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Assessment of relationship among traits and genotypes for melon (*Cucumis melo*) breeding

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Abstract. *Maghfiroh RN, Suwarno WB, Sobir, Gunawan E. 2023. Assessment of relationship among traits and genotypes for melon (*Cucumis melo*) breeding. Biodiversitas 24: 4523-4531.* Melon (*Cucumis melo* L.) is widely consumed as fresh fruit or juice, and therefore deeper analyses of fruit quality are needed for future breeding purposes. Breeders have a role in providing improved melon varieties that suit consumer ideotypes or preferences. This study aimed to (i) evaluate the performance of melon genotypes, (ii) estimate the correlation between quantitative traits, and (iii) determine the clustering among genotypes based on plant and fruit traits. This research evaluated 23 genotypes from inodorus, reticulatus, and makuwa groups that were carried out from March to June 2022 at a greenhouse in the Tajur experimental station of the Center for Tropical Horticulture Studies, Bogor, Indonesia. IPB HD 2-111 and IPB HD 2-100 had good quality based on soluble solids content, fruit shape, flesh texture, and rind color, and G30 was relatively superior on flesh thickness, fruit weight, and vitamin C. Based on the multiple regression analysis, it was known that petal width, petiole length, and days to harvest were significantly affected the proportion of fruit cavity width to the fruit diameter. The adjusted coefficient of determination (R^2) was 0.569. Cluster analysis grouped 23 genotypes into three main clusters based on the neighbor-joining method. These results may be useful for selection in melon breeding programs.

Keywords: Genetic diversity, horticulture, neighbor-joining, plant breeding

Abbreviation: FW: fruit weight, FD: fruit diameter, FT: flesh thickness, LW: leaf blade width, PEW: male flower petal width, PEL: male flower petal length, LL: leaf blade length, FL: fruit length, SL: stem (internode) length, VITC: vitamin C, SD: stem diameter, PL: petiole length, MF: days to male flowering, HF: days to hermaphrodite flowering, TTA: total titratable acidity, RT: rind thickness, SSC: soluble solids content, STD: stomatal density, DH: days to harvest, FI: firmness, CA: fruit cavity width, CDR: the proportion of fruit cavity width to the fruit diameter, R^2: coefficient of determination

INTRODUCTION

Melon (*Cucumis melo* L.) is an important horticultural commodity in the world. Melon is in great demand by consumers as a table fruit both for family consumption to fulfill daily nutrition and banquets at parties, as well as serving fruit at various restaurants. Melon has the main concern for the quality of the fruit which influences consumer choice and preferences. Fruit quality can be measured based on internal and external properties. The internal quality mainly is determined by aroma, flavor, taste, texture, nutritional quality (e.g. soluble solids content, vitamins, antioxidant activity, etc.), flesh firmness, diseases, and chemical residues. Meanwhile, external quality mainly concerns appearance, size, and color (Pathare et al. 2013; Bhargava and Bansal 2021; Felföldi et al. 2022).

Plant breeding activities were broadly classified into three main targets, namely yield and morphological traits, quality traits, breeding for resistance to diseases and pests, and breeding for resistance to abiotic stress. One objective of melon breeding is to improve quality traits. Plant breeders should understand the quality standards for melons on the market which are heavily influenced by the interaction of complex factors, for example from economic and biological factors, where these standards are very specific to the commodity (Acquaah 2018).

Flesh thickness is one of the important fruit quality traits other than high sugar content, high yield, thick flesh, crisp flesh texture, attractive flesh color, no unpleasant after-taste, hard rind for transportation, long shelf life, and good-looking dense net (for cantaloupes) (Suwarno et al. 2016). The appearance and taste of melons are influenced by varieties, besides the plant nutrition, cultivation techniques, planting dates, temperature, harvest dates, irrigation, and plant diseases factors (Vallone et al. 2013; Visconti et al. 2019; Yam et al. 2020), or in this case referred to the influence of the environment, in other words, we often say that the phenotype is a representation of the genotype, the environment, and the interaction of genotype x environment (Kyriacou et al. 2018; Ramjan and Ansari 2018). Based on these reasons, it is important for breeders to play a role in providing melons that suit consumer ideotypes/tastes through breeding for improved variety.

To improve the quality of melon varieties, it is necessary to understand the characteristics of the melons to be used as the genetic material for melon development in order to adapt them to the characteristics of the new varieties. Genetic diversity is the main capital for breeding programs (Minter et al. 2021). In addition, in the breeding stage, it is important to handle the diversity of melon genotypes very well and work out the right selection method so that the desired traits can be obtained in an efficient process. For selecting superior genotypes, we need to study the correlation between the characters. Sometimes, a trait that is rather difficult for us to see directly in the field, and is associated with other traits either directly or indirectly. Therefore, it is important to study the correlation between traits and path analysis which will be fruitful for future breeding programs. Indeed, there have been many previous studies discussing path analysis in melons, but the majority analyzed yield and yield-related traits such as fruit yield and fruit weight, and those discussed path analysis on fruit quality traits (such as proportion of fruit cavity width to the fruit diameter) were still limited (Reddy et al. 2013; Pasha et al. 2019; Nanthakumar et al. 2021; Hiremata et al. 2022; Khomphet et al. 2022; Soltani et al. 2022).

Therefore, this study presents a path analysis of the proportion of fruit cavity width to the fruit diameter, which is included in consumer tastes in choosing melons. One other important thing, it is necessary to consider the distinction between genotypes which can be studied through cluster analysis. This study aimed to (i) evaluate the performance of melon genotypes, (ii) estimate the correlation between quantitative traits, and (iii) determine the clustering among genotypes based on plant and fruit traits.

MATERIALS AND METHODS

Genetic materials

Twenty-three genotypes of melon including checks (Alisha F_1, Apollo F_1, and Glamour F_1) were planted inside the greenhouse (Table 1). The genotypes were from reticulatus, inodorus, and makuwa groups.

Experimental design

The trial was carried out from March to June 2022 inside a greenhouse at experimental station Tajur 1 of the Center for Tropical Horticulture Studies, IPB University, Bogor, West Java, Indonesia. It was located at ±250 m above sea level and is characterized by a tropical climate in latosol soil. Bogor is located in Indonesia (-6.6359ºS, 106.8235ºE), about 49 km from Jakarta. Bogor has a high rainfall rate (316 mm/month) and high relative humidity (85.16%) during this period (BMKG 2022). The trial was arranged in a randomized complete block design with three replications. Each experimental unit consisted of five polybags. The size of the polybag is 40 cm x 35 cm and spacing between plants is 60 cm between rows and 40 cm within a row. The three best fruits of each genotype were chosen to be analyzed for fruit quality traits.

Table 1. Twenty-three genetic materials of melon for evaluating fruit performance on quality-related traits

Genotype	Botanical group	Seed source
G7	Reticulatus	PKHT-IPB
G240	Reticulatus	PKHT-IPB
Glamour S_2	Reticulatus	PKHT-IPB
Alisha S_1	Inodorus	PKHT-IPB
G1	Inodorus	PKHT-IPB
G30	Inodorus	PKHT-IPB
IPB HD 1-145	Inodorus	PKHT-IPB
IPB HD 1-34	Inodorus	PKHT-IPB
IPB HD 2-100	Inodorus	PKHT-IPB
IPB HD 2-111	Inodorus	PKHT-IPB
IPB HD 4-165	Inodorus	PKHT-IPB
IPB HD 5-131	Inodorus	PKHT-IPB
IPB HD 6-130	Inodorus	PKHT-IPB
IPB HD 6-168	Inodorus	PKHT-IPB
IPB HD 6-61	Inodorus	PKHT-IPB
IPB HD 6-97	Inodorus	PKHT-IPB
M13	Inodorus	PKHT-IPB
M21	Inodorus	PKHT-IPB
M23	Inodorus	PKHT-IPB
P34	Makuwa	PKHT-IPB
Glamour F_1	Reticulatus	Sakata Seed
Apollo F_1	Inodorus	Known You Seed
Alisha F_1	Inodorus	East West Seed

Observation and data analysis

Observations and measurements were recorded for the following twenty quantitative melon fruit quality traits: fruit weight (FW), fruit diameter (FD), flesh thickness (FT), leaf blade width (LW), male flower petal width (PEW), male flower petal length (PEL), leaf blade length (LL), fruit length (FL), stem (internode) length (SL), vitamin C (VITC), stem diameter (SD), petiole length (PL), days to male flowering (MF), days to hermaphrodite flowering (HF), total titratable acidity (TTA), rind thickness (RT), soluble solids content (SSC), stomatal density (STD), days to harvest (DH), and firmness (FI). Observation of plant morphology using a ruler and caliper, total titratable acidity and vitamin C using the titration method, measurement of total soluble solids using a digital refractometer (Atago, Japan), and stomatal density was observed using a light microscope (CX 23). ImageJ software was used to help in counting the number of stomata. Microsoft Office Excel 2019, R (v. 4.1.3), and Minitab (v. 17) software packages were used for statistical analyses.

Analysis of variance (ANOVA) was calculated using the R software. The Pearson correlation was analyzed for those twenty traits that were computed based on genotype means, using the R software to obtain information on the association of the linear relationship between the two quantitative variables (Agresti et al. 2018) and this correlation coefficient will be used in path analysis. The path analysis was carried out to identify melon traits that contribute to fruit cavity width: fruit diameter ratio (proportion of fruit cavity width to the fruit diameter). A stepwise selection procedure from the full model was applied to determine the model, including determining the

traits that had to be attached and/or to be deleted. The multiple regression was obtained by the method given by Bondari (1990):

$$Y = p_1X_1 + p_2X_2 + \cdots + p_nX_n + p_eE$$

Where:

$$Y = \frac{(y-\bar{y})}{\sigma_y}; X_i = \frac{(X_i-X)}{\sigma_{x_i}}; p_i = \frac{\beta_i-\sigma_{x_i})}{\sigma_y} \quad i= 1,\ldots, n$$

Cluster analysis was performed using PBSTAT-CL (www.pbstat.com) based on the modified Gower dissimilarity matrix and neighbor-joining tree for visualizing the distance between genotypes.

RESULTS AND DISCUSSION

Phenotypic variability among melon genotypes

Means of several morphological and fruit-chemical melon traits of each genotype were subjected to analyses of variance (ANOVA) followed by Tukey honestly significant difference (HSD) mean comparisons (Tables 2 and 3). Table 2 shows the quantitative data on vegetative traits including leaf, stem, and flowering characteristics, while Table 3 performs the quantitative data on fruit traits. The results of ANOVA showed that genotype had a significant effect on all vegetative traits including SD, SL, LL, LW, PL, PEL, PEW, MF, HF, and STD; and several fruit traits including FL, FD, FT, FW, and FI (Table 2 and 3).

The coefficient of variations (CV) ranged from 4.55% (on MF) to 20.77% (on FW), indicating that the experiment is quite reliable (Gomez dan Gomez 1984). The average genotype for fruit weight ranged between 300.92 g (P34) and 1029.03 g (Glamour S_2). The average fruit weight of Glamour F_1 and Glamour S_2 were significantly (p<0.05) greater than IPB HD 2-111, IPB HD 5-131, IPB HD 6-61, M13, M23, and P34. SSC is one of the fruit traits that determine consumer tastes. Moreover, consumers will prefer thick flesh rather than thin flesh. The other criteria are fresh flesh texture, attractive flesh color (usually green, yellow, or orange), firm rind for transportation, and attractive appearance (Grumet et al. 2007; Matsumoto et al. 2014; Suwarno et al. 2016). Fruit will be accepted by the market and liked by consumers if it has a minimum of 10º Brix (Ferrante et al. 2008; Naidu et al. 2013). Based on this criteria, the genotypes IPB HD 2-111, IPB HD 2-100, IPB HD 4-165, IPB HD 5-131, M13, M21, M23, and two check varieties, namely Alisha F_1 and Apollo F_1 were classified as having good sweetness. IPB HD 2-111 and IPB HD 2-100 had similarities, namely white and thick flesh, bright yellow skin, oblate shape, and sweet taste, but there was a slight difference in the texture of the fruit flesh, namely IPB HD 2-111 was slightly more crunchy than IPB HD 2-100. All reticulatus members, namely G7, G240, Glamour S_2, and Glamour F_1, had a smaller value of rind firmness than the other genotypes from the inodorus and makuwa groups. Then, G30 was relatively superior in flesh thickness, fruit weight, and vitamin C content, had attractive flesh color (orange), fresh and crunchy texture, and good shape (round) (Figure 1), and Glamour S_2 was relatively superior in flesh thickness, fruit weight, rind firmness, and had attractive orange flesh color. Meanwhile, G1 had a unique rind pattern and color.

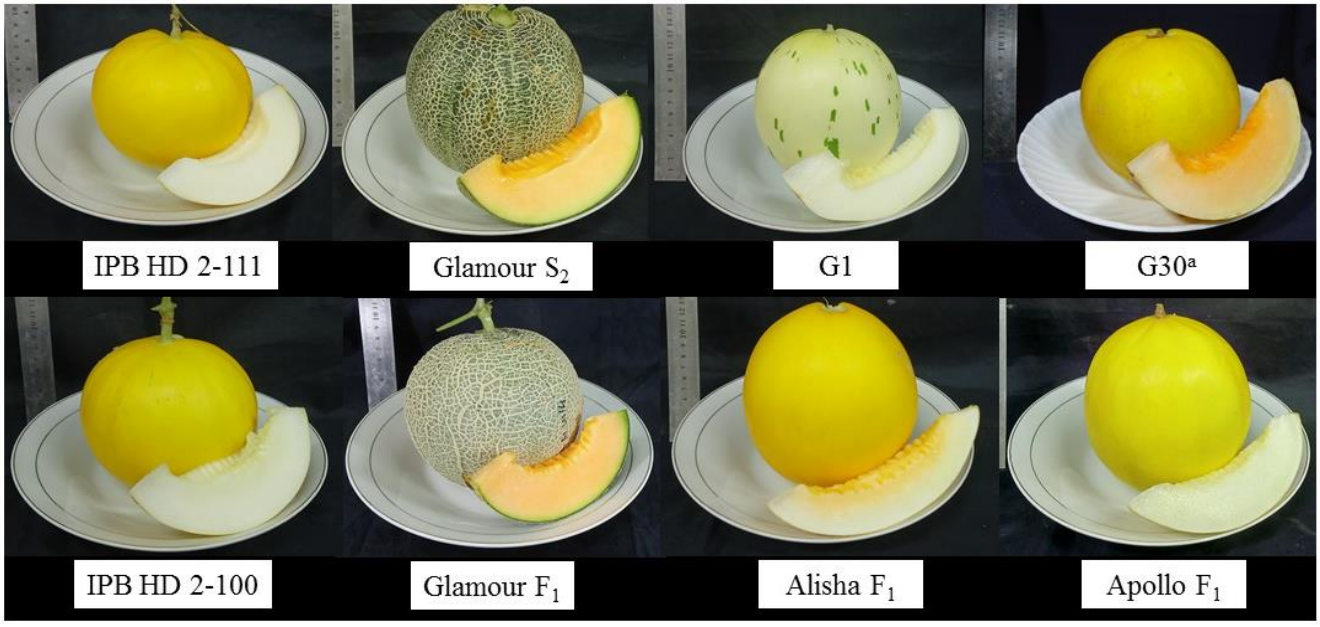

Figure 1. Fruit performance of several evaluated genotypes and three checks. a G30 was harvested from the previous planting season (2021)

Table 2. Means of vegetative traits of 23 melon genotypes

Genotype	SD	SL	LL	LW	PL	PEL	PEW	MF	HF	STD
G7	6.6 abcd	8.2 def	12.9 bcdef	17.8 abcd	14.7 defg	17.0	14.9	23 abc	29 a	498.7 ab
G240	6.8 a	7.2 f	14.5 abcd	19.6 abc	15.3 defg	19.3	16.1	22 abc	28 ab	333.3 b
Alisha S_1	6.1 abcd	8.6 cdef	14.6 abcd	17.5 abcd	14.7 defg	19.1	15.6	20 c	28 abc	517.3 ab
G1	6.6 abcd	8.0 ef	14.4 abcd	16.6 abcd	17.5 abcde	17.7	14.3	22 abc	28 abc	432.0 b
G30	6.5 abcd	10.0 abcde	16.1 abc	18.7 abcd	20.4 ab	23.3	17.1	21 abc	27 abc	405.3 b
Glamour S_2	6.8 ab	9.8 abcde	15.7 abc	19.8 abc	17.7 abcde	21.1	18.7	23 abc	27 abc	392.0 b
IPB HD 1-145	5.4 bcd	10.9 abc	16.6 a	22.2 a	17.1 abcdefg	21.9	17.7	21 abc	27 abc	597.3 ab
IPB HD 1-34	5.2 d	11.4 ab	15.6 abc	20.4 abc	16.8 abcdefg	21.4	15.4	24 ab	27 abc	357.3 b
IPB HD 2-100	5.3 d	9.6 abcde	13.5 abcdef	18.1 abcd	17.2 abcdef	19.8	16.0	22 abc	27 abc	402.7 b
IPB HD 2-111	5.4 cd	9.4 abcdef	14.1 abcd	17.0 abcd	19.2 abcd	17.3	14.2	23 abc	26 abc	432.0 b
IPB HD 4-165	5.7 abcd	11.4 a	16.3 ab	19.2 abc	16.3 bcdefg	22.8	19.3	22 abc	26 abc	432.0 b
IPB HD 5-131	5.6 abcd	8.8 cdef	12.8 cdef	16.7 abcd	21.3 a	17.4	14.3	21 abc	26 abc	360.0 b
IPB HD 6-130	6.3 abcd	8.6 cdef	11.8 def	16.0 bcd	13.6 efg	20.1	16.6	22 abc	26 abc	618.7 ab
IPB HD 6-168	6.0 abcd	10.2 abcde	11.8 def	15.7 cd	12.5 g	19.8	15.8	24 ab	26 abc	586.7 ab
IPB HD 6-61	6.1 abcd	8.8 cdef	10.6 ef	14.4 cd	12.6 fg	21.8	17.9	21 bc	26 abc	792.0 a
IPB HD 6-97	6.6 abcd	10.6 abcd	13.6 abcdef	17.8 abcd	13.8 efg	21.7	19.4	21 bc	25 abc	514.7 ab
M13	6.7 abc	8.9 cdef	13.9 abcde	17.1 abcd	20.1 abc	18.1	14.6	23 abc	25 bc	496.0 b
M21	5.6 abcd	8.4 def	13.2 abcdef	16.0 cd	16.2 bcdefg	17.9	14.6	22 abc	25 bc	498.7 ab
M23	6.1 abcd	9.0 bcdef	15.2 abcd	17.3 abcd	15.7 cdefg	22.1	17.6	22 abc	24 c	461.3 b
IPB Meta 9	5.6 abcd	8.2 def	16.6 a	20.0 abc	16.6 bcdefg	17.3	14.1	21 bc	24 c	464.0 b
Alisha F_1	5.7 abcd	8.8 cdef	14.6 abcd	17.7 abcd	16.0 bcdefg	20.0	15.9	22 abc	28 ab	437.3 b
Apollo F_1	6.4 abcd	9.8 abcde	16.0 abc	19.9 abc	17.9 abcde	21.0	16.6	22 abc	28 abc	501.3 ab
Glamour F_1	6.6 abcd	9.2 abcdef	16.1 abc	22.0 ab	16.7 bcdefg	19.2	16.2	22 abc	27 abc	442.7 b
F-test	**	**	**	**	**	**	**	**	**	**
CV (%)	7.33	8.12	7.81	10.51	8.90	10.56	10.50	4.55	4.70	19.69

Note: SD: stem diameter, SL: stem (internode) length, LL: leaf blade length, LW: leaf blade width, PL: petiole length, PEL: male flower petal length, PEW: male flower petal width, MF: days to male flowering, HF: days to hermaphrodite flowering, STD: stomatal density. CV: coefficient of variation, *significant at α= 0.05, **significant at α= 0.01, nsnot significant. Means followed by the same letter in the same column are not significantly different at α = 0.05 based on the Tukey test

Table 3. Means of fruit quality traits of 23 melon genotypes

Genotype	FL	FD	FT[a]	FW	RT[a]	SSC[a]	FI[a]	TTA[a]	VITC[b]
G7	10.3 ab	11.1 abc	2.3 ab	678.0 ab	1.0	4.2	8.6 b	32.2	34.6
G240	10.4 ab	11.4 ab	2.8 ab	651.6 ab	1.5	6.9	8.6 b	34.0	26.2
Alisha S_1	11.9 ab	10.5 abc	2.7 ab	629.9 ab	1.2	9.1	11.8 ab	20.0	22.5
G1	13.3 ab	10.5 abc	2.5 ab	667.7 ab	1.0	8.0	18.3 a	27.3	33.0
G30	11.2 ab	13.0 a	2.9 ab	896.7 ab	1.0	9.1	11.0 ab	28.4	38.3
Glamour S_2	12.9 ab	12.8 a	3.7 a	1029.0 a	1.0	7.9	8.5 b	29.1	26.1
IPB HD 1-145	12.4 ab	11.0 abc	3.0 ab	742.6 ab	1.3	9.9	11.2 ab	28.4	29.8
IPB HD 1-34	10.2 ab	10.4 abc	2.7 ab	603.8 ab	1.3	8.3	10.5 ab	21.2	16.6
IPB HD 2-100	11.6 ab	10.4 abc	2.9 ab	630.8 ab	1.3	10.0	11.3 ab	22.1	25.5
IPB HD 2-111	10.5 ab	10.4 abc	2.7 ab	530.9 b	1.0	11.9	10.6 ab	29.6	19.7
IPB HD 4-165	10.8 ab	9.8 abc	2.9 ab	601.3 ab	1.0	10.7	-	33.7	25.9
IPB HD 5-131	9.4 b	8.9 abc	2.3 ab	385.9 b	1.3	12.3	10.7 ab	32.8	15.8
IPB HD 6-130	11.1 ab	10.2 abc	2.4 ab	566.7 ab	1.5	9.3	10.8 ab	21.5	21.4
IPB HD 6-168	13.4 ab	11.1 abc	2.5 ab	710.3 ab	2.0	9.1	9.3 ab	23.1	16.6
IPB HD 6-61	11.0 ab	10.1 abc	2.5 ab	516.4 b	1.8	9.1	11.4 ab	25.0	25.3
IPB HD 6-97	11.6 ab	9.8 abc	2.4 ab	549.4 ab	1.3	8.2	11.3 ab	18.7	14.6
M13	11.4 ab	9.7 abc	2.6 ab	521.4 b	1.0	10.2	13.3 ab	48.4	34.8
M21	12.0 ab	10.2 abc	2.4 ab	596.5 ab	1.0	12.8	13.7 ab	31.8	20.4
M23	10.6 ab	8.1 bc	2.3 ab	353.5 b	1.5	11.0	11.8 ab	33.4	30.1
IPB Meta 9	10.4 ab	7.8 c	1.3 b	300.9 b	1.0	6.7	16.8 ab	31.0	19.3
Alisha F_1	13.7 ab	11.1 abc	2.7 ab	786.2 ab	1.5	10.9	11.8 ab	29.7	27.6
Apollo F_1	13.8 a	11.1 ab	3.0 a	729.8 ab	1.3	10.7	13.6 ab	27.1	19.6
Glamour F_1	13.1 ab	12.4 a	3.3 a	1011.3 a	1.0	8.2	9.3 ab	24.2	32.5
F-test	*	**	*	**	ns	ns	*	ns	ns
CV (%)	9.83	8.37	5.21	20.77	12.90	8.64	8.54	16.59	11.22

Note: FL: fruit length, FD: fruit diameter, FT: flesh thickness, FW: fruit weight, RT: rind thickness, SSC: soluble solids content, FI: firmness, TTA: total titratable acidity, VITC: vitamin C. CV: coefficient of variation, [a]Data were transformed into $\sqrt{(x+0.5)}$ prior to ANOVA, [b]data were transformed into log(x+1) prior to ANOVA, *significant at α: 0.05, **significant at α= 0.01, nsnot significant. Means followed by the same letter in the same column are not significantly different at α = 0.05 based on the Tukey test

Correlation among fruit quality traits

The correlation analysis results revealed that there were both positive and negative correlations among the fruit quality traits in 23 genotypes of melon (Figure 2). The strength and significance of the correlation were displayed on the purple-green color scale (only significant correlations were colored). The darker the green color indicates a stronger positive correlation, and the darker the purple color indicates a stronger negative correlation. The fruit quality traits that had a positive and significant correlation among the traits were fruit weight correlation is stronger to fruit diameter (r= 0.95; p<0.01) than flesh thickness (r= 0.80; p<0.01) and fruit length (r= 0.65; p<0.01) fruit weight. The correlations of fruit diameter to flesh thickness (r= 0.77; p<0.01) and fruit length (r= 0.5; p<0.05) were highly significant. Meanwhile, the correlation of the flesh thickness was positively-significant to petal width (r= 0.43; p<0.05), petal length (r= 0.42; p<0.05), and fruit length (r= 0.45; p<0.05), even though their correlation were relatively weak. Whereas the traits that had a negative and significant correlation were leaf blade length to rind thickness (r= -0.54; p<0.01), and stomatal density (r= -0.56; p<0.05), petiole length to rind thickness (r= -0.60; p<0.01) and stomatal density (r= -0.61; p<0.01). If the correlation coefficient is in the range of -0.5 to 0.5, then the association is considered weak (Gogtay and Thatte 2017).

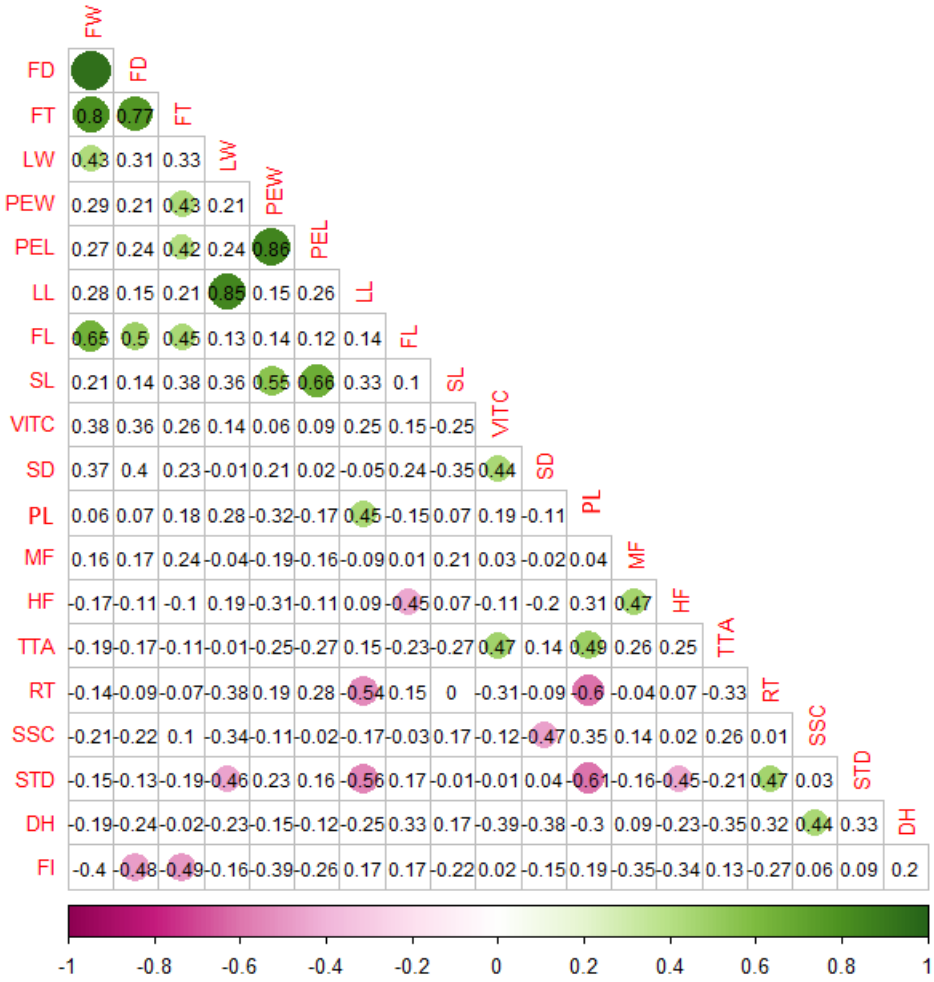

Figure 2. Correlation coefficients of twenty traits for melon fruit quality. FW: fruit weight, FD: fruit diameter, FT: flesh thickness, LW: leaf blade width, PEW: male flower petal width, PEL: male flower petal length, LL: leaf blade length, FL: fruit length, SL: stem (internode) length, VITC: vitamin C, SD: stem diameter, PL: petiole length, MF: days to male flowering, HF: days to hermaphrodite flowering, TTA: total titratable acidity, RT: rind thickness, SSC: soluble solids content, STD: stomatal density, DH: days to harvest, FI: firmness, CA: fruit cavity width, CDR: the proportion of fruit cavity width to the fruit diameter. Colored rounds indicate a significant correlation ($p \leq 0.05$)

Regression and path analysis for the proportion of fruit cavity width to the fruit diameter

The principle of path analysis is to partition the correlation coefficient into direct and indirect effects (Singh and Chaudhary 1985). The correlation coefficient above will be the total effects on the path analysis. A melon breeding program could be directed toward developing varieties with good quality fruit with flesh thickness as one of the selection traits. In this study, the related trait to the flesh thickness was explained by the proportion of the fruit cavity width to the fruit diameter. Based on the multiple linear regression analysis, it is known that petal width, petiole length, and days to harvest are significant (Table 4). The adjusted coefficient of determination (R^2) in this multiple linear regression analysis is 0.569. The coefficient of determination shows the proportion of the total variation of the dependent variable that can be explained by the model (Walpole 1982), and therefore in this case, 56.9% of the variation of the proportion of fruit cavity width to fruit diameter can be explained by the model (Singh and Chaudhary 1985). Furthermore, this partial regression coefficient is used in the formulation of the model, which is as follows:

CDR = 2.534 - 0.039 PEW - 0.016 PL - 0.011 DH - 0.014 MF + 0.022 SL - 0.011 HF

Path analysis elucidates the effect of independent variables individually and in combination with other characters on the dependent variable (Gupta et al. 2015). Path analysis separated the direct and indirect effects by partitioning the correlation coefficients of the contributing traits (Sarkar et al. 2021). The path analysis has been considerably used in the field trial of plants, animals, and fisheries breeding (Khalil et al. 2016; Chen et al. 2021; Chang et al. 2022; Lal et al. 2023). For melon breeding itself, correlation and path analysis have been widely used to understand the effects of various traits on yield, fruit yield per plant, and fruit weight (Pasha et al. 2019; Priyanka et al. 2020; Nanthakumar et al. 2021; Hiremata et al. 2022; Khomphet et al. 2022; Soltani et al. 2022).

The proportion of the direct effects to the correlation coefficient (*r*) is shown by the barplot in Figure 3. The correlation between days to male flowering and the proportion of fruit cavity width to the fruit diameter is -0.17, very similar to the direct effect (-0.27). The value of the direct effect which has a value close to the correlation coefficient indicates that the direct effect of days to male flowering has a large meaning on the proportion of fruit cavity width to the fruit diameter (Singh and Chaudhary 1985), so selecting genotypes of melon with small the fruit cavity width to fruit diameter ratio through the days to male flowering may be effective. On the other hand, the correlation coefficient of internode length is negative (-0.44), but the direct effect of internode length is positive (0.46), so it seems that the indirect effects were the cause of the correlation. Under these circumstances, the indirect factors must be considered simultaneously (Singh and Chaudhary 1985).

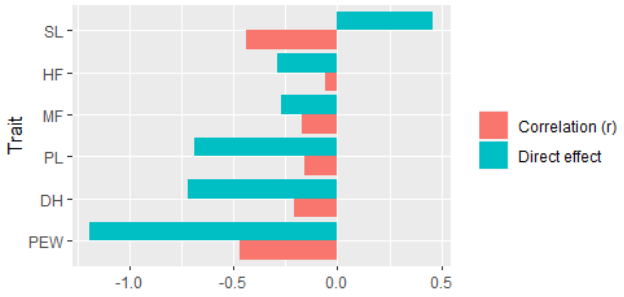

Figure 3. Proportion of the direct effect to the coefficient of correlation of each trait, in path analysis of the proportion of fruit cavity width to the fruit diameter. PEW= male flower petal width, PL= petiole length, DH= days to harvest, MF= days to male flowering, SL= stem (internode) length, HF= days to hermaphrodite flowering.

Table 4. Path analysis of six traits (independent variables) on the proportion of fruit cavity width to the fruit diameter (dependent variable) of 23 genotypes of melon

Trait	MS multiple regression	Direct effect	Indirect effect						Total effect (r)
			PEW	PL	DH	MF	SL	HF	
PEW	0.025**	-1.19		0.22	0.11	0.05	0.25	0.09	-0.47
PL	0.017**	-0.69	0.38		0.21	-0.01	0.03	-0.09	-0.16
DH	0.016**	-0.72	0.18	0.21		-0.02	0.08	0.07	-0.21
MF	0.003ns	-0.27	0.23	-0.03	-0.06		0.10	-0.14	-0.17
SL	0.004ns	0.46	-0.65	-0.05	-0.12	-0.06		-0.02	-0.44
HF	0.003ns	-0.29	0.37	-0.21	0.16	-0.12	0.03		-0.06
Adjusted R^2	**56.91%**								

Note: PEW: male flower petal width, PL: petiole length, DH: days to harvest, MF: days to male flowering, SL: stem (internode) length, HF: days to hermaphrodite flowering. MS: mean square, R^2: coefficient of determination.

The effect of days to male flowering on the proportion of fruit cavity width to the fruit diameter was negative, which means that we need to choose the longer or later days to male flowering, likewise on the petal width, petiole length, days to harvest, and days to hermaphrodite flowering. In producing melons, we need to harvest the fruit until the optimum harvest age. Surprisingly, the three variables related to the ages or stages (days to male flowering, days to hermaphrodite flowering, and days to harvest) were included in the model that was chosen by the stepwise selection. If we harvest the fruit before the proper harvest time, the enlargement of the fruit (the thickness of the fruit flesh, the diameter of the fruit, and the length of the fruit) is not optimal, so the cavity in the fruit will be bigger than if it is maintained until the optimum harvest age. The results of previous studies showed that the size of the fruit cavity in various varieties did not develop significantly from the beginning of growth to the fruit maturity phase, with the development of fruit weight, length, and diameter showing an upward trend until the maturity stage. This means that the time of fruit development greatly influences the proportion of cavities to fruit diameter with a negative association (Miccolis and Jr Saltveit 1991). The fruit quality including size, flavor, color, and texture of the fruit was affected by the maturity at harvest, and the maturity at harvest itself was affected by the harvest time (Ramjan and Ansari 2018).

The data on path coefficient analysis at the phenotypic level showing direct and indirect effects of significant characters over the proportion of fruit cavity width to the fruit diameter is tabulated in Table 4. The path analysis revealed that all traits included in the model were negatively correlated to the proportion of fruit cavity width to the fruit diameter, with negative direct effects except on the internode length. Petal width (-1.19) had a maximum negative direct effect on the proportion of fruit cavity width to the fruit diameter followed by days to harvest (-0.72), petiole length (-0.69), days to hermaphrodite flowering (-0.29), and days to male flowering. Meanwhile, the positive direct effect on the proportion of fruit cavity width to the fruit diameter was only internode length (0.46).

Maximum positive indirect effects on the proportion of fruit cavity width to the fruit diameter were exhibited by petiole length via petal width (0.38), followed by days to hermaphrodite flowering via petal width (0.37), petal width via internode length (0.25), days to male flowering through petal width (0.23), and days to harvest through petiole length (0.21). Meanwhile, the maximum negative indirect effects on the proportion of fruit cavity width to the fruit diameter were shown by internode length via petal width (0.65), days to male flowering via days to hermaphrodite flowering (-0.14), petiole length through days to hermaphrodite flowering (0.09), and days to harvest through days to male flowering (-0.02).

Cluster analysis of melon genotypes

Cluster analysis employing phenotypic data resulted in a dendrogram with three main branches (Figure 4) following the neighbor-joining method. Genotypes belonging to inodorus, reticulatus, and makuwa groups were partitioned into different nodes after cluster analysis. The first cluster was divided into two subclusters with the first subcluster containing IPB HD 1-145, Apollo F_1, M13, and IPB HD 4-165, while the second subcluster containing IPB HD 1-34, G30, IPB HD 2-100, IPB HD 2-111, P34, and IPB HD 5-131. The second cluster is divided into two subclusters too where the first subcluster includes four genotypes: IPB HD 6-97, IPB HD 6-61, IPB HD 6-168, and IPB HD 6-130, while the second subcluster includes two genotypes: Alisha S_1 and Alisha F_1. Then the third cluster is divided into two subclusters, where the first subcluster contains only two genotypes: M21 and G1, and the second subcluster contains four genotypes: G7, G240, Glamour S_2, and Glamour F_1. Neighbor-joining is one of the clustering methods for phylogenetic tree reconstruction which shows an unrooted tree with branched lengths as the output. This clustering method is useful because it is statistically consistent and can provide a sub-optimal solution that is close to the optimal tree.

Also, in this clustering analysis, the neighbor-joining method had the highest coefficient of correlation with the dissimilarity. The application of the NJ method is advantageous because it is quite efficient in determining the most appropriate tree topology, as well as displaying different branch lengths between individuals based on their close dissimilarity to one another. Indeed, NJ clustering is able to show the similarity genotypes with the same origins but different generations, for example, Glamour F_1 & Glamour S_2, and Alisha F_1 & Alisha S_1 (Figure 4). Nevertheless, the clustering of the twenty-three genotypes still shows a close relationship which can be seen from the short branches at the base of the phylogenetic tree that indicated all genotypes are closely related to each other (Saitou and Nei 1987).

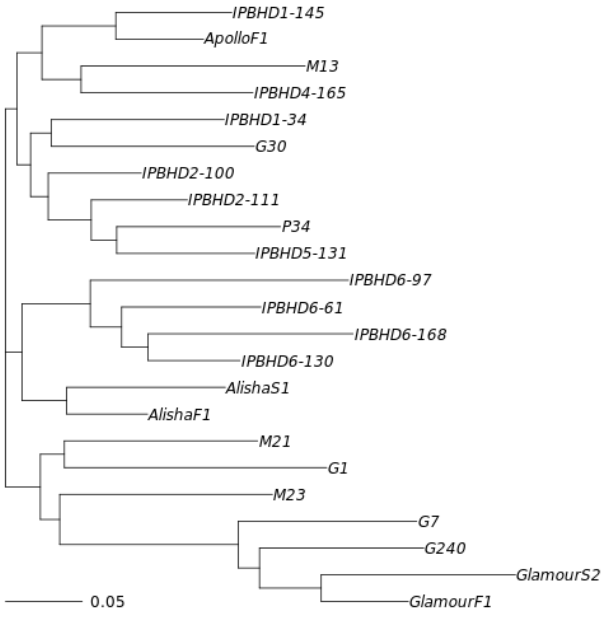

Figure 4. Neighbor-joining tree of 23 melon genotypes, generated from 20 quantitative traits and 15 qualitative traits

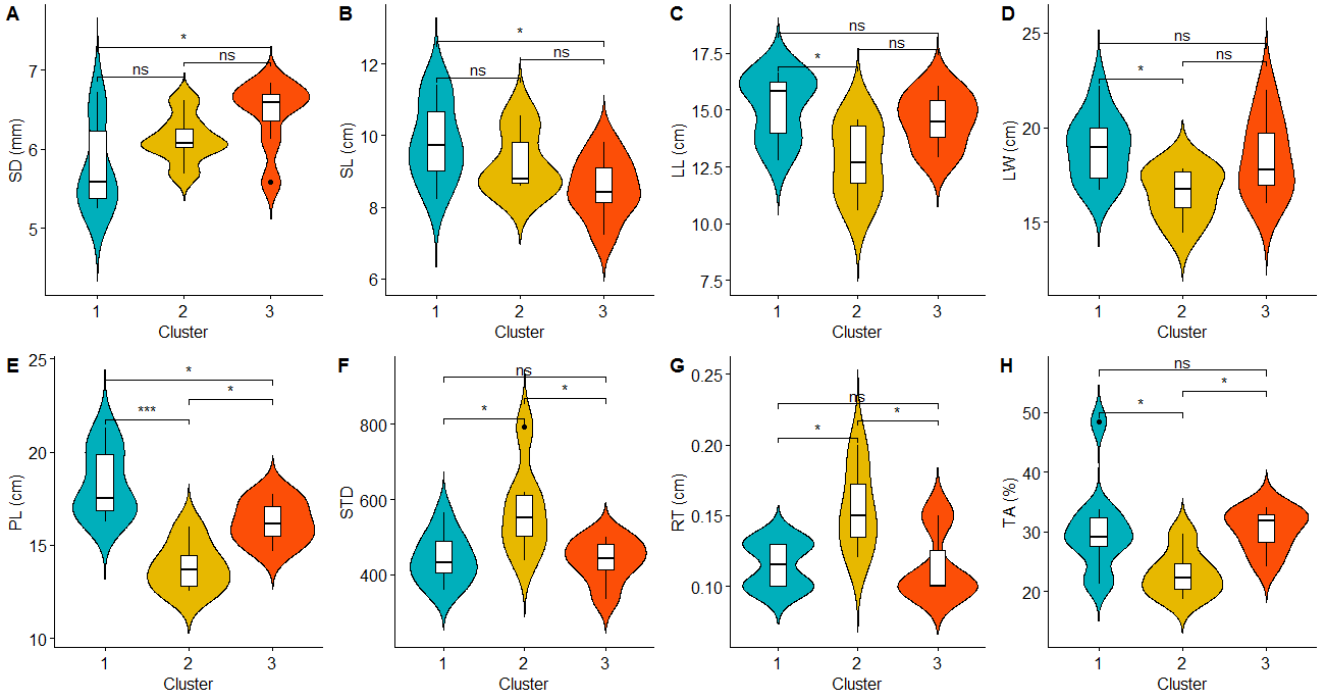

Figure 5. Distribution of genotype means in each cluster. A. SD: stem diameter, B. SL: stem (internode) length, C. LL: leaf blade length, D. LW: lead blade width, E. PL: petiole length, F. STD: stomatal density, G. RT: rind thickness, H. TA: titratable acidity. *significant at α=0.05, **significant at α= 0.01, nsnot significant based on Kruskal-Wallis test

The NJ method determines the neighbor pairs of each member (operational taxonomic units/OTU, or genotype) based on the distance matrix and pairs them gradually starting from the sum of the smallest S_{ij} branch lengths to the last neighbor pair, following the algorithm from Saitou and Nei (1987). At least there are six tree-making methods: unweighted pair-group mean average method (UPGMA), distance Wagner (DW) method, modified Farris (MF), Sattath and Tversky (ST method), Fitch-Margoliash, and neighbor-joining method (NJ) (Saitou and Nei 1987). Compared to the other five methods, NJ showed better performance. In general, we don't know which OTU pairs are actually neighbors. Therefore, the sum of the branch lengths is calculated for all OTU pairs, and the pair showing the smallest distance value is selected (concluded) as the neighbor pair. In practice, even these couples may not be real neighbors, however, for pure summation trees without backward and parallel substitution, this method may be able to show pairs of true neighbors (Saitou and Nei 1987).

The classification of genotypes into clusters is interesting because readers and researchers can retrieve information related to specific characteristics of a cluster. For distinguishing characters, the variability of genotypes within a cluster should be lower than the variability of genotypes between clusters. Based on the cluster validation presented in Figure 5, stem diameter (p= 0.035), petiole length (p= 0.001), stomatal density (p= 0.024), rind thickness (p= 0.021), and total titratable acidity (p= 0.035) were distinguishing traits between clusters, with the distribution of means depicted through boxplots. Among the three clusters, cluster 2 exhibited relatively lower leaf blade length, leaf blade width, petiole length, and total titratable acidity compared to the other two clusters, while having relatively denser stomata and thicker rind compared to cluster 1 and cluster 3 (Figure 5).

In conclusion, IPB HD 2-111, IPB HD 2-100, and G30 were non-netted melons which relatively superior in their taste and appearance, and Glamour S_2 was good quality netted melon. The proportion of fruit cavity width to the fruit diameter on melon was dependent on various traits that were mutually related. Internode length showed a significantly-negative correlation with the proportion of fruit cavity width to the fruit diameter, and hence the indirect factors must be considered simultaneously. The regression model from stepwise selection resulted from the final model for the proportion of fruit cavity width to the fruit diameter which included six traits with adjusted R^2= 0.569. Cluster analysis employing 23 evaluated genotypes into three main clusters based on neighbor-joining clustering.

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