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Research Article

The Analysis of Morphological Diversity and Polyphenols Content of *Celosia cristata* in M2 Population Induced by Ethyl Methane Sulphonate

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ABSTRACT

Celosia cristata, an edible ornamental plant, is a potential floricultural commodity that needs further improvement to increase its agro-morphological characters and polyphenol content. Induced mutagenesis using ethyl methane sulphonate (EMS) is an effective tool to increase genetic diversity that has been applied in many plant species. This study aimed to assess the morphological diversity, polyphenol content, and antioxidant activities of *C. cristata* mutagenized by EMS in the M2 generation. A total of 230 M2 plants generated from the M1 generation were evaluated in this study and the polyphenols content and antioxidant activities analysis were conducted on fifteen selected M2 plants. Polyphenols content was analyzed using the Folin-Ciocalteu method and colorimetric method with slight modification, and the antioxidant activities investigated using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant power (FRAP) assay with minor changes. There are six subpopulations with the highest diversity of quantitative characters in the M2 population in quantitative characters, i.e. C2-17-1.0%, C2-1-0.7%, C2-20-2.0%, C2-25-0.7%, C2-1-0.9%, and C2-1-0.7%. Changes in the shape and color of leaves, stems, and flowers of *C. cristata* were also observed in the M2 population. C2.1, C2.6, and C2.12. 2.1, C2.6, and C2.12 are potential plants derived from EMS mutagenesis with the highest polyphenol content and antioxidant capacity in the M2 population. In conclusion, induced mutation using EMS can enhance the agro-morphological diversity, polyphenols content along with the antioxidant activities of *C. cristata*, and demonstrate the successful mutation breeding program.

Keywords: EMS mutagenesis, Ornamental plant, Polyphenols content

Introduction

Floriculture is one of the important commodities with a selling value based on attractiveness, uniqueness, and comfort. *Celosia cristata* is one of the floriculture commodities known for its unique flower, similar to a cockscomb. Besides as a landscape plant, *C. cristata* is utilized as potted and cut flowers [1]. *C. cristata* is abundant in secondary metabolites such as flavonoids, saponins, betalains, and phenolic glycosides [2]. In addition, one of the medical advantages that can be obtained from *C. cristata* is its antioxidant activity to subdue free radicals [3]. Antioxidants in *C. cristata* are beneficial for countering various acute and chronic ailments [4]. Based on these facts, *C. cristata* is a 'versatile' floriculture commodity because of its usefulness as an ornamental and medicinal plant.

To date, the challenge faced by the floriculture industry is to provide ornamental plants with prominent traits. Particularly, in terms of morphological variations of *C. cristata*, Muhallilin et al. [5] stated that the morphological diversity of *C. cristata* is limited. Consumer demands for the availability of new, unique, and attractive shapes and colors from ornamental plants can be provided through a plant breeding program. Plant breeding

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plays a major role in producing new varieties with desirable traits.

One of the breeding methods that can be applied to increase morphological diversity is artificial mutation. Mutation techniques have produced potential genotypes in various floricultural commodities [6, 7]. One of the mutation sources that mutation breeders have widely used is ethyl methane sulphonate (EMS). Several studies have reported an increase in genetic diversity caused by EMS treatment. Lenawaty et al. [8] reported changes in the agro-morphological character of *Tagetes* sp. due to EMS treatment, changes in stem morphology of *Chrysanthemum indicum* in the M3 generation due to induced mutation using EMS were also reported by Purente et al. [9]. Besides being proven to increase plant phenotypic variation, EMS mutagenesis is well known to be involved in modifying the plant's secondary metabolite. Induced mutation using EMS increased the quantity of scopolamine and hyoscyamine in *Hyoscyamus niger* [11]. Gurdon et al. [12] also stated the potency of EMS in increasing the flavonoid content in red lettuce.

Recently, mutation breeding in *C. cristata* has been carried out using gamma-ray irradiation and regenerated for up to three generations (M3 generation) [5, 10, 13]. In this study, the agro-morphological characters and polyphenol content of *C. cristata* need to be observed further; thus, this study aims to evaluate the diversity of agro-morphological traits, polyphenol content, and antioxidant activities of *C. cristata* putative mutants in the M2 population.

Material and Methods

Study area and genetic material

This research was held from June to October 2021 at the experimental field of Pasir Sarongge IPB, Ciputri, West Java (latitude (S): 6° 04' 22", longitude (E): 106° 03' 08", altitude (m): 1026). The genetic material in this study was M2 seeds of *C. cristata* derived from EMS treatment. A total of 210 seeds of the M2 generation and 20 wild-type seeds (M0), obtained from the previous researcher [13], were planted as the M2 population. The seeds of M1 were obtained through EMS treatment (0.5%-4% v/v). Maintenance in this work contained watering, pest and disease control, and fertilizing. Observation of agro-morphological characters includes quantitative and qualitative traits. Observation of quantitative traits consisted of plant height, stem diameter, number of branches, flower length, flower width, and number of flowers, while the qualitative traits consisted of the intensity of anthocyanin coloration at the base of the stem, shape in cross-section of the stem, leaf shape, and leaf color. Documentation in photos was conducted to compare the variation of flower shape between M2 plants and M0 plants.

Extraction and Biochemical analysis

Fifteen healthy *C. cristata* plants were selected to analyze the polyphenol content and antioxidant activity. The main and lateral flowers of *C. cristata* were used as samples in this study. Extraction of *C. cristata* samples using the maceration method referred to Khumaida et al. [14] with slight modifications.

The total phenolic analysis referred to Rindita et al. [15] with a minor change, and the total flavonoid analysis referred to Calvindi et al. [16] with a minor change. Measurement of absorbance values of total phenolic and total flavonoids at wavelengths of 750 nm and 415 nm, respectively. The procedure for analyzing antioxidant activity using the DPPH method in consonance with Suleria et al. [17] with a slight modification, absorbance was determined at a wavelength of 515 nm. The antioxidant analysis using the FRAP method following Sahid et al. [18] with a slight modification determined absorbance at a wavelength of 593 nm. The instrument used to measure the absorbance of the test sample was a nano-spectrophotometer (SPECTROstarNano BMG LABTECH).

Data analysis

Quantitative character data visualized by boxplot using R Statistics software with ggplot2 package, and biochemical data were analyzed using Analysis of Variance (ANOVA) in R Statistics software with the Expdes package. Qualitative data analysis was performed by calculating the percentage of qualitative characters in each subpopulation using Microsoft Excel.

Results and Discussion

Quantitave traits

The data visualization of several quantitative traits in the M2 population of *C. cristata* is attached in Figure 1. Visualizing the distribution of

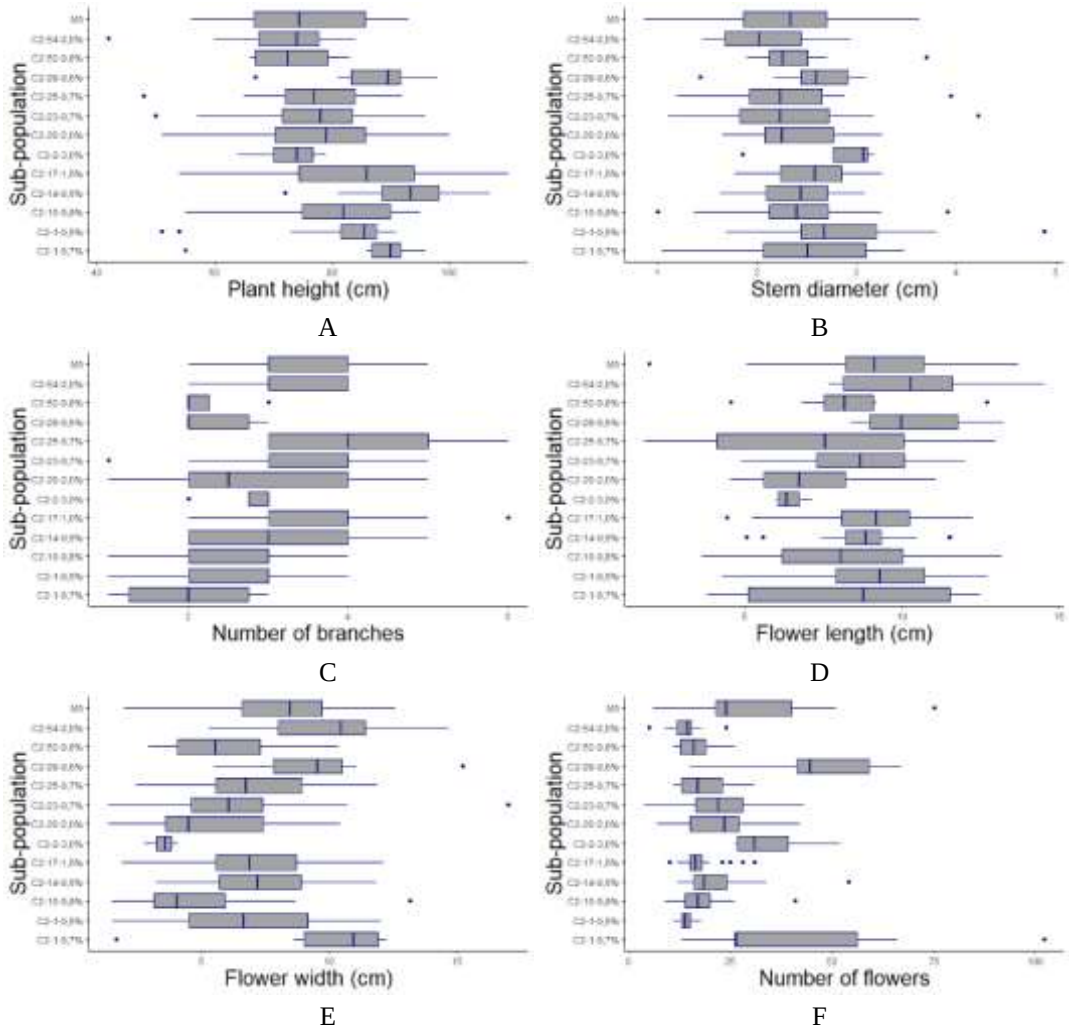

Figure 1. Boxplot of quantitative traits of *C. cristata* in M2 population. Plant height (A), Stem diameter (B), Number of branches (C), Flower length (D), Flower width (E), Number of flowers (F).

data using boxplots can reveal information about the diversity among populations [19]. Box size (refers to the interquartile range), whiskers length, and the appearance of outliers are important indicators for comparing characteristics between populations [5, 10]. The sub-population C2-17-1.0% showed the largest variation in population M2 in the character of plant height, whereas the subpopulation C2-1-0.7% pointed with the smallest diversity on plant height character. Moreover, outliers in several subpopulations include C2-54-2.0%, C2-28-0.6%, C2-25-0.7%, C2-23-0.7%, C2-14-0.9%, C2-1-0.9%, and C2-1-0.7% have lower values than M0, denoting that these plants are shorter than M0 plants. In stem diameter, subpopulation C2-1-0.7% was recorded with the highest diversity, while C2-2-3.0% was recorded with the lowest diversity. Four subpopulations have outliers greater than M0, namely C2-52-0.8%, C2-25-0.7%, C2-23-0.7%, and C2-1-0.9%. This occurrence indicates that some plants have thicker stem diameters than M0.

The boxplot on the number of branches showed that the subpopulation C2-20-2.0% had the highest variousness, followed by C2-25-0.7% and C2-14-0.9%. C2-17-1.0% had the highest outlier value among M2 subpopulations, while C2-23-0.7% had the lowest outlier value. The C2-25-0.7% had the highest flower length variation, and C2-3-3.0% showed the lowest. Four recorded subpopulations had outliers in flower length, including M0, C2-52-0.8%, C2-17-1.0%, and C2-14-0.9%. The highest diversity of flower width is found in C2-1-0.9%, while the lowest was in C2-2-3.0%. Four subpopulations had outliers, including C2-28-0.6%, C2-23-0.7%, C2-1-0.7%, and C2-10-0.8 Subpopulation C2-1-0.7% had the highest diversity in the number of flowers, and C2-

1-0.9% showed the lowest. Six subpopulations had outliers were C2-17-1.0%, C2-14-0.9%, C2-10-0.8%, and C2-1-0.7%. Interestingly, C2-17-1.0% had five outliers and was recorded as the subpopulation with the most outliers in population M2.

This study indicates that induced mutation using EMS can increase the diversity of quantitative traits of *C. cristata* in the M2 population. Several studies also reported an increase in morphological diversity induced by EMS. Siddique et al. [21] reported an increased morphological diversity in pepper in the M2 population derived from EMS mutagenesis. Espina et al. [20] also investigated a phenotypic improvement of mutated soybean in the M2 population through induced mutation using EMS. In accordance with Joya-Dávila and Gutiérrez-Miceli [22], the highest genetic diversity in mutation breeding programs arises in the M2 generation. In this regard, the M2 generation is an important generation to gain as much diversity as is feasible.

Qualitative traits

Changes in the qualitative traits of *C. cristata* were observed in the M2 population. In this study, observations of stem qualitative traits comprising the intensity of anthocyanin coloration at the base of the stem and shape in the cross-section of the stem are summarized in Table 1. The subpopulation M0 exhibited medium and strong anthocyanin intensities of 15.7% and 84.3%, respectively. In comparison to M0, there are nine subpopulations with weak anthocyanin intensity, including C2-1-0.9%, C2-10-0.8%, C2-28-0.6%, C2-14-0.9%, C2-23-0.7%, C2-17-1.0%, C2-54-2.0%, and C2-25-0.7%. The subpopulation C2-17-1.0% was recorded with the highest proportion of weak anthocyanin intensity, the highest proportion of medium anthocyanin intensity was found at C2-54-2.0%, and the highest proportion of strong anthocyanin intensity was found at C2-54-2.0%. Shape in the cross-section of the stem of entire M0 plants appeared circular shape (100%). Shape in the cross-section of the stem of entire M0 plants appeared circular shape (100%), while four subpopulations were noted to have a flattened shape in the cross-section of the stem, including C2-14-0.9% (4.2%), C2-20-2.0% (9.1%), C2-17-1.0% (8.1%), and C2-54-2.0% (9.1%).

Table 2 presents the proportion of leaves shape diversity of *C. cristata* in the M2 population. In this study, the original leaves shape of *C. cristata* was ovate, as indicated by the proportion of ovate leaves in M0 (100%). Elliptic (11.1%) and lanceolate (3.7%) leaves were found in subpopulation C2-1-0.9%. Additionally, in the subpopulation C2-17-1.0%, 2.7% of plants have lanceolate leaves. Alteration in leaf color of *C. cristata* in the M2 population is presented in Table 3. Akin to the proportion of leaves shape, all plants in subpopulation M0 show green leaf color (100%). Six subpopulations had greenish-red leaves color comprised C2-1-0.9% (3.7%), C2-52-0.8% (12.5%), C2-20-

Table 1. Stem traits percentage of *C. cristata* in M2 population.

Subpopulation	Percentage of intensity of anthocyanin coloration at the base of the stem (%)			Percentage of shape in cross-section of stem (%)	
	Weak	Medium	Strong	Circular	Flattened
M0	0	15.7	84.3	100	0
C2-1-0.9%	14.8	33.4	51.8	100	0
C2-10-0.8%	30	20	50	100	0
C2-1-0.7%	0	50	50	100	0
C2-28-0.6%	33.4	22.3	44.3	100	0
C2-52-0.8%	0	25	75	100	0
C2-20-2.0%	40	0	60	100	0
C2-14-0.9%	29.2	20.8	50	95.8	4.2
C2-20-2.0%	0	27.3	72.7	90.9	9.1
C2-23-0.7%	3.7	4	92.3	100	0
C2-17-1.0%	51.3	16.2	32.5	91.9	8.1
C2-54-2.0%	27.3	36.4	36.3	90.9	9.1
C2-25-0.7%	14.3	0	85.7	100	0

Remarks: The description for the qualitative character of *C. cristata* refers to UPOV 2002.

Table 2. Leaf shape percentage of *C. cristata* in the M2 population.

Subpopulation	Percentage of leaf shape (%)				
	Narrow Elliptic	***Elliptic***	***Ovate***	***Broad ovate***	***Lanceolete***
M0	0	0	100	0	0
C2-1-0.9%	0	11.1	85.2	0	3.7
C2-10-0.8%	0	0	100	0	0
C2-1-0.7%	0	0	100	0	0
C2-28-0.6%	0	0	100	0	0
C2-52-0.8%	0	0	100	0	0
C2-20-2.0%	0	0	100	0	0
C2-14-0.9%	0	0	100	0	0
C2-20-2.0%	0	0	100	0	0
C2-23-0.7%	0	0	100	0	0
C2-17-1.0%	0	0	97.3	0	2.7
C2-54-2.0%	0	0	100	0	0
C2-25-0.7%	0	0	100	0	0

Remarks: The description for the qualitative character of *C. cristata* refers to UPOV 2002.

Table 3. Leaf color percentage of *C. cristata* in the M2 population.

Subpopulation	Percentage of leaf color (%)		
	Green	***Greenish red***	***Red purple***
M0	100	0	0
C2-1-0.9%	96.3	3.7	0
C2-10-0.8%	100	0	0
C2-1-0.7%	100	0	0
C2-28-0.6%	100	0	0
C2-52-0.8%	87.5	12.5	0
C2-20-2.0%	80	20	0
C2-14-0.9%	91.7	8.3	0
C2-20-2.0%	100	0	0
C2-23-0.7%	92.3	7.7	0
C2-17-1.0%	100	0	0
C2-54-2.0%	100	0	0
C2-25-0.7%	90.5	9.5	0

Remarks: The description for the qualitative character of *C. cristata* refers to UPOV 2002.

2.0% (20%), C2-14-0.9% (8.3 %), C2-23-0.7% (7.7%), and C2-25-0.7% (9.5%).

Changes in the main inflorescence of *C. cristata* were noticeable in the M2 population (Figure 2). The flower shape is the most eminent character of *C. cristata*. The flower shape of seven novel plants in the M2 population showed a striking variation compared to M0 plants. In line with the results of this study, Aisyah et al. [10] and Muhalilin et al. [5] have reported the success of artificial mutations, using gamma ray irradiation, to increase the diversity of agro-morphological characters of *C. cristata*. However, these potential genetic resources are needed to be evaluated further for the stability of its agro-morphological characters. Simanjuntak et al [13] assessed the stability of the agro-morphological characters of *C. cristata* in the M3 generation.

Polyphenols content and antioxidant capacities

Concerning the *C. cristata* potency as a traditional medicinal plant, we analyzed the polyphenols and antioxidant activity of selected *C. cristata* in the M2 population. The polyphenol content and antioxidant activity analysis of *C. cristata* in the M2 population are presented in Table 4. The mean of total phenolics ranged from 10.90 – 16.63 mg GAE g-1 DW. Meanwhile, total flavonoids ranged from 1.01 to 1.41 mg QE g-1 DW. Evaluation of antioxidant activity using the DPPH method

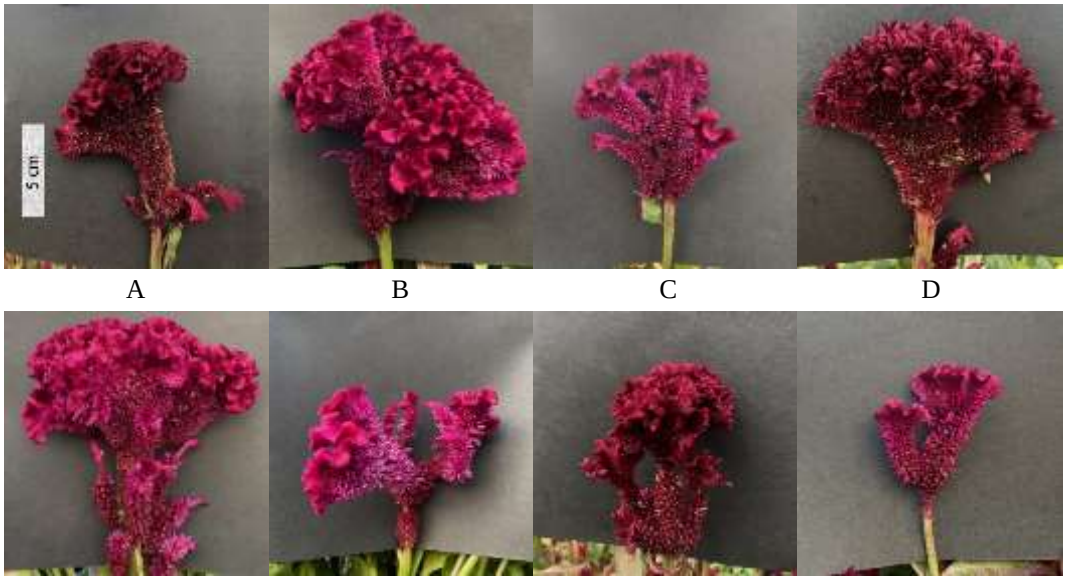

Figure 2. Flower shape variation of *C. cristata* in M2 population. M0 (A), C2-28-0.6%-2 (B), C2-1-0.7%-1 (C), C2-25-0.7%-7 (D), C2-10-0.8%-9 (E), C2-1-0.7%-2 (F), C2-1-0.9%-2 (G), C2-14-0.9%-15 (H).

Table 4. Polyphenols content and antioxidant activities of selected *C. cristata* in M2 population.

Plants	Phenolics (mg GAE g^{-1} DW)	Flavonoids (mg QE g^{-1} DW)	DPPH (µmol TE g^{-1} DW)	FRAP (µmol TE g^{-1} DW)
M0	11.66^{fg}	1.13^{b-d}	0.82^{ab}	26.27^{ef}
C2.1	**16.63^{a}**	**1.41^{a}**	0.23^{de}	47.00^{ab}
C2.2	14.19^{bc}	1.31^{ab}	0.50^{b-e}	37.79^{a-e}
C2.3	13.55^{c-f}	1.39^{a}	0.55^{b-d}	31.30^{c-f}
C2.4	10.69^{g}	1.04^{cd}	0.84^{ab}	23.67^{f}
C2.5	11.60^{fg}	1.14^{b-d}	0.59^{bc}	37.18^{a-f}
C2.6	10.90^{g}	1.01^{d}	**0.93^{a}**	33.48^{b-f}
C2.7	10.93^{g}	1.23^{a-d}	0.71^{a-c}	28.58^{d-f}
C2.8	12.08^{d-g}	1.21^{a-d}	0.43^{c-e}	33.85^{a-f}
C2.9	12.02^{d-g}	1.09^{b-d}	0.73^{a-c}	29.79^{d-f}
C2.10	14.14^{bc}	1.24^{a-c}	0.73^{a-c}	36.09^{a-f}
C2.11	14.04^{cd}	1.18^{a-d}	0.17^{e}	41.85^{a-d}
C2.12	16.14^{ab}	1.23^{a-d}	0.44^{c-e}	**47.67^{a}**
C2.13	11.88^{e-g}	1.13^{b-d}	0.83^{ab}	30.88^{c-f}
C2.14	13.82^{c-e}	1.23^{a-d}	0.20^{e}	44.70^{a-c}

Note: *Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to Honest significant difference (HSD) Test.

showed means varied from 0.17 – 0.93 mol TE g-1 DW. The means of antioxidant activity using the FRAP method was varied from 23.67 – 47.67 mol TE g-1 DW. The highest phenolic and flavonoid contents were found in C2.1, the highest antioxidant activity according to the DPPH method was recorded at C2.6, whereas C2.12 has the highest antioxidant capacity based on the FRAP method. These finding states that the mutagenized *C. cristata* in the M2 population showed a remarkable variation in the polyphenols content jointly with the antioxidant capacities.

Of most interest, plant C2.6 has the highest antioxidant activity based on the DPPH method but has the lowest phenolics and flavonoids. Thus, C2.6 needs to be evaluated further to study the metabolites that are actively involved in the antioxidant mechanism of DPPH. Lama-Muñoz et al. [23] stated that uncovering the mechanism of antioxidants in a plant is important because each

plant has a specific mechanism to repress free radicals. The test using the stable free radical DPPH aims to determine polyphenols' mechanism of electron donation against free radicals [24]. The mechanism of polyphenols to reduce electrons from free radicals to become stable molecules can be grasped through FRAP analysis [17]. This study also revealed the mechanism of *C. cristata* polyphenols through the reduction of free radical capacity. These findings agree with the results of Calvindi et al. [16] which reported the polyphenols' mechanism in the winged bean.

Conclusion

EMS mutagenesis successfully increased the diversity of agro-morphological characters (quantitative and qualitative traits) polyphenols content, and antioxidant activities of *C. cristata* in M2 generation. Further research is needed to evaluate the stability of agro-morphological characters of *C. cristata* and metabolite profiling to explore potential metabolites of *C. cristata*.

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