

Identification of Cytoplasmic Male Sterility (CMS) and Maintainer Line through Testcross and Self-Pollination in Chili Peppers (*Capsicum annuum* L.)

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Abstract

Hybrid seed production in chili is more efficient when using male-sterile plants. Cytoplasmic male sterility (CMS) produces 100% male sterile progeny and eliminates the need for emasculation, making it highly advantageous for hybrid seed production and enhancing the genetic purity of hybrid seeds. Indonesia possesses a wide range of commercial hybrid chili pepper varieties that can serve as alternative sources of CMS genetic resources. Although CMS germplasm is available from the World Vegetable Center (AVRDC), the utilization of commercial hybrid varieties as CMS sources is more practical because their seeds are widely available domestically. In this study, nine commercial hybrid chili varieties were used as female parents and testcrossed with IPB379 as a candidate maintainer line and self-pollination was performed for each hybrid. The resulting progenies were evaluated based on segregation patterns of male sterility, floral morphology, and correlation among flower traits and fertility status. This study reports that the chili pepper hybrids IPB432, IPB433, IPB434, IPB435, IPB436, IPB437, IPB439, and IPB440 are presumed to carry a CMS gene based on the 1:1 segregation ratio observed in the testcross populations and the 3:1 segregation ratio observed in the self-pollinated populations. In addition, the chili pepper line IPB379 has the potential to serve as a maintainer line because it consistently produced a 1:1 testcross segregation ratio across the eight hybrid genotypes. Furthermore, CMS chili

pepper plants exhibited smaller anther length and width than fertile plants due to reduced pollen production.

Keywords: *anther, emasculation, fertility segregation ratio, hybrid seed production*

Introduction

Hybrid varieties are produced by crossing genetically diverse parental lines with superior traits, resulting in heterosis; in this phenomenon, hybrid progeny outperform their parents (Fujimoto et al., 2018). Higher average yields in hybrid chili compared with parental lines have been reported by Syukur et al. (2024). Heterosis for fruit yield per plant in chili has been reported to reach 67.81% over the mid-parent, 49.34% over the better parent, and 106.77% over the standard check (Bhut et al., 2023). Capsaicin content in hybrids can even exceed that of their parental genotypes, as reported by Naves et al. (2022) and Sahid et al. (2020). These advantages have made hybrid varieties widely used in chili pepper cultivation. Hybrid seed production in chili pepper requires emasculation, or the removal of the male reproductive organs, from the female parent before self-pollination occurs (Syukur et al., 2012). This is because chili pepper has perfect flowers, in which both female and male reproductive organs are present within the same flower. In general, commercial hybrid seeds can be produced through manual emasculation or by utilizing male sterility systems (Berke, 1999). Cytoplasmic male sterility (CMS) is widely utilized

in hybrid seed production because it produces 100% male sterile progeny. This advantage makes the CMS system highly valuable for seed producers by simplifying hybrid seed production. Hybrid seed production through emasculation or manual pollination increases production costs, resulting in hybrid seeds that are less affordable for farmers (Swamy et al., 2019). In contrast, the use of the male sterility system can reduce labor requirements and production time, decrease production costs by up to 46.9%, and improve labor efficiency by 57.6% per unit area (Yang et al., 2008). In addition to improving production efficiency, the male sterility system also plays an important role in maintaining hybrid seed purity by preventing self-pollination. In the CMS system, the female parent does not produce viable pollen, allowing fertilization to occur only through pollination by the selected male parent. This ensures that all resulting seeds are derived from the intended cross. Therefore, the use of CMS represents an effective strategy for maintaining the genetic purity and quality of hybrid seeds.

CMS is a maternally inherited trait resulting from the interactions between cytoplasmic genes (S or N) and nuclear male sterility genes known as rf. CMS lines possess sterile cytoplasm (S) combined with the genotype rrf, while fertility restoration is controlled by dominant alleles designated as Rf, known as restorer-of-fertility genes (Peterson, 1958; Shifriss, 1997). CMS produces 100% male sterile progeny and is widely utilized because it facilitates efficient hybrid seed production and eliminates the need for manual emasculation. CMS can be maintained through crossing with maintainer or B lines (N rrf), allowing CMS lines to be directly used as A lines in hybrid seed production systems. Hybrid formation in CMS systems is achieved by crossing CMS or A lines (S rrf) with restorer or R lines (S/N RfRf), producing hybrids with the genotype S Rrf, as described by Xu et al. (2022). This genetic mechanism enables further development of CMS lines through CMS identification using hybrid varieties as female parents (S Rrf), which are subsequently testcrossed with maintainer lines as male parents

(N rrf), or through self-pollination. Data from the Directorate of Horticultural Seed (2026) indicate that approximately 77% of released curly red chili and large red chili cultivars are hybrid varieties. The large number of hybrid varieties available in Indonesia indicates that the country possesses a substantial source of genetic material that could potentially be used as a source of CMS. Although CMS genetic resources are available at the World Vegetable Center (AVRDC), using commercial hybrid varieties as a CMS source is more practical because the seeds are readily available locally. Exploration of CMS through commercial hybrid varieties offers an efficient alternative strategy for identifying CMS lines, maintainer lines, and even restorer lines without reliance on CMS genetic resources from international institutions, while simultaneously supporting the independent development of hybrid chili breeding programs. Commercial hybrid exploration can be conducted by utilizing the segregation of the Rf/rf gene present in hybrid seeds produced through the CMS system. Previous studies have shown that maintainer and restorer lines can be identified from hybrid germplasm through testcrosses with CMS lines (Mankare & Korekar, 2017), confirming the potential of hybrid varieties in CMS-based breeding systems. Nikornpun et al. (2023) successfully developed CMS lines from superior hybrid varieties through crossing and self-pollination. Based on these findings, further studies are needed to develop CMS and maintainer lines for hybrid seed production by utilizing commercial hybrid varieties available in Indonesia. Therefore, this study used nine commercial hybrid varieties as female parents and one IPB maintainer line as the male parent to identify and evaluate CMS and maintainer genotypes through testcross and self-pollination techniques.

Materials and Methods

The study was conducted from April to September 2025 at the experimental field of CV. Benih Dramaga, Bogor Regency, West Java, Indonesia, located at approximately 200 m above sea level (asl). During the experimental

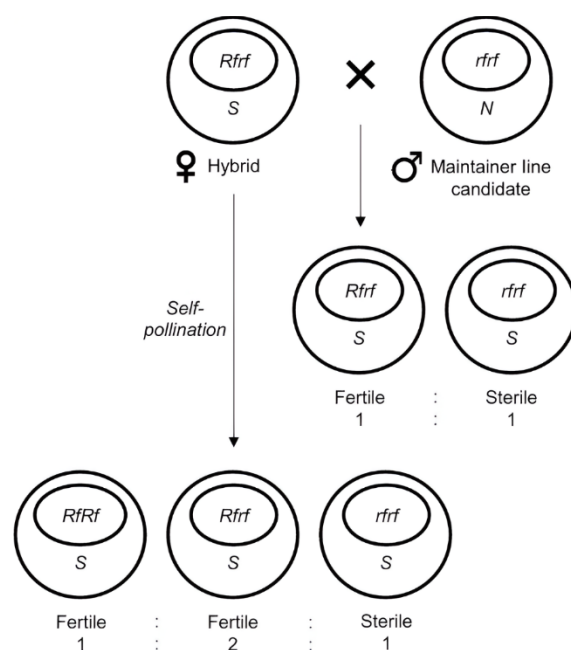
period, daily temperatures ranged from 22.73 to 32.25 °C, with an average relative humidity of 84.22% and a mean rainfall of 11.09 mm per day (Meteorology, Climatology, and Geophysics Agency, 2025). This experiment began with the development of F1 and F2 populations in the greenhouse of CV Benih Dramaga. The F1 populations were generated by testcrossing nine commercial hybrid varieties as female parents with a maintainer line candidate collection IPB (IPB379) as the male parent. These crosses produced nine genotypes, namely IPB432 × IPB379, IPB433 × IPB379, IPB434 × IPB379, IPB435 × IPB379, IPB436 × IPB379, IPB437 × IPB379, IPB438 × IPB379, IPB439 × IPB379, and IPB440 × IPB379. The F2 populations were generated by self-pollinating the nine hybrid varieties and producing the following genotypes, namely, F2-IPB432, F2-IPB433, F2-IPB434, F2-IPB435, F2-IPB436, F2-IPB437, F2-IPB438, F2-IPB439, and F2-IPB440. The crossing and self-pollination schemes are presented in Figure 1. Seeds obtained from both testcrossing and self-pollination were sown and transplanted simultaneously in the experimental field. Crop management practices included irrigation, replanting, shoot pruning, fertilization, and control of plant pests and diseases.

Plant fertility status was determined by examining the presence or absence of pollen in fully opened flowers, with ten flowers observed per plant. The F1 testcross populations had an expected segregation ratio 1:1 (50% fertile : 50% sterile), whereas the F2 populations had an expected segregation ratio 3:1 (75% fertile : 25% sterile). Anther length and width, filament length, as well as the stamen length, were measured by averaging ten samples. Stamen length, defined as the combined length of filament and anther, was measured to describe overall stamen size. Measurements were taken from representative flowers on different plants in the F1 populations and from the same plant in the F2 populations. Fertility status was coded as binary (1 = fertile, 0 = sterile) for correlation analysis. Stigma exsertions were assessed qualitatively according to the descriptors of International Plant Genetic Resources Institute (1995). The

experiment employed a randomized complete block design with genotype as a single factor. The treatments consisted of nine genotypes derived from testcrossing and nine genotypes derived from self-pollination, resulting in 18 treatment levels. Each treatment was replicated three times, producing a total of 54 experimental units. Each experimental unit consisted of ten plants, giving a total population of 540 plants. Data analysis was performed using RStudio software version 4.4.3, Microsoft Excel, and ImageJ. Quantitative data were analyzed using *t* test and the relationship between floral traits and fertility status was analyzed using point-biserial correlation. Segregation data were evaluated using the Chi-square (χ^2) test based on frequency data and stigma exsertion was analyzed descriptively. All analyses assumed independence of observations.

Figure 1

Schematic Diagram of Cross-Pollination and Self-Pollination in the Formation of F1 and F2 Populations and the Expected Ratio of Fertile and Sterile Plants in Cross-Pollination and Self-Pollination Results



Results and Discussion

Fertility Status Segregation Ratio

The segregation patterns observed in both cross and self-pollinated progenies were evaluated for conformity with CMS inheritance. The results in Table 1 demonstrated that eight hybrid cross–maintainer combinations, namely IPB432 × IPB379, IPB433 × IPB379, IPB434 × IPB379, IPB435 × IPB379, IPB436 × IPB379, IPB437 × IPB379, IPB439 × IPB379, and IPB440 × IPB379, exhibited segregation ratios that did not differ significantly from the expected 1:1 ratio (50% fertile and 50% sterile plants). The F2 populations derived from self-pollination of these eight hybrids, namely, IPB432, IPB433, IPB434, IPB435, IPB436, IPB437, IPB439, and IPB440 also followed the expected 3:1 segregation

ratio (75% fertile and 25% sterile plants). The 3:1 segregation ratio of fertile to sterile plants observed in the F2 chili population is consistent with previous findings reported in other crops, including commercial pearl millet hybrids (Kanchana & Kalra, 2024) and cotton (Pei et al., 2024). The segregation ratios observed in both populations showed that eight of the nine hybrid varieties used as female parents were indicated to carry the CMS gene, suggesting that they were likely developed using a CMS-based hybridization system. The 1:1 segregation ratio in the testcross populations and the 3:1 segregation ratio in the selfing populations indicated that most of the tested varieties likely had the S Rf₁ genotype. This inheritance model was based on the assumption that fertility was controlled by a single Rf gene locus with a dominant Rf allele and a recessive rf allele. The presence of

Table 1

Results of Fertility Status Ratio Analysis of Nine Genotypes in F1 Testcross and F2 Populations Using Chi-Square (X^2)

Genotype	Categories		Expected ratio	X^2	X^2 table	P
	Fertile	Sterile				
IPB432 × IPB379	11	19		2.13 ^{ns}		0.14
IPB433 × IPB379	9	7		0.25 ^{ns}		0.62
IPB434 × IPB379	5	3		0.50 ^{ns}		0.48
IPB435 × IPB379	15	15		0.00 ^{ns}		1.00
IPB436 × IPB379	15	15	1:1	0.00 ^{ns}	3.84	1.00
IPB437 × IPB379	19	11		2.13 ^{ns}		0.14
IPB438 × IPB379	30	0		30.00 ^{**}		<0.0001
IPB439 × IPB379	19	11		2.13 ^{ns}		0.14
IPB440 × IPB379	17	13		0.53 ^{ns}		0.47
F2-IPB432	25	5		1.11		0.29
F2-IPB433	19	11		2.18		0.14
F2-IPB434	21	8		0.10 ^{ns}		0.75
F2-IPB435	20	10		1.11 ^{ns}		0.29
F2-IPB436	22	8	3:1	0.04 ^{ns}	3.84	0.83
F2-IPB437	18	11		2.59 ^{ns}		0.11
F2-IPB438	30	0		10.00 ^{**}		0.0016
F2-IPB439	20	10		1.11 ^{ns}		0.29
F2-IPB440	23	7		0.04 ^{ns}		0.83

Notes. Calculated X^2 < Table X^2 , meaning accept H0, the observed results are not significantly different from the expected ratio (1:1 or 3:1). ** = significant at α = 1%; ns = not significant at α = 5%.

a single dominant locus for fertility restoration in chili pepper has also been reported, namely *CaRf032* on chromosome 6, with the candidate gene *CA00g82510* (Zhang et al., 2020).

The cross IPB438 × IPB379 was the only combination that deviated from the expected segregation pattern, as all progeny were fertile. The results showed that IPB379 did not act as a maintainer line in the cross combination with IPB438. This phenotypic outcome suggests that IPB438 may not have been developed using a CMS-based hybridization system, resulting in no detectable evidence of CMS expression in its progeny. Another possible explanation is that this result is influenced by the specificity of the interaction between the CMS source and the fertility restorer (*Rf*) genes, such that a maintainer line might not necessarily be able to maintain CMS expression across all CMS sources with different genetic backgrounds. Similar phenomena have been widely documented in rice (*Oryza sativa* L.), where interactions between CMS cytoplasm and fertility restorer genes (*Rf*) are highly specific and play a decisive role in determining male sterility or fertility restoration (Reddy et al., 2025). In addition, the differences in genetic background between IPB379 (curly red chili) and IPB438 (large red chili) might also have affected the interaction between the CMS cytoplasm and the fertility restorer (*Rf*) genes, even though both belong to the same species (*Capsicum annuum* L.). However, the selfing results in the F₂ population also showed that all IPB438 progeny remained fertile. In this test, the maintainer line was no longer involved, so the observed fertility reflected the genetic background of IPB438 without being influenced by interactions with the maintainer line. Therefore, the results of this study suggested that IPB438 was not developed through a CMS-based hybridization system, indicating that it did not carry the cytoplasm responsible for male sterility.

Evaluation of F₁ testcross populations from other testcross combinations confirmed that IPB379 is indicated to function as a maintainer line by consistently maintaining CMS expression patterns in eight tested hybrid varieties. These results were supported by

evaluations of the F₂ populations, which also showed segregation patterns consistent with the CMS inheritance model in those eight genotypes. This observation aligns with findings by Nikornpun et al., (2023), who reported that maintainer lines are capable of sustaining CMS inheritance in chili progeny. Gaswanto (2021) also reported that male parent genotypes can be classified as maintainer lines when their progeny consistently exhibit male sterility. The availability of stable CMS and maintainer lines derived from commercial hybrid varieties provides a practical strategy for improving seed purity and reducing the need for manual emasculation in hybrid seed production. This approach has the potential to improve seed production efficiency and support the development of hybrid chili varieties for both industrial-scale breeding programs and smallholder seed producers. In addition, the method used in this study can serve as an effective approach for exploring CMS sources and identifying maintainer lines using commercial hybrid varieties.

Quantitative Morphological Traits of Flowers in Fertile and Sterile Plants

Nikornpun et al. (2023) reported that flowers of CMS lines or sterile plants possess smaller anthers than those of fertile plants. This observation agrees with the results presented in Figures 2 and 3, where sterile flowers (Figure 3) display visibly smaller anthers compared with fertile flowers (Figure 2). Variation in anther size and shape between fertile and sterile plants is also evident in these figures. Anthers of sterile plants appear slender, reduced, or extremely small relative to those of fertile plants. Based on these morphological differences, a *t* test was conducted for anther width, anther length, filament length, and stamen length to assess differences between sterile and fertile plants. The results of the *t* test (Table 2) demonstrated that anther width, anther length, and stamen length differed significantly between sterile and fertile plants in both the F₁ and F₂ populations. Sterile plants exhibited a mean anther width of 0.88 mm in the F₁ population and 0.71 mm in the F₂ population,

Figure 2

Appearance of Several Flowers on Fertile Plants



Figure 3

Appearance of Several Flowers on Sterile Plants



Table 2

Results of t test for Several Characteristics in the F1 Testcross and F2 Populations

Population	Variable	Mean sterile (mm)	Mean fertile (mm)	P value
F1	Anther width	0.88	1.31	**
	Anther length	2.69	3.31	**
	Filament length	1.57	1.61	ns
	Stamen length	4.26	4.92	**
F2	Anther width	0.71	1.09	**
	Anther length	2.03	2.60	**
	Filament length	1.43	1.32	ns
	Stamen length	3.46	3.92	**

Note. ** = significant at $\alpha = 1\%$; ns = not significant at $\alpha = 5\%$.

values that were lower than those observed in fertile plants, which averaged 1.31 mm in the F1 and 1.09 mm in the F2. Mean anther length was also reduced in sterile plants, measuring 2.69 mm in F1 and 2.03 mm in F2, whereas fertile plants showed longer anthers with mean values of 3.31 mm in F1 and 2.60 mm in F2. Stamen length followed a similar trend, with fertile plants exhibiting significantly greater values than sterile plants, averaging 4.92 mm versus 4.26 mm in the F1 population and 3.92 mm versus 3.46 mm in the F2 population. Filament length showed no significant differences between sterile and fertile plants in either the F1 or F2 populations ($P > 0.05$). Differences in anther, filament, and stamen traits are illustrated using boxplots in Figures 4 and 5. Boxplot visualizations in Figures 4 and 5 were used to support the t test results. Clear differences in anther width, anther length, and stamen length between fertile and sterile plants are evident in the F1 population (Figures 4a, 4b, and 4d), as reflected by higher median values and wider interquartile ranges in fertile plants. Comparable patterns were observed in the F2 population for the same traits (Figures 5a, 5b, and 5d). These patterns are consistent with the significant differences detected by the t test in both populations. Based on visual observation, the anthers of sterile plants did not produce pollen and were therefore smaller than those of fertile plants, which produced normal pollen. This observation is consistent with previous findings by Pei et al. (2022), who reported that anthers of sterile plants either lack pollen entirely or contain damaged, non-viable cells, whereas fertile plants produce highly viable pollen as determined by acetocarmine staining. In contrast, filament length showed inconsistent patterns between fertile and sterile plants in both the F1 (Figure 4c) and F2 (Figure 5c) populations, with overlapping median values and interquartile ranges, supporting the non-significant t test results for this trait.

At the molecular level, several genes have been identified as key regulators of anther and pollen development in pepper (*Capsicum annuum* L.). Genes such as *PAP3*, *CaMF3*, and *CaAMS* exhibit specialized functions in male reproductive

tissues. Disruption or downregulation of these genes leads to impaired anther development, pollen abortion, and male sterility, as evidenced by pollen shrinkage, malformed or indehiscent anthers, and reduced pollen viability (Guo et al., 2018; Hao et al., 2012; Ma et al., 2016). In CMS systems, these developmental abnormalities are closely linked to mitochondrial dysfunction. Brownfield (2021) demonstrated that pollen non-viability in CMS plants is caused by the expression of chimeric mutant open reading frames (ORFs) that disrupt the mitochondrial electron transport chain and ATP synthesis in the inner mitochondrial membrane, resulting in oxidative stress and insufficient energy supply for normal anther and pollen development. In addition, according to Wang et al. (2026), male sterility occurs due to low or stable expression patterns of key genes such as *SAUR*, *A-ARR*, *GH3*, *CKX7*, *CISZOG*, *CRE1*, *AHP*, and *TIR1*, which lead to an imbalance of auxin and cytokinin hormones, thereby causing pollen development to be inhibited or become abnormal. These molecular and physiological disruptions are likely to affect anther development, resulting in the smaller anther size observed in sterile plants. Therefore, the pronounced differences in anther size between fertile and sterile plants may support early identification of plants consistent with CMS expression, enabling more efficient selection during breeding and line development.

Correlation Analysis

Correlation analysis was also performed for anther width, anther length, filament length, and stamen length to assess the relationships among traits and their association with fertility status. According to Gomez and Gomez (1984), simple linear correlation measures the strength and direction of a linear relationship between two variables. The correlation coefficient (r) ranges from -1 to +1, with values approaching +1 or -1 indicating strong positive or negative linear relationships, respectively, while values near zero indicate the absence of a linear relationship. The proportion of variation in one variable that can be explained by variation in another variable

Figure 4

Comparison of Flower Traits Between Fertile and Sterile Plants in the F1 Testcross Populations: (a) Anther Width, (b) Anther Length, (c) Filament Length, and (d) Stamen Length

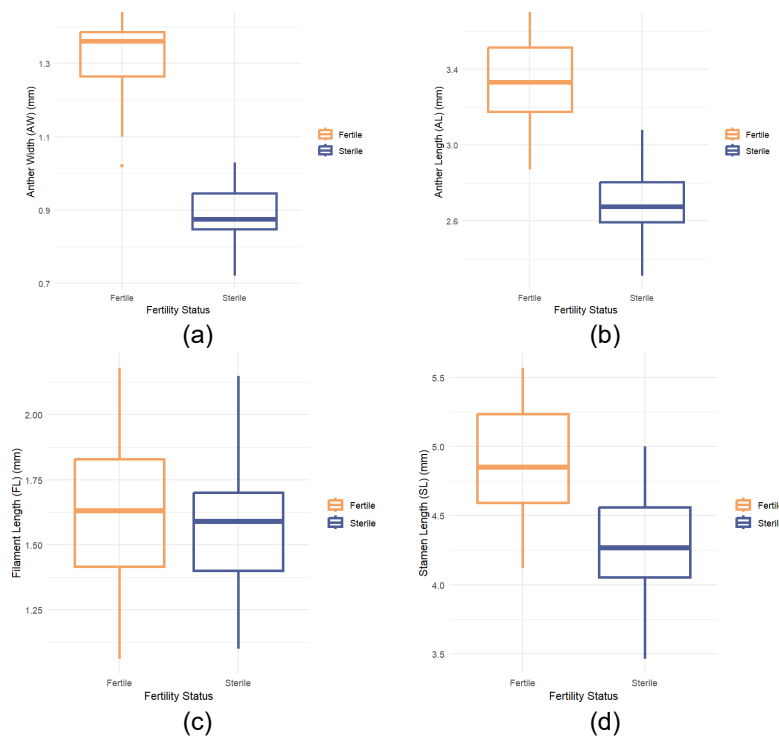
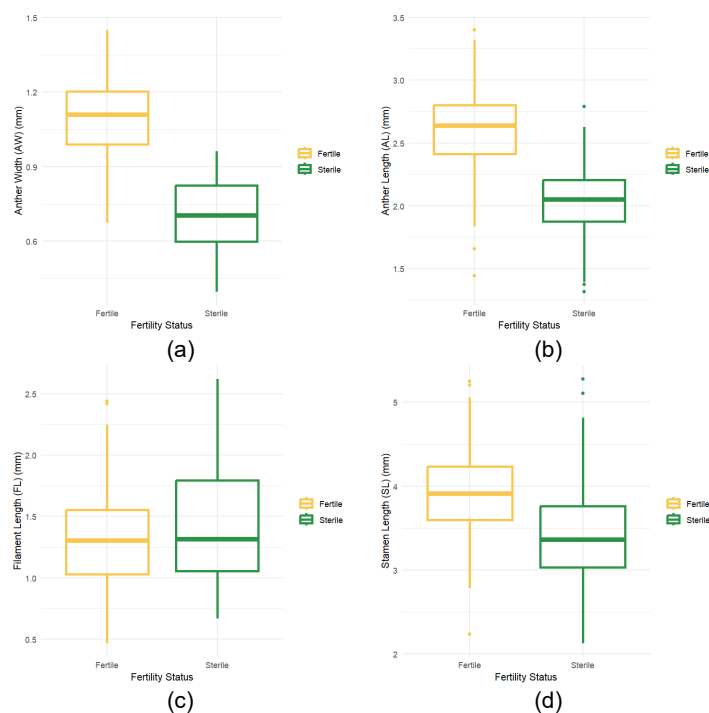


Figure 5

Comparison of Flower Traits Between Fertile and Sterile Plants in the F2 Populations: (a) Anther Width, (b) Anther Length, (c) Filament Length, and (d) Stamen Length



is expressed as the coefficient of determination (r^2). Calculation of the correlation coefficient does not require prior identification of independent or dependent variables.

In the F1 population (Figure 6a), fertility status exhibited strong positive correlations with anther width ($r = 0.91$), anther length ($r = 0.83$), and stamen length ($r = 0.65$). The highest coefficient of determination was observed for anther width ($r^2 = 82\%$), followed by anther length ($r^2 = 68.9\%$). A similar pattern was observed in the F2 population (Figure 6b), where fertility status exhibited strong positive correlations with anther width ($r = 0.74$) and anther length ($r = 0.64$), whereas the correlation between stamen length and fertility status was weak. The coefficient of determination in the F2 population was also highest for anther width, explaining 54% of the variation in fertility status, followed by anther length, which accounted for 40.1%. These results indicate that anther width has the strongest association with fertility status, followed by anther length, compared with other measured traits in both populations. Stamen length showed strong and positive correlations with anther length, anther width, and filament length in both the F1 and F2 populations. Anther width was also strongly and positively correlated with anther length. Filament length exhibited weak positive to negative correlations with anther width, anther length, and fertility status in both populations. Similar results were reported by Manjunath et al. (2024), who found that long filament length in CMS cauliflower lines showed weak and non-significant correlations with anther length and long stamen length, whereas short filament length was strongly and significantly correlated with short stamen length. The strong associations between anther width, anther length, and fertility status indicate that these traits can be used as potential selection criteria for distinguishing fertile and sterile plants in CMS-based chili breeding programs.

The Types of Stigma Exsertion in Fertile and Sterile Plants

According to International Plant Genetic Resources Institute (1995), chili flowers exhibit three types of stigma exsertion (Figure 7); exserted, in which the stigma is positioned above the anthers; same level, in which the stigma and anthers are at the same height; and inserted, in which the stigma is positioned below the anthers. Observations of stigma exsertion in fertile and sterile plants in the F1 and F2 populations are presented in Figure 8. Similar patterns of stigma exsertion were observed in both populations. Fertile plants exhibited all three stigma types, including exserted, same level, and inserted. Sterile plants displayed only exserted and same level stigma types. The absence of the inserted stigma type in sterile plants is likely associated with reduced anther size, which prevents the stigma from being positioned below anthers. In the F1 population, sterile plants showed 89% exserted and 11% same level stigma types, whereas fertile plants showed 64% exserted, 18% same level, and 18% inserted stigma types. In the F2 population, sterile plants exhibited 98% exserted and 2% same level stigma types, while fertile plants showed 71% exserted, 18% inserted, and 11% same level stigma types. The exserted stigma type was the most frequently observed in both fertile and sterile plants across both populations. Similar observations were reported by Htwe et al. (2017), who found that exserted stigma types were more prevalent than other stigma types in the F2 segregation populations. The predominance of the exserted stigma type facilitates cross-pollination and increases crossing efficiency. This characteristic is advantageous for hybrid seed production in CMS-based systems, because it can improve the efficiency of hybridization. Ritonga et al. (2018) reported that stigma exsertion length was positively correlated with the percentage of natural cross-pollination; therefore, the greater the stigma exsertion, the higher the rate of natural cross-pollination.

Figure 6

Correlation Matrix Between Fertility Status (FS), Anther Length (AL), Anther Width (AW), Filament Length (FL), and Stamen Length (SL) on F1 Testcross Population (a) and F2 Population (b)

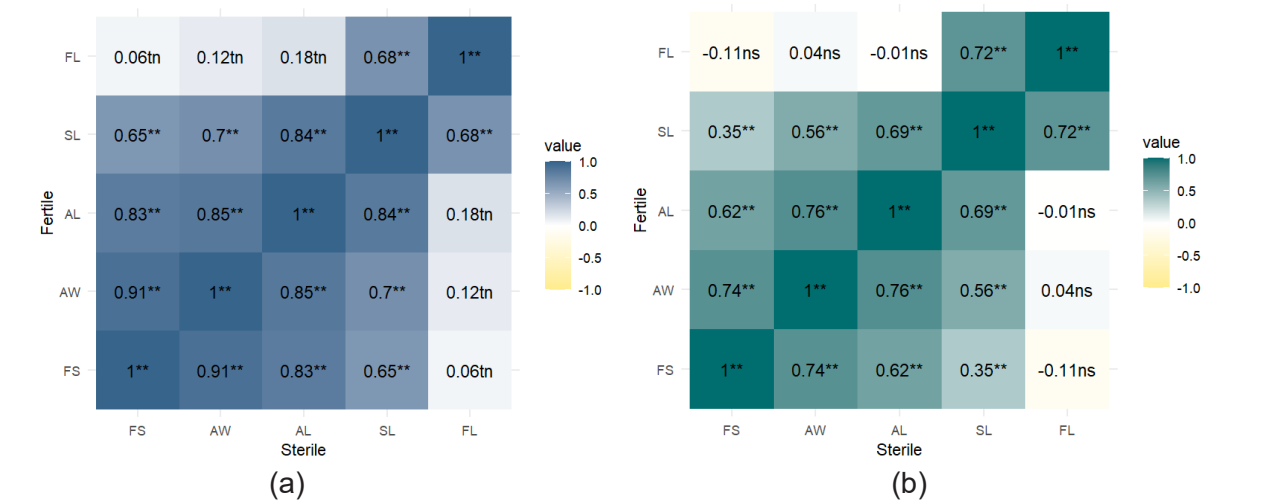


Figure 7

Types of Stigma Exsertion Based on International Plant Genetic Resources Institute (1995): Exserted (a), Same Level (b), Inserted (c)

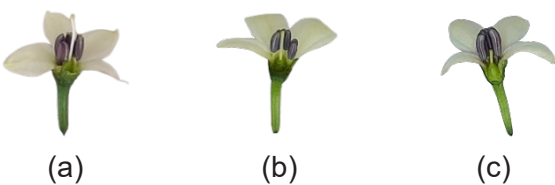
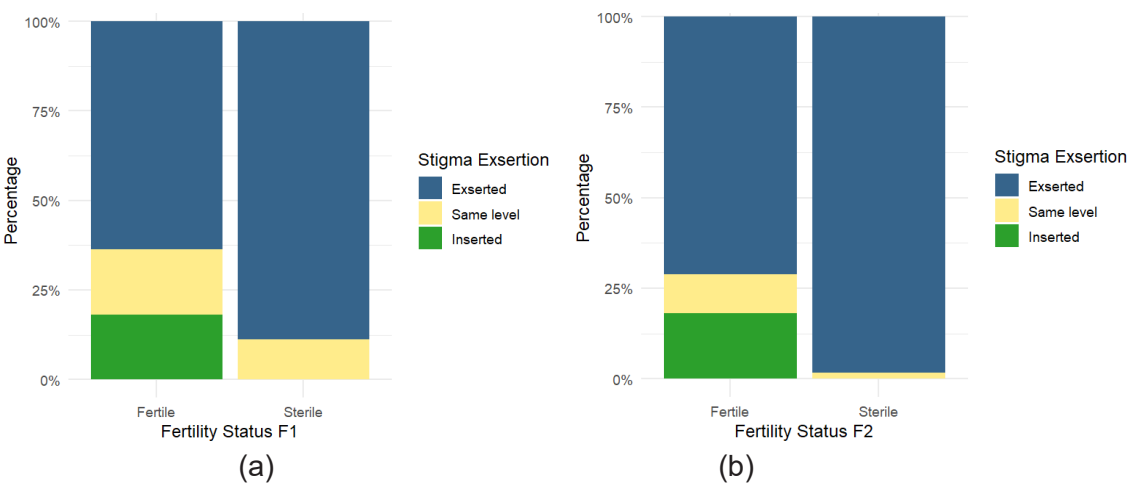


Figure 8

Percentage of Stigma Exsertions Based on Fertility Status of F1 Testcross Population (a) and F2 Population (b)



Conclusions

The chili pepper hybrids IPB432, IPB433, IPB434, IPB435, IPB436, IPB437, IPB439, and IPB440 were presumed to carry the CMS gene based on the 1:1 segregation ratio in the testcross populations and the 3:1 segregation ratio in the selfing populations. The chili pepper line IPB379 has the potential to serve as a maintainer line because it produced a 1:1 testcross segregation ratio in eight hybrid combinations. In addition, CMS chili pepper plants had smaller anther length and width than fertile plants due to reduced pollen production.

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References

- Berke, T. G. (1999). Hybrid seed production in *Capsicum*. *Journal of New Seeds*, 1(3–4), 49–67. https://doi.org/10.1300/J153v01n03_02
- Bhut, N. M., Chauhan, R. M., Parmar, B. R., Patel, P. T., Prajapati, K. N., Viradiya, Y. A., & Vavdiya, P. A. (2023). Heterosis studies in chilli (*Capsicum annuum* L.) by using CMS lines. *The Pharma Innovation Journal*, 12(12), 3950–3955.
- Brownfield, L. (2021). Plant breeding: Revealing the secrets of cytoplasmic male sterility in wheat. *Current Biology*, 31(11), R724–R726. <https://doi.org/10.1016/j.cub.2021.04.026>
- Directorate of Horticultural Seed. (2025). *Daftar varietas terdaftar hortikultura*.
- Fujimoto, R., Uezono, K., Ishikura, S., Osabe, K., Peacock, J., & Dennis, E. S. (2018). Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems. *Breeding Science*, 68, 145–158. <https://doi.org/10.1270/jsbbs.17155>
- Gaswanto, R. (2021). Sterility of 20 F1 genotypes derived from hybridization of several chili lines with M1 male sterile. *IOP Conference Series: Earth and Environmental Science*, 807, 032030. <https://doi.org/10.1088/1755-1315/807/3/032030>
- Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research* (2nd ed.). John Wiley & Sons.
- Guo, J., Liu, C., Wang, P., Cheng, Q., Sun, L., Yang, W., & Shen, H. (2018). The aborted microspores (AMS)-like gene is required for anther and microspore development in pepper (*Capsicum annuum* L.). *International Journal of Molecular Sciences*, 19, 1341. <https://doi.org/10.3390/ijms19051341>
- Hao, X., Chen, C., Chen, G., Cao, B., Chen, Q., & Lei, J. (2012). Isolation and characterization of CaMF3, an anther-specific gene in *Capsicum annuum* L. *Genetics and Molecular Biology*, 35(4), 810–817. <https://doi.org/10.1590/S1415-47572012005000057>
- Htwe, C. S. S., Thwe, A. A., Htwe, N. M., Aung, M. Z. N., & Myint, K. T. (2017). Variation and segregation in F2 population of hot pepper (*Capsicum annuum*). *Journal of Agricultural Research*, 4(2), 86–94.
- International Plant Genetic Resources Institute. (1995). *Descriptors for Capsicum (Capsicum spp.)*. International Plant Genetic Resources Institute.
- Kanchana, S. K., & Kalra, N. (2024). Inheritance pattern of A1 cytoplasmic-nuclear male sterility system in commercial pearl millet hybrids [*Pennisetum glaucum* (L.) R. Br.] in summer and post-rainy seasons. *Indian Journal of Agricultural Research*, 58(2), 195–201. <https://doi.org/10.18805/IJARE.A-6158>

- Ma, N., Liu, C., Yang, W., & Shen, H. (2016). PAP3 regulates stamen but not petal development in *Capsicum annuum* L. *Horticultural Plant Journal*, 2(2), 91–96. <https://doi.org/10.1016/j.hpj.2016.02.009>
- Manjunath, K. S., Singh, S., Kalia, P., Mangal, M., Sharma, B. B., Singh, N., Ray, M., Rao, M., & Tomar, B. S. (2024). Commercial suitability and characterization of newly developed *Erucastrum canariense* (Can) sterile cytoplasm-based cytoplasmic male sterile (CMS) lines in Indian cauliflower. *Scientific Reports*, 14, 2346. <https://doi.org/10.1038/s41598-024-52714-z>
- Mankare, S., & Korekar, S. L. (2017). Identification of restorer and maintainer lines in chilli (*Capsicum annuum* L.). *International Journal of Pure and Applied Bioscience*, 5, 1255–1257.
- Meteorology, Climatology, and Geophysics Agency. (2025). *Daily data from the West Java climatology station for April–September 2025*.
- Naves, E. R., Scossa, F., Araujo, W. L., Nesi, A. N., Fernie, A. R., & Zsögön, A. (2022). Heterosis for capsaicinoids accumulation in chili pepper hybrids is dependent on parent-of-origin effect. *Scientific Reports*, 12, 14450. <https://doi.org/10.1038/s41598-022-18711-w>
- Nikornpun, M., Sukwiwat, K., Wongsing, K., & Kumchai, J. (2023). Development of male sterile lines of CMS chillies (*Capsicum annuum* L.) from F1 hybrids. *Breeding Science*, 73(2), 158–167. <https://doi.org/10.1270/jsbbs.22042>
- Pei, H., Xie, H., Wang, X., Yan, X., Wang, B., Feng, H., Zhao, Y., Gao, J., & Gao, J. (2022). Proteomic analysis of differential anther development from sterile/fertile lines in *Capsicum annuum* L. *PeerJ*, 1–24. <https://doi.org/10.7717/peerj.13168>
- Pei, Q., Liu, J., Guo, C., Ma, X., Liu, X., You, C., Lin, H., Li, Z., Zhao, R., Zhu, B., Wu, Y., Pan, Z., & Nie, X. (2024). Morphological and cytological assessments reveal pollen degradation causes pollen abortion in cotton cytoplasmic male sterility lines. *Journal of Cotton Research*, 7, 26. <https://doi.org/10.1186/s42397-024-00189-8>
- Peterson, P. A. (1958). Cytoplasmically inherited male sterility in Capsicum. *The American Naturalist*, 92(863), 111–119. <https://doi.org/10.1086/282017>
- Reddy, V. S., Reddy, V. R., Srinivas, B., Balram, N., & Balram, M. (2025). Identification of potential restorers and maintainers with high zinc in hybrid rice (*Oryza sativa* L.). *Journal of Advances in Biology and Biotechnology*, 28(10), 687–695. <https://doi.org/10.9734/jabb/2025/v28i103092>
- Ritonga, A. W., Syukur, M., Yunianti, R., & Sobir. (2018). Assessment of natural cross pollination levels in chili pepper (*Capsicum annuum* L.). *IOP Conference Series: Earth and Environmental Science*, 196, 012008. <https://doi.org/10.1088/1755-1315/196/1/012008>
- Sahid, Z. D., Syukur, M., & Maharijaya, A. (2020). Diversity of capsaicin content, quantitative, and yield components in chili (*Capsicum annuum*) genotypes and their F1 hybrid. *Biodiversitas*, 21(5), 2251–2257. <https://doi.org/10.13057/biodiv/d210555>
- Shifriss, C. (1997). Male sterility in pepper (*Capsicum annuum* L.). *Euphytica*, 93(1), 83–88. <https://doi.org/10.1023/A:1002947907046>
- Swamy, B. N., Sharma, V. K., Arpita, S., Kumar, R., Behera, T. K., Manisha, M., Chandresh, C., & Mukesh, K. (2019). Maintenance and fertility restoration of CMS system in sweet pepper (*Capsicum annuum*). *Indian Journal of Agricultural Sciences*, 89(11), 103–107. <https://doi.org/10.56093/ijas.v89i11.95314>
- Syukur, M., Maharijaya, A., Nurcholis, W., Ritonga, A. W., Pangestu, A. Y., Hatta, A. N. N. L., Istiqlal, M. R. A., Hakim, A., & Sahid, Z. D. (2024). Combining ability of *Capsicum annuum* hybrid for antioxidant activities, polyphenol content, α-glucosidase inhibitory, yield, and yield components. *Current Issues in Molecular Biology*, 46, 11700–11713. <https://doi.org/10.3390/cimb46100695>

- Syukur, M., Sujiprihati, S., & Yuniarti, R. (2012). *Teknik pemuliaan tanaman*. Penebar Swadaya.
- Wang, M., Zhao, H., Wu, X., Li, Z., Zhao, Z., Gong, M., Huang, L., & Wang, R. (2026). Comparative transcriptome analysis provides insights into the gene regulation network of cytoplasmic male sterility in chili pepper. *AoB Plants*, 18, 1–12. <https://doi.org/10.1093/aobpla/plaf070>
- Xu, F., Yang, X., Zhao, N., Hu, Z., Mackenzie, S., Zhang, M., & Yang, J. (2022). Exploiting sterility and fertility variation in cytoplasmic male sterile vegetable crops. *Horticulture Research*, 9, 1–10. <https://doi.org/10.1093/hr/uhab039>
- Yang, C. J., Chen, B. J., Liu, J. G., & Li, S. (2008). Study on the superiority of hybrid breeding by male sterility in hot pepper. *Journal of China Capsicum*, 1, 36–3
- Zhang, Z., Zhu, Y., Cao, Y., Yu, H., Bai, R., Zhao, H., Zhang, B., & Wang, L. (2020). Fine mapping of the male fertility restoration gene CaRf032 in *Capsicum annuum* L. *Theoretical and Applied Genetics*, 133(4), 1177–1187. <https://doi.org/10.1007/s00122-020-03540-0>