

Original Article

Agronomic responses of rice to putative polyploid-inducing plant extracts: a screening study

Respostas agronômicas do arroz a extratos vegetais supostamente indutores de poliploidia: um estudo de triagem

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Abstract

Rice productivity improvement is an urgent priority to secure food availability in Indonesia, and polyploid induction using antimitotic compounds provides a promising strategy. This study evaluated agronomic responses of four rice genotypes (Cibogo, F4 Sb × Cb, F4 Sb × Ch, and Ciherang) following in vivo seed soaking with natural antimitotic extracts from *Gloriosa superba*, *Catharanthus roseus*, and *Elephantopus scaber* at varying concentrations. Seeds were treated with standardized antimitotic solutions and key traits—tiller number, plant height, panicle production, leaf number, panicle weight, and stomatal characteristics—were assessed. The results revealed clear genotype-specific and dose-dependent responses. The 10% *G. superba* treatment consistently enhanced tiller formation, plant height, and panicle production across most genotypes, and produced “giant rice” phenotypes in F4 Sb × Cb. Cibogo showed the heaviest panicles under 10% *E. scaber* and 15% *G. superba*, while Ciherang performed best at lower doses, particularly 5% *C. roseus* and 5–10% *G. superba*. In contrast, stomatal density and size remained relatively stable, indicating that stomatal traits are not reliable standalone markers of polyploidization. Collectively, these findings highlight the agronomic potential of *G. superba*—particularly at ~10%—as a candidate natural antimitotic extract to improve yield-related traits. However, because direct cytological verification (e.g., flow cytometry and/or chromosome counting) was not performed in this study, the observed responses should be interpreted as polyploid-like or putative outcomes that require validation in future work.

Keywords: antimitotic compounds, *Gloriosa superba*, polyploid rice, productivity, food security.

Resumo

O aumento da produtividade do arroz é uma prioridade urgente para garantir a disponibilidade de alimentos na Indonésia, e a indução de poliploides utilizando compostos antimitóticos oferece uma estratégia promissora. Este estudo avaliou as respostas agronômicas de quatro genótipos de arroz (Cibogo, F4 Sb × Cb, F4 Sb × Ch e Ciherang) após a imersão in vivo das sementes em extratos antimitóticos naturais de *Gloriosa superba*, *Catharanthus roseus* e *Elephantopus scaber* em diferentes concentrações. As sementes foram tratadas com soluções antimitóticas padronizadas e características-chave – número de perfilhos, altura da planta, produção de panículas, número de folhas, peso da panícula e características estomáticas – foram avaliadas. Os resultados revelaram respostas claras, específicas para cada genótipo e dependentes da dose. O tratamento com 10% de *G. superba* aumentou consistentemente a formação de perfilhos, a altura da planta e a produção de panículas, na maioria dos genótipos, e produziu fenótipos de “arroz gigante” em F4 Sb × Cb. Cibogo apresentou as panículas mais pesadas com 10% de *E. scaber* e 15% de *G. superba*, enquanto Ciherang apresentou melhor desempenho em doses mais baixas, particularmente com 5% de *C. roseus* e 5–10% de *G. superba*. Em contraste, a densidade e o tamanho dos estômatos permaneceram relativamente estáveis, indicando que as características estomáticas não são marcadores confiáveis de poliploidização por si só. Em conjunto, essas descobertas destacam o potencial agronômico de *G. superba* – particularmente em concentrações de aproximadamente 10% – como um extrato antimitótico natural candidato para melhorar características relacionadas à produtividade. No entanto, como a verificação citológica direta (por exemplo, citometria de fluxo e/ou contagem de cromossomos) não foi realizada neste estudo, as respostas observadas devem ser interpretadas como resultados do tipo poliploide ou putativos que requerem validação em trabalhos futuros.

Palavras-chave: compostos antimitóticos, *Gloriosa superba*, arroz poliploide, produtividade, segurança alimentar.

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Received: September 16, 2025 – Accepted: March 5, 2026

Editor: Takako Matsumura Tundisi



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1. Introduction

Rice, as the primary staple food for the Indonesian population, plays a crucial role in ensuring national food security. Therefore, enhancing rice productivity has become an imperative effort, pursued through various strategies such as intensification using high-yielding varieties, fertilizers, and modern cultivation technologies, as well as extensification through agricultural land expansion (Ren et al., 2023; Thakur et al., 2018). Although these approaches have shown significant results, the continuously increasing food demand necessitates further innovations capable of achieving more optimal outcomes (Riaz et al., 2025; Wu et al., 2024). One promising scientific breakthrough is the development of polyploid rice, characterized by possessing more than two sets of chromosomes. Polyploid plants generally exhibit hybrid vigor, larger organ size, and higher productivity compared to their diploid counterparts (Chen et al., 2021). This is consistent with the findings of Madani et al. (2021), which demonstrated that in several medicinal plants, the levels of secondary metabolites increased significantly in polyploid variants compared to their normal varieties. Consequently, the development of polyploid rice is expected not only to enhance yield quantitatively but also to potentially improve nutritional quality and strengthen resistance to environmental stresses, thereby serving as a strategic solution to address future food security challenges (Sun et al., 2025; Yang et al., 2024).

Polyploid plants generally exhibit accelerated growth due to genome duplication, which increases gene dosage and often induces the so-called gigas effect, characterized by larger cell size and altered metabolic activity that enhances the rate of cell division and tissue expansion. The elevated accumulation of cell wall constituents such as cellulose, hemicellulose, pectin, and other components is most likely driven by the upregulation of cell wall biosynthetic enzymes (resulting from duplicated gene dosage) and a metabolic shift toward the synthesis of structural polysaccharides (Amos and Mohnen, 2019; Shao and Duan, 2022). Polyploid plants tend to develop thicker cell walls and stronger mechanical tissues, contributing to greater biomass accumulation and enhanced stem mechanical strength. Furthermore, polyploidy frequently alters gene regulation and secondary metabolism, leading to increased concentrations of defensive phytochemicals a phenomenon reported in several medicinal plant studies that explain their enhanced resistance to biotic stresses such as pathogens and herbivores. From an abiotic perspective, genetic redundancy and potential neo-/sub-functionalization of genes provide polyploid plants with greater physiological flexibility in coping with drought, salinity, and temperature fluctuations, thereby improving tolerance to abiotic stresses (Li et al., 2024; Zhong et al., 2024). Enhanced adaptive traits may also enable certain polyploids to establish more easily in new habitats, form colonies, and potentially become invasive an ecological and biosafety concern that warrants careful consideration (Islam et al., 2022; Moura et al., 2021; Tossi et al., 2022).

In rice breeding, *in vivo* polyploid induction represents a promising strategy as it is applied directly to actively

growing plant tissues, such as root tips, shoots, or germinating seeds. The basic principle involves treatment with specific chemical agents, such as colchicine or oryzalin, which inhibit spindle fiber formation during cell division. As a result, chromosomes that should normally segregate during anaphase fail to migrate to opposite poles, leading to chromosome duplication within the nucleus without subsequent cytoplasmic division. This process generates polyploid cells, which are generally characterized by larger sizes, more complex nuclei, and enlarged organelles. Rice plants obtained through polyploid induction often display distinct phenotypic traits compared to diploid plants, including increased leaf, stem, and grain size, thicker cell walls, and more efficient metabolism. These characteristics are highly advantageous for rice improvement, as they offer the potential to enhance grain yield, improve grain quality, and strengthen tolerance to environmental stresses, both abiotic (drought, salinity, extreme temperatures) and biotic (pest and pathogen attacks). Thus, *in vivo* polyploid induction is not merely a technical approach but also a strategic breakthrough in developing high-yielding and adaptive rice cultivars.

Based on this background, research on rice breeding through *in vivo* polyploid induction becomes highly relevant as a long-term strategy to address food security challenges in Indonesia. This approach is expected to generate superior rice varieties that not only exhibit high yield potential but are also adaptive to climate change, possess enhanced resistance to both biotic and abiotic stresses, and contribute significantly to achieving national food self-sufficiency.

Exposure to plant-derived antimetabolic extracts at optimized concentrations may induce putative polyploidy and/or polyploid-like phenotypes in rice, resulting from disrupted spindle formation during mitosis and subsequent chromosome doubling in a subset of treated individuals. In this context, polyploidization is expected to generate polyploid-associated vigor (gigas effect), which may be reflected in improved vegetative growth and yield-related performance, including increased tiller production, greater plant height, enhanced panicle development, and higher panicle weight. Nevertheless, because morphological and agronomic improvements can also arise from non-cytological physiological effects of bioactive compounds present in plant extracts, such phenotypic responses should be interpreted as preliminary or putative evidence. Therefore, definitive confirmation of polyploid induction requires cytological validation through established assays, particularly flow cytometry to quantify relative nuclear DNA content and/or direct chromosome counting to verify genome duplication. This study was therefore conducted under the hypothesis that exposure of rice seeds to plant-derived antimetabolic extracts at optimized concentrations can disrupt mitotic spindle formation during early development, leading to chromosome duplication and the induction of putative polyploidy or polyploid-like phenotypes.

This study aimed to evaluate whether *in vivo* seed-soaking treatments with plant-derived antimetabolic extracts from *Gloriosa superba*, *Catharanthus roseus*, and *Elephantopus scaber* at appropriate concentrations could induce putative polyploidization or polyploid-like cellular responses in rice.

To assess the effects of these treatments, we quantified key vegetative performance parameters—including tiller number, plant height, and leaf number—as indicators of early vigor and biomass accumulation, which are expected to correlate with enhanced vegetative growth and improved yield-related agronomic traits. Specifically, this study also aimed to examine stomatal density characteristics as preliminary anatomical proxies for potential genome-level changes, given that polyploidization may affect cell size and epidermal traits. Collectively, this experimental framework was designed to identify the most responsive genotype-extract-concentration combinations and to provide baseline evidence for the agronomic potential of natural antimitotic extracts in rice improvement programs.

2. Research Method

2.1. Preparation of antimitotic compound extracts

The extraction of mutagenic compounds from *Gloriosa superba* tubers was carried out using the maceration method. Tubers with a diameter of 1.0–1.5 cm were sliced into 2 mm sections, dried in an oven at 75 °C for 6 hours, ground into powder, and sieved. A total of 100 g of fine tuber powder was placed into a 500 mL beaker, mixed with 70 mL of 90% ethanol and 30 mL of distilled water, and heated at 70 °C until a brownish precipitate was formed. According to Panday and Bandik (2012), 1 g of ethanolic extract of *G. Superba* tubers contains 0.6% pure colchicine. To prepare a 0.1% ethanolic extract solution, 4.175 g of extract was dissolved in 25 mL of distilled water, while concentrations of 0.2% and 0.3% were prepared using the same proportional increments.

Extraction of *Catharanthus roseus* mature leaves were oven-dried at 70 °C for 72 hours, ground into powder, sieved, and macerated in a mixture of 70 mL of 90% ethanol and 30 mL of water for 24 hours. The filtrate was strained through muslin cloth and subsequently heated at 70 °C until a green precipitate was obtained. According to Gupta et al. (2005), 1 g of dried *C. roseus* leaves contains 4 mg (0.04%) of total alkaloids, mainly vincristine and vinblastine; therefore, 2.5 g of dried leaf powder was required to prepare a 0.1% concentration. Extraction of antimitotic compounds from *Elephantopus scaber* (Tapak liman) was performed using the same procedure as applied to *C. roseus*.

2.2. Seed soaking in antimitotic extracts

Rice seeds were selected for viability by soaking them in water, with only the seeds that sank being used for further treatment. The antimitotic extracts applied for soaking were standardized to a colchicine-equivalent concentration of 0.15%, with an immersion duration of 24 hours.

2.3. Measurement of agronomic traits

The agronomic traits measured included plant height, number of leaves per clump, number of tillers, survival rate, and germination percentage. Measurements were conducted using appropriate instruments, and observations were carried out through direct assessment.

3. Results and Discussion

3.1. Rice seedlings per variety and per treatment

The initial response of rice varieties to antimitotic treatments can be observed from the number of seedlings successfully developed under different extract types and concentrations. This parameter provides an early indication of genotype sensitivity and adaptability to mitotic disruption, which is crucial for determining the effectiveness of polyploid induction. As shown in Figure 1, each rice variety displayed distinct responses, reflecting both genetic background and treatment dosage.

The diagram illustrates that each rice variety exhibited a distinct response to different types and concentrations of antimitotic compounds. The Cibogo variety produced the highest number of tillers under the 5% *Gloriosa superba* treatment. The F4 Sb × Cb variety showed the highest tiller production under both *Catharanthus roseus* and 10% *G. superba* treatments. The F4 Sb × Ch variety produced the greatest number of tillers with the 10% *G. superba* treatment, whereas the Ciherang variety responded most effectively to the 5% *Elephantopus scaber* treatment. Overall, the 10% *G. superba* treatment demonstrated a generally positive effect in stimulating tiller formation across nearly all rice varieties tested.

The response of rice to antimitotic treatments varies depending on the type of compound, genotype, and concentration applied. Different antimitotic agents, such as colchicine, trifluralin, oryzalin, and various plant-derived extracts, exhibit distinct effects on rice growth (Hooghvorst et al., 2020). Colchicine is widely recognized for its effectiveness in chromosome doubling, although it can also be toxic, often reducing shoot growth and causing abnormal development. Similarly, dinitroanilines such as trifluralin and oryzalin may inhibit root growth and induce abnormal shoot morphology, including helical growth and sprouting abortion. Moreover, the response to these treatments is highly genotype-dependent, with certain rice varieties showing increased tiller numbers under specific treatments, while others display no significant response or may even be negatively affected. Concentration also plays a crucial role, as moderate levels of antimitotic compounds can promote tiller formation, whereas excessive doses tend to result in toxicity and abnormal growth (Fang et al., 2020).

3.2. Height of rice plants at harvest time

Plant height is an important agronomic trait that reflects both vegetative growth and the overall vigor of rice under different treatments. It serves as an indicator of how effectively plants can utilize resources and adapt to environmental as well as chemical interventions. In the context of polyploid induction, variations in plant height may reflect polyploid-like responses; however, without cytological validation they cannot be attributed conclusively to chromosome doubling, which is often associated with larger organs and increased biomass. As illustrated in Figure 2, each rice variety responded differently to the application of antimitotic compounds, demonstrating clear genotype-dependent and concentration-specific effects.

These differences highlight the necessity of optimizing treatment dosages for each variety to achieve maximum growth without inducing toxicity or growth abnormalities.

It can be stated that each rice variety responded differently to various types and concentrations of antimimetic compounds, resulting in variations in plant height.

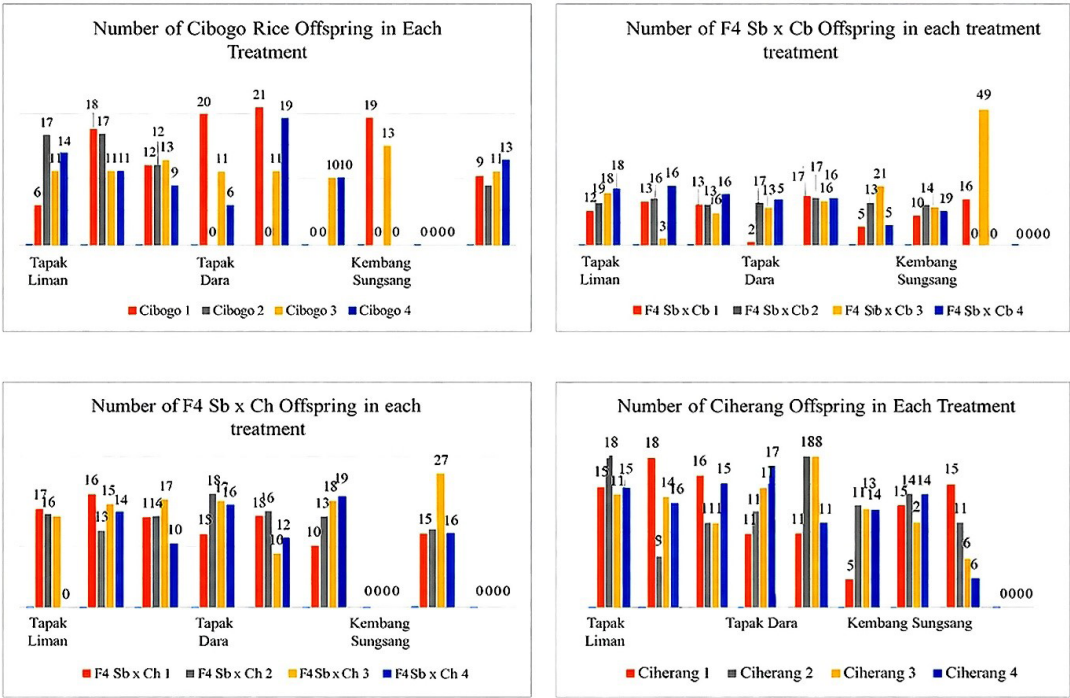


Figure 1. Number of rice seedlings per variety and per treatment.

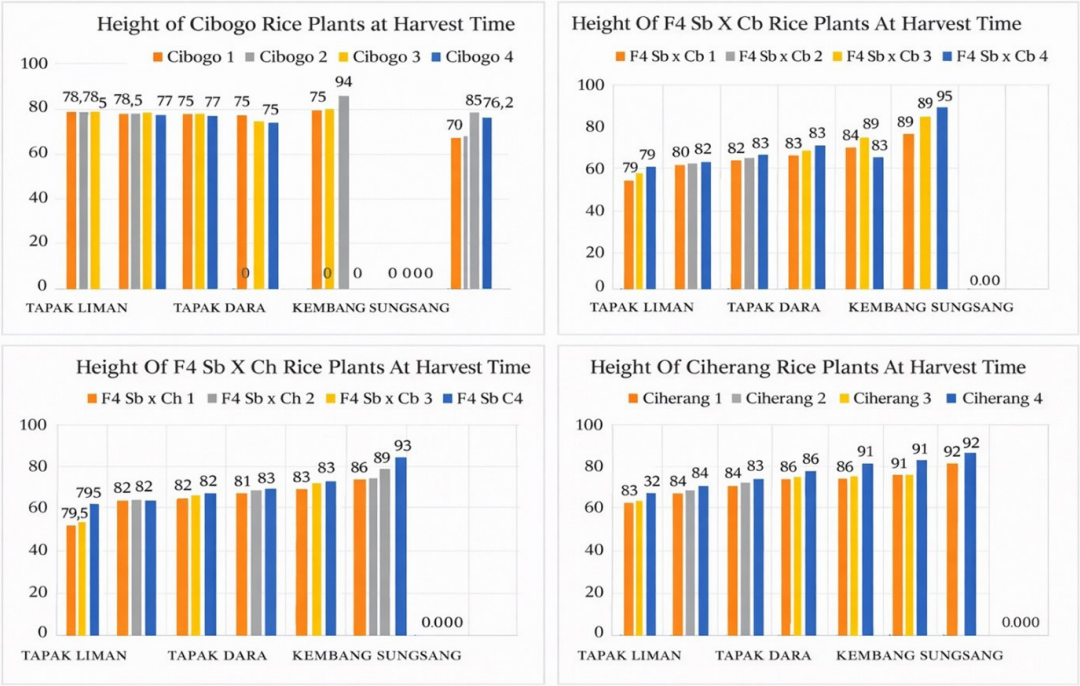


Figure 2. Height of rice plants at harvest time.

The Cibogo variety exhibited the greatest plant height under *Elephantopus scaber* treatments at concentrations of 5-15%. The F4 Sb × Cb variety produced the tallest plants under the 15% *Catharanthus roseus* treatment, and notably developed “giant rice” under the 10% *Gloriosa superba* treatment. The F4 Sb × Ch variety achieved the highest plant height under both the 10% *G. superba* and 15% *C. roseus* treatments. Meanwhile, the Ciherang variety showed the tallest growth with 5% *C. roseus* and 10% *G. superba* treatments. Consistent with the results for tiller number, the 10% *G. superba* treatment was generally well responded to in terms of plant height across nearly all rice varieties examined in this study.

Rice varieties respond differently to antimetabolic compounds, both in terms of chromosome doubling efficiency and plant growth characteristics. The effectiveness and toxicity of antimetabolic agents such as colchicine, trifluralin, and oryzalin can vary significantly between rice genotypes. For example, colchicine at specific concentrations (500-625 mg/L) was most effective for chromosome doubling, but also more toxic to plantlets compared to dinitroanilines, which caused distinct morphological changes like helical shoot growth and root inhibition 1. These differences highlight that the optimal antimetabolic treatment is variety-specific and must balance efficacy with phytotoxicity (Kirchner and Pianowski 2022).

Antimetabolic compounds not only induce chromosome doubling but also impact plant development. Colchicine treatments, while effective for chromosome doubling, can suppress shoot growth, especially when combined with DMSO. Dinitroanilines, on the other hand, tend to impede root growth and cause abnormal shoot morphology 1. These growth responses are critical for selecting appropriate

treatments in rice breeding programs, as excessive toxicity or abnormal development can reduce the viability of doubled haploid plants (Olson et al., 2017).

3.3. Number of panicles

The number of panicles per plant is a critical yield component that directly determines the grain production capacity of rice. As a reproductive trait, panicle formation is highly sensitive to genetic background and external treatments, including the application of antimetabolic compounds for polyploid induction. Evaluating this parameter provides valuable insight into how different rice varieties allocate assimilates toward reproductive development under varying concentrations of plant-derived extracts. As presented in Figure 3, each variety exhibited distinct responses, with certain treatments stimulating panicle initiation more effectively than others.

It can be stated that the number of panicles per plant varied considerably among the four rice varieties tested in this study. The Cibogo variety produced the highest number of panicles under *Catharanthus roseus* treatments at 5% and 10% concentrations. The F4 Sb × Cb variety showed the greatest panicle production under the 10% *Gloriosa superba* treatment, while also responding positively to 10% *C. roseus* and *Elephantopus scaber* treatments. The F4 Sb × Ch variety produced the highest number of panicles under the 10% *G. superba* treatment and also responded well to 5% *C. roseus* and 10% *E. scaber*. Meanwhile, the Ciherang variety achieved the highest number of panicles under 10% *C. roseus* and 5% *E. scaber* treatments. Overall, the 10% *G. superba* treatment appeared to provide the most favorable effect on panicle production across nearly all rice varieties evaluated.

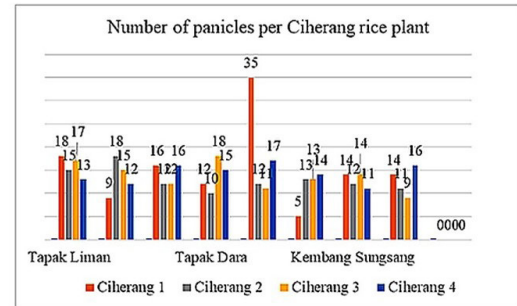
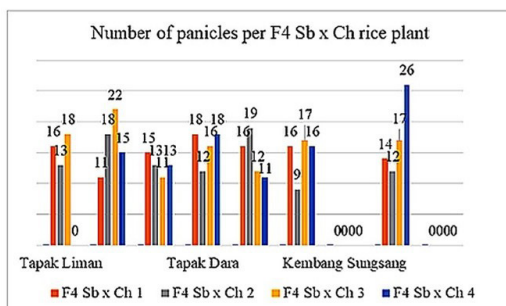
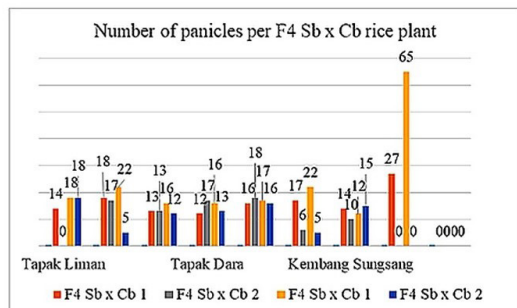
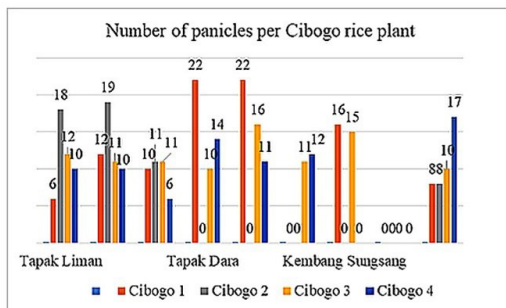


Figure 3. Number of panicles per tree.

Current research provides no direct evidence regarding the effects of *Catharanthus roseus*, *Gloriosa superba*, or *Elephantopus scaber* treatments on rice panicle production (Das et al., 2020; Kumar et al., 2022; Nourozi et al., 2025). Most available studies predominantly emphasize the medicinal properties, phytochemical composition, and biotechnological applications of *C. roseus*, particularly its alkaloid content and pharmaceutical potential, rather than its role in enhancing agronomic traits such as yield or panicle number. The literature largely discusses the improvement of secondary metabolite production and bioactive compound accumulation through approaches like polyploidization and elicitor treatments, yet does not extend these findings to cereal crops such as rice. To date, no studies have explored the application of *C. roseus* or related plant extracts in improving rice yield-related traits, indicating a clear gap in research. Therefore, further investigations are required to determine whether these plant-based treatments could contribute to enhanced panicle production and overall productivity in rice cultivation.

3.4. Number of leaves

The number of leaves is a fundamental indicator of vegetative growth that reflects photosynthetic capacity and overall plant vigor. Leaves function as the primary sites of carbohydrate synthesis, and their abundance strongly influences the assimilate supply for reproductive development. In the context of polyploid induction, variations in leaf production may signal changes in cell

division rates and metabolic activity triggered by antimetabolic compounds. As illustrated in Figure 4, each rice variety displayed distinct responses to the type and concentration of treatments, with some varieties producing a higher number of leaves under specific extracts.

It can be stated that each rice variety responded differently to the type and concentration of antimetabolic compounds applied. The Cibogo variety exhibited the highest number of leaves under *Elephantopus scaber* and *Catharanthus roseus* treatments at 10% concentration. The F4 Sb × Cb variety produced the greatest number of leaves under the 10% *Gloriosa superba* treatment, a response also observed in the F4 Sb × Ch variety. Meanwhile, the Ciherang variety showed the highest leaf number under the 5% *E. scaber* and 10% *C. roseus* treatments. These findings indicate that the hybrid varieties were more sensitive to *G. superba* treatments.

Large-scale genomic studies have revealed extensive genetic diversity in rice, including millions of single nucleotide polymorphisms (SNPs), structural variations (SVs), and gene presence-absence variations (PAVs) across different varietal groups and subpopulations (Qin et al., 2021; Wang et al., 2018). These genetic differences underpin distinct regulatory mechanisms for cell division, chromosome segregation, and stress responses, which can modulate how rice varieties respond to antimetabolic agents. For example, certain subpopulations (e.g., *Indica*, *japonica*, *aus*) possess unique gene sets and regulatory elements that may alter their cellular sensitivity to compounds affecting mitosis

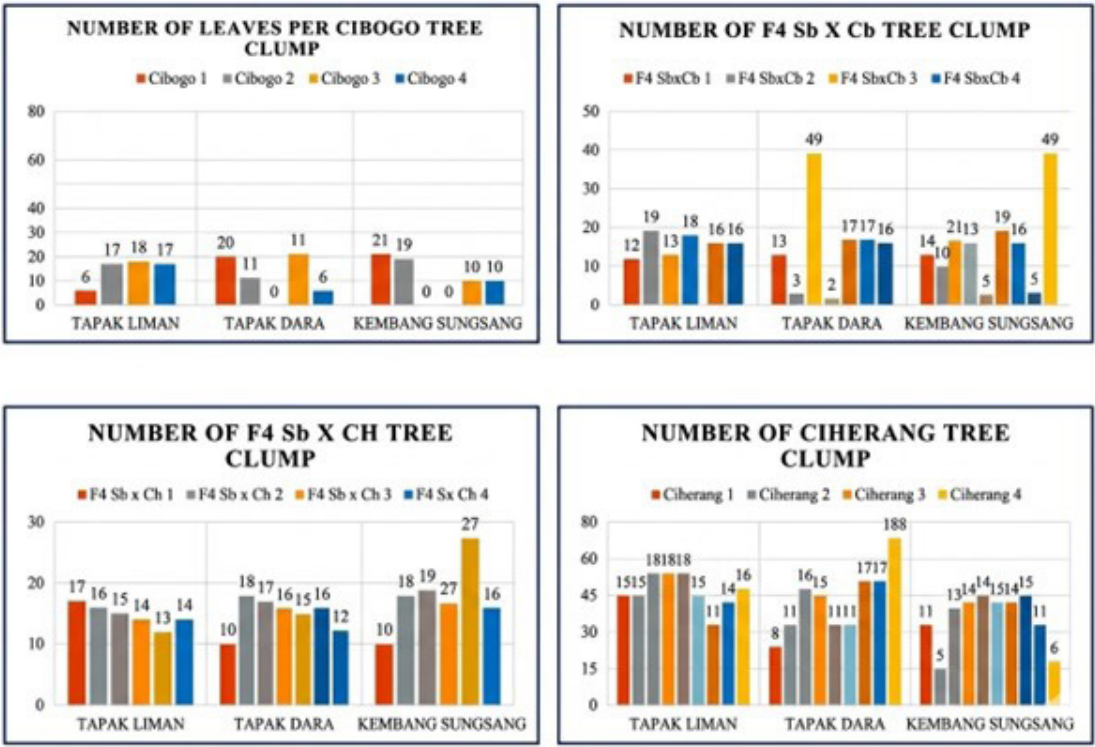


Figure 4. Number of leaves per tree clump.

Chromosome duplication efficiency and mitotic activity vary considerably among plant varieties and are influenced by both genetic background and the specific antimitotic protocols or compounds employed. Some genotypes exhibit higher rates of successful chromosome duplication, while others are more prone to abnormal mitotic events that can reduce plant vigor and delay development. In maize, for instance, colchicine-based protocols demonstrate variable duplication efficiency, with genetic background playing a stronger role in haploid induction than in duplication itself; nevertheless, significant differences in self-fertilization rates and field performance among hybrids suggest that certain germplasms are inherently more effective at producing viable doubled haploids (Couto et al., 2015; Hassan et al., 2020).

Spontaneous chromosome duplication (aneuploidy) rates in unicellular algae and yeast are highly variable, both across species and among strains. In unicellular algae, mutation accumulation experiments estimate duplication rates between 1×10^{-4} and 1×10^{-3} per genome per generation, which is 5–70 times less frequent than point mutations but still significant given the large genomic regions affected. In yeast, duplication rates are similarly variable and strongly influenced by genetic background, with some lineages or hybrids exhibiting elevated frequencies of whole-genome duplication or aneuploidy, particularly under conditions of increased genomic instability (Krasovec et al., 2022, 2023). Moreover, tolerance to large-scale duplications differs among species, as seen in marine yeasts such as *Scheffersomyces spartinae*, which show lower tolerance and frequency of chromosomal duplications compared to model organisms like *Saccharomyces cerevisiae*. These findings underscore

that genomic background plays a critical role in determining duplication dynamics, a principle that is equally relevant to higher plants. In the context of rice breeding, such variability suggests that the success of induced polyploidization or chromosome duplication through antimitotic treatments will be highly genotype-dependent, requiring careful optimization of protocols to ensure stable polyploid lines with desirable agronomic traits (Bai et al., 2025; Marsit et al., 2021).

Similarly, in plants, colchicine and other antimitotic agents may induce chromosome doubling but simultaneously disrupt normal mitotic activity, leading to growth inhibition or morphological abnormalities in sensitive varieties. These insights highlight the importance of considering varietal differences when applying antimitotic treatments in rice breeding programs, as the success of polyploid induction in rice will largely depend on genotype-specific responses that balance chromosome doubling efficiency with overall plant growth and agronomic performance.

3.5. Weight per panicle in four rice varieties due to treatment

Panicle weight is a decisive yield determinant, as it directly reflects the efficiency of assimilate partitioning into the grain-bearing structure. Heavier panicles generally indicate stronger sink strength and greater grain-filling capacity, traits often associated with successful polyploid induction and the gigas effect. The application of antimitotic compounds can influence this trait by altering cell size, organ development, and biomass allocation. As depicted in Figure 5, each rice variety responded differently to various extracts and concentrations, revealing clear genotype-specific and dose-dependent effects.

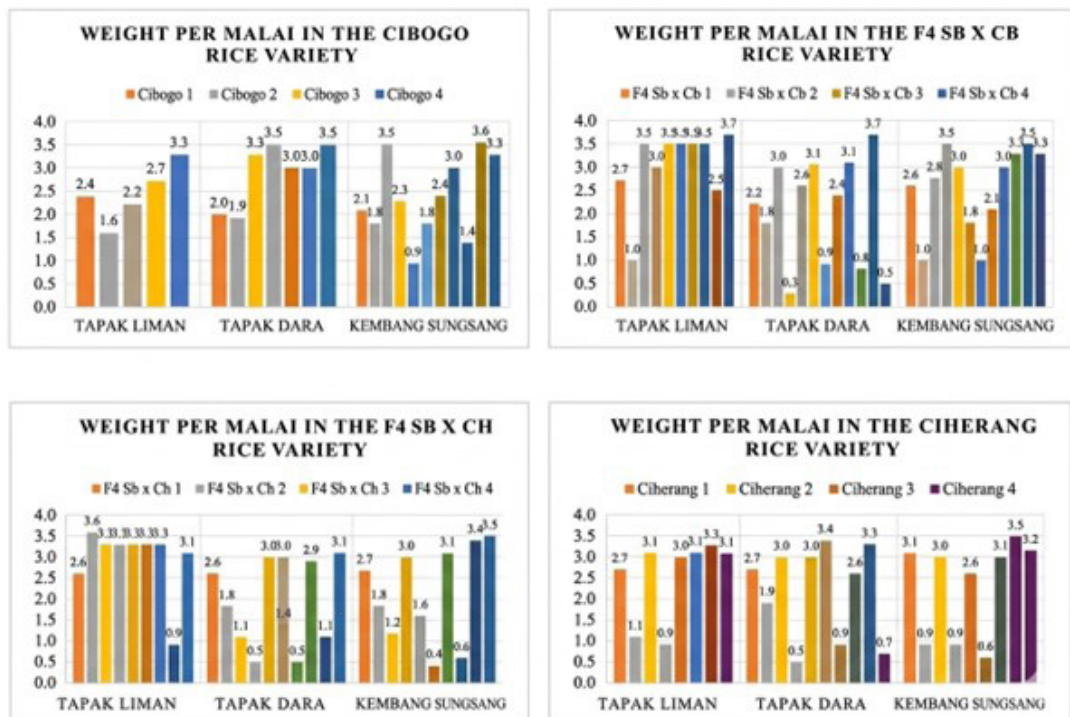


Figure 5. Weight per panicle in four rice varieties due to treatment with various types of antimitotic compounds at various concentrations.

The varietal patterns indicate clear genotype-by-treatment interactions and dose response effects. Cibogo achieved its greatest panicle mass under *Elephantopus scaber* 10% and *Gloriosa superba* 15%, suggesting this genotype tolerates relatively higher antimitotic pressure while still allocating assimilates efficiently to reproductive sinks. F4 Sb × Cb reached maximal panicle weight with *Catharanthus roseus* 15% and *G. superba* 5%, implying a narrower optimal window in which mitotic inhibition is sufficient to trigger beneficial cytological changes (e.g., chromosome doubling) without incurring growth penalties. F4 Sb × Ch performed best under *G. superba* 10% and *C. roseus* 10–15%, consistent with an intermediate optimum for microtubule disruption. Ciherang showed its heaviest panicles under *G. superba* 5–10% and *C. roseus* 5%, pointing to greater sensitivity to antimitotic toxicity at higher doses. Taken together, *G. superba*—a colchicine-bearing species emerges as the most consistently favorable agent across backgrounds, with ~10% frequently near-optimal; *C. roseus* (vincristine/vinblastine) shows genotype-specific optima at low (5%) or higher (10–15%) levels; and *E. scaber* markedly benefits Cibogo at 10% but does not dominate in the other varieties for panicle mass.

Mechanistically, these outcomes are consistent with a balance between effective spindle inhibition (promoting chromosome duplication and the gigas phenotype) and phytotoxic thresholds that depress vegetative vigor or reproductive development. The heavier panicles observed under the best treatments likely reflect polyploid-

associated increases in cell size and sink strength, but the dose that maximizes this effect is clearly genotype dependent. Practically, these results nominate *G. superba* at approximately 10% as a lead treatment for broader screening, while highlighting the need to fine-tune concentrations by variety and to verify cytological endpoints (flow cytometry/chromosome counts, stomatal and pollen size) to confirm stable polyploidy in selected lines.

3.6. Number of stomata on rice leaves under various treatments

Stomata play a central role in regulating gas exchange and transpiration, thereby influencing photosynthetic efficiency and plant water use. In studies of polyploid induction, stomatal density and size are often evaluated as potential morphological markers of chromosomal duplication. However, their responses may differ from other agronomic traits due to stronger genetic and developmental regulation. As shown in Figure 6, the number of stomata on rice leaves under various antimitotic treatments exhibited relatively limited variation across genotypes and concentrations.

Stomatal characteristics in rice leaves appeared to be relatively unaffected by the application of different antimitotic compounds and their varying concentrations. The density, size, and distribution of stomata did not exhibit significant alterations across treatments, indicating that stomatal development is more genetically conserved and less sensitive to mitotic disruption compared to other agronomic traits such as tiller number, leaf production, or panicle weight.

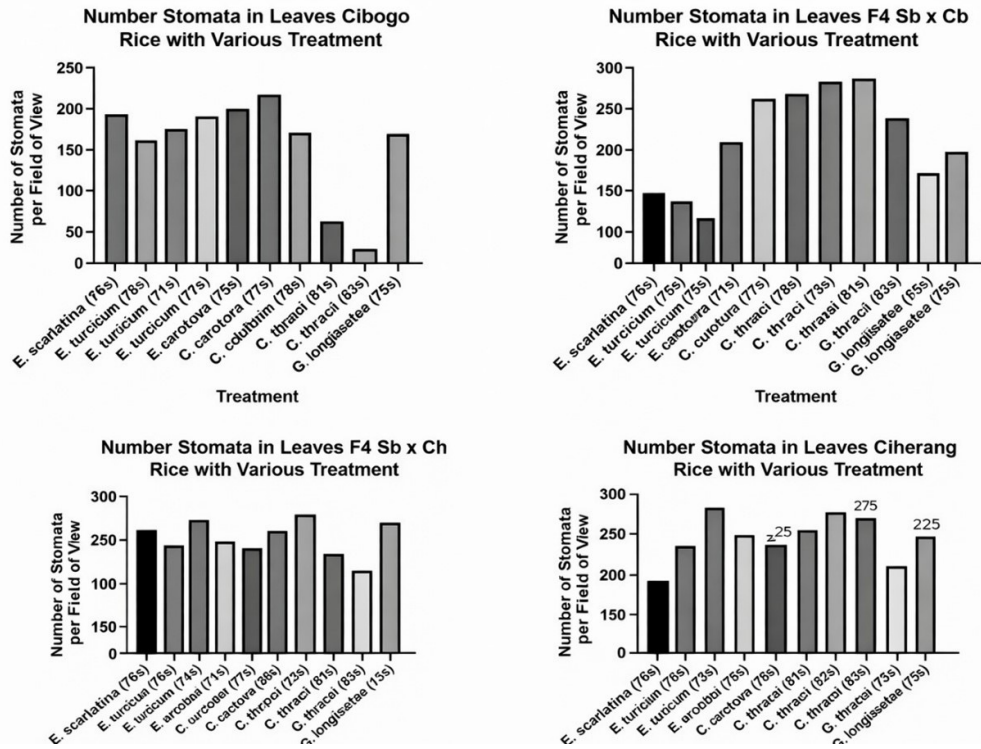


Figure 6. Number of stomata on rice leaves under various treatments.

This stability suggests that stomatal traits may not serve as a reliable morphological marker for assessing the effectiveness of antimitotic-induced polyploidization in rice. Instead, the limited response of stomata highlights the possibility that polyploidization effects induced by antimitotic agents are expressed more strongly in reproductive and growth-related parameters, while stomatal development remains under stricter genetic and developmental regulation.

Stomatal characteristics in rice, including density, size, and distribution, generally remain stable across treatments with antimitotic compounds, although slight modifications may occur at higher concentrations. These modest effects suggest that stomatal development is under strong genetic control and is less responsive to external mitotic disruption compared to traits such as plant height, tiller number, or panicle production. Even when exposed to antimitotic agents capable of altering chromosomal behavior and inducing polyploidy, the fundamental regulation of stomatal formation appears to be buffered by developmental and genetic mechanisms, thereby maintaining stability across a wide range of conditions. This stability indicates that stomatal traits should not be regarded as primary indicators for evaluating the success of antimitotic-induced polyploidization in rice. Instead, they can serve only as complementary observations within a broader suite of morphological and cytological markers. The more pronounced and reliable indicators of successful polyploid induction are typically expressed in growth-related and reproductive traits, such as increased organ size, panicle weight, and biomass accumulation (Lu et al., 2019; Phetluan et al., 2023; Setyati et al., 2024).

3.7. Enhancing productivity and a strategic solution to food security

The present findings demonstrate that *in vivo* polyploid induction can enhance multiple yield-related traits in rice, positioning this approach as a credible route to productivity gains under diverse agroecological contexts. Diagram-based evaluations of agronomic performance revealed clear genotype-specific and dose-dependent responses to antimitotic agents. For instance, the Cibogo variety produced the highest number of tillers under *Gloriosa superba* (kembang sungsang) 5% and attained maximum plant height under *Elephantopus scaber* (tapak liman) 10-15%. It also generated the heaviest panicles under *E. scaber* 10% and *G. superba* 15%, suggesting that this variety tolerates relatively higher concentrations while still allocating resources efficiently to reproductive sinks. The F4 Sb × Cb hybrid displayed the greatest tiller and panicle numbers under *C. roseus* (Tapak dara) 15% and *G. superba* 10%, with panicle weight maximized at lower levels of *G. superba* (5%). Interestingly, “giant rice” phenotypes were observed in this hybrid under 10% *G. superba*, suggesting a polyploid-like vigor response that warrants cytological confirmation to verify chromosome doubling. The F4 Sb × Ch variety responded strongly to *G. superba* 10% and *C. roseus* 10-15%, while the Ciherang variety showed optimal performance at lower doses, particularly *C. roseus* 5% and *G. superba* 5-10%.

Across these varietal patterns, the 10% *G. superba* treatment consistently yielded favorable effects on tillering, plant height, and panicle production, underscoring its consistency as an agronomically effective antimitotic extract with putative polyploid-like outcomes.

These outcomes are consistent with traits often reported in chromosome-duplicated plants, although alternative physiological mechanisms from plant extracts may also contribute: increased gene dosage, larger cell and organ size, altered metabolism, and strengthened source-sink dynamics (Dimude et al., 2015; Panigrahi et al., 2024). The heavier panicles and increased tiller numbers observed under optimal treatments are indicative of enhanced sink strength, while thicker stems and greater biomass accumulation may contribute to improved lodging resistance. Importantly, stomatal traits remained largely stable across treatments, confirming that polyploidization effects are expressed more prominently in reproductive and growth-related traits rather than epidermal development (Anatskaya and Vinogradov, 2022; Sabooni and Gharaghani, 2022).

From an agronomic perspective, these results illustrate how polyploid induction can directly contribute to food security. By increasing tiller number, panicle weight, and overall biomass, yield potential is improved without requiring expansion of agricultural land. Furthermore, varietal differences in response emphasize the importance of genotype-specific optimization to balance efficacy with toxicity. The diagrams provide clear visual evidence that productivity gains are not uniform but depend on the correct matching of genotype and treatment concentration. For example, while hybrids such as F4 Sb × Cb and F4 Sb × Ch demonstrated strong responses to *G. superba* 10%, Ciherang required lower doses to avoid growth inhibition. This indicates that breeding programs must integrate dose optimization into selection pipelines to ensure stable gains (Covarrubias-Pazaran et al., 2022; Merrick et al., 2022).

At a broader level, the consistent performance of *G. superba* across varieties suggests its potential as a lead candidate for natural, plant-derived antimitotic compounds in rice polyploidization. This is particularly relevant for sustainable breeding strategies, as *G. superba* offers a plant-derived alternative to conventional colchicine; nevertheless, cytogenetic equivalence must be demonstrated through cytological assays. By coupling cytological verification (chromosome counts, stomatal and pollen size analysis) with agronomic evaluations, future research can ensure that polyploid lines are both cytogenetically stable and agronomically superior (Niazian and Nalousi, 2020; Touchell et al., 2020; Trojak-Goluch et al., 2021).

The diagram-based findings reinforce that *in vivo* polyploid induction, especially through *G. superba* at optimized concentrations, can generate rice cultivars with enhanced yield components and stress tolerance. When translated into breeding programs, these improvements offer a practical and strategic solution for strengthening national food security by raising productivity, ensuring adaptability to climate change, and reducing vulnerability to biotic and abiotic stresses.

4. Conclusion and Recommendation

This study shows that rice genotypes exhibit differential agronomic responses to natural antimutagenic extracts, reflecting strong genotype-by-treatment interactions. Among the tested extracts *Gloriosa superba* extract, particularly around 10% concentration, consistently improved several yield-related traits (tiller number, panicle production, plant height, and panicle weight) across multiple genotypes. In contrast, *Catharanthus roseus* and *Elephantopus scaber* showed more genotype-specific response patterns. Stomatal traits remained relatively stable across treatments, suggesting limited reliability as standalone morphological indicators of genome-level changes.

Therefore, our recommendation for future experiments are strongly recommended to validated as polyploid through cytological assays for subsequent agronomic evaluation and compared with diploid controls, thereby ensuring that observed improvements in vegetative vigor or yield-related traits are genuinely associated with chromosome doubling rather than non-cytological physiological effects of plant-derived extracts. Cytological approach to confirm genome duplication and to distinguish true polyploids from phenotypically enhanced diploids, such as flow cytometry and/or chromosome counting. As complementary support, pollen size and viability assays may be included, given that polyploidization frequently influences pollen grain diameter and fertility. However, these indicators should be interpreted as supportive rather than definitive. Additionally, molecular characterization and multi-environment field evaluations are recommended to assess trait inheritance, agronomic consistency, and practical breeding potential. Such integrative approaches are essential before the application of natural antimutagenic extracts can be reliably advanced within rice improvement and food security programs.

Acknowledgements

Thank you to LPPM Unesa for funding this research through the RKI JATIMPRO scheme.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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