Sains Malaysiana 52(8)(2023): 2175-2190
http://doi.org/10.17576/jsm-2023-5208-02

Genetic Relationship Analysis of Chrysanthemum Genotypes Based on Quantitative and Qualitative Characters

(Analisis Hubungan Genetik Genotip Chrysanthemum Berdasarkan Sifat Kuantitatif dan Kualitatif)

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Received: 12 January 2023/Accepted: 11 July 2023

ABSTRACTS

Genetic improvements through biotechnological approaches have been successfully employed in many economically important crops, including ornamentals. The gamma-ray particle bombardment has been applied in the chrysanthemum variety Puspita Nusantara and superior mutants has successfully generated without degrading its important marketable characteristics. The vegetative and reproductive performances of 47 superior mutant genotypes from Puspita Nusantara are evaluated and compared with three references varieties, i.e., Puspita Nusantara, Stangkon and Arosuko Pelangi based on the quantitative and qualitative characteristics. The evaluation was carried out to select superior mutants with better characteristics. The results show that all chrysanthemum genotypes exhibited variations in quantitative characteristics, except in node length, the width of the widest point of inflorescence, the number of flowers per plant, and floret width. Six qualitative characters, i.e., non-glossy leaves, medium indentation depth, the existence of keel, inner and outer floret color, disc color before anther dehiscence, and disc color after anther dehiscence were similar in all genotypes. Mutant clones G6, G8, C1, KA7, G9, AG0, N9, and Q5 show preferable quantitative performances than the reference varieties. Clone W5 has comparative characteristics to Stangkon and can be further evaluated for alternative reference. The selected mutant genotypes provide better choices for farmers to plant more competitive varieties.

Keywords: Chrysanthemum; mutant clones; mutation breeding; qualitative characteristics; quantitative characteristics

ABSTRAK

Penambahbaikan genetik melalui pendekatan bioteknologi telah berjaya diguna pakai dalam pelbagai tanaman penting daripada segi ekonomi, termasuklah tanaman hiasan. Pembedilan zarah sinar gamma telah digunakan dalam kekwa Puspita Nusantara dan mutan superior telah berjaya dijana tanpa merendahkan ciri pemasaran penting. Prestasi vegetatif dan reproduktif 47 genotip mutan superior daripada Puspita Nusantara dinilai dan dibandingkan dengan tiga varieti semakan iaitu Puspita Nusantara, Stangkon dan Arosuko Pelangi berdasarkan ciri kuantitatif dan kualitatif. Penilaian telah dijalankan bagi memilih mutan superior dengan ciri yang lebih baik. Keputusan menunjukkan bahawa semua genotip kekwa menunjukkan variasi dalam ciri kuantitatif, kecuali panjang nod, lebar poin terluas perbungaan, bilangan bunga setiap pokok dan lebar floret. Enam ciri kualitatif iaitu daun tidak berkilat, kedalaman lekukan pertengahan, kewujudan tunas, warna floret dalam dan luar, warna cakera sebelum dehisen anter dan warna cakera selepas dehisen anter adalah serupa bagi kesemua genotip. Klon mutan G6, G8, C1, KA7, G9, AG0, N9 dan Q5 menunjukkan prestasi kuantitatif yang lebih baik daripada varieti semakan. Klon W5 mempunyai ciri bandingan dengan Stangkon dan boleh dinilai selanjutnya untuk semakan alternatif. Genotip mutan terpilih dapat memberi lebih pilihan kepada petani untuk menanam varieti yang lebih kompetitif.

Kata kunci: Ciri kualitatif; ciri kuantitatif; kekwa; klon mutan; pembiakbakaan mutase

Introduction

Chrysanthemum (*Dendranthema grandiflora* Tzvelev) is a dicotyledonous genus belonging to the family Asteraceae. These herbaceous annual or perennial plants originated in East Asia and are of great ornamental, medicinal, environmental, and industrial value. Chrysanthemum is amongst the most valuable floricultural crops in the world, and compared to other commercial cut flowers, chrysanthemum has a wide range of variations in flower color and shape, leaf shapes, flowering responses to short-day conditions, and resistance to pests and diseases (Hadizadeh, Samiei & Shakeri 2022). This genus has also displayed multiple therapeutic potentials, phytochemistry, and pharmacological features, including antioxidant, antimicrobial, anti-inflammatory, anticancer, anti-allergic, anti-obesity, immune regulation, hepatoprotective, and nephroprotective activities (Gu et al. 2022; Li et al. 2020; Liang, Gong & Zhang 2021). As an ornamental crop, chrysanthemum has replaced roses as the most marketed cut flowers in Indonesia since 2006 in the form of cut flowers and potted plants. The plant is also studied and processed for snacks, tea, and biopesticide (Hutapea et al. 2020; Puspitasari & Indradewa 2018).

Efforts to make chrysanthemum production more efficient and profitable have been carried out in several ways, including breeding activities. Breeding activities since the 2000's have released superior cut flower and potted chrysanthemum varieties with various petal colors, resistant to important diseases and adaptive to medium elevation. Among the released varieties, the spray-type chrysanthemum 'Puspita Nusantara' has more competitive market demand than the imported varieties. Having bright yellow petal color with a strong peduncle and being more tolerant to white rust (*Puccinia horiana* Henn.) has made the cultivar merely preferred by consumers and growers (Hanudin & Marwoto 2012). Several years later, however, several reports indicated that the tolerant characteristics of this cultivar had been broken (Nuryani et al. 2018).

Up to now, growers are still planting the said cultivar among other commercial cultivars and expecting high-frequency fungicides to reduce the disease or even prevent the plants from white rust attacks. These unwise cultural practices will harm human health and the environment and make the production process uncompetitive (Hanudin, Budiarto & Marwoto 2017). One way to deal with such a situation is genetic improvement of the chrysanthemum variety Puspita Nusantara through a biotechnological approach, gamma ray particle bombardment (Sadewi & Khumaida, 2013; Kurniasih et al. 2016). The method is expected to produce little variations on the solid mutant genotypes from 'Puspita Nusantara' without degrading its important marketable characteristics.

About 47 mutant genotypes have been visually selected based on the similarity of qualitative characters to Puspita Nusantara, like petal color, the strength of peduncle, resistance to white rust, etc. The observations on quantitative characters are needed to support the qualitative parameters in differentiating or grouping the mutant genotypes (Anne & Lim, 2021; Song et al. 2018). Analysis of the quantitative characters is also dedicated as a primary selection of mutant genotypes with superior traits to the reference varieties. The genotype represents the preferred vegetative characters with fast apical growth, short to medium internode length, and a strong stem. At the same time, the essential reproductive characteristics include larger flower size, a higher number of flowers per plant, attractive petal color, and long shelf-life. The research aims to evaluate the vegetative and reproductive performances of 47 essential mutant genotypes from Puspita Nusantara with three reference varieties, i.e., the parent' Puspita Nusantara, Stangkon and Arusuka Pelangi based on the quantitative dan qualitative characteristics.

Materials and Methods

The gamma-ray induction was carried out in 2016 on the callus of chrysanthemum variety Puspita Nusantara producing more than 600 M_1 potential mutant progenies. About 47 mutant genotypes were selected and further evaluated based on their qualitative and quantitative characteristics. The research was conducted under plastic house conditions at Cipanas Research Station, Indonesian Ornamental Crops Research Institute (IOCRI) at 1100 masl from July to December 2021. The planting materials were 25 rooted cuttings of 50 chrysanthemum genotypes, comprising 47 superior mutant progenies of Puspita Nusantara and three reference varieties, i.e., the parent Puspita Nusantara, Arosuko Pelangi and Stangkon. The use of Stangkon and Arosuko Pelani as the reference varieties for Puspita Nusantara mutant progenies is based on their similarities in leaf shape, flower type, and petal color. The Indonesian PVP office has recommended these two as the reference for the double spray type of bright yellow petal chrysanthemum evaluation. The 50 chrysanthemum genotypes are presented in Figure 2. The planting bed was constructed of 100 cm wide and 25 cm high. As much as 10 tons/ha horse manure, 300 kg/ha NPK (16:16:16) fertilizer, and 2 kg/m^2 carbonized rice husk were adequately mixed with the soil of the planting bed. A day before planting time, 10 l/m^2 water was gently poured into the planting bed to facilitate sufficient humidity when the cuttings were planted.

The cuttings were planted with a density of 25 per $m^{2,}$ and the arrangement of genotype plots was random among the tested genotypes based on a nested design. After planting, long day conditions were provided using 4 h of 18-watt-white LED lamp lighting every night (10.00 pm to 02.00 am) for 30 days to stimulate vegetative growth. Standard cultural practices were employed for maintaining the plant until the flowering period. During the cultures, a half dosage of synthetic insecticides followed the recommended frequency, but no fungicide was applied.

The parameters observed were quantitative traits based on TG to conduct the DUS test from UPOV (No. 26/5-17 December 2020). All the parameters were analyzed using the F test according to Scott and Milliken (1993). If there were significant differences among the tested factors (mutant genotypes and reference varieties), the evaluation was continued by using the Least Significant Increase (LSI) (Kurniasih et al. 2018). The UPGMA dendrogram was constructed using NTSY Spc version 2.0.

Results and Discussion

Quantitative expressions of tested genotypes

Based on the quantitative parameters, the mutant progenies show different attributes, including their respective parents (Table 1). Of the 19 quantitative characters, only the values of node length, the ratio of leaf length and width, the width of the widest point of inflorescence, the number of flowers per plant, and floret width showed negligible differences among the tested genotypes. The average values of the quantitative characters of the mutants are also within the ranges of the average values of references varieties, the parents, Arosuko Pelangi and Stangkon, except in days for floret coloring, and optimum flower opening, node length, ratio of leaf length and width and width of the widest point of inflorescence. The average values of days for floret coloring and optimum flower opening leaf length and width ratio of the mutant were lower, while on node length and width of the widest point of inflorescence, the average values were higher than the average values of the reference varieties.

In terms of coefficient of variation (CV), the value ranged from 4.55% (number of florets) to 19.01% (number of nodes). CV represents the variation within a population. The value was determined by the heterogeneity of the tested genotypes, reference varieties, number of treatments, and replications (Chanda et al. 2018; Mehdi & Ahsan, 1999; Ziegler & Tambarussi 2022). The low CV value in a character indicated that the character variation within the population was common or homogenous, or the genetic variability governing the respected nature was narrow. These inferred that the selection process for genetic improvement of the low-genetic variability character would be less fruitful (Adhikari et al. 2018; Karavolias et al. 2020). On the other hand, a high genetic variability character would make the selection process more efficient and give higher opportunities to get a preferred genotype (Chanda et al. 2018; Hegde et al. 2022).

TABLE 1. Quantitative attributes of the mutant clones and their parents

No.	Quantitative parameters	LSD (α≤5%)	CV (%)	Average value of mutant clones	Value range of mutant clones	Average of 'Puspita Nusantara'	Average of 'Arosuko Pelangi'	Average of 'Stangkon'
1.	Floret coloring (from the date of the neutral day)	1.14*	8.64	41.9	39.2-46.6	46.1	47.0	48.7
2.	Optimum flower opening (from the date of the neutral day)	1.26*	5.12	57.3	50.6-62.2	61.3	57.6	62.8
3.	Plant height (cm)	5.15*	8.11	95.4	68.6-114.6	89.4	98.6	74.0
4.	Number of nodes	0.95*	19.01	31.3	23.3-38.3	25.0	40.6	30.5
5.	Node length (cm)	0.42^{ns}	14.17	3.3	2.7-3.9	3.0	2.8	2.7
6.	Leaf length including petiole (cm)	1.32*	13.26	12.8	9.4-14.8	13.7	13.7	11.2
7.	Leaf width (cm)	1.92*	14.42	9.6	7.6-11.6	10.2	7.7	6.1
8.	Leaf length : width ratio	6.41*	7.92	1.3	1.2-1.5	1.4	1.8	1.9
9.	Width of the widest point of inflorescence (cm)	0.55^{ns}	13.53	18.2	13.8-23.9	15.0	16.9	15.9
10.	Number of flower /plant	0.42^{ns}	15.81	12.1	8.3-15.6	9.5	13.6	12.0
11.	Flower diameter (cm)	2.69*	9.14	6.5	5.6-7.7	7.1	4.9	5.6
12.	Lenght of flower peducle (cm)	2.62*	11.04	5.8	3.5-8.2	3.4	8.8	7.6
13.	Number of florets	8.06*	4.55	30	23.9-35.3	28.9	33.5	32.2
14.	Floret lenght (cm)	1.91*	9.25	3.2	2.8-3.6	3.3	2.6	2.8
15.	Floret width (cm)	0.44^{ns}	14.48	1.1	0.9-1.18	1.23	1.01	0.93
16.	Floret lenght : width ratio	1.13*	13.22	3.0	2.5-3.6	2.3	2.6	3.3
17.	Disc diameter (cm)	2.30*	13.93	1.9	1.5-3.4	1.9	1.8	1.6
18.	Diameter of disc relative to flower diameter	10.08*	7.52	0.3	0.2-0.6	0.28	0.37	0.30
19.	Vase life (days)	2.52*	11.75	21.6	14.8-27.1	17.6	18.8	16

* = significantly different under LSD (α≤5%), ns = significantly different under LSD (α≤5%)

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Vegetative characteristics

No accession has superior characteristics in all preferred traits among the tested mutants. In floret coloring (from the date of the neutral day), the average values of all tested genotypes ranged from 39.2 to 46.6 days, and Puspita Nusantara had the shortest among the reference varieties (Table 1). The mutant population has a shorter average period for floret coloring than reference varieties, and clone G6 has the fastest among the mutant population (Table 2). The longest period for floret coloring in mutant genotypes is A12, though it is still shorter than Arosuko Pelangi and Stangkon.

Regarding the period for optimum flower opening starting from the date of neutral day, 23 mutants accession has a faster period than Puspita Nusantara and Stangkon, and 24 genotypes have an earlier flower opening period than Arosuko Pelangi. Clone G8 was observed to have the shortest period for optimum flower opening at 50.6 days, and clone B1 was the longest with 62.2 days after the date of neutral day. The mutant genotypes tend to have a 5 to 12 days faster period for floret coloring and flower opening than the reference varieties. Related to these phenomena, gamma-ray irradiation seemed to affect accelerating the flowering period. Similar findings were also reported in rice and dendrobium (Hanifah et al. 2020; Sherpa et al. 2022). The shorter flowering period gives several advantages to the production process, i.e., input cost reduction due to the shorter plant maintenance, the increment of production cycles, and land use efficiency (Bosila, Hamza & Abdel-Gawad 2020).

The mutant clones generally have taller plants than Puspita Nusantara and Stangkon, and some mutants are taller than Arosuko Pelangi. Clone C1 was observed to be the most elevated (114.6 cm). Nineteen mutant clones, including C1, were taller than Arosuko Pelangi. Additional 15 clones were taller than Puspita Nusantara, and the other 13 were taller than Stangkon. The taller plant is an essential and preferred character in chrysanthemum-cut flowers. These findings are not following the study of Anne and Lim (2021) and Susila, Susilowati and Yunus (2019) that mutant clones derived from gamma-ray bombardment tend to have shorter plants than their parents. These different results implied that the genetic construction of the parent and randomly mutated genes might express other phenotypic performances of the mutants (Puripunyavanich et al. 2019).

Concerning the number of nodes, most mutant clones have negligible differences with Puspita Nusantara and Stangkon, yet none has more than Arosuko Pelangi. Clone O3 is the only mutant accession with more nodes than Puspita Nusantara and Stangkon. Arosuko Pelangi has the highest number of nodes among the tested chrysanthemum genotypes (40.6). The number of nodes represented the plant growth response, especially during the early planting stage. The more developed nodes indicated the slower growth response of young plants due to the poor quality of seedlings. Since the seedlings of all the tested chrysanthemum genotypes were managed correctly and prepared, thus the different numbers of nodes among the chrysanthemum genotypes were presumably related to the other genetic constructions among the tested chrysanthemum genotypes (Ahmed & Afsal 2003). According to Heins and Wilkins (1979), the light supply during a long day also influences the number of nodes. Though the sensitivity of each genotype might be different to the light, the plants provided by continuous light (more light) tended to have more auxiliary branches with a higher number of and shorter nodes.

Mutant clone C1 was observed to have the longest and the widest leaves among the tested chrysanthemum genotypes. This clone, thus, was also considered to have the most expansive leaf area. Other 30 mutant clones have longer leaves than Puspita Nusantara and Arosuko Pelangi, while 13 clones have wider leaves than Puspita Nusantara. According to several reports, gamma ray irradiation might induce longer or shorter leaves and leaf areas depending on the doses of gamma irradiation (Kumari & Kumar 2015). The leaf area reduction was usually observed on the plants with higher amounts of gamma-ray due to the disturbance in the auxin biosynthesis (Anne & Lim 2021). Regarding the leaf shape, most mutant clones have an almost circle type. This was referred to as the lower ratio of leaf length and width. Among the reference varieties, Puspita Nusantara is the only accession with a low leaf length and width ratio, while Stangkon and Arosuko Pelangi have more elliptic-leaf shapes.

Reproductive characteristics

The average inflorescence diameter of mutant clones was slightly lower than Puspita Nusantara yet higher than Arosuko Pelangi and Stangkon. Regarding flower diameter, only 6 clones have bigger flower diameters than Puspita Nusantara; most of the rest were smaller though the differences were negligible. The highest flower

TABLE 2. Comparative chrysanthemum performance based on quantitative attributes

No.	Quantitative traits	Ranking of chrysantemum genotypes						
		1	2	3	4	5	6	7
1.	Floret coloring (the shortest period)	G6	O3	C1	Y8	E1	A1	Q5
2.	Optimum flower opening (the shortest period)	G8	J3	O3	U2	S8	Y8	Q5
3.	Plant height (the tallest)	C1	H7	H8	Z3	A1	I9	Y6
4.	Number of nodes (the highest)	Arosuko Pelangi	O3	Z3	Q5	Y8	AF4	U2
5.	Leaf length (the longest)	C1	Y6	Z3	U2	H7	S7	Y8
6.	Leaf width (the widest)	C1	H7	E1	A1	Y6	R7	G7
7.	Leaf length: width ratio (the highest)	Stangkon	Arosuko Pelangi	Y8	O3	AB5	Y6	Z4
8.	Flower diameter (the highest)	KA7	C1	H8	H7	A1	AC4	Puspita Nusantara
9.	Length of flower peduncle (the longest)	Arosuko Pelangi	U2	O3	Stangkon	Y8	Z3	J3
10.	Number of florets (the highest)	G9	H7	C1	H8	G7	X7	J3
11.	Floret length (the longest)	C1	KA7	AG0	K8	AC4	H7	A1
12.	Floret width (the widest)	AG0	O3	KA7	Stangkon	Z4	W5	P0
13.	Floret length: width ratio (the highest)	N9	I4	G9	H8	Z3	I9	E1
14.	Disc diameter (the widest)	N9	I4	G9	H8	Z3	I9	E1
15.	Diameter of the disc relative to the flower diameter	N9	Arosuko Pelangi	I9	I4	J4	X7	Z3
16.	Vase life (days)	Q5	S8	L9	Y8	R7	T6	T5

diameter of mutant clones was detected at KA7. These findings indicate that gamma irradiation tended to have various effects on flower size. Similar results were reported by Lamseejan et al. (2000) and Wu et al. (2020) in their studies using different chrysanthemum types. They found that a specific dose of gamma-ray induced bigger flower, yet smaller flower mutant progenies were merely produced in higher gamma-ray doses than 30 to 35 Gy.

Unlike the standard type, flower size is less critical in spray-type chrysanthemums than the number of flowers per plant. The number of flowers per plant and uniformity of flowers would determine the compactness of the flowers constructed in a flower arrangement.

According to several reports, other physiological and morphological attributes affect flower size in spray chrysanthemums, like the growth quality and number of flowers. Apu et al. (2019) reported that removing the auxiliary branches would increase the terminal flower size but reduce the number of flowers per plant.

The average length of the flower peduncle of mutant clones was longer than Puspita Nusantara. Among the tested chrysanthemum genotypes, Arosuko Pelangi has the longest flower peduncle, and only two mutant genotypes, U2 and O3, have longer flower peduncles than Stangkon. The short flower peduncle was preferred (Kentelky et al. 2021). The shorter flower peduncle is related to the easiness of workers handling the inflorescence during packing and transportation. The shorter peduncle makes the flower more compact, easy to wrap and has less incidence of peduncle broken. The flower defect would determine the quality and the price on the market (Wijayani, Muafi & Sukwadi 2017).

Among the mutant clones, 27 had more florets than Puspita Nusantara, 12 were higher than Stangkon, and five were higher than Arosuko Pelangi. The highest number of florets was detected in clone G9, with a value of 35.3. The non-decorative type chrysanthemum generally has less than 40 florets, and all the mutant clones and the reference varieties have less than 40 florets. Thus, all the tested chrysanthemums were considered single or semi-double spray-type chrysanthemums, in which the flower has 2 to 3 layers of florets (Su et al. 2019). According to some reports, the number of florets was governed by a few simple genes, indicating that the trait was less affected by the environment (Huang et al. 2016; Liu et al. 2016).

The average floret length of the mutant clones was not significantly different from that of Puspita Nusantara but longer than Arosuko Pelangi and Stangkon. Thirty-eight mutant clones have shorter, yet nine clones have longer florets than Puspita Nusantara, i.e., C1, KA7, AG0, K8, AC4, H7, A1, H8, and R7. Only one clone (Y2) has a floret length in between Arosuko Pelangi and Stangkon. Respecting the ratio of floret length and width, most of the mutant clones have a higher floret length and width ratio than Puspita Nusantara and Arosuko Pelangi, and only three clones are higher than Stangkon. Based on the observation above, no specific clue can be drawn from comparing the mutant clones with the reference varieties. These inferred that the floret length was less affected by gamma ray treatment.

The average disc diameter of the tested genotypes were not significantly different with Puspita Nusantara, slightly lower than Arosuko Pelangi but higher than Stangkon. Most mutant clones have higher disc diameters than Stangkon, except clone T6. In terms of the relative value of disc diameter to flower diameter, the differences among the tested genotypes ranged between 0.2 and 0.6, which was categorized as slightly high. The higher the value of disc diameter relative to the flower diameter, the flower was considered as an anemone type (Lee et al. 2022). In contrast, the smaller values would categorize the flower into Santini types.

All the mutant clones have longer vase life than the reference varieties under room temperature. Clone Q5 has the longest, which can freshly withstand up to 27.10 days, while the shortest was clone J4, which has only 14 days of vase life. All the mutant clones have vase life characteristics above the Indonesian National Standard for chrysanthemum cut flowers that should exceed 10 days for grade A. Aside from the environmental factors like temperature, light, and humidity that affected the respiration and water uptake (Ferrante et al. 2005; Sharma & Srivastava 2014), the vase life of cut flower was also determined by the accumulation of assimilate on the cut flower before the flower was harvested. This carbohydrate source would be used for cut flower respiration and to maintain cell turgidity to facilitate the physiological and biochemical processes (Budiarto, Zamzami & Endarto 2022). Disregard the physical damages and improper handling during the harvesting process, the genotype with higher carbohydrate accumulation might have longer vase life since they can support the living cells longer (Mekapogu et al. 2022; Onozaki, Ikeda & Yamaguchi 2001)

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All chrysanthemum genotypes have similarities in six characters from the 27 observed characteristics (Table 3). These included non-glossy leaves, medium indentation depth, the existence of keel, similar inner and outer floret color, similar disc color before anther dehiscence, and similar disc color after anther dehiscence. The mutant accessions have 4 similar characteristics to Puspita Nusantara, yet different from Arosuko Pelangi and Stangkon. The relative length of the leaf stalk and blade was categorized as a medium in Puspita Nusantara, Stangkon, and the mutant genotypes, but it was short for Arosuko Pelangi. The leaf base shape and number of indentations were similar on Puspita Nusantara and the mutants. They have cordate-like leaf bases with few leaf indentations. Arosuko Pelangi has an obtuse, while

Stangkon has a rounded leaf base. Both genotypes show medium leaf indentation. Lastly, a single floret color was observed in all mutants, Puspita Nusantara and Stangkon, while Arosuko Pelangi has more than one floret color.

Gamma irradiation induced qualitative variations in the mutant population from the parent, Puspita Nusantara. There are at least 16 characteristics of the mutants to be different from Puspita Nusantara. The stem color of Puspita Nusantara is green, while varied from green, green-brown, or green-purple green in mutant genotypes. The leaf colors of mutants were lighter or darker, and the stipule size was smaller and larger than the parent. The angle of leaf stalk orientation was narrower or broader from the stem on mutants, while medium/flat orientation was observed in Puspita Nusantara. In the mutant population, the depth of the leaf sinus on the lateral leaf blade was medium to deep, with various forms of inflorescence. Puspita Nusantara has a shallow leaf sinus with umbrella-form inflorescence. Unlike Puspita Nusantara, which has single outer floret color with two keels, the mutant population has more variation from light yellow to orange with more than two keels. The floret longitudinal orientation, predominant inner floret colors, shape of floret tip, and type of flower head were diverse in the mutant population. Puspita Nusantara was categorized as susceptible to white rust. At the same time, mutant populations have susceptible, moderately resistant, and immune genotypes based on several pustules and disease indexes referring to Zeng et al. (2013) categorization. Similar findings were reported by Momin et al. (2012), Patil, Karale & Gaidhani (2019), and Rajasekar, Kanna and Kumar (2019) that gamma-ray induced variation in flower morphology and other reproductive characters in chrysanthemum.

KINSHIP ANALYSIS AMONG CHRYSANTHEMUM GENOTYPES

Analysis of kinship was carried out through a constructed dendrogram to evaluate the genetic relationship among the chrysanthemum genotypes based on the quantitative characters. The value of the coefficient of similarity represents the proximity of kinship among the tested chrysanthemum genotypes (Li et al. 2018; Thakur et al. 2022). The higher the similarity coefficient, the more similarities in the observed quantitative parameters, thus expectedly to have closer kinship (Naz et al. 2015). The genetic relationship of the mutant clones and the three reference varieties varied with a range of coefficient 70 to 100%. The detailed descriptions of relatedness among the tested genotypes are presented in Table 4.

Based on the constructed dendrogram (Figure 1) and description of similarity (Table 4), all chrysanthemum genotypes were fallen under the coefficient of similarity >70%. At a 70% similarity coefficient, two clusters (a and b) were observed. Cluster a included Stangkon, clones W5, P0, and J4, while cluster b was made from all genotypes except cluster a's members. At 78% similarity coefficient, cluster a was divided into clusters a1 and a2. Stangkon and clone W5 were fallen under cluster a1 with a coefficient similarity of 91.3%, while clone P0 and J4 were members of cluster a2 with a coefficient similarity of 83.4%. At 71.4% similarity, 2 clusters (b1 and c) were detected. Arosuko Pelangi was the only member of cluster b1, while cluster c contained all genotypes in cluster b, except Arosuko Pelangi. At 76.3 % similarity, cluster d1 was fallen into the same group as cluster e. Puspita Nusantara was the only member of cluster d1, while cluster e1 was made from all genotypes in cluster d, except Puspita Nusantara. At 77.7 % similarity, Puspita Nusantara was separated from those of cluster e.

At 100% similarity, clone T6 was classified in the same group as clones B1, T5, R7, and K8 based on the quantitative characters that served as the basis for the dendrogram construction. This means even though the detected similarity reached 100%, the quantitative phenotypic performances among these clones were not the same. Other characteristics have not been adequately described and are not included in constructing the dendrogram. These undescribed characteristics involved genetic constitution, secondary metabolite containment, and resistance to abiotic and biotic stresses (Gogoláková & Paganová, 2020; Hongbo et al. 2008; Kumar, Singh & Bhakuni 2005).

The constructed dendrogram gave important information for breeders to select promising clones with better phenotypic performance than the commercial reference varieties. These promising clones can be registered as commercial varieties and replace the respected reference variety. Clone W5, for instance, the coefficient similarity reached 91.3% to Stangkon. This accession has better characters than Stangkon, as presented in Table 5.

TABLE 3. Qualitative attributes of the mutant clones and their parents

No.	Qualitative traits	Mutant clones	Puspita Nusantara	Arosuko Pelangi	Stangkon
1.	Stem color	Green; green-brown; green-purple	Green	Green-brown	Green
2.	Leaf blade: Green color intensity	Light green; green; dark green	Green	Green	Dark green
3.	Size of leaf stipule	Small; medium; large	Medium	Large	Small
4.	Petiole attitude	moderately upright, horizontal, moderately downward	moderately upright	moderately downward	horizontal
5.	Relative length of leaf stalk to leaf blade	Medium	Medium	Short	Medium
6.	The depth of the leaf sinus on the lateral leaf blade	Shallow; Medium; Deep	Shallow	Deep	Medium
7.	Sinus position on the lateral leaf	Overlapping; parallel; touching	Overlapping	parallel	Touching
8.	Shape of leaf base	Cordate	Cordate	Obtuse	Rounded
9.	Leaf blade: glossiness	Absent	Absent	Absent	Absent
10.	Number of indentation	Few	Few	Medium	Medium
11.	The depth of indentation	Medium	Medium	Medium	Medium
12.	Form inflorescence	Dome; Convex Dome; Cylindrical; Umbrella; Flat umbrella	Umbrella	Cylindrical	Flat Umbrella
13.	Angle of petiole and stem	Narrow; medium; wide	Medium	wide	Medium
14.	Color of outer floret	Varied (Yellow 5A to Yellow Orange 24A)	Yellow orange 20A	Yellow orange 22A	Yellow 7A
15.	Floret surface	Keeled	Keeled	Keeled	Keeled
16.	Number of keels	Two; more than two	Two	Two	Two
17.	Longitudinal axis of floret	Reflexing; incurving; Straight	Reflexing	Straight	Straight
18.	Floret color	Light Yellow; Yellow; Dark Yellow; Orange	Dark Yellow	Orange	Light Yellow
19.	Length of Corolla tube	Shot; Medium; Long	Short	Short	Short
20.	Predominant inner color of floret	Varied (Yellow 3A to Yellow Orange 14A)	Yellow 7A	Yellow 9A spotted with Yellow Orange 13A	Yellow 4A
21.	Color of inner and outer parts of floret	Similar	Similar	Similar	Similar
22.	Type of flower head	Single; semi-double; double	Semi-double	Semi-double	Semi-double
23.	Shape of floret tip	Rounded; emarginate; pointed; dentate; mamillate	Rounded	Rounded	pointed
24.	Number of floret color	One	One	Two	One
25.	Disc color group before anther dehiscence	Yellow Green N144A	Yellow Green N144A	Yellow Green N144A	Yellow Green N144A
26.	Disc color group after anther dehiscence	Yellow	Yellow	Yellow	Yellow
27.	Disease index	Immune; monderate; susceptible	Susceptible	Monderate	Monderate

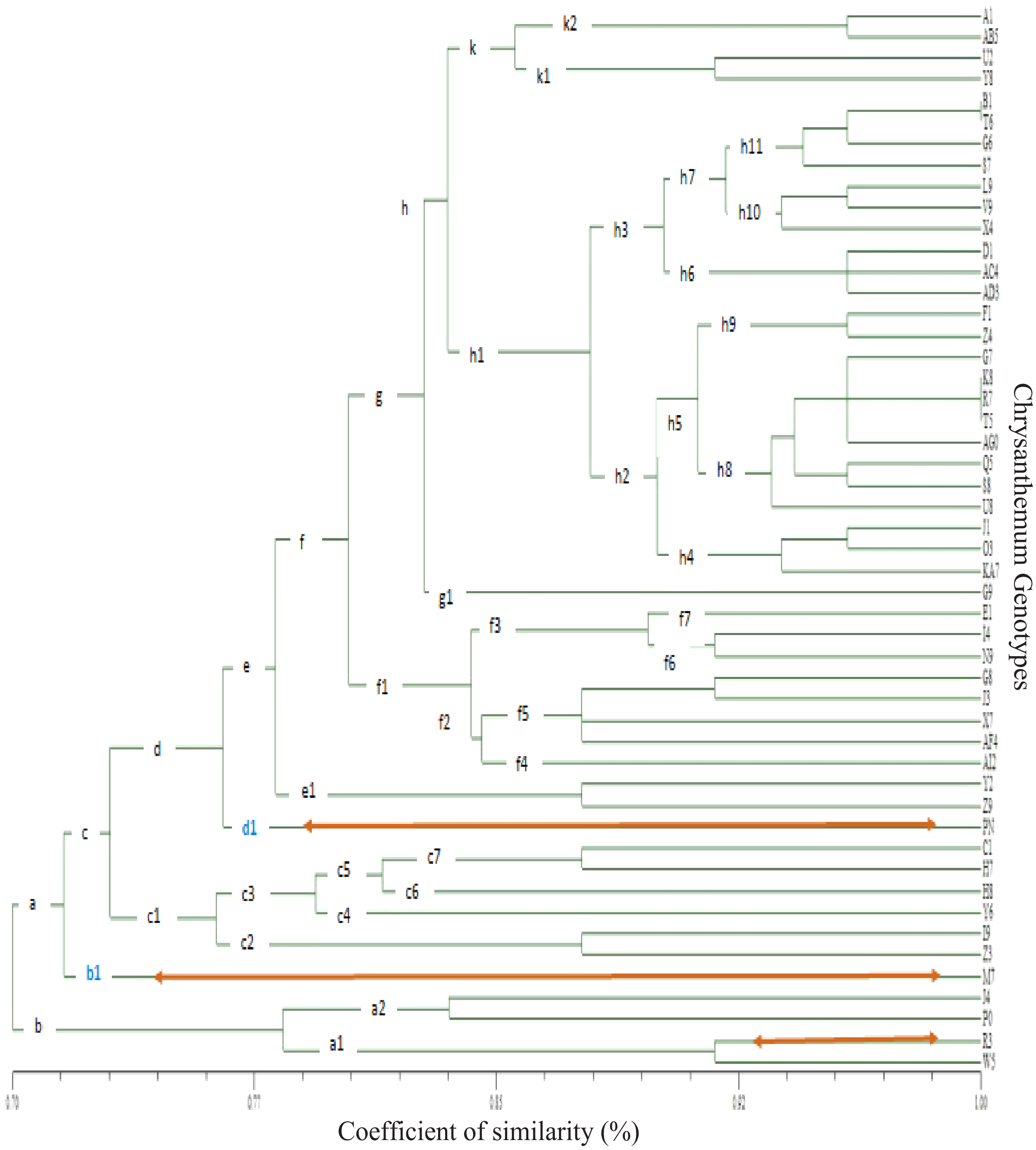

FIGURE 1. The constructed dendrogram of the tested chrysanthemum genotypes based on quantitative characters

FIGURE 2. The chrysanthemum genotypes used in the study
*A= Puspita Nusantara, B= Arosuko Pelangi , C= Stangkon , D1-D47= Mutant clones

TABLE 4. Description of similarity coefficient of the tested chrysanthemum genotypes

CV (%)	Cluster	Chrysanthemum genotypes
70 - <75		
70	a = b	Cluster a = clone R3, W5, P0, J4. Cluster b = all genotypes except clone R3, W5, P0, J4.
71.4	b1 = c	Cluster b1 = Arosuko Pelangi Cluster c = all genotypes in cluster b, except Arosuko Pelangi
72.8	c1 = d	Cluster c1 = clone Z3, I9, Y6, H8, H7, C1. Cluster d = all genotypes in cluster c, except clone Z3, I9, Y6, H8, H7, C1.
75 - < 80		
76	c2 = c3	Cluster c2 = clone Z3 and I9. Cluster c3 = clone Y6, H8, H7, C1.
76.3	d1 = e	Cluster d1 = Puspita Nusantara, Cluster e = all genotypes on cluster d, except Puspita Nusantara
77.7	e1 = f	Cluster e1 = clone Z9, Y2. Cluster f = all genotypes in cluster e, except clone Z9 and Y2.
78	a1 = a2	Cluster a1 = Stangkon and clone W5 Cluster a2 = clone P0 and J4
78.7	c4 = c5	Cluster c4 = clone Y6 Cluster c5 = clone klon H8, H7, C1.
80 - < 85		
80.2	f1 = g	Cluster f1 = clone A12, AF4, X7, J3, G8, N9, I4, E1. Cluster g = all genotypes in cluster f, except clone A12, AF4, X7, J3, G8, N9, I4, E1.
81.3	c6 = c7	Cluster c6 = clone H8 Cluster c7 = clone H7 and C1
82.6	g1 = h	Cluster g1 = clone G9 Cluster h = all genotypes in cluster g, except clone G9
83.4	h1 = k	Cluster h1 = all genotypes in cluster h, except clone Y8, U2, AB5, A1. Cluster k = clone Y8, U2, AB5, A1.
	-	clone *P0* = clone J4
84.0	f2 = f3	Cluster f2 = clone A12, AF4, X7, J3, G8. Cluster f3 = clone N9, I4, E1.
84.4	f4 = f5	Cluster f4 = clone A12. Cluster f5 = clone AF4, X7, J3, G8.
85 - < 90		
85.6	k1 = k2	Cluster k1 = clone Y8 and U2 Cluster k2 = clone AB5 and A1.
87.5	-	clone Z3 = clone I9
	-	clone H7 = clone C1
	-	clone Z9 = clone I2
	-	clone AF4 = clone X7 = clone J3 and G8.
87.8	h2 = h3	Cluster h2 = clone KA7, O3, J1, U8, S8, Q5, AG0, T5, R7, K8, G7, Z4, F1. Cluster h3 = clone AD3, AC4, D1, X4, V9, L9, S7, G6, T6, B1.

89.2	f6 = f7	Cluster f6 = clone N9 and I4. Cluster f7 = clone E1
89.6	h4 = h5	Cluster h4 = clone KA7, O3: J1. Cluster h5 = clone U8, S8, Q5, AG0, T5, R7, K8, G7, Z4, F1.
90 - < 95		
90	h6 = h7	Cluster h6 = clone AD3, AC4, D1. Cluster h7 = clone X4, V9, L9, S7, G6, T6, B1.
90.8	h8 = h9	Cluster h8 = clone U8, S8, Q5, AG0, T5, R7, K8, G7. Cluster h9 = clone Z4 and F1.
	-	Stangkon = clone W5
91.3	-	clone J3 = clone G8
	-	clone N9 = clone I4
	-	clone Y8 = clone U2
91.6	h10 = h11	Cluster h10 = clone X4, V9, L9. Cluster h11 = clone (S7, G6, T6, B1.
93.1	-	clone U8 = clone (S8, Q5, AG0, T5, R7, K8, G7.
93.4	-	clone KA7 = clone O3 and J1
	-	clone X4 = clone V9 and L9
93.8	-	clone S8 and Q5 = clone AG0, T5, R7, K8, G7.
94.2	-	clone S7 = clone G6, T6, B1
95 - 100		
95.2	-	clone O3 = clone J1
	-	clone S8 = clone Q5
	-	clone AG0 = clone T5, R7, K8 = clone G7
	-	clone Z4 = clone F1
	-	clone AD3 = clone AC4 = clone D1
	-	clone V9 = clone L9
	-	clone G6 = clone T6 and B1
	-	clone AB5 = clone A1
100	-	clone T5 = clone R7 = clone K8
	-	clone T6 = clone B1

TABLE 5. Characteristics of three promising clones dan thre reference variety, Stangkon

Characteristics	Chrysanthemum genotypes			
	Stangkon	W5	PO	J4
Floret coloring (the period from the neutral day)	48.70	43.84	43.14	41.98
Optimum flower opening (the period from the neutral day)	62.77	57.33	57.23	59.57
Plant heiight (cm)	74	74.41	90.69	80.97
Flower diameter (cm	5.59	5.75	5.59	5.64
Flower neck (cm)	7.75	6.37	5.46	5.09
Number of florets	32.17	28.14	32.94	28.68
Floret length (cm)	2.81	2.85	2.84	2.93
Diameter of disc (cm)	1.63	1.86	1.63	1.836
Vase life (days)	15.936	19.70	22.90	14.83

Conclusions

The chrysanthemum genotypes showed variations in quantitative characteristics, except in node length, the widest point of inflorescence, the number of flowers per plant, and floret width. Among the evaluated chrysanthemum, six out of 27 qualitative parameters were observably similar. These included non-glossy leaves, medium indentation depth, the existence of keel, similar inner and outer floret color, disc color before anther dehiscence, and disc color after anther dehiscence. Several mutant clones, i.e., clone G6, G8, C1, KA7, G9, AG0, N9, and Q5 have better quantitative performance than the reference varieties. The good clone W5 has more preferable characteristics than Stangkon and thus can be further promoted as s commercial variety and replace Stangkon as a reference variety.

ACKNOWLEDGMENTS

The authors would like to thank the Director of the Indonesian Agency for Agricultural Research and Development (IAARD), through Center for Horticultural Research and Development (ICHORD), Indonesian Ornamental Crops Research Institute (IOCRI) that financed, gave suggestions and criticisms in the planning and implementation of research. The authors also wish to thank the people who helped and worked during the research and report.

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