Assessing genetic divergence in interspecific hybrids of *Aechmea gomosepala* and *A. recurvata* var. *recurvata* using inflorescence characteristics and sequence-related amplified polymorphism markers

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ABSTRACT. Conventional hybridization and selection techniques have aided the development of new ornamental crop cultivars. However, little information is available on the genetic divergence of bromeliad hybrids. In the present study, we investigated the genetic variability in interspecific hybrids of *Aechmea gomosepala* and *A. recurvata* var. *recurvata* using inflorescence characteristics and sequence-related amplified polymorphism (SRAP) markers. The morphological analysis showed that the putative hybrids were intermediate between both parental species with respect to inflorescence characteristics. The 16 SRAP primer combinations yield 265 bands, among which 154 (57.72%) were polymorphic. The genetic similarity was an average of 0.59 and ranged from 0.21 to 0.87, indicating moderate genetic divergence among the hybrids. The unweighted pair group method with arithmetic average (UPGMA)-based cluster analysis distinguished the

hybrids from their parents with a genetic distance coefficient of 0.54. The cophenetic correlation was 0.93, indicating a good fit between the dendrogram and the original distance matrix. The two-dimensional plot from the principal coordinate analysis showed that the hybrids were intermediately dispersed between both parents, corresponding to the results of the UPGMA cluster and the morphological analysis. These results suggest that SRAP markers could help to identify breeders, characterize F_1 hybrids of bromeliads at an early stage, and expedite genetic improvement of bromeliad cultivars.

Key words: *Aechmea*; Interspecific hybrid; Genetic divergence; SRAP

INTRODUCTION

Ornamental bromeliad (Bromeliaceae) is one of the most popular potted flowers worldwide. *Aechmea* constitutes an important component of bromeliad industry due to its varying flower color and type and increased cold resistance compared to other bromeliads (*Neoregelia*, *Guzmania*, and *Vriesea*). Thus, it is important to develop new *Aechmea* hybrids for enriching the bromeliad cultivars.

The conventional hybridization and selection system is the most efficient method of breeding new bromeliad varieties. The Florida Committee of Bromeliads Society (http://www.fcbc.org) reported that more than 90% of the ornamental bromeliad varieties are inter-specific hybrids, underscoring the importance of developing novel bromeliad hybrids in order to meet market demands. Therefore, elucidating and quantifying the genetic variability in a population is important for breeders to study the genetic relationships between hybrids and parents. This information could also guide the selection of promising materials and new crosses and elucidate the mechanism by which horticultural traits are inherited. Previous studies have assessed the genetic diversity among various bromeliad species (Izquierdo and Pinero, 2000; Barfuss et al., 2005; Sousa et al., 2008; Palma-Silva et al., 2009; Boisselier-Dubayle et al., 2010); however, little information is available on the genetic divergence among interspecific hybrids in *Aechmea*.

The sequence-related amplified polymorphism (SRAP) technique preferentially targets exon sequences and promoter regions (Li and Quiros, 2001). SRAP profiling has been used to assess genetic diversity (Han et al., 2008; Mishra et al., 2011), construct genetic maps (Zhang et al., 2011), and gene tagging (Li et al., 2003). These varied applications demonstrate its resolving capacity in genetic studies. The present study was designed to evaluate genetic variability among F_1 hybrids derived from an interspecific cross between *Aechmea gomosepala* and *A. recurvata* var. *recurvata*.

MATERIAL AND METHODS

Plant materials and DNA isolation

Two *Aechmea* species, *A. gomosepala* and *A. recurvata* var. *recurvata*, were used to construct an interspecific hybrid population. The artificially controlled hybridization was car-

ried out according to the method as described by Vervaeke et al. (2003). Both parents and the derived F_1 hybrids were maintained at the Flower Research and Development Centre, Zhejiang Academy of Agricultural Sciences.

Young leaves were collected from each accession, frozen with liquid nitrogen, and ground into a powder. Genomic DNA was isolated following a cetyltrimethylammonium bromide (CTAB)-based procedure (Murray and Thompson, 1980). DNA concentration was estimated by comparison with known concentrations of lambda DNA on 0.8% agarose gels.

SRAP profiling

A total of 18 SRAP primer combinations of 6 forward primers and 3 reverse primers (Table 1) were initially used to screened for polymorphisms. As a result, 16 SRAP primer combinations were selected for their high level of polymorphism and clear banding patterns. All PCR amplifications were performed in a 10-µL reaction volume containing 1X PCR buffer, 3 mM Mg^{2+}, 200 µM dNTP, 0.5 U Taq DNA polymerase (Takara, Dalian, China), 10 µM of each primer, and 25 ng DNA template. The amplification program was based on the report by Li and Quiros (2001) as follows: an initial denaturing step at 94°C for 5 min followed by 5 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 1 min, followed by 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 7 min. PCR products were electrophoresed on 8% non-denaturing polyacrylamide gels, run at 300 V for 2.5 h in 0.5X TBE buffer, and visualized by silver staining.

Table 1. Primer sequences for sequence-related amplified polymorphism analysis.

Forward primer	Sequence (5'→3')	Reverse primer	Sequence (5'→3')
Me20	GAGTATCAACCCGGATT	Em1	GACTGCGTACGAATTAAT
Me21	GTACATAGAACCGGAGT	Em2	GACTGCGTACGAATTTGC
Me22	TACGACGAATCCGGACT	Em3	GACTGCGTACGAATTGAC
		Em4	GACTGCGTACGAATTTGA
		Em5	GACTGCGTACGAATTAAC
		Em6	GACTGCGTACGAATTGCA

Data scoring and statistical analysis

Amplified bands were scored as present or absent (1 or 0) across the 2 parental species for each primer combination. For each primer combination, the total number of amplified bands (TB), the number of polymorphic bands (PB), the percentage of polymorphic bands (PPB), and polymorphic information content (PIC) were calculated by PowerMarker v3.25 (Liu and Muse, 2005). The resolving power (Rp) of a primer combination was estimated through a formula Rp = ΣIb. Band informativeness (Ib) was calculated as Ib - 1 - (2 x |0.5 - p|), where *p* is the proportion of the 46 accessions that contain the bands (Prevost and Wilkinson, 1999). The PopGene 1.32 software (Yeh, 2000) was used to calculate Nei's genetic diversity and Shannon's information index. A dendrogram was constructed based on Nei's similarity coefficients using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of NTSYS-pc 2.2 (Rohlf, 2005). The cophenetic value (COPH) routine and MXCOP modules were used to assess the goodness-of-fit between the cluster analysis and original matrix. The principal coordinated analysis (PCoA) was also conducted to differentiate

the hybrids and parents in a two-dimensional array of eigenvectors using the DCENTER and EIGEN modules of the NTSYS-pc program.

RESULTS

Comparative morphology of parental species and their hybrids

Both parental species *A. gomosepala* and *A. recurvata* var. *recurvata* had spike-like inflorescence. The spikes of the former were slender and those of the latter were denser. The hybrid plants also had a spike-like inflorescence, but the spikes were morphologically distinct from either of the parental species (Figure 1). The spikes of the hybrids varied in size and were intermediate between both parental species. In addition, the bracts of the hybrids were homogenously scarlet, similar to *A. recurvata* var. *recurvata*, while the florets were homogenously blue, similar to *A. gomosepala*.

Figure 1. Inflorescence characteristics of *Aechmea gomosepala* (**A**), *A. recurvata* var. *recurvata* (**B**), and their interspecific F_1 hybrids (**C**).

SRAP performance

The result of SRAP analysis is shown in Table 2. For the 40 interspecific hybrids and 2 parental species, the 16 SRAP primer combinations yielded 265 bands, among which 154 (57.72%) were polymorphic. The TB generated by each primer combination ranged from 11 to 23, while the PB ranged from 3 to 17. The PIC was generally low, with an average of 0.31 and ranging from 0.25 to 0.36. The Rp of the informative primer combinations ranged from 1.81 to 12.04 with an average of 6.20. The primer combination Me1Em20, Me3Em21, and Me5Em21 showed the highest Rp values (10.38, 10.40, and 12.04, respectively).

Table 2. Characteristics of examined sequence-related amplified polymorphism primer combinations in the *Aechmea* hybrids.

PC	TB	PB	PPB (%)	PIC	Rp	h	I
Me21Em1	21	14	66.67	0.35	10.38	0.45	0.64
Me21Em1	15	5	33.33	0.31	3.05	0.40	0.58
Me22Em1	13	9	69.23	0.32	5.86	0.41	0.60
Me20Em2	21	13	61.91	0.32	9.33	0.45	0.65
Me21Em2	23	10	43.48	0.29	6.10	0.37	0.54
Me22Em2	17	16	94.12	0.29	8.14	0.30	0.47
Me20Em3	17	6	35.29	0.32	3.33	0.35	0.53
Me21Em3	19	15	78.95	0.32	10.40	0.35	0.52
Me22Em3	10	4	40.00	0.30	2.52	0.28	0.44
Me20Em4	14	11	78.57	0.36	8.67	0.39	0.57
Me21Em4	19	8	42.11	0.28	4.24	0.26	0.41
Me20Em5	17	3	17.65	0.30	1.81	0.26	0.42
Me21Em5	23	17	73.91	0.32	12.04	0.34	0.51
Me20Em6	11	5	45.46	0.25	2.09	0.33	0.49
Me21Em6	12	7	58.33	0.27	3.66	0.28	0.43
Me22Em6	13	11	84.62	0.33	7.62	0.36	0.54
Average	16.56	9.63	57.72	0.31	6.20	0.35	0.52
Total	265	154	-	-	99.25	-	-

PC = primer combination; TB = number of total bands; PB = number of polymorphic bands; PPB = percentage of polymorphic bands; PIC = polymorphic information content; Rp = resolving power; h = Nei's (1973) gene diversity; I = Shannon's information index (Lewontin, 1972).

Of the 154 polymorphic bands, 62 were verified in *A. gomosepala*, 63 in *A. recurvata* var. *recurvata*, 12 in both parental species, and 17 in hybrids but not in parents. A typical amplification profile is shown in Figure 2.
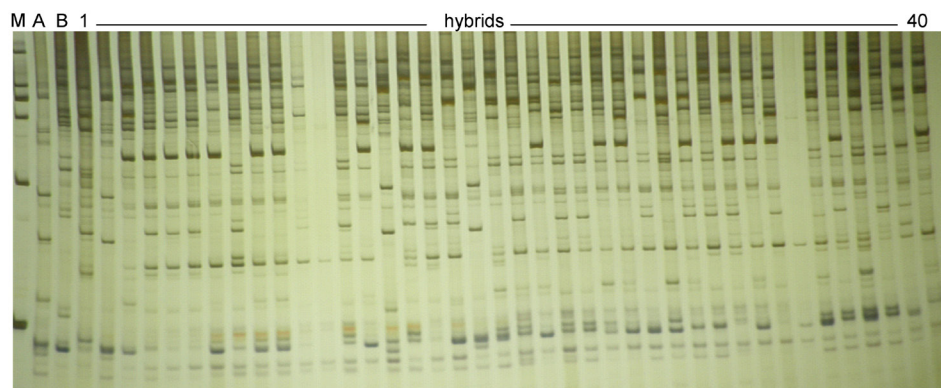
Figure 2. A typical example of sequence-related amplified polymorphism profiles of the 40 interspecific hybrids together with both parental species, obtained from Me21Em5. *Lane M* = DNA marker ladder; *lane A = Aechmea gomosepala*; *lane B = A. recurvata* var. *recurvata*; *lanes 1* to *40* = the interspecific hybrids derived from *A. gomosepala* x *A. recurvata* var. *recurvata*.

Genetic divergence

The h ranged from 0.26 to 0.45 with an average of 0.35, while the I varied from 0.41 to 0.65 with an average of 0.52 (Table 2). The genetic similarity ranged from 0.21 to 0.87 with an average of 0.59 for the whole accessions under study. Additionally, the genetic similarity was normally distributed (Figure 3).
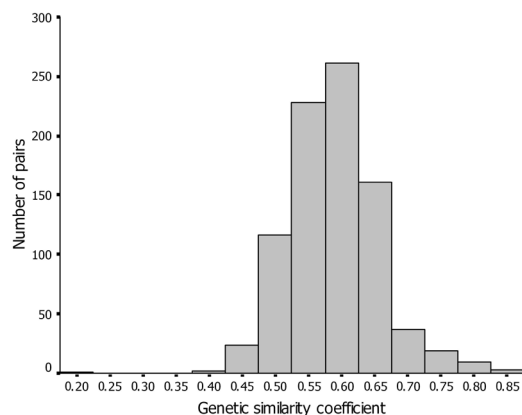
Figure 3. Frequency distributions of sequence-related amplified polymorphism-based genetic similarity estimates for possible pairs of the interspecific hybrids together with both parental species.

The dendrogram generated by UPGMA cluster analysis based on Nei's genetic distance is shown in Figure 4. The cophenetic correlation was estimated to be 0.93, indicating a good fit between the dendrogram and the original distance matrix. According to the dendrogram, the 40 hybrids were grouped into 2 main clusters, and the 2 parental species were clustered into 2 separate clades with a genetic distance of 0.54. The hybrids were distinguishable from the 2 parental species.
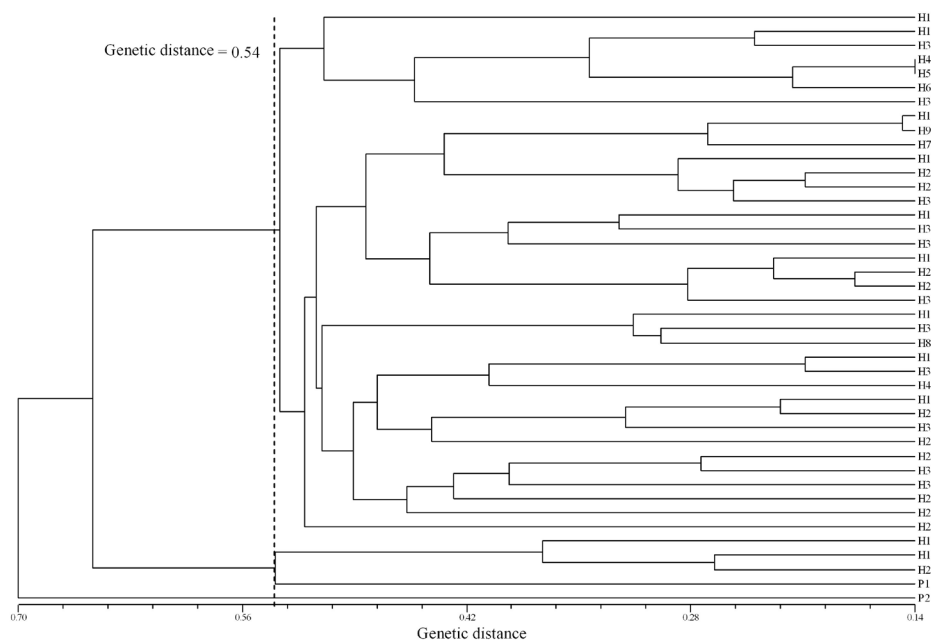
Figure 4. Dendrogram of the interspecific hybrids and both parental species, derived from the UPGMA cluster analysis based on Nei's genetic distance matrix. The codes, P1, P2, and H1 to H40, listed on the right of the dendrogram refer to *Aechmea gomosepala*, *A. recurvata* var. *recurvata*, and the interspecific hybrids, respectively.

A two-dimensional plot of the PCoA based on the similarity matrix is shown in Figure 5. The first 2 ordinates (PC1 and PC2) accounted for 26.8 and 22.0%, respectively, of the total variation in SRAP markers among the accessions. Generally, the two-dimensional plot demonstrated the expected tendency with an intermediate distribution of the interspecific F_1 hybrids relative to parents and that the F_1 hybrids dispersed in 2 clusters. As such, the PCoA results generally reinforced the cluster analysis as mentioned above.
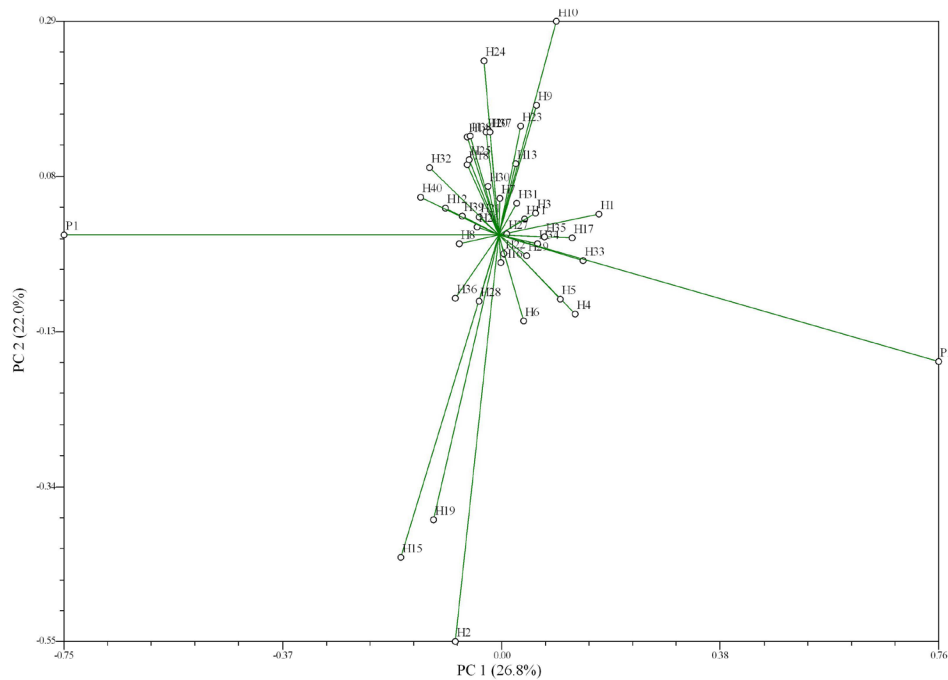
Figure 5. Two-dimensional plot with vectors of principal coordinate analysis (PCoA) of the interspecific hybrids relative to both parental species. The first two principal coordinates (PC1, PC2) accounted for 26.8 and 22.0% of the molecular variations. The codes, P1, P2 and H1 to H40, refer to Figure 4.

DISCUSSION

Conventional artificial hybridization and selection techniques have contributed to the development of new cultivars of ornamental crops, including bromeliad. Since bromeliad is a perennial crop that needs about 4 years after seeding to show its typical flower traits, it is time-consuming to identify the hybrids based on phenotype alone. Additionally, the traditional method of hybrid identification based on morphological traits can be influenced by environmental factors and thus lacks the resolution to identify hybrids at an early stage. Advances in DNA fingerprinting techniques have provided diagnostic molecular markers for facilitating the identification of hybrids, including RAPD (Oliveira et al., 2003; Dhillon et al., 2009; Bharathi et al., 2011), ISSR (Bianco et al., 2011), AFLP (Divakaran et al., 2006), and SSR (Saxena et al., 2010; Fu et al., 2011). Recently, many studies have confirmed the high infor-

mativeness and reproducibility of SRAP markers for identifying and authenticating hybrids in different crops (Liu et al., 2007; Hao et al., 2008; Mishra et al., 2011). In order to provide information on hybridization and selective breeding programs for *Aechmea* bromeliads, this study investigated the genetic divergence and hybrid nature of interspecific offspring of *Aechmea* bromeliads using inflorescence characteristics and SRAP profiles. The putative hybrids had inflorescence traits of both parents, suggesting their hybrid nature and the possibility of improving *Aechmea* bromeliads through artificial hybridization. The PPB of SRAP primer combinations ranged from 17.65 to 94.12% with an average of 57.72%, and the marker-based genetic similarity ranged from 0.21 to 0.87 with an average of 0.59. This showed moderate genetic divergence among the inter-specific hybrids investigated.

In this study, most of the primer combinations did not show specific bands in all hybrids despite their presence in the parental species. This demonstrates that the hybrid nature of the offspring could not be determined based on a single locus. However, their hybrid nature could be confirmed by combination of several loci. This result is similar to a previous report that evaluated cabbage hybrids (Liu et al., 2007). Here, 17 (~11%) bands of non-parental origin were observed in some hybrids. Similar results have also been reported in maize (Abdel-Mawgood et al., 2006), *Jatropha* (Dhillon et al., 2009), and grapes (Bianco et al., 2011). As discussed previously, these hybrid-specific bands may result from recombination, mutation, and rearrangement during the crossing-over phase of meiosis (Dabkevičiene et al., 2008). Similarly, chromosomal crossing-over during meiosis may result in the loss of priming sites, causing markers that are present in parents but not in hybrids (Smith et al., 1996).

The clustering of accessions by UPGMA demonstrates that the hybrids were grouped into 2 subgroups distinct from the 2 parental species. The cluster analysis is more sensitive to closely related individuals, whereas the PCoA is more informative for distances among major groups (Hauser and Crovello, 1982). Therefore, the multivariate approach, PCoA, is often used to complement the cluster analysis (Bianco et al., 2011; Tantasawat et al., 2011). Compared to the UPGMA cluster analysis in this study, the two-dimensional PCoA plot separated the hybrids and both parents into more distinct groups, in agreement with the UPGMA clustering and the morphological analysis. Thus, the present study confirms that SRAP profiling is a practical approach for unequivocally showing the relationship among hybrids and their parents.

In conclusion, this study showed that SRAP profiling was efficient and was able to identify the moderate genetic divergence among the inter-specific hybrids of *Aechmea* bromeliads. These results suggested that SRAP markers could help identify breeders, characterize the F_1 offspring of bromeliads at an early stage, and expedite the genetic improvement of bromeliad cultivars.

ACKNOWLEDGMENTS

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