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RESEARCH ARTICLE

Mapping of qualitative traits and inheritance patterns on cayenne F_4 lines derived multiple crosses based on frequency and multivariate analysis

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ABSTRACT

The assembly of cayenne pepper (*Capsicum frutescens* L.) cannot be separated from the direction of qualitative character development. This is based on the role of qualitative characters in determining nutritional content and market segments. Therefore, the analysis of qualitative characters in the F_4 multiple cross cayenne pepper population needs to be done in determining the direction of line development in the next generation. The purpose of this study was to identify the inheritance pattern of qualitative traits and genotype grouping from a population of F_4 multiple cross lines. The research was conducted at the Experimental Field of Hasanuddin University, Makassar, Indonesia. The study was designed with the concept of observation on 90 F_4 multiple cross chili lines and three parental cultivars (Dewata F1, Bara and Ungara). Observations focused on 10 qualitative characters. The analysis showed that there are two types of diversity in this population, namely centralized diversity and distributed diversity. Centralized diversity consists of leaf color, book color, leaf shape, canopy density, stem color, fruit shape, and fruit tip shape. In contrast, characters that have a diverse distribution are crown color, young and old chili fruit color. The distribution results show three main groups, namely the Dewata-Bara group (27 genotypes), the Ungara group (16 genotypes) and the rest are groups outside of these two groups. Based on the whole study, the results of this evaluation are recommended as a consideration for the selection of F_5 lines in supporting the direction of releasing chili cultivars from multiple cross.

Key words: Anthocyanin, biofortification, *Capsicum frutescens*, HCA, multiple cross, PCoA.

INTRODUCTION

Cayenne pepper (*Capsicum frutescens* L.) is a horticultural commodity that affects economic stability (Li et al., 2017; Syukur et al., 2023). Although this commodity is not part of food products, it plays an important role in complementing the diet and nutrition of consumers (Zhang et al., 2016). This makes this product in high demand along with the increase in population (Syukur et al., 2023). Based on Santika (2023), cayenne pepper consumption in 2022 reached 569.65 thousand tons and increased 7.86% from 2021. But on the other hand, the instability of production is the main problem of this commodity (Deviyanto et al., 2023), so that these conditions affect erratic price fluctuations. Fluctuations in chili prices will have an impact on consumers and farmers. Therefore, product development and innovation for chili are always prioritized in supporting economic stability in various countries.

The development of cayenne pepper innovation can be done with a plant breeding approach. This approach will improve the genetic traits of cayenne pepper which are adapted to the crop climate change and market dynamics (Amas et al., 2023; Wang et al., 2023). Several research reports show the effectiveness of cayenne pepper development through plant breeding programs (Hafsah et al., 2020; Amas

et al., 2023). In increasing production, both through recombination improvement and the assembly of new traits that have not existed before. This indicates that innovation in the assembly of cayenne pepper cultivars need to be encouraged in solving the problems of cayenne pepper production and quality. One of the cayenne pepper breeding programs that has taken place is the biofortification chili breeding program by Amas et al. (2023).

Biofortified chili breeding is synonymous with adding additional value related to nutritional potential for human health (Li et al., 2017; Olasupo et al., 2021; Meng et al., 2022). This is a form of prevention against covid-19 or similar pandemics, so the development of this product has always been an important part of the development of cayenne pepper cultivars (Avnee et al., 2023; Syukur et al., 2023). Amas et al. (2023) have developed cayenne pepper lines from multiple crosses, where one of the elders has a high anthocyanin content (Ungara elder). Anthocyanin content is strongly reflected in the purple color of plants, including leaves, stems, flowers and fruits (Palma et al., 2020; Meng et al., 2022). However, the parent variety with high anthocyanins (Ungara) is relatively low in pungency or capsaicin compared to commercial chili cultivars in general. This is a particular reason for Amas et al. (2023) to cross Ungara elders with several cayenne pepper cultivars with high capsaicin levels, such as Dewata F1, Bara and Katokkon.

The development of Amas et al. (2023) has entered the F_4 generation. However, the potential and inheritance of qualitative traits have not been studied in depth because selection is only centered on their productivity potential. This indicates that selection on qualitative characters is still random, so the assessment of qualitative characters in the F_4 generation can still be optimized to estimate the dominance pattern. Although the observation results in this generation are not as good as the diversity in the F_2 generation population (Cheng et al., 2018; Benowicz et al., 2020; Amas et al., 2023). However, the assessment of qualitative characters in this generation is important to determine the direction and concept of the expected variety release. This is also in accordance with Sampaio et al. (2023), where the determination of qualitative characters in chili will affect the quality of its market products. Based on this, identification of qualitative inheritance patterns of F_4 multiple cross lines of Amas et al. (2023) is very important in supporting the development of biofortification of cayenne pepper cultivars.

Identification of inheritance patterns needs to be approached with statistical analysis. There are several analyses that can help map the inheritance patterns, such as frequency analysis and multivariate analysis. Both analyses can facilitate the interpretation of data from populations with large objects and dimensions (Akyavuz et al., 2018; Haq et al., 2022; Rubiyo et al., 2022). This has also been done by Anshori et al. (2022) for their frequency analysis and Shi et al. (2020) and Wang et al. (2022) for multivariate analysis in diversity identification in chili. Therefore, identification of qualitative inheritance patterns of F_4 multiple cross lines of Amas et al. (2023) based on frequency and multivariate analysis needs to be done. The purpose of this study was to identify the inheritance pattern of qualitative traits and grouping of genotypes from the population of F_4 Amas et al. (2023) multiple cross lines.

MATERIALS AND METHODS

Experimental procedures

The research was conducted from April to September 2023 at the Experimental Garden of the Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, Indonesia. The research was designed with the concept of observation on 90 genotypes consisting of 90 F_4 multiple cross chili lines from eight multiple cross families (Table 1) and three parental cultivars (Dewata F1, Bara and Ungara). Each genotype consisted of eight plants that were observed as a whole sample per genotype, so that the total number of genotypes observed was 744 plants.

The research procedure included several aspects of cultivation, namely seeding, planting, maintenance and harvesting. Seeding was carried out using a planting medium consisting of soil, compost and burnt husk with a volume ratio (1:1:1). Cayenne pepper (*Capsicum frutescens* L.) seeds were planted in seeding trays that had previously been germinated in containers. Each planting hole consisted of one seed that was treated with water and foliar fertilizer (20% N, 15% P_2O_5, 15% K_2O, 1% $MgSO_4$; Gandasil D, PT Saprodi Sinergi Indonesia, Yogyakarta, Indonesia) at a concentration of 1 g L^{-1} until the seedlings were 30 d after sowing (DAS). After 30 DAS, the seedlings were planted into beds with a size of 9 m × 1 m per bed. The

beds were mulched with black silver mulch and hollowed out with a planting distance of 60 × 50 cm in a zigzag manner. Each planted chili seedling was given a stake to strengthen the plant stem, especially against the wind. Then, the chili seedlings were nurtured with several activities until harvest.

Table 1. The detail of multiple cross hybridization families in this study.

Label	Detail of multiple cross hybridizations	Abbreviation	Number of lines
G1	Ungara/Bara//Dewata F1/Katokkon	U/B//D/K	10
G2	Ungara/Dewata F1//Bara/Ungara	U/D//B/U	6
G4	Ungara/Katokkon//Dewata F1/Bara	U/K//D/B	13
G5	Ungara/Dewata F1//Dewata F1/Bara	U/D//D/B	21
G6	Ungara/Bara//Dewata F1/Ungara	U/B//D/U	6
G7	Ungara/Bara//Dewata F1/Bara	U/B//D/B	12
G8	Ungara/Dewata F1//Bara	U/D//B	5
G10	Dewata F1/Ungara//Bara	D/U//B	17
	Total lines		90

Maintenance of chili seedlings includes watering, replanting, weeding, fertilizing, and pest and disease control. Watering was done twice a day, in the morning and evening. Watering was done until the soil looks moist. Replanting was done by replacing plants that experience abnormal growth, wilt, and are attacked by pests or diseases at the age of 14 d after transplanting and was done in the afternoon so that the chili seedlings do not wilt. Plants were replaced based on the same genotype and age. Weeding was done by removing small shoots on the book below the dichotomous. This aims to focus the growth of chili on the main stem (main stem). Weeding was done manually by hand to remove weeds that grew around the plants and disturbed plant growth. Conversely, weeds that grow in the area outside the beds were weeded using a hoe or by applying paraquat dichloride herbicide (Gramoxone 276SL, Syngenta, Indonesia) at a dose of 2 g L^{-1} water. Fertilization was carried out periodically with several methods, namely dissolving and spraying methods. Fertilizer dissolution uses NPK (NPK Mutiara 16-16-16; PT. Meroke Tetap Jaya, Indonesia) fertilizer with a concentration of 10 g L^{-1}. Then each plant is given 200 mL fertilizer solution each week. In addition, fertilization also uses the Paten liquid fertilizer (17% organic calcium, 7 C/N, 2.5% N, 0.83% P_2O_5, 2.16% K_2O, 6546 ppm Fe and 6.98 pH) and KNO_3 spray fertilizer with a concentration of 5 g L^{-1}. These fertilizers are also given every week in between fertilizing solutions. Pest and disease control was carried out when pest and or disease attacks occurred; application was done using a sprayer. Pest and disease control uses a mixture of insecticides and fungicides. The last activity was the harvesting process. Harvesting was carried out in accordance with the objectives in the observation of the qualitative characteristics of cayenne pepper.

Data observation and analysis

Observations focused on qualitative chili characters modified from chili descriptors. Details of these observations are in Table 2. The data obtained in these observations were then analyzed based on the distribution frequency with Excel software. In addition, the data were also analyzed with circular-based Hierarchical Cluster Analysis (HCA) and Principal Coordinates Analysis (PCoA). Both analyses used Euclidean similarity with Rstudio 4.3.1 software with Factoextra R Package (Shi et al., 2020; Rubiyo et al., 2022; Wang et al., 2022).

RESULTS

The results of observations of qualitative characters in the multiple cross cayenne chili F_4 population were shown in Table 3. Observation data in Table 3 were analyzed in stages based on the characters and research objectives. Based on the frequency analysis of leaf color (Figure 1A) and leaf shape (Figure 1B), the distribution of leaf color was mostly found in scores 3 and 4. These scores are very identical to the

green color of young leaves and dark green leaves. In addition, there is one line from G5 that has a purple leaf color. Based on leaf shape (Figure 1B), almost all crossing families have leaf shape 5. Although in some crossing families, there are lines scattered in leaf shape 1 (G5 3 lines; G7 3 lines; and G10 1 line), leaf shape 3 (G1 1 line), and leaf shape 4 (G4 1 line and G7 1 line).

The results of frequency analysis on canopy density and stem color are shown in Figures 2A and 2B, respectively. Based on Figure 2A, almost all crossing families have a canopy density distribution dominated at score 3 (semi-dense), except G6, and G7. Both crossing families have the highest distribution in score 1 (tight). Canopy score 1 also has a large distribution after canopy score 1. In contrast, score 5 (sparse) has a very low distribution, with 1 line in each family G2, G4, G5, and G8. Based on stem color (Figure 2B), almost all crossing families have green stem color forms (score 1), except G4, and G8 which are relatively dominant in purple-green striped stem color. The yellow-green striped stem form also has a large distribution after the green stem color. Meanwhile, the purple stem color is only found in 1 line of G8 cross.

The results of frequency analysis on book and crown color are shown in Figures 3A and 3B, respectively. Based on Figure 3A, all cross families have book color score 9 (purple). Very little green book color was found in the F_4 population of the multiple cross. Based on crown color (Figure 3B), there is a spread of color in almost all scores, except for color score 2. Color score 1 (white) has dominance in almost all crossing families, except G4 and G8. Crossing family G4 is more dominant in score 5, while crossing family G8 is more dominant in crown color score 7. Color scores 3, 4 and 6 are the colors with the lowest distribution, where the average consists of only 1 line from several crossing families.

Table 2. Scoring of qualitative character observations of chili peppers.

	Qualitative characters									
Score	Leaf color	Leaf shape	Canopy density	Node color	Stem color	Corolla color	Fruit shape	Fruit tip shape	Fruit immaturity color	Fruit maturity color
1	Yellow	Deltoid	Sparse	Green	Green	White	Elongate	Pointed	White	White
2	Light green					Light yellow			Yellow	Lemon-yellow
3	Green	Ovate	Semi dense		Green with purple line	Yellow	Almost round	Blunt	Green	Pale orange yellow
4	Dark green					Yellow green			Orange	Orange yellow
5	Light purple	Lanceolate	Dense		Purple	Purple with white base	Triangular	Sunken	Purple	Pale orange
6	Purple					White with purple base				Orange
7						White with purple margin	Campanulate	Sunken and pointed		Light red
8						Purple				Red
9				Purple			Blocky			Dark red
10										Purple
11										Brown
12										Black

Table 3. The scoring qualitative characters of all genotypes of cayenne multiple cross F_4 generation. LC: Leaf color; LS: leaf shape; CD: canopy density; NC: node color; SC: stem color; CC: corolla color; FS: fruit shape; FTS: fruit tip shape; FIC: fruit immaturity color; FMC: fruit maturity color; PCoA: principal coordinate analysis.

Label	Genotype	Group	LC	LS	CD	NC	SC	CC	FS	FTS	FIC	FMC	Dendrogram	Den_group	PCoA	PC_group
Bara	Bara	Bara	4	5	1	1	1	1	1	1	3	7	Yellow	Dewata	Gray	Dewata
Dewata	Dewata	Dewata	4	5	1	1	1	1	1	1	1	7	Yellow	Dewata	Gray	Dewata
Geno01	G1.12.7-4	G1	3	5	1	9	1	1	1	1	3	8	Red	Dewata	Gray	Dewata
Geno02	G1.12.9-1	G1	4	5	1	1	1	1	1	1	3	9	Yellow	Dewata	Blue	Middle
Geno03	G1.12.9-7	G1	4	5	1	9	3	5	5	1	6	9	Green	Ungara	Yellow	Ungara
Geno04	G1.12.9-8	G1	4	5	1	1	1	1	1	1	3	7	Yellow	Dewata	Gray	Dewata
Geno05	G1.9.2-10	G1	4	5	1	1	1	1	1	1	3	7	Yellow	Dewata	Gray	Dewata
Geno06	G1.9.2-3	G1	4	5	1	9	3	5	5	1	6	9	Green	Ungara	Yellow	Ungara
Geno07	G1.9.5-10	G1	3	5	3	9	1	1	1	1	2	7	Red	Dewata	Gray	Dewata
Geno08	G1.9.5-3	G1	4	5	5	1	1	5	1	1	6	9	Green	Ungara	Yellow	Ungara
Geno09	G1.9.5-5	G1	3	5	3	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno10	G1.9.5-9	G1	3	5	3	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno11	G10.2.4-1	G10	4	5	3	9	1	1	5	1	3	9	Blue	Middle	Blue	Middle
Geno12	G10.2.4-3	G10	3	5	3	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno13	G10.2.4-6	G10	4	5	3	9	3	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno14	G10.5.-3	G10	3	5	1	9	3	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno15	G10.5.4-11	G10	3	5	1	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno16	G10.5.4-6	G10	3	5	3	1	1	1	1	1	3	7	Red	Dewata	Red	Middle
Geno17	G10.5.8-3	G10	4	5	1	9	1	5	1	1	6	9	Green	Ungara	Blue	Middle
Geno18	G10.5.8-4	G10	3	5	3	9	1	1	1	1	2	7	Red	Dewata	Gray	Dewata
Geno19	G10.5.8-5	G10	4	5	1	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno20	G10.5.8-5	G10	4	5	1	9	1	1	1	1	2	9	Blue	Middle	Blue	Middle
Geno21	G10.7.5-1	G10	4	5	1	1	1	1	1	1	3	7	Yellow	Dewata	Gray	Dewata
Geno22	G10.7.5-11	G10	3	5	1	1	1	1	1	1	3	7	Red	Dewata	Red	Middle
Geno23	G10.7.5-5	G10	3	5	1	9	3	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno24	G10.7.5-6	G10	3	5	3	9	1	1	5	1	6	9	Blue	Middle	Blue	Middle
Geno25	G10.7.5-7	G10	4	5	3	9	3	1	1	1	6	9	Green	Ungara	Blue	Middle
Geno26	G10.9.6-1	G10	3	1	3	9	1	1	5	1	3	7	Blue	Middle	Red	Middle
Geno27	G10.9.6-11	G10	4	5	3	9	3	7	1	1	5	8	Green	Ungara	Blue	Middle
Geno28	G2.1.10-1	G2	3	5	1	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno29	G2.1.10-2	G2	4	5	5	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno30	G2.1.10-4	G2	3	5	1	9	3	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno31	G2.1.10-9	G2	3	5	3	9	1	1	5	1	3	7	Blue	Middle	Red	Middle
Geno32	G2.6.10-1	G2	3	5	3	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno33	G2.6.10-2	G2	4	5	3	9	3	7	5	1	6	9	Green	Ungara	Yellow	Ungara
Geno34	G4.11.1-10	G4	3	5	3	9	3	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno35	G4.11.1-11	G4	4	5	1	9	3	5	1	1	5	7	Green	Ungara	Blue	Middle
Geno36	G4.11.1-12	G4	3	3	3	1	3	5	1	1	5	7	Green	Ungara	Yellow	Ungara
Geno37	G4.11.1-13	G4	3	5	3	9	1	1	5	1	3	8	Blue	Middle	Red	Middle
Geno38	G4.11.1-4	G4	4	5	1	9	3	1	1	1	6	9	Green	Ungara	Blue	Middle
Geno39	G4.11.1-6	G4	4	5	3	9	5	5	1	1	6	9	Green	Ungara	Yellow	Ungara
Geno40	G4.11.1-6	G4	4	5	3	9	3	5	1	1	5	8	Green	Ungara	Blue	Middle
Geno41	G4.3.8-10	G4	4	5	3	9	3	7	5	1	6	9	Green	Ungara	Yellow	Ungara
Geno42	G4.3.8-10	G4	4	5	1	9	1	7	5	1	6	9	Green	Ungara	Yellow	Ungara
Geno43	G4.3.8-11	G4	4	5	5	9	1	1	5	1	6	8	Blue	Middle	Blue	Middle
Geno44	G4.3.8-12	G4	4	5	1	9	3	5	5	1	3	7	Green	Ungara	Blue	Middle
Geno45	G4.3.8-3	G4	4	5	3	9	1	5	1	1	6	9	Green	Ungara	Blue	Middle
Geno46	G4.5.2-7	G4	4	5	3	9	1	1	1	1	5	9	Blue	Middle	Blue	Middle
Geno47	G5.12.1-2	G5	4	5	1	9	3	5	5	3	6	9	Green	Ungara	Yellow	Ungara
Geno48	G5.12.1-4	G5	4	5	3	9	1	8	1	1	6	9	Green	Ungara	Blue	Middle
Geno49	G5.12.1-6	G5	3	5	3	9	3	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno50	G5.12.1-8	G5	3	5	3	9	1	1	5	1	3	7	Blue	Middle	Red	Middle
Geno51	G5.12.4-1	G5	4	5	3	9	3	1	5	1	3	7	Red	Dewata	Blue	Middle
Geno52	G5.12.4-2	G5	4	5	5	9	3	5	5	1	6	9	Green	Ungara	Yellow	Ungara
Geno53	G5.12.4-3	G5	5	5	3	9	1	1	5	1	3	8	Blue	Middle	Red	Middle
Geno54	G5.12.4-4	G5	4	5	1	9	1	7	5	1	6	9	Green	Ungara	Yellow	Ungara
Geno55	G5.3.3-1	G5	3	1	3	9	1	1	5	1	3	7	Blue	Middle	Red	Middle
Geno56	G5.3.3-2	G5	3	5	3	9	1	1	5	1	3	7	Blue	Middle	Red	Middle
Geno57	G5.3.3-2	G5	3	5	3	9	1	1	5	1	6	9	Blue	Middle	Blue	Middle
Geno58	G5.3.3-3	G5	3	5	3	3	3	5	1	1	6	9	Green	Ungara	Yellow	Ungara
Geno59	G5.3.3-7	G5	3	1	3	9	1	1	1	1	3	7	Red	Dewata	Red	Middle

Cont. Table 3.

Geno60	G5.3.3-8	G5	3	1	1	9	1	1	1	1	3	7	Red	Dewata	Red	Middle
Geno61	G5.5.8-1	G5	4	5	3	9	3	5	1	1	6	7	Green	Ungara	Blue	Middle
Geno62	G5.5.8-3	G5	4	5	3	9	1	1	1	1	2	8	Blue	Middle	Blue	Middle
Geno63	G5.5.8-5	G5	4	5	3	9	1	7	5	1	6	7	Blue	Middle	Blue	Middle
Geno64	G5.5.8-7	G5	3	5	3	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno65	G5.5.9.3	G5	4	5	1	1	1	1	5	1	1	8	Yellow	Dewata	Yellow	Ungara
Geno66	G5.5.9-5	G5	3	5	3	9	1	1	5	1	2	8	Blue	Middle	Red	Middle
Geno67	G5.7.1-3	G5	3	5	3	9	1	1	5	1	3	7	Blue	Middle	Red	Middle
Geno68	G6.11.2-1	G6	4	5	1	9	3	5	5	1	6	9	Green	Ungara	Yellow	Ungara
Geno69	G6.11.2-2	G6	3	5	1	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno70	G6.11.2-3	G6	4	5	1	9	3	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno71	G6.11.2-4	G6	4	5	1	1	1	1	1	1	6	8	Yellow	Dewata	Blue	Middle
Geno72	G6.3.5-8	G6	4	5	3	9	3	7	1	1	5	8	Green	Ungara	Blue	Middle
Geno73	G6.5.10-6	G6	3	5	3	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno74	G7.3.4-2	G7	4	1	1	9	1	7	1	1	6	9	Green	Ungara	Yellow	Ungara
Geno75	G7.12.3-3	G7	4	5	3	9	3	5	1	1	5	9	Green	Ungara	Blue	Middle
Geno76	G7.12.5-10	G7	3	1	1	9	1	1	5	1	3	7	Blue	Middle	Red	Middle
Geno77	G7.3.4-3	G7	4	5	3	9	3	1	5	1	3	7	Red	Dewata	Blue	Middle
Geno78	G7.3.4-4	G7	3	5	3	1	1	1	1	1	2	8	Blue	Middle	Red	Middle
Geno79	G7.3.4-5	G7	3	5	5	9	1	1	1	1	2	8	Blue	Middle	Red	Middle
Geno80	G7.3.4-6	G7	3	5	1	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno81	G7.6.3-1	G7	4	5	3	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno82	G7.6.3-11	G7	3	1	1	9	1	1	1	1	3	7	Red	Dewata	Red	Middle
Geno83	G7.7.10-3	G7	4	5	1	9	3	7	1	1	5	8	Green	Ungara	Blue	Middle
Geno84	G7.7.10-8	G7	4	5	1	9	3	5	1	1	6	7	Green	Ungara	Blue	Middle
Geno85	G7.7.10-8	G7	3	1	1	9	1	1	5	1	2	7	Blue	Middle	Red	Middle
Geno86	G8.3.9-10	G8	4	5	9	3	9	5	5	5	1	6	Green	Ungara	Yellow	Ungara
Geno87	G8.3.9-12	G8	3	5	3	9	1	1	1	1	3	7	Red	Dewata	Gray	Dewata
Geno88	G8.3.9-14	G8	4	5	3	9	3	5	5	1	6	9	Green	Ungara	Yellow	Ungara
Geno89	G8.3.9-5	G8	4	5	1	9	3	1	1	1	6	8	Green	Ungara	Blue	Middle
Geno90	G8.3.9-6	G8	4	5	3	9	3	7	1	1	5	7	Green	Ungara	Blue	Middle
Ungara	UNGARA	UNGARA	4	5	1	9	5	8	5	1	6	9	Green	Ungara	Yellow	Ungara
	Sig. Kruskal-Wallis		0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**				

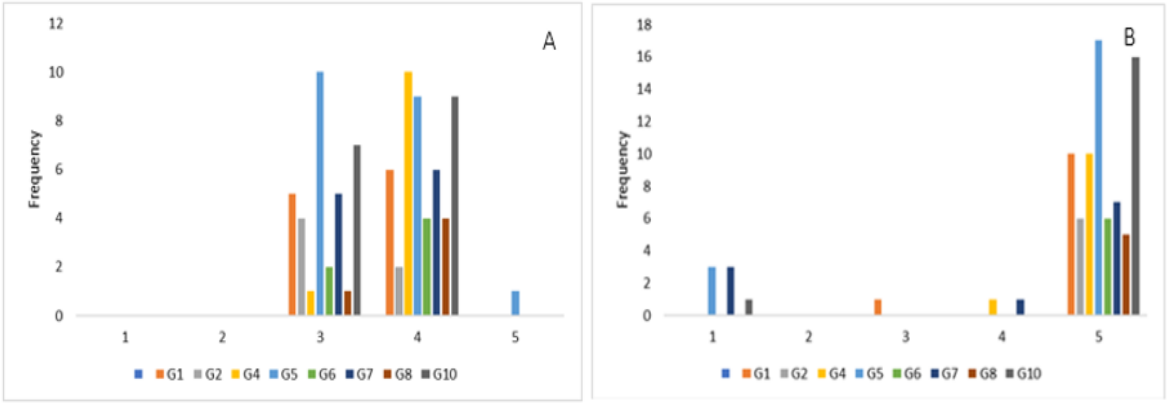

Figure 1. Frequency distribution of leaf color (A) and leaf shape (B) in F_4 multiple cross chili populations. G1 = U/B//D/K; G2 = U/D//B/U; G4 = U/K//D/B; G5 = U/D//D/B; G6 = U/B//D/U; G7 = U/B//D/B; G8 = U/D//B; G10 = D/U//B; U: 'Ungara'; B: 'Bara'; D: 'Dewata F1'; K: 'Katokkon'.

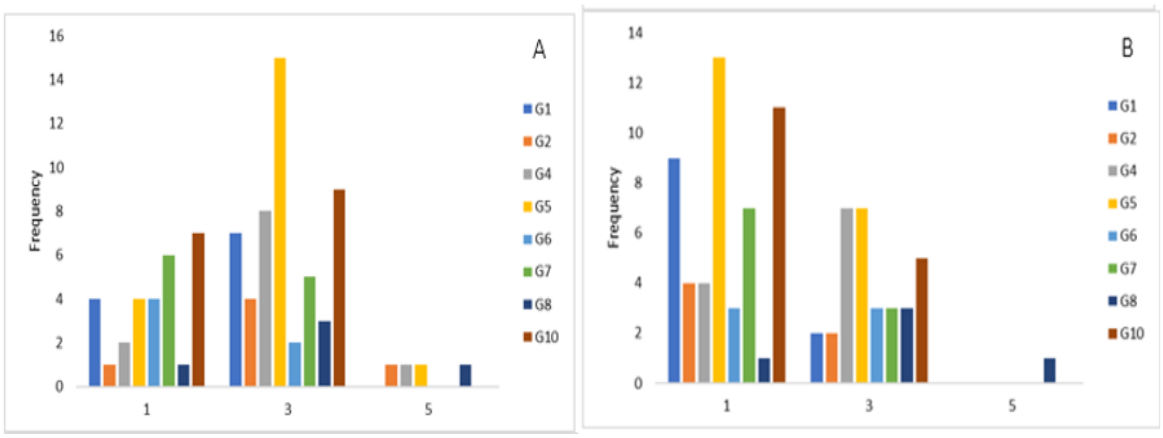

Figure 2. Frequency distribution of canopy density (A) and stem color (B) in F_4 multiple cross chili populations. G1 = U/B//D/K; G2 = U/D//B/U; G4 = U/K//D/B; G5 = U/D//D/B; G6 = U/B//D/U; G7 = U/B//D/B; G8 = U/D//B; G10 = D/U//B; U: 'Ungara'; B: 'Bara'; D: 'Dewata F1'; K: 'Katokkon'.

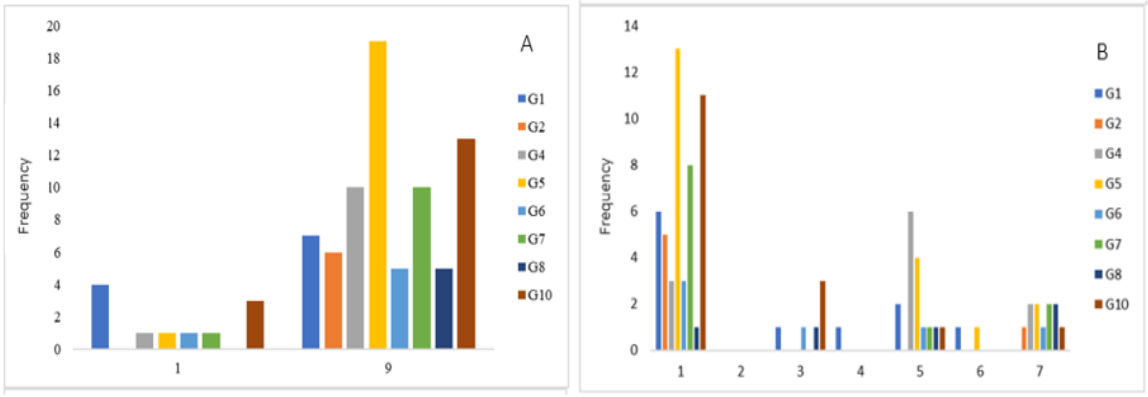

Figure 3. Frequency distribution of book color (A) and crown color (B) in F_4 multiple cross chili populations. G1 = U/B//D/K; G2 = U/D//B/U; G4 = U/K//D/B; G5 = U/D//D/B; G6 = U/B//D/U; G7 = U/B//D/B; G8 = U/D//B; G10 = D/U//B; U: 'Ungara'; B: 'Bara'; D: 'Dewata F1'; K: 'Katokkon'.

The results of the frequency analysis of fruit shape and fruit tip are shown in Figures 4A and 4B, respectively. Based on Figure 4A, the distribution of fruit shape is only spread over two scores, namely score 1 and score 5. Almost all crossing families are dominated by the shape of score 1. However, specifically for crossing family G5, the distribution of fruit shape is dominated by score 5. Meanwhile, the score 3 form is found in 3 cross families (G1, G6, and G7), each of which consists of 1 line. Based on fruit tip shape (Figure 4B), all crossing families were dominated by fruit tip shape score 1. Although, there are some outliers from crossing families that have leaf tip shapes outside of the score 1 shape. Score 2 shape consists of 1 line from crossing families G1 and G5. Form score 3 consists of 1 line from the crossing family G5. Finally, the score 5 form consists of 1 line from the G8 cross family.

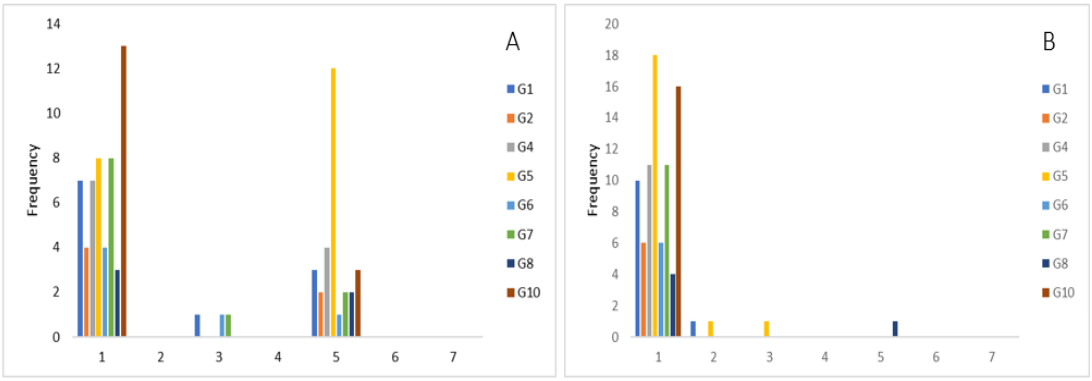

Figure 4. Frequency distribution of fruit shape (A) and fruit tip shape (B) in F_4 multiple cross chili populations. G1 = U/B//D/K; G2 = U/D//B/U; G4 = U/K//D/B; G5 = U/D//D/B; G6 = U/B//D/U; G7 = U/B//D/B; G8 = U/D//B; G10 = D/U//B; U: 'Ungara'; B: 'Bara'; D: 'Dewata F1'; K: 'Katokkon'.

The results of frequency analysis on the color of young fruit and ripe fruit are shown in Figures 5A and 5B, respectively. Based on Figure 5A, the distribution of young fruit color is almost spread across all color scores, except 7. Color score 3 almost dominates in all crossing families. Crossing families G2, G5, G10, and G7 have young fruit color that is highly dominated by score 3. Families G1 and G6 also have young fruit color dominance in score 3, but the distribution is not significantly different from young fruit color scores 5 and 6. Crossing families G4 and G8 have young fruit color dominated by score 6. Although, the distribution of both families is also widely distributed in fruit color score 5. Based on the color of ripe fruit (Figure 5B), the color distribution is dominated by scores 7, 8, and 9. Although, there is one line from the crossing family G8 that has ripe fruit color score 6. Ripe fruit in crossing families G2, G5, G7 and G10 are significantly dominated by color score 7. Ripe fruit in lines G6 and G7 are also dominated by color score 7, but the distribution difference is nonsignificant. Crossing family G4 has ripe fruit color dominated by score 8, although the difference in distribution dominance is not very significant. Meanwhile, crossing family G1 has ripe fruit dominated by color score 9, although the difference in distribution dominance is also not very significant.

Based on the Spearman correlation results (Figure 6), the highest correlation was in leaf color followed by young fruit color, stem color, and ripe fruit color. Leaf color had a significant positive correlation with stem color (0.35), leaf shape (0.30), ripe fruit color (0.44), crown color (0.51) and young fruit color (0.44). Young fruit color was also significantly positively correlated with leaf color (0.44), ripe fruit color (0.62), and crown color (0.67). Stem color was also significantly positively correlated with crown color (0.51) and fruit tip shape (0.24). Ripe fruit color was also correlated with crown color (0.46) and fruit shape (0.21). Another significant positive correlation also occurred with fruit tip shape and fruit shape (0.20).

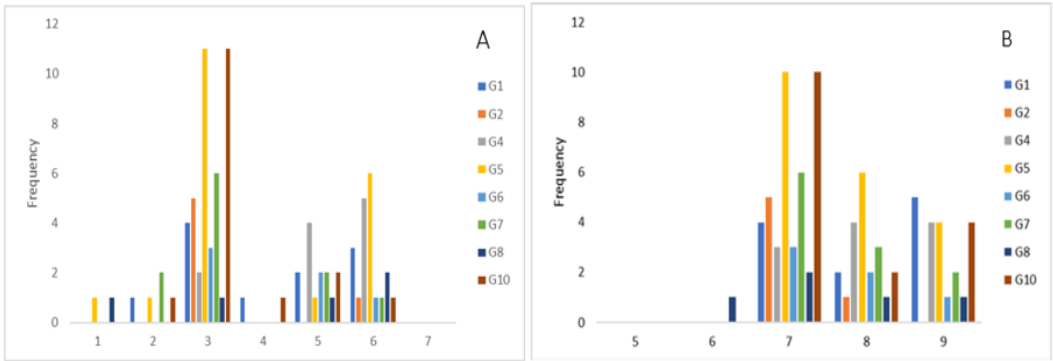

Figure 5. Frequency distribution of young fruit color (A) and ripe fruit color (B) in F_4 multiple cross chili populations. G1 = U/B//D/K; G2 = U/D//B/U; G4 = U/K//D/B; G5 = U/D//D/B; G6 = U/B//D/U; G7 = U/B//D/B; G8 = U/D//B; G10 = D/U//B; U: 'Ungara'; B: 'Bara'; D: 'Dewata F1'; K: 'Katokkon'.

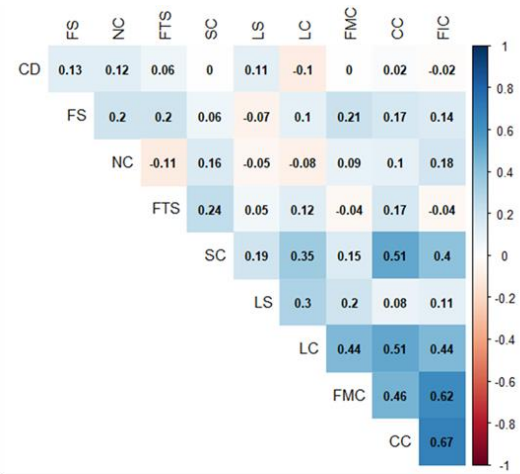

Figure 6. Spearman correlation between qualitative characters. LC: Leaf color; LS: leaf shape; CD: canopy density; NC: node color; SC: stem color; CC: corolla color; FS: fruit shape; FTS: fruit tip shape; FIC: fruit immaturity color; FMC: fruit maturity color.

Based on the results of the dendrogram analysis (Figure 7), there are four main groups based on all qualitative character components. The first largest group (green) has 33 members and one of them is 'Ungara'. The second largest group (red) consists of 31 genotypes. The third largest group (blue) has 21 genotypes. The last group (yellow) has eight genotypes with two of them being 'Dewata' and 'Bara'. The red and yellow groups have a closer kinship and are followed by the blue color. Meanwhile, the green color has a distant kinship between the three groups.

Based on the results of the Principal Coordinate Analysis (PCoA), there are also four main groups (Figure 8). The first largest group (gray) consists of 29 genotypes and two of them are 'Dewata' and 'Bara'. This group has a relatively close or tight density between its members with the center point located at Geno69. The second largest group is the blue group consisting of 28 genotypes. This group has a fairly loose closeness or density compared to the first group with the center point located at Geno48. Meanwhile, the red and yellow groups have the same group members, namely 18 genotypes. However, the yellow group has a very loose closeness or density between its member genotypes compared to other groups. In addition, 'Ungara' is also included in this group with the group center located at Geno8. In contrast, the red group has a density that is almost the same as the gray group, where the group center is located at Geno37.

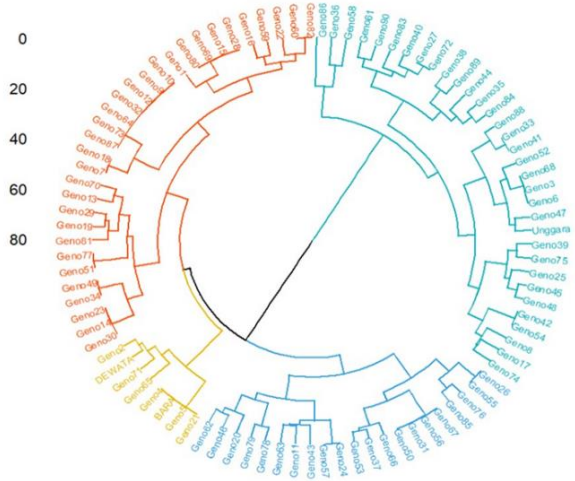

Figure 7. Dendrogram of all genotypes in the F_4 multiple cross chili population.

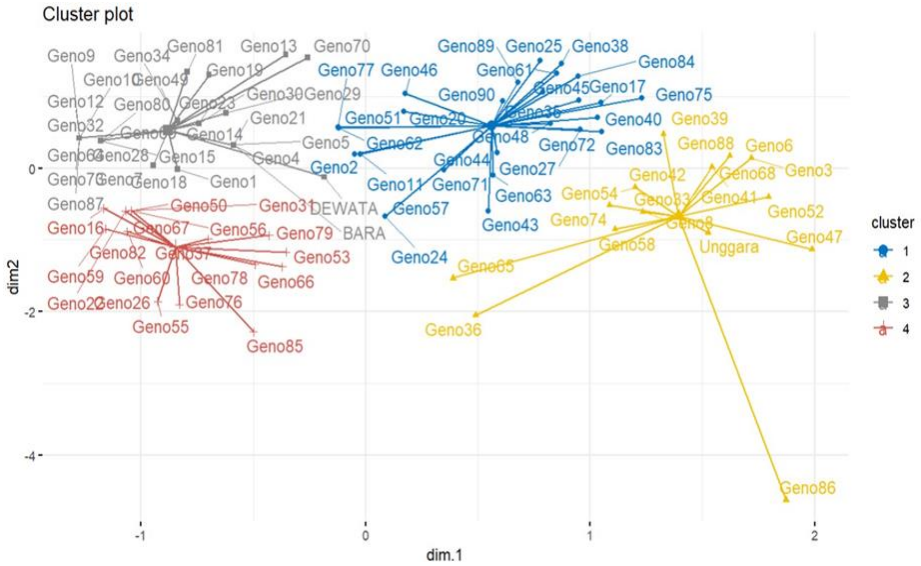

Figure 8. Principal Coordinates Analysis (PCoA) of all genotypes in F_4 multiple cross chili population.

DISCUSSION

Based on the results of qualitative analysis, there are two types of characters, namely characters with centralized distribution and relatively even distribution. Differences in the distribution of a qualitative trait are also related to the origin of the crossing parents, number of genes involved, interactions between alleles, both within the same locus and at different loci, and selection (Sobir and Syukur et al., 2011; Anshori et al., 2022). This also proves that the F_4 generation is still worthy of in-depth analysis regarding the potential inheritance of qualitative traits.

The characters with centralized distribution consist of leaf color, book color, leaf shape, canopy density, stem color, fruit shape, and fruit tip shape. The seven characters have different backgrounds in the distribution of their character phenotypes. Canopy density, stem color and book color are groups with centralized distribution due to the option of sparse population distribution. This was also reported by Gurung et al. (2020) and Guo et al. (2023). Few options indicate that the genes involved in the induction of these characters are also small, about 1-2 genes only (Sobir and Syukur et al., 2011). In stem and book color, there is an interesting dominance effect, where Ungara elders are the only elders that have black stem and book color. However, the distribution results show that the black color in both has dominance over the green color. This was also reported by Sahid et al. (2022). However, in stem color characters, the purple color does not have full dominance or is partially dominant. This is different from the book color which has relatively full dominance over green. Meanwhile, the distribution of canopy density characters is a combination of few options with characteristics between elders that are not much different. This is due to the characteristics of 'Dewata', 'Ungara', and 'Katokkon' elders, which tend to be dense and semi-dense (Anshori et al., 2022). In addition, the selection process for productivity also optimizes short internodes (Amas et al., 2023). This is because the chili fruit appears on the branching of the internodes, so that a wide canopy with short internodes will correlate with productivity. Therefore, the distribution in this population is focused on two categories, namely dense and semi-dense, while the sparse canopy density is an outlier from the segregation pattern of the cross.

The character of leaf shape and fruit tip is a character that has many options but is highly concentrated on one performance. This was also reported by Gurung et al. (2020) and Guo et al. (2023). In general, this phenomenon is due to the fact that the basis of the crossing parents is relatively similar in shape, except for a few crosses involving 'Katokkon' (G1 and G4). However, the influence of 'Katokkon' only had a minor effect on both characters, so the influence only resulted in a few outliers of both characters. Although, the population has undergone a selection process. Meanwhile, leaf color and fruit shape are characters that have many options (Gurung et al., 2020; Sampaio et al., 2023; Guo et al., 2023). However, in this population, diversity is centered

on two characters. These character differences are also caused by the considerable differences between the crossing elders, especially against 'Ungara'. The 'Ungara' elder has a purple leaf color and a flattened fruit shape, while the other elders have a common shape, namely green leaf color and slender fruit (Anshori et al., 2022). The results of these crosses indicate that green color and pointed fruit shape are believed to have dominance over the influence of 'Ungara'. This is due to the proportion of the parents which is more towards the general condition of cayenne pepper fruit. However, 'Ungara' still has a major influence, although in terms of the proportion of elders its characterization is minor. This was also conveyed by Andrade et al. (2020) and Wang et al. (2023), where the effect of anthocyanins provides a high diversity of interactions on the color of plant parts, including chili peppers. In addition, the effect is also clearly visible from the partial effect on the darker green leaf color and relatively cheeky fruit shape in the population frequency distribution. Based on this, this phenomenon can indicate that the influence of purple color and flat fruit is quite dominant as an allele compared to leaf color and fruit shape in cayenne pepper in general.

Characters that have a diverse distribution are crown color, young and old chili fruit color. In general, the color of the crown of chili fruit in general is white (Guo et al., 2023). However, the effect of the crown color of 'Ungara' which is purple causes a segregation pattern with several distribution classes, namely white, purplish white, whitish purple, and purple. However, the white color has a large dominance over other crown colors. This phenomenon was also reported by Gurung et al. (2020), Anshori et al. (2022), and Guo et al. (2023). In general, a white crown will indicate a green color in young fruit (Guo et al., 2023). However, if there is a slight interrogation of purple color, the young fruit will have a variety of colors, such as brown, light purple, dark purple, black and rainbow colors. Conversely, if the crown has a full purple color, it will produce black fruit color (Anshori et al., 2022). This indicates that the color of the crown affected by the color of purple will correlate with the color of the young fruit (Andrade et al., 2020; Anshori et al., 2022; Sampaio et al., 2023; Guo et al., 2023).

Young fruit color is the most variable character. This is due to the presence of three main components in the formation of young fruit color, namely chlorophyll, anthocyanins and carotenoids (Andrade et al., 2020; Sampaio et al., 2023). This makes the color of chili young fruit has a high diversity compared to other qualitative traits and associated with nutrient content (Moon et al., 2023; Sampaio et al., 2023; Wang et al., 2023). This is further diversified by the large number of background elders in this population, despite selection. Green color in young fruits is a common color that dominates chili pepper crops due to high chlorophyll content (Gómez-García et al., 2013). However, as with other color effects, the color of the pigeon pea plays a major role in influencing segregation of young fruit color. The black color of pigeon pea young fruit provides color variation as reviewed in the crown color. The varied color of young fruit will correlate with potential differences in metabolites, including the interaction of capsaicin content as an authentic content in chili fruit (Wang et al., 2023). Therefore, the distribution of potential young fruit is very good as a basis for selection considerations.

Ripe fruit color is the last class that has considerable diversity. This color is very related to young fruit (Anshori et al., 2022; Sampaio et al., 2023). In general, chili fruit with high chlorophyll levels will form a red color in its mature phase (Kuai et al., 2018; Wang et al., 2023). However, there are some classes of ripe fruits that do not turn red. This is due to the basic pigment of young fruit of these genotypes (Tian et al., 2015; Li et al., 2021). Fruit pigments with high anthocyanins will turn into a blackish red color in the ripe fruit, so the more anthocyanins that form the purple color will correlate with anthocyanins in the ripe fruit (Wang et al., 2023). This phenomenon is the reason why the class distribution pattern in this population is considered quite a lot, namely orange, bright red, dark red or dark and blackish red. Meanwhile, the orange color in this population is a segregating outlier from various combinations of elders. This color is a combination of carotenoids and chlorophyll (Tian et al., 2015; Kuai et al., 2018; Wang et al., 2023). This is the basis of genetic diversity among the elders of the multiple cross, so that there are outliers in the segregation pattern. This phenomenon is an important indication in the direction of future cayenne pepper development, especially towards its biofortification potential.

Based on the results of the correlation analysis, the correlation between color characters is the dominating correlation of this study, except for the book color. Book color has a very narrow class, so the correlation to characters with many classes is difficult to be significant. The color correlation is centered on leaf color characters, where the darker the leaves, the impact on anthocyanin color changes on various other

plant organs, such as plant stems, crowns, young fruit and ripe fruit. In addition, this character also has a good correlation with leaf shape. The overall correlation further strengthens the assumption of the great influence of anthocyanins in 'Ungara' in providing color diversity in the population (Anshori et al., 2022; Wang et al., 2023). Although, this variety is the only parent that has high anthocyanin levels in the base formation of the multiple cross population. Therefore, color characters can be an important indicator, both in knowing the segregation pattern of the multiple cross population and in grouping between lines in the population.

Grouping lines in this population can be done using a combination of dendrogram and PCoA. Both are multivariate analyses that have similarities in the basic structure of their analysis (Pambabay-Calero et al., 2021). Both use similarity data between lines on overall characters, such as Euclidean or Gower analysis (Watson et al., 2019; Wang et al., 2022). However, both have different concepts of data processing and interpretation. Dendrogram analysis performs visualization directly based on the accumulation of similarities between each object based on the grouping method (Chehreghani and Chehreghani, 2020). In contrast, PCoA analysis does not directly use these similarity values, but these values are converted into compressed eigenvalues like principal component analysis (Shi et al. 2020; Wang et al., 2022). This compression makes the mapping formed objective and does not overlap in grouping between objects (Shi et al., 2020; Rubiyo et al., 2022; Wang et al., 2022). Integration between the two will produce robust and systematic grouping of objects. Several researchers have reported the use of this analysis in the mapping process of non-categorical data (González-Pérez et al., 2014; Akyavuz et al., 2018; Haq et al., 2022). Therefore, the integration of the two is effectively used to increase the strength and precision of grouping.

Grouping results integration of dendrogram and PCoA analysis showed that there were 64 consistent genotypes from the two sections (Table 3). The 64 genotypes were categorized into three groups. The first group is genotypes with consistent grouping of the parents of the 'Dewata' and 'Bara'. This group is a group with a general display of spicy cayenne peppers released in Indonesia. This group consists of 27 consistent genotypes and three of them (Geno 4, Geno 5, and Geno 21) have very close similarities to the two comparisons cultivars. The second group is genotypes that have a consistent grouping of 'Ungara'. This group is a group that is consistent in its biofortification properties through plant anthocyanin content. Based on this grouping, there were 16 genotypes that consistently grouped with the parent 'Ungara'. Meanwhile, the last group is a group whose consistency is outside the two main groups or genotype groups with variations between the two parent groups. This group consists of 21 genotypes which are divided into two subgroups based on PCoA analysis, namely eight genotypes in the blue subgroup and 13 genotypes in the red subgroup. The blue subgroup is the middle variation between the two main groups (while the red subgroup is more dominated by Dewata and Bara elder groups).

CONCLUSIONS

Evaluation based on qualitative characters shows that the multiple cross chili F_4 population still has quite high diversity. There are two types of diversity in this population, namely centralized diversity and distributed diversity. Centralized diversity consists of leaf color, node color, leaf shape, canopy density, stem color, fruit shape and fruit tip shape. On the other hand, the characters that have a diverse distribution are the color of the crown, the color of young and old chilies. The distribution of this diversity is greatly influenced by the nature of the anthocyanin or purple color found in the Ungara elder. However, the influence of 'Dewata' and 'Bara' (27 grouped genotypes) dominates the grouping of F_4 lines compared to the influence of 'Ungara' (16 grouped genotypes). Apart from that, there are also genotypes that consistently cluster outside the two main groups. This group consists of 21 genotypes which are divided into two subgroups, namely eight genotypes which are identical to the combination of Dewata-Bara group and Ungara group; and 13 genotypes that are more inclined towards the Dewata-Bara group. Meanwhile, characters related to color, especially leaf color, are characters with a dominant correlation compared to other qualitative character groups. Based on the entire study, the results of this evaluation are recommended as material for consideration in selecting F_5 lines to support the direction of releasing chili cultivars from multiple crosses.

Author contribution

Conceptualization: A.Y.M., M.F., F.U. Methodology: A.Y.M., M.F., M.F.A. Software: M.F.A. Validation: M.F. Formal analysis: A.Y.M., M.F.A. Investigation: A.Y.M. Resources: M.F., M.F.A, F.U. Data curation: M.F.A, F.U. Writing-original draft: A.Y.M. Writing-review & editing: M.F.A. Visualization: A.Y.M., M.F.A. Supervision: M.F., F.U. Project administration: M.F.A. Funding acquisition: M.F., M.F.A. All co-authors reviewed the final version and approved the manuscript before submission.

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