The identification of phylogenic relationship of six orchid species and hybrids using amplified fragment length polymorphism (AFLP) fingerprinting method

The materials include three species of Cyc. warscewiczii, Cyc. haagii, and Morm. badium and three hybrids of Cycd. Jumbo Puff, Cycd. Taiwan Gold, and Cycd. Jumbo Jewel used in this study. First of all, DNA was isolated from leaves or stems of these species and hybrids for DNA fingerprinting using amplified fragment length polymorphism (AFLP) method (Vos et al., 1995), which is a reliable method for biological studies such as genetic mapping (Mackill et al., 1996) and phylogenic relationship (Chang and Veilleux, 2009). The AFLP procedure consists of DNA digestion with two restriction enzymes of *Eco*RI and *Mse*I, ligation of specific adaptors to digested DNA fragments, two rounds of DNA amplifications each with pre-selective and specific primers using polymer chain reaction (PCR), amplified products subjected to polyacrylamide gel electrophoresis for separation of different sizes of DNA fragments, and DNA image development by silver staining.

The fingerprinting result with 5 combinations of primer pairs (E-TGA combined with each of M-CAA, CAT, CAC, CTA, or CTT) is shown in Fig. 1. Each set of primer pairs can exhibit its own DNA pattern among six samples. Otherwise, in every set of primers, some of species have more common DNA fragments to each other than the others. For an example of the E-TGA/M-CTT primer combination (highlighted in maroon color in Fig. 1), there are more common bands between Cycd. Jumbo Puff and Cycd. Taiwan Gold than to others; the same situation also exists between Cycd. Jumbo Jewel and Cyc. haagii. In a proposed pedigree, Cyc. haagii was assumed to be a grandparental line for Cycd. Taiwan Gold (Fig. 2). However, based on comparison of fingerprint patterns, unique DNA fragments of Cyc. haagii especially cannot find a match in that of Cycd. Taiwan Gold (indicated by arrows under E-TGA/M-CTT in Fig. 1). Therefore, this evidence indicates that there could be no pedigree relationship between Cyc. haagii and Cycd. Taiwan Gold. In contrast, there is a strong pedigree relationship between Cycd. Jumbo Jewel and Cyc. haagii to know that Cycd. Jumbo Jewel is derived from the cross of Cyc. haagii and Morm. badium (Fig. 2).

The fingerprinting data of six samples from all of 5 primer combinations can be used for phylogenic analysis by Bio++ program to generate similarity matrix (Table 1) and dentrogram (Fig. 3). In Table 1, the similarity level of Cyc. haagii is about 37% and 36% to Cycd. Taiwan Gold and Cycd. Jumbo Puff, respectively, and that of Cycd. Taiwan Gold to Cycd. Jumbo Puff is up to 83%. This result seems to support that Cyc. haagii is highly diverse from both of Cycd. Taiwan Gold and Cycd. Jumbo Puff. The dentrogram also shows that two of Cycd. Taiwan Gold and Cycd. Jumbo Puff are clustered together in a subgroup with far distance from Cyc. haagii (Fig. 3).

Overall evidence seems to reject that Cycd. Taiwan Gold is a progeny from the pedigree of Cyc. haagii. Moreover, Cycd. Taiwan Gold could have a sibling relationship with Cycd. Jumbo Puff because they seem to be originated from the cross of Cyc. warscewiczii and Morm. badium.

Literature cited

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Mackill D. J. et al. (1996) Level of polymorphism and genetic mapping of AFLP markers in rice. Genome 39: 969-977.

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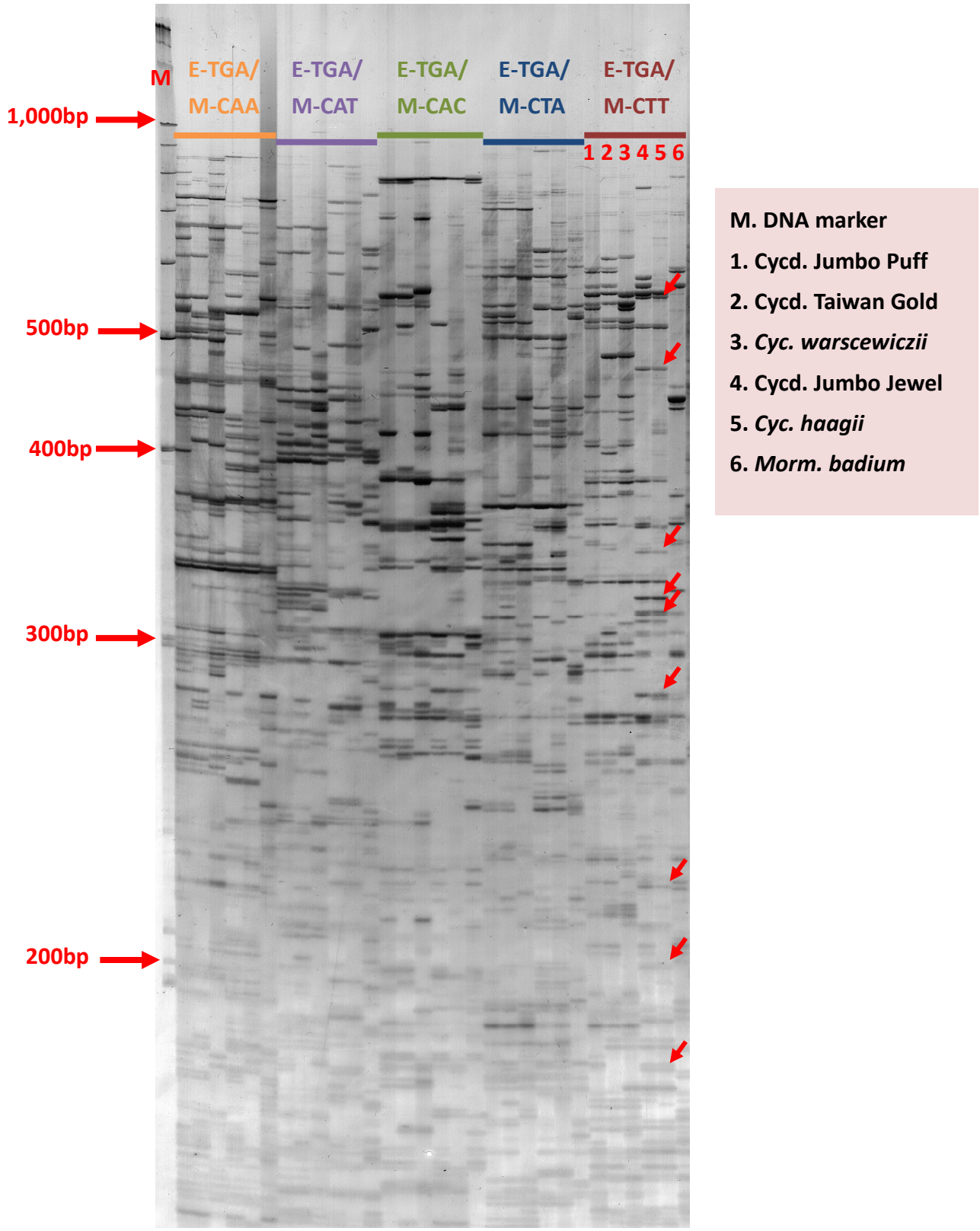

Fig. 1. The fingerprints of six orchid species and hybrids generated by AFLP method. In the E-TGA/M-CTT set, unique DNA fragments for Cyc. haagii are indicted by arrows.

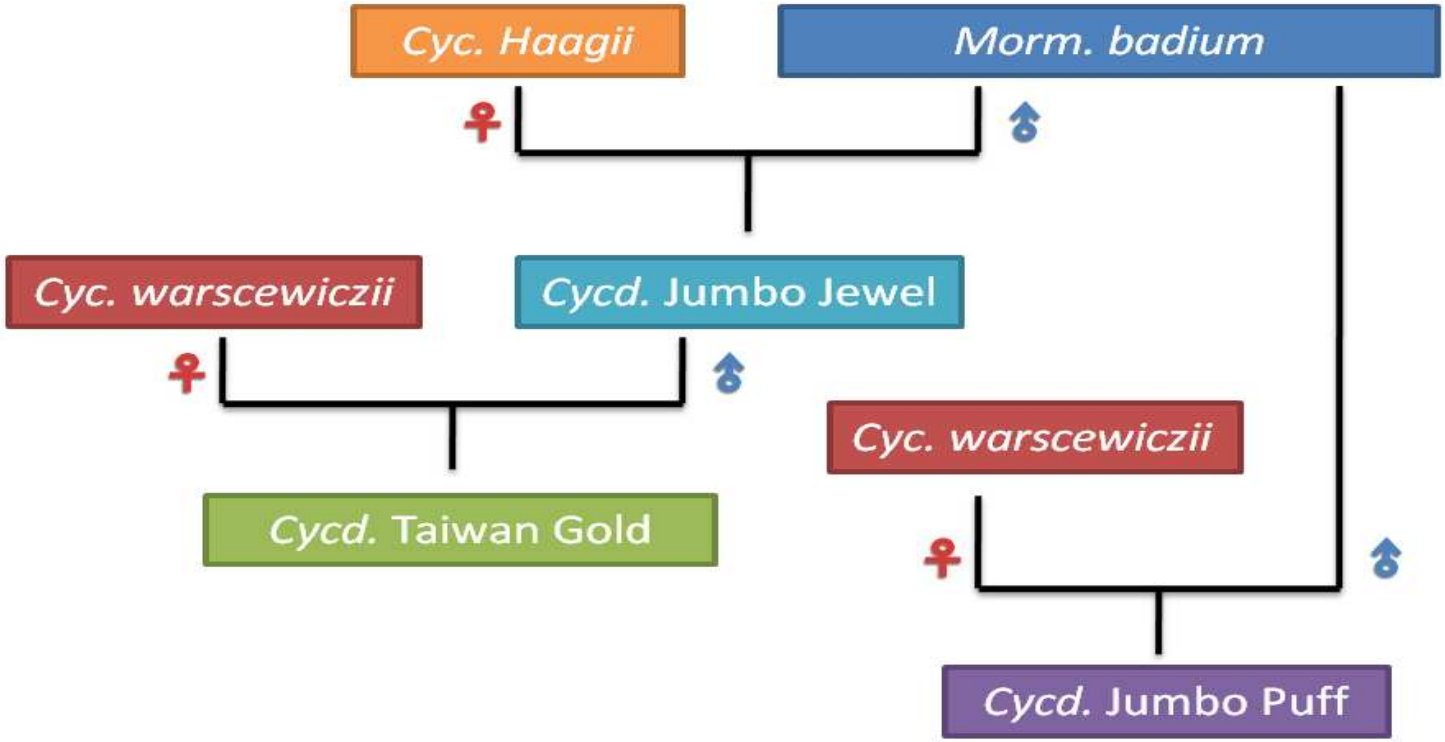

Fig. 2. The proposed pedigree of six orchid species and hybrids used in this study.

Table 1. Similarity matrix for six orchid species and hybrids used in this study.

	L1	L2	L3	L4	L5	L6	L7
CYCD.TAIWAN-GOLD(L1)	1.00						
CYC.HAAGII(L2)	0.37	1.00					
CYC.WARSCEWICZII(L3)	0.70	0.51	1.00				
CYCD.JUMBO-JEWEL(L4)	0.61	0.66	0.42	1.00			
CYCD.JUMBO-PUFF(L5)	0.83	0.36	0.64	0.66	1.00		
MARKER(L6)	0.00	0.00	0.00	0.00	0.00	1.00	
MORM.BADIUM(L7)	0.58	0.33	0.21	0.72	0.61	0.00	1.00

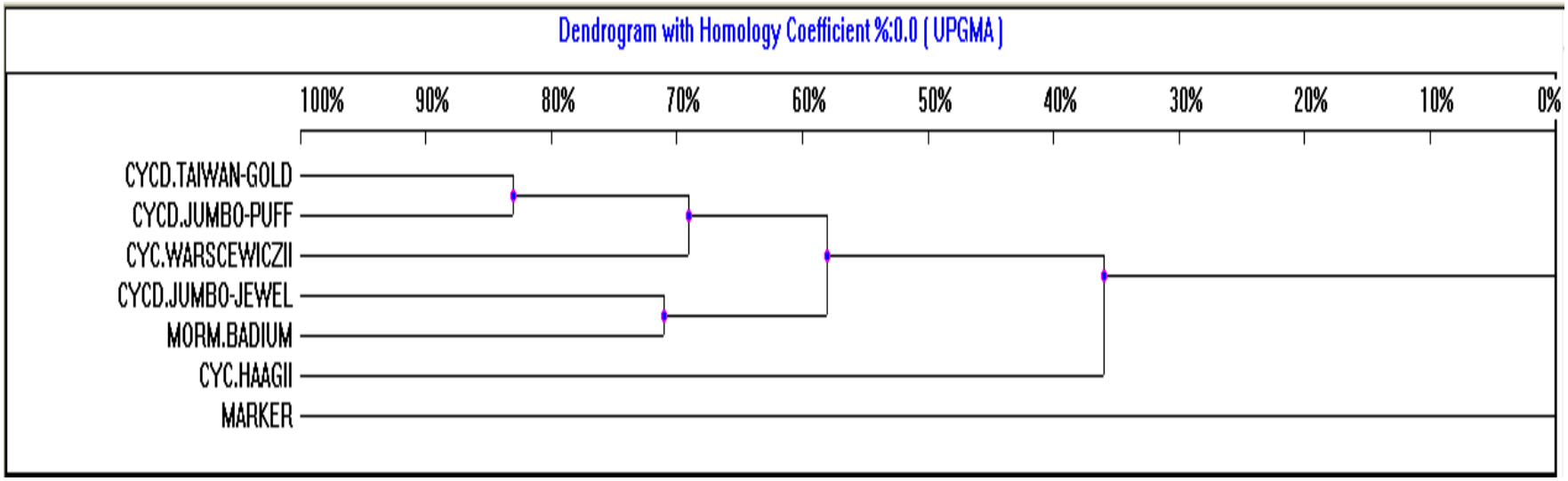

Fig. 3. Dendrogram for six orchid species and hybrids generated by unweighted pair group method with arithmetic mean analysis (UPGMA) based on amplified fragment length polymorphism data.