

USE OF CANONICAL CORRELATION ANALYSIS FOR DETERMINATION OF RELATIONSHIPS BETWEEN PLANT CHARACTERS AND YIELD COMPONENTS IN WINTER SQUASH (*Cucurbita maxima* Duch.) POPULATIONS

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Abstract

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Canonical correlation analysis is one of the most popular multivariate analysis techniques. In this study, a canonical correlation analysis (CCA) was used to estimate relationships between plant characters [*X set* - fruit length (FL), fruit diameter (FD), flesh thickness (FT), fiber weight per fruit (FW), length of seed cavity (LSC), skin thickness (ST), vine length (VL), brunch number per plant (BN), leaf length (LL), leaf width (LW), female flowering time (50%) (FFT), and time to maturity (TM)], and yield components [*Y set*- total fruit weight per plant (FW/P), average fruit weight (AFW) and number of fruits per plant (FN/P)] of 117 winter squash (*Cucurbita maxima* Duch.) in populations collected from the Black Sea Region of Turkey. In this study, three canonical correlation coefficients (CCCs) were estimated, and the first two of them were significant (0.903 and 0.571, $p < 0.001$) with respect to the likelihood ratio test while third CCC was no significant (0.340, $p > 0.218$). The findings obtained from the CCA indicate that FW/P had the largest contribution for the explanatory capacity of canonical variables estimated from yield components of 117 Turkish winter squash (*Cucurbita maxima* Duch.) populations when compared with other yield components. FD and FL had largest contribution for the explanatory capacity of canonical variables estimated from plant characters when compared with the other characters. The obtained results show that FD and FL should be used with the aim of increasing yield per plant in winter squash (*Cucurbita maxima* Duch.) populations in this study.

Key words: winter squash, population, canonical correlation coefficient, canonical variable, variation
Abbreviations: AFW (average fruit weight), BN (brunch number per plant), CCA (canonical correlation analysis), CCCs (canonical correlation coefficients), FD (fruit diameter), FL (fruit length), FT (flesh thickness), FFT [female flowering time (50 %)], FN/P (number of fruits per plant), FW (fiber weight per fruit), FW/P (total fruit weight per plant), LL (leaf length), LSC (length of seed cavity), LW (leaf width), VL (vine length), ST (skin thickness), TM (time to maturity)

Introduction

Canonical correlation analysis can be used in wide field of study (plant science, biology, chemistry, meteorology, demography, artificial intelligence, cognitive science, political science, sociology, psychometrics, educational research, economics and management science) to analyze multidimensional relationships between multiple independent and dependent variable sets (Akbas and Takma, 2005; Martin et al., 2005). Canonical correlation analysis is a procedure to determine the strength of relationships among physiological, morphological or chemical traits of plants or animals (Keskin and Yasar, 2007). The goal of canonical correlation is to determine simultaneous relationships between two sets of variable such as X and Y (Glahn, 1968; Haroon et al., 2003).

Correlation coefficients generally show relationships among independent characters and the degree of linear relation between these characteristics (Guler et al., 2001). In plant breeding research, usually more than one character or variable is taken for measurement from the same plant. To determine the degree and direction of linear relationships belonging to these obtained measurements, simple correlation analysis is usually preferred by researchers (Cankaya and Kayaalp, 2007). It is important to determine the relationship between two or more characters measured at an early time and a later time (Akbas and Takma, 2005; Cankaya et al., 2010) since early selection is one of the vegetable breeding methods applied for a higher yield the plants. If there is a relationship between plant characters and yield components, multivariate analyses such as canonical correlation analysis (CCA) - a technique for describing the relationship between two variable sets simultaneously and for producing both structural and spatial meanings (Thompson, 1984) - can provide pumpkin and squash breeders information based on indirect selection.

The Cucurbita species, pumpkin and winter squash are grown all over the world. *Cucurbita* spp. collectively ranks among the 10 leading

vegetable crops worldwide. China and India lead world production. Other major producers are the USA, Egypt, Mexico, Ukraine, Cuba, Italy, Iran and Turkey (FAOSTAT, 2008). Winter squash and pumpkins are two of the most important Cucurbit vegetable crops in Turkey. Turkey is one of the important diversity centers for cultivated cucurbits because of their adaptation to diverse ecological conditions as a result of natural selection and also farmers' selections (Sari et al., 2008). Only one improved cultivar of winter squash is currently grown commercially in Turkey. Farmers have maintained local populations of winter squash and mainly at local markets. It is a traditional vegetable often grown in small gardens. Growers have selected the high-yielding genotypes. However, for decades, the old winter squash landraces have progressively been replaced by new cultivars, including Arican 97 v, which ensure higher yields and incomes, and meet the requirements of processors and consumers (Sari et al., 2008, Balkaya et al., 2009).

As in all cultivated plants, the main objective of winter squash growing is to grow high yield and high quality crops. Breeding for improved fruit colour and morphology, for increased productivity and pest and disease resistance are still major objectives of current winter squash breeding (Ferriol and Pico, 2008). Fruit quality is a major concern of current squash breeders. Squash and pumpkins are important horticultural crops worldwide, but there has been relatively little research to systematically describe yield components and improve productivity in this species (Loy, 2004). With the canonical analysis, the following descriptors were selected in the pumpkin accessions: seed and fruit length, biggest fruit diameter, node of the first male flower, vine length, number of days for flowering first female flower, internode, fruit weight, soluble solids, number of days for flowering 1st male flower, and number of seeds/g (Ramos, 1997). Five morphological and agronomic characteristics were evaluated in seven summer squash (*Cucurbita moschata*

L.) lines (Bezerra Neto et al., 2006). Simple and canonical correlations revealed that fruit average weight increased together with increments in pulp thickness and longitudinal and transversal fruit length in the biometric analysis of summer squash lines. On the other hand, plants with more fruits produced smaller fruits (longitudinal length), with thinner pulp, but with higher transversal length. In this population, promising commercial genotypes, producing small fruits with thick pulps, can be obtained using selection indexes.

Squash and pumpkin's yield are affected by genotypic and environmental factors. The size of mature fruit is especially influenced by genetics, environment, and plant conditions during development of pistillate flower and fruit (Maynard, 2007). For this reason, determination of the effects of genotypic factors in winter squash breeding is a primary concern. The correlation of economic yield components with yield and the partitioning of the correlation coefficient into its components of direct and indirect effects. Correlation studies between characters have also been of great value in the determination of the most effective breeding procedures (Ariyo et al., 1986).

Determining fruit characters and yield components will provide important benefits in winter squash breeding programs. More information is needed to clarify the relationships between plant and fruit characteristics.

The application of CCA in plant breeding began to increase with the availability of related computer packages (Carvalho et al., 1988; Ramos, 1997; Tavares et al., 1999; Bezerra Neto et al., 2006). To our knowledge, however, the applications of CCA have not been founded for the estimation of relationships between plant characters and yield components in winter squash populations.

Accordingly, the objectives of the present study were twofold: i) to estimate the interrelationships between plant characters and yield components measured from 117 winter squash populations collected from Turkey; and ii) to determine which variables can be used as early selection criteria

for increasing the yield of winter squashes using CCA.

Material and Methods

Data collection

Winter squash seeds from four provinces (Samsun, Amasya, Sinop, and Bolu) of the Black Sea region of Turkey were collected before and during harvest time between September 2004 and February 2006. A total of 117 populations were used in this study. These genetic materials were collected from well-established, traditional, open-pollinated populations grown by local farmers. The field component of this study was carried out in the Bafra district of Samsun province in 2006 and 2007. The experimental site was located at 41° 35' N, 35° 54' E. This site is situated in the north of Turkey, and has a humid climate with annual relative humidity of 72.0% and average annual rainfall of 780.2 mm (TSMS, 2008). The soil of the experimental area was sandy loam with a pH of 6.5.

The seeds of all populations were sown into plug trays containing peat, organic manure and sand mixed in the ratio of 4:2:1 on 20 April 2006 and 10 April 2007. Twenty seedlings from each population were field planted at the 4-5 leaf stage at a spacing of 2.8 m x 2.0 m in the middle of May of each year. Standard fertilization and weed control practices were applied. The fruits were harvested when they reached full maturity. Harvesting started at the end of September and lasted to the middle October of each year (the investigated populations have different harvest periods). Measurements and observations of examined characters were done on 16 plants, which had been randomly chosen in the mid-row of each plot.

The following measurements and observations were made: female flowering time (50 %) (FFT); time to maturity (TM); vine length (VL); brunch number per plant (BN); leaf length (LL); leaf width (LW); fruit length (FL); fruit diameter (FD); flesh thickness (FT); skin thickness (ST); length

of seed cavity (LSC); number of fruits per plant (FN/P); total fruit weight per plant (FW/P); and average fruit weight (AFW). Female flowering time was determined as sowing to 50% female flowering of plants. Time to maturity was recorded as the time from sowing to fruit maturity. Vine length (cm); brunch number per plant (unit), leaf length (cm); and leaf width (cm) were measured at first harvest for each population. The other characters were measured at or after harvest. To standardize the interpretation of fruit characters, the same researcher did all the classifications in this study. The number of fruits per plant and total fruit weight per plant values were determined for each population during the harvest period. Mean fruit weight was found by dividing the total fruit weight by the fruit number. The field experiment was established as a randomized block design with three replications. Because the effects of year were not significant on plant characters and yield, according to two-way ANOVA, two-year data were combined before the analysis used in this study.

Canonical correlation analysis (CCA)

The relationships of several morphological characters with yield and their components across winter squash populations were also investigated using canonical correlation analysis. The CCA, developed by Harold Hotelling in 1935, focuses on the correlation between a linear combination of the variables in the plant characters variable set (X -set) - called canonical variable U - and a linear combination of the variables in the yield components variable set (Y -set) - called canonical variable V - such that the correlation between the two canonical variables is maximized (Gunderson and Muirhead, 1997). Canonical variables (U and V), which in this study are needed to represent the association between the different plant characters from 117 winter squash populations, are so formed that the first pair has the largest correlation of any linear combination of the original variables.

Subsequent pairs also have maximized correlations subject to the constraint that they are

uncorrelated with each previous pair (Johnson and Wichern, 2002). Symbolically, given $X_{n \times p}$ and $Y_{n \times q}$, then $U_i = Xa_i$ and $V_i = Yb_i$, where a_i and b_i are standardized canonical coefficients that can be used to determine which variables are redundant in interpreting the canonical variables (Cankaya and Kayaalp, 2007). These coefficients indicate the relative importance of the variable set of plant measurements at the harvest period in determining the value of the variable set of the plant characteristics at the yield components for winter squash populations, with $i=1, \dots, \min(p, q)$.

However, the coefficients can be unstable because of the presence of multicollinearity in the data. For this reason, the canonical loadings are considered to provide a substantive meaning of each variable for the canonical variables (Akbas and Takma, 2005).

The result satisfies $Corr(U_p, V_j) = 0$, $Corr(U_p, U_j) = 0$, $Corr(V_p, V_j) = 0$ for $i \neq j$ and $Corr(U_p, V_j) = \rho_i$ for $i = j$ (Al-Kandari and Jolliffe, 1997). The canonical correlation coefficient (ρ_i) is the measure of the interrelationship between two variable sets. Let $\rho_1^2, \dots, \rho_p^2$ ($0 \leq \rho_p^2 \leq \dots \leq \rho_1^2 \leq 1$) be min

(p, q) ordered eigenvalues (λ_i) of the matrix

$$\Sigma_{11}^{-1} \Sigma_{12} \Sigma_{22}^{-1} \Sigma_{21}, \text{ where } \Sigma = \begin{bmatrix} \Sigma_{11} & \Sigma_{12} \\ \Sigma_{21} & \Sigma_{22} \end{bmatrix}$$

Their positive roots ρ_1, \dots, ρ_p are the population of canonical correlation coefficients between U and V .

$$\rho_{U, V_i} = r_i = \sqrt{\lambda_i} = \frac{Cov(U, V)}{\sqrt{Var(U)Var(V)}} = \frac{a' \Sigma_{12} b}{\sqrt{(a' \Sigma_{11} a)(b' \Sigma_{22} b)}} \\ i = 1, 2, \dots, q$$

Interpretations of canonical correlation analysis (CCA)

The null and alternative hypotheses for assessing the statistical significance of the canonical correlation coefficients (CCCs) are,

$$H_0 : \rho_1 = \rho_2 = \dots = \rho_r = 0$$

$$H_1 : \rho_i \neq 0 \text{ at least one } i = 1, 2, \dots, r$$

The F test statistic for the statistical significance of ρ_i^2 is

$$F = \frac{1 - \lambda_1^{1/t}}{\lambda_1^{1/t}} \frac{sd_2}{sd_1} \sim F_{sd_1, sd_2, \alpha}$$

Here,

$$\lambda_1 = \prod_{i=1}^s (1 - r_i^2);$$

$$s = \min(p, q); \quad sd_1 = pq; \quad sd_2 = wt - \frac{1}{2}pq + 1;$$

$$w = n - \frac{1}{2}(p + q + 3) \quad t = \sqrt{\frac{p^2q^2 - 4}{p^2 + q^2 - 5}}$$

where, n is the number of cases, p is number of variables in the X set, q is the number of variables in the Y set, and r_i^2 : represents the eigenvalues of $\sum_{11}^{-1} \sum_{12} \sum_{22}^{-1} \sum_{21}$ or the squared canonical correlations.

CCCs do not identify the amount of variance accounted for in one variable set by other variable sets. Therefore, it is important to calculate the redundancy measure for each canonical correlation to determine how much of the variance in one set of variables is accounted for by the other set of variables (Sharma, 1996). The redundancy measure can be formulated as below

$$RI_{U, V_i} = OV(Y|V_i)r_{uv}^2 \quad OV(Y|V_i) = \frac{\sum_{i=1}^q LY_{ij}^2}{q}$$

where $OV(Y|V_i)$ is the averaged variance in Y variables that is accounted for by the canonical variate V_i , LY_{ij} which is the loading of the j^{th} Y variable on the i^{th} canonical variant; and q is the number of traits in canonical variants mentioned.

Applications of canonical correlation analysis

While the first twelve characters were included in the first variable set ($X_{n \times p}$: plant characters), the latter three characters were included in the second variable set ($Y_{n \times q}$: yield components). All of the computational work was performed to examine the relationships between the two sets of traits by means of the PROC CONCORR procedure of the SAS 6.0 statistical package (SAS, 1988).

Results and Discussion

Descriptive statistics for the examined characters are presented in Table 1. Bivariate correlations displaying the relationships among the traits of winter squash populations are given in Table 2. The highest correlation was predicted between TM and FFT (0.86, $P < 0.01$), while the lowest correlations ($r = -0.01$ or 0.01 , $P > 0.05$) were between FD and LW, FD and FN/P, FW and FFT, LW and AFW for all traits. The findings supported those of Ramos (1997). Also, the highest correlations were predicted between FW/P and AFW ($r = 0.69$, $P < 0.01$) for yield components; FFT and TM ($r = 0.86$, $P < 0.01$) for plant characters; and FD and AFW ($r = 0.75$, $P < 0.01$) for the interrelationships between plant characters and yield components. The lowest correlations were predicted between AFW and FN/P ($r = -0.05$, $P > 0.05$) for yield components; FD and LW ($r = 0.01$, $P > 0.05$), FW and FFT ($r = -0.01$, $P > 0.05$) for plant characters; and AFW and LW ($r = -0.01$, $P > 0.05$) for the interrelationships between yield components and plant characters. In addition to these results, it was determined that the relationships between yield components and plant characters were similar to the results of previous studies (Ramos, 1997; Viana et al., 2003, Bezerra Neto et al., 2006; Khan et al., 2007).

Although plant characters are important indicators of yield components in winter squash populations, it is extremely difficult to explain simultaneously the relationship between the traits. Therefore, instead of interpreting the correlations given in Table 2, three canonical correlation coefficients were estimated to explain the interrelationships between the variable sets since the number of canonical correlations that need to be interpreted is the minimum number of traits within plant and fruit character variables at the X variables and the Y variables sets (Table 3).

Table 3 showed that first two of estimated three CCCs were significant (0.903 and 0.571, $p < 0.001$) with respect to the likelihood ratio test while

Table 1
Descriptive values for examined characters

Plant characters	X variable set (mean \pm SD)	Yield components	Y variable set mean \pm SD
Fruit length (cm) (FL)	38.09 \pm 0.45	Total fruit weight plant ⁻¹ (kg) (FW/P)	12.63 \pm 0.42
Fruit diameter (cm) (FD)	44.95 \pm 0.40	Average fruit weight (kg) (AFW)	7.36 \pm 0.17
Skin thickness of fruit (mm) (ST)	5.73 \pm 0.13	Fruit number plant ⁻¹ (FN/P)	1.73 \pm 0.04
Flesh thickness (mm) (FT)	34.75 \pm 0.53		
Length of seed cavity (cm) (LSC)	19.38 \pm 0.21		
Fiber weight/ fruit (g) (FW)	187.95 \pm 7.66		
Branch number/plant (BN)	2.44 \pm 0.03		
Vine length (cm) (VL)	627.56 \pm 10.07		
Female flowering time (50 %) (FFT)	74.78 \pm 0.40		
Leaf length (cm) (LL)	23.69 \pm 0.21		
Leaf width (cm) (LW)	34.71 \pm 0.29		
Time to maturity (TM)	160.12 \pm 0.39		

SD: Standard deviation

Table 2
The correlation matrix between traits

	FL	FD	ST	FT	LSC	FW	BN	VL	FFT	LL	LW	TM	FW/P	AFW
FD	0.45**													
ST	0.12	0.35**												
FT	0.36**	0.34**	0.04											
LSC	0.38**	0.44**	0.28**	0.18										
FW	0.32**	0.37**	0.23*	0.19*	0.36**									
BN	-0.20*	0.05	0.05	-0.07	-0.09	-0.21*								
VL	0.10	-0.02	0.07	-0.06	-0.09	0.04	0.08							
FFT	-0.02	-0.09	-0.14	0.08	-0.15	-0.01	-0.22*	-0.03						
LL	-0.03	-0.02	-0.02	0.02	0.04	-0.14	0.13	-0.11	-0.03					
LW	-0.11	0.01	0.03	0.03	0.03	-0.19*	0.14	-0.10	-0.04	0.92**				
TM	-0.14	-0.10	-0.14	0.03	-0.21*	-0.03	-0.15	-0.05	0.96**	0.02	0.03			
FW/P	0.33**	0.58**	0.32**	0.33**	0.52**	0.39**	0.10	0.03	-0.25**	-0.18	-0.14	-0.28**		
AFW	0.67**	0.75**	0.37**	0.49**	0.57**	0.48**	-0.12	0.03	-0.02	0.02	0.01	-0.10	0.69**	
FN/P	-0.23*	-0.01	0.03	-0.02	0.13	0.08	0.20*	0.05	-0.30**	-0.28**	-0.21*	-0.26**	0.67**	-0.05

*, **: correlation is significant at the 0.05 and 0.01 level (2-tailed), respectively. -: correlation is not statistically significant at the 0.05 level (2-tailed).

The superscript indicates that no correlation was found between the traits (for example between FL and FN/P).

Bold figures present the highest and lowest correlation between the traits.

Table 3
Summary results for the canonical correlation analysis

Pair of canonical variables	Canonical correlation	Squared canonical correlation	Eigenvalue	DF	Likelihood ratio	Probability Pr>F
U_1V_1	0.903	0.815	4,397	36	0.111	<0.001
U_2V_2	0.571	0.326	0.483	22	0.597	<0.001
U_3V_3	0.340	0.115	0.130	10	0.885	0.218

Table 4
Standardized canonical coefficients for canonical variables

	X – Variable set												Y – Variable set			
	FL	FD	ST	FT	LSC	FW	BN	VL	FFT	LL	LW	TM	FW/P	AFW	FN/P	
U_1	0.33	0.50	0.15	0.16	0.16	0.10	-0.01	-0.01	0.26	0.09	-0.01	-0.21	V_1	0.82	0.39	-0.64
U_2	-0.49	0.03	-0.05	0.17	0.40	0.16	0.32	0.04	-0.69	-0.78	0.25	0.22	V_2	1.3	-0.87	0.06

Table 5
Canonical loadings of the original variables with their canonical variables

	X – Variable set												Y – Variable set			
	FL	FD	ST	FT	LSC	FW	BN	VL	FFT	LL	LW	TM	FW/P	AFW	FN/P	
U_1	0.75	0.86	0.42	0.54	0.63	0.52	-0.12	0.02	-0.04	0.04	0.01	-0.11	V_1	0.66	0.99	-0.11
U_2	-0.30	0.17	0.17	0.03	0.31	0.17	0.41	0.05	-0.58	-0.48	-0.35	-0.51	V_2	0.74	0.02	0.97

third CCC was no significant (0.340, $p>0.218$). Based on this result, we interpreted the relationship between the first pair of canonical variables (U_1 and V_1), which had a maximum coefficient. Standardized canonical coefficients (canonical weights) and canonical loadings were given for the first pair of canonical variables (U_1 and V_1), as shown in Tables 4 and 5, respectively. Magnitudes of the canonical coefficients signify their relative contributions to the correlated variant. That is, the coefficients indicate the effects of plant characters on the yield components for winter squash populations. Therefore, the canonical variants (U_1 and V_1) representing the optimal linear combinations of dependent and independent variables can be defined by using the standardized canonical coefficients (given in Table 4) as,

$$U_1 = 0.33(\text{FL}) + 0.50(\text{FD}) + 0.15(\text{ST}) + 0.16(\text{FT}) + 0.16(\text{LSC}) + 0.10(\text{FW}) - 0.01(\text{BN}) - 0.01(\text{VL}) + 0.26(\text{FFT}) + 0.09(\text{LL}) - 0.01(\text{LW}) - 0.21(\text{TM})$$

$$V_1 = 0.82(\text{FW/P}) + 0.39(\text{AFW}) - 0.64(\text{FN/P})$$

Accordingly, if the values of the plant characters (except for BN, VL, LW and TM) increase, the fruit weight per plant and average fruit weight will increase, and the fruit number per plant will decrease. Variables with larger canonical loadings contributed more to the multivariate relationships between yield components and plant characters (Table 5). The loadings for the yield components suggested that AFW and FW/P were more influential than FN/P in forming V_1 . The loading for plant

Table 6
Cross loading of the original variables with opposite canonical variables

	X – Variable set													Y – Variable set		
	FL	FD	ST	FT	LSC	FW	BN	VL	FFT	LL	LW	TM		FW/P	AFW	FN/P
V_1	0.67	0.78	0.38	0.49	0.56	0.47	-0.11	0.01	-0.03	0.03	0.01	-0.10	U_1	0.59	0.89	-0.10
V_2	-0.17	0.10	0.10	0.02	0.18	0.10	0.23	0.03	-0.33	-0.27	-0.20	-0.29	U_2	0.42	0.01	0.56

Table 7
The explained total variation ratio by canonical variables for the variable sets

X – Variable set				Y – Variable set			
	Variance extracted		Redundancy		Variance extracted		Redundancy
U_1	0.204	V_1	0.167	V_1	0.514	U_1	0.418
U_2	0.116	V_2	0.038	V_2	0.473	U_2	0.154

FD and FL were more influential than other plant characters in forming U_1 . According to the cross loadings, FD and AFW contributed the most to canonical variants V_1 and U_1 , respectively. There is a relationship between fruit diameter and average fruit weight; the findings indicated that fruit diameter and average fruit weight should be used with the aim of increasing yield per plant in winter squash populations (Table 6).

In the present study, it was found that 51.4 and 47.3% of total variation in the yield components set was explained by all canonical variables V_i , while the redundancy measure of 0.418 for the first canonical variable suggests that about 41.8% of the ratio was explained by canonical variable U_1 .

Also, it was found that 20.4% of total variation in the plant characters set was explained by the first canonical variable U_1 , while the redundancy measure of 0.167 for first canonical variable suggests that about 16.7% of the ratio was explained by canonical variable V_1 (Table 7).

Determining the relationship between characters affecting optimum output is very important for increasing yield components in winter squash populations. Larger fruit dimensions are desirable for both farmers. To this end, this study has

revealed the relationships between the yield components and plant characters of the winter squash. Fruit diameter and fruit length were the most influential factors in this relation. Ramos (1997) and Bezerra Neto et al. (2006) have reported that fruit diameter and fruit length, vine length, number of days for flowering first female flower and total fruit weight have strong positive correlations with yield. Therefore, the results obtained from this work will advance plant breeding practices and research on yield components by guiding *Cucurbita* breeders in selecting the best plant characters in winter squashes. Some of them were identified as having specific characteristics to be used in winter squash breeding strategies. In conclusion, this will lead to an increase in desirable yield values by decreasing the number of studied characters, which will in turn increase selection efficiency in winter squash production.

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