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Original Research Paper

Pollination phenotypes in *Phalaenopsis* crosses: Guiding selection for optimal breeding

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ABSTRACT

Phalaenopsis orchid is one of the most popular flowers that have a large share of global flower and plant market due to its attractiveness, color varieties, and medicinal properties. In this study, the morphological (capsule length, full weight of capsule, empty weight of capsule, the weight of seeds in capsule and capsule volume) and phenological (time of pollination symptom, time of capsule swelling, and time of capsule ripening) traits of pollination were evaluated in 5 varieties of *Phalaenopsis* by the first plant pollination syringe of orchids in 5 types of self-pollination and 20 combinations of cross-pollination during 2019-2023. The results showed that 92% of the total variations were explained by the first 4 components in the analysis of the main components. The first and second components accounted for about 57.5% and 19.6% of the total data. Using cluster analysis results, pollination types were classified into three groups based on pollination factors. Discriminant analysis showed that clustering based on the Euclidean distance criterion was better than other distance criteria and was obtained favorably clustering based on it. Low heritability and genetic improvement were observed for time to petal wilting after pollination (TPS) characteristic. Nottingham × Nottingham pollination has the highest amount of capsule length, weight of seeds per capsule, capsule volume, and fresh weight of capsule. Capsule length had the most phenotypic and genotypic variation coefficient among all of the examined characteristics in 25 pollination types. The results of this study are used to select parents and favorable crosses to produce new varieties and capsules containing seeds.

Keywords: Capsule, discriminant analysis, orchid, pollination syringe, seed

INTRODUCTION

The Orchidaceae family is one of the largest and most diverse among angiosperms, encompassing 30,000 to 35,000 species of terrestrial plants (Singh et al., 2008). *Phalaenopsis* ranks as the world's second most important cut flower and potted orchid, gaining popularity due to its ease of cultivation, diverse flower colours, shapes, sizes, and delicacy. It is commercially grown in Germany, Japan, the Netherlands, Taiwan, and the United States. In the USA alone, approximately 13.5 million *Phalaenopsis* plants were sold in 2005, currently accounting for 75% of purchased orchids (Sarmah et al., 2017).

The selection of suitable cultivars in breeding programs depends on various traits, which may exhibit positive or negative correlations (Zelterman, 2016). Since, correlation studies reveal linear relationships between traits, principal component analysis is often used to decompose these relationships under both balanced and stressed conditions. Numerous studies have explored descriptive statistics (Ye et al., 2008; Shao et al., 2010; Erzurumlu et al., 2018), trait correlations (Sheela et al., 2006; Rakonjac et al., 2014), cluster analysis (Rakonjac et al., 2014; Shao et al., 2010; Erzurumlu et al., 2018), and principal component analysis (Erzurumlu et al., 2018) in ornamental plants. Slipper orchids, for example, are often derived from *in vitro* germinated seeds of hybrid species, resulting in highly variable and unpredictable traits, with some hybrids producing up to 50% albino plants (Koopowitz, 2008). Seeja et al. (2019) reported that a high genetic variation coefficient, combined with high heritability and genetic progress, provides a clear basis for selecting genotypes, as confirmed by Roychowdhury & Tah (2011).

This study aims to evaluate the morphological and phenological characteristics associated with pollination in 5 self-pollinated and 20 cross-pollinated combinations. The focus is on their effects on capsule

Nottingham (W) Andorra (K) Bucharest (R) Dubrovnik (Y) Memphis (P)

Fig. 1 : Five varieties of *Phalaenopsis* orchid

structure, seed weight, and seed formation physiology for use in breeding and hybridization programs. Principal component analysis, a multivariate statistical method, is employed to group and cluster a large number of traits, linking them to a small number of principal components. This approach helps to determine the relationship between pollination factors and specific morphological structures, identifies hidden traits that influence pollination, and introduces key characteristics for breeding. Notably, this study presents the first reported use of orchid pollination syringes to examine the correlation between phenological and morphological traits in orchids globally.

MATERIAL AND METHODS

This research was conducted in the greenhouse of the Agriculture Institute, Research Institute of Zabol, Zabol, Iran, during 2019-2023. Five *Phalaenopsis* orchid varieties were used *viz.*, Nottingham (W), Dubrovnik (Y), Bucharest (R), Memphis (P), and Andorra (K) (Fig. 1). These varieties were subjected to 5 self-pollination and 20 cross-pollination treatments. Since, *Phalaenopsis* orchids are typically pollinated by specific bees and insects, manual pollination was employed in this study.

A simple and innovative device, named the orchid pollination syringe, was used for manual pollination (Fig. 2) that it includes the components of toothpicks, two size of empty medical syringes, lamps, phase and null wires and battery. This device pulls the pollinia into itself and then releases them into the ovarian tube. Several morphological and phenological traits were measured to compare the differences between treatments (Fig. 3). Pollination symptoms, such as sepal and petal wilting, appeared a few days after pollination (TPS). Non-pollinated flowers dried out shortly after pollination. The onset of pollination symptoms was recorded for all treatments.

The time of capsule swelling (TCS) was recorded for each cross. Capsule lengths (CL) were measured by a normal ruler and seed weights (WSC), the empty weight of capsule (EWC) and the full weight of capsule (FWC) were calculated by Japanese weighing scales (AND model EK610i). Data were analysed using SAS software, and Duncan's method was employed for mean comparison.

Fig. 2 : Orchid pollination syringe

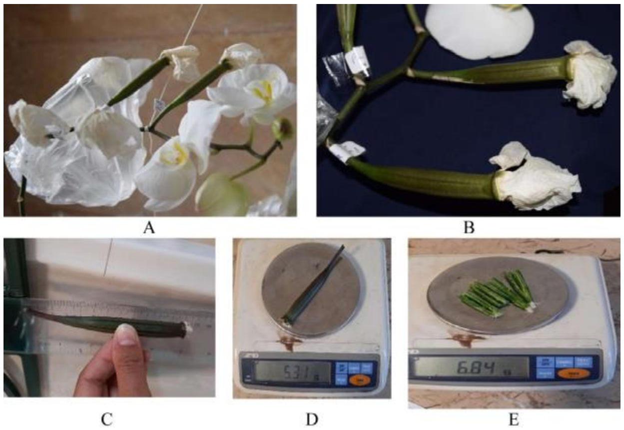


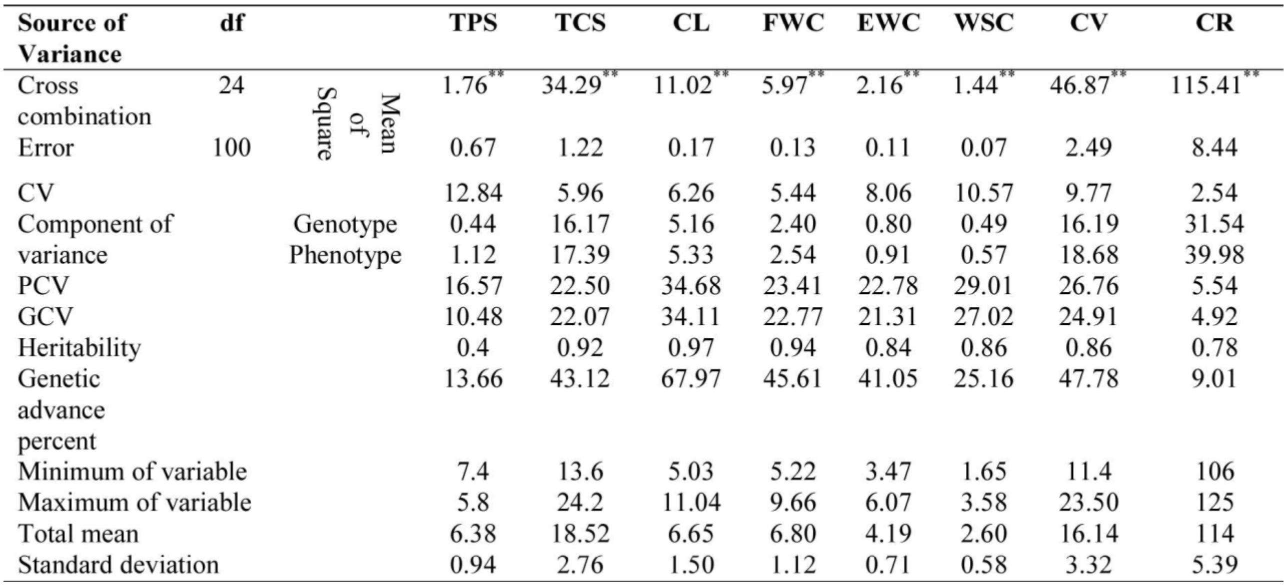
Fig. 3 : Measuring of characteristics in different treatments: A) wilting of petals a few days after pollination (TPS); B) symptoms of capsule swelling after pollination (TCS); C) lengths of the capsules measured by a ruler (CL); seeds weights (WSC); D) empty weight of the capsule (EWC); E) full weight of the capsule (FWC)

RESULT AND DISCUSSION

Genetic parameters

The results of this study revealed that the type of pollination (self-pollination vs. cross-pollination) had a significant effect on all of measured traits at the P

Table 1 : Some phenotype and genotype parameters from self and cross-pollination of 5 varieties of *Phalaenopsis*



Source of Variance	df		TPS	TCS	CL	FWC	EWC	WSC	CV	CR
Cross combination	24	Mean of Square	1.76**	34.29**	11.02**	5.97**	2.16**	1.44**	46.87**	115.41**
Error	100		0.67	1.22	0.17	0.13	0.11	0.07	2.49	8.44
CV			12.84	5.96	6.26	5.44	8.06	10.57	9.77	2.54
Component of variance		Genotype	0.44	16.17	5.16	2.40	0.80	0.49	16.19	31.54
		Phenotype	1.12	17.39	5.33	2.54	0.91	0.57	18.68	39.98
PCV			16.57	22.50	34.68	23.41	22.78	29.01	26.76	5.54
GCV			10.48	22.07	34.11	22.77	21.31	27.02	24.91	4.92
Heritability			0.4	0.92	0.97	0.94	0.84	0.86	0.86	0.78
Genetic advance percent			13.66	43.12	67.97	45.61	41.05	25.16	47.78	9.01
Minimum of variable			7.4	13.6	5.03	5.22	3.47	1.65	11.4	106
Maximum of variable			5.8	24.2	11.04	9.66	6.07	3.58	23.50	125
Total mean			6.38	18.52	6.65	6.80	4.19	2.60	16.14	114
Standard deviation			0.94	2.76	1.50	1.12	0.71	0.58	3.32	5.39

TPS: time to petal wilting after pollination, TCS: time to capsule swelling, CL: capsule length, FWC: full capsule weight, EWC: empty capsule weight, WSC: seed weight, CV: capsule volume, CR: capsule ripening

[1] In all of cross-pollination, the first variety is male parent and the second variety in female parent.

$\leq$ 0.01 level (Table 1). The self-pollination type WW exhibited the highest values for several characteristics, including capsule length (CL) at 11.04 cm, capsule volume (CV) at 23.5 cm³, seeds weight (WSC) at 3.58 g, empty weight of capsule (EWC) at 6.07 g, and full weight of capsule (FWC) at 9.66 g. Conversely, the lowest values for these traits were observed in parent crossing of K×Y[1] (CL at 5.03 cm), Y×P (CV at 11.4 cm³), K×Y (WSC at 1.65 g), Y×Y (EWC at 3.47 g), and K×Y (FWC at 5.22 g).

Regarding the time to petal wilting after pollination (TPS), the K×W parent crossing exhibited the longest duration (7.4 days), while, P×P parent crossing had the shortest (4.6 days). For the time to capsule swelling (TCS), the Y×P parent crossing required the longest period (24.2 days), whereas, W×W parent crossing was the quickest (13.6 days). Additionally, the capsule ripening (CR) duration was longest in the K×W parent crossing (125 days) and shortest in P×P parent crossing (106 days). The mean comparison results suggested that parent crossing of Y×P, K×Y, and W×W pollination types are particularly suitable for breeding programs, given their advantageous values across multiple traits. These findings indicate that parent crossing of Y×P, K×Y, and K×W demonstrate hybridization effects distinct from the parent varieties.

The genotypic variation coefficient was employed (Table 1) to assess variation within traits. Analysis of variance revealed significant differences among pollination types for all studied traits, indicating diverse responses and high variability among the varieties under self and cross-pollination conditions. Most traits exhibited a coefficient of variation above 20%, with the highest phenotypic and genotypic variation coefficients observed in capsule length (CL) during the trait appearance period. Traits such as TCS, FWC, EWC, WSC, and CV displayed high variation coefficients, whereas TPS and CR had the lowest variation coefficients. This suggests that genetic factors contributed more significantly than environmental factors to the variation observed in these traits. These findings contrast with those of Shao et al. (2010), who reported that a genetic variation coefficient of less than 20%. However, our results align with studies by Ye et al. (2008), Roychowdhury & Tah (2011), and Seeja et al. (2019), which also identified a high genetic variation coefficient. Notably, there was minimal difference between genotypic and phenotypic coefficients across all traits except TPS.

Characteristics correlation

The correlation analysis results (Table 2) revealed several significant relationships among the measured

Table 2 : Phenotypic correlation among 8 characters in 5 varieties of orchids

Character	WSC	TPS	TCS	FWC	CL	EWC	CV	CR
WSC	1							
TPS	-0.27^{ns}	1						
TCS	-0.79^{**}	0.22^{**}	1					
FWC	0.83^{**}	-0.27^{ns}	-0.72^{**}	1				
CL	0.73^{**}	-0.44^{**}	-0.68^{**}	0.83^{**}	1			
EWC	0.14^{ns}	-0.60^{**}	0.11^{ns}	0.14^{ns}	0.33^{ns}	1		
CV	0.64^{**}	0.25^{ns}-	-0.73^{**}	0.71^{**}	0.73^{**}	0.23^{ns}	1	
CR	0.34^{ns}	0.19^{ns}	-0.47^{*}	0.46^{*}	0.40^{*}	0.14^{ns}	0.62^{**}	1

WSC: seed weight, TPS: time to petal wilting after pollination, TCS: time to capsule swelling, FWC: full capsule weight, CL: capsule length, EWC: empty capsule weight, CV: capsule volume, CR: capsule ripening, ns: not significant, *: significant **: highly significant

traits. Seed weight (WSC) had a strong negative correlation with the time to capsule swelling (TCS) (-0.77), while showing a significant positive correlation with capsule length (CL), full capsule weight (FWC), and capsule volume (CV), all at 0.75 ($P \leq 0.01$). TCS demonstrated a negative correlation with CL (-0.71, $P \leq 0.01$), as well as with FWC (-0.77) and CV (-0.80) at the $P \leq 0.01$ level. Additionally, TCS was negatively correlated with capsule ripening (CR) (-0.40, $P \leq 0.05$) but showed a weak positive correlation with the time to petal wilting after pollination (TPS) (0.20, $P \leq 0.01$).

FWC was positively correlated with CL (0.73) and WSC (0.83) at $P \leq 0.01$, and also with CR (0.46, $P \leq 0.05$). CL displayed a positive correlation with CV (0.73, $P \leq 0.01$) and CR (0.40, $P \leq 0.05$), while it was negatively correlated with TPS (-0.44, $P \leq 0.01$). Notably, empty capsule weight (EWC) did not show any significant positive correlation with other traits ($P \leq 0.05$), but it had a negative correlation with TPS (-0.60, $P \leq 0.01$). CV was positively correlated with WSC (0.64) and CR (0.62) at $P \leq 0.01$.

The highest positive correlation coefficient observed was 0.83 between WSC and FWC, indicating a strong association between these traits. Conversely, the most significant negative correlation was found between WSC and TCS (-0.79). Overall, the analysis revealed a strong positive correlation among WSC, CL, CV, and FWC, suggesting that these traits are closely related and may be influenced by similar factors in the breeding process.

Cluster analysis

The dendrogram partitioning divided the self-pollination and cross-pollination treatments of *Phalaenopsis* into three main clusters based on the farthest average linkage method, with the dendrogram cutting between 0.11 and 0.25 (Fig. 4). The first cluster was distinctly separated from the second and third clusters based on the characteristics of TPS, TCS, CL, FWC, EWC, and WSC. The first cluster only showed overlap with the second and third clusters in terms of CR.

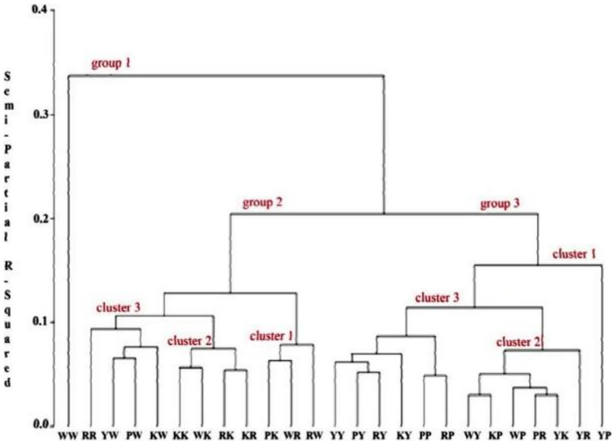
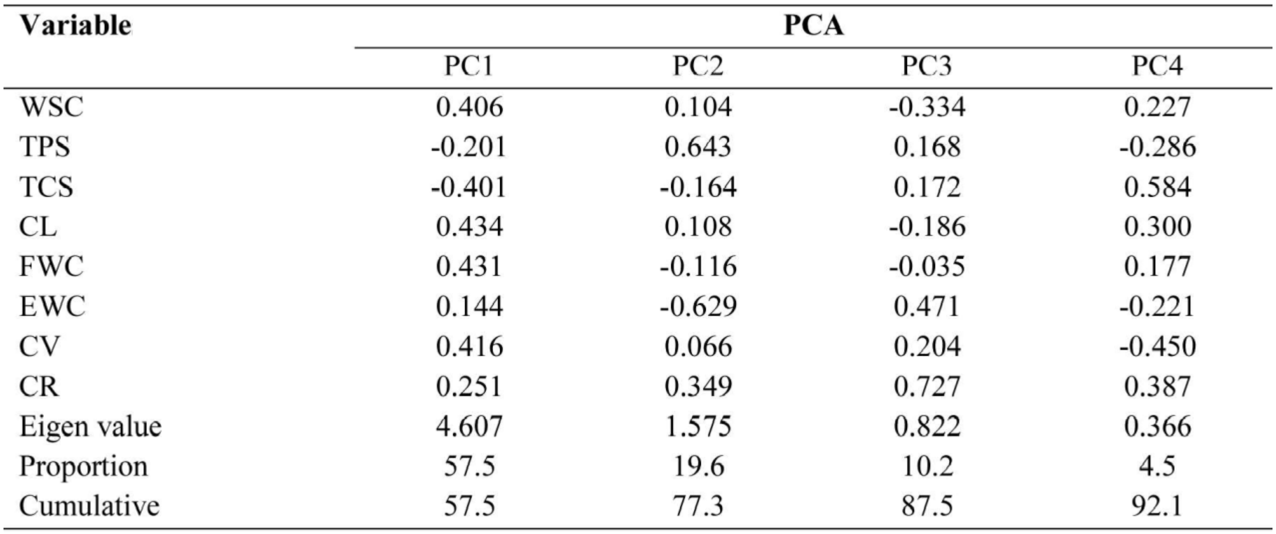


Fig. 4 : Grouping of different pollination types based on morphological and phenological pollination characteristics

The second and third clusters exhibited overlap within the average range for TPS, CL, FWC, WSC, EWC, CV, and CR. This overlap explains why these two clusters jointly separated from the first cluster in the analysis. However, the second and third clusters did not overlap in TCS was a key factor in their separation, which it was along with the low overlap in CL. In the third main cluster, the first sub-cluster was separated from the second and third sub-clusters primarily based on TCS and CV characteristics. Overlaps were observed among the first, second, and

Table 3 : Analysis of eigen value and cumulative variances of the principal component in all characteristics



Variable	PCA			
	PC1	PC2	PC3	PC4
WSC	0.406	0.104	-0.334	0.227
TPS	-0.201	0.643	0.168	-0.286
TCS	-0.401	-0.164	0.172	0.584
CL	0.434	0.108	-0.186	0.300
FWC	0.431	-0.116	-0.035	0.177
EWC	0.144	-0.629	0.471	-0.221
CV	0.416	0.066	0.204	-0.450
CR	0.251	0.349	0.727	0.387
Eigen value	4.607	1.575	0.822	0.366
Proportion	57.5	19.6	10.2	4.5
Cumulative	57.5	77.3	87.5	92.1

WSC: seed weight, TPS: time to petal wilting after pollination, TCS: time to capsule swelling, FWC: full capsule weight, CL: capsule length, EWC: empty capsule weight, CV: capsule volume, CR: capsule ripening

third sub-clusters (within the third main cluster) for TPS, CL, FWC, EWC, WSC, and CR, which is why they were grouped together within the third main cluster, the second and third sub-clusters showed overlap for TPS, TCS, CL, FWC, WSC, EWC, and CR characteristics, but they did not overlap for CV, leading to their separation into two distinct sub-clusters. Similarly, in the second main cluster, the first, second, and third sub-clusters overlapped on TPS, TCS, FWC, WSC, EWC, and CR, placing them within the same cluster. However, the first sub-cluster was separated from the second and third sub-clusters based on the average range of CL, with the second and third sub-clusters not overlapping on these traits. Additionally, these sub-clusters did not overlap on CV, further justifying their separation. Overall, the first, second, and third sub-clusters in the second main group were distinguished by mean differences in CL and CV. The cluster analysis also revealed that the self-pollinated variety Nottingham × Nottingham was grouped in the first cluster, Andorra × Andorra and Bucharest × Bucharest were in the second cluster, and Dubrovnik × Dubrovnik and Memphis × Memphis were in the third cluster.

Principal component analysis

The principal component analysis (PCA) results are detailed in Table 3, which includes eigen values, eigen vectors, and the cumulative and relative variance explained by each principal component. PCA was performed on the correlation matrix derived from the mean data of five self-pollination and twenty cross-pollination types. The analysis revealed that the first two principal components explained 77% of the variation in the pollination characteristics. Specifically, the first principal component (PC1) accounted for 57.5% of the variance, while the second principal component (PC2) accounted for 19.6%. The third component contributed an additional 10%, with subsequent components contributing minimally.

The high coefficient values in PC1 and PC2 highlight their significance in explaining the qualitative and quantitative variations in pollination types. The principal components had specific coefficients of 0.48 for PC1 and -0.24 for PC2 (Table 3). Notably, the traits with high coefficients in PC1 include capsule length (CL), seed weight (WSC), full capsule weight (FWC), and time to petal wilting (TPS), indicating their major influence on the variation between pollination types.

These equations indicate that PC1, with its higher coefficients, plays a significant role in differentiating between pollination types. The visualization of the data using a bi-plot diagram based on PC1 and PC2 (Fig. 5) further demonstrated the division of pollination types into three distinct groups.

Our findings align with previous studies, such as Erzurumlu et al. (2018), where the first three components explained 55% of the variance in native

The linear combinations for the first and second principal components are as follows:

$$PC1 = 0.406Ws - 0.201Syi - 0.401Ss + 0.434Lc + 0.431Wfc + 0.144Wec + 0.416Bc + 0.251Rc$$

$$PC2 = 0.104Ws + 0.643Syi - 0.164Ss + 0.108Lc - 0.116Wfc - 0.629WEC + 0.066Bc + 0.349Rc$$

orchid species, and Ashish et al. (2012), where the first two components accounted for 75% of the variance in *Dactylorhiza hatagirea.* This study's PCA results suggest that the first two principal components are effective for clustering and analysing variations in pollination types.

The distribution of pollination types based on the first two principal components of the principal component analysis is shown in the Fig. 5. At the bottom right of the chart, the self-pollination of Nottingham × Nottingham (parent crossing of W×W) is positioned in the first group, while the cross-pollination of Bucharest × Nottingham (parent crossing of R×W) is in the second group, which is characterized by high amounts in morphological characteristics (CL, CV, WSC, and FWC) but a lower amount in capsule ripening (CR).

In the top-right position, the self-pollinations of Bucharest × Bucharest (parent crossing of R×R) and Andorra × Andorra (parent crossing of K×K), as well as the cross-pollinations of Nottingham × Andorra (parent crossing of W×K) and Andorra × Nottingham (parent crossing of K×W), are located in regions with high yields for both morphological traits (CL, CV, and FWC) and phenological traits (TPS, TCS, and CR). Therefore, these combination crosses are more desirable compared to others.

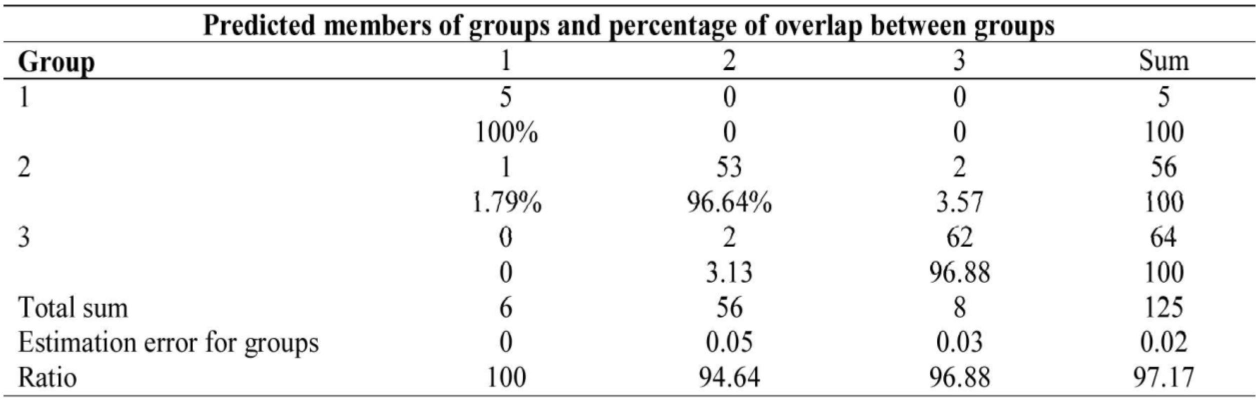
The second group includes pollination types such as Bucharest × Andorra (parent crossing of R×K), Andorra × Bucharest (parent crossing of K×R), Nottingham × Bucharest (parent crossing of W×R), Memphis × Andorra (parent crossing of P×K), Dubrovnik × Nottingham, and Memphis × Nottingham, which are situated in areas with intermediate yields for both morphological and phenological characteristics.

The third group consists of the self-pollinations of Memphis × Memphis (P×P) and Dubrovnik × Dubrovnik (Y×Y), along with cross-pollinations of Memphis × Bucharest (P×R), Dubrovnik × Andorra (Y×K), Nottingham × Memphis (W×P), Nottingham × Dubrovnik (W×Y), Andorra × Memphis K×P), Memphis × Dubrovnik (P×Y), Bucharest × Dubrovnik (R×Y), Bucharest × Memphis (R×P), and Andorra × Dubrovnik (K×Y). These are found in the lower region of the chart concerning the first and second components.

The results from the discrimination function analysis, indicating a 97.1% accuracy in grouping obtained from the cluster analysis and principal component analysis is presented in Table 4. The first group achieved 100% accuracy. Among the four multivariate tests conducted, Roy's Greatest Root yielded the highest F value (52.92, $P \leq 0.01$), demonstrating significance. Other tests, including Wilks' Lambda, Pillai's Trace, and Hotelling-Lawley Trace, also showed significant results ($P \leq 0.01$).

Heritability is crucial for selecting desirable traits, as high heritability suggests effective selection based on phenotypic traits. However, high heritability alone does

Table 4 : Discrimination function for grouping confirmation of evaluated pollination types



Predicted members of groups and percentage of overlap between groups				
Group	1	2	3	Sum
1	5	0	0	5
	100%	0	0	100
2	1	53	2	56
	1.79%	96.64%	3.57	100
3	0	2	62	64
	0	3.13	96.88	100
Total sum	6	56	8	125
Estimation error for groups	0	0.05	0.03	0.02
Ratio	100	94.64	96.88	97.17

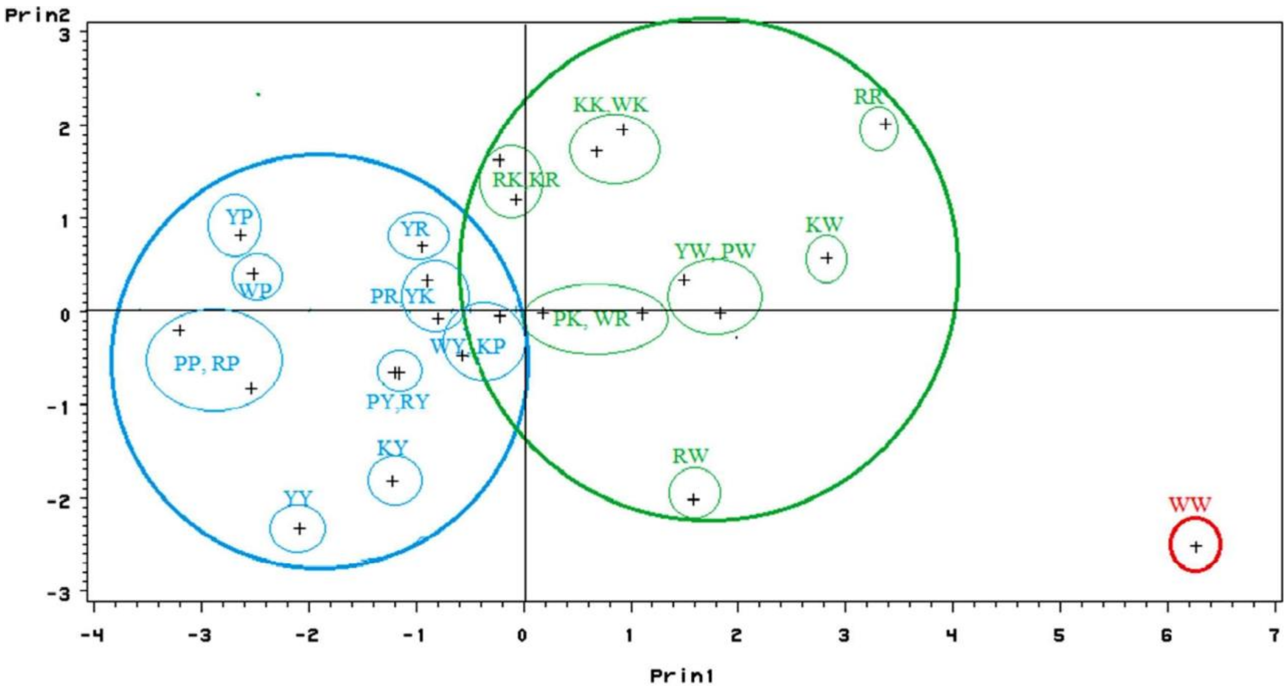


Fig. 5 : Distribution diagram of pollination types (bi-plot) based on the two first and second components of principal components analysis in five varieties of orchid *Phalaenopsis*

not guarantee genetic progress, which is essential for selecting the best individuals. High heritability coupled with significant genetic progress indicates that traits are controlled by additive genes, making them amenable to improvement through selective breeding. High variation coefficients observed in this study suggest substantial genetic diversity among the pollination types, facilitating precise selection for desired characteristics.

Overall, the study suggests that hybridization programs should focus on enhancing traits with high heritability and genetic progress, utilizing varieties with superior morphological and phenological characteristics. The PCA, cluster analysis, and discrimination function analysis provide a comprehensive approach for evaluating and selecting pollination types, with minimal overlap between groups, confirming the reliability of the classification and highlighting the potential for improved pollination yield through strategic hybridization.

CONCLUSION

Notably, capsule length (CL) exhibited the highest phenotypic and genotypic variation, while, capsule ripening (CR) showed high heritability (0.78) but minimal genetic progress (9%), indicating the involvement of non-additive genes. The cross Nottingham×Nottingham (parent crossing of W×W) pollination recorded highest values for CL, WSC, CV, and FWC, indicating its potential for use in hybridization programs. Cluster analysis and principal component analysis (PCA) revealed that Nottingham×Nottingham (parent crossing of W×W) and Dubrovnik×Memphis (parent crossing of Y×P) are particularly suitable for breeding due to their favourable morphological and phenological traits.

The bi-plot illustrated that pollination types with high first and second component values are advantageous for hybridization. The Nottingham variety, with high morphological yield, and the Andorra variety, with both high morphological yield and quality, do not significantly influence the quality and yield of Dubrovnik and Memphis varieties, suggesting recessive genes in these traits. Cross-pollinations such as Dubrovnik×Memphis and Dubrovnik×Bucharest were positioned in distinct clusters due to their varied component values.

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