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PERFORMANCE AND RELATIONSHIP OF VARIOUS LOCAL GARLIC GENOTYPES AND PUTATIVE MUTANTS RESULTING FROM MV4 IRRADIATION USING MORPHOLOGICAL MARKER

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ABSTRACT

Garlic (*Allium sativum* L.) is used as a medicinal plant or seasoning, but its national production can only meet people's needs by 6.4%, and 95% is imported from China. The development rate of new garlic varieties is relatively slow because garlic is generally propagated vegetatively through cloves; therefore, its diversity has become relatively narrow. Character and diversity can be improved by inducing mutations using gamma-ray irradiation. Experiments on garlic subjected to gamma-ray irradiation have shown physiological changes in the putative 3rd (MV3) mutant offspring. The experiment's 4th mutant progeny (MV4) was re-planted and compared with other local genotypes. The aim was to perform clustering based on the yielding characteristics and the intensity of the anthocyanin content. The experiment used a single-plant design with 21 experimental units, namely 16 MV4 genotypes and 5 control genotypes. The analysis of variance showed that the MV4 Lumbu Kuning 1 Gy and Ciwidey 2 Gy genotypes had better tuber weight and diameter than the other genotypes, including the control genotypes. Cluster analysis clustered garlic genotypes into five groups at a cophenetic distance 0.4. Principal component analysis (PCA) also divided the garlic genotypes into four quadrants. Genotypes in quadrant 2 had anthocyanin content intensities superior to those of other genotypes.

1. INTRODUCTION

Garlic (*Allium sativum* L.) is a species in the genus Allium with the second-largest production rate in the world after onions (*Allium cepa* L.) (Ammarellou et al., 2022). The main part used is clove for medicine and cooking seasoning (Chanchan et al., 2013). Garlic production in 2020 is expected to reach 81,805 tons (BPS, 2021); however, it is only able to meet the needs of the community by 6.4%, with the total national consumption of garlic reaching 1,275,224.08 tons (KEMENTAN, 2021). 95% of the people's need for garlic is imported from China. Indonesians also prefer imported garlic because the price is lower and the bulb size is larger than local garlic. Garlic production in China averages 25.3 t ha-1, making it less expensive than local garlic. The average productivity of garlic in Indonesia is 8.7 t ha-1 (Hernita et al., 2019).

The solution to increasing garlic production is to expand the planting area, but another problem is the limited availability of high-quality garlic seeds (higher varieties). The development of new garlic varieties is still relatively slow because garlic is generally propagated vegetatively through cloves; therefore, its diversity is relatively narrow (Parreño et al., 2023). Conventional garlic breeding is difficult in Indonesia because garlic is a long-day plant (Draghici & Lagunovschi-luchian, 2015). The formation of garlic flowers requires 14-16 hours of light exposure, whereas, in Indonesia, the light duration is only ± 12 h.

Mutation induction can improve the characteristics and increase plant diversity (Lestari, 2021). Mutations can be artificially induced using physical mutagen gamma rays. A previous study has shown that gamma irradiation can affect the longevity of wheat plants (Dwinanda et al., 2020), and changes in leaf color occur in *C. blumei* plants (Aisyah et al., 2015). Gamma irradiation also increases oleoresin levels in several genotypes of ginger plants (Abdullah et al., 2018) and causes genetic diversity in bananas based on ISSR molecular markers (Due et al., 2019).

Pangestuti et al. (2020) tested the effect of gamma irradiation on three local garlic genotypes, Ciwidey, Lumbu Kuning, and Tawangmangu Baru, and obtained the LD50 dose for each genotype. The third derivative (MV3) from Pangestuti et al. (2020) was tested again to observe physiological changes, and several new metabolite compounds appeared in the MV3 generation (Winarni et al., 2022). This is the basis for further research to confirm these changes in the 4th generation garlic mutant (MV4). In addition to the MV4 genotype, this study also used several other local garlic genotypes as a comparison, including the Lumbu Putih, Lumbu Hijau, Jawa Lama, Siam, and Sangga Sembalun genotypes.

This study analyzed morphological markers using the UPOV guide for garlic (Geneva, 2022). This study aimed to determine the relationship between the MV4 garlic and control genotypes. The characteristic that became the primary marker was anthocyanin intensity; therefore, garlic genotypes with superior anthocyanin intensity were obtained for use in plant breeding and cultivation.

2. METHODS

2.1. Genetic Material

This study used planting material of 16 genotypes of local garlic of the 4th generation resulting from gamma-ray irradiation (MV4), namely Lumbu Kuning 0 Gy (LK0G), Lumbu Kuning 1 Gy (LK1G), Lumbu Kuning 2 Gy (LK2G), Lumbu Kuning 4 Gy (LK4G), Lumbu Kuning 6 Gy (LK6G), Lumbu Kuning 8 Gy (LK8G), Tawangmangu Baru 0 Gy (TM0G), Tawangmangu Baru 2 Gy (TM2G), Tawangmangu Baru 4 Gy (TMB4G), Tawangmangu Baru 6 Gy (TM6G)), Tawangmangu Baru 8 Gy (TM8G), Ciwidey 0 Gy (CW0G), Ciwidey 1 Gy (CW1G), Ciwidey 2 Gy (CW2G), Ciwidey 4 Gy (CW4G), and Ciwidey 6 Gy (CW6G). Five other local garlic genotypes were used for comparison: Lumbu Putih (LP), Lumbu Hijau (LH), Old Java (JL), Siam (SI), and Sangga Sembalun (SS).

2.2. Characterization of The Morphological Characteristics

The experiment was conducted during one growing season from April to August 2021. Planting was carried out at the Pasir Sarongge Experimental Garden, Pacet District, Cianjur Regency, West Java, at 1200 m asl. The planting material used is Cloves from garlic bulbs that had passed 4-5 months of dormancy.

Land preparation was carried out by liming (3 t ha^{-1}) two weeks before planting. Then, manure doses of 20 tons ha^{-1}, NPK 15-15-15 200 kg ha^{-1}, and ZA 475 kg ha^{-1} were applied one week before planting. The beds were made with a height of 30 cm, width of 90 cm, and length adjusted to the length of the land, and a silver black mulch was installed. Plant spacing was 15 × 15 cm. Planting is performed by sticking ¾ the cloves into the ground with the disc (tree stem) facing down. A sprinkler of 3% carbofuran insecticide was administered to avoid pest attacks. Plant maintenance includes watering, fertilizing, weeding, and pest and disease control. Harvest time ranges from 90-120 DAP, marked by the leaves and stems of the plants withered and dried by approximately 50-60%, the stems began to harden, and the tubers appeared to the ground.

The study used a single-plant design without repetition and had 21 experimental units. Each experimental unit had a different plant population depending on the availability of plants from previous generations. The experimental unit and its population consisted of 51 plants LK0G, 54 plants LK1G, 52 plants LK2G, 54 plants LK4G, 52 plants LK6G, 51 plants LK8G, 27 plants TM0G, 51 plants TM2G, 52 plants TMB4G, TM6G 52 plants, TM8G 47 plants, CW0G 51 plants, CW1G 52 plants, CW2G 51 plants, CW4G 51 plants, CW6G 13 plants, LP 55 plants, LH 51 plants, JL 52 plants, SI as many as 53 plants, and SS as many as 53 plants. A total of 1 025 plants were observed, starting from the tuber formation phase at 8 WAP.

2.3. Data Analysis

The growth data obtained were tested using an analysis of variance (F test) with a 5% significance level, followed by a 5% HSD test if there was a significant effect. Qualitative data were analyzed using PBSTAT-CL to produce a cluster dendrogram using the dissimilarity method (Gower) and clustering method (average linkage). In addition, principal component analysis (PCA) was also used using R-studio software.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. *Growth characteristics of garlic MV4 and control genotypes*

The results in Table 1 show that the character of plant height with the highest average value was the MV4 Lumbu Kunig 1 Gy genotype, but not significantly different from the comparison genotypes Jawa Lama, Sangga Sembalun, MV4 Ciwidey 2 Gy, Lumbu Kuning 0 Gy, and Lumbu Kuning 2 Gy. The number of leaves with the highest average value was the MV4 Tawangmangu Baru 2 Gy genotype but was not significantly different from the Tawangmangu Baru 0 Gy genotype. The tuber weight character with the heaviest average value was the MV4 Lumbu Kuning 1 Gy genotype, but it was not significantly different from Ciwidey 2 Gy. The average character value of the widest tuber diameter was the MV4 Ciwidey genotype 2 Gy, but not significantly different from the 13 MV4 genotypes and other comparison genotypes, successively including Lumbu Kuning 1 Gy, Tawangmangu Baru 2 Gy and Lumbu Kuning 4 Gy. Lumbu Kuning 2 Gy, Lumbu Kuning 8 Gy, Ciwidey 0 Gy, Sangga Sembalun, Lumbu Kuning 0 Gy, Lumbu Kuning 6 Gy, Ciwidey 1 Gy, Siam, Tawangmangu Baru 0 Gy, and Tawangmangu Baru 4 Gy.

Table 1 Growth and performance of garlic genotypes

Genotype	Plant height (cm)	Number of leaves	Tuber weight (g)	Tuber Diameter (mm)
LK0G	69.08 a-c	11.50 e-h	16.62 d-i	34.15 a-e
LK1G	79.00 a	12.50 c-e	37.93 a	41.28 a
LK2G	68.42 a-d	11.42 e-i	26.59 b-d	39.86 ab
LK4G	65.00 c-e	11.33 e-i	22.78 b-g	39.90 ab
LK6G	62.67 c-e	10.42 h-j	23.27 b-f	33.42 a-e
LK8G	60.58 c-e	10.58 g-j	24.34 b-e	36.13 a-c
TM0G	67.42 b-e	13.83 ab	20.96 c-h	32.47 a-e
TM2G	67.42 b-e	14.75 a	26.75 b-d	40.52 a
TM4G	65.33 c-e	12.83 b-d	13.53 f-i	32.40 a-e
TM6G	57.00 de	12.42 c-f	9.80 i	26.54 c-e
TM8G	56.08 e	12.08 c-f	12.33 g-i	27.48 c-e
CW0G	59.83 c-e	7.83 e	25.62 b-e	35.73 a-d
CW1G	63.50 c-e	10.25 ij	27.33 bc	33.27 a-e
CW2G	71.08 a-c	13.25 bc	32.16 ab	41.42 a
CW4G	61.00 c-e	12.33 c-f	22.57 b-g	30.68 b-e
CW6G	56.17 e	10.58 g-j	15.17 e-i	30.12 c-e
LP	63.50 c-e	11.25 f-i	7.84 i	26.12 de
LH	57.42 de	10.83 g-j	6.98 i	26.78 c-e
JL	77.00 ab	9.50 j	11.18 hi	25.53 e
SI	65.08 c-e	11.67 d-g	13.54 f-i	32.85 a-e
SS	71.21 a-c	10.25 ij	17.16 c-i	35.21 a-e

Information : Numbers in the same column followed by the same letter are not significantly different according to the HSD test at the 5% significance level.

3.1.2. *Qualitative characteristics and anthocyanins in garlic MV4 and control genotypes.*

Local garlic genotypes, consisting of 16 MV4 and five control genotypes, had tuber yields, as shown in Figure 1. Each genotype was differentiated based on yield characteristics, especially anthocyanin intensity.

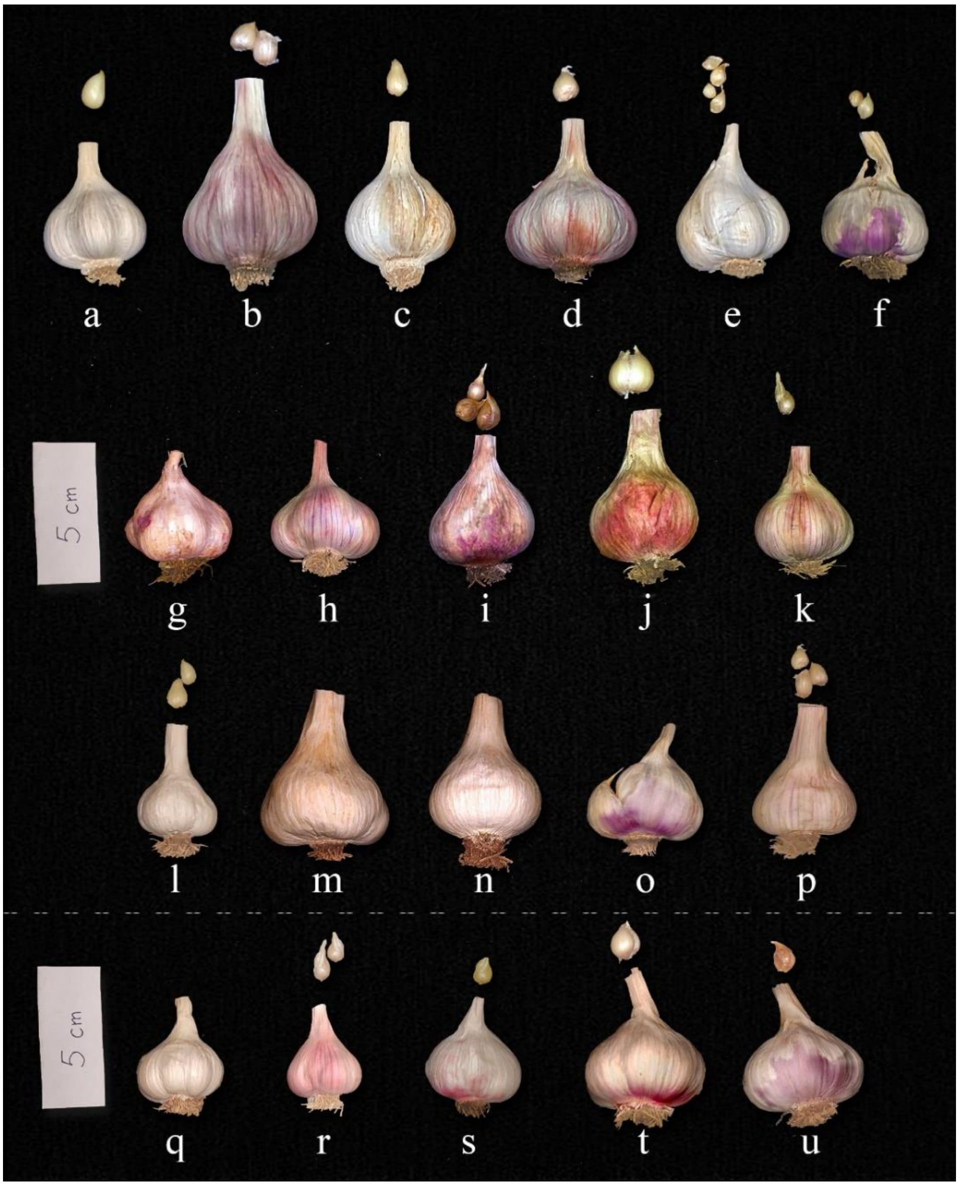


Figure 1 Garlic Bulbs of Various Genotypes MV4 (a-p) and the Control Genotypes (q-u) a) LK0G, b) LK1G, c) LK2G, d) LK4G, e) LK6G, f) LK8G, g) TM0G, h) TM2G, i) TM4G, j) TM6G, k) TM8G, l) CW0G, m) CW1G, n) CW2G, o) CW4G, p) CW6G, q) LP, r) LH, s) JL, t) SI, u) SS

Cluster analysis was performed to determine the cophenetic distance between garlic genotypes. Cluster analysis was performed based on qualitative characteristics according to the UPOV for Garlic guidelines (Geneva, 2022). Qualitative characteristics observed were the upright position of leaves, presence of bulbils on stems, tuber shape longitudinal position, disc position, tuber base shape, clove density, epidermis color, anthocyanin presence, anthocyanin intensity, epidermal anthocyanin lines, epidermal strength, thickness of epidermis, number of tuber cloves, presence of outer cloves, clove skin color, clove skin color intensity, flesh color, and clove distribution. Cluster analysis was carried out on 84 plants, four of which were taken from each genotype with the criteria of having the heaviest weight and highest anthocyanin intensity, consisting of 64 MV4 plants and 20 control genotype plants. The results showed that 84 plants were divided into five clusters at a cophenetic distance 0.4 (Figure 2).

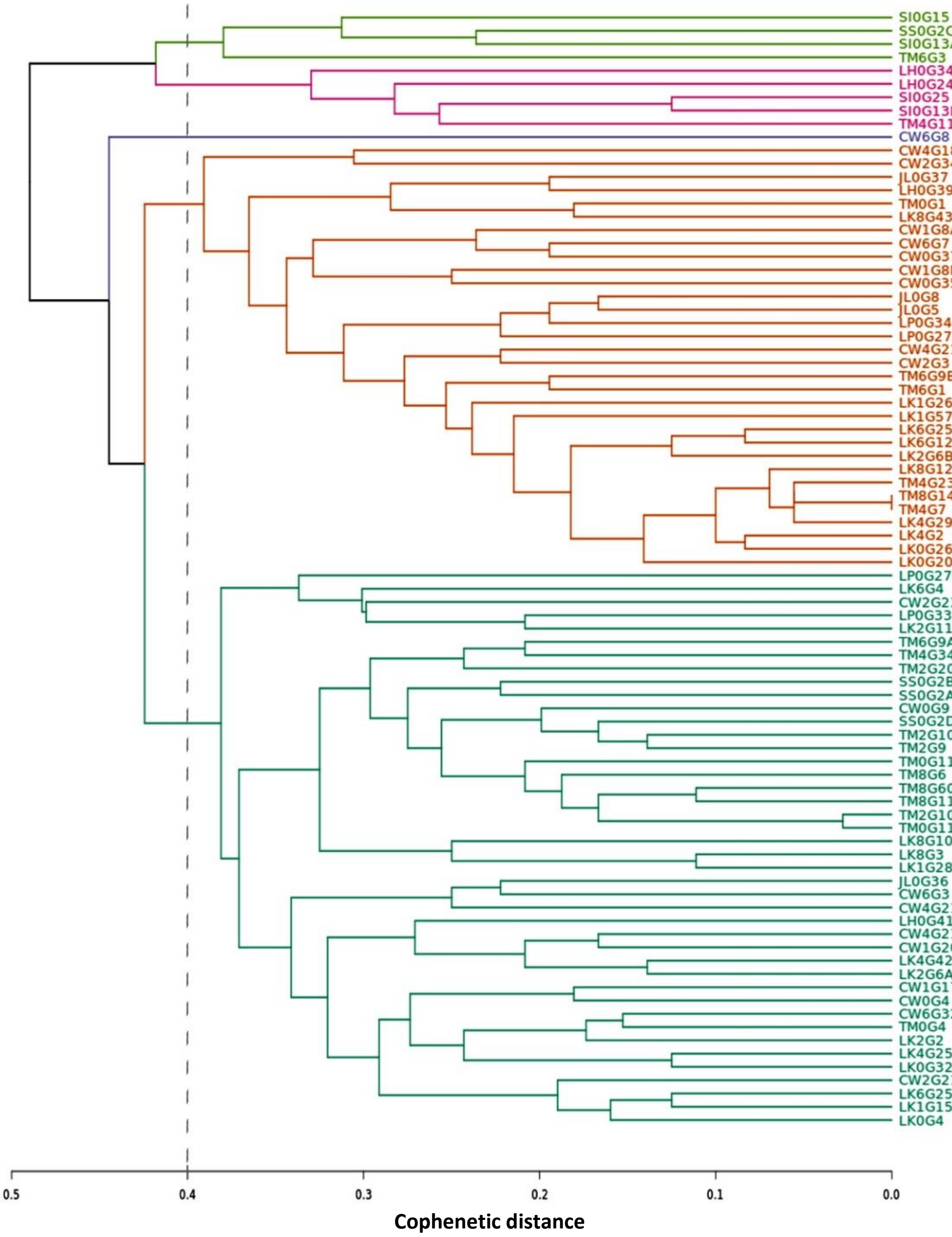
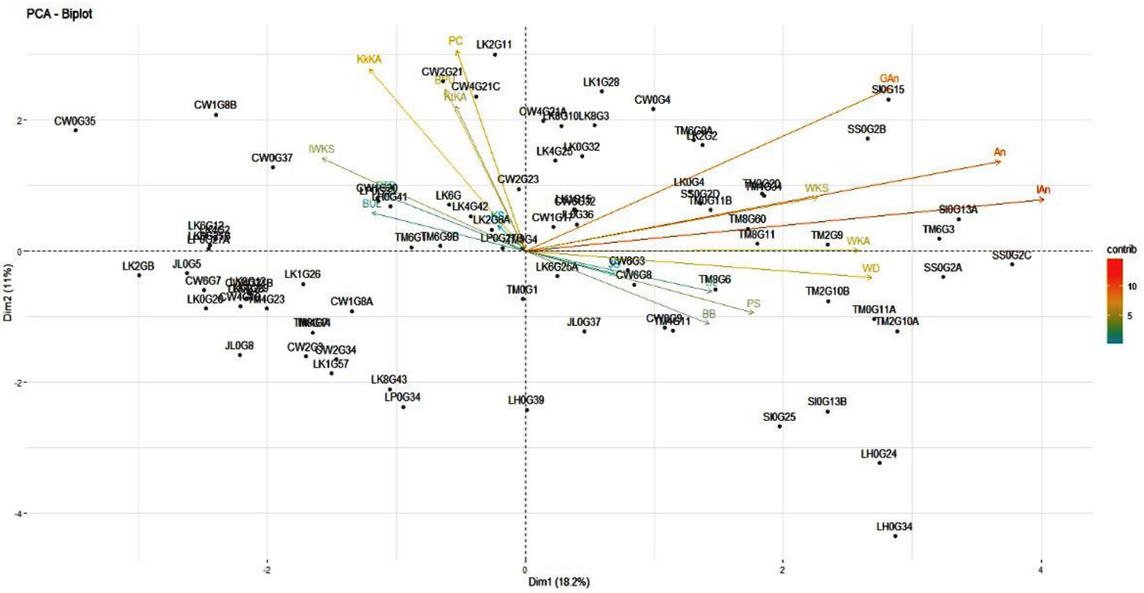


Figure 2 Dendograms of 84 garlic plants based on qualitative characteristics.

Cluster 1 consisted of 35 MV4 plants and seven control genotype plants. Cluster 2 consisted of 26 MV4 plants and six control genotype plants. Cluster 3 consisted of one MV4 plant. Cluster 4 consisted of one MV4 plant and four plants of the control genotype. Cluster 5 consisted of one MV4 plant and three plants of the control genotype. The suitability level of grouping is determined based on the agglomerative coefficient value, namely the suitability of grouping with the criteria of strong ($0.71 \leq AC \leq 1.00$), appropriate ($0.51 \leq AC \leq 0.70$), weak ($0.26 \leq AC \leq 0.50$), and very unsuitable ($AC \leq 25$) (Kaufman & Rousseeuw, 2005). The clustering formed on the dendrogram (Figure 2) has an agglomerative coefficient value of 0.73, indicating that the groupings formed meet the strong criteria.

Principal component analysis (PCA) was performed to determine each plant genotype's superior characteristics. PCA is a reliable technique for extracting structure from a data set with many dimensions (Tazi et al., 2019; Greenacre et al., 2023). The results of the PCA of

84 garlic plants based on qualitative characteristics were grouped into four quadrants, as shown in the biplot (Figure 3).



Quadrant 1 :	CW0G35, CW0G37, CW1G20, CW1G8A, CW1G8B, CW2G21, CW2G23, CW4G21C, LH0G41, LK1G15, LK2G11, LK2G6A, LK4G2, LK4G42, LK6G4, LK6G12, LK6G25B, LP0G27A, LP0G27B, LP0G33, TM6G1, TM6G9B
Quadrant 2 :	CW0G4, CW1G17, CW4G21A, CW6G32, JL0G36, LK0G32, LK0G4, LK1G28, LK2G2, LK4G25, LK8G10, LK8G3, SI0G13A, SI0G15, SS0G2B, SS0G2D, TM0G11B, TM2G20, TM2G9, TM4G34, TM6G3, TM6G9A, TM8G11, TM8G60
Quadrant 3 :	CW0G9, CW6G3, CW6G8, JL0G37, LH0G24, LH0G34, LH0G39, LK6G25A, SI0G13B, SI0G25, SS0G2A, SS0G2C, TM0G11A, TM2G10A, TM2G10B, TM4G11, TM8G6
Quadrant 4 :	CW2G3, CW2G34, CW4G18, CW4G21B, CW6G7, JL0G5, JL0G8, LK0G20, LK0G26, LK1G26, LK1G57, LK2G6B, LK4G29, LK8G12, LK8G43, LP0G34, TM0G1, TM0G4, TM4G23, TM4G7, TM8G14

Figure 3 Principal component analysis (PCA) of 84 garlic plants based on qualitative characters

Plants in quadrant 1 were grouped with more striking characters in the upright leaf position, tuber shape, longitudinal position, disc position, tuber base shape, clove density, epidermis strength, epidermis thickness, and clove skin color intensity. Plants in quadrant 2 were grouped with more striking characters in the epidermis color, presence of anthocyanins, intensity of anthocyanins, anthocyanin lines of the epidermis, and skin color of the cloves. Plants in quadrant 3 clustered with more striking characteristics in the presence of bulbils on the stem. Also, the number of tuber cloves, the presence of outer cloves, the color of the flesh, and the distribution of cloves. Plants in quadrant 4 were grouped with no striking features in any of the qualitative characteristics.

3.2. Discussion

The growth characteristics of plant height and number of leaves influence tuber weight and diameter yield characteristics. Table 1 shows several MV4 genotypes with plant height and number of leaves, with higher average values able to increase tuber weight and diameter. However, in the comparison genotypes, even though the average plant height was not significantly different from some of the MV4 genotypes, it did not increase tuber weight. What happened to The comparison genotype was in line with the research conducted by Siswadi et al. (2019) and Efendi et al. (2020), who found that garlic plants that were given various treatments with high yields of plants and a higher number of leaves had lighter tuber weight compared to plants with lower vegetative growth.

The tuber weight of the MV4 genotype, which had a higher average value, was likely influenced by the effect of gamma irradiation in the previous generation. Jankowicz-Cieslak

& Till (2016) stated that the nature of gamma-ray mutations is random and cannot be directed at a particular gene or trait in an organism, so the resulting mutants cannot be predicted. Providing an appropriate dose of irradiation can have a positive effect on plant growth. Gamma-ray irradiation at a dose of 8 Gy positively affected the height gain of Doulu cultivar garlic plants, which was better than that of the control plants (Gultom et al., 2020).

Gamma-ray irradiation reduces the percentage of living plants and causes slow seed germination (Santosa et al., 2014). The higher the irradiation dose, the higher the deterministic effect (Fadli et al., 2018). Mutations can cause sterility, physiological disorders, or death (lethality) (Due et al., 2019; Du et al., 2022). As shown in Table 1, the MV4 genotype of garlic treated with higher irradiation doses had a lower average tuber weight.

Referring to the research objective of obtaining plants with superior anthocyanin intensity, based on the results of PCA analysis, grouped garlic genotypes in quadrant 2 can be used as a source of plant propagation material. Anthocyanins are a class of phenolic pigments soluble in water and constitute plants' largest group of pigments. Anthocyanins can exhibit different colors, such as purple, red, and blue, expressed in fruits, flowers, leaves, and seeds (Alvarez-Suarez et al., 2021). Other plant parts are generally found more often in the skin (Metrani et al., 2020). Anthocyanin in plants is not only a color pigment regulator, but the anthocyanin content is also closely related to human health benefits. The roles of anthocyanins include anticancer (Wang et al., 2018), hepatoprotectors (Peng et al., 2017), antioxidants (Lee et al., 2014), anti-inflammatory (Khajah et al., 2019), and neuroprotective (Raggi, 2015).

4. CONCLUSION

The results showed that some MV4 garlic genotypes had better tuber weight and diameter than the control genotypes. Irradiation significantly affected the MV4 genotype at the appropriate varieties and doses, as seen in Lumbu Kuning 1 Gy and Ciwidey 2 Gy. Cluster analysis at a cophenetic distance of 0.4 showed that the MV4 garlic and control genotypes were divided into five clusters. PCA also showed that garlic genotypes spread into four quadrants. Garlic genotypes that can be used as a source of plant propagation with superior anthocyanin intensity characteristics are clustered in Quadrant 2.

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