

Canonical Correlation Analysis for Determination of Relationship between Morphological and Physiological Pollinated Characteristics in Five Varieties of *Phalaenopsis*

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Phalaenopsis is an important genus of orchids that is grown for economical production of cut flower and potted plants. The objective of this study is the evaluation of correlation between morphological and physiological traits of self and cross-pollination of 5 varieties of *Phalaenopsis* orchid. Some morphological traits were measured: Capsule length (CL), capsule volume (CV), weight of seeds in capsule (WSC), full weight of capsule (FWC) and empty weight of capsule (EWC) and physiological traits: Time to first pollination symptom (TPS), time to capsule swelling (TCS) and capsule ripening (CR). The results showed that correlation (canonical r) was 0.91 in first pair canonical variables; second and third pair canonical variables had correlation 0.68 and 0.30, respectively. The first canonical variable in physiological traits revealed 66.1% variation of physiological variables. Special values were 0.820, 1.388 and 4.850 for Wilks' Lambda, Pillai's Trace and Roy's Greatest Root, respectively. Canonical correlation was significant for physiological and morphological characteristics in all statistical tests. The first canonical variables in physiological characteristics had positive correlation with TCS and TPS, so it was resulted that if TCS was fewer, TPS and CR characteristics were fewer. In pollinated varieties, the lowest TCS belonged to Nottingham× Nottingham and Bucharest× Nottingham; and the most CR belonged to Dubrovnik× Dubrovnik and Memphis× Dubrovnik of pollination states; Therefore, it can be concluded that white and yellow female parent require fewer and more days for capsule swelling respectively.

Abstract

Keywords: Capsule, Nottingham, Orchid, Pollination, Seed.

INTRODUCTION

The *Phalaenopsis* (Orchidaceae) is concern as the most popular orchid genus in the horticultural industry because of the appreciation of beauty value and durability of the flowers, as well as the adaptability of the plants to room environment (Kosir *et al.*, 2004). Size of orchid plants vary from 2-3 mm to 15-20 cm or more in diameter. They may be of almost any color from pure white to almost pitch black (although none are pure black).

Insects, birds, rodents and numerous other biotic factors have been used as pollen vectors. Increasing evidence suggests that pollinators have a key function in generating novel floral forms and maintaining species diversity in orchids (Xu *et al.*, 2012). Another research direction in the study of the phylogenetic evolution of orchid pollination using genomic, transcriptomic, and proteomic methods (Clare *et al.*, 2013; Sedeek *et al.*, 2013).

One of the causes of the breeding barriers is due to abnormal pollen meiosis which may lead to ineffective Sporades (Bolaños-Villegas and Chen, 2007; Bolaños-Villegas *et al.*, 2008).

Pollinator effectiveness can be assessed with different measures, including pollen load carried and transferred, pollinator visits (relative abundance, visitation timing and duration), pollen viability and quality, probability of contact with stigma and anthers, fruit set, seed set, seed viability and germinability, and seed or fruit characteristics such as size, mass and number (Sahli and Conner, 2007; Thesis *et al.*, 2007). Convenient indicators of seed production were leaf width and capsule size. Capsule size is often assumed to be linked to plant vigor. Self-pollinated flowers tended to produce more seeds than did crossed flowers, indicating that the behavior of insects at flowers should be examined (Petit *et al.*, 2009).

The first sign of success in pollination is petals wilt, which differed greatly between treatments in Balilashaki *et al.* (2015) research. Studies show though orchid flowers not pollinating can stay healthy for a long time, but they are going to wilt with pollination and ethylene production in a short time; and two factors of ethylene sensitivity and ethylene production are involved (Arditti, 1993). Gonzalez-Diaz and Ackerman (1988) said that self-pollinated flower in orchids by on pollinium produces fruits with 619 seeds fruit⁻¹ and weight of 23 mg fruit⁻¹; and self-pollinated flowers by two pollinia produces 93% fertile seeds with 623 seeds per each fruit and weight of 46.2 mg fruit⁻¹. Correlation coefficient commonly displays relationship between characters and the degree of linear relation between these characteristics (Guler *et al.*, 2001). Determination of the relationship between two or more characters measured at an early time and a later time is important (Akbas and Takma, 2005; Cankaya *et al.*, 2008) since early selection is one of the vegetable breeding methods used for a higher yield the plants. Canonical correlation analysis is a method for delineation the relation between two variables sets together and for producing both structural and spatial concepts (Cankaya *et al.*, 2010).

The importance of canonical correlation analysis is that the new variables derive from the linear combination of a collection of main variables, and the optimizing criterion for this analysis is to maximize the relationship between two variables groups. Also, canonical correlation analysis can quantify the response of a group of variables collected at different time of a season (Nicolas *et al.*, 2005). The performance of this innovative technique has already been reported by Ursem *et al.* (2008), DiLeo *et al.* (2011), and Silva *et al.* (2016). Phenotypic correlation networks simplify the explanation of the correlations between characteristics, and therefore make it easier to verify which characteristics can be applied for indirect selection.

The goal of this study is the evaluation of relationship between two variable groups such as physiological and morphological characteristics in self and cross-pollination of 5 varieties of *Phalaenopsis* by canonical correlation analysis and determination of relative contribution of each variable.

MATERIALS AND METHODS

This study was carried out in a greenhouse of Pardis Township around the Tehran city, Iran

country in 2018-2019. Five variety of *Phalaenopsis* orchid (Nottingham, Andorra, Memphis, Dubrovnik and Bucharest) were pollinated via 5 states of selfing and 20 states of cross-pollination (Fig. 1). It was used 5 replication (5 flowers for pollination) in each state of selfing and cross-pollination. Since orchid pollination is normally done by specified bees and insects, pollination in this research was performed manually. The symptoms of pollination can be seen with petals wilt, a few days after pollination. Also Non-pollinated flowers dry out shortly after pollination. The beginning of the pollination symptoms was recorded for all varieties (TPS) in Fig. 2. The required time was recorded for the onset of primary symptoms of capsule swelling after pollination for each variety (TCS). The lengths of capsules were measured by ruler (CL). Then the seeds weights (WSC) were measured by reduction of capsule's empty weight (EWC) from capsule's full weight (FWC). The first symptom of capsule ripening was determined with browning the outer shell of the capsule (CR), that it was different between various varieties. Data analyzed by SAS software to determine canonical coefficient. Canonical correlation analysis was performed by 4 ways: Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root.



Fig. 1. Five varieties of *phalaenopsis* used in the research.

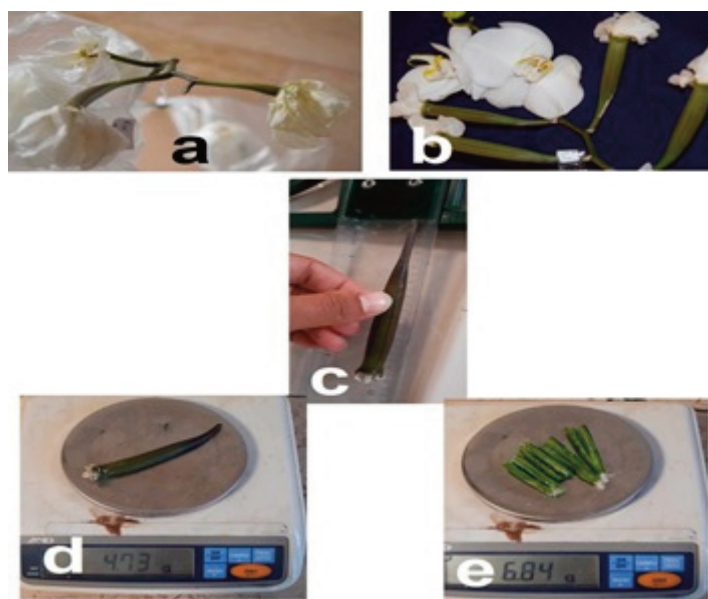


Fig. 2. Measuring the characteristics. a) TPS; b) TCS; c) CL; d) FWC and e) EWC.

RESULT AND DISCUSSION

Canonical correlation analysis

The result of canonical correlation analysis for morphological and physiological characteristics showed two canonical variables were significant, but third variable wasn't significant (Table 1). Significant canonical variables in physiological characteristics fit 87% of the total variance of physiological characteristics. Significant canonical variables in morphological characteristics showed 96% of the variance in pollination characteristics. Correlation was 0.91 in first pair canonical variables; second and third pair canonical variables had correlation 0.68 and 0.30, respectively. Significant canonical correlation between two variables groups showed relationship between physiological and morphological pollination characteristics.

Table 1. Canonical correlation and probability of significant level in physiological and morphological characteristics of *Phalaenopsis*.

Canonical variable	Canonical correlation	Square of canonical correlation	Special value	Percentage	Accumulative percentage	P value
1	0.91	0.82	4.85	83.3	83.3	0.0001
2	0.68	0.46	0.86	14.9	98.2	0.01
3	0.30	0.09	0.10	1.7	100	0.58

The correlation coefficients evaluate the relationship between the canonical variables and the main variables, commonly called structural coefficients (Johnson and Wichern, 2002). To investigate this relationship, the correlation between the canonical variables and the main variables in canonical correlation analysis was compared for the first two pairs of canonical variables. These pair of variables fit the most correlation with morphological and physiological variables of orchid pollination. The first canonical variable in physiological characteristics revealed 66.1% variation of physiological variables (Table 1). The highest correlation in the first canonical variables was about TCS ($r = 0.73$). The first canonical variables in physiological characteristics had positive correlation with TCS and TPS, so it was resulted that if TCS was fewer, TPS and CR characteristics were fewer. In pollinated varieties, the lowest TCS belonged to Nottingham × Nottingham and Bucharest × Nottingham; and the most CR belonged to Dubrovnik × Dubrovnik and Memphis × Dubrovnik of pollination states; Therefore, it can be concluded that white and yellow female parent require fewer and more days for capsule swelling, respectively. Mean comparison results showed that male parents of white variety reduced TCS if it pollinated with yellow parent. The yellow male parent also increases TPS when it pollinated with the white female parent.

The first canonical variable in morphological characteristics showed 45.7% variation of pollination morphological variables (Table 3). The most correlation in the first canonical variable was belonged to CV in *Phalaenopsis* ($r = 0.74$). The first canonical variables in morphological characteristics had positive correlation with CL, WSC, FWC and EWC; so the result showed if CL, WSC, FWS and EWC were more, CV was more. The lowest CV was belonged to Bucharest × Memphis, Bucharest × Dubrovnik and Dubrovnik × Memphis of pollination state. The most CV was belonged to Nottingham × Nottingham and Andorra × Nottingham. Female parent of Memphis and Dubrovnik varieties showed CV fewer than other varieties. The second canonical variable in physiological characteristics revealed 16.4% variation of physiological variables (Table 1). The most correlation in the second canonical variables was in TCS ($r = 0.75$).

The result of table 2 for investigation of significant tests of canonical correlation in pollination physiological characteristics showed that the most special values was for correlation test with the methods of Hotelling -Lawley Trace. Special values were 0.820, 1.388 and 4.850 for Wilks' Lambda, Pillai's Trace and Roy's Greatest Root, respectively. Canonical correlation was significant for physiological and morphological characteristics in all statistical tests.

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Table 2. Significant tests of canonical correlation for physiological and morphological of pollination characteristics.

Ways	Special value	F	df	P
Wilks' Lambda	0.820	4.62	15	0.0001
Pillai's Trace	1.388	3.27	15	0.0006
Hotelling-Lawley Trace	5.823	6.28	15	0.0001
Roy's Greatest Root	4.850	18.43	5	0.0001

Table 3. The correlation of morphological characteristics with two significant canonical variables.

Morphological characteristics	Canonical variables	
	CA ₁	CA ₂
WSC	**0.60	**0.61
FWC	**0.67	**0.67
CL	**0.65	**0.69
EWC	0.05 ^{ns}	**0.39
CV	**0.74	**0.75
Variance of canonical variables	45.7	31.3

** and ^{ns}: Significant at P < 0.01 and insignificant respectively.

Table 4. Correlation of physiological characteristics with two significant canonical variables pollination morphology.

Physiological characteristics	Canonical variables	
	CA ₁	CA ₂
TPS	** -0.27	** -0.84
TCS	** -0.75	** 0.45
CR	** 0.31	0.06 ^{ns}
Variance of canonical variables	54.8	17.5

** and ^{ns}: Significant at P < 0.01 and insignificant respectively.

The first correlation variable of morphological characteristics showed 54.8% of the variation of physiological variables (Table 3). The highest correlation in the first canonical variable was belonged to CR ($r=0.31$). The first canonical variable in morphological characteristics showed positive correlation with CR, and it had negative correlation with other physiological characteristics. The results showed that whatever morphological characteristics amounts have been more, CR will be more; on the other hand, TPS and TCS will be less. The second canonical variable in morphological characteristics fit 17.5% of the physiological variables variation, which it had positive correlation with TCS, but it had negative correlation with TPS. Also, it had no significant relationship with CR.

The first canonical variable in physiological characteristics displayed 37.8% of morphological variables variation (Table 4). The highest correlation in the first canonical variable was in CV ($r=0.73$). The first canonical variable in physiological characteristics had positive correlation with all of morphological characteristics. The result showed whatever CR have been more, CV will be more, too. The result of this research showed the second canonical variable in physiological

characteristics fit 14.5% of morphological variables variation. The second canonical variable in physiological characteristics had high positive correlation with CL and EWC, but it had negative correlation with CV and FWC. Also, it had no significant relationship with WSC.

Table 5. Correlation of morphological characteristics with two significant canonical variables pollination physiology.

Morphological characteristics	Canonical variables	
	CA ₁	CA ₂
WSC	**0.29	0.09**
FWC	0.09 ^{ns}	-0.44**
CL	**0.11	0.95**
EWC	0.02 ^{ns}	0.75**
CV	0.58**	-0.76**
Variance of canonical variable	37.8	14.5

** and ^{ns}: Significant at P < 0.01 and insignificant respectively.

According to the result of this research (Table 5), it can be concluded that selection by morphological characteristics of pollination in *Phalaenopsis* such as CL, CV and FWC (they had the highest correlation with first canonical variable) leads to selection of genotypes with superior physiological characteristics such as low amount of TCS and CR in self-pollination and cross-pollination states. The result of this study showed the importance of canonical correlation analysis for understanding of the relationships between physiological and morphological characteristics in pollination of *Phalaenopsis*. Simple correlation can't analyze cause and effect relationships between physiological and morphological characteristics alone and with high precision. In breeding program, the interference between pollination's characteristics should be examined to distinguish the suitable characteristics and used in breeding and hybridization programs. The selection of productive characteristics can be indirectly performed by morphological traits due to the correlation between these two groups of characteristics (Silva *et al.*, 2017). Path analysis makes it possible to study the cause and effect relationship between traits, which allows the accurate establishment of the best criteria for indirect selection (Cruz *et al.*, 2012). The results of Cankaya *et al.* (2010) study show that PH, FWT and FW should be applied with the purpose of enhancing yield per plant in red pepper genotypes. Solanki *et al.* (1986) and Basavaraj (1997) have reported that fruit length, fruit width, number of fruits per plant and total fruit weight have high positive correlations with yield.

CONCLUSION

According to the result, it can be said that selection by pollination's morphological characteristics such as CL, FWC, CV and WSC have the most correlation with the first canonical variables, and they can lead to select pollination states with high pollination's physiological characteristics for example TPS, TCS and CR. These results display the importance of canonical correlation analysis for understanding the relationship between physiological and morphological characteristics of *Phalaenopsis* orchids. It can be noticed that canonical correlation analysis is an effective way for detecting the relationships between characteristics in pollination states due to simultaneous analysis of several variables. Thus, it can be used this ways for similar studies in the future. The identification of traits that have high phenotypic correlation and high direct effect in the same direction on the main trait is desirable, since the correlated response by means of indirect selection can be effective.

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