Preliminary assessment: colchicine-induced mutants of wild *Rubus rosifolius* var. *rosifolius* for dryland adaptation

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Abstract. *Rubus rosifolius* produces fruit with various beneficial bioactive compounds, but the fruit tastes sour and not tasty, thus making it unattractive and not cultivated. This study evaluated the effects of colchicine-induced on survival rate, polyploidy induction, and dryland adaptability of *Rubus rosifolius*. The results demonstrated that colchicine concentration significantly influenced both survival rate and mutation success. Increasing concentration reduced survival, with the highest mortality (60%) observed at 7.5 mM, but enhanced mutation frequency. Polyploid mutants, including two tetraploid (2n = 4x = 28) that stem cuttings originating from the mountains Sumbing and Sindoro, as well as one aneuploid (2n = 2x − 1 = 13), were successfully obtained. Under controlled drought stress conditions, polyploid mutants consistently outperformed the control in growth parameters, showing higher plant height, fresh weight, leaf number, and root length, indicating improved vigor and adaptability. In terms of yield, tetraploid mutants especially from Sindoro, produced significantly higher fruit weight, larger fruit size, and increased °Brix content compared to 2n plants, while aneuploid plants exhibited inferior performance. Overall, the results indicate that colchicine-induced polyploidy effectively improves both growth performance and fruit quality, highlighting its potential as a rapid strategy for developing superior and adaptive genotypes.

1 Introductions

Rubus rosifolius var. *rosifolius* or usually known as roseleaf bramble, is a species of *Rubus* which comes from the *Rosaceae* family. *R. rosifolius* is a thorny perennial shrub originating from the Himalayas, Asia (including Indonesia), and Australia, characterized by its red conical fruits and white blossoms. Roseleaf bramble produces fruit that can be consumed, used as a fresh fruit, traditional medicine, and even as a garden decoration. The fruit contains phenolic acid, anthocyanin, flavonoids, and ascorbic acid which are very important for antioxidants, antimicrobials, and anti-diabetic [1], so it has good potential.

Compared to other *Rubus* species, especially those that have been widely cultivated, *R. rosifolius* is less popular for consumption and in some places is considered as a weed. Besides its good potential, the fruit has a strong sour taste with a slight sweetness, dry texture, and even bland, so it is not preferred for consumption. In tropical countries like Indonesia, *R. rosifolius* is commonly known as ucen-ucen, gucen, kemutung, and various other local names, it typically grows in highland forests, including mountainous regions of Java. The plant can flower and bear fruit almost year-round, although production still peaks during certain periods. Local people consider it as a less useful shrub, but in the desired habitat, it can grow wild and become food for existing animals, especially birds.

In the perspective of plant breeding and agronomy, *R. rosifolius* is one of the genetic resources that has high economic and nutritional potential, if research and development are conducted, especially to address the plant's fruit-related limitations or disadvantages. This plant also exhibits tolerance to dry land conditions and the ability to grow in lowland areas. However, such conditions result in suboptimal growth and lower fruit quality (worse fruit yields compared to growing in the desired location), as optimal performance is achieved in cool highlands ranging between 1000 – 2200 masl [2] with sufficient soil moisture. Thus, they have the potential to be developed as an alternative commodity in areas with limited water availability, especially in the lowland area. However, the productivity and quality of wild plants are generally low and not standardized, necessitating improvements through plant breeding approaches.

One approach that can be used to rapidly expand genetic diversity is through mutation induction, particularly through the use of antimitotic agents such as colchicine. Colchicine works by inhibiting spindle formation during cell division, thereby causing chromosome doubling or polyploidy [3]. Polyploid plants are known to have several agronomic advantages, such as increased organ size, tolerance to abiotic stresses, and the

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potential for increased yield and fruit quality. Mutation induction using colchicine has been widely used to produce superior genotypes in a relatively short time.

The success of mutation induction is influenced by genotype, colchicine concentration, and application method [4], and does not always produce the desired traits, because genetic mutations can be random. Therefore, initial evaluation is necessary to select potential individuals. In dryland areas, important parameters studied include water use efficiency, growth stability, and yield quality. Research on mutation induction in *R. rosifolius* is still relatively limited, particularly regarding increased adaptation to drought stress. The use of wild species as genetic resources plays a strategic role in supporting the development of new varieties that are adaptive to climate change and land degradation.

Based on the background and limitations of existing research, this study aims to conduct a preliminary assessment of colchicine-induced mutants in wild *R. rosifolius*, particularly regarding their potential adaptation to dryland conditions. The results are expected to serve as a basis for the initial selection of superior genotypes and support the development of further plant breeding programs.

2 Research method

The research was conducted from October 2024 to March 2026, at two plots/locations in Temanggung and Sragen Regency. The first plot was in Bansari District, Temanggung with an altitude of 1137 masl for the regeneration of mutant candidates and the second plot in Jenar District, Sragen with an altitude of 174 masl for evaluating the adaptation of mutants with dry land scheme. Observations of several variables such as ploidy and fruit quality were carried out in the Biotechnology and Plant Breeding Laboratory of Tidar University, Magelang, Central Java. Planting material in the form of stem cuttings was taken from wild populations originating from two places, namely *R. rosifolius* from Mount Sindoro and Mount Sumbing (Figure 1).

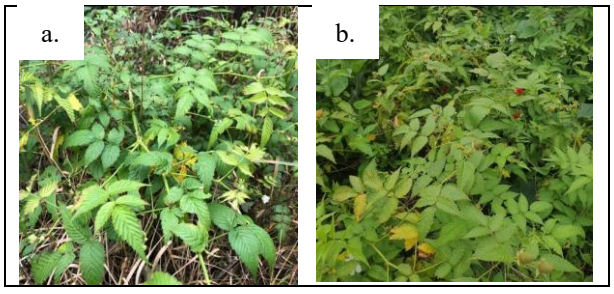

Fig. 1. *R. rosifolis* from two different locations.

Description: a: origin from Sindoro and b: from Sumbing

As the initial stage of the research, preparation of stem cuttings and application of chemical mutagens were carried out. Stem cuttings were taken from the mother plant by making the cutting material with 6 buds on each stem with a uniform length of approximately 20 cm, the upper incision was covered with cambium ointment and the lower stem was cut obliquely. The colchicine mutagen ($C_{22}H_{25}NO_6$) was obtained from Sigma-Aldrich in powder form. The initial stock solution was made using very low concentration DMSO (Dimethyl sulfoxide) solvent with an initial standard dilution to make colchicine with a concentration of 100 mM (100 ml). 3.99 grams of colchicine was added to 100 ml of DMSO solution, then a second dilution was carried out for the colchicine concentration treatment with concentrations of 0 (control), 0.1, 1.0, 5.0, and 7.5 mM.

Mutagen application was carried out by soaking the stem cuttings in each concentration with 3 different soaking durations, namely 3, 6, and 12 hours, with 3 replications (replication is a block, 5 cuttings as sub-samples), and rinsed using distilled water (H_2O) after soaking was complete. Planting cuttings for mutant regeneration was carried out in polybags measuring 20 x 20 cm with loose soil, compost, and cocopeat in a ratio of 2:1:1. To stimulate root growth, Clonex rooting hormone was used with an IBA (indole-3-butyric acid) base at the same concentration.

The mutant regeneration experiment was conducted using a factorial complete randomized block design, with 2 types of cutting sources, 5 types of colchicine concentrations, and 3 soaking durations, thus forming 30 treatment combinations with a total of 450 stem cuttings. The duration from planting to observation is approximately 6 months (from November 2024 to April 2025) and carried out at the screenhouse.

Polyploidy observations were only carried out on plants that successfully regenerated after induction using colchicine. The part of the plant observed was the meristematic root tips that was still actively dividing cells. Chromosome observation was carried out using the Squash method with staining using Aceto orcein (Loba Chemie) to find out the karyotype formula. Plants that obtain a different karyotype formula from the control will then undergo adaptation testing on dry land scheme for approximately 8 months (starting from April to November 2025, during the dry season).

Plants that have been verified as a mutant with a different karyotype formula will be tested for vigor and initial yield in a greenhouse with a non-factorial completely randomized design. The main treatment is a type of mutant with a different karyotype formula and a control in the form of the non-mutant genotype, The volume of watering is important in this experiment, but it was kept constant for each experimental unit with the volume given being a quarter (25%) of the field capacity (FC) and recalibrate the FC measurements every 3 weeks, because compaction may occur due to watering and root growth, which can change the FC. To ensure uniform stress conditions, all experimental units received the same watering volume, irrigation interval, growing medium, pot size, and greenhouse environment throughout the experiment to avoid the dynamics of environmental conditions that affect the availability of water and soil moisture in polybags.

The experiment was carried out by cuttings, with the same method as during regeneration. The replication was repeated five times, the planting media used were only soil and cow manure with a ratio of 3:1 in polybags

measuring 40 x 40 cm, the frequency of watering was the same, once every 4 days, and no pruning was carried out. Soil/media moisture monitoring was carried out using a soil tester at daily intervals, especially immediately after watering and the day after watering. Relatively, soil moisture was in the same moisture percentage range and decreased after watering but remained within the same range.

Data collection was conducted in sequential stages. In the first stage, mutant regeneration was assessed when stem cuttings exhibited stable growth and developed into fully established plants, allowing the determination of survival rates. Subsequently, root sampling was performed for chromosome observation to identify the number of plants exhibiting mutations or polyploidization. Plants verified to have undergone polyploidization or chromosomal alterations were further evaluated under controlled conditions using a drought stress scheme to assess their vigor and initial yield performance.

The data were tested for normality assumptions using PROC UNIVARIATE and homogeneity of variance using Levene's test through PROC GLM to ensure the feasibility of the analysis. The main analysis was conducted using PROC GLIMMIX in SAS. Differences between treatments were further tested using LSMEANS at a significance level of $\alpha = 0.05$ for polyploid induction and using Tukey's HSD for adaptability test, while the results of the analysis were reported as a Type III test of fixed effects. All data were analyzed using SAS (version 9.4M8) and XLSTAT software integrated with Microsoft Excel 2017.

3 Results and discussion

3.1 Survival rate and polyploidy induction

Plant breeding is an important effort in improving the plant traits, in order to obtain cultivars that have advantageous agronomic traits, but often the plant breeding process takes a long time and requires a lot of resources. Plant breeding through mutation methods is one solution that can be implemented to shorten the long plant breeding process and is effective in increasing plant diversity/variation, especially in obtaining a polyploid plants. Polyploid plants are organisms that have more than two complete sets of chromosomes in their somatic cells, resulting from genome duplication and heritable to the next generation/offspring.

Polyploid plants exhibit several agronomic advantages, including improved growth, enhanced yield, increased stress tolerance, and broader adaptability. These benefits are reflected in traits such as superior vigor, higher productivity, extended postharvest shelf life, greater resistance to both abiotic and biotic stresses [5], and more efficient metabolic performance. If considered in the context of *R. rosifolius*, which exhibits limitations such as sour fruit, small size, and suboptimal growth and yield when cultivated in lowland and dryland conditions, mutation breeding could provide a highly beneficial approach to improve these traits. Better fruit characteristics and having the desired taste preferences will make *R. rosifolius* have the opportunity to be cultivated on a mass scale.

The type of planting material, particularly the origin of the cuttings, colchicine concentration, and soaking duration, are important factors that need to be evaluated in relation to plant regeneration, as they determine the effectiveness of mutation induction methods while minimizing plants which failed to regenerate. When evaluated independently, both the type of cutting material and colchicine concentration significantly affected the survival rate of the cuttings. However, the soaking time did not show any significance. Significant interactions were also observed between the origin of the cutting material and colchicine concentration, as well as between colchicine concentration and soaking duration (Table 1).

Table 1. Type III tests of fixed effects on survival rate of cuttings.

Source of variation	df	F value	P > F
A	1	6.21	0.018^{*}
B	4	9.87	$< 0.001^{**}$
C	2	1.42	0.257^{ns}
A x B	4	4.63	0.004^{**}
A x C	2	0.88	0.423^{ns}
B x C	8	2.51	0.031^{*}
A x B x C	8	1.09	0.389^{ns}

Description: *significance at $\langle < 0.05$, **significance at $\langle < 0.01$, ns: not significant, A: cutting sources, B: colchicine concentration, C: soaking duration, and x: interaction.

Colchicine concentration is a critical determinant of induction success and survival rate, as it governs the balance between effective polyploid induction and the level of toxicity to plant tissues [6], especially those applied to parts of plants that will be propagated vegetatively. In the research conducted, increasing the concentration of colchicine (Table 2) reduced the chance of stem cuttings survival, this is in accordance with research conducted by Alisha et al. [7] where increasing colchicine concentration reduced the survival rate. In this study, the highest concentration, 7.5 mM, damage tissue/kill cuttings up to 60%

Table 2. Effect of colchicine concentration on survival rate.

Concentration (mM)	Survival rate (%)
0 (control)	93.33^{a}
0.1	86.67^{ab}
1.0	73.33^{bc}
5.0	56.67^{cd}
7.5	40.00^{d}

Description: the same letter followed behind mean, indicates no significance at $\langle < 0.05$.

Not all stem cuttings that regenerate and grow successfully become polyploid organisms, this is because the contact and interaction of colchicine is selective in certain cells. This phenomenon can also occur due to uneven influence on various plant cells. This proves that there are chimeras/mixploids rather than completely polyploid organisms due to cell selectivity, colchicine is more effective in actively dividing cells (meristematic). In

the research conducted, induction was carried out by soaking the cuttings in a colchicine solution, it is possible that the colchicine did not evenly contact the shoot buds and also that the condition of the shoot buds was not known for certain whether they were still actively dividing or in a dormant state.

The type of cutting material and the duration of soaking did not have a significant effect on polyploidy induction, but the concentration had a significant effect (Table 3), However, the interaction between the type of cutting material with the concentration of colchicine and the concentration of colchicine with the duration of immersion showed significance. The interaction between the type of cutting material and concentration can occur due to differences in the potential vigor and cell division of the shoots in the stem, so that shoots with good cell division will be easier to induce than those with slow division.

Table 3. Type III tests of fixed effects on polyploidy induction.

Source of variation	df	F value	P > F
A	1	2.14	0.152^{ns}
B	4	12.76	$< 0.001^{**}$
C	2	1.08	0.347^{ns}
A x B	4	5.92	0.001^{**}
A x C	2	0.73	0.491^{ns}
B x C	8	3.11	0.012^{*}
A x B x C	8	0.95	0.482^{ns}

Description: *significance at $\langle < 0.05$, **significance at $\langle < 0.01$, ns: not significant, A: cutting sources, B: colchicine concentration, C: soaking duration, and x: interaction.

R. rosifolius cuttings from Mount Sindoro produced more mutants than those from Mount Sumbing, even though they received the same level of colchicine concentration treatment. This can be indicated that the active meristem in the form of buds on the stem cuttings from Mount Sindoro was more optimal and sensitive to colchicine induction. In this study, increasing the concentration of colchicine increased the percentage of successful mutations (Table 4), especially in cuttings originating from Mount Sindoro, while those originating from Mount Sumbing only showed a small percentage of successful mutations (6.67%) at a concentration of 5.0 mM. Research conducted by Touchell et al. [8] also confirmed that increasing the concentration of colchicine increases the success of mutations as long as they are not at a damaging point.

Table 4. Interaction effect of cutting source (genotype) and colchicine concentration on polyploidy/mutant induction.

Concentration (mM)	Cutting sources	
	Sindoro	Sumbing
0 (control)	0.00^{c}	0.00^{c}
0.1	0.00^{c}	0.00^{c}
1.0	6.67^{bc}	0.00^{c}
5.0	20.00^{ab}	6.67^{bc}
7.5	33.33^{a}	0.00^{c}

Description: the same letter followed behind mean, indicates no significance at $\alpha < 0.05$.

This study successfully obtained two genotypes of *R. rosifolius* that polyploid and one that experienced a change in the 2n number of chromosomes (Table 5). Normal *R. rosifolius* has a karyotype formula of 2n = 2x = 14, but the tetraploid mutant from Sumbing has a karyotype formula of 2n = 4x = 28 and the tetraploid from Sindoro has a karyotype of 2n = 4x = 28. There is *R. rosifolius* which remains diploid (2n) but is indicated to have genetic aneuploidy, where one chromosome is missing from a pair of homologous chromosomes (2x-1) or monosomic. Monosomy in plants is a rare phenomenon compared to polyploidy or trisomy. Plants are more tolerant to the genetic phenomenon of aneuploidy than animals, monosomy in plants still shows decent vigor and fertility [9], but is very rare in nature, because it has weak resistance.

Table 5. karyotype formula of polyploid mutants.

Cutting sources	Karyotype formula	Code
Sindoro (control)	2n = **2x** = 14	1c
Sumbing (control)	2n = **2x** = 14	2c
Sumbing	2n = **4x** = 28	3
Sindoro	2n = **4x** = 28	4
Sumbing	2n = **2x–1** = 13	5

In this study, although verification of polyploidy mutations was only performed using karyotyping and observing changes in plant organs, it was sufficient for preliminary studies. To strengthen polyploidy verification, flow cytometry can be used to identify changes in plant genome size and their indications of ploidy level, as well as observations of stomatal anatomy, such as changes in size and density per unit area. The mutants obtained and selected will then be compared with the control (non-mutant) to obtain information on their adaptation to the drought stress scheme.

3.2 Initial evaluation of mutant growth and yield

Improving traits is one of the goals of breeding, especially to improve traits that are less profitable or less productive for agronomic purposes. In the context of *R. rosifolius*, several limitations have been identified, including sour fruit taste, relatively small fruit size, and suboptimal growth and yield under lowland dryland conditions. However, naturally *R. rosifolius* already has adaptive and tolerant properties to dry land and various altitudes, so it is hoped that these adaptive properties will still exist in mutants obtained by improving other agronomic traits.

Before discussing the adaptation results, this study has several limitations that should be considered when interpreting the results. Only a limited number of confirmed and selected mutant genotypes (two tetraploids and one monosomic) were available for evaluation because successful polyploid induction occurred at a relatively low frequency. In addition, each genotype was evaluated using a limited number of biological replicates (five replications) under controlled greenhouse conditions. Therefore, the observed differences should be regarded as preliminary evidence of the potential effects of polyploidization.

In terms of vigor, between the control and the mutant, there were significant differences in plant height, fresh weight, dry weight, number of leaves, and longest root length (Table 6). In terms of growth, mutants with polyploidy showed the highest average in all aspects observed, and were proven to be superior to the control. The highest plant height was in tetraploid *R. rosifolius* from Sindoro with an average of 33.42 cm, then the highest fresh weight was in the tetraploid mutant from Sumbing with an average of 268.96 grams, the highest number of leaves was in the tetraploid mutant from Sindoro with an average of 185.20, and the longest root length was in the tetraploid mutant from Sumbing with an average of 18.24 cm, but the highest dry weight was in the control (Sindoro) with an average of 96.20 grams.

Table 6. Mutant growth performance under drought stress.

M	Ph	Fw	Dw	Nol	Lrl
1c	24.78^{a}	228.10^{ab}	96.20^{b}	138.40^{ab}	14.36^{c}
2c	22.36^{a}	212.80^{ab}	88.72^{b}	152.80^{ab}	14.76^{abc}
3	30.69^{b}	268.96^{c}	92.97^{b}	167.40^{ab}	18.24^{c}
4	33.42^{b}	243.36^{bc}	91.50^{b}	185.20^{b}	17.38^{bc}
5	19.28^{a}	188.74^{a}	63.00^{a}	132.60^{a}	11.14^{a}
M	26.11	228.39	86.48	155.28	15.18
F	20.06	10.20	15.90	3.51	10.08
C	11.19	9.30	8.69	16.52	13.00
S	±5.85	±33.14	±13.74	±29.93	±3.06

Description: the same letter followed behind mean, indicates no significance at $\langle < 0.05$, Ph: plant height (cm), Fw: fresh weight (gr), Dw: dry weight (gr), Nol: number of leaves, Lrl: longest root length (cm), M: mean, F: F-value, C: coefficient of variation, and S: standard deviation.

Plants with the highest average in drought stress conditions can be interpreted as having high adaptability, then the wet and dry weight of the plant is an important indication of physiological response, water stress, and biomass accumulation. In general, the presence of drought stress will reduce the wet and dry weight of plants, the mutants showed the highest wet and dry weight that was not significantly different from the highest control, so it was quite adaptive when compared with aneuploid *R. rosifolius*.

The number of leaves on a plant (regardless of their width) is positively correlated with growth, especially closely related to photosynthesis, the more leaves there are, the wider accumulation of leaf surface area for light interception, photosynthesis, and fresh weight accumulation [10]. The longest root length is an important indicator, especially for plants growing on dry land, The ability of plants to penetrate roots and root length is an important adaptation to reach water in deeper and wider zones, thus influencing their resistance to drought stress. Tetraploid from Sumbing and Sindoro, even though they are planted in polybags, the roots can penetrate out of the polybag into the soil below.

Fruit is an important part of the *R. rosifolius* plant, optimal fruit production in terms of quantity and quality in dry land, especially lowlands, will open up good cultivation and commercial opportunities. Besides being an initial strategy to overcome the problem of rising global temperatures and drought stress due to climate change. In the research conducted, the resulting mutants showed the highest average in terms of fruit quality when compared with the control, including the weight of 10 fresh fruits, the weight of 10 dry fruits, °Brix content, fruit length, and fruit width as can be observed in Table 7.

Table 7. Initial yield performance of mutants under drought stress.

M	Fwg	Dfw	Bl	Fl	Fw
1c	16.00^{b}	8.88^{b}	5.40^{a}	16.00^{b}	10.20^{a}
2c	15.64^{b}	9.06^{b}	4.90^{a}	16.60^{b}	12.60^{b}
3	19.14^{c}	13.08^{c}	7.64^{b}	21.20^{c}	16.40^{c}
4	19.50^{c}	12.68^{c}	8.32^{b}	26.40^{d}	19.00^{d}
5	11.90^{a}	6.38^{a}	10.08^{c}	9.40^{a}	8.20^{a}
M	16.44	10.02	7.27	17.90	13.30
F	50.44	90.61	108.94	70.84	65.17
C	5.91	6.62	6.30	9.40	9.22
S	±2.89	±2.60	±1.95	±0.59	±0.41

Description: the same letter followed behind mean, indicates no significance at $\langle < 0.05$, Fwg: weight of 10 fresh fruits (gr), Dfw: weight of 10 dry fruits (gr), Bl: °Brix level, Fl: fruit length (mm), Fw: fruit width (mm), M: mean, F: F-value, C: coefficient of variation, and S: standard deviation.

All tested plants were able to produce fruit, but with different characteristics, as a consequence of the uniform drought stress treatment. The fruits with the highest wet and dry weights were tetraploid mutants, indicating that even with limited water availability, the mutants can still accumulate photosynthates and water into the fruit at a higher rate compared to the control and aneuploidy plants. There are fundamental changes at the cellular and physiological levels of the plant. Polyploidization causes an increase in cell size (cell gigantism) due to the increased number of chromosome sets [11], resulting in larger cells in the fruit tissue and the ability to store more water. This condition directly contributes to increased fruit weight.

Under drought stress, polyploid plants also tend to have better osmoregulatory capacity [12], such as the accumulation of osmolytes (e.g., proline and soluble sugars), which help maintain cell turgor pressure. This allows fruit cells to remain hydrated and develop optimally despite limited water availability. The combination of larger cell size, better water use efficiency, and the ability to maintain tissue water status explains why polyploid fruits can be heavier and juicier than diploid fruits under drought conditions.

The higher °Brix levels in polyploid fruit are primarily due to increased sugar metabolism capacity due to the gene dosage effect, which increases the activity of enzymes that synthesize and accumulate sugars. Under drought stress, fruit water content decreases, resulting in a more concentrated sugar content, while polyploid plants are able to maintain photosynthesis and photosynthates supply due to better water use efficiency. Although polyploid *R. rosifolius* have high °Brix levels, monosomic mutants showed the highest °Brix levels, It is thought to be caused by a genetic imbalance that leads to a smaller fruit volume so that sugar accumulation is more dominant combined with stress conditions and decreasing volume of fruit water.

In terms of fruit dimensions, especially length and width, polyploid *R. rosifolius* showed the highest average compared to the control, especially in tetraploids from Sindoro with an average length of 26.40 mm and a width of 19.00 mm. Monosomic *R. rosifolius* showed the smallest average fruit dimensions with an average length of 9.40 mm and a width of 8.20 mm. Besides dimensions, mutants also show different shapes, especially those with aneuploidy which appears to have an almost perfectly round shape with larger surface protrusions, as can be observed in Figure 2.

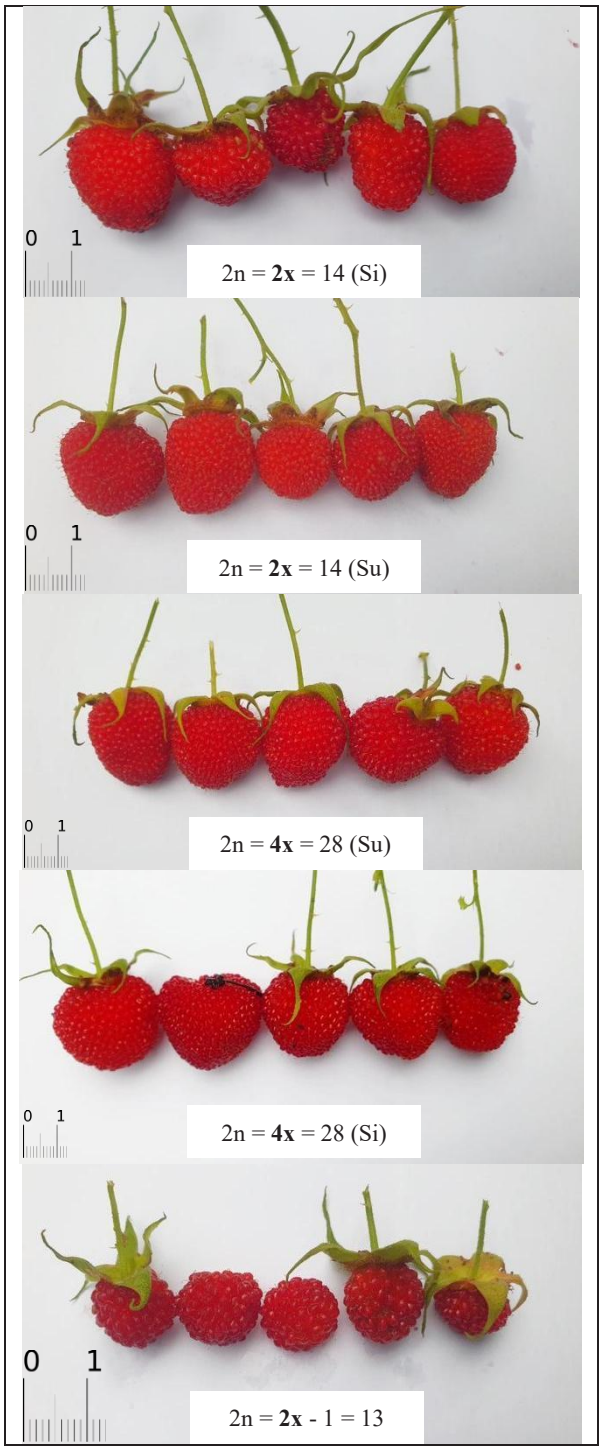

Fig. 2. Differences in fruit shape and size between control and mutant.

Mutants can produce larger fruit than normal plants due to genetic changes, particularly in cases of polyploidy or mutations that affect growth regulation and increase gene dosage and cellular metabolic activity [13], thus encouraging more intensive cell enlargement. This phenomenon is known as the gigas effect, which is organ enlargement due to larger cell size, an increased number of vacuoles, and a higher capacity for biomolecule synthesis [14]. Furthermore, certain mutants can also experience changes in the balance of hormones such as auxin and gibberellin, which enhance cell enlargement and fruit development. This combination of increased cell size, metabolic efficiency, and hormonal regulation is what causes the enlargement of fruit size.

Another change in morphology that can be observed in mutants apart from fruit and leaves is in the generative organs of flowers. Flowers of non-mutant or wild *R. rosifolius* show a relatively similar number of petals and size when compared. However, in tetraploid plants from Sumbing, there was an increase in the number of flower petals, which was originally normal at 5 to 7 and tetraploid from Sindoro becomes 8 petals with a stacked/asymmetrical arrangement accompanied by an enlarged receptacle size (figure 2). The flower size of polyploid *R. rosifolius* also shows a significant increase in size when compared with the flowers of diploid plants. The same thing also happened to *R. parvifolius* L. tetraploid which was induced by colchicine, experienced changes in the flower parts, especially the size and number of petals [15].

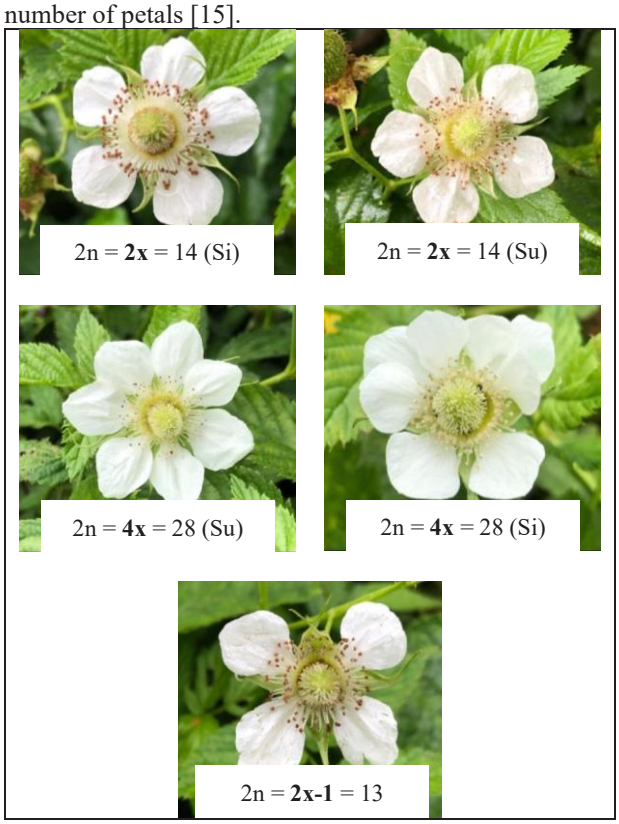

Fig. 3. Flower petals number of *R.rosifolius* between mutant and control.

Based on the evaluation conducted, overall, tetraploid *R. rosifolius* showed adaptive results when given controlled drought stress in terms of growth and initial

yield when compared to the control (2n), so it can be used as a potential candidates for cultivation. *R. rosifolius* with aneuploidy in the form of Monosomic (2x-1) can still grow and produce although not as well as the control, but still has further potential for further research in the aspects of cytogenetics and breeding, Because one chromosome is missing, the genes on the remaining chromosome become hemizygous (like haploids), so that the expression of beneficial genes, whether dominant, recessive, or epistatic, is easier to observe.

4 Conclusions and recommendation

The study confirms that colchicine induction effectively generated genetic variation, with concentration determining the balance between mutation success and plant survival. The resulting genotypes included two tetraploid from Sumbing and Sindoro (2n = 4x = 28) and aneuploid monosomic (2n = 2x − 1 = 13). Polyploid mutants consistently outperformed the control under controlled drought-stress conditions, indicating enhanced vigor and potential adaptation to water-limited environments. These results suggest that the selected tetraploid have promising potential for adaptation to water-limited environments. However, further evaluation under natural dryland field conditions and across multiple environments is required before confirming their agronomic superiority and recommending them for cultivation. It is also recommended to evaluate bioactive compound profiles and perform advanced polyploidy verification using flow cytometry or anatomy such as observing stomata.

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