

PHENOTYPIC VARIATION AND CORRELATION OF FRUIT TRAITS IN DIVERSE MUSKMELON MATERIALS

Nguyen Trung Duc^{1,*}, Pham Quang Tuan¹, Nguyen Thi Nguyet Anh¹, Vu Van Liet¹

ABSTRACT

Understanding the phenotypic variation, correlation, and identification of important agronomic traits will speed up the selection efficiency of elite melon materials for nutritious hybrid melon breeding in Vietnam. This study utilized three approaches *viz.*, correlation analysis, principal component analysis, and cluster analysis methods to investigate the primary and secondary relationships of the main agronomic traits affecting the yield and quality of 20 muskmelon materials originated from Taiwan-China, India, Thailand, and Japan in the Spring and Autumn season 2021 in Gia Lam, Ha Noi. The results showed that the highest significant positive correlation at $p < 0.001$ was observed between fruit weight (FW) and fruit length (FL) (0.744***), fruit diameter (FD) (0.716***), and fruit thickness (FT) (0.527***). A total of ten quantitative traits could be reduced to just four components, which could explain more than 90% of the total variation. The first four traits *viz.*, FW, FL, FD, and FT contributed more than 90% of their cumulative contribution, these principal component values representing genetic characteristics of eight agronomic traits. Hierarchical clustering based on principal component analysis showed that 20 muskmelon materials were divided into three distinct groups in the Spring season and four distinct groups in the Autumn season. To the best of our knowledge, this is the first report on applying dimension reduction methods to investigate phenotypic variability in muskmelon materials in Vietnam.

Keywords: *Muskmelon, variability, correlation, principal component analysis, cluster analysis.*

Received: 18 August 2022; revised: 12 September 2022; accepted: 21 November 2022.

1. INTRODUCTION

With sweetness, delicious, and nutritious, muskmelon (*Cucumis melo* L.) is widely grown around the world [1]. In Vietnam, with great economic potential, muskmelon has been cultivated in high-tech greenhouses and polyhouses with an area constantly increasing throughout the country. However, apart from some muskmelon varieties such as TL3, King KN, Queen KN, most of the high-quality cantaloupe varieties are imported from Thailand, Taiwan, and Japan. This poses a

challenge to the selection and breeding of domestic melon varieties, it is necessary to develop domestic melon varieties soon to help agricultural production have more choices and actively produce seeds. As a result of high growth vigor, uniform fruit size and shape, and high yield with suitable quality, all muskmelon growth in Vietnam were hybrid varieties. The fundamentals in hybrid muskmelon breeding were the collection, screening, classification, development, and selection of muskmelon inbred lines. Melons have been bred and grown in nearly all of the warmer regions of the world, leading to the wide diversity of phenotypic traits, especially

¹ Vietnam National University of Agriculture, Ha Noi, Vietnam

* Corresponding author: ntduc@vnua.edu.vn

in terms of fruit traits such as size, shape, rind thickness, flesh thickness, sugar content, acidity, texture and aroma [2, 3, 4]. This wide variety is a source for ongoing breeding and genetic research aimed at mapping QTLs and identifying genes that influence key horticultural traits and consumer needs [5, 6, 7]. Many genetic studies using diverse collections or isolated populations have focused on fruit quality traits in melons, including fruit size and shape [8].

Phenotypic evaluation is an important method for selecting pure melon lines. Keeping observed of all phenotypic traits across a large breeding population is a challenge for breeders. The principal component analysis is an effective analytical method to extract important information from highly correlated phenotypic complex traits while retaining the original information [9, 10]. Principal component analysis and cluster analysis are believed to be effective in multidimensional data processing, helping to get the best approximation of the dataset from which to draw important results in agricultural research [10]. Understanding the correlation

and identification of important agronomic traits improves selection efficiency and more accurately identifies promising melon lines for target breeding programs.

This study applied principal component analysis and cluster analysis to investigate the primary and secondary relationships of the main agronomic traits affecting yield and quality and phenotypic diversity of 20 muskmelon materials in the Spring and Autumn season 2021 in Gia Lam, Ha Noi. The result of this study provided valuable information for muskmelon pre-breeding in Vietnam.

2. MATERIALS AND METHODS

2.1. Plant materials

Plant materials included 19 muskmelon materials viz., ML01, ML02, ML03, ML04, ML05, ML06 originated from Taiwan, China; ML07, ML08, ML09, ML10, ML11 originated from India; ML12, ML13, ML14, ML15 originated from Thailand; and ML16, ML17, ML18, ML19 originated from Japan, with check variety TL3 (Chanh Phong Agriculture Co., Ltd distributed).

2.2. Experimental design

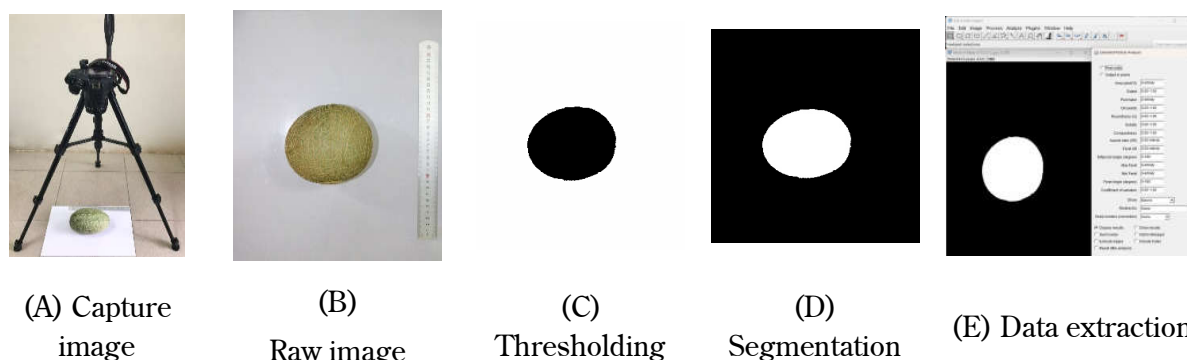


Figure 1. Image-based phenotyping on muskmelon fruits

The experiment was arranged in a randomized complete block design (RCBD), with 3 replications in a polyhouse at Vietnam National University of Agriculture, Ha Noi, Vietnam in Spring and Autumn seasons of

2021. Experimental plot area was 12 m² (1.2 m x 10 m), each seed sample was planted in 2 rows of 10 m long. Planting spacing was 60 cm between rows, 55 cm between plants (24,000 plants/ha). Fertilizer application per ha viz,

1,400 kg of manure + 600-800 kg of superphosphate + 1,000 kg of NPK (15-5-20) + 50 kg of urea + 50 kg of potassium chloride. Each plant kept only one fruit for about 7-10 days after hand pollination. Ten important agronomic traits were monitored namely: harvest time (HT, day), fruit weight (FW, kg), fruit length (FL, cm), fruit diameter (FD, cm), fruit flesh thickness (FT, cm), rind thickness (RT, cm), total soluble solids (TSS, °Brix), seed number (SN, number of seeds/fruit), seeds weight/fruit (SW, g), and thousand seed weight (TSW, g).

Fruit length and fruit diameter were extracted from the image taken by Canon EOS 1500D, by BioVoxel Toolbox on ImageJ software. The main steps were described in Figure 1.

2.3. Data analysis

Data were handled by Microsoft Excel, and analyzed by the one-way ANOVA method in Statistix 10. Descriptive statistics using “*rstatix*” package, correlation analyzed by “*GGally*” package in R 4.1.1. Principal component analysis and hierarchical clustering based on principal component analysis were performed by “*factoextra*” and “*FactoMineR*” packages in the R 4.1.1 [11].

3. RESULTS AND DISCUSSION

3.1. Descriptive statistics and correlation analysis between investigated traits

Descriptive parameter results included mean, minimum, maximum, standard deviation, and coefficient of variation, and statistically significant of 10 traits over two seasons (Table 1). ANOVA showed that there were statistical significant at $p \leq 0.01$ for all investigated traits. Overall, these yield traits showed high variability, and muskmelon materials have a longer duration in Autumn season compared with Spring season. HT of

muskmelon materials ranged from 73.0 days to 79.0 days in the Spring season and ranged from 78.0 days to 87.0 days in the Autumn season. Fruit-related traits namely FW, FL, FD, FT are important economical parameters in melon breeding program, affected both demand and supply [12, 7]. FW of muskmelon materials ranged from 1.4 kg to 2.9 kg in Spring season and ranged 1.3 kg to 3.2 kg in the Autumn season. FL fluctuated from 13.5 cm to 19.0 cm in Spring season and ranged between 12.6 cm and 20.4 cm in the Autumn season. FD of muskmelon materials ranged from 9.2 cm to 14.5 cm in the Spring season and ranged from 8.0 cm to 16.7 cm in the Autumn season. FT ranged between 2.7 cm and 4.2 cm in Spring season and ranged between 2.5 cm and 3.9 cm in the Autumn season. RT is affected by both genetic [13] and cultivation methods [14]. RT ranged from 0.4 to 0.7 cm in Spring season and ranged 0.4 to 0.8 cm in the Autumn season. TSS is a vital quality trait, governed by additive effects, can be improvement through recurrent selection [15, 4]. TSS ranged between 11.1 and 15.0 cm in Spring season and ranged 12.1 to 16.0 in Autumn season. SN, SW and TSW are valuable traits of the fruit in terms of taxonomy and evolution, also had a significant effect the commercial product for melon seed companies [16]. SN trait of muskmelon materials ranged between 158.0 and 258.0 seeds/fruit in Spring season and ranged from 130.0 to 270.0 seeds/fruit in the Autumn season. SW varied from 5.0 to 7.7 g in Spring season and 5.8 to 8.3 g in the Autumn season. TSW ranged from 20.9 to 45.9 g in Spring season and ranged from 22.1 to 46.9 g in Autumn season. Three traits *viz*, FW, SN, and TSW showed highest variability with coefficient of variation of FW reached 20.2 in Spring season and 27.3 in the Autumn season, SN had a coefficient of variation reached 22.0

in Spring season and 19.5 in the Autumn season. Thus, selection on those traits can be applied to select more suitable genotypes have more phenotypic variation.

Table 1. Descriptive statistics of eight agronomic traits over two seasons

Trait	Season	Min	Max	Mean	Range	Standard deviation	Coefficient of variation	Statistical significance
HT	Spring 21	73.0	79.0	75.5	6.0	1.8	2.3	**
	Autumn 21	78.0	87.0	82.4	9.0	2.7	3.3	**
FW	Spring 21	1.4	2.9	1.8	1.5	0.4	20.2	**
	Autumn 21	1.3	3.2	1.6	1.9	0.4	27.3	**
FL	Spring 21	13.5	19.0	16.2	5.5	1.6	9.8	**
	Autumn 21	12.6	20.4	15.4	7.8	2.2	14.5	**
FD	Spring 21	9.2	14.5	11.1	5.3	1.7	15.2	**
	Autumn 21	8.0	16.7	10.4	8.7	1.9	18.4	**
FT	Spring 21	2.7	4.2	3.4	1.5	0.4	12.9	**
	Autumn 21	2.5	3.9	3.2	1.4	0.4	13.3	**
RT	Spring 21	0.4	0.7	0.5	0.3	0.1	11.2	**
	Autumn 21	0.4	0.8	0.5	0.4	0.1	17.4	**
TSS	Spring 21	11.1	15.0	13.1	3.9	1.2	9.2	**
	Autumn 21	12.1	16.0	13.7	3.9	1.3	9.7	**
SN	Spring 21	108.0	258.0	216.4	150.0	47.5	22.0	**
	Autumn 21	130.0	270.0	226.2	140.0	44.2	19.5	**
SW	Spring 21	5.0	7.7	6.4	2.7	0.9	13.8	**
	Autumn 21	5.8	8.3	6.9	2.6	0.8	11.8	**
TSW	Spring 21	20.9	45.9	30.6	25.0	6.7	21.7	**
	Autumn 21	22.1	46.9	31.6	24.8	6.7	21.1	**

Note: ** mean statistically significant at $p \leq 0.01$.

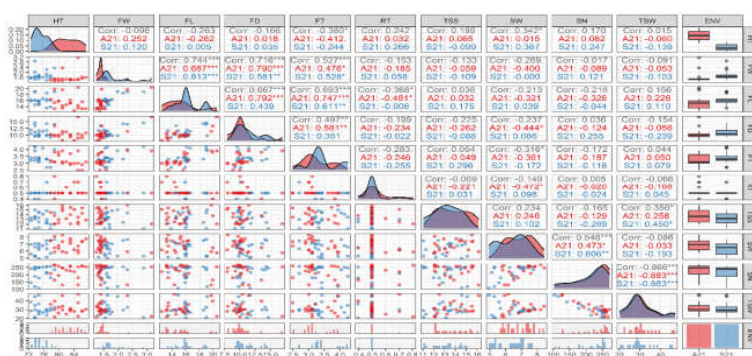


Figure 2. Correlogram chart for ten agronomic traits over two seasons

Note: ** mean statistically significant at $p \leq 0.01$; A21: Autumn season 2021; S21: Spring season 2021.

Correlogram chart for ten agronomic traits over two seasons was presented in Figure 2. The density distribution of each trait is shown diagonally with blue color indicating

Spring season 2021 and pink color indicating Autumn season 2021. At the top-right of Figure 2, a two-variable scatter plot is shown while at the top, with the symbols *, **, *** indicating statistical significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively. The boxplot on the right illustrates the difference between two seasons. Knowledge of the correlation between yield and its components can help breeders to decide the selection pressures on the traits involved in order to simultaneously improve the yield traits [17]. FW is a polygenic inherited trait and governed by many genes, located on different chromosomes [4]. Overall, in this study, the highest significant positive correlation at $p < 0.001$ was observed between FW and FL (0.744***), FD (0.716***), and FT (0.527***), suggesting that simultaneous selection of these characters would lead to yield improvement of muskmelon (Figure 2).

Significant positive correlations at $p < 0.001$ were also found between FL and FD (0.667***), FT (0.693***). This information can be useful for future melon breeding programs to obtain high yield muskmelon varieties. For example, crosses can be made between cultivars with large fruit. The above results have served as a basis for the selection of elite muskmelon materials by multivariate method. Despite of HT of those muskmelon materials in Autumn season were longer than Spring season, the variation and FW and it related traits were higher than Spring season. The quality traits *viz.*, TSS were also higher in Autumn season compared with Spring season. These results might due to the difference in temperature between day time and night time in the Autumn season in the North of Vietnam.

3.2. Principal component analysis

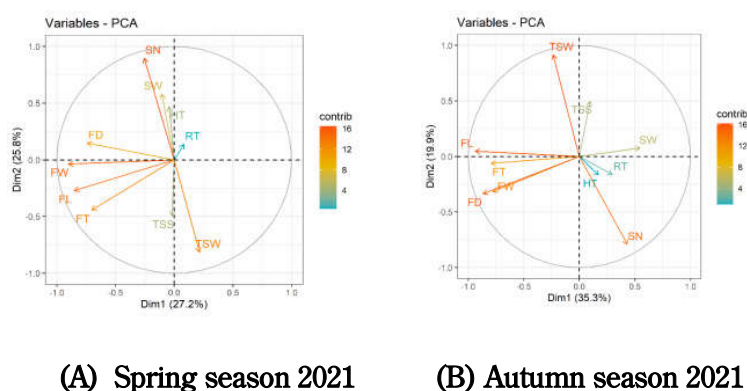


Figure 3. Contribution of traits on the first two major components

By applying principal component analysis (PCA), the most important characteristics in the data set were revealed. PCA is a technique that falls within the data framework of multivariate statistical analysis. To determine genetic variation and relationships between muskmelon materials, PCA was used. Total of 8 quantitative traits could be reduced to just four components, which could explain more

than 90% of the total variation. A PCA diagram is presented based on the first two components (Figure 3). The arrangement of traits on PC1 and PC2 demonstrates that some traits are highly correlated with each other as seen in the overlap pattern of traits. Variables on the same PC and next to each other indicate a positive correlation between them, and an increase in one will lead to an increase

in the other. However, the traits at the opposing ends are not correlated and an increase in one point will decrease the other traits and vice versa. In both seasons, based on the first component, accessions exhibit high positive component values representing relatively high fruit weight (FW), fruit length (FL), fruit diameter (FD), and fruit thickness (FT).

The market value of melons is influenced by fruit quality in terms of fruit size, and fruit shape [18, 1]. In both seasons, FW, FL, FD, and FT which were fruit-related traits strongly contributed to the total variation of 20 muskmelon materials. However, in the Autumn season, the total of the two first components achieved 55.2%, which was slightly higher than the Spring season (54.0%) (Figure 3). The first component, accounting

for 27.2% of the total variation in the Spring season and 35.3% in the Autumn season, was closely related to fruit weight and fruit shape, including fruit length, fruit diameter, and fruit thickness (Figure 4). The first two components accounted for 54% of the morphological variation, and fruit length, being the most important variable constituting PC1. Kaur *et al.* (2022) [12] highlighted the importance of fruit traits in muskmelon breeding for yield improvement. The second component, which accounted for 25.8% of the total variation in the Spring season and 19.9% in the Autumn season, was mainly correlated with the characteristics of seed number (SN), one-thousand seed weight (TSW), and seed weight/fruit (SW) (Figure 5).

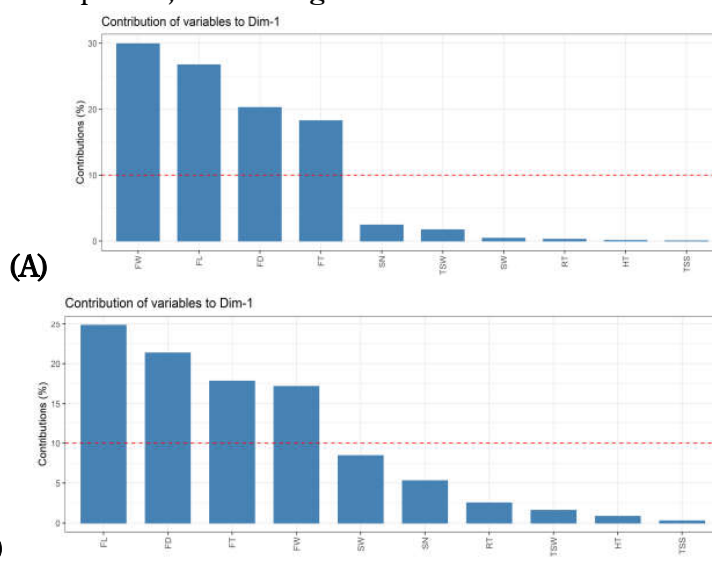
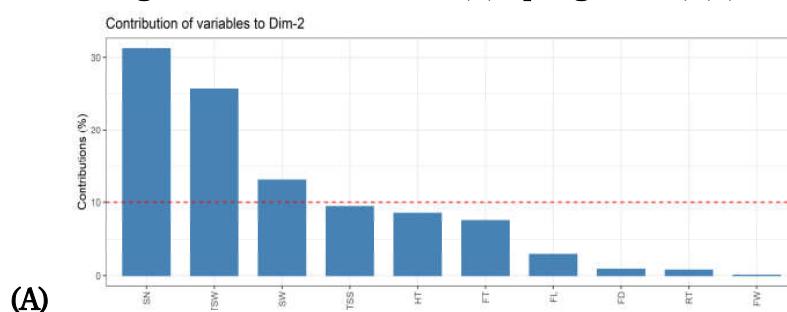


Figure 4. Contribution of agronomic traits to PC1 in (A) Spring season, (B) Autumn season



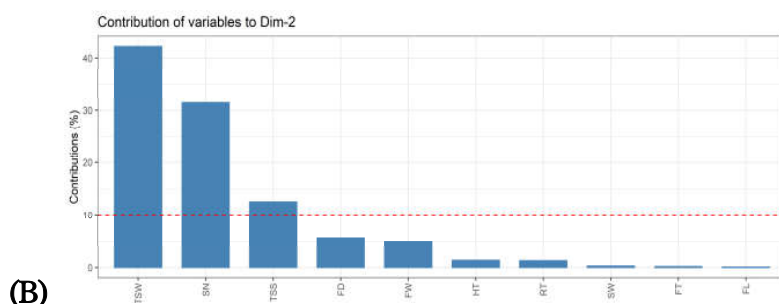


Figure 5. Contribution of agronomic traits to PC2 in (A) Spring season, (B) Autumn season

3.3. Cluster analysis

Diversity and the relationship between traits are essential information in crop improvement programs, and the success of plant breeding programs relies heavily on the existence of phenotypic variation and inheritance for specific traits. In this study, hierarchical clustering based on principal component analysis was used to investigate

genetic similarity. The euclidean distance was used as a measure to measure this phenotypic similarity among 20 muskmelon materials based on 8 investigated traits. The dendrogram showed 20 muskmelon materials were divided into three distinct groups in the Spring season and four distinct groups in the Autumn season (Figure 6).

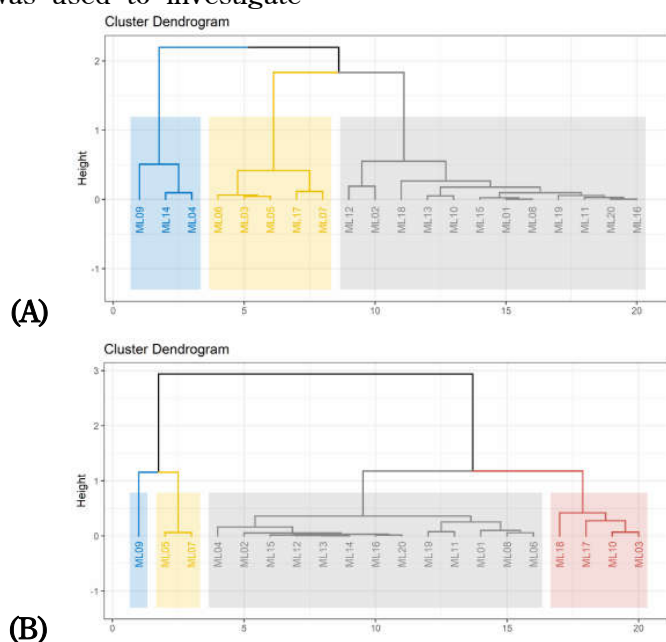


Figure 6. Cluster dendrogram of 20 muskmelon materials in (A) Spring season, and (B) Autumn season

Based on the phenotypic traits in the Spring season, hierarchical clustering based on principal component analysis showed three major groups *viz.*, the first group included 3 muskmelon materials (ML09, ML14, and ML04), the second group included 5

muskmelon materials (ML06, ML03, ML05, ML07, and ML17) and the largest third group included 12 muskmelon materials along with the check variety TL3. On the other hand, based on phenotypic traits in the Autumn season, hierarchical clustering based on

principal component analysis showed four major groups *viz.*, the first group included only one material ML09 which showed the highest fruits weight, the second group included 2 muskmelon materials (ML05, and ML07), the third group included 4 muskmelon materials (ML18, ML17, ML10, and ML03) and the largest fourth group included 13 muskmelon materials along with check variety TL3 (Figure 5). Those muskmelon lines responded dynamically under two seasons, responsiveness to the weather conditions, and presented useful information for breeding high adaptation muskmelon varieties in Vietnam. Recently, hierarchical clustering based on principal component analysis has been used by Gong *et al.* (2014) [19] to study diversity in apples. Iqbal *et al.* (2014) [20] studied genetic diversity in tomato. To the best of our knowledge, this is the first study applied hierarchical clustering based on principal component analysis to investigate phenotypic diversity on the agronomic traits in muskmelon in Vietnam.

4. CONCLUSION

This study investigated the phenotypic diversity of 20 muskmelon materials in the Spring and Autumn seasons based on ten agronomical traits in Hanoi, Vietnam. Three quantitative traits *viz.*, fruit weight, seed number, and one-thousand seed weight showed the highest variability. Fruit weight, fruit length, fruit diameter, and fruit thickness contributed more than 90% of their cumulative contribution suggesting that those traits can be used to classify muskmelon materials. The result of this study is highly useful for effective utilization of muskmelon germplasm, provided a foundation breeding population for the hybrid muskmelon breeding program in Vietnam.

ACKNOWLEDGEMENTS

This study is part of a Key Science and Technology project at the Vietnam National University of Agriculture: "A pilot study on image-based phenotyping, building big data and application in plant breeding" - code "T2021-41-15TD". The authors acknowledge financial support from Vietnam National University of Agriculture.

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