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ASSESSMENT OF *TAGETES PATULA* MUTANTS AND ITS WILD TYPE FOR FLOWER MORPHOLOGY, POLYPHENOL CONTENTS, AND ANTIOXIDANT ACTIVITY

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SUMMARY

Mutation breeding can improve the flower color and biochemical content. French marigold (*Tagetes patula* L.) is an ornamental and edible flower plant used for medicinal purposes. The latest study aimed to obtain potential genotypes with modified flower morphology, which contain high polyphenol content and antioxidant activity induced by gamma irradiation in M4 populations. The plant material was a wild type (MG21 genotype), a local genotype from Takengon, Aceh, Indonesia, with red-orange tubular and ligulate flower types and its five mutants with different flower morphologies. The wild type and its mutant genotypes vegetative propagation reached planting from October 2022 until March 2023 at an altitude of 1100 m asl (6° 46′ 6.268″ N latitude, 107° 2′ 57.703″ E longitude). Flower morphology assessment ensued through various observations based on UPOV and RHSCC criteria, and phenotypic measurement employed a colorimeter. The polyphenol analysis determined the total anthocyanin content (TAC), total phenolic content (TPC), and total flavonoid content (TFC). The antioxidant activity estimation of *T. patula* used the Ferric Reducing Antioxidant Power (FRAP) assay. The results also showed the diversity of mutant flower morphology, followed by different polyphenol contents and antioxidant activity. The highest TAC, TPC, and TFC were evident in Type-C (all-ligulate, red group), which was also higher than the wild type (tubulate and ligulate, red group). In addition, the highest FRAP occurred in Type-A (tubuligulate and ligulate, orange-red group), Type-B (all-ligulate, orange-red group), and Type-C, while the wild type was the lowest. The research revealed two potential mutant genotypes, type A and C, with high polyphenol content and antioxidant capability due to gamma irradiation in the M4 populations.

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Keywords: French marigold (*Tagetes patula* L.), ornamental plant mutation, polyphenol, flavonoids, antioxidants, color analysis

Key findings: Two promising mutant genotypes characterized by different flower morphology and high biochemical content were obtainable through mutation in the local *T. patula* genotype. The latest research outcome offers insights into the potential traits of these mutants compared with the wild type. It also establishes correlation, encompassing the quantification of flower color using the CIELAB system to polyphenol content and antioxidant activity.

INTRODUCTION

French marigold (*Tagetes patula* L.), classified as an annual botanical species, belongs to the family Asteraceae originating in South America and gaining widespread cultivation globally in contemporary times (Soule, 1994). The plants of this species have typical characteristics of small stature (20–60 cm) and small flowers (approximately 3–5 cm) in varying shades of yellow, orange, or a combination thereof (Ördögh, 2021; Cicevan *et al*., 2022). In addition to its aesthetically pleasant flowers, *T. patula* occurrence of secondary metabolites makes it useful for culinary and medicinal purposes (Chitrakar *et al.*, 2019; Dujmović *et al.*, 2022). Numerous plant components, including their flowers, serve as wealthy innate antioxidants (Prabawati *et al.*, 2021) and exhibit phenolic compounds, flavonoids, and anthocyanins (Skrajda-Brdak *et al.*, 2020).

One of the *T. patula* local genotypes, the MG21, found in Takengon, Aceh, Indonesia, shows distinct traits, i.e., massive bushy form reaching up to 124 cm, numerous large flowers, and noteworthy red flower coloration (Lenawaty et al., 2022). However, there is a scope for improving the MG21 genotype that can work effectively by applying advanced plant breeding methods, including induced mutation. The induction of plant mutation considerably enhances and improves the diversity of the characteristics by utilizing various mutagens. Gamma irradiation, a commonly employed physical mutagen frequently used, helps achieve a broad spectrum of character variations (Faiz *et al.*, 2018). Previous studies have demonstrated the effectiveness of gamma irradiation in enhancing flower size and color diversity in chrysanthemum, *Celosia plumosa*, and *Tagetes erecta* and influencing phytochemical properties, such as polyphenols and antioxidant activities in *Catharanthus roseus*, *Tulipa gesneriana*, and *Calendula officinalis* (Anne and Lim, 2021; Aisyah *et al*., 2022; Li *et al*., 2022; Choudhary and Singh, 2023). Comprehensive investigations have progressed on pigment content and its interplay with other metabolic aspects in distinct flower types, both *T. erecta* and *T. patula* (Manivannan *et al.*, 2021). However, a lack of precise information exists regarding the phenolics, flavonoids, and antioxidant potential of *T. patula*, especially in ligulate florets displaying different colors due to mutation.

Consequently, the latest study aimed to attain the potential *T. patula* genotypes with modified flower morphology, containing the highest polyphenol content and antioxidant activity induced through gamma irradiation. The potential mutant genotypes discovered in this research stand as a promising new genetic resource suitable for accomplishment as breeding material in French marigold plants. Their value extends beyond ornamental use, offering significant potential both as consumption and medicinal plants due to their sole flower shapes and prominent biochemical content. These mutants represent a valuable asset for developing French marigolds that serve ornamental purposes and hold promise for their application in consumable and medicinal contexts.

MATERIALS AND METHODS

Plant material

The French marigold (*T. patula* L.) wild type 'MG21 genotype,' a local from Takengon, Aceh,

Indonesia, and its five mutants with different flower morphologies came from the M4 populations through gamma irradiation-induced by 600 Gy in M0. Describing the *T. patula* wild type and its five mutant flower characteristics relied on the UPOV for Marigold and Royal Horticultural Society (RHS) Color Chart Sixth Edition (Table 1, Figure 1). The wild type and its mutant genotypes vegetative propagation and planting began from October 2022 until March 2023 at the Pasir Sarongge Experimental Field, Cianjur, Indonesia, located at an altitude of 1100 masl (6° 46′ 6.268″ N latitude, 107° 2′ 57.703″ E longitude).

Flower agro-morphology observation

Observations based upon the agro-morphological traits of each flower type proceeded at the stage of full bloom. Assessment of each plant's first three bloomed flowers continued by measuring the size of flower diameter, outer floret length, and width. Each observation unit included five plants with three replications.

Floret color measurement

Employing a portable colorimeter (WR10, Shenzhen WaveGD Photoelectric Technology Co., Shenzhen, China) helped assess the color space values of ligulate florets, both in the *T. patula* wild type and its mutants. Each flower type consisted of three outer ligulate florets taken from different plants. The ligulate floret's placement on a clean white paper had the light source alignment at the midpoint of the floret for CIELAB measurement (Lu *et al.*, 2021). Specifically, L* represents a color's degree of darkness and lightness, spanning the spectrum from black to white with ranges 0 to 100, while a* and b* denote the distinct color directions within the color system. The a* describes a range from green to red, with negative to positive values, and the b* spans from blue to yellow, similar to negative to positive values (Durmus, 2020).

Preparation of extraction samples

In the presented study, the extraction method was a modification by Shehata *et al.* (2020). Approximately 5 g of fresh ligulate floret samples sustained mashing and mixing with ethanol: 0.1 M HCl (85%:15%, v/v). The sample-to-extraction solvent ratio was 1:2, and the mixture incubation reached one hour at room temperature with 200 rpm in an orbital shaker. The floret extract solution mixture's filtering used a filter paper (0.45 µm) to obtain the liquid extract. The liquid extract storage was at 4 °C until used for further analysis.

Determination of total anthocyanin content

Samples for determining total anthocyanins began by diluting them separately with 1.0 buffer (0.025 M potassium chloride) and pH 4.5 buffer (0.4 M HCl). Combining each sample with the supernatant had a 1:4 volume ratio (v/v) of extract sample to buffer, mixed in a 50 mL volumetric flask. The absorbance measurement for the 2-test solutions at pH 1.0 and 4.5 used a spectrophotometer at 520 nm. Absorbance was at 700 nm for haze correction. Calculating the concentration of anthocyanin pigments (cyanidin-3-glucoside equivalents) employed the methodology of Lee *et al.* (2005) using the following equation:

$$Total\ anthocyanin\ content\ (mg\ Cy3G\ 100\ g^{-1}\ FW) = \frac{A.MW.DF.10^3}{\varepsilon.L}$$

Where:

A = $(A_{520\ nm}-A_{700\ nm})$ pH1.0 - $(A_{520\ nm}-A_{700\ nm})$ pH4.5

MW = 449.2 g moL^{-1} for cyanidin-3-glucoside

DF = Dilution factor

L = Path-length (cm)

ε = 26.900 *molar extinction coefficient*, in L mol^{-1} cm for cyanidin-3-glucoside

10^3 = Factor for conversion from g to mg

Table 1. The flower type characteristics in each mutant of the M4 generation and wildtype of *T. patula.*

Characteristics	Flower Type					
	Type-A	Type-B	Type-C	Type-D	Type-E	Wildtype
Floret type	Tubuligulate and ligulate	All ligulate	All ligulate	Tubuligulate and ligulate	Tubulate and ligulate	Tubulate and ligulate
Number of ligulate floret whorls	Medium	Many	Many	Medium	Few	Very few
Number of adaxial ligulate color	Two	Two	Two	Two	Two	Two
Distribution of color type	Two	Two	One	One and two	One	One
Size of central color zone	Medium to large	Medium to large	Very small	Very small to medium	Large	Very small
Primary adaxial ligulate color (RHSCC)	Orange-red group (N34A)	Orange-red group (N34A)	Red group (46A)	Orange-red group (N34A)	Orange-red group (N34A)	Orange-red group (N34A)
Secondary adaxial ligulate color (RHSCC)	Orange group (N25D)	Orange group (N25C)	Orange group (N25D)	Orange group (25C)	Yellow-orange group (14A)	Orange group (N25D)

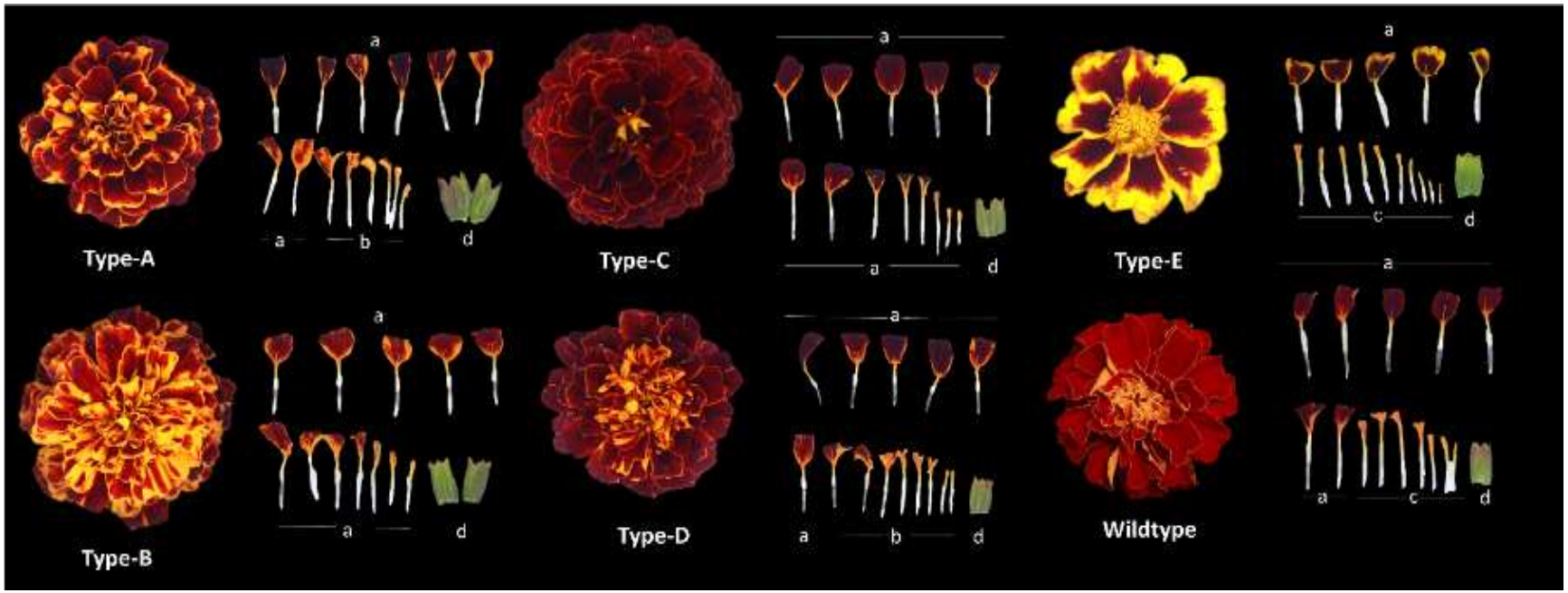

Figure 1. Performance of flower type in M4 generation with floret characteristics consisting of ligulate (a), tubuligulate (b), and tubulate (c), whereas sepal (d) has the same shape.

Determination of phenolic content

Total phenolic content (TPC) analysis used the Folin-Ciocalteu modified method (Nurcholis *et al.*, 2022). Adding an extracted sample that used the same technique for anthocyanin analysis to 120 µL of 10% Folin-Ciocalteu reagent ensued. The sample solution incubated for 5 min had 80 µL of 10% Na_2CO_3 added to a 96-well microplate and incubated for 30 min. Analysis of samples used a nano-spectrophotometer (SPECTROstar Nano, BMG LABTECH, Germany) with a wavelength of 750 nm. The test sample consisted of three replicates expressed as mg gallic acid equivalent per gram of fresh weight extract (mg GAE g^{-1} FW). The measured gallic acid concentrations were in the 0-300 ppm concentration range.

Determination of flavonoid content

Total flavonoid content (TFC) determination used the aluminum chloride solution method (Yudha *et al.*, 2022). A total of 10 µL of the sample, added to 50 µL of ethanol, 10 µL of $AlCl_3$ (10%), and 120 µL of distilled water, comprised mixing to a 96-well microplate and incubated for 30 min. Sample absorbance measurement used a nano-spectrophotometer (SPECTROstar Nano, BMG LABTECH, Germany) at a wavelength of 409 nm. The test sample had three replicates expressed as mg quercetin equivalent per gram of fresh weight extract (mg QE g^{-1} FW). The produced quercetin had a concentration range of 0-500 ppm.

Antioxidant assay

The antioxidant activity determination used the Ferric Reducing Antioxidant Power (FRAP) method, preparing a reagent of 10 mM TPTZ (in 40 mM HCl), 20 mM FeCl3 (in distilled water), and acetate buffer (pH 3.6) (Nurcholis *et al.,* 2022). The FRAP reagent preparation had a ratio of TPTZ, $FeCl_3$, and acetate buffer (1:1:10), after which the reagent gained incubation for 30 min at 37 °C. A total of 10 µL of the sample and 300 µL of FRAP reagent, injected into a 96-well microplate, received incubation in the dark at 37 °C for 30 min. Analyzing the test samples used a nano-spectrophotometer (SPECTRO star Nano, BMG LABTECH, Germany) with three replicates, with the absorbance measured at a wavelength of 593 nm. Measuring the antioxidant activity utilized Trolox equivalents (TE mol TE g^{-1} FW). Trolox preparation had concentrations ranging from 0-600 µM.

Statistical analysis

All conducted experiments used a randomized complete block design (RCBD) with different flower types as the main factors (wildtype, Type A, Type B, Type D, and Type E). Each treatment also had three replicates, resulting in 18 experimental units. The expression of the recorded values was means ± standard deviation. Variability among the different flower types for color values, total anthocyanin, TPC, TFC, and antioxidant activity underwent analysis of variance (ANOVA) using SAS OnDemand for Academics (https://welcome.oda.sas.com). The least significant difference ($LSD_{0.05}$) test helped determine the substantial differences among the treatment means for various analyzed parameters. Construction of the heatmap and correlation analysis used the R-software.

RESULTS

Flower agro-morphological traits

Consequently, variations observed in the mutants regarding flower size had probable influences from the arrangement and proliferation of the ligulate florets (Table 1). In the evaluated flower types, significant ($P \leq 0.01$) variations existed across the wild type and its five mutants in the flower diameter (Table 2). Type C manifested the largest flower diameter, and Type E exhibited the smallest value for the said feature for flower types. This discrepancy indicates that the mutant genotypes showed an increase and decrease in flower size compared with the wild type. However, the size of the outer ligulate florets showed nonsignificant differences among the flower types of the genotypes.

Table 2. Agro-morphological traits of *T. patula* flower M4 mutants and its wild type flower.

Flower Type	Flower diameter (cm)	Floret length (cm)	Flower width (cm)
Type-A	4.73±0.19cd	3.20±0.14	2.23±0.02
Type-B	5.13±0.13ab	3.04±0.07	2.36±0.05
Type-C	5.23±0.11a	3.49±0.21	2.41±0.14
Type-D	4.96±0.17bc	3.36±0.18	2.30±0.06
Type-E	4.25±0.14e	3.07±0.08	2.43±0.11
Wildtype	4.57±0.15d	3.15±0.20	2.31±0.16
Pr > F	<.0001**	<.0522^{ns}	0.6671^{ns}
Coeff Var (%)	2.594	5.247	4.705

According to the Least Significant Different test, different letters in the column indicate a mean difference at $P < 0.05$. ****:** Significant at 1% level, ***:** Significant at 5% level, and ^{ns}: not significant at 5% level.

Color spaces of *T. patula* outer ligulate floret

The *T. patula* mutants and the wild type revealed significant differences for all the color parameters (Table 3, Figure 1). Visual observation of the outermost primary color of the ligulate florets on several flower types was the same; however, the color quantification showed varied values. The visual color of Type C differs from that of the other flower types, where this color (RHS 46A) was the darkest, as indicated by the lowest L* value. Type B has the same visual primary colors as Types D, E, and the wild type; however, it has significant differences in all the parameters, confirming that Type B has the brightest, redder, and yellower colors.

Polyphenol contents and antioxidant capacity

Observations on the five mutant flower types and their wild type for the total anthocyanin, phenolic, flavonoid contents, and antioxidant activity are available in Table 4. The five mutant flower types and their wild type also significantly differed ($P < 0.01$) for polyphenol contents and antioxidant activities. The total anthocyanin content (TAC) ranged from 24.767 to 61.051 mg of cyanidin-3-glucoside equivalent per 100 grams of fresh ligulate florets in flower types, with the most substantial accumulation observed in Type C flowers. The TAC in Type C flowers corresponded to the phenotypic variation characterized by a deep red flower color (Figure 1). On the contrary, the flowers with a broader distribution of yellow-orange hues also showed a decreased level of anthocyanin.

The TPC in the mutant flower and the wild types ranged from 6.827 to 10.346 mg gallic acid equivalent per gram of fresh weight. However, in comparison, the TFC ranged from 3.054 to 5.706 mg quercetin equivalent per gram fresh weight, and the antioxidant activity measured by FRAP varied between 93.630 and 125.168 µmol Trolox equivalent per gram fresh weight. The heatmap visualization enunciated the division of three distinct clusters based on the tested biochemical characteristics (Figure 2). Notably, two potential mutant flower types, Type A and Type C, showed superior biochemical properties compared with the wild type. It suggests that genetic improvement in the M4 populations resulting from the induction of mutation through gamma irradiation can enhance the biochemical content of *T. patula*. Furthermore, the referred mutation also demonstrated the potential to decrease the biochemical content, as evident in mutants with Types B and E, displaying lower biochemical characteristics than the wild type.

The heatmap visualization can also assess the relationships among the evaluated biochemical characteristics (Figure 2). Within a subcluster, the TPC and TFC exhibited a close relationship with anthocyanins compared with FRAP. The Pearson correlation test corroborated the proximate relationship among these biochemical attributes (Table 5). The test further revealed a strong positive correlation

Table 3. Color parameters L*, a*, and b* on the adaxial outer ligulate floret of *T. patula* mutants and their wild type compared to RHSCC.

Flower Type	L*	a*	b*	Primary outer ligulate floret color
Type-A	36.15±1.06^{b}	58.17±2.10^{b}	116.52±4.81^{a}	Red group (N45A)
Type-B	45.49±9.89^{a}	65.40±23.37^{ab}	121.73±6.16^{a}	Orange-red group (N34A)
Type-C	23.71±1.41^{c}	76.62±6.16^{a}	92.036±5.18^{b}	Red group (46A)
Type-D	31.91±5.34^{bc}	50.75±11.03^{b}	78.143±9.27^{b}	Red group (N45A)
Type-E	29.24±1.92^{bc}	48.32±5.59^{b}	86.264±9.24^{b}	Orange-red group (N34A)
Wildtype	32.98±2.83^{b}	61.93±7.59^{ab}	80.936±4.05^{b}	Orange-red group (N34A)
Pr > F	0.0072**	0.0429*	0.0001**	
Coeff Var (%)	15.282	4.0592	8.357	

According to the Least Significant Different test, different letters in the column indicate a mean difference at p < 0.05. ****:** Significant at 1% level, ***:** Significant at 5% level, and ^{ns}: not significant at 5% level.

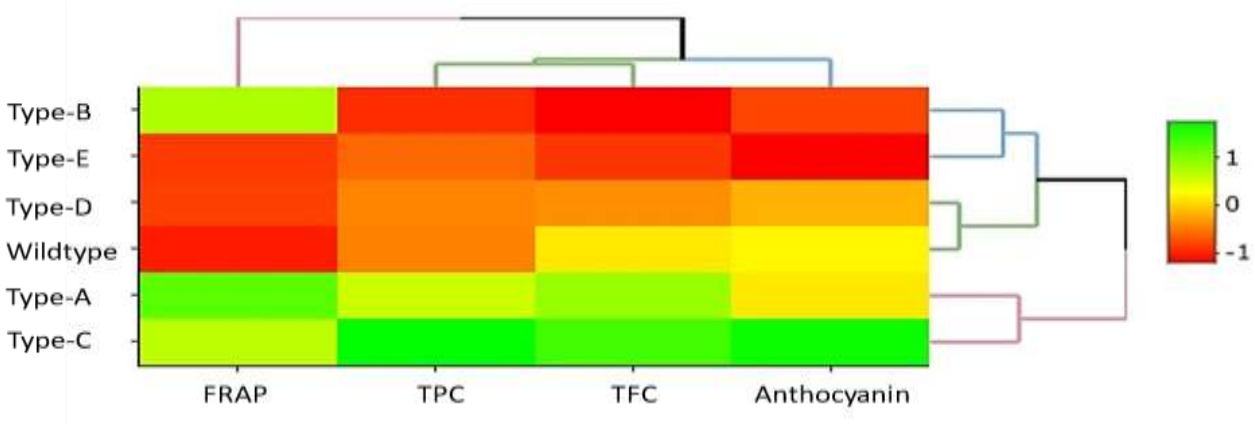

Figure 2. Heatmap total anthocyanin, phenolic, flavonoid content, and antioxidant properties in five types of mutant flower and wild type.

Table 4. Total anthocyanin, phenol, flavonoid content, and antioxidant capacities of ligulate floret of *T. patula* mutants and wild type.

Flower type	TAC (mg Cy3G 100 g^{-1} FW)	TPC (mg GAE g^{-1} FW)	TFC (mg QE g^{-1} FW)	FRAP (µmol TE g^{-1} FW)
Type-A	41.525±0.139^{c}	8.805±0.308^{b}	5.187±0.080^{b}	125.168±1.517^{a}
Type-B	29.747±0.054^{e}	6.827±0.231^{c}	3.054±0.047^{f}	117.989±4.887^{b}
Type-C	61.051±0.187^{a}	10.346±0.543^{a}	5.706±0.069^{a}	116.656±7.461^{b}
Type-D	37.695±0.079^{d}	7.494±0.238^{c}	3.900±0.052^{d}	94.912±2.561^{c}
Type-E	24.767±0.152^{f}	7.267±0.167^{c}	3.375±0.037^{e}	94.194±4.308^{c}
Wildtype	42.593±0.635^{b}	6.827±0.409^{c}	4.395±0.013^{c}	93.630±5.002^{c}
Pr > F	<.0001**	<.0001**	<.0001**	<.0001**
Coeff Var (%)	1.339	4.609	1.363	3.513

According to the Least Significant Different test, different letters in the column indicate a mean difference at p < 0.05. ****:** Significant at 1% level, ***:** Significant at 5% level, and ^{ns}: not significant at 5% level. TAC= total anthocyanin content, TPC= total phenolic content, TFC= total flavonoid content, FRAP= Ferric Reducing Antioxidant Power.

Table 5. Pearson correlation among the tested biochemical characteristics of the mutants and wild type flower.

	TPC	TFC	FRAP
TAC	0.87200	0.90432	0.30688
	<.0001**	<.0001**	0.2155
TPC		0.89510	0.46525
		<.0001**	0.0517
TFC			0.38519
			0.1144

TAC= total anthocyanin content, TPC= total phenolic content, TFC= total flavonoid content, FRAP= Ferric Reducing Antioxidant Power.

Table 6. Pearson correlation among the color parameters L*, a*, and b* and the biochemical properties.

Biochemical properties	L*		a*		b*	
	R	P value	R	P value	r	P value
TAC	-0.4767	0.0455*	0.5198	0.0270*	0.0014	0.9954
TPC	-0.5523	0.0175*	0.4529	0.0591	0.0660	0.7946
TFC	-0.4934	0.0375*	0.3868	0.1128	0.0848	0.7379
FRAP	0.2918	0.2399	0.2684	0.2815	0.7904	<.0001**

TAC= total anthocyanin content, TPC= total phenolic content, TFC= total flavonoid content, FRAP= Ferric Reducing Antioxidant Power.

among the total anthocyanin, TPC, and TFC qualities. However, the antioxidant activity of FRAP displayed a positive correlation that did not reach the significance level for the other three biochemical traits.

Correlation between the color space and biochemical properties

The Pearson correlation coefficient (r) usage examined the association among the biochemical properties and color space values, specifically L*, a*, and b* (Table 6). The lightness (L*) has a moderate negative correlation with anthocyanin, TPC, and TFC. The L* value decline indicated a darker flower color, corresponding to an increase in anthocyanin, TPC, and TFC content, particularly notable in potential mutants of flower Type C (Figure 2). In contrast, the a* displayed a moderate positive correlation with anthocyanin content. Higher positive a* values indicated a redder coloration, closely joined to the higher anthocyanin level. The b* value exhibited a strong positive correlation with the FRAP. An elevated b* value indicated a shift toward the more yellow coloration in the visual representation (Figure 1, Table 3).

DISCUSSION

Gamma irradiation in mutation plant breeding of French marigolds has shown assurance in boosting the agro-morphological and biochemical traits, particularly those associated with local genotypes. In the latest study perspective, the mutants from the M4 generation, the flower types B, C, and D, showed an increase in flower size compared with the mother wild-type genotype. Interestingly, reports of similar occurrences of considerably larger flowers through gamma irradiation have emerged in subsequent generations of other crop plants, i.e., *Chrysanthemum*, *Celosia*, and *Portulaca* (Susila *et al.,* 2019; Anne and Lim, 2021; Aisyah *et al.*, 2022). As reported in a mutant of *Arabidopsis thaliana* by Nhat *et al.* (2021) and Li *et al.* (2019), it has possible attributes with the synergistic effects of gene disruption, which promotes the enlargement of these floral structures. On the contrary, the observed

reduction in flower size in Type-E mutants can refer to the adverse effects of radiation. Gamma irradiation potentially impacts gene expression patterns, reducing flower size (Mahapatra and Roy, 2020).

The gamma irradiation has a genuine impact on the phenotype of the flowers as well. Variations that occur can comprise the shape and color of the flowers. The genetic material used in the presented research contains the mutants with different flower types from the wild type to screening the advanced genotypes with unique flower colors and shapes, along with potential phytochemical content. The inimitability of the flower color is a particularly prominent trait, influencing the consumer perceptions of flower petals, and color values help in understanding the amount and type of pigment found in flower petals. One accurate method for categorizing and characterizing the flower color phenotype is the CIELAB color system (Lei *et al.*, 2017; Cui *et al.*, 2019). A mutant with Type-C flower attained categorization within the red group, characterized by the darkest color intensity, denoted by the lowest L* and highest a* values (Table 3, Figure 1). In contrast, the flower types with high L* and b* values received grouping in the orange-red color. Remarkably, similar trends have also been evident in comparative studies involving flower color in related Asteraceae members, such as Gerbera, Chrysanthemum, and Tagetes, where white, yellow, and orange flower groups exhibit high L* and b* values relative to the purple and red flower groups and characterized by higher a* values (Lu *et al.*, 2021; Manivannan *et al.*, 2021; Zhou *et al.*, 2022).

The superior characteristics of the mutant flower types were also evident in their polyphenolic compounds. Among the mutant candidate genotypes, the flower type C had the highest anthocyanin content compared with other mutants and the mother wild-type genotype. Typically, anthocyanins contribute to pink shades, whereas carotenoids predominantly give rise to orange and yellow tones, and a combination of both contributes to red hues (Kishimoto *et al*., 2019; Yamagishi, 2020; Li *et al*., 2022). In *T. patula* flowers, the observed red coloration may be due to the presence of xanthophylls and anthocyanins, with constituents being cyanidin-3-galloyl sophoroside, cyanidin-3-glucoside, and cyanidin-3-sophoroside (Teixeira *et al.*, 2023). Furthermore, other polyphenolic compounds, such as TPC and TFC, acquired enhancements in mutant flower types A and C compared with the wild type. The potential of mutation through gamma irradiation to produce genetic material with heightened polyphenolic content could serve as valuable resources in the study of medicinal plants for their contribution to global health by resisting oxidative stress caused by reactive oxygen species (ROS) (Juan *et al.*, 2021).

Evaluating antioxidant activity through the FRAP method provides insights into mutants' capability to reduce free radicals' strength (Calvindi *et al.*, 2020). The pertinent results also revealed the mutants with high FRAP values among types A, B, and C, indicative of their superior free-radical quenching potential compared with mutants of types D, E, and the wild type. Cluster analysis of polyphenol characteristics and antioxidant capacity identifies two potential mutants, specifically those with flower types A and C. It is worth noting that the TFC in Type C and the TPC and FRAP in specific flower-type mutants exceeded the levels observed in the past study (Manivannan *et al.*, 2021), which examined TPC, TFC, and antioxidant activities in dried samples of a French marigold genotype and nine African marigold genotypes. In the *T. patula* mutants and the wild-type genotype, the relationship between the polyphenol contents and antioxidant activity also had probing, which exhibited a close relationship through cluster analysis. Anthocyanins, phenolics, and flavonoids have a strong positive correlation. Remarkably, phenolics and flavonoids stand as the principal secondary metabolites within the polyphenol family (Tungmunnithum *et al.*, 2018), while anthocyanins had a subset of flavonoids classification themselves (Mattioli *et al.*, 2020). The antioxidant activity test through the FRAP method showed no significant correlation with the polyphenol component. The same study results came from Manivannan *et al.* (2021), wherein FRAP also had no significant

correlation with TPC. However, Chen *et al.* (2023) stated that FRAP did not correlate well with the phenolics, including TPC, TFC, and TAC.

The quantification of color values obtained from the color space system further illuminated the relationship between polyphenol contents, antioxidant activity, and the color of mutant and wild-type flowers. The L* value showed a significant negative correlation with the polyphenol, while the a* value has a positive correlation solely with TAC. This correlation aligns with previous studies that consistently established a strong association between the color values, such as L* and a*, and anthocyanin content in various flowers (Wang *et al.*, 2023). Following anthocyanin accumulation, the value of L* decreases while a* gradually increases. This trend, observed in various anthocyanins, such as cyanidin-3-O-(6''-O-malonyl-glucoside), cyanidin-3-O-(3'', 6''-O-dimalonyl-β-glucopyranoside), and other anthocyanins, is indicative of the phenotypic darker and redder flower colors (Lu *et al.*, 2021).

The FRAP analysis exhibited a nonsignificant correlation with either the polyphenol component or the L* and a* values. However, FRAP has a strong positive correlation with b* values. The b* value showed positive correlations with lutein and total carotenoids, as also confirmed by Lu *et al.* (2021). Previous research by Ingkasupart *et al.* (2015) involving 11 marigold cultivars also demonstrated a positive correlation between lutein and various antioxidant assays, including FRAP. The antioxidant activity could also closely link with the plant carotenoid content, a known contributor to the yellow-orange hues observed in flowers (Chensom *et al.*, 2019). Moreover, the presented study also highlights the potential of the mutant marigold genotypes to offer unique and beneficial traits, such as enhanced polyphenol content, which may have implications for the ornamental and medicinal plant fields.

CONCLUSIONS

The mutation through gamma irradiation effectively modified the morphological traits and biochemical properties of the *T. patula* wild-type flower, leading to the imminent mutants possessing ornamental values and serving as a source of high TAC, TFC, TPC, and antioxidants. Potential mutants resulted within Type-C and Type-A mutant flowers. The darkest red color of Type-C mutant adaxial florets was due to the highest TAC (61.051 mg Cy3G 100 g^{-1} FW), TPC (10.346 mg GAE g^{-1} FW), and TFC (5.706 mg QE g-1 FW) compared with other flower types, including the wild type. In comparison, the Type-A mutant flowers also displayed the maximum level of antioxidant activity (125.168 µmol TE g^{-1} FW).

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