RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 59 Issue 3: 357-365 (March 2025)

Effects of Colchicine on the Morphology and Agricultural Characteristics of RD.43 Thai Rice Variety

Suntaree Surson[1], Suphasit Sitthaphanit[2], Jakkapat Prachachit[3], Tharadol Jitjak[3], Khumpanat Wongkerson[3]

10.18805/IJARe.AF-906

ABSTRACT

Background: Rice (*Oryza sativa* L.) is the staple food of the Asian population. Rice breeding will help the rice to withstand unsuitable environmental conditions while providing greater yield and palatability. Induction of rice polyploids can help improve desirable characteristics in rice. The objective of the study was to induce polyploids in Thai rice, which is genetically different from foreign rice.

Methods: Colchicine concentrations of 0.0, 0.1, 0.15, 0.2 and 0.25 per cent had been applied to 2-day-old germinated rice grains for 2, 4 and 6 hours. After that, rice agronomic characteristics and yield component were studied. To indicate polyploid rice, flow cytometry technique and awn seed characteristics were applied.

Result: The results showed that colchicine concentration and exposure time influenced germination and seed abnormalities. Colchicine concentration also influenced leaf number, shoot number, leaf width, leaf length and SPAD value. Exposure time influenced the number of leaves. In light of yield component characteristics, colchicine concentration influenced the total number of seeds, full seed number and seed width. Exposure time influenced number of grains, total number of seeds, number of whole seeds, number of atrophied seeds, full seed weight and seed width. Rice plants that had awn seeds (8.33-58.33 per cent) were grown from the seeds that received colchicine. The experiments had shown that colchicine influenced on morphology and yield component traits. T15 (colchicine 0.25%, exposure time 6 h.) had the most plants with awn seeds, followed by T9 (colchicine 0.5%, exposure time 6 h) and T11 (colchicine 0.2%, exposure time 4 h), respectively.

Key words: Colchicine, Mutation, *Oryza sativa* L., Polyploidy, Tetraploid.

INTRODUCTION

Polyploidy significantly impacts plant growth and breeding (Chen, 2010). Recent genomic studies show that over 70% of angiosperms have duplicated their genomes (Rieseberg and Willis, 2007). A polyploid organism possesses three or more full sets of chromosomes in its somatic cells. Polyploid plants are huge and have high nutrient and secondary metabolite levels. Vitality, flexibility and cold and drought tolerance are among its numerous benefits (Chen *et al*., 2021). Chromosome duplication causes whole-genome duplication (WGD), or polyploidy. Polyploidy drives evolution and speciation in all flowering plants (Van de Peer *et al*., 2017). Compared to diploid plants, polyploid plants have larger leaves and more nutrients and secondary metabolites. Autotetraploid rice hybridization, which combines the best features of heterosis and polyploidy to yield better harvests, has become a powerful rice breeding technique (Chen *et al*., 2021). People have recognized rice as an ancient polyploidy since the discovery of tetraploid rice in 1933. In terms of productivity and stress resistance, polyploid rice breeding has demonstrated notable benefits since its inception in China in 1953 (Cai *et al*., 2007; Nakamori, 1933; Bao and Yan, 1956). Since the 1960s, Asian researchers have focused mostly on genetic improvement and practical application while studying autotetraploid rice (Chen *et al*., 2021; Xiong *et al*., 2022). Colchicine is used to double the number of chromosomes in diploid rice, resulting in autotetraploid rice (*Oryza sativa*), a rich genetic resource. Compared to diploid hybrids, intersubspecific autotetraploid rice hybrids (ssp. indica 3; ssp. japonica) have more biological advantages, such as increased adaptability and the potential for larger yields (Wu *et al*., 2013). Currently, tetraploid rice has been cultivated in China, such as PMeS-1 white (Sg99012) and PMeS-2 white (HN2026) which have polyploid meiosis

[1]Plant Science, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon Province-47000, Thailand.
[2]Department of Agriculture and Resources, Faculty of Natural Resources and Agro-Industry, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus-47000, Thailand.
[3]Animal Science, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon Province-47000, Thailand.

Corresponding Author: Suntaree Surson, Plant Science, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon Province-47000, Thailand.
Email: suntaree.s@snru.ac.th ORCIDs: 0009-0003-4251-3154

How to cite this article: Surson, S., Sitthaphanit, S., Prachachit, J., Jitjak, T. and Wongkerson, K. (2025). Effects of Colchicine on the Morphology and Agricultural Characteristics of RD.43 Thai Rice Variety. Indian Journal of Agricultural Research. 59(3): 357-365. doi: 10.18805/IJARe.AF-906

Submitted: 06-08-2024 **Accepted:** 28-11-2024 **Online:** 28-12-2024

stability (PMeS) genes and the offspring of these two tetraploid rice varieties with other tetraploid rice varieties (Cai *et al.,* 2007). In other countries, polyploid rice is being studied and developed, such as Indonesia and Thailand. Polyploid rice has many good agronomic traits, such as panicle length and grain length (Koide *et al.,* 2020) and improved grain quality (Gong *et al.*, 2023).

The characteristics of Thai rice varieties are different from Chinese or other foreign rice varieties. Furthermore, it has also been well-accepted that polyploidy rice has many outstanding characteristics. For example, polyploid rice has the characteristics of salt and drought tolerance, panicle length and large grain size (Chen *et al.,* 2021). In addition, there are many interesting characteristics of polyploid rice that have not been seriously studied, such as cooking quality, palatability, aroma and nutritional value, *etc*. For this reason, the concept of breeding Thai polyploid rice has attracted the attention of the research team, where the research team will use the genetic source of Thai rice, which is different from Chinese rice varieties, to induce polyploid cultivation. However, research on polyploid rice in Thailand is still scarce, so in this study, a study was conducted to find an effective method for inducing polyploid rice in Thailand and to examine RD. 43 variant rice in terms of morphology and initial yield components to determine whether these colchicine-induced rice are worthy of further breeding. After that, breeders will continue to search for Thai polyploid rice varieties that have the potential to produce seeds for further use in Thai rice breeding.

MATERIALS AND METHODS

Plant material

The study was conducted in March-September 2023. The RD.43 rice variety studied was a diploid *Oryza sativa* L. (2n = 2× = 24). RD43 rice was selected from a single cross between the Suphan Buri fragrant rice variety and Suphan Buri1 variety at the Suphan Buri Rice Research Center of Thailand. Normally, RD.43 rice should be planted in irrigated rice fields, areas with long-term flooding, or areas where farmers have less time to farm than other rice-growing areas. This rice variety has moderate resistance to leaf blast disease and brown planthopper problems. RD.43 rice has a long and slender grain shape, with little chalky grains. The quality of the rice grains for cooking and eating is good. The glycemic index is 57.5, which is classified as a food with a moderate to low glycemic index. (Thadamatakul *et al*., 2021).

Colchicine treatment in seed

Factorial in completely randomized experiment was designed. The study considered colchicine concentration (0.0, 0.1, 0.15, 0.2 and 0.25 per cent) and treatment time (2, 4 and 6 hours). The experiment had 15 treatments of 4 repeating 60 seeds. Bleaching the seeds began with a 3-minute rinse in tap water. Next, rinse the seeds with dish soap (sunlight) and tap water for 3 minutes. Bleach and disinfect the seed shell for 10 minutes with 10% Hyter bleach. Three times, rinse with drinking water (Singha water) for 2 minutes. Soak grains overnight in simple water. Then, put the seeds on seed paper in 5×8×2 cm plastic trays for 2 days. For all 15 treatments, the seeds were steeped in various colchicine concentrations.

Germination test

After the treatment of the seed germination of the RD.43 variety in various treatments, the rice was cultivated in a 60-hole seeding tray filled with peat moss. After 10 working days of cultivation, germination (percentage) was checked by counting all germinated rice plants and calculating them using the formula: (Surson *et al.,* 2021).

$$\text{Germination percentage} = \frac{\text{Number of germinated rice plants}}{\text{Total number of seeds planted}} \times 100$$

Abnormality seedling

After cultivating rice in various treatments for 10 days, the rice germinated. The characteristics of the rice seedlings were classified into two types: normal rice seedlings and abnormal rice seedlings. Normal rice seedlings have tall, slender stems. All leaves are slender. Normal seedlings germinate and grow quickly. The abnormal seedling has short, stout stems at the base of which are slightly swollen. The first pair of true leaves are characterized by short and spreading leaves that are rather round. Abnormal seedlings germinate and grow slowly. The normal and abnormal plants were counted and calculated using the following formula: (Surson *et al.,* 2021).

$$\text{Abnormal rice plant percentage} = \frac{\text{Abnormal number of plants}}{\text{Number of seeds planted}} \times 100$$

Agro-morphological characteristics

After separating abnormal seedlings from normal ones based on their characteristics, these were transplanted into 8-inch pots for a comparative study of each treatment's morphology in months 1, 2 and 3. These variables included plant height (cm), number of leaves, number of shoots/ plant, plant circumference, leaf width, leaf lengthand SPAD values. From base to highest point, the plant height was measured in cm. All enormous, fully spread leaves were counted. All plant shoots were counted for shoot number characteristics. The circumference of the initial shoots (cm) is measured 5 cm above ground. The 3rd order leaf of the initial stem is measured for leaf width characteristics (cm). The middle and tip of the 3rd leaf of the first shoot from the base were measured for SPAD.

Yield component characteristics

After analyzing agro-morphology, yield component characteristics were examined. Several variables were

investigated, including the number of ears, total seeds, full seeds, atrophied seeds, full seed weight (g), seed length (cm) and seed width (cm). Total ears born in the rice plant were counted. The plant's total number of atrophied seeds was counted. Include all atrophied and full seeds in the plant when counting seeds/plants. Count only full seeds in the plant to count the full seeds. Sort then weigh only full seeds/plant for full seed weight/plant (g). The average seed length (cm) was calculated from 10 measurements. After measuring 10 seed widths (cm), the average was calculated per seed.

Characterization of polyploid rice

Flow cytometer analysis

Random abnormal rice plants to be examined with a flow cytometer which has the following steps: The ploidy of the aberrant plants was assessed after the seeds had been sown for a period of three months. The leaves, which ranged in weight from 0.1 to 0.5 grams apiece and measured 1-2 cm in size, were finely minced using 500 µL of Quantum Stain NA UV 2 (A) on a plastic Petri plate. In order to eliminate waste or solid particles, a solution containing roughly 0.05 grams of polyvinyl-pyrrolidone (PVP) was dropped into the mixture. Afterwards, the mixture underwent filtration using a 30-micron strainer to remove any particles and preserve the nuclei. Subsequently, a volume of 500 µL of Quantum Stain NA UV 2 (B) was put into the tube holding the sampled nuclei. Prior to evaluating the combination using the Quantum Analysis Flow Cytometer from Germany, it was necessary to vigorously shake the tube in order to achieve complete homogenization of the contents. Polyploid plant percentage were calculated by using the formula below:

$$\text{Polyploid plant percentage} = \frac{\text{Polyploid plant}}{\text{Polyploid plant measured}} \times 100$$

Determination of polyploid rice by the awn seed characteristic

Studies in several reports have shown that rice treated with colchicine, which is genetically converted to polyploids, has a seed with awn (Song *et al*., 2014). In this study, the rice variety used in the study was rice variety RD.43, a diploid rice whose seed does not have awn seed. After the treatment of germinated rice seeds, the number of plants whose seeds had awns in each treatment were counted. The percentage of rice with awn seeds were calculated by using the formula underneath:

$$\text{Percentage of rice plants with awn} = \frac{\text{Number of rice plants with awn seed}}{\text{Total number of plants}} \times 100$$

Statistical analysis

Factorial in completely randomized experiment was designed. Factor A is the concentration of colchicine (0.0, 0.1, 0.15, 0.2 and 0.25 per cent) and factor B is the duration of colchicine exposure (2, 4 and 6 hours). Data analyzed using two way ANOVA (5 colchicine concentrations × 2 treatment duration time). Subsequently, wherever the F-test was significant, mean comparison were conducted using DMRT. All analyses were performed using the SPSS version 16 package.

RESULTS AND DICUSSION

Effect of colchicine on germination and abnormality of rice variety RD.43:

Germination of rice variety RD.43

Germinated rice seed variants RD.43 were induced in colchicine concentrations of 0.0, 0.1, 0.15, 0.2 and 0.25 per cent for 2, 4 and 6 hours. Rice grains with higher colchicine concentrations and times had lower rice

Table 1: Effect of colchicine on rice of RD.43 variety (germination, abnormality, plant height number of leaves) at the age of 1 month.

Factor	Germination	Abnormality	Plant height (cm)	Number of leaf
Concentration (A)				
0.0	93.89±5.04^{a}	0.00±0.00^{c}	23.09±1.81^{a}	3.37±0.19^{a}
0.1	54.44±21.58^{b}	21.80±8.39^{a}	12.90±4.40^{b}	2.91±0.50^{b}
0.15	43.33±24.07^{c}	18.20±10.62^{a-b}	11.51±3.75^{b}	2.70±0.49^{b}
0.2	44.17±19.47^{c}	16.95±5.63^{a-b}	11.81±3.70^{b}	2.70±0.33^{b}
0.25	20.97±21.40^{d}	13.75±15.47^{b}	8.28±2.17^{c}	1.81±1.12^{c}
F-test	**	**	**	**
Time (B)				
2	67.42±20.75^{a}	18.75±13.19^{a}	15.98±5.85^{a}	3.09±0.41^{a}
4	45.58±30.43^{b}	12.75±11.83^{b}	12.91±5.50^{b}	2.80±0.43^{b}
6	41.08±33.69^{b}	10.92±9.63^{b}	11.65±6.07^{b}	2.21±1.06^{c}
F-test	**	**	**	**
Treatment (A×B)	**	**	**	**
C.V (%)	20.82	47.34	15.46	13.35

Different superscripts in each row indicate significant ($p<0.05$) differences; ns = Non-significant; ** = Highly significant ($p<0.01$); * = Significant ($p<0.05$).

germination. However, high colchicine concentrations inhibited rice germination, resulting in fewer aberrant rice plants (Table 1). After analyzing colchicine concentration and duration, T3 (colchicine 0.0%, 6 h) had the highest rice germination (95.84%). Increasing colchicine doses and durations diminish seed germination in several trials (Surson *et al*., 2024 a, b).

Abnormality of rice variety RD.43

After evaluating colchicine concentration and duration, T13 (0.25%, 2h) had the most anomalies (33.75%). In numerous experiments by Surson *et al*. (2024 a, b), increasing doses and durations of colchicine treatment in several plant species caused more aberrant plants.

Morphology of rice variety RD. 43

Morphology of rice at the age of 1 month

The effects of colchicine concentrations and exposure duration were examined. Rice leaf number and height were affected by colchicine content and exposure period. When colchicine concentration rose, plant height and leaves dropped. Germination, abnormalities, plant heightand leaf number were statistically significantly affected by colchicine concentration and treatment period (Table 1).

Morphology of rice at the age of 2 months

Rice at 2 months old demonstrated a statistically significant effect of colchicine concentration and exposure period on height, number of leaves, number of shoots per plant and shoot circumference. ($p \leq 0.01$). Different exposure times do not statistically affect height, leaves, shoots, or shoot circumference. The treatment with the highest rice plant height was T1 (colchicine 0.0%, duration 2 h.) (74.05 cm). The lowest height is T15 (colchicine 0.25%, 6 h). The greatest shoot circumference was T12 (3.47 cm, colchicine 0.2%, 6 h) and the smallest was T11 (2.76 cm, 4 h). No significant difference was observed in leaf and shoot numbers per treatment. Different levels of colchicine caused statistically significant variations in leaf width, lengthand SPAD in RD.43 rice leaves. Colchicine at various times did not statistically affect leaf width, length, or SPAD. When concentration and duration of colchicine exposure were considered, leaf width, lengthand SPAD values did not change across treatments (Table 2).

Morphology of rice at the age of 3 months

Different colchicine concentrations and exposure time had significant effects on rice variety RD.43, including leaf and shoot numbers, but not on height or shoot circumference (Table 3). Number of shoots/plant varied statistically and dramatically with exposure duration. Shoot circumference, leaf number and height were not significantly different. However, longer colchicine exposure increases plant height, shoot number and shoot circumference (Table 3). Rice had statistically significant differences in plant height, number of leaves/plant, number of shoots/plantand plant circumference when exposed to different colchicine concentrations and times. In rice varieties RD.43, varying concentrations of colchicine showed statistically significant changes in leaf width, length nand SPAD. There was no significant difference in leaf width, length, or SPAD between colchicine exposure times. There was not a significant difference in leaf width, length, or SPAD values when colchicine concentration and exposure duration were considered (Table 3). Colchicine affected rice plant morphology at 3 months old by influencing leaf number, shoots/plant, leaf width, leaf length and leaf SPAD values. Surson *et al*. (2021) found that colchicine concentration affected black sesame plant height and leaf number/plant. In watermelons, colchicine concentration and exposure duration impact leaf width, length, petiole length, stem diameterand plant height (Khan *et al*., 2023). Plant genetics and polyploid induction mechanisms also have an effect on plant morphology, germination and the percentage of polyploid plants (Ewald, 2009; Jokari, 2022; Khan, 2023; Zeinullina, 2023). This study investigated how colchicine concentration and exposure duration affected germination, awn seed plant percentage and morphology.

Effects of colchicine on yield component characteristics of RD.43 rice variety

The effects of colchicine concentration and exposure time on the yield component characteristics of the rice variety RD. 43 showed that there were no significant differences in the number of ears per plant, but there were significant differences in the number of seeds and full seeds per plant ($P \leq 0.05$). Studying varied concentrations of colchicine exposure duration revealed significant changes in ear, seedand full seed numbers ($p \leq 0.01$). The number of ears, total seedsand the full seeds/plant increase with colchicine duration time (Table 4). Colchicine concentration and exposure time had a significant effect on the number of ears/plant and total seeds/plant ($p \leq 0.01$ and $p \leq 0.05$, respectively), but not on the number of full seeds/plant. The effects of colchicine on the number of atrophied seeds/plant, full seed weight, seed lengthand seed width showed that different concentrations did not affect atrophied seeds/plant, full seed weight, or seed width, but did affect seed length ($p \leq 0.01$). Different exposure times statistically affected atrophied seed/plant, full seed weight/plant and seed length. Colchicine concentration and exposure duration caused statistically significant differences in whole seed weight/plant, seed width and seed length. Table 4 shows no statistical difference in the quantity of atrophied seeds/plant ($p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.05$). Colchicine concentration affected total seed number/plant, full seed number/plant, seed widthand seed length in RD.43 rice. Exposure duration influenced the number of ears, total seeds, full seeds, atrophied seeds, full seed weightand seed length. Administering colchicine for 2, 4, or 6 hours improved the yield. Extending the colchicine treatment from 6 to 12 hours led to an increase in millet (*Panicum miliaceum* L.) grain production. However, colchicine lowered grain production in practically all millet types after 24 hours (Zeinullina *et al*., 2023). The exposure duration

Table 2: Effect of colchicine on rice of the RD. 43 variety (plant height, number of leaves, shoot number/plant, first shoot circumference, leaf width, leaf length, SPAD value) at 2 months of age.

Factor	Pant height (cm)	Number of leaves	Shoot number/ plant	First shoot circumference (cm)	Leaf width (cm)	Leaf length (cm)	SPAD value
Concentration (A)							
0.0	73.55±2.44 ^{a}	22.52±6.49 ^{b}	4.56±1.39 ^{c}	3.15±0.28 $^{a-b}$	1.12±0.05 ^{a}	46.87±1.89 ^{a}	35.02±1.16 ^{a}
0.1	71.02±3.42 ^{b}	30.65±7.64 ^{a}	6.25±1.62 $^{a-b}$	3.01±0.30 ^{b}	1.04±0.05 ^{b}	44.73±3.57 $^{a-b}$	32.62±1.81 ^{b}
0.15	70.20±2.76 ^{b}	33.13±7.75 ^{a}	6.79±1.50 $^{a-b}$	3.17±0.34 $^{a-b}$	1.04±0.06 ^{b}	43.97±2.53 ^{b}	33.24±0.92 ^{b}
0.2	70.73±3.52 ^{b}	28.94±7.68 ^{a}	6.02±1.55 $^{a-b}$	3.26±0.47 ^{a}	1.06±0.05 ^{b}	45.35±1.82 $^{a-b}$	33.65±1.45 $^{a-b}$
0.25	65.00±3.75 ^{c}	22.06±5.53 ^{b}	5.40±1.62 $^{b-c}$	2.78±0.28 ^{c}	1.03±0.04 ^{b}	41.22±2.19 ^{c}	35.05±2.29 ^{a}
F-test	**	**	**	**	**	**	**
Time (B)							
2	70.01±4.31	26.23±8.58	5.45±1.76	3.06±0.43	1.06±0.06	44.21±3.50	33.92±2.07
4	70.19±3.90	28.44±9.09	5.88±1.73	3.15±0.32	1.07±0.06	44.34±2.77	34.10±1.45
6	70.10±4.58	27.71±6.91	6.09±1.55	3.02±0.36	1.04±0.05	44.74±2.96	33.72±1.97
F-test	ns	ns	ns	ns	ns	ns	ns
Treatment (A×B)	*	ns	ns	**	ns	ns	ns
C.V (%)	4.31	25.21	25.34	7.98	5.17	5.89	4.85

Different superscripts in each row indicate significant (p<0.05) differences; ns = Non-significant; ** = Highly significant (p<0.01); * = Significant (p<0.05).

Table 3: Effect of colchicine on rice variety RD. 43 (plant height, number of leaves, number of shoots/plant, first shoot circumference, leaf width, leaf length, SPAD value) at the age of 3 months.

Factor	Plant height (cm)	Number of leaves	Number of shoots/ plant	First shoot circumference (cm)	Leaf width (cm)	Leaf length (cm)	SPAD value
Concentration (A)							
0.0	94.57±5.25	28.89±9.23 ^{b}	6.33±2.26 ^{c}	2.69±0.16	1.13±0.09 ^{a}	40.64±4.45 $^{b-c}$	40.48±2.26 ^{a}
0.1	94.66±5.77	35.14±4.81 $^{a-b}$	9.31±1.65 ^{a}	2.89±0.30	1.00±0.07 ^{b}	40.24±4.85 $^{b-c}$	36.96±2.05 ^{b}
0.15	90.46±4.92	32.58±7.78 ^{b}	9.39±1.11 ^{a}	2.84±0.37	1.03±0.10 ^{b}	38.90±5.41 ^{c}	36.47±3.00 ^{b}
0.2	94.68±6.16	35.92±10.72 $^{a-b}$	7.69±2.33 $^{b-c}$	2.79±0.21	1.15±0.10 ^{a}	45.38±5.65 ^{a}	38.59±2.91 $^{a-b}$
0.25	97.78±12.70	41.92±12.91 ^{a}	9.06±2.89 $^{a-b}$	2.82±0.23	1.11±0.10 ^{a}	43.67±3.16 $^{a-b}$	38.27±2.36 $^{a-b}$
F-test	ns	**	**	ns	**	**	**
Time (B)							
2	92.03±8.24	31.85±8.37	7.47±2.40 ^{b}	2.74±0.29	1.09±0.11	41.51±4.18	38.28±2.44
4	94.89±5.47	34.68±9.84	8.35±2.31 $^{a-b}$	2.84±0.25	1.07±0.11	41.25±6.59	37.88±2.58
6	96.37±8.58	38.13±11.45	9.25±2.22 ^{a}	2.83±0.25	1.10±0.10	42.53±4.73	38.30±3.50
F-test	ns	ns	**	ns	ns	ns	ns
Treatment (A×B)	**	*	**	**	ns	ns	ns
C.V (%)	6.00	23.70	20.68	8.94	7.72	11.23	7.03

Different superscripts in each row indicate significant (p<0.05) differences; ns = Non-significant; ** = Highly significant (p<0.01); * = Significant (p<0.05).

Table 4: Effect of colchicine at different concentrations and times on number of ears/plant, total number of seeds/plant, number of full seeds/plant, number of atrophied seeds/plant, full seed weight, seed length, seed width of rice variety RD. 43.

Factor	Number of ears/plant	Total number of seeds/plant	Number of full seeds/plant	Number of atrophied seeds/plant	Full seed weight (g)	Seed length (cm)	Seed width (cm)
Concentration (A)							
0.0	4.67±1.46	291.83±107.35^{a-b}	196.33±81.83^{a-b}	91.14±31.78	5.74±2.82	1.052±0.009^{a}	0.265±0.005
0.1	5.03±2.10	227.64±92.45^{b}	145.89±61.79^{b}	90.03±48.82	4.78±1.95	1.049±0.009^{a}	0.266±0.004
0.15	4.38±1.00	309.82±116.41^{a-b}	212.99±67.21^{a}	93.06±52.57	6.24±2.36	1.060±0.020^{a}	0.266±0.009
0.2	4.61±0.91	358.31±153.57^{a}	242.81±97.90^{a}	93.50±52.30	7.13±2.48	1.026±0.018^{b}	0.266±0.008
0.25	4.67±1.27	296.51±94.91^{a-b}	201.68±76.52^{a-b}	92.67±33.50	6.11±2.03	1.030±0.027^{b}	0.265±0.006
F-test	ns	*	*	ns	ns	**	ns
Time (B)							
2	4.20±0.81^{b}	247.56±73.01^{b}	165.27±60.30^{b}	81.00±32.19^{b}	4.88±1.67^{b}	1.038±0.020^{b}	0.264±0.006
4	4.15±1.12^{b}	287.45±143.55^{b}	191.00±92.91^{b}	80.82±45.67^{b}	5.66±2.46^{b}	1.044±0.020^{b}	0.265±0.006
6	5.66±1.57^{a}	355.46±108.01^{a}	243.54±71.73^{a}	114.42±43.52^{a}	7.46±2.30^{a}	1.049±0.025^{a}	0.268±0.007
F-test	**	**	**	*	**	*	ns
Treatment (A×B)	**	*	ns	ns	*	**	**
C.V (%)	19.39	33.41	33.44	44.52	31.57	0.00	1.99

Different superscripts in each row indicate significant (p<0.05) differences; ns = Non-significant; ** = Highly significant (p<0.01); * = Significant (p<0.05).

increasing from 2, 4 and 6 hours did not impact rice production in this experiment.

Identification of polyploid plants from flow cytometry analysis and awn seed characteristic

Randomization of 10 aberrant rice seedlings under various treatment showed 0% polyploid rice plants (Fig 1). Rice plants with awn seeds, which suggest polyploidy (Song *et al*., 2014), were identified in all treatments (colchicine of all concentrations and exposure durations). T15 (colchicine 0.25%, time 6 h.) had the most awn seed plants, T4 (colchicine 0.1%, time 2 h.) had the fewestand treatments without colchicine did not find any (Table 5; Fig 2). Screening aberrant rice plants before polyploid seed categorization minimizes the number of rice plants that need flow cytometer (FCM) and awn seed characteristics monitoring, reducing polyploid monitoring budget and manpower. A study of aberrant rice plants from awn-characteristic seed indicated 8.33-58.33 per cent polyploid. It depends on colchicine concentration. Many plant studies have revealed that vivo polyploid induction concentrations and exposure times vary. This study found that treatments T9, T11 and T15 (colchicine 0.15%, 6 h.) induced polyploidy in seeds with awns. The results were different when polyploidy was analyzed by flow cytometry. Flow cytometry showed that colchicine-treated rice did not have polyploidy, even if the plants presented seed with awn, according to Song *et al*. (2014). Rice plants may be chimera, with diploid and polyploid stems. Plant leaf diploid sections showed no polyploidy by flow cytometry. However, rice plants that produced specific awn seeds generated diploid and tetraploid progeny during M2 polyploidy research. Thus, rice plants treated with colchicine, which produced awn seeds, may generate tetraploid progeny despite no ploidy

Table 5: Influence of concentration level and time of exposure to different colchicine on the percentage of rice plants with awn seed of rice varieties RD. 43.

Treatment	Rice plants with awn seed (%)
T1 (0.0,2)	0.00
T2 (0.0,4)	0.00
T3 (0.0,6)	0.00
T4 (0.1,2)	8.33
T5 (0.1,4)	16.67
T6 (0.1,6)	33.33
T7 (0.15,2)	33.33
T8 (0.15,4)	33.33
T9 (0.15,6)	50.00
T10 (0.2,2)	33.33
T11 (0.2,4)	50.00
T12 (0.2,6)	16.67
T13 (0.25,2)	25.00
T14 (0.25,4)	25.00
T15 (0.25,6)	58.33

change in the M1 generation. Colchicine treatment has also been shown to induce genetic variation in plants (Zeinullina *et al*., 2023; Fathurrahman *et al*., 2023; Valenzuela *et al*., 2022; Yan *et al*., 2022; Kasmiyati *et al*., 2021; Cabahug, 2021). Colchicine treatment does not produce polyploid plants, but it can generate plant variety, which may create novel characteristics that are better than the parent plants, comparable to mutations. Plant breeding programs benefit from it. Rice polyploid induction can occur *in vivo* or *in vitro* (Chen *et al*., 2021). Many research have used polyploid induction in vivo, including Chen *et al*. (2021) and Bao and Yan (1956). Colchicine is the most common polyploid inducer. Colchicine modifies tubules. Colchicine inhibits microtubule activity for cell division in metaphase

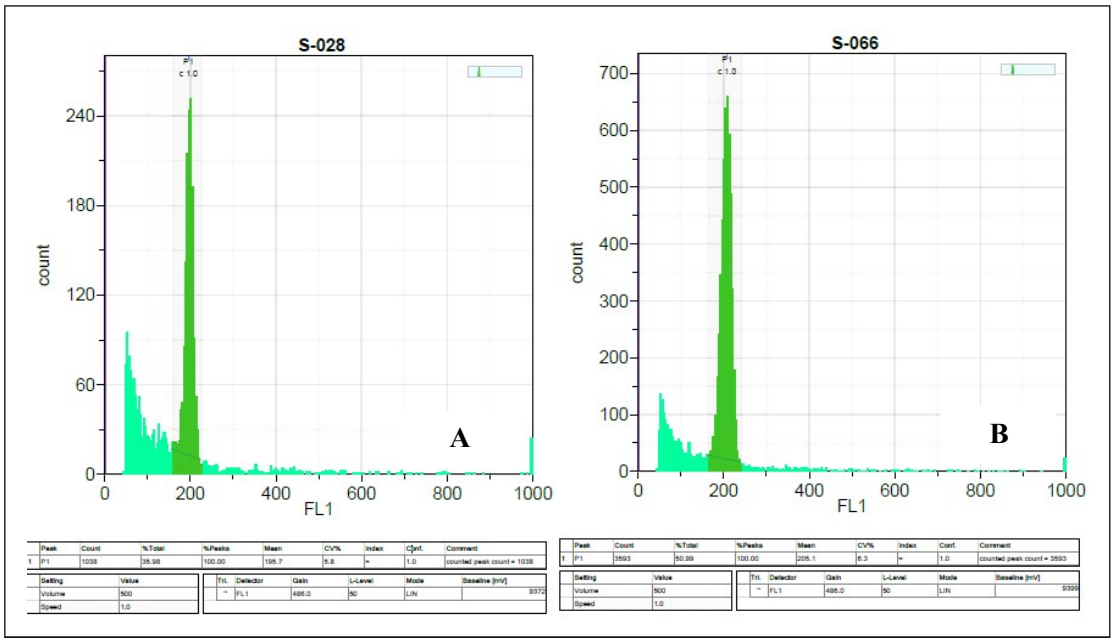


Fig 1: Flow cytometry analysis of rice plant: Normal rice plant (A) and abnormal rice plant (B).

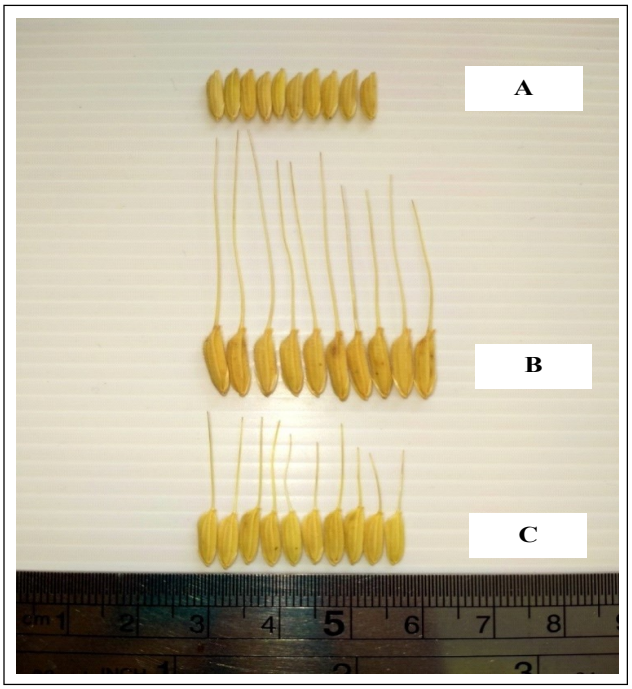


Fig 2: Rice seeds of variety RD.43 treated without colchicine (A) and treated with colchicine (B and C).

(Jokari *et al*., 2022). Colchicine promotes aberrant cell division and increases chromosome number generating polyploids. Cai *et al*. (2007) found that tissue culture induces rice polyploids better than *in vivo*. *Vivo* technique is a simple approach that induces polyploid rice types without tissue culture knowledge, funds, laboratory, or supplies. Thus, this work intended to improve polyploid rice induction *in vivo* and screen out aberrant rice before sorting polyploid rice with costly instruments.

CONCLUSION

Colchicine was tested as the best way to produce polyploidy in rice RD43. Different colchicine concentrations (0.0, 0.1, 0.15, 0.2 and 0.25%) were used for 2, 4 and 6 hours. The most aberrant plants were seen in rice RD43 treated with 0.25% colchicine for 2 hours. The study sought the best polyploidy method. It found that 0.15% colchicine for 6 hours was best. Aberrant plants survived this treatment at high rates. Additionally, a large percentage of rice plants in the experimental group had awn seeds, indicating polyploidy. The height, leaf number, shoot number, breadth, leaf lengthand chlorophyll content of 3-month-old rice RD43 varied significantly with colchicine concentration. The number of shoots per plant varied greatly with colchicine duration. When yield components were examined, colchicine at different doses caused significant differences in seed number and seed development.

ACKNOWLEDGEMENT

The authors would like to extend their gratitude to the Faculty of Agricultural Technology and the Research Institution, of Sakon Nakhon Rajabhat University; and Kasetsart University-Chalermphrakiat Sakon Nakhon Province Campus for their support by allowing us to use the tools, materials, supplies, offices and places to conduct this study.

Disclaimers

The opinions and findings articulated in this article are exclusively those of the writers and do not always reflect the perspectives of their connected institutions. The writers bear responsibility for the truth and completeness of the material presented; nevertheless, they disclaim all duty for direct or indirect damages arising from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest.

REFERENCES

Bao, W.K. and Yan, Y.R. (1956). A preliminary report on investigations of autopolyploids and amphidiploid in some cereal crops. Acta. Bot. Sin. 5: 297-316.

Cabahug, R.A.M., Khanh, H.T.T.M., Lim, K.B., Hwang, Y.J. (2021). Phenotype and ploidy evaluation of colchicine-induced Echeveria 'Peerless'. Toxicology and Environmental Health Sciences. 13: 17-24.

Cai, D.T., Chen, J.G., Chen, D.L., Dai, B.C., Song, Z.J., Yang, Z.F., Du, C.Q., Tang, Z.Q., He, Y.C., Zhang, D.S. *et al*. (2007). The breeding of two polyploid rice lines with the characteristic of polyploid meiosis stability. Sci China C Life. 50: 356-366.

Chen, R., Feng, Z., Zhang, X., Song, Z., Cai, D. (2021). A new way of rice breeding: Polyploid rice breeding. Plants. 10: 422.

Chen, Z.J. (2010). Molecular mechanisms of polyploidy and hybrid vigor. Trends in Plant Science. 15: 57-71.

Ewald, D., Ulrich, K., Naujoks, G., Schröder, M.B. (2009). Induction of tetraploid poplar and black locust plants using colchicine: Chloroplast number as an early marker for selecting polyploids *in vitro*. Plant Cell, Tissue and Organ Culture (PCTOC). 99: 353-357.

Fathurrahman, F. (2023). Effect of colchicine mutagen on phenotype and genotype of *Vigna unguiculata* var. sesquipedalis the 7th generation. Biodiversitas. 24: 1408-1415.

Gong, D., Zhang, X., He, F., Chen, Y., Li, R., Yao, J., Zhang, M., Zheng, W., Yu, G. (2023). Genetic improvements in rice grain quality: A review of elite genes and their applications in molecular breeding. Agronomy. 13: 1375.

Jokari, S., Shekafandeh, A., Jowkar, A. (2022). *In vitro* tetraploidy induction in Mexican lime and sour orange and evaluation of their morphological and physiological characteristics. Plant Cell, Tissue and Organ Culture (PCTOC). 150: 651-668.

Kasmiyati, S., Kristiani, E.B.E., Herawati, M.M., Rondonuwu, F.S. (2021). Exploring anticancer activity of wild and polyploid mutant of Artemisia cina. Biodiversitas Journal of Biological Diversity. 22: 1227-1234.

Khan, M.N.E.A., Hassan, J., Biswas, M.S., Khan, H.I., Sultana, H., Suborna, M.N., Rajib, M.M.R., Akter, J., Gomasta, J., Anik, A.A.M. (2023). Morphological and anatomical characterization of colchicine-induced polyploids in watermelon. Horticulture, Environment and Biotechnology. pp 1-14.

Koide, Y., Kuniyoshi, D., Kishima, Y. (2020). Fertile tetraploids: New resources for future rice breeding?. Front. Plant Sci. 11: 1231.

Nakamori, E. (1933). On the occurrence of the tetraploid plant of rice, *Oryza sativa* L. Pro. Imperial Academy. 9: 340-341.

Rieseberg, L.H. and Willis, J.H. (2007). Plant speciation. Science. 317: 910-914.

Song, Z.J., Du, C.Q., Zhang, X.H., Chen, D.L., He, Y.C., Cai, D.T. (2014). Studies on awns in polyploid rice (*Oryza sativa* L.) and preliminary cross experiments of a special awnless tetraploid rice. Genetic Resources and Crop Evolution. 61: 797-807.

Surson, S., Sitthaphanit, S., Wongkerson, K. (2021). Polyploidy induction of black sesame (*Sesamum indicum* L.) for yield component improvement. Songklanakarin Journal of Science and Technology. 43.

Surson, S., Sitthaphanit, S., Wongkerson, K. (2024a). Effective colchicine-induced polyploid induction in *Centella asiatica* (L.) Urban. Plant Cell, Tissue and Organ Culture (PCTOC). 159: 1-11.

Surson, S., Sitthaphanit, S., Wongkerson, K. (2024b). Effect of Colchicine on andrographis variety Phichit 4-4 for plant breeding in stem and leaf characteristics. Indian J. Agric. Res. 58: 744-751.doi: 10.18805/IJARe.AF-829.

Thadamatakul, P., Songsiri, P. and Techasukthavorn, V. (2021). Three rice of Thailand on blood glucose control. Thai JPEN. 29(1): 24-33.

Valenzuela, F., D'Afonseca, V., Hernández, R., Gómez, A., Arencibia, A.D. (2022). Validation of reference genes in a population of blueberry (*Vaccinium corymbosum*) plants regenerated in colchicine. Plants. 11: 2645.

Van de Peer, Y., Mizrachi, E., Marchal, K. (2017). The evolutionary significance of polyploidy. Nature Reviews Genetics. 18: 411-424.

Wu, J.W., Hu, C.Y., Shahid, M.Q., Guo, H.B., Zeng, Y.X., Liu, X.D., Lu, Y.G. (2013). Analysis on genetic diversification and heterosis in autotetraploid rice. Springer Plus. 2: 1-12.

Xiong, Z., Xiong, D., Cai, D., Wang, W., Cui, K., Peng, S., Huang, J. (2022). Effect of stomatal morphology on leaf photosynthetic induction under fluctuating light across diploid and tetraploid rice. Environmental and Experimental Botany. 194: 104757.

Yan, Y.J., Qin, S.S., Zhou, N.Z., Xie, Y., He, Y. (2022). Effects of colchicine on polyploidy induction of *Buddleja lindleyana* seeds. Plant Cell, Tissue and Organ Culture (PCTOC). 149: 735-745.

Zeinullina, A., Zargar, M., Dyussibayeva, E., Orazov, A., Zhirnova, I., Yessenbekova, G., Zotova, L., Rysbekova, A., Hu, Y.G. (2023). Agro-morphological traits and molecular diversity of proso millet (*Panicum miliaceum* L.) affected by various colchicine treatments. Agronomy. 13: 2973.