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Effect of Colchicine and Bio-catharantin on the DNA Relative Content and Stomatal Structure of Black Rice (*Oryza sativa* L. var. Jeliteng)

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Abstract

Black rice (*Oryza sativa* L. var. Jeliteng), known for its health benefits compared to white rice, faces challenges in productivity. Among varieties, this black rice is popular in Indonesia but shows low yield. Research on improving black rice through genetic manipulation with antimitotic substances is limited. Therefore, this study aims to compare the effects of colchicine and Bio-catharantin on the germination rate, DNA relative content, and stomatal structure of *O. sativa* L. var. Jeliteng. Seeds were treated with colchicine (0.1%, 0.2%, and 0.3%) and Bio-catharantin (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) at soaking durations of 12, 24, and 48 hours. Germination was assayed, ploidy was determined using flow cytometry, and stomatal traits, including size and density, were examined microscopically. The results showed that Bio-catharantin did not exhibit any toxic effects on germination rates, whereas colchicine reduced germination starting at 0.2% concentration. Both chemical agents modified the DNA relative content of Jeliteng black rice. Colchicine generally increased stomatal length and width while decreasing stomatal density, with significant changes at 0.3% concentration for 24 hours. Bio-catharantin also altered stomatal traits, enhancing length and width in most cases but significantly reducing density under certain conditions. Bio-catharantin emerged as a promising alternative to colchicine for inducing chromosomal mutations in plants, offering benefits in altered stomatal structures without the toxic effects on germination, compared to colchicine.

Keywords: antimitotic; crop improvement; flow cytometry; germination

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INTRODUCTION

Rice serves as the primary staple food for 95% of the Indonesian population (Paiman et al., 2020). Based on its pigment content, rice is categorized as either pigmented or non-pigmented. Pigmented rice contains bioactive compounds in the pericarp layer of the rice that produce color pigments of brown, black, purple, or red (Xia et al., 2021; Nabilah et al., 2022). Certain bioactive compounds, such as polyphenols, flavonoids, and antioxidants are present in black rice (Suryanti et al., 2020; Tyagi et al., 2022). The consumption of black rice as a functional food was increasingly popular as people became more health-conscious (Suryanti et al., 2020; Nabilah et al., 2022). Black rice offers various health benefits, such as cancer prevention, free radical defense, improved digestion, cholesterol reduction, and anti-inflammatory effects (Luo et al., 2014; Thepthanee et al., 2021).

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The black rice *Oryza sativa* L. var Jeliteng is a superior variety with a fluffy texture and an amylose content of 19.6% (Nandariyah et al., 2023; Nurhidajah et al., 2024). This black rice variety originated from the hybridization of black sticky rice (*ketan hitam*) and commercial rice seed (Pandan Wangi Cianjur) (Sitaresmi et al., 2023). It contained high levels of phenolic content (29±3.6 mg FAE g^{-1}) (Wijayanti et al., 2023). The harvest age of Jeliteng was 113 days with a productivity of 6.18 tons (Nandariyah et al., 2022). The agronomic advantages of this rice were susceptibility to rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) and moderate susceptibility to *Nilaparvata lugens* or brown planthopper (Indonesian Rice Research Center, 2019).

Although Jeliteng rice was considered a superior variety, it was not extensively cultivated. Conventional farmers prefer to cultivate the common commercial white rice due to its shorter lifespan of 90 days and higher productivity of 8.99 tons (Nandariyah et al., 2022; 2023). Therefore, breeding efforts are necessary to enhance the qualities of the Jeliteng black rice. In rice breeding strategies, mutation breeding using chemical agents such as colchicine, ethyl methanesulfonate (EMS), and sodium azide (SA) induces random mutations that can enhance specific traits while minimizing undesirable changes and to some extent induced polyploid plants. Irradiation methods like fast-neutron irradiation and ion beam radiation are also employed to generate genetic variability, targeting important traits such as disease resistance and abiotic stress tolerance (Viana et al., 2019). Genetic manipulation through cell treatment with antimitotic induction could be performed to enhance the qualities of rice (Chen et al., 2021) for sustainable agriculture in Indonesia (Hafeez et al., 2023).

Genetic manipulation can be induced either physically, biologically or using antimitotic chemical agents, where the latter is the most widely applied method (Chen et al., 2021). Colchicine is an antimitotic agent that impedes anaphase, hence preventing the separation and transfer of chromosomes to the opposite pole. This mechanism produces individuals that contain multiple sets of chromosomes or in another case altered variations in phenotypes (Eng and Ho, 2018). The alternation of phenotypic features such as the enlargement of roots, stems, leaves, fruits, and flowers (Ermayanti et al., 2018), as well as stomatal size (Rohmah et al., 2017; Wu et al., 2022). The application of chemical compounds was desirable due to their effectiveness, lower detrimental characteristics, and long-lasting characteristic changes (Chen et al., 2021).

The application of colchicine in plant breeding induced genetic mutation resulted in metabolite alternation (Samadi et al., 2022), and enhanced stomatal size by increasing chloroplasts in guard cells, leading to a larger stomatal size (Yao et al., 2023). Stomatal size and density have an inverse relationship, as larger stomatal size results in decreased stomatal density (Tossi et al., 2022). Nonetheless, the use of colchicine as a means to reduce stomatal density is limited due to its toxicity, which can also negatively affect plant germination (Kurniawan et al., 2023). Moreover, these compounds possess toxic properties for humans, particularly when used in high concentrations (Eng and Ho, 2018). Natural antimitotic compounds, such as Bio-catharantin extracted from *Catharanthus roseus*, have the potential to serve as a polyploid inductor and avoid these toxic effects (Muarifin et al., 2021; Billa et al., 2022; Kurniawan et al., 2023; Kasim et al., 2024). Bio-catharantin was successfully used to enhance the chromosome number of *Allium cepa* L. var. ascalonicum 'Tajuk' from diploid (2n) into triploid (3n) and tetraploid (4n) at concentrations of 0.2% and 0.4%, respectively (Billa et al., 2022). Bio-catharantin was also reported to improve the growth performance of *Alternanthera amoena* Voss., even in the absence of polyploid individuals (Shafura et al., 2022). Another study reported that Bio-catharantin altered the stomata and chromosome structure of *O. sativa* var. Cempo Ireng without negative effect on the seed germination (Kurniawan et al., 2023).

The research on the improvement of black rice through genetic modification using antimitotic compounds in Indonesia remains limited (Dwiningsih and Alkahtani, 2023). By employing a natural compound, Bio-catharantin, which has no toxic impact, this research has paved the way for the development of effective antimitotic compounds in the genetic modification of black rice. This study aims to compare the impact of genetic modification by using antimitotic compounds, specifically colchicine and Bio-catharantin, on the germination rates, DNA relative, and stomatal structure of the black rice *O. sativa* L. var Jeliteng. This study investigated the toxic effects of combined treatments of colchicine and Bio-catharantin at various

concentrations and soaking durations on seed germination rates. Subsequently, the paddy leaves were subjected to a flow cytometric analysis to ascertain the impact of the treatments on the chromosomes of the black rice's somatic cells. Further observations of the stomatal structure were conducted to compare the changes in size and density of the induced rice.

MATERIALS AND METHOD

Materials

The black rice *O. sativa* L. var. Jeliteng seeds were obtained from the Center for Rice Agriculture Research and Development, Ministry of Agriculture with registration number 167/HK.540/C/01/2019. The Bio-catharantin was a commercial product from the research group of the Faculty of Biology at Universitas Gadjah Mada, Indonesia. Colchicine was the commercial product of C9754 Sigma-Aldrich ≥ 95% (High performance liquid chromatography/HPLC) powder.

Polyploid induction and germination rate

The Jeliteng rice seeds were peeled and sterilized in 70% ethanol for 5 minutes, then diluted in 0.1% NaCl solution for 10 minutes. Rice seeds were subsequently rinsed with sterile distilled water 4 times and dried with sterile filter paper for 2 hours (Prasetyo et al., 2018). The polyploidy induction was carried out following the method proposed by Gaafar et al. (2017). A total of 100 seeds were soaked in each concentration and duration series using 2 antimitotic substances, colchicine and Bio-catharantin. Colchicine is a highly toxic chemical compound (Chen et al., 2021); therefore, the lower concentration was applied. The colchicine solution concentrations were 0.1%, 0.2%, and 0.3%, and Bio-catharantin solution concentrations were 0.1%, 0.2%, 0.3%, 0.4%, and 0.5%. The soaking times for colchicine were 24 and 48 hours and for Bio-catharantin were 12, 24, and 48 hours. The seeds were then germinated in a tray containing clay soil in the Biology Department greenhouse (Universitas Jember) for 14 days. Observations of germination were made by counting the number of seeds that germinated normally within 7 to 14 days. The normal shoots were indicated by the presence of a root system with primary and seminal roots, well-developed hypocotyl without tissue damage, perfect plumula growth with green leaves emerging from the coleoptile, and epicotyl growth with normal buds. The germination rate was calculated based on the proportion of germinated seeds among the total seeds of each treatment (Kurniawan et al., 2023). All treatments were replicated a minimum of 3 times.

Flow cytometry test

Fresh rice leaves were cut into 0.5 cm^2 pieces and placed on a petri dish. Next, 250 µl of CyStain PI Absolute (nuclei extraction buffer) was dripped onto the leaves. Using a razor blade, the leaves were finely chopped and filtered with a 30 µm Millipore sieve. The filtered filtrate was transferred to a cuvette tube and mixed with 350 µl staining solution, consisting of propidium iodine and RNAse. Finally, the DNA relative content was measured using the BD Accuri™ C6 Plus Flow cytometer for ploidy analysis (Hodač et al., 2016).

Stomatal size and density

The abaxial leaves were cleaned and coated with transparent nail polish. After the polish had dried, a strip of tape was applied to the polished area. The tape was slowly removed, causing the epidermis to peel off and adhere to a slide. Stomatal characteristics were observed using a Nikon Eclipse E100 LED MV R microscope connected to Optilab Advance by Miconos at 400x magnification. Stomatal length and width were analyzed utilizing Image Raster software. Stomatal density was computed using Equation 1 (Chatterjee et al., 2020).

$$\text{Stomatal density} = \frac{\text{Number of stomata}}{\text{Field of view area in mm}^2} \quad (1)$$

Statistical analysis

Data were analyzed using R version 4.1.2 for Windows (R Foundation for Statistical Computing). Statistical data were visualized with ggplot2 (Wickham et al., 2016). Significant differences were assessed using either parametric (ANOVA) or non-parametric (Kruskal Wallis) tests. Post-hoc analysis was conducted using either the Student's T-test, Wilcox test, or Duncan's test, with the 'agricolae' package (de Mendiburu and de Mendiburu, 2019).

RESULTS AND DISCUSSION

Colchicine and Bio-catharantin effect on seed germination

Colchicine at a higher concentration (more than 0.2%) reduced the germination rate of black rice seeds, whereas Bio-catharantin showed no adverse effects on the germination rate (Figure 1). The germination rate of Jeliteng black rice seeds

was 91.29% (Supplementary Table 1). The statistical analysis confirmed that the germination rate of the seeds under Bio-catharantin treatments remained within the range of the control treatment (83.33 to 93.33%). In contrast, the colchicine toxicity to the seeds became apparent at a concentration of 0.2% with a 24-hour soaking duration, resulting in a noticeable decline in germination. The negative effect of colchicine increased with higher concentrations and soaking durations (Figure 1).

Colchicine is one of the most common chemical mutagens for inducing polyploidy in plants (Chen et al., 2021). Polyploidization with colchicine has been utilized in horticultural crops for more than 8 decades and continues to be a leading antimitotic agent for polyploid production. A total of 46 plant mutants with colchicine had been registered in FAO in 2018. As research on the use of colchicine in the polyploidization of horticultural crops grows, its effectiveness remains prominent. The success of polyploidization with this mutagenic was related to the concentration and duration of treatment (Eng and Ho, 2018). The consideration of concentration affected the seedling viability. Application of colchicine in a higher concentration reduced the seed germination in related family Poace, i.e. *Dendrocalamus brandisii* (Munro) Kurz by inhibiting cell division, DNA synthesis, and sucrose degradation (Lv et al., 2021). Another report also confirmed the negative effect of colchicine in high concentration that reduced the seed germination and plant growth of the medicinal plant *Salvia hains* (Grouh et al., 2011). Toxic effects can be circumvented by employing natural antimitotic compounds, for instance, Bio-catharantin from *Catharanthus roseus* extract. Bio-catharantin has the potential to serve as a polyploid inductor (Kurniawan et al., 2023). It was reported successfully inducing polyploidization with no toxicity effect on *Arachis hypogaea* (Muarifin et al., 2021), *A. cepa* (Billa et al., 2022), and *A. amoena* (Shafura et al., 2022).

Colchicine and Bio-catharantin effect on DNA relative

The DNA relative of the black rice was measured using flow cytometry analysis as presented in Figure 2. The flow cytometer data revealed that none of the treatments exhibited polyploid in the leaf samples. However, the antimitotic compounds affected the chromosome structure, resulting in a slight shift in the peak index (PI). The relative DNA content of the control was 50.95, while the Bio-catharantin-treated seeds exhibited the greatest alteration in PI at 55.85 (Figure 2e), followed by colchicine at 54.85 (Figure 2i). The flow cytometer's X axis

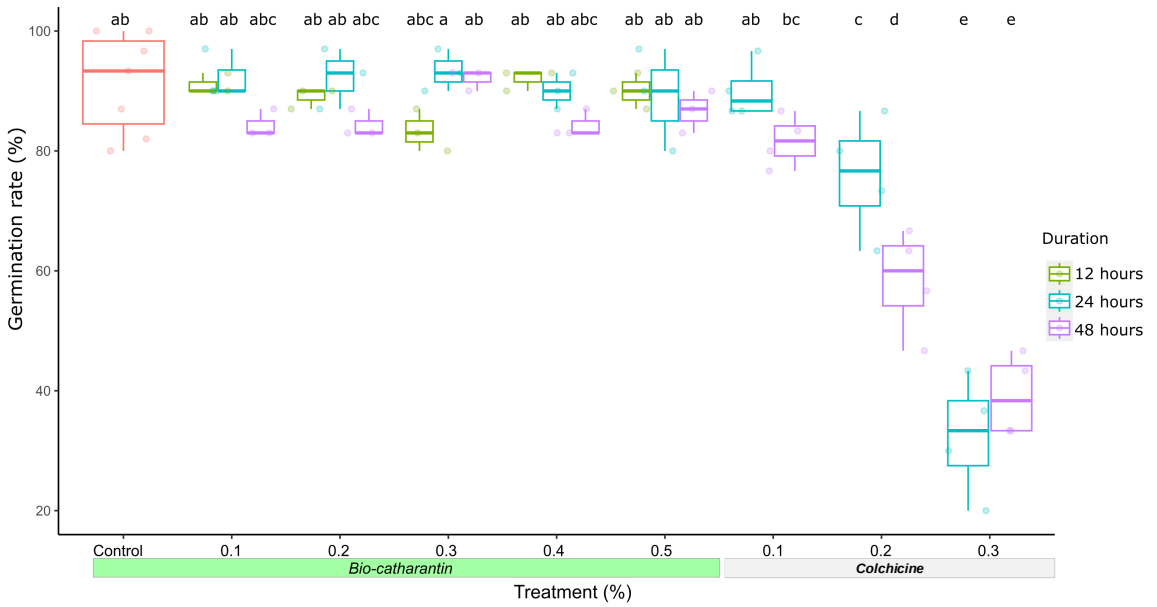

Figure 1. Germination rates for *O. sativa* L. var. Jeliteng seeds under Bio-catharantin and colchicine treatment

Note: The box and whisker plots display the distribution across the 25th and 75th percentile range and median. The jitter plot illustrates the distribution of the data. Significantly different values of the Duncan test ($p < 0.005$) were indicated by different letters. Detailed data were provided in Supplementary Table 1

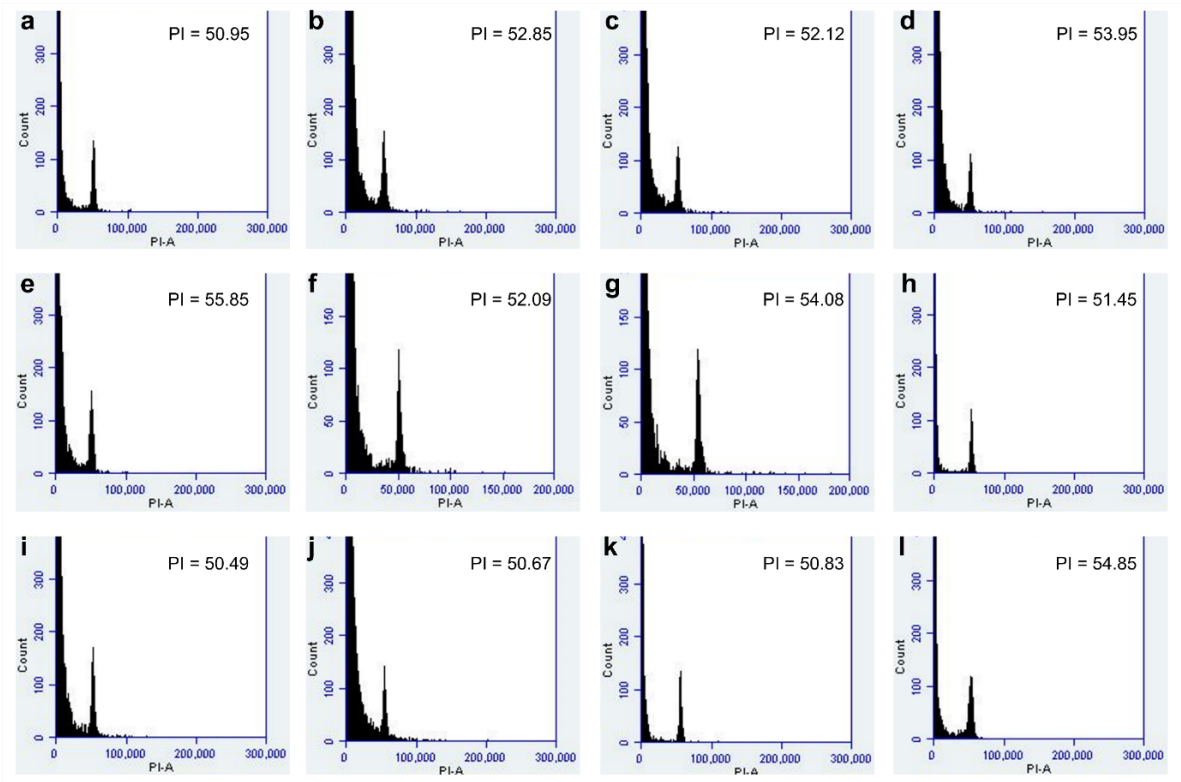

Figure 2. The ploidy level of leaf samples of *O. sativa* L. var. Jeliteng using a flow cytometer. Samples were categorized as a) Control; b-f = Bio-catharantin with shocking duration 48 hours, b) 0.1 %; c) 0.2 %; d) 0.3 %; e) 0.4 %; and f) 0.5 %; g-j = colchicine: g) 0.1% for 24 hours, h) 0.1 % for 48 hours; i) 0.2% for 48 hours; j) 0.2% for 48 hours; k) 0.3% for 48 hours; l) control. DNA relative to the mean value PI was recorded for each sample

displays the sample's relative DNA content, while the Y axis indicates the number of cells detected by the device.

The flow cytometer is essential for analyzing the DNA relative content. This high-throughput machine can assess a large number of nuclei (100 to 10,000 cells per second) and evaluate multiple samples in a single run (Hodač et al., 2016). Polyploid plants exhibit an increase in total DNA content in the cell nucleus while doubling chromosomes, and thus total DNA content serves as an indicator for ploidy determination in plants (Moghbel et al., 2015). Each plant type has a distinct optimal time and concentration for genetic mutation (Eng and Ho, 2018). Polyploid induction on diploid *Mentha spicata* L. with 40 µM oryzalin for 48 hours produced a stable hexaploid genotype with a higher essential oil yield (Bharati et al., 2023). In *Colocasia esculenta*, soaking with 0.1% and 0.2% colchicine for 24 hours resulted in mixoploid and tetraploid plants, but longer exposure (48 hours) did not lead to polyploidy (Ermayanti et al., 2018). Another study reported the genotype mutation of *Passiflora foetida*, where treatment of Bio-catharantin on the concentration of 1 to 1.5% for 24 to 72 hours only produced mixoploid, while a significantly lower concentration of colchicine led to the formation of tetraploid (Kasim et al., 2024). If the mutagenic solution concentration is too low, the polyploidy properties cannot be obtained. Even so, chromosomes will still polymerize into microtubules, resulting in spindle thread formation and diploid properties (Eng and Ho, 2018). On the other hand, if the concentration is too high or the soaking time is too long, the cell differentiation process will be hindered (Trojak-Goluch and Skomra, 2013), and chromosome doubling cannot occur. Cells undergo plasmolysis, which prevents the formation of polyploid properties (Eng and Ho, 2018). Additionally, the Jeliteng variety of black rice contains high levels of phenolic compounds (Nandariyah et al., 2022; Nurhidajah et al., 2024), which have antimutagenic properties that might inhibit colchicine-induced mutations in chromosomes.

Colchicine and Bio-catharantin effect on stomatal size and densities

The stomata of *O. sativa* var. Jeliteng exhibits typical graminae characteristics, with 2 dumbbell-shaped guard cells flanked by 2 lateral subsidiary cells (Figure 3). The experiment showed that altering the concentration of colchicine and soaking time affected the length, width, and density of stomata (Figure 4). In almost all colchicine-treated seeds, stomata were significantly wider and longer compared to the control group (Figure 4a and 4b). The control group exhibited an average length of 20.6 µm and a width of 13.9 µm. In treated seeds, the longest and widest stomata were observed at a concentration of 0.3% for 24 hours, measuring 24.7 µm in length and 16.5 µm in width. Conversely, the shortest stomatal length was at 0.2% for 24 hours, with a length of 21 µm, and the shortest width was at a concentration of 0.2%, measuring 14.5 µm (Supplementary Table 2). The stomatal density of the control group was higher than some colchicine-treated plants (Figure 4c). The control group had an average stomatal density of 330 mm², whereas the treated group had values ranging from 159 to 281 mm². The treatment with 0.2% concentration for 48 hours showed the highest stomatal density value of 281 mm², while the treatment with 0.3% concentration for 24 hours showed the lowest value of 159 mm² (Supplementary Table 2). The reduction of stomatal density under colchicine treatments was significantly observed in 3 treatments, i.e., at the concentrations of 0.1% for 48 hours, 0.3% for 24 hours, and 0.3% for 48 hours.

On the other hand, the Bio-catharantin altered the stomatal length, width, and density of black rice in several treatments (Figure 4). The average stomatal length treated with Bio-catharantin was within the range of 15.3 to 23.7 µm, while the average stomatal width was within the range of 13.6 to 20.3 µm (Supplementary Table 2). The stomatal length under Bio-catharantin of 0.1% varied based on different soaking durations. A 12-hour treatment reduced the stomatal length, a 24-hour treatment resulted in a length similar to the control group, and the 48-hour treatment significantly increased the stomatal length

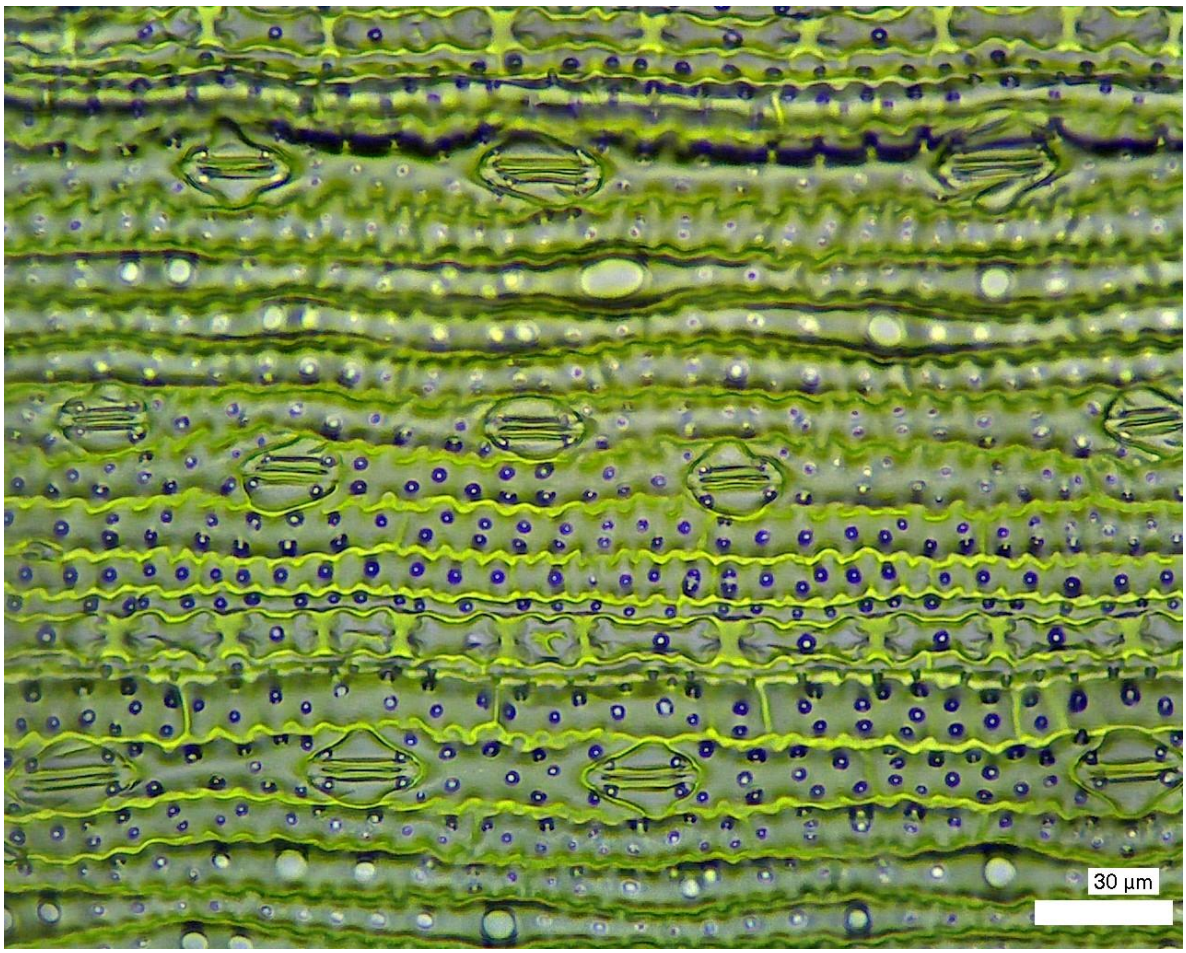

Figure 3. Stomatal structure of *O. sativa* L. var. Jeliteng under control treatment

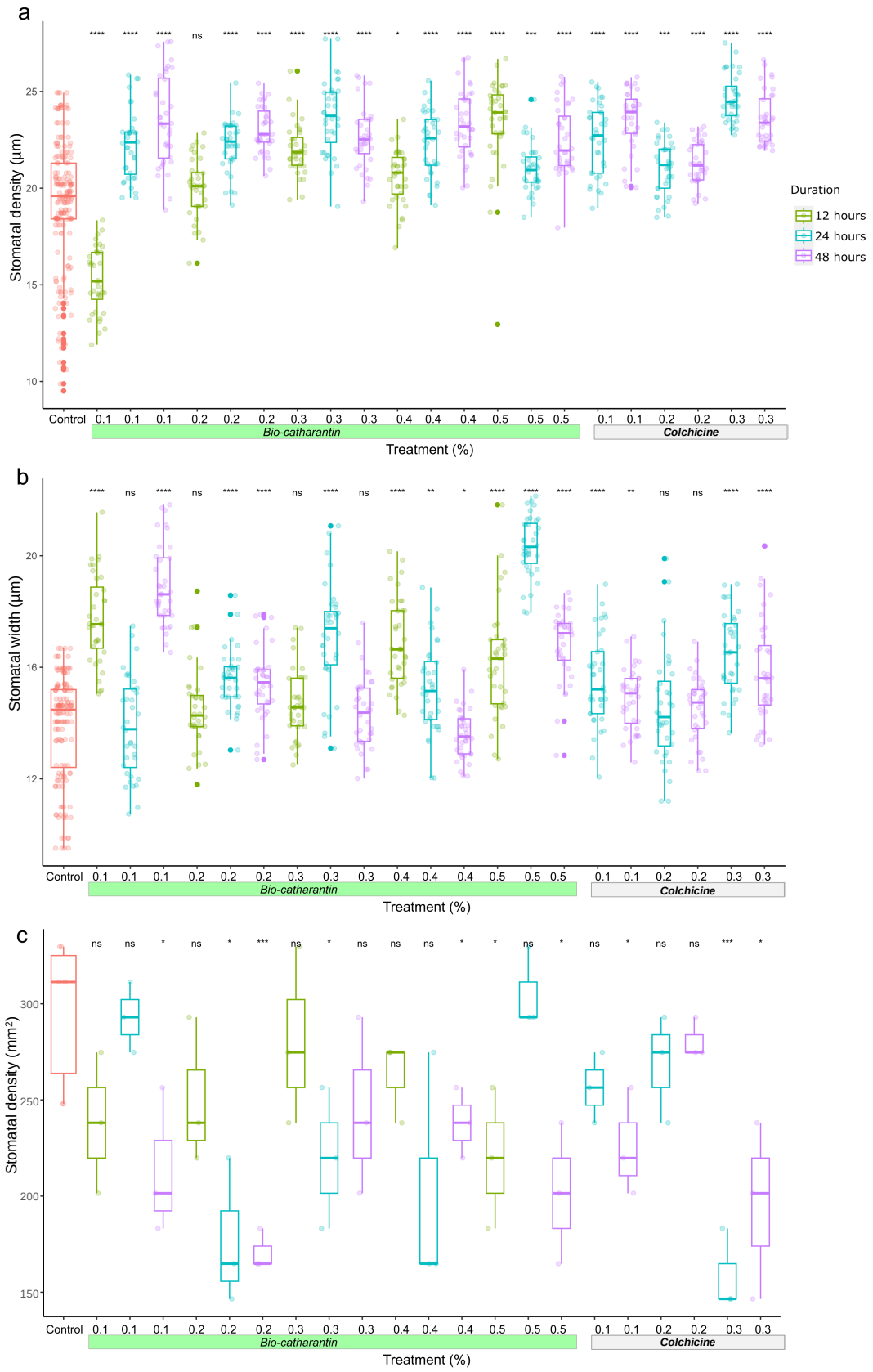

Figure 4. Stomatal structure of *O. sativa* L. var. Jeliteng under Bio-catharantin and colchicine treatment. a) Stomatal length; b) Stomatal width; c) Stomatal density

Note: The box and whisker plots display the distribution across the 25th and 75th percentile range and median. The jitter plot illustrates the distribution of the data. Significantly different values of the Duncan test ($p < 0.005$) were indicated by different letters. Detailed data were provided in Supplementary Table 2

(Figure 4a). The rest of the higher concentrations in all soaking durations extended the stomatal size (Figure 4a). The majority of treatments also led to an increase in stomatal width, except for 4 cases: 0.1% concentration for 24 hours, 0.2% for 12 hours, and 0.3% for both 12 and 48 hours. The reduction of stomatal density under Bio-catharantin treatments was significantly observed in 8 treatments, i.e., at the concentrations of 0.1% for 24 and 48 hours, 0.2% for 24 and 48 hours, 0.3% for 24 hours, 0.4% for 48 hours, 0.5% for 12 and 48 hours.

Based on research conducted by Wardana et al. (2019), the treatment of 0.1% colchicine for 24 hours in *Zephyranthes rosea* increased leaf area from 9.6 cm² in the control group to 13.92 cm². Similarly, a study by Manzoor et al. (2019) on *Gladiolus grandiflorus* Andrews found that immersion in 0.3% colchicine for 24 hours increased the length of stomata from 22.6 to 25.6 µm and width from 18 to 21 µm. There is a variation in the stomatal length and width in the control and treatment combination of the black rice variety Jeliteng. As detailed in Figure 4a and 4b, the treated black rice Jeliteng exhibited longer and wider stomata than the control group. Stomatal size typically influences density, as tissues with smaller cell sizes exhibit higher stomatal density in a given area compared to tissues with larger cells (Bharati et al., 2023; Kurniawan et al., 2023). The stomatal size and density are critical factors of water conductivity (Rathnasamy et al., 2023; Phunthong et al., 2024). The cultivation of rice is water-intensive and vulnerable to drought and high temperatures, both of which are expected to increase in frequency due to climate change. Despite reduced photosynthesis in some cases, the low-stomatal-density rice produced equivalent or even higher yields (Caine et al., 2019). The data of lower stomatal densities suggested that *O. sativa* L. var. Jeliteng induced by the 2 antimitotic compounds would become the genetic resource for mitigation of the negative impacts of climate change on food security by improving water-use efficiency and drought tolerance.

Genetic changes induced by antimitotic compounds led to alterations in cellular structure, regardless of whether polyploid individuals were formed. In this study, none of the polyploids occurred, although the antimitotic compound altered the stomatal size and density, which might relate to the genetic mutation. The stomatal size and density alternation with the absence of polyploid were reported solely by a genetic mutation, such as in sugarcane with gamma irradiation (Yasmeen et al., 2020), and in rice with overexpression of OsEPF1 (Caine et al., 2019) and CRISPR/Cas9 approach (Rathnasamy et al., 2023). The high genetic diversity in local Indonesian black rice still requires efforts to develop short-lived, high-yielding varieties (Muhamad et al., 2017; Sitaresmi et al., 2023). The research on Cempo Ireng provides a reference for the potential use of non-toxic antimitotic compounds, which are safe for direct application by farmers in black rice breeding. The stomatal data in this study might contribute to the stomatal diversity and development among rice (Trojak-Goluch and Skomra, 2013; Nofitahesti and Daryono, 2016; Chatterjee et al., 2020; Kurnianingsih et al., 2024). Plant breeding using antimitotic compounds requires optimization of concentration and soaking duration (Viana et al., 2019; Bhuvaneswari et al., 2020; Chen et al., 2021; Kurniawan et al., 2023). Another approach to enhance the success rate of genetic modification with antimitotic compounds was *in vitro* methods. With tissue culture technology, somatic cells can be more effectively exposed to antimitotic compounds (Udall and Wendel, 2006; Eng and Ho, 2018; Chen et al., 2021; Bharati et al., 2023). Bio-catharantin has greater potential compared to colchicine due to its lower toxicity, which does not harm the cells in rice calluses. Moreover, somatic embryo growth tests have been widely reported (Nabilah et al., 2022) so it has potential for further studies with the application of Bio-catharantin.

CONCLUSIONS

The present study highlights that Bio-catharantin and colchicine have distinct impacts on the germination and genetic characteristics of Jeliteng black rice. While Bio-catharantin did not affect the germination rate, colchicine at a concentration of 0.2% significantly reduced it. Both agents induced changes in the DNA relative content, suggesting their effectiveness in genetic manipulation. Variations in the treatment concentrations and soaking durations influenced the stomatal size and density, with Bio-catharantin uniquely affecting stomatal size without altering density. Importantly, Bio-catharantin emerged as a promising agent for chromosome doubling due to its lack of toxic effects, highlighting its potential for use in plant

breeding programs. This study underscores the viability of Bio-catharantin as a safer alternative to traditional agents like colchicine for inducing polyploidy in crops.

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Appendices

Table 1. Summary statistic values of germination rate of *O. sativa* L. var. Jeliteng after colchicine and Bio-catharantin treatment

Treatment		N	Mean	SD	Median	Min	Max
Concentration (%)	Duration (Hour)						
Bio-catharanthine							
0.1	12	3	91.00	1.73	90.00	90.00	93.00
	24	3	92.33	4.04	90.00	90.00	97.00
	48	3	84.33	2.31	83.00	83.00	87.00
0.2	12	3	89.00	1.73	90.00	87.00	90.00
	24	3	92.33	5.03	93.00	87.00	97.00
	48	3	84.33	2.31	83.00	83.00	87.00
0.3	12	3	83.33	3.51	83.00	80.00	87.00
	24	3	93.33	3.51	93.00	90.00	97.00
	48	3	92.00	1.73	93.00	90.00	93.00
0.4	12	3	92.00	1.73	93.00	90.00	93.00
	24	3	90.00	3.00	90.00	87.00	93.00
	48	3	84.33	2.31	83.00	83.00	87.00
0.5	12	3	90.00	3.00	90.00	87.00	93.00
	24	3	89.00	8.54	90.00	80.00	97.00
	48	3	86.67	3.51	87.00	83.00	90.00
Colchicine							
0.1	24	4	90.00	4.71	88.33	86.67	96.67
	48	4	81.67	4.30	81.67	76.67	86.67
0.2	24	4	75.83	9.95	76.67	63.33	86.67
	48	4	58.33	8.82	60.00	46.67	66.67
0.3	24	4	32.50	9.95	33.33	20.00	43.33
	48	4	39.17	6.87	38.33	33.33	46.67
Control		7	91.29	8.34	93.33	80.00	100.00

Note: N = 150 seeds

Table 2. Summary statistic values of stomatal size and density of *O. sativa* L. var. Jeliteng after colchicine and Bio-catharantin treatment

Data	Concentration (%)		Soaking duration (hr)	N	Mean	SD	Median	Min	Max	CV
Length	Control			108	18.32	3.70	19.06	9.51	24.94	20.18
	Bio-catharantin	0.1	12	36	15.29	1.69	15.19	11.90	18.34	11.05
			24	36	22.26	1.77	22.37	19.50	25.86	7.96
			48	36	23.52	2.35	23.34	18.88	27.58	9.99
		0.2	12	36	19.98	1.55	20.11	16.12	22.86	7.75
			24	36	22.23	1.33	22.41	19.13	25.44	5.98
			48	36	23.03	1.20	22.79	20.64	25.43	5.19
		0.3	12	36	21.99	1.30	21.86	19.41	26.06	5.92
			24	36	23.73	1.92	23.75	19.05	27.73	8.09
			48	36	22.62	1.60	22.53	19.31	25.83	7.06
		0.4	12	36	20.59	1.42	20.81	16.92	23.56	6.87
			24	36	22.35	1.64	22.59	19.12	25.56	7.32
			48	36	23.37	1.70	23.20	20.03	26.76	7.27
		0.5	12	36	23.36	2.48	23.92	12.95	26.69	10.63
			24	36	20.99	1.22	20.94	18.49	24.58	5.83
			48	36	22.49	1.73	21.95	17.96	25.76	7.71

	Colchicine	0.1	24	36	22.54	1.84	22.73	18.96	25.49	9.38
			48	36	23.52	1.59	23.94	20.04	25.74	8.15
		0.2	24	36	21.04	1.37	21.21	18.49	23.40	6.74
			48	36	21.29	1.10	21.18	19.21	23.19	6.50
		0.3	24	36	24.68	1.20	24.47	22.75	27.52	5.14
			48	36	23.58	1.35	23.39	21.95	26.64	4.87
Width	Control			72	13.87	1.89	14.48	9.51	16.68	5.73
	Bio-catharantin	0.1	12	36	17.71	1.59	17.55	15.05	21.56	13.61
			24	36	13.87	1.78	13.78	10.74	17.48	8.96
			48	36	18.93	1.45	18.62	16.53	21.83	12.81
		0.2	12	36	14.47	1.45	14.27	11.79	18.73	7.68
			24	36	15.59	1.06	15.62	13.03	18.58	10.00
			48	36	15.39	1.29	15.46	12.69	17.90	6.79
		0.3	12	36	14.68	1.24	14.56	12.50	17.42	8.36
			24	36	17.10	1.92	17.40	13.10	21.07	8.42
			48	36	14.34	1.29	14.38	12.01	17.59	11.22
		0.4	12	36	16.86	1.55	16.64	14.28	20.16	9.02
			24	36	15.21	1.56	15.15	12.03	18.86	9.22
			48	36	13.60	0.87	13.52	12.09	15.92	10.28
		0.5	12	36	16.21	1.97	16.31	12.71	21.83	6.41
			24	36	20.34	1.09	20.32	17.95	22.14	12.13
			48	36	16.79	1.26	17.22	12.84	18.67	5.38
	Colchicine	0.1	24	36	15.52	1.63	15.21	12.06	18.98	7.52
			48	36	14.86	1.08	15.07	12.59	17.10	13.61
		0.2	24	36	14.51	1.97	14.22	11.20	19.90	10.52
			48	36	14.50	1.13	14.74	12.29	16.92	7.24
		0.3	24	36	16.50	1.44	16.54	13.65	18.98	13.56
			48	36	15.81	1.81	15.61	13.23	20.35	7.81
Density	Control			3	296.34	42.86	311.36	247.99	329.67	8.73
	Bio-catharantin	0.1	12	3	238.10	36.63	238.10	201.47	274.73	11.45
			24	3	293.04	18.32	293.04	274.73	311.36	14.46
			48	3	213.68	38.13	201.47	183.15	256.41	15.38
		0.2	12	3	250.31	38.13	238.10	219.78	293.04	6.25
			24	3	177.05	38.13	164.84	146.52	219.78	17.84
			48	3	170.94	10.57	164.84	164.84	183.15	15.23
		0.3	12	3	280.83	46.09	274.73	238.10	329.67	21.53
			24	3	219.78	36.63	219.78	183.15	256.41	6.19
			48	3	244.20	46.09	238.10	201.47	293.04	16.41
		0.4	12	3	262.52	21.15	274.73	238.10	274.73	16.67
			24	3	201.47	63.45	164.84	164.84	274.73	18.87
			48	3	238.10	18.32	238.10	219.78	256.41	8.06
		0.5	12	3	219.78	36.63	219.78	183.15	256.41	31.49
			24	3	305.25	21.15	293.04	293.04	329.67	7.69
			48	3	201.47	36.63	201.47	164.84	238.10	16.67
	Colchicine	0.1	24	3	256.41	18.32	256.41	238.10	274.73	6.93
			48	3	225.89	27.97	219.78	201.47	256.41	18.18
		0.2	24	3	268.62	27.97	274.73	238.10	293.04	14.46
			48	3	280.83	10.57	274.73	274.73	293.04	7.14
		0.3	24	3	158.73	21.15	146.52	146.52	183.15	12.38
			48	3	195.36	46.09	201.47	146.52	238.10	10.41

Note: N = 3-5 individual