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Effects of colchicine on polyploid induction, morphology, and yield components of several Thai rice varieties

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Abstract. *Surson S, Sitthaphanti S, Prachachit J, Jitjak T, Wongkerson K. 2024. Effects of colchicine on polyploid induction, morphology, and yield components of several Thai rice varieties. Biodiversitas 25: 4677-4689.* Rice (*Oryza sativa*) is a Thai staple food and is different from foreign rice in its characteristics. By producing polyploids, rice cultivation becomes resistant to unfavorable climatic conditions and produces good yields and good tastes. Inducing polyploidy can improve Thai rice. This research aimed to induce polyploid formation in Thai rice to be used as a breeding source for improving Thai polyploid rice in the future. This study created polyploids from 10 Thai rice varieties to test colchicine's impact on morphology and yield. Colchicine-treated rice plants were tested for polyploidy at 4 months of age using flow cytometry and awn seed characteristics. The results showed that colchicine treatment and varied exposure periods at 1 month of age significantly affected germination and abnormalities in rice varieties. Variations in rice varieties and exposure times resulted in varying morphological and yield component attributes at 3 months. All colchicine-treated rice types had 5-90% awn seeds. This study reveals that rice varieties and exposure durations affect the morphology and features of yield components. Awn seeds determined rice polyploidy, which varied by variety. Blackberry has the most awn-seed plants (90%), followed by Maejo 2 (60%), and Homnaka (60%).

Keywords: Colchicine, mutation, *Oryza sativa,* polyploidy, tetraploid, Thai rice

INTRODUCTION

Polyploidy or whole-genome duplication (WGD) is caused by chromosome doubling (Rao et al. 2023) and results in a change in growth and productivity (van de Peer et al. 2017; Rao et al. 2023). Polyploid occurrence in plants is recognized for its important role in the process of plant evolution and plant breeding. WGD has occurred several times during the evolution of Angiosperms over the last 200 million years. WGD is a process of chromosomal doubling, with both a sudden increase in genome size and a new set of genes. The common ancestors of Poaceae, including important crop species, such as corn, rice, wheat, and sorghum, shared WGD about 70 million years ago (Lee et al. 2020). Polyploid induction can be performed both in vivo and in vitro. Polyploid induction can be carried out in various plant parts, such as seeds, seedlings, and plants (Zhang and Gao 2020; Maru et al. 2021; Shariat and Sefidkon 2021; Kim et al. 2022). Polyploid technology is widely used in plant breeding, especially for increasing the size of the organ in the vegetative part or increasing the biomass (Chen et al. 2021; Shariat and Sefidkon 2021; Kim et al. 2022).

Rice (*Oryza sativa*) is an important crop used as a staple food for the world's population, especially in Asia. More than 90% of Asian people consume rice as a staple food (Xiong et al. 2022). Rice breeding can help solve the global food shortage (Sun et al. 2020; Xiong et al. 2022). Polyploid breeding is one of the approaches used in rice breeding. More recently, autopolyploid rice breeding has become a powerful application in rice breeding because it combines the advantages of polyploidy and heterosis to improve the characteristics of polyploid rice (Chen et al. 2021; Rao et al. 2023). Compared with diploid rice, polyploid rice has more advantages in terms of agricultural characteristics (Chen et al. 2021), such as having larger seeds, heavier seeds, stronger stems, better resistance to various stresses, and better adaptability.

The first polyploid rice, Nakamori, was created by Japanese scientists in 1933. Later, in the 1960s, Japanese, Indian, and Filipino scientists conducted autoploid rice studies with an interest in genetics and their use and hybridization between wild and cultivated rice varieties. However, autoploid rice has been found to have few grain production problems, making it difficult to apply this research to polyploid rice production. Therefore, most researchers have not researched polyploid rice breeding. China has created two highly fertilized tetraploids, the polyploidy meiosis stability (PMeS) gene, which is purebred. Rice with the PMeS gene eliminates the problem of low seed set in polyploid rice. The indica-japonica hybrid polyploid rice, which has heterosis, has been selected and successfully grown (Chen et al. 2021).

The breeding of polyploid rice in Thailand is a challenge that has led to the development of Thai rice varieties. A preliminary study in Thai rice RD.43 variety polyploid induction has revealed a method for inducing morphological changes in Thai rice using colchicine. Such an experimental approach has the potential to revolutionize rice breeding in Thailand. Subsequently, this study aimed to study the effects of colchicine on polyploidy induction, morphological characteristics, and yield components of several Thai rice varieties.

MATERIALS AND METHODS

Plant materials

Ten rice varieties, all awnless seed diploid rice (2n = 2x = 24) bred in Thailand, were used in the study, namely Kularbdang, Tuptim chumpae, Malidum, Riceberry, Homnil, RD. 79, Maejo2, Homnaka, Nan 59, and Blackberry. These rice varieties are sourced from local farmer through online platform in Thailand.

Procedures

Research design

The experiment was conducted in a completely randomized design (CRD), and the experiment had 2 factors. The first factor was the rice varieties (Kularbdang, Tuptim chumpae, Malidum, Riceberry, Homnil, RD. 79, Maejo2, Homnaka, Nan 59, and Blackberry). Factor 2 was a duration of colchicine exposure of 0.1% (0 and 6 hours). The trial included a total of 20 treatments. There were 4 repeats of 60 seeds each.

Colchicine induction

Colchicine induction was performed according to the method of Jena et al. (2016), Kumar et al. (2019), and Surson et al. (2021). The seeds were washed in dishwashing liquid (Sunlight) for 3 minutes. Afterward, the grains were rinsed in tap water for 3-5 minutes, and the grain surface was disinfected with a 10% concentration bleach (Hyter) for 10 minutes. Afterward, the seeds were rinsed with drinking water (Singha water) 3 times for 3 minutes each. The seeds were soaked in plain water for 1 day. Afterward, the seeds were seeded in seed paper packed in transparent plastic boxes measuring 5 × 7 × 2 cm for 2 days. The germinated grains were treated with 0.1% colchicine for 6 hours. At the end of the colchicine induction, the germinated grains were washed in drinking water 2 times for 1 minute each, after which the seeds were planted in a 60-well seedling tray filled with peat moss.

Germination and abnormalities of the seedlings

Germinated seeds and the number of aberrant plants were recorded after 10 days by counting the seedlings in each of the 20 treatments, each replicated 4 times. Seedling germination was calculated following formula described by Verma et al. (2017) and Surson et al. (2021) as follows:

Germination percentage = (Number of germinated seeds/total number of seeds) × 100%

We have adopted the following categories and criteria to classify normal and abnormal seeds. Normal rice seeds usually have tall, slender stems with slender first-order leaves close to the base of the plant and germinate quickly. On the other hand, abnormal seedlings have short stems, namely the first order of leaves that are short and spread near the base of the plant and germinate more slowly than usual (Dwinanda et al. 2020; Wang et al. 2020; Surson et al. 2021). The number of abnormal seeds is calculated to determine the percentage of abnormal plants using the following formula described by Mori et al. (2016), Dwinanda et al. (2020), and Surson et al. (2021) as follows:

Abnormal seedling percentage = (abnormal number of seedlings/total number of seeds) × 100

Agro-morphological traits

The agro-morphological traits were collected and refined from several experimental studies (Gaafar et al. 2017; Saha et al. 2019; Surson et al. 2021). When the rice seedlings are 1 month old, the abnormal plants are moved into pots with a diameter of 20.32 cm. The number of seeds planted in each treatment was 4 seeds, with the number of plants per treatment being 7 plants. Morphological data were collected at months 1, 2 and 3, and seven characteristics were collected, i.e.: plant height, number of leaves/plant, number of shoots/plant, plant circumference, leaf width, leaf length, and SPAD (Surson et al. 2015; Saha et al. 2019; Surson et al. 2021). Every sprawling leaf on the plant was counted to determine the number of leaves/plant. For the number of shoots/plant, every shoot in the plant was also counted. Therefore, to determine the shoot circumference, the first shoot of the plant at a height of 5 cm above the soil was measured, whereas the 3rd leaf of the first stem was measured from its center to determine the width of the leaf. The length of the base of the 3rd leaf of the first stem was measured. SPAD values are measured from the leaf tip, leaf center, and leaf tip of the 3rd leaf of the first shoot.

Yield component trait

When the seeds turned golden yellow, 7 yield components were collected, i.e.: number of ears/plant, full seed weight/plant, number of full seeds/plant, number of atrophied seeds/plant, total number of seeds/plant, seed width, and seed length (Devi et al. 2017; Gaafar et al. 2017; Saha et al. 2019). Every seed from every shoot in the plant was counted to determine the number of ears/plant, while only all full seeds were sorted and then weighed to obtain the full seed weight/plant. Only full seeds from every shoot in the plant were counted, and only atrophied seeds from every shoot in the plant were counted to determine the number of atrophied seeds from every shoot in the plant. Both full seeds and atrophied seeds from every shoot in the plant were counted. Next, to determine the width and length of the seeds, 10 seeds were measured, and the average width and length per seed were subsequently calculated.

Polyploid validation

Rice plants were checked for polyploid status via flow cytometry analysis and for the appearance of grains with awns (Song et al. 2014; Kumar et al. 2019; Surson et al. 2024). Rice plants possessing awn seeds will be categorized as polyploid (Song et al. 2014; Kumar et al. 2019). Abnormal rice plants from all the treatments were randomly selected to measure the effectiveness of the polyploids for flow cytometry analysis. The percentage of polyploids was calculated via formula described by Mori et al. (2016), Wang et al. (2020), and Surson et al. (2021) as follows: The percentage of polyploid rice plants = (Number of polyploid rice plants/total number of rice plants used for flow cytometry analysis) × 100%.

Flow cytometry analysis was performed according to the following methods (Kumar et al. 2019; Surson et al. 2024). After the rice seeds had been planted for three months, the ploidy of the abnormal plants was determined. The leaves, weighing between 0.1 and 0.5 g each (1-2 cm), were finely chopped in a drop of 500 µL of Quantum Stain NA UV 2 (A) on a plastic Petri dish. Next, to remove waste or solid particles, a solution of approximately 0.05 g of polyvinyl pyrrolidone (PVP) was added to the mixture. The mixture was subsequently passed through a 30-micron strainer to eliminate any debris and retain the nuclei. Afterward, 500 µL of Quantum Stain NA UV-2 (B) was added to the tube containing the sampled nuclei. Therefore, to ensure thorough blending of the substances, the tube was shaken before the mixture was assessed by a flow cytometer (Quantum Analysis Flow cytometer from Germany).

Every rice plant with awn seeds from each treatment was counted for the analysis of the awn seeds. Afterward, the percentage of rice plants with awn seeds of each rice variety was calculated via formula described by Song et al. (2014) and Kumar et al. (2019) as follows: The percentage of rice plants with awn seeds = (Number of rice plants with awn seeds/total number of rice plants) × 100%. However, it has been reported in a few cases in China that no awn seeds formed in some varieties of polyploid rice (Song et al. 2014).

Data analysis

A factorial, completely randomized design was used. The data were analyzed via two-way ANOVA (10 variety × 2 duration). Subsequently, whenever the F test was significant, mean comparisons were conducted via DMRT. All analyses were performed via the SPSS Version 16 package.

RESULTS AND DISCUSSION

Effects of rice variety and 0.1% colchicine exposure time on germination, abnormalities, height, and number of leaves/plant of 1-month-old rice

This study revealed that different rice varieties given a colchicine concentration of 0.1% had a statistically significant influence on germination, abnormalities, plant height, and the number of leaves/plant. The variety with the highest germination percentage was Tuptim chumpae (83.54%), whereas the lowest germination rate was Riceberry (59.58%). After the rice was given 0.1% colchicine for 0 and 6 hours, rice variety with the most abnormality was Homnaka (25.63%), and the least abnormality was Riceberry (8.75%). The rice variety that received 0.1% colchicine and presented the greatest plant height was Kularbdang (15.96%), whereas the shortest plant height was Riceberry (10.68 cm). The variety with the greatest number of leaves/plant was Blackberry (3.46 leaves/plant), and the lowest number of leaves was Maejo2 (2.44 leaves/plant).

Comparing rice that received colchicine for 6 hours with rice that did not receive colchicine, germination, anomalies, plant height, and the number of leaves/plants differed significantly ($p \leq 0.01$). Considering the effects of different varieties and the degree of exposure time to colchicine, the variety and colchicine exposure time had statistically significant ($p \leq 0.01$) influences on germination, abnormalities, plant height, and the number of leaves/plants. Rice varieties that were given 0.1% colchicine for 0 and 6 hours presented reduced germination, plant height, and number of leaves/plant but more abnormalities (Table 1).

Effects of colchicine exposure time on plant height, number of leaves/plant, number of shoots/plant, and plant circumference in 2-month-old rice varieties

A study examining the impact of rice variety and colchicine exposure duration on the height, leaf number per plant, shoot number per plant, and shoot circumference of 2-month-old rice plants demonstrated that various rice varieties significantly affected these factors ($p \leq 0.01$). The variety with the greatest plant height was Tuptim chumpae (66.06 cm), and the least plant height was Riceberry (51.72 cm). The rice variety with the greatest number of leaves/plant was Malidum (53.47 leaves/plant), and the lowest number of leaves/plant was Blackberry (23.94 leaves/plant). The rice variety with the greatest number of shoots/plants was Nan 59 (14.78%), and the lowest number of shoots/plants was Blackberry (5.63 shoots/plant). The variety with the greatest shoot circumference was Homnil (3.05 cm), and the least shoot circumference was RD.79 (2.48 cm) (Table 2).

The rice seeds of different varieties that were exposed to colchicine were compared with those that were not exposed to colchicine, statistically significant differences in height, number of leaves/plant, number of shoots/plant, and plant circumference were detected ($p \leq 0.01$) (Table 2). The study of the effects of variety combined with the period of exposure to colchicine revealed that rice varieties with different periods of exposure to colchicine presented statistically significant differences in height and shoot circumference. Moreover, different rice varieties and colchicine exposure periods did not significantly affect the number of leaves/plants or the number of shoots/plants of different rice varieties (Table 2).

The study of the effects of rice variety and colchicine exposure time on the leaf width, leaf length, and SPAD values of 2-month-old rice revealed that different rice varieties caused statistically significant differences in leaf width, leaf length, and SPAD values (p≤0.01). The variety with the greatest leaf width was Blackberry (1.20 cm), and the least leaf width was Tuptim chumpae (0.86 cm). The rice variety with the greatest leaf length was Tuptim chumpae (42.83 cm), and the shortest leaf length was Riceberry (31.72 cm). The variety with the highest SPAD value was RD.79 (39.14), and the lowest SPAD value was Kularbdang (30.89) (Table 3). When the effects of different durations of colchicine exposure were studied, it was found that different durations of colchicine exposure significantly affected the leaf width and length of 2-month-old rice (p≤0.01). Different durations of colchicine exposure did not significantly affect SPAD values (Table 3). Moreover, the effects of different varieties combined with different levels of colchicine exposure were studied, and it was found that different rice varieties that received colchicine at different times presented statistically significant differences in terms of leaf width and leaf length (p≤0.01) but not significantly different SPAD values (Table 3).

Effects of rice variety and duration of exposure to 0.1% colchicine on the height characteristics, number of leaves/plant, number of shoots/plant, and plant circumference of 3-month-old rice

The impact of rice variety on the height, leaf number per plant, shoot number per plant, and shoot circumference of 3-month-old rice plants showed statistically significant differences in height, leaf number per plant, shoot number per plant, and shoot circumference (p≤0.01). The variety with the greatest plant height was Tuptim chumpae (120.06 cm), and the least plant height was Nan 59 (83.71 cm). The variety with the greatest number of leaves/plants was Maejo 2 (99.25 leaves/plant), and the lowest number of leaves/plants was Blackberry (47.69 leaves/plant). The variety with the greatest number of shoots/plants was Maejo 2 (29.88 shoots/plant), and the lowest number of shoots/plants was Blackberry variety (8.78 shoots/plant). The variety with the greatest shoot circumference was Tuptim chumpae (4.59 cm), and the least plant circumference was Homnil (3.40 cm) (Table 4).

Table 1. Effect of rice varieties and time of colchicine exposure on germination, abnormality, plant height, number of leaves at 1 month of age

Factor	Germination	Abnormality	Plant height (cm)	Number of leaves
Varieties				
Kularbdang	12.0±69.581^{b}	27.52±24.38^{a}	6.34±15.96^{a}	0.52±2.93^{c}
Tuptim	16.70±83.54^{a}	28.52±25.42^{a}	8.60±15.65^{ab}	0.49±2.85^{c}
Malidum	18.37±79.17^{ab}	15.25±13.96^{bcd}	5.71±14.75^{b}	0.60±2.76^{cd}
Riceberry	27.53±59.58^{c}	11.50±8.75^{d}	4.59±10.68^{e}	0.84±2.57^{de}
Homnil	77.29±15.38^{ab}	20.79±18.13^{ab}	3.81±12.05^{cd}	0.16±2.88^{c}
RD.79	33.23±69.99^{b}	11.99±00..10^{cd}	6.60±14.73^{b}	0.90±2.92^{c}
Maejo2	15.90±78.13^{ab}	21.53±20.00^{ab}	3.94±10.76^{e}	0.56±2.44^{e}
Homnaka	13.33±72.92^{b}	25.58±25.63^{a}	4.23±11.44^{de}	0.61±3.44^{a}
Nan 59	27.40±71.67^{b}	20.16±17.50^{abc}	±12.794.61^{c}	0.63±3.17^{b}
Blackberry	15.38±77.50^{ab}	21.62±19.37^{ab}	2.60±15.24^{ab}	±3.460.30^{a}
F-test	**	**	**	**
Time				
0	9.25±90.04^{a}	0.00±0.00^{a}	3.24±18.11^{a}	0.39±3.42^{a}
6	15.25±57.79^{b}	14.70±36.62^{b}	2.06±8.70^{b}	0.48±2.46^{b}
F-test	**	**	**	**
Treatment				
Kularbdang/0	5.93±76.67^{cde}	0.00±0.00^{f}	1.74±21.78^{b}	0.17±3.40^{ce}
Kularbdang/6	5 12.9±62.50 ^{e}	13.50±48.75^{ab}	0.66±10.15^{g}	0.17±2.46^{hi}
Tuptim/0	1.59±97.92^{a}	0.00±0.00^{f}	1.26±23.64^{a}	0.24±3.24^{ef}
Tuptim/6	9.86±69.17^{de}	13.22±50.84^{a}	0.86±7.67^{hi}	0.31±2.46^{hi}
Malidum/0	4.91±95.00^{ab}	0.00±0.00^{f}	1.56±20.00^{c}	0.35±3.28^{e}
Malidum/6	9.72±63.34^{e}	4.78±27.92^{c-d}	0.34±9.50^{g}	0.08±2.25^{ij}
Riceberry/0	6.85±83.75^{abc}	0.00±0.00^{f}	0.91±15.28^{e}	0.21±3.34^{d-e}
Riceberry/6	12.79±35.42^{f}	10.23±17.50^{e}	0.88±6.09^{i}	0.08±1.80^{k}
Homnil/0	5.16±89.59^{abc}	0.00±0.00^{f}	0.81±15.53^{e}	0.19±2.93^{fg}
Homnil/6	11.06±65.00^{e}	11.50±36.25^{c-d}	0.87±8.57^{gh}	0.13±2.83^{g}
RD.79/0	1.36±98.33^{a}	0.00±0.00^{f}	1.19±20.80^{bc}	0.25±3.74^{ab}
RD.79/6	20.06±41.25^{f}	8.28±20.00^{e}	1.40±8.66^{gh}	0.26±2.11^{jk}
Maejo2/0	5.53±92.50^{ab}	0.00±0.00^{f}	0.73±14.40^{ef}	0.17±2.95^{fg}
Maejo2/6	2.85±63.75^{e}	3.85±40.00^{abc}	0.69±7.12^{hi}	0.15±1.93^{jk}
Homnaka/0	6.46±82.50^{bd}	0.00±0.00^{f}	0.90±15.34^{e}	0.06±4.00^{a}
Homnaka/6	11.30±63.33^{e}	12.42±51.25^{a}	0.67±7.54^{hi}	0.15±2.88^{g}
Nan 59/0	2.36±96.67^{ab}	0.00±0.00^{f}	0.27±17.04^{d}	0.17±3.72^{ac}
Nan 59/6	8.92±46.67^{f}	11.47±35.00^{cd}	1.12±8.54^{gh}	0.30±2.62^{gh}
Blackberry/0	15.72±87.50^{abc}	0.00±0.00^{f}	1.24±17.34^{d}	0.27±3.62^{bd}
Blackberry/6	6.16±67.50^{e}	9.47±38.75^{bcd}	1.61±13.15^{f}	0.25±3.30^{de}
F-test	**	**	**	**
CV (%)	12.70	40.05	7.89	7.61

Notes: Different superscripts in each row indicate significant (p<0.05) differences; ^{ns}: non-significant; **: highly significant (p<0.01); *: significant (p<0.05)

The study of the effects of different colchicine exposure periods revealed that different colchicine exposure periods resulted in statistically significant differences in height, number of leaves/plants, and number of shoots/plants ($p \leq 0.01$), but shoot circumference was not significantly different (Table 4). When the effects of variety and colchicine exposure period were studied, it was found that different rice varieties and colchicine exposure durations caused statistically significant differences in plant height and number of shoots/plants ($p \leq 0.05$). There was no significant difference in leaf count/plant or shoot circumference. Concerning plant height characteristics, some varieties of rice that did not receive colchicine had taller plant heights than did the other varieties that received colchicine. With respect to the number of shoots/plants, most rice varieties that received colchicine presented a smaller number of shoots/plants than did rice varieties that did not receive colchicine. However, some rice varieties that received colchicine, such as Tuptim chumpae and Riceberry, presented greater numbers of shoots/plants than rice varieties that did not receive colchicine. It was also found that some varieties, whether their seeds were treated with colchicine or not, presented the same number of shoots/plants. e.g., Maejo 2 (Table 4).

Different rice varieties had statistically significant differences in terms of leaf width, leaf length, and SPAD values ($p \leq 0.01$). The rice variety with the greatest leaf width was Blackberry (1.53 cm), and the smallest leaf width was Homnil (1.02 cm). The variety with the greatest leaf length was Tuptim chumpae (71.46 cm), and the shortest leaf length was Homnil (42.41 cm). The variety with the highest SPAD value was Homnil (46.68), and the lowest SPAD value was Tuptim chumpae (36.34) (Table 5).

Table 2. Effect of rice varieties and time of colchicine exposure on plant height, number of leaves, number of shoots/plant, first shoot circumference at 2 months of age

Factor	Plant height)cm)	Number of leaves	Number of shoots/plant	First shoot circumference)cm)
Varieties				
Kularbdang	55.69±1.69^{de}	50.53±4.69^{ab}	11.44±1.47^{b}	2.57±0.21^{de}
Tuptim	66.06±8.00^{a}	34.56±10.48^{c}	8.52±2.80^{c}	2.92±0.29^{ab}
Malidum	60.70±1.37^{bc}	53.47±6.95^{a}	14.50±2.38^{a}	2.86±0.16^{ac}
Riceberry	51.72±4.40^{f}	43.50±10.99^{b}	11.16±3.28^{b}	2.78±0.33^{bd}
Homnil	61.59±1.32^{b}	42.06±8.55^{bc}	10.94±2.31^{b}	3.05±0.13^{a}
RD.79	58.14±4.81^{cd}	35.38±11.22^{c}	8.69±2.80^{c}	2.48±0.22^{e}
Maejo2	57.94±3.40^{d}	48.97±9.30^{ab}	11.91±2.84^{b}	2.55±0.22e
Homnaka	55.27±3.18d^{e}	47.31±9.33^{ab}	13.22±2.73^{ab}	2.69±0.19cde
Nan 59	54.54±2.57^{e}	47.81±13.02^{ab}	14.78±4.08^{a}	2.63±0.10de
Blackberry	57.59±5.30^{d}	23.94±10.73^{d}	5.63±2.62^{d}	2.61±0.27de
F-test	**	**	**	**
Time				
0	60.37±5.30^{a}	48.47±9.69^{a}	12.79±3.25^{a}	2.79±0.28^{a}
6	55.48±4.56^{b}	37.04±12.73^{b}	9.37±3.54^{b}	2.64±0.25^{b}
F-test	**	**	**	**
Treatment				
Kularbdang/0	56.48±2.05^{cde}	53.19±3.70	12.31±0.77	2.65±0.28^{defgh}
Kularbdang/6	54.89±0.90^{e}	47.88±4.33	10.56±1.55	2.50±0.07^{fgh}
Tuptim/0	72.42±2.13^{a}	42.19±7.45	10.42±2.20	3.10±0.19^{a}
Tuptim/6	59.71±6.10^{bcd}	26.94±6.76	6.63±1.96	2.74±0.27^{cdefg}
Malidum/0	61.57±1.04^{b}	57.00±7.08	16.25±1.66	2.93±0.20^{abcd}
Malidum/6	59.83±1.13^{bcd}	49.94±5.41	12.75±1.51	2.80±0.11^{bcdef}
Riceberry/0	55.54±1.42^{e}	52.88±5.30	13.75±2.01	3.04±0.13^{abc}
Riceberry/6	47.91±2.10^{f}	34.13±4.40	8.56±1.78	2.51±0.23^{fgh}
Homnil/0	62.09±1.05^{b}	48.88±6.39	13.00±1.02	3.06±0.10^{ab}
Homnil/6	61.10±1.51^{b}	35.25±2.47	8.88±0.32	3.04±0.17^{abc}
RD.79/0	61.91±2.15^{b}	43.75±4.17	10.75±0.74	2.52±0.15^{efgh}
RD.79/6	54.37±3.39^{e}	27.00±9.45	6.63±2.54	2.45±0.30^{gh}
Maejo2/0	60.14±2.51^{bc}	52.38±9.02	12.56±3.34	2.47±0.19^{gh}
Maejo2/6	55.75±2.80^{de}	45.56±9.46	11.25±2.56	2.63±0.25^{defgh}
Homnaka/0	55.35±4.50^{e}	48.56±4.74	14.00±1.91	2.64±0.28^{defgh}
Homnaka/6	55.19±1.81^{e}	46.06±13.28	12.44±3.48	2.75±0.03^{cdefg}
Nan 59/0	56.28±1.83^{cde}	54.44±12.12	17.31±2.66	2.61±0.09^{efgh}
Nan 59/6	52.80±2.00^{e}	41.19±11.47	12.25±3.85	2.65±0.13^{defgh}
Blackberry/0	61.90±2.10^{b}	31.44±8.67	7.50±2.12	2.83±0.08^{abcde}
Blackberry/6	53.27±3.39^{e}	16.44±6.58	3.75±1.46	2.40±0.19^{h}
F-test	**	ns	ns	**
CV (%)	4.50	17.79	19.57	6.90

Notes: Different superscripts in each row indicate significant ($p<0.05$) differences; ^{ns}: non-significant; **: highly significant ($p<0.01$); *: significant ($p<0.05$)

The investigation of colchicine exposure at several durations revealed statistically significant variations in leaf width and length. Nonetheless, the SPAD values of the rice samples exhibited no significant differences (Table 5). When the influence of the combination of colchicine exposure duration on rice varieties was studied, it was found that the duration of colchicine exposure significantly affected the leaf width and length in each study. However, the SPAD values of each treatment did not significantly differ. Exposure to colchicine resulted in a lower leaf width and leaf length only in rice (Table 5).

Effects of rice variety and duration of 0.1% colchicine exposure on rice yield characteristics

Different rice varieties caused statistically significant differences in the number of ears/plant, full seed weight/plant, and number of full seeds/plant ($p \leq 0.01$). The variety with the greatest number of ears/plant was Maejo 2 (9.98 cm), and the smallest number of ears/plant was Blackberry (4.28 ears/plant). The variety with the highest full seed weight/plant ratio was Maejo 2 (18.14 g/plant), and the lowest full seed weight/plant ratio was the Homnil (4.70 g/plant). The variety with the greatest number of full seeds/plant was Maejo 2 (676.78 seeds/plant), and the lowest number of full seeds/plant was Homnil (164.52 seeds/plant) (Table 6).

The investigation of colchicine exposure duration revealed that varying periods of exposure led to statistically significant differences in full seed weight per plant and the number of full seeds per plant ($p \leq 0.01$), whereas the number of ears per plant showed no significant variation (Table 6). When the effects of the combination of different rice varieties and the colchicine exposure period were studied, the number of ears/plant and full seed weight/plant ratio did not significantly differ among the rice varieties. Studies have shown that rice varieties given colchicine lead to fewer full seeds/plant, except Malidum variety (Table 6).

Table 3. Effect of rice varieties and time of colchicine exposure on leaf width, leaf length, SPAD value at 2 months of age

Factor	Leaf width (cm)	Leaf length (cm)	SPAD value
Varieties			
Kularbdang	$0.97{\pm}0.07^{de}$	$35.63{\pm}1.09^{bcd}$	$30.89{\pm}2.31^{e}$
Tuptim	$0.86{\pm}0.10^{f}$	$42.83{\pm}5.35^{a}$	$33.72{\pm}2.83^{cde}$
Malidum	$1.05{\pm}0.05^{c}$	$36.54{\pm}2.84^{bc}$	$32.54{\pm}3.49^{de}$
Riceberry	$0.95{\pm}0.09^{de}$	$31.72{\pm}2.82^{f}$	$32.77{\pm}2.52^{de}$
Homnil	$1.00{\pm}0.03^{cde}$	$37.34{\pm}1.07^{b}$	$35.51{\pm}1.90^{bcd}$
RD.79	$1.13{\pm}0.09^{b}$	$35.89{\pm}2.38^{bcd}$	$39.14{\pm}2.27^{a}$
Maejo2	$0.94{\pm}0.05^{e}$	$32.91{\pm}2.50^{ef}$	$36.29{\pm}1.85^{abc}$
Homnaka	$0.95{\pm}0.02^{e}$	$33.96{\pm}2.52^{def}$	$33.90{\pm}3.10^{cde}$
Nan 59	$1.01{\pm}0.02^{cd}$	$34.22{\pm}2.25^{cde}$	$36.12{\pm}2.84^{bc}$
Blackberry	$1.20{\pm}0.09^{a}$	$36.34{\pm}3.62^{bcd}$	$37.54{\pm}3.34^{ab}$
F-test	**	**	**
Time			
0	$1.04{\pm}0.11^{a}$	$37.31{\pm}4.06^{a}$	$35.26{\pm}3.61$
6	$0.98{\pm}0.11^{b}$	$34.16{\pm}3.25^{b}$	$34.43{\pm}3.38$
F-test	**	**	ns
Treatment			
Kularbdang/0	$1.02{\pm}0.03^{def}$	$36.37{\pm}0.58^{bcdefg}$	$32.22{\pm}1.96$
Kularbdang/6	$0.93{\pm}0.07^{fgh}$	$34.89{\pm}0.99^{defgh}$	$29.57{\pm}1.98$
Tuptim/0	$0.92{\pm}0.09^{gh}$	$46.92{\pm}3.73^{a}$	$33.09{\pm}1.17$
Tuptim/6	$0.80{\pm}0.08^{i}$	$38.75{\pm}2.90^{bc}$	$34.36{\pm}4.04$
Malidum/0	$1.09{\pm}0.05^{cd}$	$38.39{\pm}2.15^{bcd}$	$33.80{\pm}3.44$
Malidum/6	$1.02{\pm}0.03^{def}$	$34.70{\pm}2.28^{efgh}$	$31.27{\pm}3.50$
Riceberry/0	$1.02{\pm}0.02^{def}$	$33.72{\pm}2.21^{fgh}$	$31.91{\pm}3.43$
Riceberry/6	$0.88{\pm}0.07^{h}$	$29.71{\pm}1.70^{i}$	$33.62{\pm}1.07$
Homnil/0	$0.99{\pm}0.03^{efg}$	$37.56{\pm}0.58^{bcde}$	$36.34{\pm}2.22$
Homnil/6	$1.00{\pm}0.02^{defg}$	$37.12{\pm}1.48^{bcdef}$	$34.68{\pm}1.31$
RD.79/0	$1.19{\pm}0.03^{ab}$	$37.14{\pm}0.72^{bcdef}$	$39.85{\pm}1.93$
RD.79/6	$1.08{\pm}0.10^{cde}$	$34.64{\pm}2.92^{efgh}$	$38.44{\pm}2.64$
Maejo2/0	$0.94{\pm}0.03^{fgh}$	$34.23{\pm}0.45^{efgh}$	$36.74{\pm}1.83$
Maejo2/6	$0.94{\pm}0.07^{fgh}$	$31.58{\pm}3.12^{hi}$	$35.83{\pm}2.02$
Homnaka/0	$0.94{\pm}0.01^{fgh}$	$34.43{\pm}3.00^{efgh}$	$33.69{\pm}4.15$
Homnaka/6	$0.96{\pm}0.03^{fgh}$	$33.50{\pm}2.28^{fgh}$	$34.11{\pm}2.24$
Nan 59/0	$1.01{\pm}0.03^{defg}$	$35.33{\pm}1.77^{cdefg}$	$35.91{\pm}3.11$
Nan 59/6	$1.01{\pm}0.02^{defg}$	$33.12{\pm}2.32^{gh}$	$36.33{\pm}3.00$
Blackberry/0	$1.25{\pm}0.06^{a}$	$39.02{\pm}1.32^{b}$	$39.01{\pm}3.18$
Blackberry/6	$1.14{\pm}0.09^{bc}$	$33.65{\pm}3.10^{fgh}$	$36.07{\pm}3.17$
F-test	*	*	ns
CV (%)	5.42	6.16	7.81

Notes: Different superscripts in each row indicate significant ($p<0.05$) differences; ns: non-significant; **: highly significant ($p<0.01$); *: significant ($p<0.05$)

Different rice varieties had statistically significant differences in the number of atrophied seeds/plant, the total number of seeds/plant, seed width, and seed length (p≤0.01). The rice variety with the greatest number of atrophied seeds/plant was Maejo 2 (637.05 seeds/plant), and the least atrophic seeds was Homnil (95.46 seeds/plant). The variety with the highest total number of seeds/plant was Maejo 2 (1,316.89 seeds/plant), and the lowest total number of seeds/plant was Homnil (261.36 seeds/plant). The variety with the greatest width was Malidum (0.294 cm), and the smallest width was Blackberry (0.247 cm). The variety with the greatest seed length was RD.79 (1.065 cm), and the shortest seed length was Homnil (0.984 cm) (Table 7).

The study of the influence of colchicine exposure duration on atrophied seed number/plant, total number of seeds/plant, and seed width and length revealed that different colchicine exposure periods resulted in statistically significant differences in the total number of seeds/plant and seed width (p≤0.01) but did not significantly affect atrophied seed number/plant or seed length (Table 7).

The number of atrophied seeds/plant, total number of seeds/plant, and seed width (p≤0.01) were significantly different among the rice varieties. Still, statistically significant differences in seed length were not detected (Table 7). Studies have shown that most colchicine-treated rice varieties have a reduced number of atrophied seeds, except Malidum, Riceberry, and Homnil varieties. Rice varieties that received colchicine presented a decrease in the total number of seeds/plants, except Malidum, Riceberry, and Homnil varieties. All the rice varieties that received colchicine presented increased seed width (Table 7).

Table 4. Effect of rice varieties and time of colchicine exposure on plant height, number of leaves, number of shoots/plant, first shoot circumference at 3 months of age

Factor	Plant height)cm)	Number of leaves	Number of shoots/plant	First shoot circumference)cm)
Varieties				
Kularbdang	92.00 ± 2.06^{cd}	94.28 ± 12.19^{a}	21.59 ± 2.12^{b}	3.41 ± 0.29^{e}
Tuptim	120.06 ± 4.26^{a}	70.63 ± 5.49^{b}	16.84 ± 2.52^{cd}	4.59 ± 0.32^{a}
Malidum	104.72 ± 2.96^{b}	74.00 ± 12.76^{b}	18.34 ± 2.47^{cd}	3.87 ± 0.20^{bcd}
Riceberry	90.90 ± 9.19^{de}	71.50 ± 10.55^{b}	17.00 ± 3.45^{cd}	4.09 ± 0.27^{b}
Homnil	90.75 ± 8.69^{de}	54.75 ± 7.07^{c}	15.38 ± 2.55^{d}	3.40 ± 0.24^{e}
RD.79	93.22 ± 5.03^{cd}	66.91 ± 13.36^{b}	16.78 ± 3.79^{cd}	3.46 ± 0.19^{e}
Maejo2	85.82 ± 4.56^{cf}	99.25 ± 12.48^{a}	29.88 ± 3.72^{a}	3.81 ± 0.24^{cd}
Homnaka	88.98 ± 3.37^{de}	94.31 ± 10.80^{a}	22.75 ± 4.34^{b}	3.74 ± 0.15^{d}
Nan 59	83.71 ± 3.24^{f}	96.09 ± 13.29^{a}	18.81 ± 2.48^{c}	4.11 ± 0.21^{b}
Blackberry	96.33 ± 6.88^{c}	47.69 ± 19.37^{c}	8.78 ± 2.80^{e}	4.03 ± 0.27^{bc}
F-test	**	**	**	**
Time				
0	96.73 ± 11.62^{a}	82.03 ± 18.47^{a}	19.32 ± 5.63^{a}	3.88±0.43
6	92.57 ± 10.95^{b}	71.85 ± 22.14^{b}	17.91 ± 6.31^{b}	3.82±0.42
F-test	**	**	*	ns
Treatment				
Kularbdang/0	93.07 ± 2.14^{def}	104.19±8.47	22.63 ± 1.64^{bc}	3.46±0.41
Kularbdang/6	90.92 ± 1.51^{defgh}	84.38±3.67	20.56 ± 2.23^{bcd}	3.35±0.15
Tuptim/0	122.78 ± 3.53^{a}	74.44±2.49	15.06 ± 1.09^{efgh}	4.61±0.14
Tuptim/6	117.34 ± 3.17^{a}	66.81±5.04	18.63 ± 2.29^{cdef}	4.57±0.46
Malidum/0	104.72 ± 3.58^{b}	83.88±9.78	20.06 ± 2.10^{cd}	4.02±0.03
Malidum/6	104.73 ± 2.78^{b}	64.13±4.92	16.63 ± 1.42^{defg}	3.73±0.19
Riceberry/0	94.83 ± 11.80^{cde}	68.63±7.03	15.13 ± 2.78^{efgh}	4.09±0.25
Riceberry/6	86.98 ± 4.12^{fghi}	74.38±13.71	18.88 ± 3.28^{cdef}	4.09±0.32
Homnil/0	97.69 ± 2.98^{bcd}	58.38±7.72	16.75 ± 2.36^{defg}	3.49±0.30
Homnil/6	83.81 ± 6.26^{hi}	51.13±4.70	14.00 ± 2.12^{gh}	3.31±0.17
RD.79/0	95.59 ± 3.05^{cd}	74.06±8.80	19.06 ± 4.11^{cde}	3.35±0.16
RD.79/6	90.84 ± 5.89^{defgh}	59.75±14.23	14.50 ± 1.62^{fgh}	3.57±0.17
Maejo2/0	85.72 ± 6.85^{fghi}	98.69±18.38	29.38 ± 4.62^{a}	3.70±0.23
Maejo2/6	85.91 ± 1.25^{fghi}	99.81±4.98	30.38 ± 3.22^{a}	3.93±0.22
Homnaka/0	87.37 ± 2.33^{efghi}	94.50±5.67	24.75 ± 3.03^{b}	3.72±0.18
Homnaka/6	90.58 ± 3.77^{defgh}	94.13±15.48	20.75 ± 4.91^{bcd}	3.75±0.15
Nan 59/0	84.77 ± 2.42^{ghi}	102.38±12.14	19.31 ± 2.06^{cde}	4.22±0.09
Nan 59/6	82.65 ± 3.95^{i}	89.81±12.63	18.31 ± 3.08^{cdefg}	4.01±0.26
Blackberry/0	100.71 ± 2.14^{bc}	61.19±6.43	11.06 ± 1.28^{h}	4.15±0.19
Blackberry/6	91.95 ± 7.39^{defg}	34.19±18.67	6.50 ± 1.67^{i}	3.90±0.30
F-test	*	ns	*	ns
CV (%)	4.99	12.53	14.80	6.26

Notes: Different superscripts in each row indicate significant (p<0.05) differences; ^{ns}: non-significant; **: highly significant (p<0.01); *: significant (p<0.05)

Table 5. Effect of rice varieties and colchicine exposure time on leaf width, leaf length, SPAD values at 3 months of age

Factor	Leaf width (cm)	Leaf length (cm)	SPAD values
Varieties			
Kularbdang	1.19±0.16[d]	51.45±2.93[de]	37.22±1.73[bc]
Tuptim	1.26±0.15[c]	71.46±7.78[a]	36.34±1.79[c]
Malidum	1.50±0.07[a]	65.07±2.57[b]	36.99±2.13[bc]
Riceberry	1.31±0.13[bc]	53.30±3.88[cd]	38.48±3.48[bc]
Homnil	1.18±0.13[d]	42.41±3.51[f]	46.68±2.20[a]
RD.79	1.37±0.18[b]	55.28±7.35[c]	39.19±1.44[b]
Maejo2	1.14±0.10[d]	49.48±2.57[e]	37.52±1.62[bc]
Homnaka	1.02±0.05[e]	49.30±1.96[e]	37.74±2.39[bc]
Nan 59	1.31±0.20[bc]	48.08±4.85[e]	39.27±1.42[b]
Blackberry	1.53±0.12[a]	55.86±8.34[c]	39.18±1.89[b]
F-test	**	**	**
Time			
0	1.33±0.19[a]	56.19±10.65[a]	39.04±3.48
6	1.23±0.20[b]	52.14±7.64[b]	38.68±3.40
F-test	**	**	ns
Treatment			
Kularbdang/0	1.33±0.06[de]	53.54±2.26[cd]	36.30±0.78
Kularbdang/6	1.05±0.02[hi]	49.37±1.83[cf]	38.14±2.03
Tuptim/0	1.39±0.07[cd]	78.00±2.19[a]	36.17±1.27
Tuptim/6	1.13±0.06[gh]	64.91±4.70[b]	36.52±2.40
Malidum/0	1.52±0.08[ab]	65.06±2.65[b]	37.68±2.54
Malidum/6	1.47±0.06[bc]	65.08±2.89[b]	36.29±1.69
Riceberry/0	1.21±0.09[fg]	54.03±5.66[c]	40.09±2.01
Riceberry/6	1.41±0.06[bcd]	52.58±1.29[cde]	36.88±4.17
Homnil/0	1.28±0.05[ef]	42.00±3.67[h]	47.38±1.47
Homnil/6	1.09±0.11[hi]	42.81±3.85[gh]	45.99±2.80
RD.79/0	1.52±0.08[ab]	60.10±4.13[b]	38.62±1.12
RD.79/6	1.22±0.11[efg]	50.47±6.88[cde]	39.77±1.64
Maejo2/0	1.23±0.05[efg]	50.43±2.41[cde]	37.89±0.82
Maejo2/6	1.05±0.04[hi]	48.54±2.70[cdef]	37.15±2.26
Homnaka/0	1.05±0.06[hi]	50.86±0.34[cde]	37.98±3.46
Homnaka/6	1.00±0.04[i]	47.74±1.53[efg]	37.50±1.11
Nan 59/0	1.14±0.08[gh]	44.47±2.87[fgh]	39.40±2.04
Nan 59/6	1.47±0.11[bc]	51.69±3.44[cde]	39.15±0.73
Blackberry/0	1.62±0.06[a]	63.47±1.16[b]	38.94±1.74
Blackberry/6	1.44±0.08[bcd]	48.24±2.51[def]	39.42±2.27
F-test	**	**	ns
CV (%)	5.52	6.13	5.42

Notes: Different superscripts in each row indicate significant ($p<0.05$) differences; [ns]: non-significant; **: highly significant ($p<0.01$); *: significant ($p<0.05$)

Polyploid validation

Polyploid validation via flow cytometry analysis

Twenty-one rice plants with abnormal seedling characteristics were randomly sampled and analyzed. A total of 9.52% of them were mixoploids (Figure 1). Twenty-one rice plants were randomly selected for polyploidy analysis using a flow cytometer. It was found that 19 plants (90.48%) showed diploid histogram (Figure 1.A) and 2 plants (9.52%) showed mixoploid histogram (Figure 1.B).

Determination of polyploidy from awn seed characteristics of different varieties

Studies have shown that exposure to colchicine has led to the discovery of rice plants whose seeds include awn seeds of all varieties. The percentage of rice plants with awn seeds of different varieties is different. The rice variety with the highest percentage of rice plants with awn seeds was Blackberry (90%), followed by Maejo 2 (65%), Homnaka (60%), Riceberry (35%), Malidum (30%), Homnil (10%), RD.79 (10%), and Kularbdang (5%) (Table 8; Figure 2).

Discussion

Polyploid rice induction has been conducted for a long time. Therefore, several methods for inducing polyploids in rice have been studied. China has genetically improved more than 150 varieties of domestic polyploid rice. At present, China has improved polyploid rice varieties to the extent that the yield characteristics are much better, such as effective seed attachment and the discovery of awnless polyploidy rice (Chen et al. 2021).

Table 6. Influence of rice varieties and time of colchicine exposure on number of ears/plant, full seed weight/plant, and full seed number/plant

Factor	Number of ears/plant	Full seed weight/plant (g)	Full seed number/plant
Varieties			
Kularbdang	5.99±1.54[b]	11.88±2.53[b]	442.14±102.53[b]
Malidum	9.75±1.95[a]	11.47±4.57[b]	326.64±110.67[c]
Riceberry	6.23±1.62[b]	11.11±3.36[b]	431.67±87.01[b]
Homnil	4.77±0.99[bc]	4.70±1.48[c]	164.52±59.60[d]
RD.79	8.98±1.68[a]	16.96±5.05[a]	588.58±167.97[a]
Maejo2	9.98±1.10[a]	18.14±3.31[a]	676.78±121.74[a]
Homnaka	9.17±1.10[a]	17.58±2.58[a]	597.97±144.49[a]
Blackberry	4.28±1.56[c]	6.40±2.26[c]	316.69±119.50[c]
F-test	**	**	**
Time			
0	7.71±2.59	13.53±6.04[a]	485.47±222.14[a]
6	7.08±2.60	11.04±5.15[b]	400.78±160.28[b]
F-test	ns	**	**
Treatment			
Kularbdang/0	6.89±1.31	12.30±2.21	491.34±84.36[b]
Kularbdang/6	5.10±1.30	11.46±3.09	392.95±104.69[bc]
Malidum/0	9.85±2.73	12.47±6.60	268.00±126.52[cd]
Malidum/6	9.64±1.19	10.47±1.63	385.28±58.31[bc]
Riceberry/0	5.88±2.07	12.40±4.60	443.50±130.39[b]
Riceberry/6	6.58±1.23	9.83±0.88	419.83±17.02[bc]
Homnil/0	4.96±1.13	4.81±2.08	153.00±72.28[d]
Homnil/6	4.58±0.96	4.60±0.87	176.04±52.06[d]
RD.79/0	9.88±1.58	20.25±2.71	709.34±104.06[a]
RD.79/6	8.08±1.40	13.68±4.83	467.83±126.95[b]
Maejo2/0	9.95±1.57	18.81±2.83	708.18±103.02[a]
Maejo2/6	10.00±0.61	17.48±4.04	645.39±146.07[a]
Homnaka/0	9.09±0.96	18.88±2.65	704.98±114.22[a]
Homnaka/6	9.25±1.37	16.28±2.00	490.96±71.63[b]
Blackberry/0	5.19±1.79	8.29±1.38	405.40±107.10[bc]
Blackberry/6	3.37±0.51	4.51±0.73	227.98±29.41[d]
F-test	ns	ns	**
CV (%)	19.66	25.44	22.00

Notes: Different superscripts in each row indicate significant ($p<0.05$) differences; [ns]: non-significant; **: highly significant ($p<0.01$); *: significant ($p<0.05$)

Table 7. Influence of rice varieties and colchicine exposure time on atrophied seeds/plant, total seed number/plant, seed width, and seed length

Factor	Atrophied seeds/plant	Total seed number/plant	Seed width)cm)	Seed length)cm)
Varieties				
Kularbdang	296.87±128.26[c]	748.84±192.78[c]	0.266±0.007[c]	1.054±0.014[a]
Malidum	355.82±148.36[c]	685.23±231.97[c]	0.294±0.009[a]	1.023±0.006[b]
Riceberry	265.13±114.98[cd]	703.15±166.47[c]	0.254±0.010[e]	1.012±0.026[b]
Homnil	95.46±18.05[e]	261.36±66.14[e]	0.268±0.009[c]	0.984±0.017[c]
RD.79	451.35±148.55[b]	1018.80±283.52[b]	0.261±0.012[d]	1.065±0.014[a]
Maejo2	637.05±85.05[a]	1316.89±151.77[a]	0.274±0.005[b]	1.019±0.027[b]
Homnaka	516.94±123.38[b]	1102.57±253.45[b]	0.274±0.009[b]	0.992±0.011[c]
Blackberry	182.97±67.49[d]	505.10±172.21[d]	0.247±0.005[f]	0.986±0.008[c]
F-test	**	**	**	**
Time				
0	361.30±218.20	848.31±417.09[a]	0.261±0.014[a]	1.017±0.032
6	339.10±180.51	737.17±317.24[b]	0.273±0.016[b]	1.016±0.034
F-test	ns	**	**	ns
Treatment				
Kularbdang/0	380.16±119.77[de]	871.50±188.49[b]	0.261±0.006[f]	1.051±0.020
Kularbdang/6	213.58±74.42[fg]	626.18±105.20[cd]	0.272±0.003[de]	1.056±0.002
Malidum/0	225.90±52.33[fg]	498.90±152.44[de]	0.286±0.004[b]	1.024±0.010
Malidum/6	485.74±60.10[bcd]	871.57±98.65[b]	0.302±0.003[a]	1.023±0.003
Riceberry/0	198.15±45.92[fg]	641.65±165.64[cd]	0.247±0.005[g]	0.999±0.012
Riceberry/6	332.11±129.52[ef]	764.65±164.72[bc]	0.261±0.009[f]	1.026±0.031
Homnil/0	92.71±17.76[g]	245.71±77.63[f]	0.262±0.008[f]	0.993±0.009
Homnil/6	98.21±20.60[g]	277.00±59.41[f]	0.274±0.005[cde]	0.975±0.019
RD.79/0	524.38±174.30[abc]	1233.71±163.69[a]	0.250±0.005[g]	1.068±0.009
RD.79/6	378.33±83.01[de]	803.89±193.92b[c]	0.271±0.003[de]	1.062±0.018
Maejo2/0	650.04±117.22[a]	1357.93±175.36[a]	0.270±0.002[de]	1.023±0.037
Maejo2/6	624.06±51.84[a]	1275.85±136.03[a]	0.279±0.004[bcd]	1.015±0.018
Homnaka/0	608.71±71.52[ab]	1313.90±110.28[a]	0.268±0.009[cdef]	0.996±0.015
Homnaka/6	425.17±89.12[cde]	891.25±136.53[b]	0.281±0.004[bc]	0.988±0.005
Blackberry/0	210.34±89.59[fg]	623.19±173.57[cd]	0.248±0.007[g]	0.985±0.009
Blackberry/6	155.61±24.62[g]	387.01±43.4[ef]	0.246±0.005[g]	0.987±0.007
F-test	**	**	**	ns
CV (%)	24.86	17.84	2.09	0.00

Notes: Different superscripts in each row indicate significant (p<0.05) differences; [ns]: non-significant; **: highly significant (p<0.01); *: significant (p<0.05)

Table 8. Influence of rice varieties and colchicine exposure time on percentage of rice plants with awn seed

TMT	Treatment	Plants with awn seed
K1	Kularbdang/0	0.00
K2	Kularbdang/6	5.00
K5	Malidum/0	0.00
K6	Malidum/6	30.00
K7	Riceberry/0	0.00
K8	Riceberry/6	35.00
K9	Homnil/0	0.00
K10	Homnil/6	10.00
K11	RD.79/0	0.00
K12	RD.79/6	10.00
K13	Maejo2/0	0.00
K14	Maejo2/6	65.00
K15	Homnaka/0	0.00
K16	Homnaka/6	60.00
K19	Blackberry/0	0.00
K20	Blackberry/6	90.00

In Thailand, the induction of polyploid rice has not been extensively conducted. Therefore, polyploid rice breeding is not yet widespread and successful. The development of Thai polyploid rice still has a long way to go. In this research, efforts were made to develop various Thai rice varieties into polyploid rice with the hope that the induction of polyploidy in Thai rice could increase heredity, just like other types of rice. The potential benefits of polyploid rice in Thai agriculture could significantly improve Thai rice production.

A Chinese study employed an effective technique to produce polyploidy in rice. The study did not specify the success rate of polyploidy induction in rice, but it indicated that polyploidy could be achieved through in vivo doubling (cutting rice seedlings with 1-2 tillers at half-depth and immersing them in a 0.3% colchicine solution for 48-56 hours) and in vitro doubling (cultivating young panicle tissue in an induction medium and subsequently transferring it to a liquid induction medium containing 0.05% colchicine for 48 hours), after which PMeS lines of super rice with better seed set compared to normal polyploid rice were selected (Chen et al. 2021).

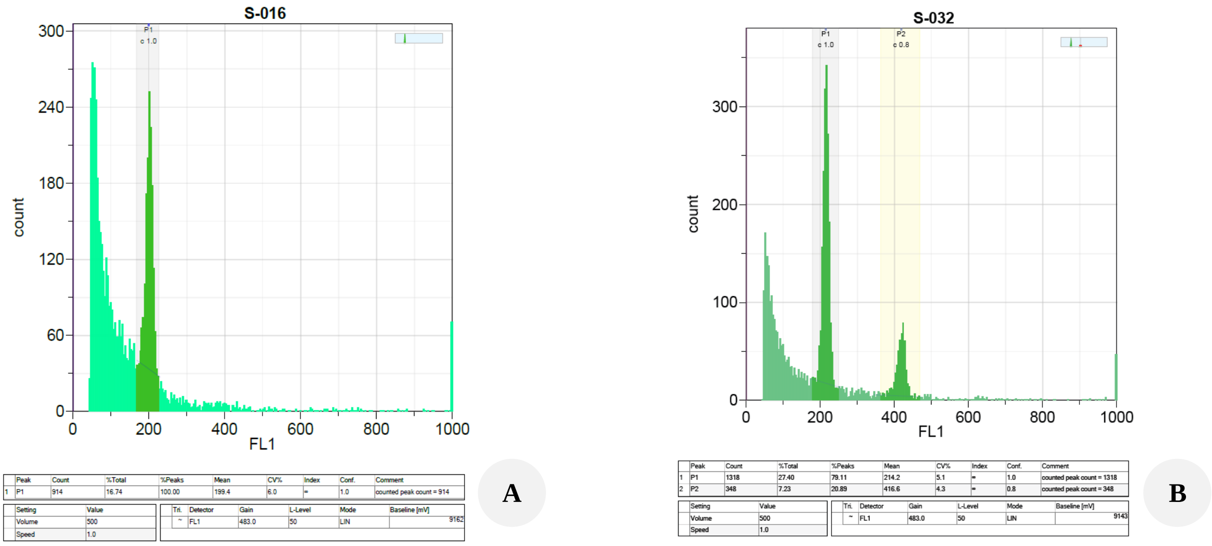

Figure 1. Flow cytometry analysis of normal rice plants. A. Diploid rice plant; B. Mixoploid rice plant treated with 0.1% colchicine for 6 hours

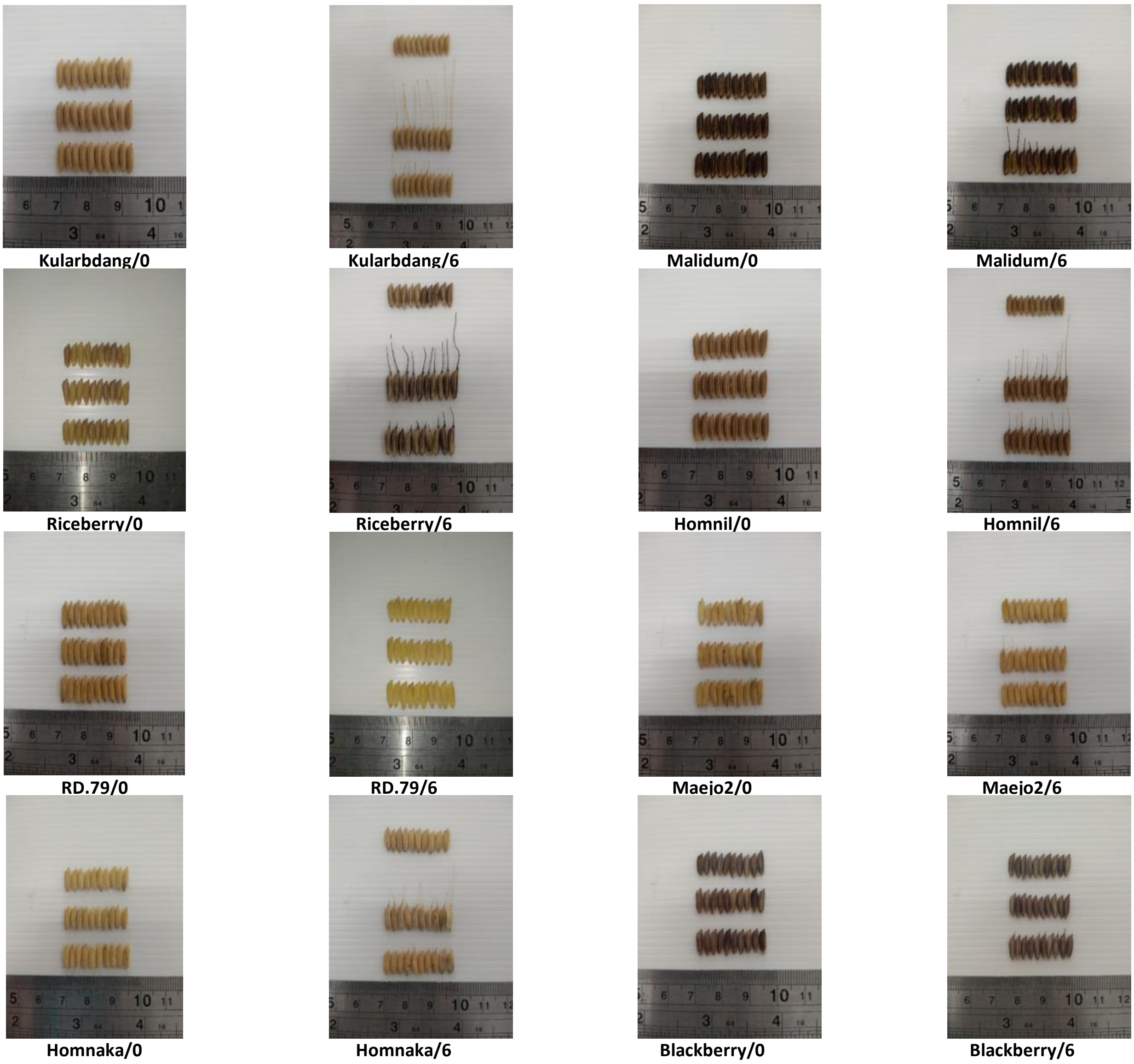

Figure 2. Different rice varieties were treated with 0.1% concentration of colchicine for 0 and 6 hours

This study employed an alternative approach from China for inducing polyploidy. The studies employed 2-day-old rice seedlings subjected to 0.1% colchicine for 6 hours, resulting in unsatisfactory polyploidization induction, likely due to a short period of treatment. Nonetheless, it was observed that the rice plants exhibited several morphological alterations and a significant quantity of rice plants with awned seeds emerged.

The induction of polyploids in rice favors the use of colchicine (Chen et al. 2021). By studying polyploid induction methods in many plants, it was found that colchicine was most effective in inducing polyploids (Jokari et al. 2022). This is because data from previous studies revealed that colchicine at intensities of 0.1%, 0.15%, and 0.25% resulted in rice plants whose awn seeds presented the most polyploid nature. At a concentration of 0.1%, there are anomalies in the morphological changes when colchicine is most commonly given. The use of 0.1% colchicine for 0 and 6 hours in 10 different rice varieties in this study revealed that the seedlings presented several abnormalities. The percentage of abnormal rice plants was 17.50-51.25%, depending on the variety.

An examination of the morphology of different rice varieties that were given 0.1% colchicine for 0 and 6 hours revealed that exposure to colchicine caused early height characteristics. The number of leaves/plant, number of shoots/plant, plant circumference, leaf width, leaf length, and SPAD values had a marked downward trend. However, some rice varieties, such as Tuptim Chumpae, Riceberry, and Maejo 2, tended to have an increased number of shoots/plants. This finding is consistent with the findings of studies of Mexican lime and sour orange, which revealed that exposure to colchicine tends to decrease the values of certain morphological traits, such as the heights of Mexican lime and sour orange (Jokari et al. 2022). However, Jokari et al. (2022) reported that chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and relative water content (RWC) decreased. In comparison, electrolyte leakage (EI), the leaf proline content, and the root proline content increased. In addition, the stem diameter, number of branches, number of leaves, leaf area, leaf thickness, stem fresh weight, and root fresh weight were also determined.

The yield characteristics of different rice varieties that received colchicine decreased, such as full seed weight/plant, number of full seeds/plant, number of atrophied seeds/plant, total number of seeds/plant, seed width and seed length (Tables 6 and 7). However, some rice varieties, such as Riceberry, Maejo 2, and Homnaka, tended to yield more panicles. Some rice varieties tended to have a relatively large number of full grains per plant, such as the Malidum and Homnil varieties. Some rice varieties tended to have an increase in the total number of seeds/plant, such as Malidum, Riceberry, and Homnil. All the rice varieties that received 0.1% colchicine for 6 h presented increased grain width. Some rice varieties tended to increase grain lengths, such as Kularbdang, Homnil, and Blackberry. When studying rice with awn seeds individually, it was found that some rice plants in many varieties had good yield potential because of colchicine treatment. In addition to inducing polyploid rice properties, receiving colchicine leads to mutation-inducing properties, chromosome aberration (Le et al. 2020), chromosome loss, rearrangement or gene mutation, and DNA methylation (Wang et al. 2021; Rao et al. 2023). Studies in millet (*Panicum miliaceum*) have also revealed that different concentrations of colchicine affect molecular DNA (Zeinullina et al. 2023).

The abnormal rice plants were subsequently transplanted, and further studies were carried out by conducting examinations to identify polyploid plants. An examination with FCM revealed that 9.52% of the abnormal rice plants were mixoploid. However, when the awn characteristics of the seeds were examined, it was found that each rice variety had a different awn seed (Blackberry was 90%, Maejo 2 was 65%, Homnaka was 60%, Riceberry was 35%, Malidum was 30%, Homnil was 10%, and Kularbdang was 5%). This finding is consistent with previous studies in cotton (*Gossypium herbaceum*) (Maru et al. 2021), Mexican lime and sour orange (Jokari et al. (2022), and millet (*P. miliaceum*) (Zeinullina et al. 2023), that different plant species react differently to colchicine.

Experiments involving the treatment of several rice varieties with colchicine revealed that in the M1 generation, some rice plants maintained their original morphology, rice plants produced seeds with awns, and rice plants exhibited mixoploidy. Additional examination of these rice plants (unavailable data) revealed other intriguing discoveries, for example, rice containing awn seeds, which do not exhibit polyploidy in the M1 generation, can generate offspring with both diploid and tetraploid characteristics. Thus, it is plausible that these rice plants in the M1 generation, which include awn seeds, might be chimeras composed of both diploid and polyploid stem tissues. A flow cytometry examination of the diploid portion revealed that it did not exhibit polyploidy. Nevertheless, no tetraploid offspring were observed when the M2 generation of mixoploid plants was examined. Various options are available, including reproductive tissue that only generates flowers from diploid cells. While mixoploid rice plants can generate diploid, triploid, and tetraploid M2 offspring, insufficient planting and examination of M2 plants may fail to identify triploid and tetraploid rice plants.

Colchicine treatment often does not produce polyploid plants in several species, it can cause genetic variation that may provide features superior to those of the parent plants. Colchicine has been employed in several plant species studies to promote variety for diverse objectives. Weihmüller et al. (2014) hypothesized that sequence variation in *Paspalum plicatulum* arises quickly following autopolyploidization and that these changes are particular rather than random. Münzbergova (2017) sought to investigate the subsequent inquiries. What are the differences in seed size, performance, and flowering phenology between natural and synthetic tetraploids and natural diploids? Do the discrepancies vary among source populations? Can the differences between diploids and tetraploids be ascribed to initial variations in seed size? A significant occurrence of diploid seeds was noted in the synthetic tetraploid mother plants, facilitating the investigation of colchicine administration effects in the second generation devoid of polyploidization impacts. Consequently, an additional query was raised: what are the impacts of colchicine administration on mother plants regarding offspring performance?

De Carvalho Santos et al. (2019) reported genetic diversity in autotetraploid banana populations by inducing chromosomal doubling with colchicine, focusing on agronomic traits and utilizing SSR and IRAP molecular markers. Cabahug et al. (2020) seek to ascertain the optimal amounts of colchicine treatment and to furnish phenotypic data concerning its impacts on *Echeveria* species, including growth and development. Cabahug et al. (2021) presented impact of colchicine treatment on the phenotypic and ploidy level of *Echeveria* 'Peerless'. Valenzuela et al. (2022) verified GAPDH, ATP1, NADH, and COX2 genes, which exhibited good stability, for use as reference genes in gene expression experiments for blueberry plants and other *Vaccinium* species. Yan et al. (2022) investigated the impact of colchicine on the germination and mutagenesis of *Buddleja lindleyana* seeds at varying concentrations and incubation durations, in order to identify the optimal combination and offer guidance for the development of mutated populations of *B. lindleyana* for future research. Zeinullina et al. (2023) examined the impact of different colchicine concentrations and treatment durations on the agronomic characteristics of proso millet (*P. miliaceum*) grown under field conditions and evaluated the genetic diversity induced by colchicine using ISSR markers. Fathurrahman et al. (2023) assessed the impact of colchicine mutagenesis on the phenotype and genotype of *Vigna unguiculata* var. *sesquipedalis*. In many of the above studies, it was found that colchicine not only induces polyploid plants, but also induces mutations without changing the chromosome number of the plants. In addition to the morphological and yield component changes, colchicine-mutated plants also have changes at the chromosomal and molecular levels. The results of such changes are very useful for plant breeding. Similarly, in this study, it was found that colchicine induction in rice resulted in mixoploid rice plants and changes in the morphology and yield components of M1 rice in several rice varieties. This study also found that several mutant rice plants had improved traits and yield potential, which are suitable for further study. This procedure resembles induced mutation (Cabahug et al. 2021; Kasmiyati et al. 2021; Fathurrahman et al. 2023) and can be efficiently utilized in plant breeding initiatives.

In conclusion, the investigation of the effect of colchicine on inducing polyploidy in 10 Thai rice varieties revealed that colchicine induction at 0.1% concentration for 6 hours resulted in 9.52% mixoploid. However, colchicine at such concentration and time caused variation in morphological characteristics (height, number of leaves, number of shoots per plant, leaf width, leaf length, and chlorophyll content in leaves) and yield components (full seed weight/plant, number of full seeds/plant, number of atrophied seeds/plant, total number of seeds/plant, seed width and seed length) in many rice varieties. In addition, a large number of rice plants with awn seeds were also found. The results indicate that there is variation in genetics, which will increase the potential of rice germplasm for further improvement of Thai rice varieties.

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