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Colchicine mutagenesis effects on the characteristics and correlation of the M2 variant population of rice varieties

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Abstract. *Surson S, Sitthaphanit S, Prachachit J, Jitjak T, Wongkerson K, Rachapila T. 2025. Colchicine mutagenesis effects on the characteristics and correlation of the M2 variant population of rice varieties. Biodiversitas 26: 2355-2367.* Rice is a self-pollinating plant. In conventional rice breeding, variability is created in rice by crossbreeding. The method of crossbreeding rice is complicated because it requires much expertise to breed. The method of creating genetic variability in rice using chemicals, such as colchicine, is simple. This study used colchicine-induced variability in rice seeds, after which M1 generation variant plants with awn seeds were selected to create an M2 population for selection and to study the variation in the characteristics and correlation of rice. The results of the present study revealed that the M2 population of each M1 variant differed from the parental plants that did not receive colchicine in many morphologies and yield components. In addition, the M2 variant populations of each rice variety also differed. The study of the correlation values revealed interesting results for many traits, such as when rice seeds with awns appeared, the percentage of pollination was low. High tillering resulted in a high number of panicles, full grains, full grain weight, and number of leaves, but it also reduced the stomatal width, plant height, leaf width, leaf length, first shoot diameter, and SPAD value. This study selected mutant rice lines that had potent agronomic characteristics to improve Thai rice varieties.

Keywords: Colchicine, mutagenesis, *Oryza sativa*, rice, variation

INTRODUCTION

Rice breeding can be performed via many methods, such as conventional rice breeding (Das 2020; Haque et al. 2021), somaclonal variation rice breeding (Elshafei et al. 2019), interspecific hybridization rice breeding (Zhou et al. 2022), polyploid rice breeding (Koide et al. 2020; Chen et al. 2021; Wang et al. 2022), protoplast rice breeding (Kim et al. 2022), double haploid rice breeding (Das et al. 2020; Singh et al. 2022), mutation rice breeding (Zhang et al. 2022), and molecular rice breeding (Rasheed et al. 2022a; Gong et al. 2023). For developing countries, most rice breeding is based on conventional breeding. The creation of genetic variance in rice is accomplished by crossbreeding two or more rice varieties to combine the traits of both parents into offspring. The breeding method is based more on phenotype, which creates unreliability in the determination of pure lines of genes (Lamichhane and Thapa 2022). Because rice is a self-pollinating plant with small flowers, crossbreeding of rice requires delicate and specialized breeding. Therefore, the rice breeding step is complicated in the breeding program, especially in projects that require the creation of many F1 hybrids. Therefore, some rice breeding projects look for easier methods, such as polyploid rice breeding. Polyploid rice breeding was first initiated in Japan and has been very successful in China (Chen et al. 2021). Polyploid rice has many good agronomic traits, such as panicle length and grain length (Koide et al. 2020), and has improved grain quality (Gong et al. 2023). A study also revealed that Neo-tetraploid rice lines have improved seed set and pollen fertility (68% and 92%, respectively) (Koide et al. 2020). In addition, polyploid rice is also relatively resistant to adverse environmental conditions, such as salinity (Haque et al. 2021). In several plant species, colchicine has been found to induce mutagenesis in addition to producing polyploid plants (El-Nashar and Ammar 2016; Datta 2020; Cabahug et al. 2021, 2022; Gupta et al. 2021; Rasheed et al. 2022b; Samadi et al. 2022; Susrama et al. 2022; Yan et al. 2022; Twumasi et al. 2023; Zeinullina et al. 2023). Previous experiments have shown that colchicine can alter the morphological and chromosomal characteristics of plants (Bhuvaneswari et al. 2020; Li et al. 2020; Kushwah et al. 2021; Zhang and Gao 2021; Khan et al. 2023; Nirala et al. 2023). Colchicine has also been found to cause changes in plant DNA molecules (El-Nashar and Ammar 2016; Yassein et al. 2021; Zeinullina et al. 2023), further indicating that it induces mutagenesis in plants. Colchicine induces chromosomal doubling or mutagenesis differently in each plant. Colchicine is mostly used to treat the vegetative and seed parts of plants (Xiang et al. 2019; Li et al. 2020; Surson et al. 2021, 2024a). In plants that cannot be seeded, tissue culture labs treat vegetative parts with colchicine under sterile conditions. Increasing the number of chromosomes in plants without changing their genetics is its main function (Xiang et al. 2019; Li et al. 2020). Colchicine treatment of seeds of various plant species can double the chromosome number several times and create genetic variation in the plant, especially in cross-pollinated and hybrid seeds (Surson et al. 2024b, 2024c, 2025). Colchicine treatment in seeds is simple and does not require a tissue culture laboratory, which takes more resources and skills, making it acceptable for breeders without one. According to Surson et al. (2024a, 2025), colchicine treatment of rice seeds yielded M1 mutant rice lines. In that study, many M1 rice plants, the parents of M2 mutant rice, were chosen for their interesting morphological and agronomic traits. This study aimed to investigate the morphological characteristics, yield components, stomatal traits and correlations of M2 variant rice varieties derived from M1 variant plants and to investigate whether the population exhibited different variances from the parental varieties and whether it had sufficient potential to be used for further selection of new varieties.

MATERIALS AND METHODS

Rice varieties used in the study

Two groups of rice varieties were used in this study: the parent rice group, which included Kularpdang, Malidum, Riceberry, Homnil, Maejo, Homnaka, and Blackberry. The variant rice group, which was obtained from colchicine treatment in the previous experiment, consists of the Kularpdang variant, Malidum variant 1, Malidum variant 2, Riceberry variant 1, Riceberry variant 2, Homnil variant 1, Maejo2 variant 1, Maejo2 variant 2, Homnaka variant 1, Homnaka variant 2, Blackberry variant 1, and Blackberry variant 2. The studied rice varieties were obtained from an experiment in 2023 in which polyploid rice was induced with colchicine.

Generation of the M2 variant rice populations used in the study

The M2 variant rice populations were obtained from M1 variant plants that were separated. M1 variant rice plants were induced with 0.1% colchicine for 6 hours. Then, the seeds were grown, and M1 variant plants were selected by collecting seeds separately to create M2 populations from each M1 variant plant in each rice variety. After that, the seeds of the M2 variant populations were planted to compare the M2 variant populations with each other and between the M2 variant populations.

Morphological study of the M2 variant populations and parental plants

The study began with the selection of M1 variant plants and the collection of seeds separately, and the obtained seeds were M2 variant plant populations. The M2 variant plant populations of each population were subsequently planted and compared between each M2 variant plant population and its parental plants, resulting in a total of 19 treatments. The experiment was designed as CRD, with 19 treatments, 3 replications, and 15 plants per treatment. The rice was planted in 10-inch pots filled with soil:raw rice husk:manure at a ratio of 2:1:1.

Agro-morphological characteristics

After rice seeds from the different treatments were grown in trays for 1 month, the seedlings from the different treatments were transplanted into 10-inch diameter pots for a comparative study of the morphology of each treatment, with data collected at month 4. These collected characteristics included the plant height, number of leaves, number of shoots/plant, plant circumference, leaf width, leaf length, and SPAD value. The plant height was measured from the base of the plant to the apex. For the number of leaves, all the large leaves that fully spread were counted. For the characteristics of the number of shoots/plant, every shoot in the plant was counted. For the circumference characteristics of the first shoot, measurements were taken at a height of 5 cm above the ground. For leaf width characteristics, the width of the 3rd-order leaf of the first shoot of the plant was measured. For the SPAD value, the middle and tip of the 3rd leaf of the first shoot from the base were measured.

Study of the yield component characteristics of the M2 variant plant populations of various rice varieties and mother varieties

After the agro-morphology characteristics were studied, the yield component characteristics were studied. These characteristics included the number of ears/plant, the number of total seeds/plant, the number of full seeds/plant, the number of atrophied seeds/plant, full seed weight/plant, seed length, and seed width. For the number of ears/plant, the total number of ears born on each rice plant was counted. For the number of atrophied seeds/plant, the total number of atrophied seeds in each plant was counted. For the number of total seeds/plants, the total number of seeds, including all atrophied and full seeds in each plant, was counted, and only the complete seeds in each plant were counted. For full seed weight/plant, all full seeds of each plant were sorted, and only the full seeds on each plant were weighed. For seed length characteristics, 10 seed length measurements were taken, after which the average length per seed was determined. For seed width, 10 seed widths were measured, after which the average width was determined per seed.

Study of the stomatal characteristics of the M2 variant plant populations of rice varieties and parental lines

At 3 months of age, the fourth leaf of the first shoot from each treatment was collected, with 4 replicates per treatment and 10 plants per replicate. The stomatal width was measured at 50 stomata per plant, whereas the stomatal density was measured at 5 fields per plant. The stomata were determined using adhesive by applying a thin layer of adhesive to the abaxial side of the midpoint of the fourth leaf. When the adhesive was dry, it was peeled off and placed on a glass microscope slide for stomatal analyses. Stomatal characteristics were examined using a 40× microscope (Euromex, Netherlands). The stomatal width was measured from the center of the widest stomatal area. The stomatal length was measured from both ends of the stomata. Stomatal density was determined by counting the number of stomata from 5 fields (area of 1 mm^2) of each plant visible under the microscope.

Flow cytometry analysis of the M2 variant plant populations of rice varieties and parental lines

Flow cytometry analysis was performed according to the following methods. 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 into a 500 µL drop of Quantum Stain NA UV 2 (A) on a plastic Petri dish. 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. 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).

Statistical analysis

A completely randomized design was used. There were 19 treatments studied: Kularpdang, Kularpdang variant 1, Malidum, Malidum variant 1, Malidum variant 2, Riceberry, Riceberry variant 1, Riceberry variant 2, Homnil, Homnil variant 1, Maejo2, Maejo2 variant 1, Maejo2 variant 2, Homnaka, Homnaka variant 1, Homnaka variant 2, Blackberry, Blackberry variant 1, and Blackberry variant 2. Subsequently, wherever the F test was significant, mean comparisons were conducted using DMRT. All analyses were performed via the SPSS version 16 package.

Correlation of the morphological characteristics and yield components of the M2 variant rice varieties

The correlation coefficients were used to describe the relationships among agronomic traits and some phenotypic traits. Correlation analysis between traits was performed via the SPSS version 16 package.

RESULTS AND DISCUSSION

Morphological characteristics of the M2 variant populations and parental lines

After different rice varieties were planted for 4 months, data on the height, number of leaves/plant, number of shoots/plant, leaf width, leaf length, leaf thickness, first shoot diameter, and leaf SPAD value were recorded. The data were then analyzed, and the results are outlined below.

The comparative analysis of the morphological characteristics of the rice varieties Kularbdang and Kularbdang M2 variant 1 indicated that Kularbdang is a consistent parent, but Kularbdang M2 variant 1 presents variability in the examined variables. The examination of the morphological traits of the rice varieties Kularbdang and Kularbdang M2 variant 1 revealed that both presented increased height, leaf width, and leaf length but a reduction in the number of leaves per plant, number of shoots per plant, leaf thickness, and diameter of the first shoot. Nonetheless, several qualities exhibited no statistically significant differences. Compared with Malidum M2 variant 1, Malidum M2 variant 1 presented greater numbers of leaves per plant, shoots per plant, and leaf width but presented reduced plant height, leaf length, leaf thickness, first shoot diameter, and leaf SPAD value. Moreover, Malidum M2 variant 2 exhibited a greater number of shoots per plant, although it presented a lower plant height, number of leaves per plant, leaf width, leaf length, leaf thickness, first shoot diameter, and leaf SPAD value. Riceberry M2 variant 1 exhibited increased plant height, leaf width, first shoot diameter, and leaf SPAD value but reduced leaf number per plant, shoot number per plant, leaf length, and leaf thickness. Riceberry M2 variation 2 presented an increase in plant height, accompanied by a reduction in the number of leaves per plant, shoot number per plant, leaf width, leaf length, leaf thickness, first shoot diameter, and leaf SPAD value. Homnil M2 variant 1 exhibited increased plant height, shoot number per plant, leaf width, leaf length, leaf thickness, first shoot diameter, and leaf SPAD value but reduced the number of leaves per plant. Maejo2 M2 variant 1 presented increased plant height, number of leaves per plant, shoot number per plant, leaf width, leaf length, leaf thickness, and first shoot diameter but reduced leaf SPAD value. Furthermore, Maejo2 M2 variant 2 exhibited greater plant height, number of leaves per plant, shoot number per plant, leaf width, leaf thickness, and first shoot diameter. However, it presented reduced leaf length and leaf SPAD value relative to those of Maejo2. Homnaka M2 variant 1 revealed greater plant height, leaf width, leaf length, leaf thickness, first shoot diameter, and leaf SPAD value, although it had an equivalent number of leaves per plant and a reduced number of shoots per plant compared with Homnaka. Compared with Homnaka, Homnaka M2 variant 2 presented greater plant height, leaf width, leaf length, and leaf SPAD value, although it presented a lower leaf number per plant, shoot number per plant, leaf thickness, and first shoot diameter. Compared with Blackberry, Blackberry M2 variant 1 exhibited a greater number of shoots per plant and greater leaf length; however, it presented a lower plant height, fewer leaves per plant, narrower leaf width, shorter leaf thickness, smaller first shoot diameter, and lower leaf SPAD value. Blackberry M2 variant 2 exhibited greater leaf length and first shoot diameter. However, it presented greater plant height, number of leaves per plant, shoot number per plant, leaf breadth, leaf thickness, and leaf SPAD value compared with Blackberry (Table 1; Figure 1).

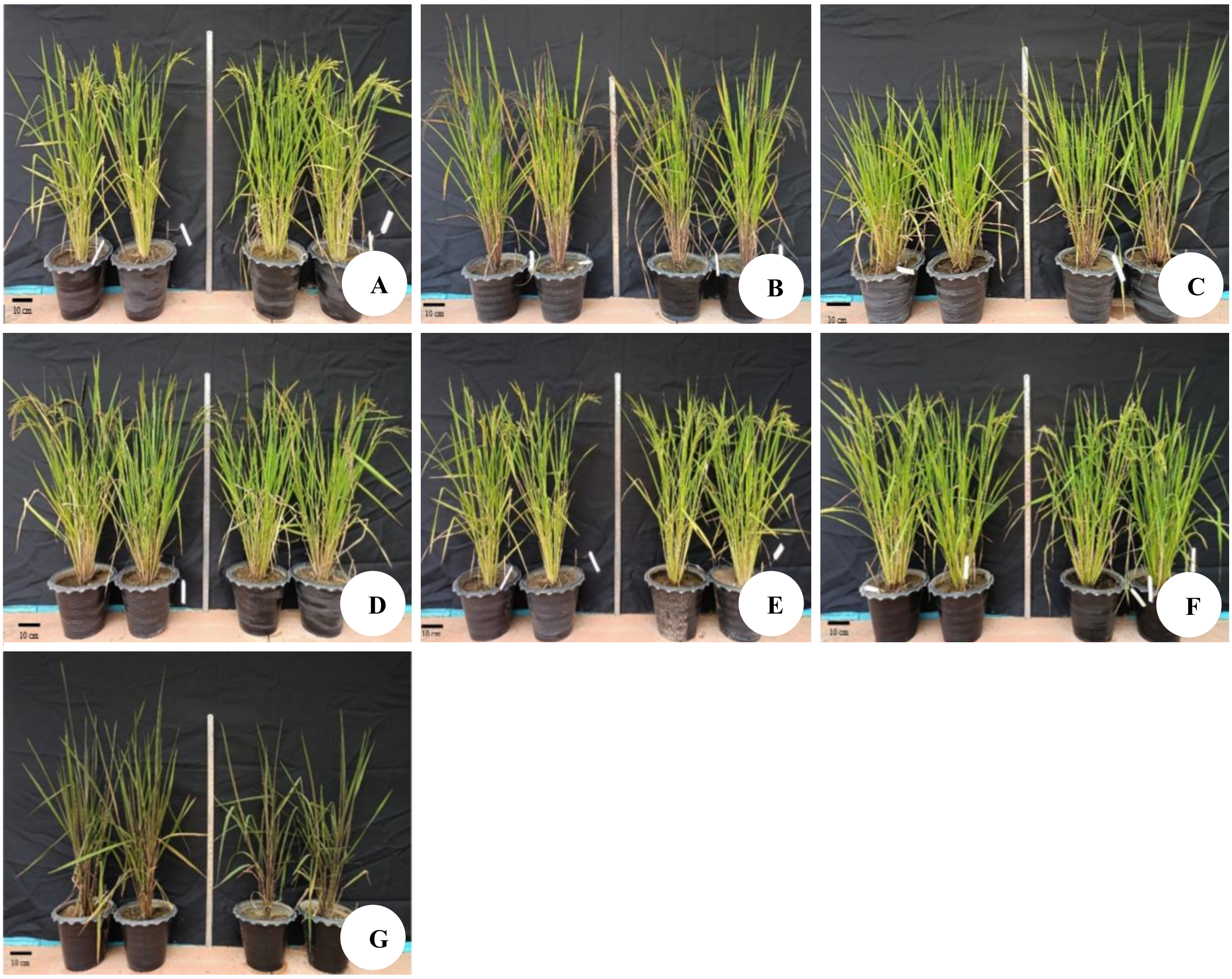


Figure 1. Rice plants: Left is a normal plant and right is variants of different varieties: A. Kularpdang; B. Malidum; C. Riceberry; D. Homnil; E. Maejo2; F. Homnaka; G. Blackberry

Yield components of the rice M2 variant plant populations of various rice varieties and parent varieties

A comparison of the yield components between the Kularbdang and Kularbdang M2 rice varieties revealed that Kularbdang M2 variant 1 exhibited a greater number of awn seeds per plant, greater full seed weight per plant, greater seed width, and greater seed length. However, it resulted in a lower number of panicles per plant, full seeds per plant, withered seeds per plant, total number of seeds per plant, and seed set percentage than Kularbdang. Compared with Malidum, Malidum M2 variant 1 presented a greater number of panicles per plant, full seeds per plant, withered seeds per plant, awn seeds per plant, total seeds per plant, weight of full seeds per plant, seed width, and seed length; however, it presented a lower percentage of seed set. Compared with Malidum, Malidum M2 variant 2 presented greater numbers of panicles per plant, full seeds per plant, withered seeds per plant, total seeds per plant, weight of full seeds per plant, seed width, and seed length. However, it had an equivalent number of awn seeds per plant and a lower seed set percentage than Malidum. Riceberry M2 variant 1 exhibited a greater number of withered seeds per plant, total number of seeds per plant, seed width, and seed length, although it had an equivalent number of awn seeds per plant, as well as a lower number of panicles per plant, full seeds per plant, weight of full seeds per plant, and seed set percentage than did Riceberry. Compared with Riceberry, Riceberry M2 variant 2 presented greater numbers of full seeds per plant, total number of seeds per plant, seed width, and seed length. However, it exhibited an equivalent number of awn seeds per plant and a reduced number of panicles per plant, withered seeds per plant, weight of full seeds per plant, and seed set percentage compared with Riceberry. Compared with Homnil, Homnil M2 variant 1 presented a greater number of withered seeds per plant, awn seeds per plant, and seed width but presented a lower number of panicles per plant, full seeds per plant, total seeds per plant, weight of full seeds per plant, seed length, and percentage of seed set. Compared with Maejo2, Maejo2 M2 variant 1 presented a greater number of panicles per plant, full seeds per plant, withered seeds per plant, awn seeds per plant, total seeds per plant, weight of full seeds per plant, seed width, and seed length; nevertheless, it presented a lower seed set percentage.

Compared with Maejo, Maejo2 M2 variant 2 exhibited a greater number of panicles per plant, full seeds per plant, withered seeds per plant, awn seeds per plant, total seeds per plant, full seed weight per plant, seed width, and seed length, although it had a lower seed set percentage. Homnaka M2 variant 1 exhibited a greater number of panicles per plant, greater numbers of withered seeds per plant, an increased number of awn seeds per plant, greater weight of full seeds per plant, wider seed sizes, and a greater percentage of seed set. However, it presented a smaller number of full seeds per plant, total number of seeds per plant, and seed length than Homnaka did. Homnaka M2 variant 2 exhibited greater numbers of awn seeds per plant, although it presented a lower number of panicles per plant, number of full seeds per plant, number of withered seeds per plant, total number of seeds per plant, weight of full seeds per plant, seed width, seed length, and seed set % than Homnaka did. Compared with Blackberry, Blackberry M2 variant 1 presented a greater number of withered seeds per plant, awn seeds per plant, and greater seed width. However, the number of panicles per plant, number of full seeds per plant, total number of seeds per plant, weight of full seeds per plant, seed length, and percentage of seed set than Blackberry. Compared with Blackberry, Blackberry M2 variant 2 presented greater numbers of withered seeds per plant and greater seed width; however, it surpassed Blackberry in terms of the number of panicles per plant, number of full seeds per plant, number of awn seeds per plant, total number of seeds per plant, weight of full seeds per plant, seed length, and percentage of seed set (Table 2).

Stomatal characteristics of the M2 variant plant populations of various rice varieties and parental varieties

The examination of stomatal characteristics among various rice varieties revealed statistically significant differences in stomatal width, length, and density. However, the rice varieties and their variants did not differ significantly in terms of stomatal width, length, or density.

It is possible that the rice variants still have the same chromosome set, so the stomatal size did not change much. A comparative study of several rice variants of Maejo2 and Honnaka revealed that several groups of variants presented stomatal sizes and densities different from those of their parent varieties (Table 3; Figure 2).

Flow cytometry analysis of the M2 variant plant populations of rice varieties and parental lines

This study revealed that all the examined rice varieties were derived from the M1 variants with awned seeds. After examination by flow cytometry analysis, only the Maejo2 M1 variants and the Homnaka M1 variants were verified as mixoploid. Upon obtaining the M2 variant progeny, five M2 variant plants from each variety were randomly chosen for polyploidy analysis. The findings indicated that one Riceberry M2 variant plant was tetraploid, whereas the others were diploid. The Riceberry M2 tetraploid plants presented nearly all seeds with awns, large leaves, elongated stems, and consistent tillering, producing 9 shoots. Unfortunately, all the seeds were shriveled. A comparison of the stomatal characteristics of normal Riceberry rice plants with those of Riceberry M2 variant diploid and tetraploid plants revealed that both the Riceberry M2 variant diploid and tetraploid plants presented larger stomata than did normal Riceberry rice (Figure 3).

Correlations between rice varieties and parental varieties

The correlations between the characteristics of rice varieties treated with colchicine revealed that the number of panicles was related to the number of full seeds, the number of withered seeds, the number of awn seeds, the number of total seeds, the weight of full seeds, seed width, seed length, stomatal density, plant height, the number of leaves, leaf width, leaf length, first shoot diameter, SPAD value, and the number of shoots. When the number of panicles increased, the plant height, leaf width, leaf length, first shoot diameter, and SPAD value decreased, whereas the number of full seeds, number of withered seeds, number of total seeds, weight of full seeds, width, length, stomatal density, number of leaves, and number of shoots increased.

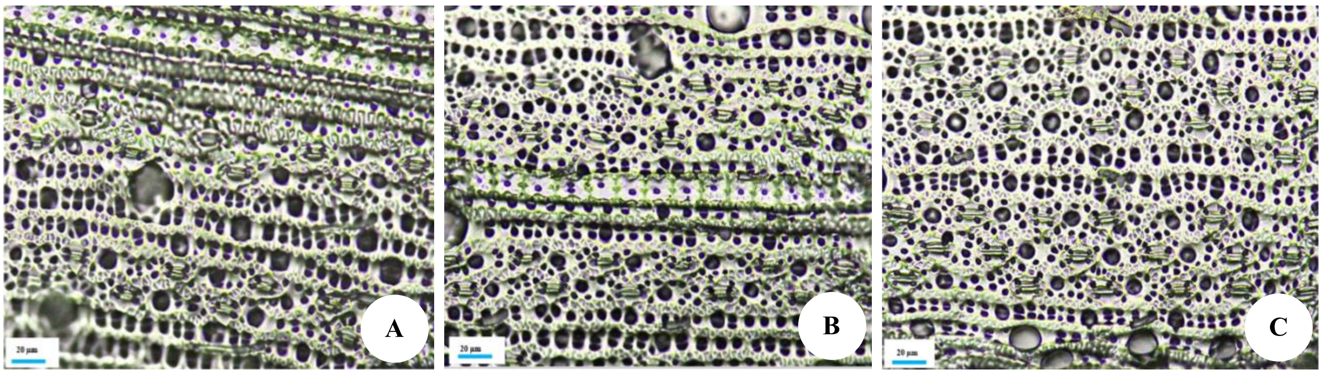
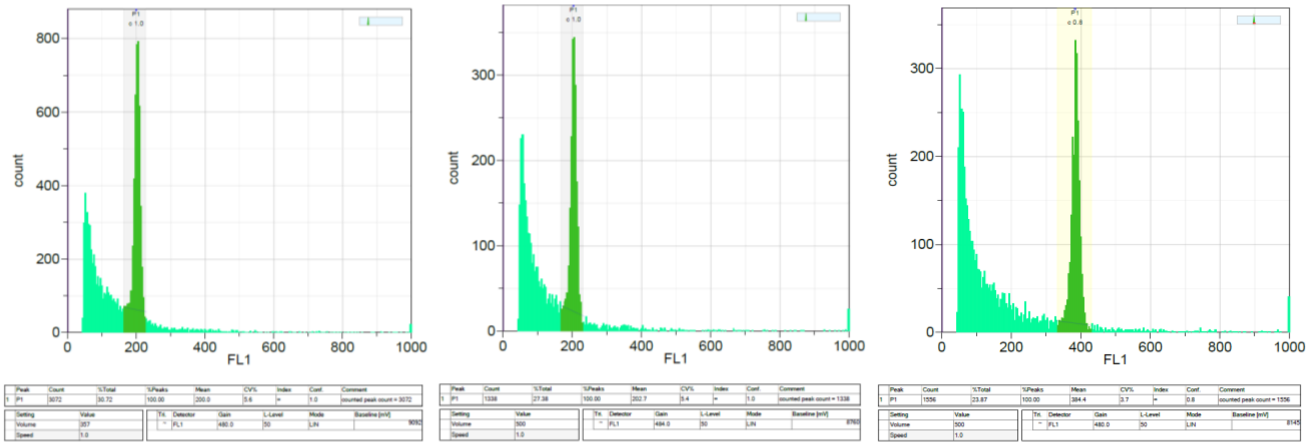


Figure 2. Stomata of riceberry plants: A. Stomata of normal plants; B. Stomata of the variant plants with awn seeds; C. Stomata of the variant plants with tailed seeds and tetraploid



A B C

Figure 3. Flow cytometry analysis: A. Normal plant; B. Variant plant with awn seeds; C. Variant plant with awn seeds and tetraploid

The number of full seeds was related to the number of panicles, number of awn seeds, number of total seeds, weight of full seeds, percentage of seed set, stomatal density, plant height, number of leaves, leaf width, leaf length, leaf thickness, first shoot diameter, SPAD value and number of shoots. When the number of full seeds increased, the number of awn seeds, plant height, leaf width, leaf length, leaf thickness, first shoot diameter and SPAD value decreased, whereas the number of panicles, number of total seeds, weight of full seeds, percentage of seed set, stomatal density, number of leaves and number of shoots increased. The number of awn seeds was related to the number of full seeds, seed length, percentage of seed set, leaf length, and leaf thickness. When the number of awn seeds increased, the number of full seeds and the percentage of seed set decreased, whereas the seed length, leaf length, and leaf thickness increased.

The total number of seeds was related to the number of panicles, the number of full seeds, weight of the full seeds, seed width, seed length, stomatal density, number of leaves, leaf width, leaf length, first shoot diameter, SPAD value, and the number of shoots. As the number of total seeds increased, the leaf width, leaf length, first shoot diameter, and SPAD value decreased. In contrast, the number of panicles, number of full seeds, number of withered seeds, weight of full seeds, seed width, seed length, stomatal density, number of leaves, and number of shoots increased. The full seed weight was related to the number of panicles, number of full seeds, number of total seeds, percentage of seed set, stomatal density, number of leaves, leaf width, leaf length, SPAD value, and number of shoots. As the weight of full seeds increased, the leaf width, leaf length, and SPAD value decreased, whereas the number of panicles, number of full seeds, total number of seeds, percentage of seed set, stomatal density, and number of shoots increased.

The seed width was related to the number of panicles, number of withered seeds, number of total seeds, seed length, leaf width, first shoot diameter, and SPAD value. As the seed width increased, the leaf width, first shoot diameter, and SPAD value decreased. As the number of panicles increased, the number of withered seeds, total number of seeds, and seeds length increased. The seed length was related to the number of panicles, number of withered seeds, number of awn seeds, number of total seeds, seed width, percentage of seed set, stomatal length, stomatal density, diameter of the first shoot, and SPAD value. As the seed length increased, the percentage of seed set, stomatal length, diameter of the first shoot, and SPAD value decreased. In contrast, the number of panicles, the number of awn seeds, the number of withered seeds, the number of total seeds, the width of the seeds, and the stomatal density increased. The percentage of seed set was related to the number of full seeds, number of fallen seeds, number of awn seeds, weight of full seeds, seed length, number of leaves, and leaf length. As the percentage of seed set increased, the number of fallen seeds, number of awn seeds, seed length, and leaf length decreased, whereas the number of full seeds, weight of full seeds, and number of leaves increased.

The stomatal length was related to the seed length and stomatal density. As the stomatal length increased, the seed length and stomatal density decreased. The stomatal density was related to the number of panicles, number of full seeds, number of total seeds, weight of full seeds, seed length, stomatal length, plant height, leaf width, leaf length, diameter of the first shoot, SPAD value, and shoot number. As the stomatal density increased, the plant height, leaf width, leaf length, diameter of the first shoot, and SPAD value decreased, whereas the number of panicles, number of full seeds, number of total seeds, weight of full seeds, seed length, and shoot number increased. The plant height was related to the number of panicles, number of full seeds, stomatal density, number of leaves, leaf width, leaf length, leaf thickness, and shoot number. As the plant height increased, the number of panicles, number of full seeds, stomatal density, number of leaves, and number of shoots decreased, whereas the leaf width, leaf length, and leaf thickness increased.

Table 1. Morphological study of M2 variant rice varieties: Kularbdang, Malidum, Riceberry, Maejo 2, Homnakha, and Blackberry at 4 months of age

Varieties	Plant height (cm)	Number of leaves/plant	Number of shoots/plants	Leaf width (cm)	Leaf length (cm)	Leaf thickness (mm)	First shoot diameter (mm)	SPAD value
Kularpdang	91.60±1.47^{c-d}	66.57±5.83^{a-b}	17.00±1.70^{c-d}	1.17±0.08^{d-e}	46.98±1.21^{b}	0.97±0.43^{a}	6.89±0.34^{b-c}	34.34±2.42^{b-d}
Kularpdang-variant1	94.63±2.84^{b-c}	43.07±5.97^{e}	10.51±1.83^{g-h}	1.24±0.06^{d}	49.56±2.65^{a-b}	0.70±0.10^{b}	6.86±0.32^{b-c}	39.46±0.74^{a}
Malidum	95.06±2.27^{b-c}	55.72±5.92^{c-d}	17.33±2.40^{b-d}	1.05±0.05^{g-h}	38.27±0.32^{c-d}	0.52±0.10^{b-e}	4.92±0.03^{e}	33.02±2.48^{c-d}
Malidum-variant1	88.57±0.32^{d-e}	57.07±1.59^{b-c}	18.93±0.97^{b-c}	1.06±0.14^{f-h}	32.68±1.44^{f-g}	0.39±0.06^{c-e}	4.69±0.44^{e}	24.62±2.50^{f}
Malidum-variant2	86.03±2.70^{e-f}	56.22±4.06^{c-d}	20.07±1.30^{b}	1.04±0.02^{g-h}	33.30±2.37^{e-g}	0.41±0.04^{c-e}	4.73±0.15^{e}	24.79±2.10^{f}
Riceberry	75.73±0.57^{i}	60.00±5.99^{b-c}	24.90±1.59^{a}	1.10±0.02^{e-h}	37.60±0.89^{c-e}	0.57±0.01^{b-d}	6.86±0.39^{b-c}	36.81±3.91^{a-c}
Riceberry-variant1	81.00±2.71^{h}	57.03±6.13^{b-c}	19.60±2.12^{b-c}	1.13±0.04^{e-g}	34.47±1.37^{d-g}	0.36±0.07^{d-e}	7.18±1.09^{a-c}	39.34±1.24^{a}
Riceberry-variant2	85.47±2.08^{e-g}	59.73±6.58^{b-c}	18.93±2.62^{b-c}	1.01±0.01^{h}	34.93±0.80^{c-g}	0.44±0.07^{c-e}	6.46±0.41^{c-d}	36.67±1.26^{a-c}
Homnil	83.33±1.06^{f-h}	46.77±2.70^{d-e}	15.50±0.53^{d-e}	1.04±0.05^{g-h}	30.94±1.47^{g}	0.33±0.01^{e}	6.45±0.37^{c-d}	27.56±4.11^{e-f}
Homnil-variant1	85.90±1.80^{e-f}	46.15±5.76^{e}	15.53±1.71^{d-e}	1.12±0.00^{e-g}	33.36±3.60^{e-g}	0.36±0.04^{d-e}	6.51±0.39^{c-d}	30.63±3.92^{d-e}
Mae Jo2	72.68±4.36^{i}	40.35±3.90^{e}	11.07±0.95^{f-h}	1.02±0.07^{h}	34.46±1.21^{d-g}	0.45±0.07^{c-e}	6.42±0.27^{c-d}	38.61±2.55^{a-b}
Maejo2-variant1	75.27±2.29^{i}	63.13±3.85^{a-c}	13.17±0.90^{e-g}	1.05±0.02^{g-h}	34.90±1.86^{c-g}	0.49±0.05^{b-e}	6.91±0.17^{b-c}	36.63±0.85^{a-c}
Mae Jo2-variant2	81.36±0.46^{h}	71.63±5.44^{a}	13.37±0.67^{e-f}	1.12±0.02^{e-g}	34.45±3.95^{d-g}	0.49±0.10^{b-e}	6.99±0.40^{a-c}	34.44±0.66^{b-d}
Homnaka	82.03±1.27^{g-h}	70.87±7.04^{a}	14.93±0.15^{d-e}	1.12±0.02^{e-g}	34.98±1.01^{c-g}	0.42±0.09^{c-e}	6.01±0.37^{d}	34.84±0.75^{b-c}
Homnaka-variant1	88.77±2.37^{d-e}	70.87±7.04^{a}	14.50±2.63^{d-e}	1.17±0.01^{d-e}	38.87±1.50^{c}	0.47±0.06^{c-e}	7.35±0.44^{a-b}	36.15±0.46^{a-c}
Homnaka-variant2	87.73±0.87^{e}	64.87±9.60^{a-c}	14.03±1.98^{e}	1.15±0.05^{d-f}	36.59±2.03^{c-f}	0.38±0.02^{c-e}	5.98±0.49^{d}	36.61±0.42^{a-c}
Blackberry	98.77±1.61^{a}	46.90±0.95^{d-e}	8.93±0.55^{h}	1.58±0.03^{a}	47.87±1.06^{a-b}	0.60±0.00^{b-c}	7.63±0.19^{a-b}	40.26±1.25^{a}
Blackberry-variant1	96.77±1.14^{a-b}	39.43±3.52^{e}	10.07±0.90^{h}	1.32±0.06^{c}	49.64±4.75^{a-b}	0.50±0.02^{b-e}	7.12±0.37^{a-c}	40.19±2.30^{a}
Blackberry-variant2	92.58±5.65^{c}	26.65±6.09^{f}	8.30±2.30^{h}	1.45±0.09^{b}	51.50±3.87^{a}	0.52±0.13^{b-e}	7.74±0.56^{a}	39.30±1.74^{a}
F-test	**	**	**	**	**	**	**	**
% CV.	2.39	9.66	10.31	3.89	5.86	23.37	6.52	6.40

Note: Different superscripts in each column indicate significant ($p<0.05$) differences. Note: ns: Non-significant; **: Highly significant ($p<0.01$); *: Significant ($p<0.05$); ±: Standard deviation

Table 2. Study of the yield components of M2 variant rice varieties: Kularbdang, Malidum, Riceberry, Maejo 2, Homnakha, and Blackberry

Varieties	NP	NFS	NWS	NAS	NTS	WFS (g)	SW (cm)	SL (cm)	PSS (%)
Kularpdang	14.43±2.31 $^{d-e}$	583.94±282.38 $^{g-h}$	635.84±29.75 $^{b-c}$	1.47±0.42 ^{b}	1209.71±327.79 $^{d-e}$	12.89±6.43 $^{e-f}$	0.265±0.01 $^{d-g}$	1.047±0.01 $^{a-b}$	46.34 ±10.67 $^{d-e}$
Kularpdang-variant1	10.89±1.12 $^{f-h}$	305.57±96.09 $^{i-j}$	529.38±134.61 $^{c-e}$	342.04±66.07 ^{a}	1149.16±132.47 $^{d-f}$	16.75±4.04 $^{d-e}$	0.276±0.00 $^{b-c}$	1.062±0.01 ^{a}	26.99 ±10.10 ^{f}
Malidum	17.39±2.13 $^{a-d}$	819.93±143.62 $^{d-g}$	560.76±97.98 $^{c-d}$	0.00±0.00 ^{b}	1383.86±236.51 $^{a-d}$	17.10±3.47 $^{d-e}$	0.285±0.00 $^{a-b}$	1.020±0.01 $^{c-e}$	59.23 ±0.54 $^{b-d}$
Malidum-variant1	19.93±1.70 ^{a}	874.50±55.06 $^{c-e}$	804.97±122.76 $^{a-b}$	0.33±0.58 ^{b}	1679.50±177.05 ^{a}	18.88±1.09 $^{c-d}$	0.292±0.01 ^{a}	1.024±0.00 $^{c-d}$	52.23 ±2.31 $^{c-e}$
Malidum-variant2	19.84±0.82 ^{a}	895.31±36.66 $^{c-e}$	803.17±93.06 $^{a-b}$	0.00±0.00 ^{b}	1698.47±114.79 ^{a}	19.29±0.53 $^{c-d}$	0.288±0.00 ^{a}	1.028±0.01 ^{c}	52.81 ±2.68 $^{c-e}$
Riceberry	19.93±1.59 ^{a}	1124.17±141.21 $^{a-b}$	425.27±24.46 $^{c-f}$	0.00±0.00 ^{b}	1579.43±75.06 $^{a-c}$	24.35±3.07 $^{a-c}$	0.243±0.00 ^{i}	1.001±0.00 $^{f-g}$	71.00 ±5.64 $^{a-b}$
Riceberry-variant1	17.88±0.50 $^{a-c}$	602.73±104.31 $^{f-h}$	1010.18±53.38 ^{a}	0.00±0.00 ^{b}	1612.77±130.18 $^{a-b}$	12.06±1.84 $^{e-f}$	0.258±0.01 $^{f-h}$	1.004±0.01 $^{e-g}$	37.22 ±3.89 $^{e-f}$
Riceberry-variant2	18.17±1.50 $^{a-b}$	1194.50±16.39 ^{a}	405.30±94.55 $^{c-f}$	0.00±0.00 ^{b}	1596.47±91.63 $^{a-b}$	23.27±0.29 $^{a-c}$	0.264±0.01 $^{e-g}$	1.006±0.00 $^{d-f}$	74.99 ±4.52 $^{a-b}$
Homnil	15.33±1.29 $^{b-e}$	1108.67±181.38 $^{a-c}$	295.63±92.34 $^{e-g}$	0.00±0.00 ^{b}	1384.30±122.70 $^{a-d}$	25.52±4.27 $^{a-b}$	0.263±0.00 $^{e-g}$	1.031±0.02 $^{b-c}$	79.73 ±6.17 ^{a}
Homnil-variant1	14.93±1.53 $^{b-e}$	881.60±162.69 $^{b-e}$	371.57±116.06 $^{d-f}$	19.23±6.90 ^{b}	1271.40±123.78 $^{b-d}$	20.46±4.38 $^{b-d}$	0.271±0.00 $^{c-e}$	1.027±0.01 ^{c}	69.13 ±9.06 $^{a-c}$
Mae Jo2	10.08±3.12 $^{f-h}$	522.41±263.37 $^{h-i}$	99.85±19.68 ^{g}	1.20±1.31 ^{b}	622.81±274.58 ^{g}	10.85±5.68 ^{f}	0.250±0.00 $^{h-i}$	0.990±0.00 $^{f-h}$	81.36 ±8.92 ^{a}
Maejo2-variant1	14.63±1.88 $^{c-e}$	845.19±129.99 $^{c-f}$	365.15±116.72 $^{d-f}$	8.76±2.14 ^{b}	1217.96±202.41 $^{d-e}$	21.09±3.93 $^{b-d}$	0.270±0.00 $^{c-e}$	1.006±0.02 $^{d-f}$	69.63 ±6.04 $^{a-c}$
Mae Jo2-variant2	12.82±0.61 $^{e-g}$	890.63±21.80 $^{b-e}$	302.40±95.49 $^{e-g}$	8.26±1.57 ^{b}	1200.84±76.66 $^{d-e}$	20.21±0.48 $^{b-d}$	0.267±0.00 $^{c-f}$	0.995±0.01 $^{f-h}$	74.45 ±6.61 $^{a-b}$
Homnaka	15.42±0.73 $^{b-e}$	1098.38±80.99 $^{a-c}$	287.39±57.75 $^{e-g}$	6.64±0.65 ^{b}	1392.07±27.75 $^{a-d}$	25.85±1.51 $^{a-b}$	0.270±0.01 $^{c-e}$	0.993±0.01 $^{f-h}$	78.85 ±4.48 ^{a}
Homnaka-variant1	15.65±2.13 $^{b-e}$	1037.34±63.72 $^{a-c}$	406.66±75.10 $^{c-f}$	9.61±0.68 ^{b}	1299.01±214.90 $^{b-d}$	27.47±2.25 ^{a}	0.275±0.00 $^{c-d}$	0.992±0.01 $^{f-h}$	81.68 ±16.88 ^{a}
Homnaka-variant2	13.17±1.80 $^{e-f}$	935.10±144.88 $^{b-d}$	291.60±54.43 $^{e-g}$	16.07±2.61 ^{b}	1228.87±139.49 $^{c-e}$	22.04±2.74 $^{a-d}$	0.267±0.00 $^{c-g}$	0.989±0.01 $^{f-h}$	75.97 ±5.40 $^{a-b}$
Blackberry	9.74±0.57 $^{g-h}$	665.93±29.52 $^{e-h}$	192.77±13.93 $^{f-g}$	5.66±1.56 ^{b}	879.20±35.37 $^{e-g}$	16.30±1.10 $^{d-f}$	0.252±0.01 $^{h-i}$	0.990±0.01 $^{f-h}$	75.80 ±3.73 $^{a-b}$
Blackberry-variant1	9.58±0.58 ^{h}	562.68±44.55 ^{h}	286.07±61.82 $^{e-g}$	13.00±11.36 ^{b}	853.84±104.62 $^{f-g}$	12.01±0.87 $^{e-f}$	0.257±0.01 $^{g-h}$	0.986±0.01 $^{g-h}$	66.13 ±3.05 $^{a-c}$
Blackberry-variant2	5.33±3.06 ^{i}	168.50±195.80 ^{j}	485.00±439.63 $^{c-e}$	1.33±2.31 ^{b}	638.17±409.21 ^{g}	4.59±3.22 ^{g}	0.258±0.00 $^{f-h}$	0.977±0.02 ^{h}	35.98 ±29.85 $^{e-f}$
F-test	**	**	**	**	**	**	**	**	**
% CV.	11.90	17.31	27.77	67.74	14.95	16.84	2.03	0.00	14.69

Note: Different superscripts in each column indicate significant ($p<0.05$) differences. Note: ns: Non-significant; **: Highly significant ($p<0.01$); *: Significant ($p<0.05$); ±: Standard deviation; NP: Number of Panicles/plant; NFS: Number of Full Seeds/plant; NWS: Number of Withered Seeds/plant; NAS: Number of Awn Seeds/plant; NTS: Number of Total Seeds/plant; WFS: Weight of Full Seed/plant; SW: Seed Width; SL: Seed Length; and PSS: Percentage of Seed Set

Table 3. Study of stomatal characteristics of M2 variant rice varieties: Kularbdang, Malidum, Riceberry, Maejo 2, Homnakha, and Blackberry

Varieties	Stomatal width	Stomatal length	Stomatal density
Kularpdang	11.70±0.08^{b-c}	21.36±0.23^{b-e}	417.51±3.82^{d-f}
Kularpdang-variant1	11.87±0.13^{b-c}	21.59±0.24^{a-e}	424.24±26.27^{d-f}
Malidum	11.68±0.22^{b-c}	21.44±0.25^{b-e}	424.91±9.11^{d-f}
Malidum-variant1	11.96±0.23^{b-c}	21.84±0.25^{a-e}	461.62±25.01^{a-c}
Malidum-variant2	12.15±0.31^{a-c}	21.64±0.20^{a-e}	439.73±23.35^{c-e}
Riceberry	12.30±0.25^{a-b}	21.40±0.30^{b-e}	438.04±8.10^{c-e}
Riceberry-variant1	12.19±0.13^{a-c}	21.30±0.04^{c-e}	440.74±7.31^{c-e}
Riceberry-variant2	11.85±0.12^{b-c}	21.23±0.21^{c-e}	443.43±14.04^{b-e}
Homnil	11.46±0.18^{c}	20.81±0.40^{e}	488.89±18.87^{a}
Homnil-variant1	11.42±0.30^{c}	20.95±0.26^{d-e}	472.06±17.39^{a-b}
Maejo2	11.70±0.28^{b-c}	22.40±2.50^{a-c}	438.72±11.08^{c-e}
Maejo2-variant1	11.62±0.02^{b-c}	21.05±0.28^{d-e}	408.75±16.45^{e-f}
Maejo2-variant2	11.98±0.24^{b-c}	21.91±0.49^{a-e}	403.70±17.42^{f-g}
Homnaka	12.87±1.52^{a}	22.64±0.26^{a-b}	427.94±13.64^{d-f}
Homnaka-variant1	12.10±0.14^{a-c}	22.88±0.85^{a}	400.67±3.55^{f-g}
Homnaka-variant2	12.18±0.34^{a-c}	22.45±0.67^{a-c}	418.85±7.31^{d-f}
Blackberry	12.15±0.14^{a-c}	22.05±0.12^{a-e}	416.83±18.96^{d-f}
Blackberry-variant1	11.66±0.40^{b-c}	21.73±0.46^{a-e}	377.44±9.16^{g}
Blackberry-variant2	11.99±0.29^{b-c}	22.24±0.36^{a-d}	319.19±30.96^{h}
F-test	*	*	**
%CV.	3.47	3.12	3.93

The number of leaves was related to the number of panicles, number of full seeds, number of total seeds, weight of full seeds, percentage of seed set, plant height, leaf width, leaf length, and number of shoots. As the number of leaves increased, the plant height, leaf width, leaf length, and number of shoots decreased, whereas the number of panicles, number of full seeds, number of total seeds, weight of full seeds, percentage of seed set, and number of shoots increased. The leaf width was related to the number of panicles, number of full seeds, number of total seeds, weight of full seeds, seed width, stomatal density, height, number of leaves, leaf length, leaf thickness, first shoot diameter, SPAD value, and number of shoots. As the leaf width increased, the number of panicles, number of full seeds, number of total seeds, weight of full seeds, seed width, stomatal density, number of leaves and number of shoots decreased, whereas the plant height, leaf length, leaf thickness, first shoot diameter and SPAD value increased. The leaf length was related to the number of panicles, number of full seeds, number of awn seeds, number of total seeds, weight of full seeds, percentage of seed set, stomatal density, plant height, number of leaves, leaf width, leaf thickness, first shoot diameter, SPAD value and number of shoots. When the leaf length increased, the number of panicles, number of full seeds, number of total seeds, weight of full seeds, percentage of seed set, stomatal density, number of leaves, and number of shoots decreased, whereas the number of awn seeds, plant height, leaf width, leaf thickness, first shoot diameter and SPAD value increased. The leaf thickness was related to the number of full seeds, the number of awn seeds, plant height, leaf width, leaf length, and the diameter of the first shoot. As the leaf thickness increased, the number of full seeds decreased, whereas the number of awn seeds, plant height, leaf width, leaf length, and diameter of the first shoot increased.

The first shoot diameter was related to the number of panicles, number of full seeds, number of withered seeds, number of total seeds, seed width, seed length, stomatal density, leaf width, leaf length, leaf thickness, SPAD value, and number of shoots. As the first shoot diameter increased, the number of panicles, number of full seeds, number of withered seeds, total number of seeds, width, seed length, and number of shoots decreased, whereas the leaf width, leaf length, leaf thickness, and SPAD value increased. The SPAD value was related to the number of panicles, number of full seeds, number of withered seeds, number of total seeds, weight of full seeds, seed width, seed length, stomatal density, leaf width, leaf length, first shoot diameter, and number of shoots. As the SPAD value increased, the number of panicles, number of full seeds, number of withered seed, number of total seeds, weight of full seeds, seed width, seed length, stomatal density, and number of shoots decreased, whereas the leaf width, leaf length, and first shoot diameter increased. The number of shoots was related to the number of panicles, number of full seeds, number of withered seeds, number of total seeds, weight of the full seed, stomatal density, plant height, number of leaves, leaf width, leaf length, diameter of the first shoot, and SPAD value. As the number of shoots increased, the plant height, leaf width, leaf length, diameter of the first shoot, and SPAD value decreased, whereas the number of panicles, number of full seeds, number of withered seed, number of total seeds, weight of full seeds, stomatal density, and number of leaves increased (Table 4).

Discussion

Colchicine has the chemical formula $C_{22}H_{25}O_6N$. Colchicine is an alkaloid derived from the amino acids phenylalanine and tyrosine of *Colchicum autumnale* (Le et al. 2020). By disrupting the orientation and structure of mitotic division and spindles, colchicine inhibits mitosis in various plant and animal cells. Colchicine inhibits metaphase, prevents the polymerization of tubulin into microtubulin and prevents tubulin from becoming a functional yarn fiber. Thus, the anaphase stage for separating chromosomes does not occur. As a result, the separation will fail to form without a bobbin tread so that chromosomes and their duplicates remain in the same cell. Cell division does not occur immediately; division begins with diploid cells, and ends with the formation of tetraploid cells can be induced artificially in plants via colchicine at the right concentration and time (Fathurrahman et al. 2023). The colchicine technique is attractive to breeders for three reasons. First, it may enable the isolation of homozygous or near-homozygous genotypes from hybrids more rapidly than conventional breeding does. Second, new genetic variation may be generated by chromosome loss, rearrangement or gene mutation, thereby enlarging the breeder's germplasm base. Third, it is a simple, inexpensive technique requiring no special equipment or expertise (Luckett 1989).

Table 4. Correlation of morphological characteristics and yield components of colchicine-treated rice cultivars

	NP	NFS	NWS	NAS	NTS	WFS	SW	SL	PSS	STW	STL	STD	PH	NL	LW	LL	LT	FSD	SPAD	NS
NP	1	0.701**	0.524**	-0.213	0.937**	0.590**	0.357**	0.304*	0.056	0.092	-0.174	0.607**	-0.311*	0.576**	-0.626**	-0.625**	-0.148	-0.534**	-0.550**	0.886**
NFS	0.701**	1	-0.139	-0.369**	0.662**	0.904**	0.107	-0.052	0.675**	0.097	-0.018	0.475**	-0.299*	0.609**	-0.484**	-0.645**	-0.268*	-0.277*	-0.424**	0.583**
NWS	0.524**	-0.139	1	0.038	0.612**	-0.174	0.384**	0.397**	-0.741**	0.083	-0.191	0.159	0.047	0.099	-0.157	-0.059	0.014	-0.316*	-0.303*	0.468**
NAS	-0.213	-0.369**	0.038	1	-0.082	-0.028	0.169	0.502**	-0.432**	-0.046	-0.039	0.003	0.257	-0.194	0.125	0.364**	0.263*	0.094	0.219	-0.259
NTS	0.937**	0.662**	0.612**	-0.082	1	0.608**	0.374**	0.357**	-0.079	0.129	-0.198	0.545**	-0.189	0.532**	-0.496**	-0.523**	-0.153	-0.469**	-0.538**	0.798**
WFS	0.590**	0.904**	-0.174	-0.028	0.608**	1	0.187	0.088	0.587**	0.102	0.024	0.451**	-0.216	0.595**	-0.390**	-0.515**	-0.186	-0.187	-0.354**	0.412**
SW	0.357**	0.107	0.384**	0.169	0.374**	0.187	1	0.450**	-0.216	0.020	-0.008	0.169	0.225	0.255	-0.331*	-0.260	-0.122	-0.668**	-0.620**	0.139
SL	0.304*	-0.052	0.397**	0.502**	0.357**	0.088	0.450**	1	-0.428**	-0.257	-0.355**	0.463**	0.147	0.007	-0.257	-0.015	0.243	-0.332*	-0.409**	0.238
PSS	0.056	0.675**	-0.741**	-0.432**	-0.079	0.587**	-0.216	-0.428**	1	0.000	0.194	0.161	-0.247	0.305*	-0.190	-0.409**	-0.224	0.055	-0.044	0.014
STW	0.092	0.097	0.083	-0.046	0.129	0.102	0.020	-0.257	0.000	1	0.209	-0.152	-0.056	0.250	0.080	0.024	-0.115	-0.087	0.077	0.066
STL	-0.174	-0.018	-0.191	-0.039	-0.198	0.024	-0.008	-0.355**	0.194	0.209	1	-0.323*	0.127	0.118	0.255	0.145	-0.046	0.057	0.251	-0.228
STD	0.607**	0.475**	0.159	0.003	0.545**	0.451**	0.169	0.463**	0.161	-0.152	-0.323*	1	-0.345**	0.195	-0.567**	-0.648**	-0.259	-.0426**	-0.525**	0.487**
PH	-0.311*	-0.299*	0.047	0.257	-0.189	-0.216	0.225	0.147	-0.247	-0.056	0.127	-0.345**	1	-0.295*	0.639**	0.670**	0.289*	0.051	0.112	-0.365**
NL	0.576**	0.609**	0.099	-0.194	0.532**	0.595**	0.255	0.007	0.305*	0.250	0.118	0.195	-0.295*	1	-0.426**	-0.467**	0.107	-0.181	-0.183	0.522**
LW	-0.626**	-0.484**	-0.157	0.125	-0.496**	-0.390**	-0.331*	-0.257	-0.190	0.080	0.255	-0.567**	0.639**	-0.426**	1	0.779**	0.261*	0.522**	0.486**	-0.593**
LL	-0.625**	-0.645**	-0.059	0.364**	-0.523**	-0.515**	-0.260	-0.015	-0.409**	0.024	0.145	-0.648**	0.670**	-0.467**	0.779**	1	0.522**	0.470**	0.561**	-0.540**
LT	-0.148	-0.268*	0.014	0.263*	-0.153	-0.186	-0.122	0.243	-0.224	-0.115	-0.046	-0.259	0.289*	0.107	0.261*	0.522**	1	0.273*	0.212	-0.061
FSD	-0.534**	-0.277*	-0.316*	0.094	-0.469**	-0.187	-0.668**	-0.332*	0.055	-0.087	0.057	-0.426**	0.051	-0.181	0.522**	0.470**	0.273*	1	0.652**	-0.406**
SPAD	-0.550**	-0.424**	-0.303*	0.219	-0.538**	-0.354**	-0.620**	-0.409**	-0.044	0.077	0.251	-0.525**	0.112	-0.183	0.486**	0.561**	0.212	0.652**	1	-0.428**
NS	0.886**	0.583**	0.468**	-0.259	0.798**	0.412**	0.139	0.238	0.014	0.066	-0.228	0.487**	-0.365**	0.522**	-0.593**	-0.540**	-0.061	-.406**	-0.428**	1

Note: **: Highly significant (p<0.01); *: Significant (p<0.05); NP: Number of Panicles; NFS: Number of Full Seeds; NWS: Number of Withered Seeds; NAS: Number of Awn Seed; NTS: Number of Total Seeds; WFS: Weight of Full Seed; SW: Seed Width; SL: Seed Length; PSS: Percentage of Seed Set; STW: Stomatal Width; STL: Stomatal Length; STD: Stomatal Density; PH: Plant Height; NL: Number of Leaves; LW: Leaf Width; LL: Leaf Length; LT: Leaf Thickness; FSD: First Shoot Diameter; SPAD: SPAD value; NS: Number of Shoot

There have been many studies on the occurrence of mutagenesis from colchicine application in plants (Luckett 1989; Datta 2020; Gupta et al. 2021; Cabahug et al. 2022; Susrama et al. 2022; Yan et al. 2022). The application of colchicine in plants has led to the discovery of considerable evidence of chromosome aberration. Studies in several plant species have revealed chromosomal aberrations in mitotic cell division, such as univalency, multivalency, chromosome stickness, unorientation, precocious chromosome movements, chromosome bridges, lagging chromosomes, acentric fragments, chain chromosomes, ring chromosomes, vagrant chromosomes and micronuclei (Barman et al. 2021; Kushwah et al. 2021; Maru et al. 2021; Alam et al. 2022; Khah et al. 2022; Samadi et al. 2022). In addition to the finding that colchicine causes chromosomal aberrations, there is also evidence that colchicine causes other molecular DNA changes (Luan et al. 2008; El-Nashar and Ammar 2016; Xiang et al. 2019; Yassein et al. 2021; Zeinullina et al. 2023). The effects of such changes include changes in the anatomical plant traits, morphology and yield components of the plants in the same way as in this study (Gaafar et al. 2017; Bhuvaneswari et al. 2020; Mo et al. 2020; Taratima et al. 2020; Cabahug et al. 2021, 2022; Gupta et al. 2021; Yassein et al. 2021; Susrama et al. 2022; Yan et al. 2022; Zeinullina et al. 2023).

This study revealed that, with the exception of Blackberry M2 variant 1 and variant 2, which were found to be taller than rice plants that did not receive colchicine, the height of the rice plants with different variants increased. Other studies that used colchicine in rice reported that colchicine caused variation in rice height, with rice plants being taller and shorter than normal rice plants (Gaafar et al. 2017; Taratima et al. 2020). It is possible that colchicine has different effects on genes that control the height of each rice plant or may have different effects on each gene that controls the height. When other characteristics of rice, such as the number of leaves/plant, the number of shoots/plant, leaf width, leaf length, leaf thickness, the diameter of the first shoot, and the SPAD value, are considered, the trend is the same; i.e., some M2 variant rice varieties have increased morphology, whereas some varieties have decreased morphology. Notably, the same M2 variant rice varieties tend to have the same increase or decrease in some characteristics, such as Riceberry M2 variant 1 and variant 2, which are taller than normal Riceberry rice that does not receive colchicine. The variability of such characteristics indicates that there is variability in the genetics of rice that has received colchicine, which is likely to have both improved and worsened characteristics, as has been previously studied in other plants (Nura et al. 2013; Gaafar et al. 2017; Bhuvaneswari et al. 2020; Rasheed et al. 2022a), depending on the plant species and variety.

A study of important yield components revealed that the Malidum variant presented greater panicle numbers than Malidum. The Maejo2 variant had a greater panicle number than did Maejo. A study of full seeds/plant revealed that Malidum variant 1 and variant 2, Riceberry variant 2, and Maejo2 variant 1 and variant 2 presented better characteristics than did the mother variety, which did not receive colchicine. A study of the weight of full seeds/plants revealed that the Kularbdang variant, Malidum variant 1 and variant 2, Maejo2 variant 1 and variant 2, and Homnaka variant 1 presented better characteristics than did the mother variety, which did not receive colchicine. In terms of seed width, almost all the rice varieties presented greater seed width than did the parent varieties, which did not receive colchicine. A study of grain length revealed that Kularbdang variant 1, Malidum variant 1 and variant 2, Riceberry variant 1 and variant 2, and Maejo2 variant 1 and variant 2 presented better characteristics than did the parent varieties that did not receive colchicine. A study of the percentage of seed set revealed that, with the exception of Homnaka variant 1, which increased the percentage of seed set, all the rice varieties presented a decreased percentage of seed set. The response of colchicine to rice characteristics depended on the genetics of each rice variety.

In addition, this study revealed that some rice plants treated with colchicine presented seeds with awn traits even though the ploidy level of the rice plants did not change. The presence of awn seeds in rice is a trait that indicates the polyploidy of colchicine-treated rice (Song et al. 2014). This study revealed that, similar to other tetraploid or polyploid plants, most rice varieties presented increased stomatal width and stomatal length and decreased stomatal density (Mo et al. 2020; Yan et al. 2022). The correlation analysis revealed that many traits were related. Interestingly, awn seeds were related to many traits, such as the number of full seeds, seed length, percentage of seed set, leaf length and leaf thickness. In particular, the number of seeds with awns was negatively related to the percentage of seed set, indicating that when the number of seeds with awns was high, the number of full seeds and percentage seed set were low.

In conclusion, a study of the morphology, yield components and stomata of M2 variants resulting from colchicine treatment of different rice varieties revealed that each M2 progeny of the same rice variant population presented various or inconsistent characteristics. When the morphology, yield components and stomatal characteristics of variant rice varieties were compared between populations of the same rice variety, significant or minor differences were detected. When the characteristics of the different rice variants varieties were compared, there was even greater variation. This difference was due to the effect of colchicine on the mutation of each rice plant and each variety. Colchicine-treated rice plants may affect chromosomes, DNA molecules or rice genes, indicating that colchicine causes genetic variation in these rice varieties. These genetically variable rice varieties can be used for selection to improve rice varieties. When the correlations between different rice traits were studied, a positive or negative relationship with yield was detected. Therefore, rice with good yield can be selected on the basis of traits related to yield.

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REFERENCES

Alam Q, Khah MA, Azad ZRAA. 2022. Comparative analysis of different chemical mutagens in inducing chromosomal aberrations in meiotic cells of *Triticum aestivum* L. Cytologia 87 (2): 99-105. DOI: 10.1508/cytologia.87.99.

Barman M, Roy S, Ray S. 2021. Mitotic abnormality inducing effects of leaf aqueous extract of *Clerodendrum inerme* Gaertn. on *Allium cepa* root apical meristem cells. Cytologia 86 (2): 113-118. DOI: 10.1508/cytologia.86.113.

Bhuvaneswari G, Thirugnanasampandan R, Gogulramnath M. 2020. Effect of colchicine induced tetraploidy on morphology, cytology, essential oil composition, gene expression and antioxidant activity of *Citrus limon* (L.) Osbeck. Physiol Mol Biol Plants 26 (2): 271-279. DOI: 10.1007/s12298-019-00718-9.

Cabahug RAM, Khanh HTTM, Lim K-B, Hwang Y-J. 2021. Phenotype and ploidy evaluation of colchicine-induced *Echeveria* 'Peerless'. Toxicol Environ Health Sci 13: 17-24. DOI: 10.1007/s13530-020-00069-z.

Cabahug RAM, Tran MKTH, Ahn Y-J, Hwang Y-J. 2022. Retention of mutations in colchicine-induced ornamental succulent *Echeveria* 'Peerless'. Plants 11 (24): 3420. DOI: 10.3390/plants11243420.

Chen R, Feng Z, Zhang X, Song Z, Cai D. 2021. A new way of rice breeding: Polyploid rice breeding. Plants 10 (3): 422. DOI: 10.3390/plants10030422.

Das P, Adak S, Majumder AL. 2020. Genetic manipulation for improved nutritional quality in rice. Front Genet 11: 776. DOI: 10.3389/fgene.2020.00776.

Datta SK. 2020. Induced mutations: Technological advancement for development of new ornamental varieties. Nucleus 63: 119-129. DOI: 10.1007/s13237-020-00310-7.

El-Nashar YI, Ammar MH. 2016. Mutagenic influences of colchicine on phenological and molecular diversity of *Calendula officinalis* L. Genet Mol Res 15 (2): gmr.15027745. DOI: 10.4238/gmr.15027745.

Elshafei A, Barakat M, Milad S, Khattab S, Al-Mutlaq M. 2019. Regeneration of rice somaclons tolerant to high level of abscisic acid and their characterization via RAPD markers. Bull Natl Res Cent 43: 107. DOI: 10.1186/s42269-019-0154-2.

Fathurrahman F, Mardaleni, Krisianto A. 2023. Effect of colchicine mutagen on phenotype and genotype of *Vigna unguiculata* var. *sesquipedalis* the 7th generation. Biodiversitas 24 (3): 1408-1415. DOI: 10.13057/biodiv/d240310.

Gaafar RM, El Shanshoury AR, El Hisseiwy AA, AbdAlhak MA, Omar AF, Abd El Wahab MM, Nofal RS. 2017. Induction of apomixis and fixation of heterosis in Egyptian rice Hybrid1 line using colchicine mutagenesis. Ann Agric Sci 62: 51-60. DOI: 10.1016/j.aoas.2017.03.001.

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 (5): 1375. DOI: 10.3390/agronomy13051375.

Gupta G, Memon AG, Pandey B, Khan MS, Iqbal MS, Srivastava JK. 2021. Colchicine induced mutation in *Nigella sativa* plant for the assessment of morpho-physiological and biochemical parameter Vis-A-Vis in vitro anti-inflammatory activity. Open Biotechnol J 15: 173-182. DOI: 10.2174/1874070702115010173.

Haque MA, Rafii MY, Yusoff MM, Ali NS, Yusuff O, Datta DR, Anisuzzaman M, Ikbal MF. 2021. Advanced breeding strategies and future perspectives of salinity tolerance in rice. Agronomy 11 (8): 1631. DOI: 10.3390/agronomy11081631.

Khah MA, Alshehri MA, Filimban FZ, Alam Q, Aloufi S. 2022. Influence of colchicine in causing severe chromosomal damage in microsporocytes of hard wheat (*Triticum durum* Desf.): Possible mechanisms and genotoxic relevance. Cytologia 87 (2): 137-143. DOI: 10.1508/cytologia.87.137.

Khan MN-E-A, Hassan J, Biswas MS, Khan HI, Sultana H, Suborna MN, Rajib MMR, Akter J, Gomasta J, Anik AAM. 2023. Morphological and anatomical characterization of colchicine-induced polyploids in watermelon. Hortic Environ Biotechnol 64 (1): 461-474. DOI: 10.1007/s13580-022-00488-6.

Kim K, Ho TS, Hwang US. 2022. Production and characterization of somatic hybrids between rice (*Oryza sativa* L.) and reed (*Phragmites communis* Trin.) obtained by protoplast fusion. J Plant Biochem Biotechnol 31: 370-379. DOI: 10.1007/s13562-021-00689-7.

Koide Y, Kuniyoshi D, Kishima Y. 2020. Fertile tetraploids: New resources for future rice breeding?. Front Plant Sci 11: 1231. DOI: 10.3389/fpls.2020.01231.

Kushwah KS, Patel S, Chaurasiya U, Wani MB. 2021. The effect of Colchicine on *Vicia faba* and *Chrysanthemum carinatum* (L.) plants and their cytogenetical study. Vegetos 34: 432-438. DOI: 10.1007/s42535-021-00204-2.

Lamichhane S, Thapa S. 2022. Advances from conventional to modern plant breeding methodologies. Plant Breed Biotechnol 10: 1-14. DOI: 10.9787/PBB.2022.10.1.1.

Le K-C, Ho T-T, Lee J-D, Paek K-Y, Park S-Y. 2020. Colchicine mutagenesis from long-term cultured adventitious roots increases biomass and ginsenoside production in wild ginseng (*Panax ginseng* Mayer). Agronomy 10 (6): 785. DOI: 10.3390/agronomy10060785.

Li S, Lin Y, Pei H, Zhang J, Zhang J, Luo J. 2020. Variations in colchicine-induced autotetraploid plants of *Lilium davidii* var. *unicolor*. Plant Cell Tiss Organ Cult 141: 479-488. DOI: 10.1007/s11240-020-01805-6.

Luan L, Wang X, Long W-B, Liu Y-H, Tu S-B, Zhao Z-P, Kong F-L, Yu M-Q. 2008. Microsatellite analysis of genetic variation and population genetic differentiation in autotetraploid and diploid rice. Biochem Genet 46 (5-6): 248-266. DOI: 10.1007/s10528-008-9156-8.

Luckett DJ. 1989. Colchicine mutagenesis is associated with substantial heritable variation in cotton. Euphytica 42: 177-182. DOI: 10.1007/BF00042630.

Maru B, Parihar A, Kulshrestha K, Vaja M. 2021. Induction of polyploidy through colchicine in cotton (*Gossypium herbaceum*) and its conformity by cytology and flow cytometry analyses. J Genet 100 (2): 1-10. DOI: 10.1007/s12041-021-01297-z.

Mo L, Chen J-H, Chen F, Xu Q-W, Tong Z-K, Huang H-H, Dong R-H, Lou X-Z, Lin E-P. 2020. Induction and characterization of polyploids from seeds of *Rhododendron fortunei* Lindl. J Integr Agric 19 (8): 2016-2026. DOI: 10.1016/S2095-3119(20)63210-5.

Nirala D, Tiwari JK, Sahu NK, Sinha SK. 2023. Morphological and physiological changes (variation) in colchicine induced tetraploids of spine gourd (*Momordica dioica* Roxb.) in comparison to their diploid counterparts. Plant Physiol Rep 28: 481-489. DOI: 10.1007/s40502-023-00758-0.

Nura S, Adamu AK, Mu'Azu S, Dangora DB, Fagwalawa LD. 2013. Morphological characterization of colchicine-induced mutants in sesame (*Sesamum indicum* L.). J Biol Sci 13 (4): 277-282. DOI: 10.3923/jbs.2013.277.282.

Rasheed A, Li H, Nawaz M, Mahmood A, Hassan MU, Shah AN, Hussain F, Azmat S, Gillani SFA, Majeed Y, Qari SH, Wu Z. 2022a. Molecular tools, potential frontiers for enhancing salinity tolerance in rice: A critical review and future prospective. Front Plant Sci 13: 966749. DOI: 10.3389/fpls.2022.966749.

Rasheed N, Ahmad I, Nafees M, Intizar-ul-Hassan M. 2022b. Assessment of mutation caused by colchicine in apple gourd (*Praecitrullus fistulosus*) based on morphological and biochemical attributes. J Glob Innov Agric Sci 10 (1): 19-24. DOI: 10.22194/JGIAS/10.972.

Samadi N, Naghavi MR, Moratalla-López N, Alonso GL, Shokrpour M. 2022. Morphological, molecular and phytochemical variations induced by colchicine and EMS chemical mutagens in *Crocus sativus* L. Food Chem 4: 100086. DOI: 10.1016/j.fochms.2022.100086.

Singh R, Kaur N, Praba UP, Kaur G, Tanin MJ, Kumar P, Neelam K, Sandhu JS, Vikal Y. 2022. A prospective review on selectable marker-free genome engineered rice: Past, present and future scientific realm. Front Genet 13: 882836. DOI: 10.3389/fgene.2022.882836.

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. Genet Resour Crop Evol 61: 797-807. DOI: 10.1007/s10722-013-0074-1.

Surson S, Sitthaphanit S, Prachachit J, Jitjak T, Wongkerson K. 2024a. Effects of colchicine on polyploid induction, morphology, and yield components of several Thai rice varieties. Biodiversitas 25 (12): 4677-4689. DOI: 10.13057/biodiv/d251204.

Surson S, Sitthaphanit S, Prachachit J, Jitjak T, Wongkerson K. 2025. Effects of colchicine on the morphology and agricultural characteristics of

RD.43 Thai rice variety. Indian J Agric Res 59 (3): 357-365. DOI: 10.18805/ijare.af-906.
Surson S, Sitthaphanit S, Wongkerson K. 2021. Polyploidy induction of black sesame (*Sesamum indicum* L.) for yield component improvement. Songklanakarin J Sci Technol 43 (4): 1049-1055.
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 (5): 744-751. DOI: 10.18805/ijare.af-829.
Surson S, Sitthaphanit S, Wongkerson K. 2024c. Effective colchicine-induced polyploid induction in *Centella asiatica* (L.) urban. Plant Cell Tiss Organ Cult 159: 33. DOI: 10.1007/s11240-024-02893-4.
Susrama IGK, Trigunasih NM, Suada IK, Mayadewi NNA. 2022. Performance evaluation of induced mutagenesis using colchicine and EMS solution on cowpea M3 purple and mung bean Vima1 to increase resistance. Caraka Tani: J Sustain Agric 37 (2): 333-343. DOI: 10.20961/carakatani.v37i2.56341.
Taratima W, Reanprayoon P, Raso S, Chantarangsee M, Maneerattanarungroj P. 2020. Physiological and anatomical changes in thai rice landrance (*Oryza sativa* L.) CV Pakaumpuel after colchicine treatment. Pak J Bot 52 (5): 1631-1638. DOI: 10.30848/pjb2020-5(24).
Twumasi P, Asare PA, Afutu E, Amenorpe G, Addy SNTT. 2023. Chemosensitivity analysis of tiger nuts (*Cyperus esculentus* L.) using Ethyl Methanesulfonate (EMS) and colchicine mutagens. Heliyon 9 (12): e22619. DOI: 10.1016/j.heliyon.2023.e22619.
Wang W, Tu Q, Chen R, Lv P, Xu Y, Xie Q, Song Z, He Y, Cai D, Zhang X. 2022. Polyploidization increases the lipid content and improves the nutritional quality of rice. Plants 11: 132. DOI: 10.3390/plants11010132.
Xiang Z-X, Liang H-H, Tang X-L, Liu W-H. 2019. Physiological and genetic diversity analysis of diploid and autotetraploid *Platycodon grandiflorm* A. De Candolle. Plant Cell Tiss Organ Cult 136: 597-603. DOI: 10.1007/s11240-018-01541-y.
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 Tiss Organ Cult 149: 735-745. DOI: 10.1007/s11240-022-02245-0.
Yassein AAM, Hassen AAHA, Abdel-Alah EM, Salim SM. 2021. Morphological and genetic diversity analysis in calendula (*Calendula officinalis* L.) influenced by mutagenic effect of colchicine. J Microbiol Biotechnol Food Sci 10 (5): e3392. DOI: 10.15414/jmbfs.3392.
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 (12): 2973. DOI: 10.3390/agronomy13122973.
Zhang X, Gao J. 2021. Colchicine-induced tetraploidy in *Dendrobium cariniferum* and its effect on plantlet morphology, anatomy and genome size. Plant Cell Tiss Organ Cult 144: 409-420. DOI: 10.1007/s11240-020-01966-4.
Zhang X, Yang F, Ma H, Li J. 2022. Evaluation of the saline-alkaline tolerance of Rice (*Oryza sativa* L.) mutants induced by heavy-ion beam mutagenesis. Biology 11: 126. DOI: 10.3390/biology11010126.
Zhou J, Yang Y, Lv Y, Pu Q, Li J, Zhang Y, Deng X, Wang M, Wang J, Tao D. 2022. Interspecific hybridization is an important driving force for origin and diversification of asian cultivated rice *Oryza sativa* L. Front Plant Sci 13: 932737. DOI: 10.3389/fpls.2022.932737.