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Morphological Characterization of *Phalaenopsis* spp. and Hybrids from Indonesia

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Abstract

The existence of *Phalaenopsis* species had declined in the wild. Efforts to preserve the species with artificial propagation through crosses to produce a better crop than the parents, often fail because of incompatibility of the parents. This could be minimized by performing characterization to determine the relationship between parental crosses. The purpose of this research was to study the morphological characters of 30 genotypes (five genotypes of *Phalaenopsis* species, i.e. *Phal. violacea*, *Phal. tetraspis*, *Phal. amboinensis*, *Phal. modesta*, and *Phal. cornu-cervi*, and 25 hybrid genotypes found in Indonesia). Morphological characterization was executed in accordance with the type of roots, stem, leaf, and flower using UPOV guidelines and Principal Coordinate Analysis (PCA). The results showed that the 70 morphological characters could be separated into 490 sub-characters that can be divided into 484 polymorphic sub-characters (98.78%) and six monomorphic sub-characters (1.22%). Data analysis with NTSYS program showed that there were eight clusters with 42% of coefficient similarity. The five *Phalaenopsis* species formed into one cluster. The similarity coefficient between *Phalaenopsis* spp. and the 25 hybrids was only 30%, 29-70% within species, and 39-64% between the hybrids. Matrix correlation of morphological markers value (r) was 0.88, showed goodness of fit for resemblance coefficient. The PCA clustering was not in line to those identified by the NTSYS tree cluster analysis.

INTRODUCTION

Phalaenopsis species in Indonesia had declined in the wild because of deforestation. Tsai (2011) classified *Phal. violacea*, *Phal. amboinensis*, and *Phal. Modesta* into section Amboinenses, *Phal. tetraspis* into section Zebrinae and *Phal. cornu-cervi* into section Polychilos. The hybrids found in Indonesia's market were mostly imported, this condition had impelled Indonesia to find the hybrids from its own breeding, so it will be adapted to local condition. Efforts to preserve the species with artificial propagation through crosses to produce a better crop than the parents, often fail because of incompatibility of the parents. This could be minimized by performing characterization to determine the relationship between parental crosses. The purpose of this research was to study the morphological characters of 30 genotypes (five genotypes of *Phalaenopsis* species, i.e. *Phal. violacea*, *Phal. tetraspis*, *Phal. amboinensis*, *Phal. modesta*, and *Phal. cornu-cervi*, and 25 hybrid genotypes found in Indonesia). Unfortunately, the hybrid genotypes do not have names because they were obtained from local traders who only classified them into standard and novelty hybrids based on the colour of the petals and the sepals.

MATERIALS AND METHODS

Morphological characterization was executed in accordance with the type of roots, stem, leaf, and flower using UPOV guidelines (2003) and Principal Coordinate Analysis (PCA) was performed further for 30 genotypes (five genotypes of *Phalaenopsis* species, i.e. no. 1 = *Phal. violacea*, 2 = *Phal. tetraspis*, 3 = *Phal. amboinensis*, 4 = *Phal. modesta*,

and 5 = *Phal. cornu-cervi*, and 25 hybrid genotypes found in Indonesia: no. 6 = H22, 7 = H21, 8 = H22, 9 = H23, 10 = H24, 11 = H25, 12 = H26, 13 =H27, 14 = H28, 15 = H29, 16 = H30, 17 =H31, 18 = H32, 19 = H33, 20 = H34, 21 = H35, 22 = H36, 23 = H37, 24 = H1, 25 = H2, 26 = H3, 27 = H4, 28 = H5, 29 = Phuket Beauty, 30 = Zauber Rose).

RESULTS AND DISCUSSION

Morphological variability was observed in the *Phalaenopsis* spp. and also within the hybrids that can be used as morphological characterization materials. The results showed that the 70 morphological characters could be separated into 490 sub-characters that can be divided into 484 polymorphic sub-characters (98.78%) and six monomorphic sub-characters (1.22%).

Main marker of *Phalaenopsis* found on six monomorphic morphological sub-characters was observed both in the species and the hybrids, and will not change with crossing.These characters were the leaf and stem growth type, leaf edge shape, leaf position on the stem, petal formation, spur, and the number of polinia.

Data analysis with NTSYS program showed that there were eight groups with 42% of coefficient similarity. *Phalaenopsis* spp., i.e. *Phal. violaceae*, *Phal. amboinensis*, *Phal. tetraspis*, *Phal. modesta*, *Phal. cornu-cervi*, formed into one cluster. This cluster formed into three clusters, i.e. *Phal. violaceae*, *Phal. amboinensis* that came from Amboinenses section that made the first cluster, while *Phal. tetrapis* from Zebrina section made another cluster, and *Phal. modesta* from Amboinenses section and *Phal. cornu-cervi* from Polychilos section clustered together as the third cluster. *Phal. modesta* and *Phal. cornu-cervi* came from different section (Christenson, 2001), but in this study they formed into 1 cluster that was consistent with the earlier study of Fatimah and Sukma (2011) that used 16 microsatellites as markers, and Niknejad et al. (2009) that formed the grouping using RAPD that reflected the fundamental heterotic patterns of *Phalaenopsis* and the widespread practice of producing new accessions by crossing species of *Phalaenopsis* for improvement of orchid, and showed a clear grouping of different species of *Phalaenopsis* according to classification in different section. More sampling of the plants with different sampling strategies and from different localities could resolve this inconsistency with previous report.The only hybrid that formed the second group is H23 that has similarity coefficient 0.32 with the first group of *Phalaenopsis* species. Padolina et al. (2006) study on phylogenetic reconstruction of *Phalaenopsis* used nuclear and chloroplast DNA sequence data and used *Phalaenopsis* as natural system for assessing methods to reconstruct hybrid evolution in phylogenetic analysis on fourteen Phalaenopsis species and seven horticultural hybrids to create a real dataset with which to test phylogenetic network reconstruction methods. Neighbor-Net was able to predict accurately the parents of the hybrids in only about half of the datasets tested, and there were so many false positives that it was impossible to distinguish the hybrids from the species.

The 3rd-8th group consisted of hybrids with morphological similarity coefficient 0.39-0.64 (Fig. 1). The third group formed by hybrids of H24, H26, H27, H25, H30, H28, H6, H7, H8, H11, and H21. The fourth group consisted of H20 and H22.The fifth group consisted of H14.The sixth group consisted of H9, H10, and H19.The seventh group consisted of H12, H15, H13, and H18.The eighth group consisted of H16 and H17.

The similarity coefficient between *Phalaenopsis* spp. and the 25 hybrids found in Indonesia was only 30%, 29-70% within species, and 39-64% within the hybrids.These data showed the distinctiveness between the species and the hybrids, within species, and within the hybrids. Matrix correlation of morphological markers value (r) was 0.88, showed goodness of fit for resemblance coefficient.

Morphological marker is influenced by environment, but this variability is important because it would have been observed by phenotypic difference after selfing, crossing between siblings and crossing. Morphological character usually is a qualitative character, which are the shape and color of plant organs, controlled by single gene (Rieseberg (1992) on maize, and Reddy et al. (2008) on plant color in sorghum due to

anthocyanin pigmentation).

The *Phalaenopsis* spp. clustered together, and the hybrid formed 7 clusters. The possibilities of crossing between *Phalaenopsis* spp. and the hybrids, within *Phalaenopsis* spp. and within the hybrids were confirmed by the low value of similarity coefficient.This large parent-plant variation is required to ensure that constantly better and new varieties can be developed.It consists of specially selected parent plants and botanical species.New varieties are developed by crossing the plants from the stock and by selecting the best of plants from the offspring, which are then allowed to reproduce (International Union for the Protection of New Varieties of Plants, 2003).

Morphological character that formed the clustering was analyzed with principal coordinate analysis. Character data that can be depicted in five principal coordinate and cluster analysis was 70% of the whole data (Fig. 2). The separation was not in line to those identified by the NTSYS tree cluster analysis. This condition was caused by the different analysis that being used.

The quantitative data showed that in *Phalaenopsis* species observed, only *Phal. tetraspis* that has longer inflorescence (23.4 cm), where as the other species has 5.7-9.7 cm inflorescence length.The hybrids that have ≤10 cm inflorescence length are H13, H14, H18, H23, and H28.The inflorescence in the shape of panicle was found on H2, H10, H11, H16, H17, H18, H21, H22, and H23, while the others in the shape of raceme (data not shown).

The petal width and length varied in some genotype wider, and others lengthier. *Phalaenopsis* species has smaller flower (2.7-5.5 cm in width, and 2.5-5.7 cm in length) than the hybrids (1.5-5.5 cm in width, and 1.1-6.8 cm in length; data not presented). Lesar et al. (2012) used seven commercial hybrids differed in size and color of flowers and number of inflorescences and flowers. He used an overlap of groups among combinations of cross crossing with small flowers × big flowers and reciprocal crossing of big flowers × small flowers and found that smallest flowers was not compatible with any test plants. The Orchid Mall (2013) stated that size of *Phalaenopsis* hybrid white that considered big is ≥13 cm, this showed that hybrids in Indonesia were considered small in size.

Efforts to preserve the species with artificial propagation through crosses to produce a better crop than the parents, often fails because of incompatibility of the parents. Stock (2005) found that almost all of U.S. breeding has been with diploids, triploids, and the aneuploids that have resulted from breeding triploid reds to diploids and tetraploids. Aneuploids were also produced through attempts to increase flower size by breeding tetraploid reds to tetraploid pinks and stripes. Most attempts to increase size and flower count with diploid red breeding lines have resulted in the production of triploids. Unfortunately, triploid *Phalaenopsis* will often probably produce seeds, and the results of using 'anything that will breed', has produced a sea of aneuploids, which are then used in further breeding attempts.The outcome of this type of breeding is the well-known 'sterility barrier' so common in today's *Phalaenopsis* breeding. Griesbach (1985) stated that most commercially valuable orchids are hybrids. In some instances, their hybridity can be quite complex involving up to four genera. Thus, both allo- and autopolyploidy could play a role in increasing fertility. Lu and Bridgen (1997) stated that sterile diploid hybrids revealed abnormal meiotic behaviors in *Alstroemeria aurea* × *A. caryophyllae* and the aneuploid chromosome numbers, ranging from $2n$=1 to $2n$=18. The sterility of this hybrid is not caused by parental chromosome differences, but other complex fertility/sterility-regulating mechanisms are involved too. Further study on chromosome number is needed to anticipate the different number of ploidy found in the existing genotypes.

CONCLUSIONS

Variability in morphological character was found in five *Phalaenopsis* species and 25 hybrids in Indonesia showed by similarity coefficient between *Phalaenopsis* spp. and the 25 hybrids found in Indonesia was 30%, 29-70% within species, and 39-64% between the hybrids. This condition would be the basis for building new varieties in Indonesia,

since it is required to ensure that constantly better and new varieties can be developed.

ACKNOWLEDGEMENTS

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Figures

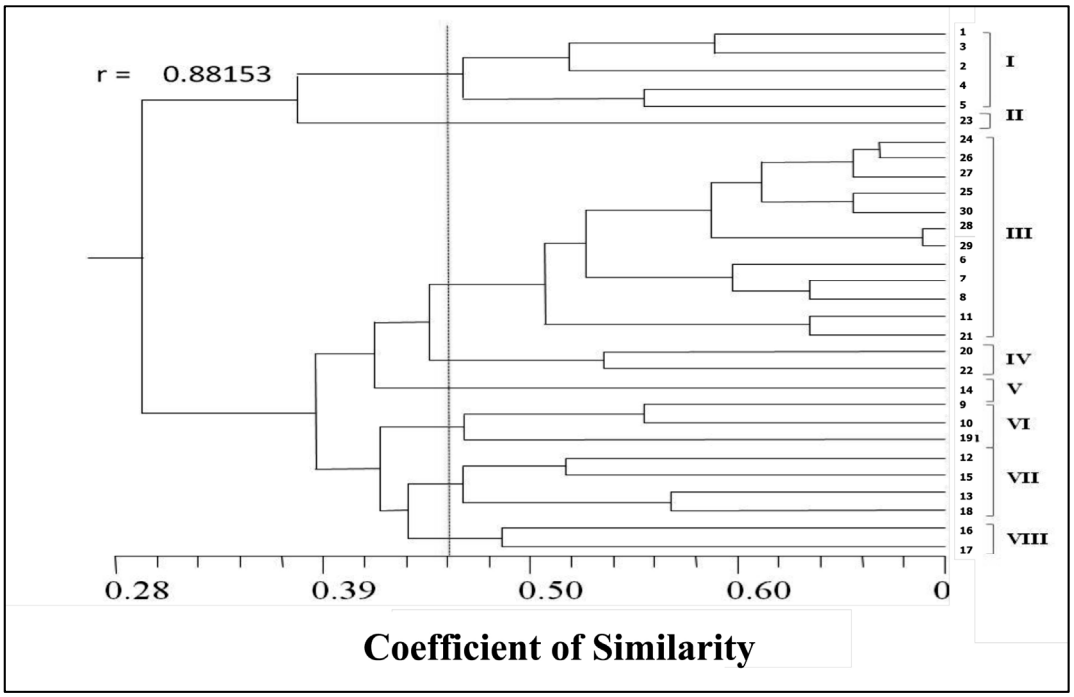

Fig. 1. Dendrogram of cluster analysis of morphological characters of 30 *Phalaenopsis* genotypes (No. 1-5 are species: 1 = *Phal. Violecea*, 2 = *Phal. tetraspis*, 3 = Phal. *amboinensis*, 4 = *Phal. Modesta*, 5 = *Phal. cornucervi*, 6-30 are hybrids: 6 = H22, 7 = H21, 8 = H22, 9 = H23, 10 = H24, 11 = H25, 12 = H26, 13 =H27, 14 = H28, 15 = H29, 16 = H30, 17 =H31, 18 = H32, 19 = H33, 20 = H34, 21 = H35, 22 = H36, 23 = H37, 24 = H1, 25 = H2, 26 = H3, 27 = H4, 28 = H5, 29 = Phuket Beauty, 30 = Zauber Rose).

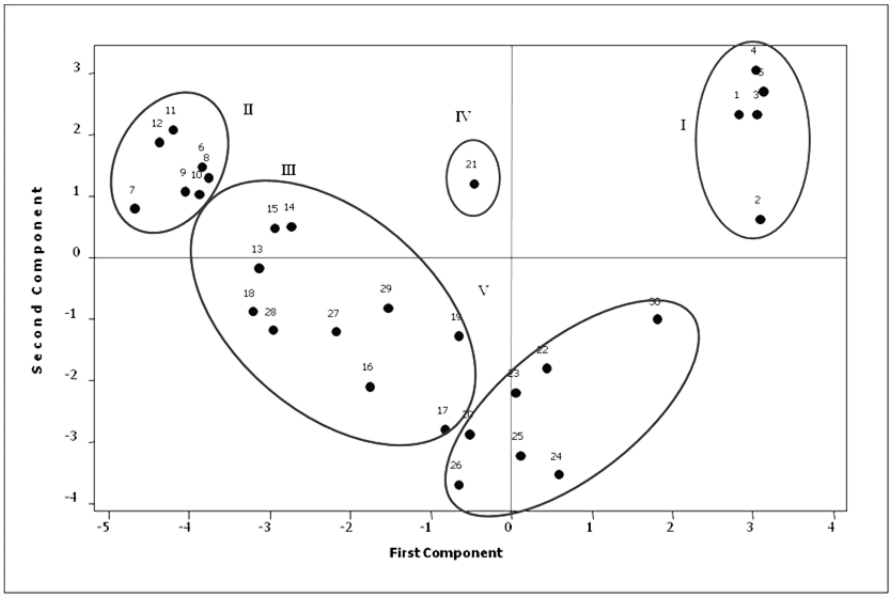

Fig. 2. Morphological relationship among 30 *Phalaenopsis* genotypes with five groups derived from principal coordinate analysis.