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Evaluation of Agronomic and Genetic Diversity in M2V1 Generation of Marigold (*Tagetes erecta* L.)

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Abstract

Marigold (*Tagetes erecta* L.) is an ornamental plant widely used as a potted or cut flower due to its vibrant colors and diverse flower shapes. High genetic diversity is essential for successful breeding programs aimed at developing superior varieties. Genetic information can be obtained by estimating variance components and heritability values, key parameters in plant breeding. A high heritability value suggests that genetic factors influence a plant's characteristics more than environmental factors. This study assessed the genetic diversity and performance of vegetative propagation from 2nd generation mutant plants (M2V1). The research was conducted at the Pasir Sarongge experimental field in Cianjur Regency from July to October 2023, using a randomized complete block design with 17 genotypes and three replicates. The genotypes included 14 from vegetative propagation of M2V1 mutant plants and three comparison varieties: "Maharani", "BAIP 2", and "BAIP 3". Data analysis was performed using PKBT-STAT 3.1 software, followed by the honest significant difference (HSD) test at the 5% level. The results revealed significant differences among genotypes in plant height, stem diameter, canopy width, leaf width, flower weight, flower diameter, number of flowers per plant, total flower weight per plant, and flower stalk length. Floret types observed were either all ligulate or all tubuligulate, with flower colors ranging from orange to yellow to white. Traits such as canopy width, flower weight, flower diameter, and stalk length, which exhibited broad genetic diversity and high heritability, can be used for selection in future marigold breeding programs.

Keywords: genotypes, heritability, ornamental, plant breeding, variance

Introduction

Marigold (*Tagetes erecta* L.), a member of the Asteraceae family, is an ornamental plant native to the Americas, now widely cultivated across Africa, Asia, and Europe (Salehi et al., 2018). With its vibrant flower colors and diverse forms, marigolds are popular for potted plants and cut flower arrangements. In Indonesia, particularly on the island of Bali, marigolds, known locally as "Gemitir," play a significant role in religious practices. Hindu communities in Bali frequently use marigold flowers in religious ceremonies. According to Suryanti et al. (2019), demand for marigolds spikes during Hindu holidays, causing prices to rise to 40,000–50,000 rupiah per kilogram. Beti (2020) reported that the daily demand for marigolds in Bali can reach up to 8 tons per day.

Beyond religious use, marigolds are also popular as ornamental plants in parks and gardens, contributing to the tourism sector. This underscores the potential for further development in marigold cultivation, particularly regarding flower color and form variations. Currently, marigold genetic diversity is mostly limited to shades of orange and yellow, highlighting a need to expand the range, especially with white flowers. Induced mutation techniques offer a way to enhance genetic diversity and create new plant variants more rapidly. In this study, the chemical mutagen ethyl methane sulfonate (EMS) was applied to the marigold genotype MG04, which produces yellowish-white flowers (initial population, M0). The resulting M1 population (first-generation mutants) included some promising variants with ray floret (ligulate) flowers that were whiter than those in the initial population (Lenawaty et al., 2022).

The primary attraction of the marigold flower lies in its ray florets (ligulate flowers), which consist entirely of ray florets and lack a central disc floret. Unlike the hermaphroditic disc florets, these ray florets

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are sterile and cannot produce seeds. As a result, vegetative propagation is required to maintain the desired characteristics of mutated plants. Vegetative propagation ensures true-to-type plants, preserving the desired traits (Dawane et al., 2015). This method is particularly useful for producing planting materials for marigolds. To evaluate the characteristics of the mutated plants, vegetative propagation from second-generation mutant plants (M2V1) must be conducted. Genetic diversity plays a critical role in the success of breeding programs aimed at developing superior varieties. To assess the genetic influence on plant characteristics, researchers estimate variance components and heritability values. Heritability is a key parameter in plant breeding, representing the proportion of phenotypic variation due to genetic factors rather than environmental influences (Syukur et al., 2010). The expression of plant traits is typically influenced by a combination of genetic and environmental factors, and heritability values help quantify the likelihood of passing traits from parents to offspring. This study aims to provide information about the appearance and genetic diversity of second-generation (M2V1) marigold mutant plants.

Material and Methods

The experiment was conducted at the IPB Pasir Sarongge Experimental Farm, at 1,200 meters above sea level in Cianjur Regency, West Java, Indonesia, from July to October 2023. The average temperature during the study ranged from 21.70-22.90°C, and air humidity ranged from 76.10-83.00%. A randomized complete block design with one genotype factor was employed. Seventeen marigold genotypes were used as planting material. Fourteen genotypes that were vegetative propagation from 2nd generation mutant plants (M2V1) and three comparison varieties, “Maharani”, “BAIP 2”, and “BAIP 3”, were also included. Each genotype was propagated using eight shoot cuttings with three replicates, resulting in 408 cuttings. Six plants from each plot were used for data collection, for 306 observed plants.

The study evaluated quantitative characters such as plant height, leaf length, leaf width, stem diameter, canopy width, flower diameter from the first flower at full bloom, stalk length, number of flowers per plant, weight per flower, and flower weight per plant. Qualitative characters were assessed following the UPOV Marigold (2007) standard for plant habitus, floret type, leaf type, leaf color, and flower color based on the Royal Horticultural Society (RHS) color chart. ANOVA was performed on quantitative data to obtain the squared mean and squared expected mean values, which are used to estimate genetic variance and heritability values. Genetic and phenotypic variance components were estimated from the ANOVA results as described by Syukur *et al.* (2015). Significant F-test results were followed by the honest significant difference (HSD) tests at the 5% level. PKBT-STAT 3.1 software was used for data analysis.

The formula used to calculate genotypic and phenotypic variance and heritability estimates were: variance of genetic (σ^2_g) = (MSG-MSE)/r, variance of environments (σ^2_e) = MSE, variance of phenotype (σ^2_p) = $\sigma^2_g + \sigma^2_e$/r. Note: MSB = mean square of block, MSG = mean square of genotype, and MSE = mean square of error. Estimated value of broad-sense heritability (h^2_{bs}) was calculated as (h^2_{bs}) = σ^2_g / σ^2_p × 100%. Heritability values are classified into three categories according to Stansfield: low ($h^2 < 20\%$); medium ($20\% \leq h^2 < 50\%$); and high ($h^2 \geq 50\%$) (Priyanto et al., 2018). Characters with broad or narrow genetic diversity can be determined based on the value of genetic variance and standard deviation using the following formula:

$$\sigma_{\sigma^2 g} = \sqrt{\frac{2}{r^2}\left(\frac{(MSG)^2}{df\,g+2} + \frac{(MSE)^2}{df\,e+2}\right)}$$

Note: df g = degree of freedom genotypes, df e = degree of freedom error.

A character has broad genetic diversity if the value of genetic variance is at least twice the standard deviation of its genetic variation ($\sigma^2 g \geq 2\sigma g$), whereas narrow genetic diversity is found if the value of genetic variance is less than twice the standard deviation ($\sigma^2 g \leq 2\sigma g$) (Syukur et al., 2010).

Result and Discussion

Analysis of variance revealed significant differences among the marigold genotypes used in this experiment for several plant characters, including plant height, stem diameter, canopy width, leaf width, weight per flower, flower diameter, number of flowers per plant, flower weight per plant, and stalk length (Table 1). This genotype diversity can be attributed to genetic and environmental influences. The mean squares from the ANOVA can be used to estimate environmental variance, genotypic variance, and phenotypic variance, providing information on the extent to which genetics contribute to the observed diversity of these characters.

Mean values for plant height, stem diameter, canopy width, and leaf characteristics of the 17 tested marigold genotypes are presented in Table 2. The “BAIP 3”

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Table 1. Analysis of variance (ANOVA) of marigold characters

Characters	Range	MSG	MSE	F-values
Plant height (cm)	31.00–64.20	54.90	22.56	2.43*
Stem diameter (cm)	0.98–2.19	0.12	0.02	7.01**
Canopy width (cm)	35.67–69.00	119.81	32.14	3.73**
Leaf length (cm)	6.00–10.42	0.83	0.54	1.54ns
Leaf width (cm)	4.17–6.48	0.38	0.18	2.09*
Weight per flower (g)	1.10–19.26	58.99	1.16	50.91**
Flower diameter (cm)	2.08–8.21	7.57	0.07	104.94**
Number of flowers per plant	26.75–337.33	7714.16	3166.68	2.44*
Flower weight per plant (kg)	0.03–1.16	0.10	0.03	3.63**
Stalk length (cm)	3.83–7.33	1.85	0.28	6.55**

Note: * and ** = significantly at 5 and 1%, respectively, ns = not significantly, MSG = mean square of genotype, MSE = mean square of error

genotype exhibited the tallest plants (56.57±5.87 cm), though it did not differ significantly from most other genotypes. However, it was notably taller than genotype 08 (41.03±12.36 cm). The variation in plant height across genotypes may be attributed to differences in genetic expression. This finding aligns with the reports of Thirumalmurugan et al. (2020), Dahal et al. (2021), Tiwari et al. (2022), and Patel et al. (2023), who also observed genotype-specific variations in plant height among marigold varieties.

“BAIP 3” also had the largest stem diameter (1.80±0.34 cm), though it was not significantly different from most other genotypes. However, it had a significantly larger stem diameter than genotypes D22, L2, L5, L7, L13, and 08, which exhibited smaller diameters. Variations in stem diameter among the genotypes can likely be attributed to both genetic differences and environmental conditions. These results are consistent with findings from Srinivas and Rajasekharam (2020), who also reported variability in stem diameter among marigold genotypes.

In terms of canopy width, “BAIP 3” had the widest canopy (59.81±4.53 cm), significantly different from genotypes L2 and L5 (Table 2). Tanya and Kaur (2023) similarly observed that genotypes with greater plant height also tended to have wider canopies, suggesting a correlation between these traits.

Leaf length and width did not show significant differences across the tested genotypes, with leaf length ranging from 6.00–10.42 cm and leaf width from 4.17–6.48 cm. Regarding flower characteristics, “Maharani”, “BAIP 2”, and “BAIP 3” had the highest values for both flower weight and diameter compared to other genotypes (Table 3). Specifically, flower weight was as follows: “Maharani” (13.94±1.14 g), “BAIP 2” (15.65±3.26 g), and “BAIP 3” (12.36±0.51 g). Flower diameter followed a similar pattern: “Maharani” (7.34±0.12 cm), “BAIP 2” (7.58±0.65 cm), and “BAIP 3” (6.67±0.16 cm).

Following these three comparison varieties, genotypes L2 (6.83±0.40 g), L5 (6.42±1.06 g), L7 (7.61±1.65 g), and L13 (7.26±0.70 g) exhibited relatively high flower weights. Tanya and Kaur (2023) reported similar trends, noting that genotypes with larger flower diameters also tended to have heavier flowers.

Genotype D17 had the highest number of flowers per plant (230.03±137.52), significantly more than genotypes L2, L5, and L7. “BAIP 2” exhibited the greatest flower weight per plant (0.71±0.23 kg), significantly differing from genotype D20, which had the lowest flower weight (0.10±0.08 kg). The “Maharani” genotype had the longest stalk length (6.65±0.81 cm), which was significantly greater than genotypes D6, D14, D17, D20, D22, D24, D27, D31, and 08, all of which had shorter stalks. These differences in flower number, flower weight per plant, and stalk length across genotypes likely reflect genetic variations. The findings align with previous studies by Firmansyah and Respatijarti (2021), Shilpa et al. (2022), and Tanya and Kaur (2023).

The qualitative traits of M2V1 marigold mutants displayed variation in both floret type and flower color (Table 4). Floret types included all ligulate and all tubuligulate forms. Based on the RHS color chart (Figure 1), the genotypes were grouped into four flower color categories: the yellow-orange group for “Maharani”, the orange group for “BAIP 2”, the yellow group for “BAIP 3” and genotypes D6, D14, D17, D20, D21, D22, D24, D27, D31, and 08, and the white

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Table 2. Mean values of plant height, stem diameter, canopy width, and leaf width characters of M2V1 generation marigold

Genotype	Plant height (cm)	Stem diameter (cm)	Canopy width (cm)	Leaf width (cm)
“Maharani”	50.81±4.84ab	1.61±0.18abc	48.64±3.12abc	5.70±0.28
“BAIP 2”	53.01±7.10ab	1.56±0.13abc	54.04±8.49abc	5.42±0.33
“BAIP 3”	56.57±5.87a	1.80±0.34a	59.81±4.53a	5.50±0.63
D6	51.84±3.33ab	1.56±0.18abc	58.33±9.88ab	5.34±0.29
D14	48.36±2.84ab	1.44±0.07abcd	52.75±6.31abc	5.46±0.97
D17	51.36±1.82ab	1.60±0.06abc	57.33±3.18abc	5.20±0.32
D20	46.89±3.53ab	1.50±0.14abc	52.44±5.75abc	4.81±0.57
D21	51.00±5.57ab	1.48±0.17abcd	55.28±10.42abc	5.17±0.30
D22	47.55±4.95ab	1.37±0.21bcd	55.75±2.12abc	5.31±0.17
D24	50.08±4.14ab	1.50±0.07abc	58.33±4.84ab	5.59±0.77
D27	55.46±8.60ab	1.69±0.06ab	56.97±5.45abc	4.85±0.38
D31	46.49±5.67ab	1.56±0.02abc	56.57±6.38abc	5.01±0.47
L2	42.69±0.49ab	1.09±0.09d	42.06±2.42bc	4.69±0.17
L5	45.46±1.33ab	1.22±0.08cd	40.52±4.20c	4.61±0.10
L7	45.20±0.49ab	1.10±0.11d	42.73±0.42abc	4.58±0.22
L13	45.40±2.82ab	1.29±0.21bcd	46.45±3.96abc	4.79±0.11
08	41.03±12.36b	1.33±0.26bcd	46.06±9.09abc	5.29±0.54

Note: values followed by the same letters in the same columns are not significantly different at the level of 5% HSD, data presented as mean values ± standard deviation

Table 3. Mean values of flower characters in M2V1 generation marigold

Genotype	Weight per flower (g)	Flower diameter (cm)	Number of flowers per plant	Stalk length (cm)	Flower weight per plant (kg)
“Maharani”	13.94±1.14a	7.34±0.12ab	71.67±28.75ab	6.65±0.81a	0.41±0.14abc
“BAIP 2”	15.65±3.26a	7.58±0.65a	121.58±42.74ab	5.86±0.84abcd	0.71±0.23a
“BAIP 3”	12.36±0.51a	6.67±0.16b	126.85±65.59ab	5.75±0.30abcd	0.64±0.36ab
D6	2.95±0.58c	3.41±0.09d	175.34±142.23ab	4.81±0.63cd	0.23±0.27abc
D14	2.77±0.60c	3.28±0.23d	151.33±41.74ab	4.99±0.36bcd	0.21±0.13abc
D17	2.14±0.55c	3.15±0.26d	230.03±137.52a	4.46±0.44d	0.25±0.17abc
D20	2.30±1.16c	2.91±0.76d	82.67±36.83ab	4.44±0.68d	0.10±0.08c
D21	2.60±0.30c	3.41±0.15d	148.54±87.43ab	5.25±0.98abcd	0.17±0.12bc
D22	2.82±0.72c	3.37±0.05d	135.05±95.31ab	4.94±0.33bcd	0.17±0.12bc
D24	2.52±0.64c	3.22±0.19d	132.42±57.51ab	4.80±0.50cd	0.15±0.10bc
D27	2.61±0.60c	3.26±0.14d	129.37±70.31ab	4.46±0.28d	0.15±0.11bc
D31	2.59±0.54c	3.34±0.08d	132.93±38.27ab	4.80±0.58cd	0.16±0.10bc
L2	6.83±0.40b	5.20±0.03c	45.72±31.42b	6.40±0.32abc	0.15±0.10bc
L5	6.42±1.06b	5.26±0.13c	52.20±2.48b	6.49±0.44ab	0.18±0.00bc
L7	7.61±1.65b	5.52±0.49c	45.91±16.69b	6.27±0.05abc	0.16±0.05bc
L13	7.26±0.70b	5.37±0.04c	70.71±20.54ab	5.77±0.45abcd	0.24±0.08abc
08	2.9±0.58c	3.36±0.18d	162.06±91.57ab	4.49±0.45d	0.49±0.58abc

Note: values followed by the same letters in the same columns are not significantly different at the level of 5% HSD, data presented as mean value ± standard deviation

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group for genotypes L2, L5, L7, and L13. Flower color is a qualitative trait, controlled by the genes present in each genotype's hereditary material. All tested genotypes had semi-upright growth habits, pinnate leaf types, and leaf colors matching RHS color chart code NN137B (greyish olive green).

also been reported by Gobade et al. (2017) that high genetic diversity in marigolds was found in the characters of weight per flower and flower diameter.

High genetic diversity within plant populations is essential for effective selection. However, relying solely on genetic diversity values for selection can be challenging. Heritability estimates provide a valuable genetic parameter to guide selection based on phenotypic variation, revealing the extent to which a phenotype is determined by its genotype (Choudhary et al., 2017). Heritability in a plant population is calculated by dividing genetic variance by phenotypic variance. In this study, heritability estimates in the broad sense ranged from 34.52% to 99.21% (Table 5). Most traits exhibited high heritability, except for leaf length, which had a medium heritability value (34.52%). As noted by Marwan et al. (2022), traits with low or medium heritability are less likely to be passed on to offspring, making selection less effective for those traits. Estimating heritability helps clarify whether genetic or environmental factors primarily influence an individual's phenotype.

Table 4. Flower color and floret-type characters of marigold flowers in the M2V1 generation

Genotype	Flower color	Floret type
"Maharani"	23A Vivid orange-yellow	All ligulate
"BAIP 2"	N25D Strong orange yellow	All ligulate
"BAIP 3"	6A Brilliant greenish-yellow	All ligulate
D6	4B Light greenish yellow	All tubuligulate
D14	4B Light greenish yellow	All tubuligulate
D17	4B Light greenish yellow	All tubuligulate
D20	4B Light greenish yellow	All tubuligulate
D21	4B Light greenish yellow	All tubuligulate
D22	4B Light greenish yellow	All tubuligulate
D24	4B Light greenish yellow	All tubuligulate
D27	4B Light greenish yellow	All tubuligulate
D31	4B Light greenish yellow	All tubuligulate
L2	155B Yellowish white	All ligulate
L5	155B Yellowish white	All ligulate
L7	155B Yellowish white	All ligulate
L13	155B Yellowish white	All ligulate
08	4B Light greenish yellow	All tubuligulate

Genetic diversity in marigolds of the M2V1 generation can be assessed based on the values of variance components, including phenotypic, genetic, and environmental variations. The value of genetic variance and its standard deviation can determine the extent or narrowness of a character's genetic diversity (Table 5). Characters with broad genetic diversity include stem diameter, canopy width, weight per flower, flower diameter, and flower stalk length. In contrast, plant height, leaf length, leaf width, flower weight per plant, and number of flowers exhibit narrow genetic diversity.

High genetic diversity in a character indicates that genetic factors strongly influence its diversity (Hermanto et al. 2017). Characters with broad genetic diversity usually have broad phenotypic diversity. Conversely, characters with narrow genetic diversity do not necessarily have narrow phenotypic diversity. This shows that phenotypes result from the relationship between genetics and the environment. (Syukur et al., 2010). The selection of characters with broad genetic diversity is more effective and offers the opportunity to obtain gene sources to improve these characters. The same research results have

The high heritability values for traits such as plant height, stem diameter, canopy width, leaf width, weight per flower, flower diameter, number of flowers per plant, flower weight per plant, and stalk length (Table 5) suggest that these traits are largely controlled by genetic factors rather than environmental conditions. According to Saputra et al. (2019), when a trait's

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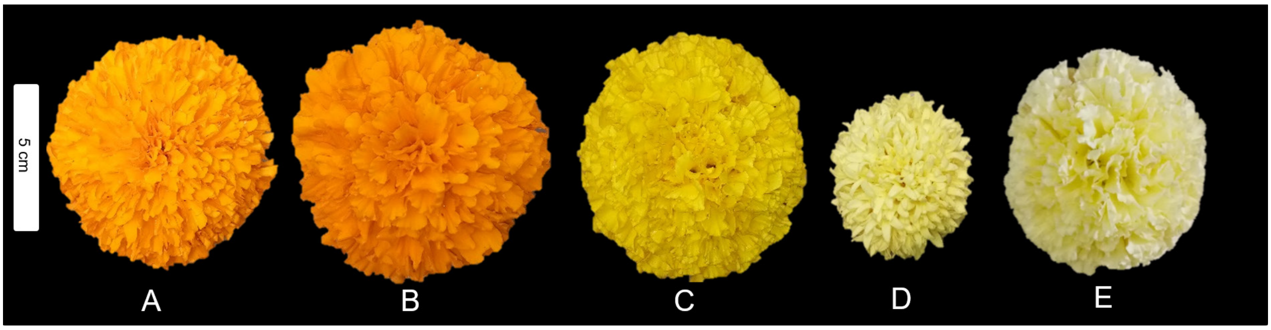

Figure 1. Flower color and floret type characters of marigold: A) “Maharani”, floret type all ligulate, yellow orange RHS 23A; B) “BAIP 2”, floret type all ligulate, orange RHS N25D; C) “BAIP 3”, floret type all ligulate, yellow RHS 6A; D) D6, floret type all tubuligulate, yellow RHS 4B; E) L2, floret type all ligulate, white RHS 155B

Table 5. Estimation of variance components and broad-sense heritability of quantitative characters in M2V1 marigold mutants

Characters	Variance components			$2\ \sigma\sigma_{^2g}$	h^2_{bs}
	σ^2_e	σ^2_g	σ^2_p		
Plant height	7.52	10.78	18.30	12.73^{N}	58.91^{H}
Stem diameter	0.01	0.03	0.04	0.03^{B}	83.33^{H}
Canopy width	10.71	29.22	39.94	27.13^{B}	73.17^{H}
Leaf length	0.18	0.10	0.28	0.20^{N}	34.52^{M}
Leaf width	0.06	0.07	0.13	0.09^{N}	51.28^{H}
Weight per flower	0.39	19.28	19.66	13.11^{B}	98.03^{H}
Flower diameter	0.02	2.5	2.52	1.68^{B}	99.21^{H}
Number of flowers per plant	1055.56	1515.83	2571.39	1789.09^{N}	58.95^{H}
Flower weight per plant	0.01	0.02	0.03	0.02^{N}	77.78^{H}
Stalk length	0.09	0.52	0.62	0.41^{B}	84.41^{H}

Note: σ^2e = environmental variance, σ^2g = genetic variance, σ^2p = phenotype variance, $\sigma\sigma^2g$ = standard deviation of genetic variance, B = broad, N = narrow, h^2bs = broad-sense heritability, H = high, M = medium.

appearance is largely influenced by genetics, it is likely to be inherited by the next generation. Conversely, if a trait’s appearance is driven by environmental factors, it is less likely to be passed on.

Several studies have reported similarly high heritability values for marigold traits, such as plant height, canopy width, and flower weight (Poulose et al., 2020), stem diameter (Choudhary et al., 2017), and flower diameter and number of flowers per plant (Sahu et al., 2021). Breeding programs can become more effective by focusing on traits with high heritability for selection and improvement in future generations.

Genotype groupings based on their similarity or genetic distance are visualized in the dendrogram (Figure 2). Genotypes with greater similarity or a shorter genetic distance between them are clustered together, while those with significant differences or longer distances are placed in separate clusters. Cluster analysis, performed using Gower’s dissimilarity measure and the average linkage method, grouped the tested genotypes into two main clusters. This analysis incorporated both quantitative and qualitative data.

Cluster 1 included the genotypes “Maharani”, BAIP2, BAIP3, L2, L5, L7, and L13, while Cluster 2 comprised genotypes D6, D14, D17, D20, D21, D22, D24, D27, D31, and 08. The genetic distance among genotypes ranged from 0.06 to 0.60. The shortest genetic distance, 0.06, was found between genotypes L7 and L5, indicating a close genetic relationship. In contrast, the largest genetic distance, 0.60, was observed between L2 and D17, suggesting a more distant genetic relationship. Thus, genotypes L2 and L5 are closely related, while L2 and D17 are genetically distinct.

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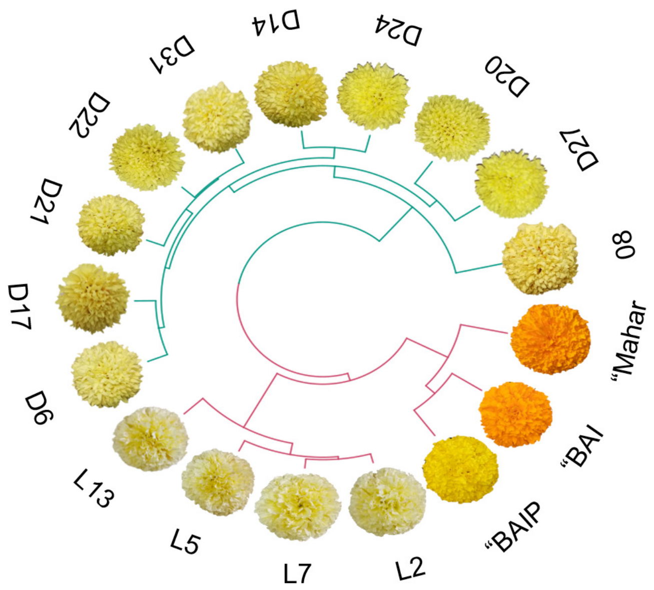

Figure 2. Cluster analysis dendrogram in M2V1 generation of marigold,

Conclusion

The vegetative propagation of second-generation mutant (M2V1) marigold genotypes revealed significant differences across most traits, except leaf length. The “BAIP 3” genotype exhibited the most notable plant height, stem diameter, and canopy width, whereas “BAIP 2” showed the highest values for weight per flower, flower diameter, and flower weight per plant. Genotype D17 stood out with the most significant number of flowers per plant. Marigolds in this study displayed two floret types—ligulate and tubuligulate—with flower colors ranging from orange and yellow to white. Traits such as stem diameter, canopy width, weight per flower, flower diameter, and stalk length demonstrated both broad genetic diversity and high broad-sense heritability, making them valuable targets for selection and further development in marigold breeding programs.

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