is also low. However, a previous report concerning the nutritional value of Pakaumpuel rice suggests that increasing the seed size would improve production capability, with induced polyploidy as an alternative protocol to increase yield.

Colchicine is widely used in agricultural experiments as a polyploidizing agent (Melchinger *et al*., 2016; Noori *et al*., 2017; Pereira *et al*., 2014). Many hypotheses have been proposed to explain the biological mechanisms of polyploidy induction by colchicine treatment in plant species including sugar beet (*Beta vulgaris* L.) (Gurel *et al*., 2000), palisade grass (*Brachiaria brizantha*) (Pinheriro *et al*., 2000), maize (Melchinger *et al*., 2016), *Buddleia globosa* (Rose *et al*., 2000), bread wheat (*Triticum aestivum* L.) (Sariano *et al*., 2007), ajowan (*Trachyspermum ammi* L.) (Noori *et al*., 2017), and Citrus (Wu & Mooney, 2004). Polyploidy leads to anatomical change in some characteristics such as guard cell size (Gu *et al*., 2005; Stanys *et al*., 2006), chloroplast content in guard cell (Sari *et al*., 1999), leaf size (Escandon *et al*., 2006; Madon *et al*., 2005), and flower size (Escandon *et al*., 2006). Although some reports have cited chromosome number and anatomical change in many plant species such responses in rice species are poorly perceived and understood. Moreover, knowledge regarding the physiological and anatomical effects of colchicine is sparse compared with information concerning the cytological effects in rice plants. No previous reports consider the physiology and anatomy of Pakaumpuel rice and effects of colchicine treatment. Thus, here, colchicine treatments on growth and leaf anatomy of the Pakaumpuel rice landrace were investigated. This information may be useful to support and confirm the existence of polyploidy in Thai rice landraces.

Materials and Methods

Plant materials: Thai rice landrace cv Pakaumpuel was collected from the Surin Rice Research Center, Surin Province, Thailand. Dehusked seeds were surface sterilized using 25% (v/v) sodium hypochlorite with 3-4 drops of Tween 20 for 20 min, prior to washing in sterilized distilled water 3 times. Sterilized seeds were then incubated in

various concentrations of colchicine (0, 0.025, 0.50, 0.75 and 1.00% (v/v)) for 24 and 48 hrs in the dark before washing with distilled water and culturing for 2 weeks. After germination, rice seedlings were transplanted using the same soil set at one seedling per pot. After 16 weeks of culture and before flowering, mature leaves were collected as explants and used in all experiments.

Plant growth: Treated and control plants were cultured at the Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. Chlorophyll content, plant height, leaf length, leaf width and tiller number of control and treated plants were recorded after culture for 16 weeks. Chlorophyll content was checked using the Konica Minolta SPAD-502 Plus. Panicle per plant, panicle length, seed set per panicle, seed set percentage, effective seed, seed length, seed width and 100 seed weight were measured after harvesting. All experiments were conducted using mature and healthy plant and leaf.

Leaf anatomy: Mature rice leaves were dissected into small pieces and soaked in 15% (v/v) sodium hypochlorite (Clorox) for 10-15 min prior to peeling. The abaxial epidermis was peeled and stained with 1% (w/v) Safranin O. Guard cell size and stomatal densities were recorded under light microscope. For leaf section, midrib and leaf blade of all treatments were transverse sectioned using free hand technique and stained with 1% (w/v) Safranin O. At least three areas were observed and scored. Three characteristics of epidermis, thirteen for midrib anatomy and fifteen for lamina anatomy were recorded. Definitions of their anatomical traits are as follows: Stomata-guard cell length (GCL), guard cell width (GCW), stomatal density (SD); Midrib-midrib thickness (MT), vertical length of vascular bundle in ventral surface of midrib (VLVVM), horizontal length of vascular bundle in ventral surface of midrib (HLVVM), xylem diameter of vascular bundle in ventral surface of midrib (XDVVM), vertical length of phloem of vascular bundle in ventral surface of midrib (VLPVVM), horizontal length of phloem of vascular bundle in ventral surface of midrib (HLPVVM), vertical length of vascular bundle in dorsal surface of midrib (VLVDM), horizontal length of vascular bundle in dorsal surface of midrib (HLVDM), xylem diameter of vascular bundle in dorsal surface of midrib (XDVDM), vertical length of phloem of vascular bundle in dorsal surface of midrib (VLPVDM), horizontal length of phloem of vascular bundle in dorsal surface of midrib (HLPVDM), vertical length of air space (VLA), horizontal length of air space (HLA); Lamina-thickness of large vascular bundle in lamina (TLVL), thickness of small vascular bundle in lamina (TSVL), vertical length of large vascular bundle in lamina (VLLVL), horizontal length of large vascular bundle in lamina (HLLVL), diameter of xylem of large vascular bundle in lamina (DXLVL), vertical length of phloem of large vascular bundle in lamina (VLPLVL), horizontal length of phloem of large vascular bundle in lamina (HLPLVL), vertical length of small vascular bundle in lamina (VLSVL), horizontal length of small vascular bundle in lamina (HLSVL), diameter of xylem of small vascular bundle in lamina (DXSVL), vertical length of phloem of small vascular bundle in lamina (VLPSVL), horizontal length of phloem of small vascular bundle in lamina (HLPSVL), vertical length of bulliform cell (VLB), horizontal length of bulliform cell (HLB) and lamina thickness (LT).

Statistical analysis

One-way analysis of variance (ANOVA) was used to investigate statistically significant differences between treatments, with LSD (Least Significant Difference) values used to compare the means of the treatments at $p<0.05$.

Results and Discussion

CT-chlorophyll content, LL-Leaf length, LW-Leaf width, TN-Tiller number, PLH-Plant height, PP-Panicle/plant, PL-Panicle length, SSP-Seed set/panicle, SS-Seed set (%), ES-Effective seed, SL-Seed length, SW-Seed width.

Plant growth: Treated plants were compared with control for all treatments and physiological parameters including chlorophyll content, plant height, leaf length, leaf width and tiller number were measured. Colchicine treatment increased some growth characteristics such as chlorophyll content (CT), plant height (PLH), seed set/panicle (SSP), effective seed (ES), seed length (SL) and seed width (SW) in 24 hrs treatment and CT, SSP and ES in 48 hrs while tiller number was clearly decreased in all treatments. When statistical analysis was considered, chlorophyll content showed a significant difference between treated plants (highest number) and control, especially in the 24 hrs treatment (Tables 1 and 2). For vegetation and yield, colchicine treatments gave significantly higher values than control plants (Tables 1 and 2). Seed set per panicle and effective seed number were higher than untreated plants for all colchicine treatments.

Values of vegetation, yield and yield components of control and colchicine treated plants harvested after culture for 16 weeks are shown in Table 1. Colchicine treatment increased all growth characteristics except tiller number (Tables 1 and 2). Chlorophyll content, plant height, seed set per panicle, seed set percentage and effective seed of colchicine treatments showed significantly higher values than control plants (Table 1).

Survival percentage was not significantly affected by colchicine concentration and treatment duration (data not shown). Our results concurred with Tiwari and Mishra (2011) who reported that colchicine concentration and incubation time did not influence second generation survival rate of *Phlox drummondi*.

Colchicine treatment may cause apomixis in Pakaumpuel rice but apomictic traits in rice are not linked to morphological features (Gaafar *et al*., 2017). Diplosporic apomictic plants are hard to distinguish from sexual ones (Reiser & Fischer, 1993), particularly in diploid and self-pollinated crops such as rice (Gaafar *et al*., 2017). Our results indicated that colchicine treated plants had the same characteristics the same as control plants. Leaf length, leaf width, tiller number, panicle per plant, panicle length, seed length, seed weight, and 100 grains weight of control and colchicine treated plants

were not significantly different, especially for tiller number. Here, tiller number decreased after colchicine treatment in agreement with Gaafar *et al*., (2017) who reported that colchicine remarkably impacted the number of tillers per plant. Colchicine may affect cytokinin activity which is necessary for plant growth and development (Deikman & Ulrich, 1995). This result proved that applying colchicine inhibited tiller numbers of Pakaumpuel rice plants. This result concurred with Paul *et al*., (1978) who found that hypocotyl length, root length, fresh weight and dry weight of mungbean seedlings dramatically decreased after treatment with various concentrations of colchicine.

Epidermal and stomatal study: Types of tissue and leaf arrangement of control and all treatments were similar. Epidermal tissue also showed similar patterns of stomatal apparatus, macro hairs, and prickle hairs. The leaf surface of Pakaumpuel rice included some types of cells and appendages including long and short cells, stomata with guard cells, macro hairs, prickle hairs, papillae and silica bodies. Long cells were the major cell type among the diverse cells (Fig. 1). The entire leaf surface had a thick cover of epicuticular wax and bore hairs. Long cells of both costal and intercostal areas had dense and moderately marked sinuous walls, while short cells were generally equal in shape, present in coastal regions but absent in intercostal areas. Papillae are prominences of diverse shapes and sizes from the epidermal cells outer walls. However, there were some notable differences in guard cell length, guard cell width and stomatal density (Fig. 2, Table 3). Micrometric observation showed that guard cell length and stomatal density of treated plants increased while guard cell width decreased compared with control plants. However, based on statistical analysis, almost treatments showed no significant differences except some characteristics. Guard cell width of 48 hrs treatment was clearly significantly decreased when compared with control (Table 3).

Table 1. Values of vegetation yield and yield components of control and colchicine treated plants harvested after culture for 16 weeks.

Characteristic	Mean ± SE				
	Colchicine concentrations (% w/v) incubation for 24 h				
	0	0.025	0.050	0.075	0.010
CT	41.7±0.8^{b}	44.2±1.0^{a}	43.8±0.9^{a}	44.4±1.2^{a}	45.5±0.9^{a}
LL (cm)	30.5±0.9^{ab}	30.3±0.3^{ab}	31.3±0.3^{a}	29.7±0.7^{b}	29.6±0.8^{b}
LW (mm)	10.7±0.2 ^{ab}	11.2±0.2^{a}	11.0±0.4^{a}	10.5±0.5^{b}	10.0±0.0^{b}
TN	32.5±3.7^{a}	27.2±2.2^{ab}	28.7±2.6^{a}	26.2±1.1^{b}	26.0±0.7^{b}
PLH (cm)	185.8±4.4^{bc}	201.2±3.4^{a}	185.8±6.1^{c}	192.0±6.6^{b}	190.0±4.5^{b}
PP	32.0±2.3^{a}	34.8±3.3^{a}	27.4±2.5^{b}	29.2±0.8^{b}	25.0±1.5^{b}
PL (cm)	28.9 ±1.0^{ab}	26.8±0.6^{b}	30.5±0.7^{a}	29.0±0.3^{a}	28.6±0.2^{ab}
SSP	142.1±5.3^{b}	153.0±5.9^{b}	171.0±6.9^{a}	147.5±6.7^{b}	174.8±3.8^{a}
SS (%)	72.1±2.5^{a}	73.6±2.8^{a}	74.4±2.8^{a}	72.9±2.6^{a}	69.1±2.5^{b}
ES	102.5±5.4^{c}	112.1±4.9^{b}	126.4±5.0^{a}	106.3±3.1^{c}	120.7±4.8^{ab}
SL (mm)	10.1±0.1^{a}	10.5 ± 0.1^{a}	10.8±0.1^{a}	10.5±0.2^{a}	10.5±0.1^{a}
SW(mm)	2.4± 0.2^{a}	2.6 ± 0.1^{a}	2.6±0.1^{a}	2.5±0.0^{a}	2.5±0.1^{a}
100 gw (g)	2.3±0.0^{a}	2.3 ± 0.0^{a}	2.4±0.0^{a}	2.4±0.0^{a}	2.4±0.0^{a}
	Various colchicine concentrations (%w/v) incubation for 48 h				
CT	41.7±0.8^{b}	43.1±1.4^{a}	42.2±0.6^{a}	44.1±1.0^{a}	43.4±0.8^{a}
LL (cm)	30.5±0.9^{a}	29.2±0.6^{a}	28.2±0.5^{b}	27.7±0.4^{b}	28.3±0.9^{b}
LW (mm)	10.7±0.2 ^{a}	10.7±0.4^{a}	10.2±0.2^{ab}	9.7±0.2^{b}	11.0±0.0 ^{a}
TN	32.5±3.7^{a}	28.0±1.6 ^{b}	31.5±1.5^{a}	30.7±0.4^{a}	28.5±0.2^{ab}
PLH (cm)	185.8±4.4^{a}	186.2±3.2^{a}	183.8±3.1^{a}	184.4±2.3^{a}	187.8±2.9^{a}
PP	32.0±2.3^{a}	28.4±1.6^{b}	28.2±2.0^{b}	33.2±0.6^{a}	33.8±0.8^{a}
PL (cm)	28.9 ±1.0^{a}	29.9±0.5^{a}	28.8±0.7^{a}	29.3±0.5^{a}	29.0±0.2^{a}
SSP	142.1±5.3^{d}	201.1±2.0^{b}	192.7±1.8^{c}	211.0±2.3^{a}	194.8±3.6^{c}
SS (%)	72.1±2.5^{b}	72.5±2.1^{b}	81.5±3.1^{a}	79.1±1.7^{a}	79.2±1.9^{a}
ES	102.5±5.4^{d}	144.5±5.7^{c}	155.6±7.7^{b}	166.1±6.5^{a}	136.7±4.3^{c}
SL (mm)	10.1±0.1^{a}	10.0±0.1^{a}	10.2±0.2^{a}	10.0±0.1^{a}	10.2±0.1^{a}
SW (mm)	2.4±0.2^{a}	2.4±0.0^{a}	2.6±0.1^{a}	2.6±0.1^{a}	2.5±0.1^{a}
100 gw (g)	2.3±0.0^{a}	2.3±0.0^{a}	2.4±0.0^{a}	2.4±0.0^{a}	2.4±0.0^{a}

Values with different superscripts within same row indicate significant differences at $p<0.05$ by LSD test; CT-chlorophyll content, LL-Leaf length, LW-Leaf width, TN-Tiller number, PLH-Plant height, PP-Panicle/plant, PL-Panicle length, SSP-Seed set/panicle, SS-Seed set (%), ES-Effective seed, SL-Seed length, SW-Seed width, 100 gw-100 grains weight

Table 2. Physiological characteristics and yield of colchicine treatments and control at minimum and maximum number harvested after culture for 16 weeks.

Characteristic	Control	Colchicine treatments					
		Lowest	Treatment* (% col., h)	Change (%)	Highest	Treatment* (% col., h)	Change (%)
Chlorophyll content	41.7±0.8^{b}	42.2±0.6^{b}	0.050, 48	101.1	45.5±0.9^{a}	0.100, 24	108.9
Leaf length (cm)	30.5 ±0.9^{a}	27.7±0.4^{b}	0.075, 48	90.9	31.37±0.3^{a}	0.050, 24	102.8
Leaf width (mm)	10.7±0.2^{b}	9.7±0.2^{b}	0.075, 48	90.6	11.2±0.2^{a}	0.025, 24	104.6
Tiller number	32.5±3.7^{a}	26.0±0.7^{b}	0.100, 24	80.0	31.5±1.5^{a}	0.050, 48	96.9
Plant height (cm)	185.8±4.4^{b}	183.8±3.1^{b}	0.050, 48	98.9	201.2±3.4^{a}	0.025, 24	108.2
Panicle/plant	32.0±2.3^{a}	25.0±1.5^{b}	0.100, 24	78.1	34.8±3.3^{a}	0.025, 24	108.7
Panicle length (cm)	28.9±1.0^{b}	26.8±0.6^{b}	0.025, 24	92.7	30.5±0.7^{a}	0.050, 24	105.5
Seed set/panicle	142.1±5.3^{b}	147.5±6.7^{b}	0.075, 24	103.8	211.0±2.3^{a}	0.075, 48	148.4
Seed set (%)	72.1±2.5^{b}	69.1±2.5^{b}	0.100, 48	95.8	81.5±3.1^{a}	0.050, 48	113.0
Effective seed	102.5±5.4^{b}	106.3±3.1^{b}	0.075, 24	103.7	166.1±6.5^{a}	0.075, 48	162.0
Seed length (mm)	10.1±0.1^{a}	10.0±0.1^{a}	0.025, 48	99.0	10.8±0.1^{a}	0.050, 24	106.9
Seed width (mm)	2.4±0.2^{a}	2.4±0.0^{a}	0.025, 48	100.0	2.6±0.1^{a}	0.025, 24	108.3
100 grains weight (g)	2.3±0.0^{a}	2.3±0.0^{a}	0.025, 24	100.0	2.4±0.0^{a}	0.050, 24	104.3

*Colchicine concentrations (% w/v), incubation time (h) Different superscripts within the same row indicate significant differences at p<0.05 by LSD test

Table 3. Stomatal size and midrib anatomy of colchicine treated plants and control.

Characteristic	Mean (µm) ± SE				
	Various colchicine concentrations (% w/v) incubation for 24 hrs				
	0	0.025	0.050	0.075	0.010
Epidermis (µm)					
GCL (µm)	21.70±0.2^{a}	19.8 ±0.2^{b}	22.5±0.2^{a}	19.7±0.2^{b}	19.3±0.2^{b}
GCW (µm)	21.00±0.5^{a}	17.7± 0.4^{b}	20.3±0.5^{a}	18.8±0.3^{b}	18.9±0.3^{b}
SD (mm^{2})	19.20±0.2^{a}	19.8±0.3^{a}	19.6±0.6^{a}	19.8±0.7^{a}	18.4±1.8^{a}
	Various colchicine concentrations (% w/v) incubation for 48 hrs				
GCL (µm)	21.70±0.2^{a}	19.8±0.4^{b}	18.9±0.3^{b}	22.3±0.3^{a}	21.0±0.3^{a}
GCW (µm)	21.00±0.5^{a}	19.6±0.4^{b}	19.1±0.4^{b}	19.2±0.3^{b}	17.5±0.2^{c}
SD (mm^{2})	19.20±0.2^{b}	20.6±1.6^{ab}	22.8±1.3^{a}	18.0±0.7^{b}	18.2±0.7^{b}
	Various colchicine concentrations (%w/v) incubation for 24 hrs				
Midrib (µm)					
MT	761.5±0.0^{b}	745.4±0.3^{b}	810.0±1.1^{a}	830.2±0.9^{a}	753.5±0.3^{b}
VLVVM	139.0±1.2^{c}	135.5±0.7^{c}	148.0±1.1^{b}	156.5±2.0^{a}	140.0±0.3^{c}
HLVVM	147.0±0.8^{c}	162.5±0.5^{a}	162.0±1.5^{a}	145.5±0.9^{c}	156.0±1.0^{b}
XDVVM	54.5±1.7^{a}	55.5±1.3^{a}	56.0±1.0^{a}	48.0±0.4^{b}	55.5±0.9^{a}
VLPVVM	53.0±0.9^{ab}	59.0±0.7^{a}	55.5±0.1^{a}	57.5±0.7^{a}	45.5±1.0^{b}
HLPVVM	72.5±1.4^{b}	74.5±0.8^{b}	83.5±0.7^{a}	76.0±1.0^{b}	74.0±0.5^{b}
VLVDM	86.5±1.3^{a}	67.5±2.0^{c}	83.0±1.0^{a}	82.0±0.8^{a}	80.0±0.9^{a}
HLVDM	74.0±0.7^{a}	62.0±1.4^{b}	65.5±0.3^{b}	67.0±0.7^{b}	68.0±2.2^{b}
XDVDM	29.0±0.5^{a}	25.0±0.7^{a}	27.5±1.7^{a}	24.0±0.4^{a}	28.5±0.9^{a}
VLPVDM	31.5±0.2^{b}	20.5±2.1^{d}	37.5±0.8^{a}	34.0±0.6^{ab}	30.0±1.5^{b}
HLPVDM	42.5±0.8^{b}	41.0±0.2^{b}	42.5±2.3^{b}	48.0±1.4^{a}	40.0±0.8^{b}
VLA	521.2±2.5^{a}	515.1±0.4^{b}	123.0±2.1^{d}	527.2±1.8^{a}	502.9±1.0^{c}
HLA	311.1±1.4^{a}	286.8±3.0^{c}	276.0±2.0^{c}	313.1±4.1^{a}	294.9±0.8^{b}
	Various colchicine concentrations (%w/v) incubation for 48 hrs				
MT	761.5±0.0^{a}	504.4±1.1^{d}	759.5±1.1^{a}	727.2±0.5^{b}	644.4±0.5^{c}
VLVVM	139.0±1.2^{b}	136.0±0.7^{c}	134.0±0.9^{c}	148.0±1.3^{a}	119.5±0.8^{d}
HLVVM	147.0±0.8^{b}	162.0±1.2^{a}	160.5±1.0^{a}	164.5±0.4^{a}	150.0±1.1^{b}
XDVVM	54.5±1.7^{a}	60.5±0.0^{a}	57.0±0.4^{a}	57.0±0.8^{a}	57.0±1.0^{a}
VLPVVM	53.0±0.9^{a}	49.0±0.8^{b}	47.5±0.4^{b}	49.5±1.0^{b}	42.5±0.8^{c}
HLPVVM	72.5±1.4^{b}	77.0±0.7^{b}	80.0±0.8^{a}	84.5±2.0^{a}	70.5±1.0^{b}
VLVDM	86.5±1.3^{a}	74.0±2.0^{b}	71.5±1.5^{b}	66.5±0.7^{c}	68.5±0.7^{c}
HLVDM	74.0±0.7^{a}	55.5±1.8^{c}	57.0±0.7^{c}	56.0±3.0^{c}	69.0±2.0^{b}
XDVDM	29.0±0.5^{a}	24.5±2.0^{b}	24.5±2.1^{b}	23.0±0.4^{b}	24.5±2.0^{b}
VLPVDM	31.5±0.2^{a}	25.0±0.1^{b}	25.0±0.9^{b}	21.5±0.4^{c}	27.5±0.7^{b}
HLPVDM	42.5±0.8^{a}	33.0±1.4^{bc}	32.0±2.0^{c}	35.5±0.8^{b}	36.0±0.4^{b}
VLA	521.2±2.5^{a}	521.2±2.1^{a}	521.1±1.5^{a}	490.9±1.0^{b}	395.9±0.6^{c}
HLA	311.1±1.4^{a}	266.6±2.7^{d}	256.5±1.0^{d}	292.9±1.3^{b}	282.8±1.5^{c}

Different superscripts within the same row indicate significant differences at p<0.05 by LSD test

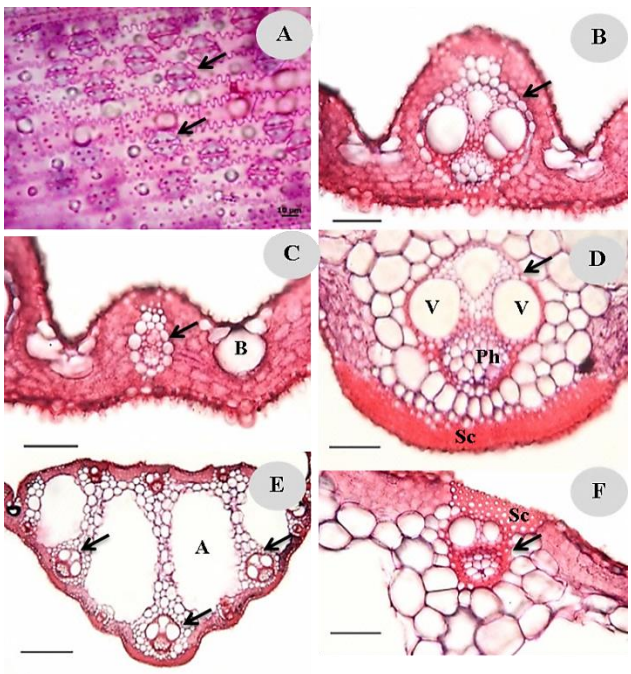

Fig. 1. Leaf anatomy of Pakaumpuel rice: *A*-stomata (*arrows*); *B*-major vascular bundle (*arrow*); *C*-small vascular bundle (*arrow*); *D*-ventral vascular bundle of midrib (*arrow*), V=vessel, Ph=phloem, Sc=sclerenchyma; *E*-midrib area shows small vascular bundles (*arrows*), A=air space; *F*-dorsal vascular bundle of midrib (*arrow*), Sc=sclerenchyma. Scale bar =200 μm.

Lamina anatomy: In transverse sectional aspect, Pakaumpuel rice leaf blade exhibited distinct iso-bilateral characteristics with prominent midrib and bulliform cells, undifferentiated mesophyll, parallel arranged vascular bundles, and conjoint collateral vascular bundles with endarch xylem. Vascular bundles in the lamina, except those in the large adaxial ribs and near the leaf margins, not conspicuously angular in outline, large vascular bundles in the tall ribs of basic type. Adaxial surface with ribs of two distinct sizes, those over the small vascular bundles being low, fairly wide, with rounded apices and separated from one another by much narrower, shallow furrows. Midrib conspicuous, owing to a prominent, rounded, abaxial and less pronounced flat adaxial projection containing several vascular bundles (Fig. 1).

Patterns of leaf anatomy, midrib, and blade anatomy of control and treated plants were not significantly different. Here, twenty-eight anatomical characteristics of Pakaumpuel rice leaf were investigated. Comparison of control and treatment in midrib anatomical characteristics showed that seven out of thirteen exhibited significant difference, especially for the highest number, while eleven characteristics of lamina anatomy showed significant difference in the highest number. However, the lowest number of all characteristics for all treatments showed significant difference compared with control plants except horizontal length of vascular bundle in ventral surface of midrib (HLVVM) and horizontal length of phloem of vascular bundle in ventral surface of midrib (HLPVVM) (Tables 3-5).

Anatomical characteristics of epidermal and stomata of Pakaumpaul rice were also similar to previous reports of light microscope studies (Metcalfe, 1960; Sarwar & Ali, 2002; Islam *et al*., 2009). Epidermal characteristics of the leaf perform significant role in typical members of the Poaceae (Metcalfe, 1960; Ellis, 1979) together with defining different rice (*Oryza sativa* L.) cultivars (Islam *et al*., 2009; Sarwar & Ali, 2002). Generally, stomatal and epidermal cell frequency per unit leaf area decreased while stomatal guard cell length enlarged with an increase in ploidy. Moreover, reduction in stomatal frequency at higher ploidy levels was principally a result of larger epidermal cells (Mishra, 1997). Here, concentration of 0.075% colchicine and 48 hrs treatment showed larger guard cell length and lower stomatal density than control. This result was similar to many reports (Karpechenko *et al*., 1928; Mishra, 1997; Abdoli *et al*., 2013; Sajjad, 2013). However, tetraploid races of some plants have larger stomata than those of the diploid, for example *Solanum* sp. (Sax & Sax, 1937). This large stomata trait has been described as an important contributor to polyploidy characteristics (Gu *et al*., 2005; Megbo, 2010; Sari *et al*., 1999). However, anatomical and cytological traits in treated plants require further investigation.

Some characteristic anatomical differences between control and treatments are speculated to be colchicine regulated. Colchicine plays an important role by activating chromosome doubling in plant cells (Deppe, 1993). Many reports indicated that anatomical aspects of colchicine treated plants were higher than control such as guard cell size, vascular bundle size, and chloroplast content in guard cells (Gu *et al*., 2005; Megbo, 2010; Sari *et al*., 1999; Stanys *et al*., 2006). However, our results showed that three out of twenty-eight characteristics exhibited highest measured data of control plants compared to treated plants for vertical and horizontal length of vascular bundle and xylem diameter of vascular bundle in dorsal surface of midrib; VLVDM, HLVDM and XDVDM. This finding, suggesting that tetraploid plants showed increased leaf thickness and leaf surface than diploid plants concurred with Abdoli *et al*., (2013), Chulalaksananukul and Chimnoi (1999), Escandon *et al*., (2006), Rauf *et al*., (2006) and Madon *et al*., (2005). Interestingly, leaf thickness of all treated plants was higher than control plants in both midrib and small vascular bundle areas. Although it is not possible to accurately indicate ploidy level from physiological and anatomical information, this knowledge may be important evidence for phenotype trait. Our results were consistent with Evan (1955), Megbo (2010) and Przywara *et al*., (2011), who reported that stomata length was an accurate indicator of the polyploid level in many plants.

Conclusions

Plant growth and leaf anatomical responses of Pakaumpuel rice were investigated. Chlorophyll content, plant height, leaf length and leaf width of treated plants were higher than control. No difference was recorded in anatomical pattern but numerical anatomy of numerous characteristics showed significant differences after colchicine treatments. Physiological and anatomical characteristics as polyploidy have been proven in some plant species but not in rice. This discovery can be implied as important evidence for phenotype trait and will be useful in research regarding other rice landraces. Response aspects by Thai rice landraces to colchicine treatment require further investigation for more detailed and comprehensive confirmation of our results.

Table 4. Lamina anatomy of colchicine treated plants and control.

Characteristic	Mean (µm) ± SE				
	Colchicine concentrations (% w/v) incubation for 24 hrs				
	0	0.025	0.050	0.075	0.010
Lamina (µm)					
TLVL	149.5±0.4^{b}	150±1.0^{b}	146.0±0.7^{c}	178.5±0.6^{a}	145.5±1.0^{c}
TSVL	84.5±2.1^{b}	83.5±2.0^{b}	80.5±1.0^{c}	95.5±2.1^{a}	75.0±1.0^{d}
VLLVL	111.0±0.0^{a}	96.0±1.1^{c}	92.5±0.5^{c}	108.0±1.1^{b}	94.5±0.6^{c}
HLLVL	121.5±0.7^{b}	124.5±2.0^{b}	122.0±1.2^{b}	130.5±0.8^{a}	123.5±2.3^{b}
DXLVL	40.0±1.4^{a}	37.0±0.0^{ab}	36.0±1.0^{b}	39.5±2.0^{a}	39.0±0.4^{a}
VLPLVL	31.5±0.5^{b}	27.0±0.6^{c}	29.5±1.2^{bc}	40.5±1.4^{a}	27.5±0.8^{c}
HLPLVL	46.0±0.9^{b}	47.5±1.1^{b}	42.5±0.5^{c}	56.0±2.0^{a}	43.5±0.6^{c}
VLSVL	52.0±1.3^{a}	50.5±2.0^{ab}	43.0±1.8^{b}	52.0±0.9^{a}	41.5±2.2^{b}
HLSVL	41.5±0.4^{b}	46.0±0.5^{a}	37.5±0.1^{c}	43.0±0.9^{ab}	35.0±0.1^{c}
DXSVL	8.5±0.7^{a}	8.0±0.0^{a}	7.5±0.4^{a}	7.0±0.9^{a}	8.0±0.1^{a}
VLPSVL	18.0±3.0^{a}	18.5±0.5^{a}	15.0±0.4^{b}	19.5±1.0^{a}	14.5±1.1^{b}
HLPSVL	21.5±0.8^{a}	25.0±1.0^{a}	18.5±0.8^{b}	23.5±2.0^{a}	17.0±0.5^{b}
VLB	35.5±2.5^{ab}	32.5±2.0^{b}	32.5±0.8^{b}	39.5±0.9^{a}	34.5±1.8^{ab}
HLB	36.5±0.1^{a}	34.5±1.0^{b}	32.0±1.2^{b}	37.0±1.1^{a}	35.0±0.3^{ab}
LT	76.0±0.0^{b}	77.5±1.1^{b}	72.5±2.3^{c}	83.5±1.1^{a}	70.0±1.2^{c}
	Various colchicine concentrations (% w/v) incubation for 48 hrs				
TLVL	149.5±0.4^{d}	164.5±2.0^{c}	171.5±2.0^{b}	187.0±1.0^{a}	149.5±0.8^{d}
TSVL	84.5±2.1^{c}	90.5±1.6^{b}	96.0±0.9^{a}	96.0±1.1^{a}	82.0±2.1^{c}
VLLVL	111.0±0.0^{b}	104.0±0.7^{b}	119.5±1.0^{a}	115.5±0.9^{a}	96.5±1.1^{c}
HLLVL	121.5±0.7^{b}	126.5±1.4^{b}	129.0±0.5^{a}	139.0±0.4^{a}	118.0±1.5^{b}
DXLVL	40.0±1.4^{ab}	37.0±0.9^{b}	44.0±0.8^{a}	42.5±1.1^{ab}	38.0±1.2^{b}
VLPLVL	31.5±0.5^{b}	30.0±0.5^{b}	36.0±0.6^{ab}	31.5±0.5^{b}	31.0±1.0^{b}
HLPLVL	46.0±0.9^{b}	48.0±0.9^{a}	51.5±1.5^{a}	48.5±1.0^{a}	46.5±2.0^{b}
VLSVL	52.0±1.3^{ab}	55.5±0.4^{a}	54.0±0.3^{a}	51.0±0.4^{ab}	45.5±1.1^{b}
HLSVL	41.5±0.4^{b}	40.0±1.0^{b}	48.5±0.7^{a}	40.5±0.3^{b}	41.0±1.0^{b}
DXSVL	8.5±0.7^{a}	8.0±0.2^{a}	7.0±0.4^{a}	9.0±0.8^{a}	9.0±0.0^{a}
VLPSVL	18.0±3.0^{a}	15.5±0.9^{b}	18.0±1.1^{a}	19.0±0.0^{a}	18.0±0.8^{a}
HLPSVL	21.5±0.8^{a}	22.0±0.7^{a}	21.0±0.0^{a}	23.0±1.0^{a}	22.5±0.4^{a}
VLB	35.5±2.5^{ab}	37.0±0.0^{a}	34.0±0.7^{ab}	39.0±0.7^{a}	31.5±2.5^{b}
HLB	36.5±0.1^{b}	39.0±1.0^{ab}	46.0±0.3^{a}	42.0±0.0^{a}	34.0±0.8^{b}
LT	76.0±0.0^{b}	75.0±1.1^{bc}	82.5±2.0^{a}	77.5±0.8^{b}	67.5±0.6^{c}

Different superscripts within same row indicate significant differences at $p<0.05$ by LSD test

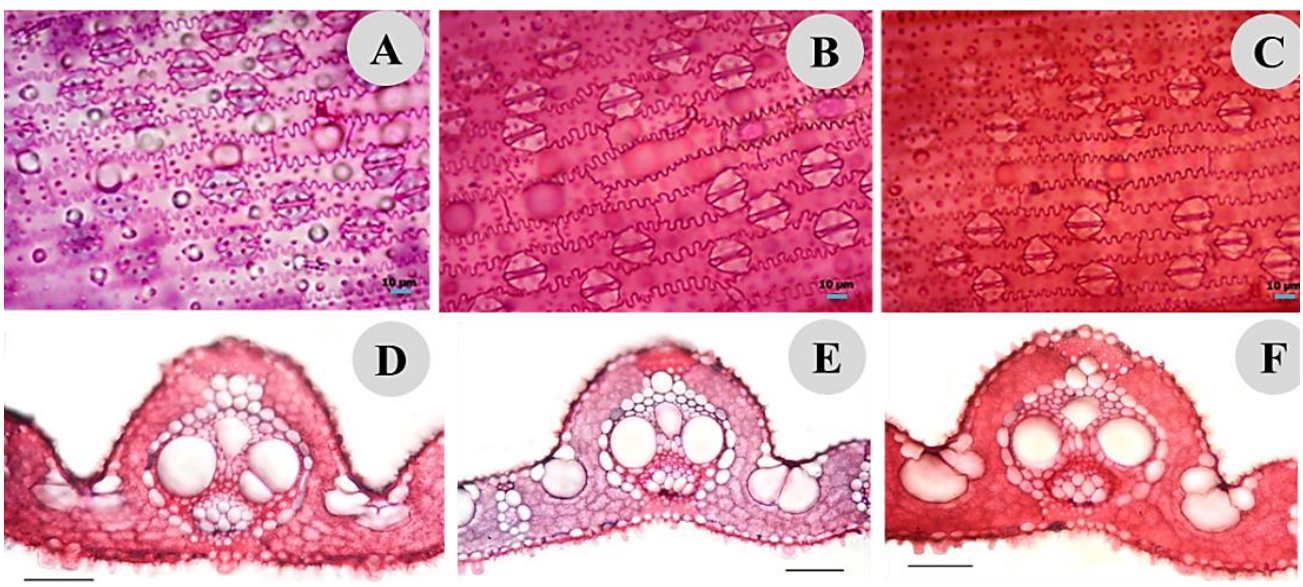

Fig. 2. Stomata and midrib comparison of control and treated plants; A and D-control, B and E- lowest measured data, C and F-highest measured data.

Table 5. Stomata and leaf blade anatomy of colchicine treated plants and control.

Characteristic	Control	Treatments					
		Lowest	Treatment* (% col., hrs)	Change (%)	Highest	Treatment* (% col., hrs)	Change (%)
Epidermis							
GCL (μm)	21.7±0.2^{a}	18.9±0.3^{b}	0.050, 48	87.09	22.5±0.2^{a}	0.050, 24	103.68
GCW (μm)	21.0±0.5^{a}	17.5±0.2^{b}	0.100, 48	83.33	20.3±0.5^{a}	0.050, 24	96.66
SD (mm^2)	19.2±0.2^{a}	18.0±0.7^{a}	0.075, 48	93.75	22.8±1.3^{a}	0.050, 48	118.75
Midrib (μm)							
MT	761.5±0.0^{b}	504.4±1.1^{c}	0.075, 24	66.24	830.2±0.9^{a}	0.025, 48	108.99
VLVVM	139.0±1.2^{b}	119.5±0.8^{c}	0.100, 48	85.97	156.5±2.0^{a}	0.075, 24	112.58
HLVVM	147.0±0.8^{c}	145.5±0.9^{c}	0.075, 24	98.98	164.5±0.4^{a}	0.075, 48	111.90
XDVVM	54.5±1.7^{a}	48.0±0.4^{c}	0.075, 24	88.07	60.5±0.0^{a}	0.050, 48	111.00
VLPVVM	53.0±0.9^{ab}	42.5±0.8^{c}	0.025, 48	80.18	59.0±0.7^{a}	0.050, 24	111.32
HLPVVM	72.5±1.4^{b}	70.5±1.0^{b}	0.050, 48	97.24	84.5±2.0^{a}	0.075, 48	116.55
VLVDM	86.5±1.3^{a}	66.5±0.7^{c}	0.075, 48	76.87	83.0±1.0^{a}	0.050, 24	95.95
HLVDM	74.0±0.7^{a}	55.5±1.8^{c}	0.025, 48	75.00	69.0±2.0^{b}	0.100, 48	93.24
XDVDM	29.0±0.5^{a}	23.0±0.4^{b}	0.075, 48	79.31	28.5±0.9^{a}	0.100, 24	98.27
VLPVDM	31.5±0.2^{b}	20.5±2.1^{c}	0.025, 24	65.07	37.5±0.8^{a}	0.050, 24	119.04
HLPVDM	42.5±0.8^{b}	32.0±2.0^{c}	0.050, 48	75.29	48.0±1.4^{a}	0.075, 24	112.94
VLA	521.2±2.5^{a}	123.0±2.1^{b}	0.050, 24	23.59	527.2±1.8^{a}	0.075, 24	101.15
HLA	311.1±1.4^{a}	256.0±1.0^{b}	0.050, 24	82.28	313.1±4.1^{a}	0.075, 24	100.64
Lamina (μm)							
TLVL	149.5±0.4^{b}	145.5±1.0^{b}	0.100, 24	97.32	187.0±1.0^{a}	0.075, 48	125.08
TSVL	84.5±2.1^{b}	75.0±1.0^{c}	0.100, 24	88.75	96.0±0.9^{a}	0.050, 48	113.60
VLLVL	111.0±0.0^{a}	92.5±0.5^{b}	0.050, 24	83.33	119.5±1.0^{a}	0.050, 48	107.65
HLLVL	121.5±0.7^{b}	118.0±1.5^{b}	0.100, 48	97.11	139.0±0.4^{a}	0.075, 48	114.40
DXLVL	40.0±1.4^{ab}	36.0±1.0^{b}	0.050, 24	90.00	44.0±0.8^{a}	0.050, 48	110.00
VLPLVL	31.5±0.5^{b}	27.0±0.6^{c}	0.025, 24	85.71	40.5±1.4^{a}	0.075, 24	128.57
HLPLVL	46.0±0.9^{b}	42.5±0.5^{c}	0.050, 24	92.39	56.0±2.0^{a}	0.075, 24	121.73
VLSVL	52.0±1.3^{ab}	41.5±2.2^{b}	0.100, 24	79.80	55.5±0.4^{a}	0.025, 48	106.73
HLSVL	41.5±0.4^{b}	35.0±0.1^{c}	0.100, 24	84.33	48.5±0.7^{a}	0.050, 48	116.86
DXSVL	8.5±0.7^{a}	7.0±0.4^{b}	0.050, 48	82.35	9.0±0.8^{a}	0.075, 48	105.88
VLPSVL	18.0±3.0^{a}	14.5±1.1^{b}	0.100, 24	80.55	19.5±1.0^{a}	0.075, 24	108.33
HLPSVL	21.5±0.8^{a}	17.0±0.5^{b}	0.100, 24	79.06	25.0±1.0^{a}	0.025, 24	116.27
VLB	35.5±2.5^{ab}	31.5±2.5^{b}	0.100, 48	88.73	39.5±0.9^{a}	0.075, 24	111.26
HLB	36.5±0.1^{b}	32.0±1.2^{b}	0.050, 24	87.67	46.0±0.3^{a}	0.050, 48	126.02
LT	76.0±0.0^{b}	67.5±0.6^{c}	0.100, 48	88.81	83.5±1.1^{a}	0.075, 24	109.86

*colchicine concentrations (% w/v), incubation time (h) Different superscripts within the same row indicate significant differences at $p<0.05$ by LSD test

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