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Growth Performance and Polyploidy Characterization of M2 Mutants of Black Soybean (*Glycine max* L.) cv. Detam-2 Induced by Colchicine

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ABSTRACT

Background: Black soybean (*Glycine max* L.) cv. Detam-2 is an important Indonesian cultivar with potential for genetic improvement. Colchicine-induced chromosome variation can generate novel phenotypic and cytogenetic traits, but their expression and chromosome constitution require evaluation in subsequent generations.

Methods: A total of 192 M2 plants comprising an untreated control and three mutant groups derived from 3,500 ppm colchicine-treated M1 plants were evaluated at the experimental field of Universitas Islam Riau, Indonesia. Agronomic traits were analyzed using analysis of variance followed by Duncan's multiple range test at $p<0.05$, while chromosome number and karyotype characteristics were assessed cytogenetically in one randomly selected plant per treatment.

Result: Mutant-1, Mutant-2 and Mutant-3 showed greater plant height than the control, reaching 61.32±15.03, 62.20±13.21 and 60.20±11.62 cm, respectively, compared with 33.20±12.30 cm. Mutant-1 and Mutant-2 produced significantly more pods per plant (192.87±32.66 and 193.32±44.51, respectively) than the control (111.12±42.01). Mutant-2 retained the purple-petiole phenotype, whereas Mutant-3 exhibited curly leaves. Cytogenetic analysis identified diploid complements in the control and Mutant-3 (2 n = 40) and triploid complements in Mutant-1 and Mutant-2 (3 n = 60). Karyotypes were predominantly metacentric, with one submetacentric chromosome observed in Mutant-3. Mutant-1 and Mutant-2 therefore represent promising materials for further breeding evaluation, although fertility, chromosome stability, heritability, seed yield and multilocation performance require further validation.

Key words: Agronomic traits, Chromosome number, Genetic variation, Mutant selection, Plant breeding, Polyploidization.

INTRODUCTION

Black soybean (*Glycine max* L.) is an important legume crop in Indonesia and is valued as a source of plant-based protein and bioactive compounds with functional and nutraceutical potential (Amalia *et al*., 2021; Kumar *et al*., 2023). Improving black soybean cultivars is therefore important for increasing productivity while maintaining desirable quality traits (Susanto *et al*., 2023). Among Indonesian cultivars, Detam-2 was developed through selection involving the introduced line 9837 and kawi and released in 2008, with a reported potential yield of 3.45 t ha^{-1} and average yield of 2.41 t ha^{-1} (Amelia *et al*., 2021; Fattah *et al*., 2024). Continued improvement requires identifying productive and stable genetic materials across environments (Susanto *et al*., 2023; Wijaya *et al*., 2022; Ridara *et al*., 2026).

Mutation breeding can generate novel genetic variation when existing germplasm lacks sufficient variation for target traits (Singer *et al*., 2021a). Colchicine is widely used for chromosome manipulation because it disrupts microtubule-mediated spindle formation and chromosome segregation, potentially producing altered chromosome complements and polyploid states (Eng and Ho, 2019; Singh *et al*., 2025). However, its effects vary with genotype, concentration, exposure conditions, developmental stage and tissue type (Eng and Ho, 2019; Touchell *et al*., 2020;

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Manzoor *et al*., 2019). Colchicine-induced morphological and chromosome variation has been reported in several crops, including strawberry, garlic, chili and *Amaranthus* (Amanah *et al*., 2016; Tacia *et al*., 2021; Novitasari *et al*., 2023; Adisty *et al*., 2024). The induced viable mutations in soybean varied in frequency and spectrum according to plant type, leaf type, seed type and pod type. The combination of gamma rays and EMS treatment exhibited significant lethality in producing both chlorophyll and viable mutations (Shekar and Pushpendra, 2017).

Evaluation of subsequent generations is essential because mutation-derived populations may remain segregating or cytogenetically unstable after mutagenic treatment (Jankowicz-Cieslak *et al*., 2017; Zhou *et al*., 2021). The M2 generation provides an opportunity to identify selected phenotypes and assess their persistence before further progeny testing. Integrating agronomic and cytogenetic characterization can help identify mutant materials with breeding potential while distinguishing phenotypic variation from chromosome-number changes (Comai, 2005). A further instance of soybean mutation research is the recently registered mutant variety "Binasoybean-6," which was created from the BARI Soybean-5 variety. The mutant line SBM-22 is recognized for generating increased yields and displaying resistance to yellow mosaic disease, along with reduced levels of insect pest invasion (Malek *et al*. 2022). Study by Nilahayati *et al*. (2026) demonstrates that gamma irradiation caused morphological and agronomic variation in the M.1.1.3 soybean line during the M, generation. Moderate radiation levels (150-250 Gy) improved branching, pod development, seed mass and yield, while excessive doses (350 Gy) negatively impacted plant growth and reproduction.

Previous work on colchicine-treated Detam-2 identified purple petioles, curly leaves, altered stomatal characteristics, differences in growth and production traits and polymorphic RAPD profiles among mutant plants (Fathurrahman *et al*., 2024). However, the persistence of selected phenotypes in M2 and their relationship with chromosome number and karyotype structure remain insufficiently characterized. Therefore, this study evaluated growth performance, pod production, chromosome number, chromosome-arm dimensions and karyotype characteristics of three M2 Detam-2 mutant groups derived from colchicine-treated plants.

MATERIALS AND METHODS

Plant materials and experimental design

The plant materials consisted of an untreated Detam-2 control and three colchicine-derived M2 mutant groups. Mutant-1, Mutant-2 and Mutant-3 originated from M1 plants previously obtained from seeds treated with 3,500 ppm colchicine according to Fathurrahman *et al*. (2024). The M2 groups were designated according to their selected M1 phenotypes: Mutant-1 with normal leaves, Mutant-2 with purple petioles and Mutant-3 with curly leaves.

The experiment was conducted from 25 October 2024 to 22 February 2025 at the experimental field of the Faculty of Agriculture, Universitas Islam Riau, Pekanbaru, Indonesia. A total of 192 plants were established, comprising 48 plants per treatment in four replicate plots of 12 plants. Each plot measured 100 × 90 cm, with 50 × 30 cm spacing. Organic NPK and Mutiara 16:16:16 NPK fertilizers were applied according to standard cultivation practices. Three plants were randomly selected from each plot for quantitative measurements, resulting in 12 sampled plants per treatment.

Phenotypic characterization

Qualitative observations focused on leaf morphology and petiole color, particularly phenotypes previously identified in the M1 generation. Quantitative traits included plant height, branch number, pod number per plant, dry seed weight per plant and 100-seed weight. Plant height was measured from the soil surface to the apical growing point, while branches and pods were counted at physiological maturity. Seeds were dried before determining dry seed weight and 100-seed weight.

Cytogenetic analysis

Cytogenetic characterization was conducted using one randomly selected plant from each treatment. Root tips were fixed in 45% glacial acetic acid at 4°C for 24 h, rinsed, hydrolyzed in 1 N HCl at 60°C for 11 min and stained with 1% aceto-orcein for 3 h following a modified procedure based on Nathewet *et al*. (2009). Root-tip cells were examined at 1,000× magnification and well-resolved metaphase cells were used for chromosome counting. Chromosome complements were classified as diploid at 2 n= 40 and triploid at 3 n= 60.

Karyotype analysis was performed using clear prometaphase or metaphase preparations. Image processing was conducted using Image Raster 3, while IKAROS (MetaSystems Hard and Software GmbH, Germany) and CytoVision (Leica Microsystems, Germany) were used for chromosome analysis. The centromere index (CI) was calculated as:

$$CI\ (\%) = \frac{p}{p + q} \times 100$$

Where,
p= The short-arm length.
q= The long-arm length.

The arm ratio was calculated as:

$$AR = \frac{q}{p}$$

Chromosome morphology was classified according to centromere position following Levan *et al*. (1964). Three clear cells from each selected plant were measured descriptively; because they originated from a single plant per treatment, they were not considered independent biological replicates and were not subjected to inferential statistical analysis.

Statistical analysis

Quantitative phenotypic data were analyzed using one-way ANOVA, followed by Duncan's multiple range test (DMRT) at $p<0.05$ when treatment effects were significant. Analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and data are presented as mean±standard deviation (SD).

RESULTS AND DISCUSSION

Phenotypic variation in M2 Detam-2

Distinct morphological phenotypes were observed among the M2 populations (Fig 1). The control and Mutant-1 exhibited normal leaves, whereas Mutant-2 had purple petioles and Mutant-3 had curly leaves. The purple-petiole and curly-leaf phenotypes were also observed in the corresponding M1 materials, indicating their persistence following generation advancement (Fathurrahman *et al*., 2024).

Phenotypic expression was not uniform within the mutant populations, as purple petioles were not observed in all Mutant-2 plants, indicating continued segregation in the M2 generation. Similar morphological variation following colchicine treatment has been reported in strawberry, *Amaranthus* and other crops (Tacia *et al*., 2021; Adisty *et al*., 2024; Wulansari *et al*., 2016). Such variation is expected in mutation-derived populations and requires repeated selection and progeny testing to establish phenotypic stability (Jankowicz-Cieslak *et al*., 2017; Zhou *et al*., 2021).

The persistence of the selected phenotypes from M1 to M2 supports their use for preliminary mutant selection but does not demonstrate genetic fixation or stable inheritance. Successive-generation evaluation is therefore required to distinguish heritable variation from residual segregation and environmental effects.

Growth and pod production

Significant differences were observed among treatments for plant height, branch number and pod production (Table 1). Plant height increased from 33.20±12.30 cm in the control to 61.32±15.03, 62.20±13.21 and 60.20±11.62 cm in Mutant-1, Mutant-2 and Mutant-3, respectively, representing increases of approximately 81-87%. Branch number increased from 4.21±0.23 branches plant^{-1} in the control to 8.38±1.25 and 8.17±0.89 in Mutant-1 and Mutant-2, respectively, while Mutant-3 produced 6.82±1.26 branches plant^{-1}. These findings are consistent with previous reports of colchicine-associated phenotypic variation (Fathurrahman, 2016, 2023; Herman *et al*., 2013; Molla *et al*., 2021).

Pod production showed the clearest differentiation. Mutant-1 and Mutant-2 produced 192.87±32.66 and 193.32±44.51 pods plant^{-1}, respectively, compared with 111.12±42.01 in the control, representing increases of approximately 74%. Mutant-3 produced 149.89±35.63 pods plant^{-1}, approximately 35% higher than the control, but the difference was not significant. These results agree with previous findings for colchicine-derived Detam-2 and other mutant populations (Fathurrahman *et al*., 2024; Anwar *et al*., 2024).

Mutant-1 and Mutant-2 are therefore promising for preliminary selection based on plant height and pod production. However, pod number does not directly indicate grain yield, which also depends on seed number per pod, pod filling and seed weight. Furthermore, persistence from M1 to M2 does not establish genetic fixation or high heritability; successive-generation testing is required to confirm trait stability and breeding value (Fathurrahman *et al*., 2024).

Chromosome number and mitotic characteristics

Mitotic stages, including prophase, prometaphase, metaphase, anaphase and telophase, were observed in root-tip preparations (Fig 2). Chromosome counting showed 2 n = 40 in the control and Mutant-3 and 60 chromosomes in Mutant-1 and Mutant-2 (Fig 3), identifying the selected Mutant-1 and Mutant-2 plants as triploid and Mutant-3 as diploid. Colchicine-induced chromosome variation and triploid formation have been reported across several crops, with responses influenced by genotype and treatment conditions (Herman *et al*., 2013; Fathurrahman, 2023; Novitasari *et al*., 2023; Amanah *et al*., 2016; Yang *et al*., 2018).

The coexistence of diploid and triploid plants indicates non-uniform chromosome responses among the colchicine-derived materials (Eng and Ho, 2019; Touchell *et al*., 2020). Mutant-1 and Mutant-2 were triploid and also



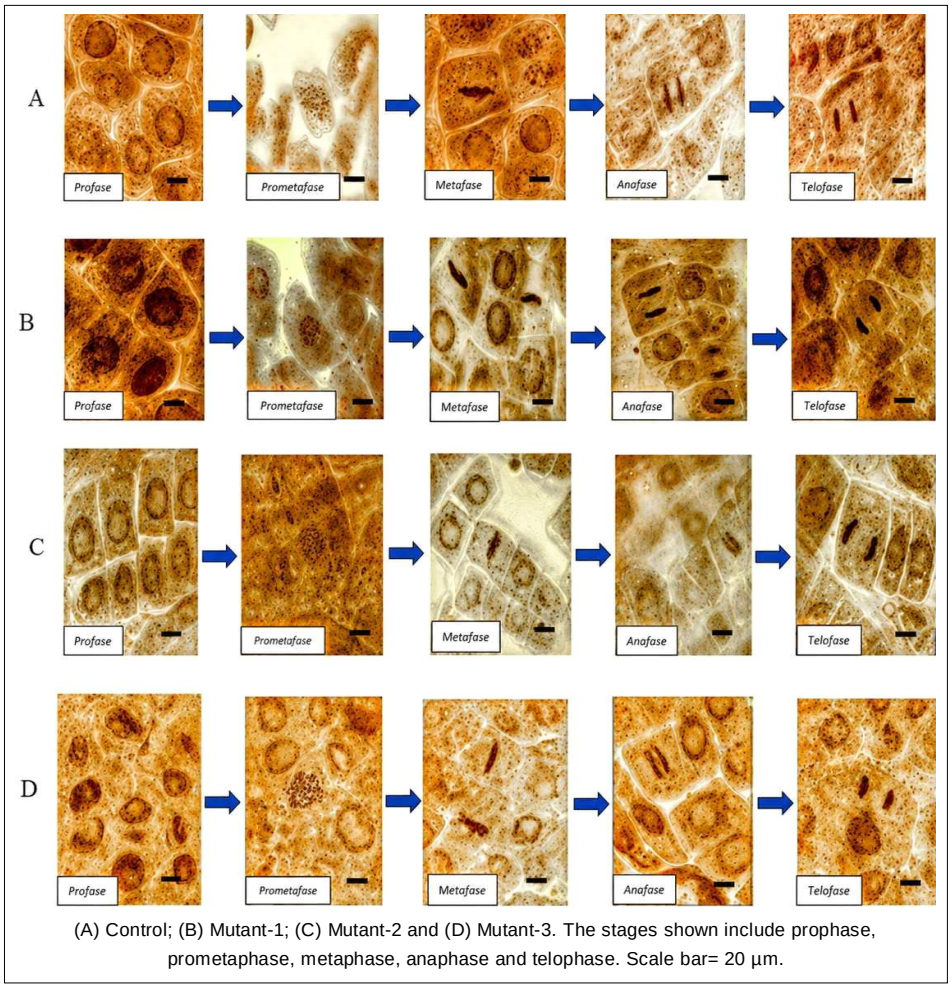
(A) Control with normal leaves; (B) Mutant-2 with purple petioles and (C) Mutant-3 with curly leaves.

Fig 1: Leaf morphology of M2 black soybean cv. Detam-2.

Table 1: Growth and production characteristics of M2 black soybean cv. Detam-2.

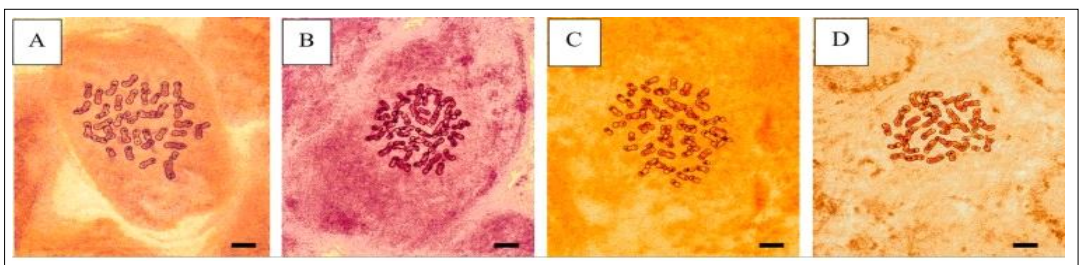
Traits	Control	Mutant-1 (Normal)	Mutant-2 (Purple petiole)	Mutant-3 (Curly leaves)
Plant height (cm)	33.20±12.30^{b}	61.32±15.03^{a}	62.20±13.21^{a}	60.20±11.62^{a}
Number of branches	4.21±0.23^{b}	8.38±1.25^{a}	8.17±0.89^{a}	6.82±1.26^{a}
Number of pods plant^{-1}	111.12±42.01^{b}	192.87±32.66^{a}	193.32±44.51^{a}	149.89±35.63^{ab}

Note: Values are means±SD. Different superscript letters within a row indicate significant differences according to DMRT at p<0.05.



(A) Control; (B) Mutant-1; (C) Mutant-2 and (D) Mutant-3. The stages shown include prophase, prometaphase, metaphase, anaphase and telophase. Scale bar= 20 µm.

Fig 2: Mitotic stages observed in root-tip cells of M2 black soybean cv. Detam-2.



(A) control (2 n = 40); (B) Mutant-1 (3 n = 60); (C) Mutant-2 (3 n = 60) and (D) Mutant-3 (2 n = 40). Scale bar = 5 µm.

Fig 3: Chromosome number of M2 black soybean cv. Detam-2.

showed the greatest plant height and pod production, suggesting a possible association between chromosome constitution and agronomic performance. However, because cytogenetic analysis was limited to one selected plant per treatment, this association cannot be generalized to the entire mutant populations or interpreted as a causal effect of triploidy.

Colchicine-induced variation may involve chromosome-number changes as well as other genetic or structural alterations (Singer *et al*., 2021b). Therefore, broader cytogenetic screening, together with meiotic analysis, pollen viability and fertility assessment, is required to determine the stability and reproductive consequences of the triploid condition (Eng and Ho, 2019; Singh *et al*., 2025; Comai, 2005).

Chromosome length and karyotype structure

Total chromosome length was 171.17 µm in the control, 167.99 µm in Mutant-1, 130.84 µm in Mutant-2 and 348.26 µm in Mutant-3 (Table 2). Mutant-3 therefore had the greatest measured chromosome length despite retaining the diploid chromosome number (2n = 40). Because chromosome dimensions can vary with condensation, mitotic stage and preparation quality, these values should be considered descriptive cytological characteristics rather than direct indicators of genome size or chromosome duplication.

Variation in chromosome structure has been reported in soybean and other polyploid plants (Yuan and Song, 2023; Yahui *et al*., 2022; Li *et al*., 2024; Manzoor *et al*., 2019). The greater chromosome length in Mutant-3 may reflect differences in chromosome condensation or structural organization rather than ploidy. However, because measurements were obtained from one selected plant per treatment and a limited number of cells, they cannot be interpreted as statistically validated treatment effects.

Karyotype analysis showed predominantly metacentric chromosomes in the control, Mutant-1 and Mutant-2, whereas Mutant-3 contained one submetacentric chromosome and the remaining chromosomes were metacentric (Fig 4). The diploid plants showed 20 chromosome types, while the triploid Mutant-1 and Mutant-2 showed 30 types corresponding to their chromosome complements. The presence of a submetacentric chromosome in Mutant-3 indicates karyotypic variation without a change in chromosome number, although its stability requires confirmation in additional individuals.

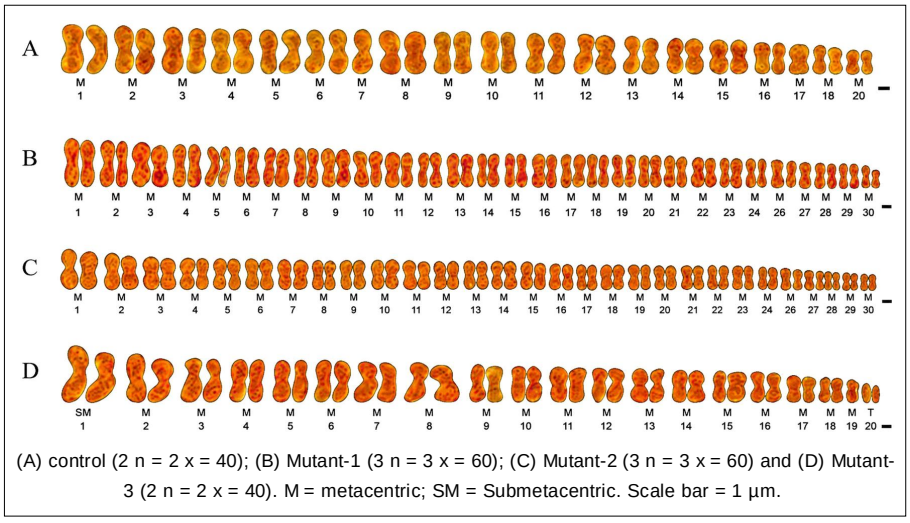
Overall, the karyotype results demonstrate chromosome-number and structural variation among the selected M2 plants. The triploid karyotypes of Mutant-1 and Mutant-2 confirm their chromosome status but do not establish that triploidy caused their superior agronomic performance. Broader cytogenetic screening is therefore needed to determine the stability and distribution of these characteristics within the mutant populations.

Breeding implications

Mutant-1 and Mutant-2 were the most promising materials identified in this study, combining greater plant height and approximately 74% higher pod production than the control

Table 2: Total length of chromosome arms in M2 black soybean cv. Detam-2.

Treatments	Short arm, *p* (µm)	Long arm, *q* (µm)	Total length, *p* + *q* (µm)
Control (2n = 40)	80.16	91.01	171.17
Mutant-1 (3n = 60)	79.19	88.80	167.99
Mutant-2 (3n = 60)	61.99	68.85	130.84
Mutant-3 (2n = 40)	154.61	193.65	348.26



(A) control (2 n = 2 x = 40); (B) Mutant-1 (3 n = 3 x = 60); (C) Mutant-2 (3 n = 3 x = 60) and (D) Mutant-3 (2 n = 2 x = 40). M = metacentric; SM = Submetacentric. Scale bar = 1 µm.

Fig 4: Karyotypes of M2 black soybean cv. Detam-2.

with triploid chromosome complements (3 n = 60). These characteristics support their potential for further selection within the Detam-2 breeding population, although they cannot yet be considered superior genotypes. Their breeding value requires cautious interpretation because triploidy may affect reproductive stability and the mechanisms underlying their enhanced growth and pod production were not evaluated (Comai, 2005; Mangena and Mushadu, 2023; Mangena, 2023; Li *et al*., 2024). Moreover, cytogenetic characterization was based on one plant per treatment, preventing the observed association between triploidy and agronomic performance from being generalized to the entire mutant populations.

Further evaluation should include chromosome screening of multiple individuals, meiotic behavior, pollen viability, seed set, progeny chromosome stability and trait inheritance across successive generations. Multilocation testing should then assess the stability of agronomic performance across environments before these materials are advanced in breeding programs (Susanto *et al*., 2023; Wijaya *et al*., 2022; Ridara *et al*., 2026).

CONCLUSION

Colchicine-derived M2 black soybean cv. Detam-2 exhibited substantial phenotypic and cytogenetic variation, highlighting its potential as a source of novel breeding materials. Mutant-1 and Mutant-2 showed significantly greater plant height and pod production than the control and in the cytogenetically analyzed plants, possessed triploid chromosome complements (3n = 60), whereas Mutant-3 remained diploid (2n = 40) and exhibited a distinctive curly-leaf phenotype with karyotypic variation. The combination of enhanced agronomic traits and triploid chromosome complements makes Mutant-1 and Mutant-2 promising candidates for further selection; however, the limited cytogenetic sampling does not establish a causal relationship between ploidy and agronomic performance. Successive-generation evaluation is therefore required to confirm chromosome stability, fertility, trait inheritance and agronomic performance across environments before these materials can be advanced in breeding programs.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES

Adisty, A., Nita, E. and Suratman. (2024). Effect of colchicine on chromosome number, morphological character and β-carotene production of *Amaranthus tricolor*'s red giti cultivar. *Journal of Cell Biology and Development*. **5:** 18-24. doi: 10.13057/cellbioldev/t050103.

Amalia, A., Kusumawinahyu, R. and Rohenti, I.R. (2021). Study of the potential anti-aging properties of black soybean extract [*Glycine max* (L.) Merrill] variety Detam-1 through antioxidant testing. *Warta Akab*. **45:** 43-50. doi: 10.55075/wa.v45i2.29.

Amanah, H.A., Arumingtyas, E.L. and Indriyani, S. (2016). Chromosome analysis of cayenne pepper (*Capsicum frutescens* L.) in colchicine-induced mutation. *Journal of Applied Horticulture*. **18:** 217-220. doi: 10.37855/jah.2016.v18i03.38.

Amelia, R., Beandrade, M.U. and Hasmar, W.N. (2021). Formulation and physical characterization of black soybean (*Glycine max* L.) variety of Detam II tablets with dry granulation method. *International Journal of Natural Science and Engineering*. **5:** 30-38. doi: 10.23887/ijnse.v5i1.3344.

Anwar, S., Kusmiyati, F., Lukiwati, D.R., Sas, M.G.A. and Arifah, Y.N. (2024). Growth and production of M6 and M7 black soybean mutant genotypes. *Biodiversitas Journal of Biological Diversity*. **25(6).** doi: 10.13057/biodiv/d250624.

Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*. **6(11):** 836-846. doi: 10.1038/nrg1711.

Eng, W.H. and Ho, W.S. (2019). Polyploidization using colchicine in horticultural plants: A review. *Scientia Horticulturae*. **246:** 604-617. doi: 10.1016/j.scienta.2018.11.010.

Fathurrahman, F. (2016). Effect of colchicine application on the growth and yield of black soybean plants [*Glycine max* (L.) Merr.]. *Journal of Agricultural Dinamika*. **32:** 21-26. (Indonesian).

Fathurrahman, F. (2023). Growth and genetic characteristics of cucumber (*Cucumis sativus* L.) cultivar mercy F1 hybrid and mutant populations. *SABRAO Journal of Breeding and Genetics*. **55:** 485-494. doi: 10.54910/sabrao2023.55.2.20.

Fathurrahman, F., Ulpah, S., Sodiq, N.A.M. and Mahadi, I. (2024). The effect of colchicine treatment on phenotype and genotype characteristics of Detam-2 variety of soybean *Glycine max*. *Biodiversitas*. **25:** 1230-1238. doi: 10.13057/biodiv/d250339.

Fattah, A., Negara, A., Supriadi, K., Hannan, M.F.I., Ardjanhar, A., Beding, P.A., Najamuddin, E., Pustika, A.B., Susilawati, S., Nonci, N., Latifah, E., Arifin, Z.I.N., Udiarto, B.K. and Dewayani, W. (2024). Characteristics of several soybean varieties (*Glycine max* L.) and weed management systems in an effort to increase productivity in low land rice. *Frontiers in Sustainable Food Systems*. **8:** 1-10. doi: 10.3389/fsufs.2024.1418759.

Herman, Malau, I.M. and Roslim, D.I. (2013). The effect of colchicine mutagen on green bean seeds (*Vigna radiata* L.) on chromosome number and growth. *Proceedings of the National Seminar on Biodiversity and Ecology Tropica Indonesia (BioETI)*. **4:** 1-12.

Jankowicz-Cieslak, J., Mba, C. and Till, B.J. (2017). Mutagenesis for Crop Breeding and Functional Genomics. In: Biotechnologies for Plant Mutation Breeding. Springer International Publishing. pp. 3-18. doi: 10.1007/978-3-319-45021-6_1.

Kumar, V.A., Pravitha, M., Yadav, A., Pandiselvam, R. and Srivastav, P.P. (2023). Influence of ultrasonic application on soybean aqueous extract based composite edible film: Characterization and their food application. *Food Hydrocolloids*. **135.** doi.org/10.1016/j.foodhyd.2022.108210.

Levan, A., Fredga, K. and Sandberg, A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas*. **52:** 201-220.

Li, X., Zhang, L., Wei, X., Datta, T., Wei, F. and Xie, Z. (2024). Polyploidization: A biological force that enhances stress resistance. *International Journal of Molecular Sciences*. **25(4):** 1957. doi: 10.3390/ijms25041957.

Malek, M.A., Emon, R.M., Khatun, M.K., Bhuiyan, M.S.H., Nevame, M.Y.A. and Alam, A.M. (2022). Binasoybean-6: A high yielding mutant soybean variety developed through sustainable mutation breeding. *Legume Research*. **45(2):** 143-148. doi: 10.18805/LRF-651.

Mangena, P. (2023). Impact of polyploidy induction for salinity stress mitigation in soybean [*Glycine max* (L.) Merrill]. *Plants*. **12:** 1356. doi: 10.3390/plants12061356.

Mangena, P. and Mushadu, P.N. (2023). Colchicine-induced polyploidy in leguminous crops enhances morpho-physiological characteristics for drought stress tolerance. *Life*. **13:** 1966. doi: 10.3390/life13101966.

Manzoor, A., Ahmad, T., Bashir, M.A., Hafiz, I.A. and Silvestri, C. (2019). Studies on colchicine-induced chromosome doubling for enhancement of quality traits in ornamental plants. *Plants*. **8:** 194. doi: 10.3390/plants8070194.

Molla, G.H., Kahsay, T.M., Sylvère, N. and Sony, S. (2021). Garlic micro-propagation and polyploidy induction *in vitro* by colchicine. *Plant Breeding and Biotechnology*. **9:** 1-19. doi: 10.9787/PBB.2021.9.1.

Nathewet, P., Yanagi, T., Hummer, K.E., Iwatsubo, Y. and Sone, K. (2009). Karyotype analysis in wild diploid, tetraploid and hexaploid strawberries, *Fragaria* (Rosaceae). *Cytologia*. **74:** 355-364. doi: 10.1508/cytologia.74.355.

Nilahayati, Dewi, A.D., Nanda, R., Khaidir, D.K. and Donepudi, S. (2026). Identification of promising mutants in gamma-irradiated M2 generation of the soybean line based on morpho-agronomic traits. *Agricultural Science Digest*. **46(4):** 580-585. doi: 10.18805/ag.DF-832.

Novitasari, A., Damanhuri, Soetopo, L. and Adiredjo, A.L. (2023). Induction of polyploidy using colchicine on garlic (*Allium sativum* L.) var. lumbu kuning and lumbu hijau. *Agricultural Journal*. **6:** 648-658. doi: 10.37637/ab.v6i3.1369.

Ridara, F., Ustari, D., Wicaksono, A.A., Algina, A., Amien, S., Susanto, G.W.A., Koerniati, S., Hastilestari, B.R., Concibido, V. and Karuniawan, A. (2026). Integrating multi-trait selection and environmental stability for soybean [*Glycine max* (L.) Merr.] genotype improvement in Indonesia. *Plant, Cell and Environment*. **49(7):** 4241-4253. doi: 10.1111/pce.15662.

Shekar, G.C. and Pushpendra. (2017). Induced mutations in soybean (*Glycine max* L.). *Legume Research*. **40(6):** 1012-1019. doi: 10.18805/LR-3783.

Singer, A., Grinshpun, C.J. and Sagi, D.L. (2021a). Colchicine treatment increases the risk for fetal chromosomal aberrations: An observational study and systematic literature review. *Rheumatology*. **14:** 2342-2347. doi: 10.1093/rheumatology/keaa602.

Singer, S.D., Laurie, J.D., Bilichak, A., Kumar, S. and Singh, J. (2021b). Genetic variation and unintended risk in the context of old and new breeding techniques. *Critical Reviews in Plant Sciences*. **40:** 68-108. doi: 10.1080/07352689.2021.1883826.

Singh, B., Yun, S., Gil, Y. and Park, M.H. (2025). The role of colchicine in plant breeding. *International Journal of Molecular Sciences*. **26:** 6743. doi: 10.3390/ijms26146743.

Susanto, G.W.A., Maulana, H., Putri, P.H., Purwaningrahayu, R.D., Wijaya, A.A., Sekti, B.A. and Karuniawan, A. (2023). Stability analysis to select the stable and high yielding of black soybean [*Glycine max* (L.) Merr.] in Indonesia. *International Journal of Agronomy*. **2023:** 1-14. doi: 10.1155/2023/7255444.

Tacia, B.A., Damanhuri. and Agisimanto, D. (2021). Morphological diversity of strawberry shoots (*Fragaria ananassa* L.) due to colchicine treatment in *in vitro* media. *Journal of Crop Production*. **9:** 86-95.

Touchell, D.H., Palmer, I.E. and Ranney, T.G. (2020). *In vitro* ploidy manipulation for crop improvement. *Frontiers in Plant Science*. **11:** 722. doi: 10.3389/fpls.2020.00722.

Wijaya, A.A., Haris, M., Susanto, G.W.A., Karuniawan, A., Sumardi, D., Amien, S. and Ruswandi, D. (2022). Grain yield stability of black soybean lines across three agroecosystems in West Java, Indonesia. *Open Agriculture*. **7:** 749-763. doi: 10.1515/opag-2022-0137.

Wulansari, A., Martin, A.F. and Ermayanti, T.M. (2016). Induction of polyploid taro plants (*Colocasia esculenta* L.) with oryzalin treatment *in vitro*. *Indonesian Journal of Biology*. **12:** 297-305.

Yahui, X., Bi, M., Dong, L., Yu, T., Qiwei, Z. and He, N. (2022). Chromosome restructuring and number change during the evolution of *Morus notabilis* and *Morus alba*. *Horticulture Research*. **9:** uhab030. doi: 10.1093/hr/uhab030.

Yang, J., Wang, J., Liu, Z., Xiong, T., Lan, J., Han, Q., Li, Y. and Kang, X. (2018). Megaspore chromosome doubling in *Eucalyptus urophylla* S.T. Blake induced by colchicine treatment to produce triploids. *Forests*. **9:** 728. doi: 10.3390/f9110728.

Yuan, J. and Song, Q. (2023). Polyploidy and diploidization in soybean. *Molecular Breeding*. **6:** 51. doi: 10.1007/s11032-023-01396-y.

Zhou, H., Tang, K., Li, G., Liu, W., Yu, H., Yuan, X., Yang, S., Bhattacharyya, M.K. and Feng, X. (2021). A robust and rapid candidate gene mapping pipeline based on M2 populations. *Frontiers in Plant Science*. **12:** 681816. doi: 10.3389/fpls.2021.681816.