Morphological and DNA analysis of a wild-collected and a cultivated Epidendrum O’brienianum on Oahu, Hawaii: preliminary study

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Abstract

Naturalized Epidendrum O’brienianum are found on a rocky hillside in Naunau, Oahu, Hawaii. A preliminary study was conducted from Summer 2006 to Spring 2007 to look for polymorphism between a wild-collected and a cultivated E. O’brienianum cultivar. Distinctive morphological differences between the pollinia, petals, leaves, and root tip of the two species were observed. DNA from fresh leaf tissues was extracted using Wizard Genomic DNA Purification Kit (Promega, Madison, WI). A restriction enzyme reaction of each restriction enzyme reaction was performed for 1 cycle of 2 minutes at 95°C, 5 cycles of 1 minute at 95°C, 1 minute at 52°C, 2 minutes at 72°C and 1 cycle of 3 minutes at 72°C. PCR products were loaded in 2% agarose gel containing 0.5 µg/mL ethidium bromide. Electrophoresis was run in 1x TAE bufer.

Pollen collection and restriction analysis

Two reaction mixtures containing each ITS and TrnTL primers were prepared. One reaction mixture contained of 15 µL autoclaved distilled water, 3 µL of 10x Multisource bufer of Promega, 0.2 µL of BSA, and 0.5 µL of the corresponding restriction enzyme. A total of 15 µL of each restriction enzyme reaction mixture was added to 5 µL of each corresponding PCR product. The SacI, HaeIII, and TrnTL – digested products were incubated for 1 hour at 37°C. The SacI – digested products were incubated for 1 hour at 63°C. Each 47 µL of each PCR product per well was loaded on 2% agarose gel containing 0.5 µg/mL ethidium bromide. Electrophoresis was run in 1x TAE bufer.

Results and Discussion

SIU photograph of pollen from each cultivated E. O’brienianum shows a length of 830.31 µm and a width of 462.58 µm (Fig. 3A). The length of the pollen from the cultivated cultivar, by comparison, is 724.60 µm and the width is 276.17 µm (Fig. 3B). A more deeply lobed and rugose stigma belongs to the wild-collected E. O’brienianum (Fig. 3C). Its width is also greater (178.81 µm), compared to 84.15 µm in the cultivated cultivar (Fig. 3D).

In the wild cultivar might have been a result of an adaptation to the windy climate. Similar bulbous cell structures are found on the upper layer of petals from both cultivars (Fig. 4A & 4B). Differing cell structures are seen on the lower surface of the petals. Polygonal concave cells with numerous dimples belong to E. O’brienianum from Pali (Fig. 4C), while elongated flattened cells are seen on the cultivated cultivar (Fig. 4D).

The two cultivars showed no detectable differences in leaf and/or root tip cell structures other than a much thinner veination of leaf tissue (146.33 µm) from the wild-collected E. O’brienianum (Fig. 5A), compared to a 305.83 µm thickness in the cultivated cultivar (Fig. 5B). Differences in the thickness of leaf tissues were determined by differences in the size of cells as seen in each of their vascular bundles (Fig. 5C & 5D). A thinner root tip (1094.19 µm) is also seen in the wild-collected E. O’brienianum, as opposed to the thicker root tip (1636.34 µm) of the cultivated cultivar (Fig. 6A & 6B). The greater thickness of leaf and root tissues of the cultivated cultivar is due to abundant and constant water received during its growth in the climate-controlled greenhouse equipped with sprinkler systems.

DNA bandings with molecular weight slightly higher than 1 kb were detected in 1% agarose gel stained with methylene blue. PCR products run in 2% agarose gel containing 0.5 µg/mL ethidium bromide show bandings at 1000 bp (primed with TrnTL) and 700 bp (primed with ITS). All four base - recognizing enzymes: SacI, HaeIII, and TrnTL produce restriction fragments (Fig. 7 & 8). Gel results, however, were inconclusive due to the presence of smears. It is suggested for the next experiment to load only 1 µL digested PCR product per well, and to use 3.5% Maha Phor TM (FMC Bio Products, Rockland, ME) agarose gels to resolve restriction fragments smaller than 200 bp in size that were not visualized clearly on conventional 2% agarose gels.

Morphological differences in cell structures found on the stipes and lower surfaces of petals, as well as different restriction sites generated by the 3 enzymes indicate that the wild-collected and the cultivated E. O’brienianum might have genetically divergent origins.

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Literature Cited


Prepared by I. White

References

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