Occurrence of Vesicular Arbuscular Mycorrhizal Associations in Species of *Aeonium* From the Canary Islands

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**ABSTRACT**

Aeonium roots were collected on the Canary Islands in August 2000 and fixed in a Formalin-Acetic Acid-Alcohol (FAA) solution. Roots from each population were cleared and stained (Koske and McGinnis 1982) for the presence of VAM and were considered mycorrhizal if they contained vesicles, arbuscules or both. Fixed roots were cleared in a 10% KOH at room temperature for 3-6 days to remove the host cytoplasm and nuclei as well as allow for stain penetration. The KOH solution was poured off, the specimens were rinsed three times in distilled water, and bleached in alkaline HCl for 20 min. The fixed roots were then rinsed thoroughly with three changes of distilled water to remove the HCl. The specimens were washed in 1% lactic acid for 3-4 minutes before the HCl solution was poured off. The fixed roots were not rinsed but were covered with a 0.05% acid fuchsin-lactic acid staining solution for 5 days. After staining was complete the root specimens were mounted on glass slides in a lactic acid/glycerin solution for mycorrhizal assay using a compound microscope. Results demonstrate that VAM fungi colonize more than 80% of the roots of *Aeonium* species on the Canary Islands.

**INTRODUCTION**

*Aeonium* (Crassulaceae) comprises approximately 32 species that are confined to Macaronesia (Fig. 2). The vast majority of these species (30) are endemic to the Canary Islands, which comprise 7 major islands located off the western coast of Morocco (Fig. 2). Species of Aeonium display a high degree of variation in morphology, growth form, physiology and ecology and have been termed the botanical equivalent to Darwin’s finches (Lemis 1990). Recent studies of Aeonium have produced a robust estimate of evolutionary relationships that may now be used to study the evolution of diversity in this genus (Mes 1995, Mort et al. 2002). As the seeds of Aeonium lack endosperm (Spongberg 1978), it is likely that a symbiotic association is needed for seed germination and establishment. Since symbiosis has yet to becertified, an important piece in the puzzle regarding the ecology and diversification of Aeonium was missing. The goal of this research project was to examine the roots of Aeonium and determine the presence or absence of a vesicular-arbuscular mycorrhizal (VAM) symbiont.

**MATERIALS AND METHODS**

*Aeonium* Crassulaceae) species on the Canary Islands. On a recent trip to the Canary Islands, the roots of Aeonium species from several different sites on several of the islands were collected and fixed in FAA. After return to the laboratory, plant roots were cleared, stained, and scored for the presence and abundance of VAM associates. Preliminary results demonstrated that VAM fungi colonize more than 80% of the roots of Aeonium species on the Canary Islands.

**RESULTS**

The roots of fifteen species of Aeonium were collected from several locations on the Canary Islands (Table 1). Several roots were examined from each population in order to confirm the presence or absence of arbuscules (Fig. 3) and vesicles (Fig. 4). Although, 80% of the fifteen species exhibited both arbuscules and vesicles in the roots, the roots of *A. goochiae* and *A. lindleyi* only contained vesicles. Additional exceptions to this pattern include *A. phyllanthus*, where both arbuscules and vesicles were found in the roots of two populations while a third population contained only arbuscules and *A. urbicum*, where the roots of one population contained both arbuscules and vesicles and the roots of a second population contained only arbuscules. The roots of *Aeonium rubrolineatum* did not contain arbuscules or vesicles and the occurrence of a mycorrhizal associate could not be demonstrated in this species. Overall, 23% of the roots had arbuscules, 48% had vesicles, and 19% had both arbuscules and vesicles.

**DISCUSSION**

This study represents the first attempt to examine the potential role of symbiotic fungi in the adaptive radiation of a plant genus on oceanic islands. Although previous studies have reconstructed evolutionary relationships within Aeonium (Mes 1995, Mort et al. 2002), this study is the first to examine the distribution of fungal symbionts in Aeonium. The data gathered in this study will provide the basis for more detailed studies of the roles of mycorrhizal fungi in Aeonium ecology and the potential co-evolution of these plants and VAM fungi. Now that we have confirmed the presence of VAM fungi in the roots of Aeonium, we can ask a more specific question; Is the presence of a fungal symbiont phylogenetically informative in Aeonium? Since permits to collect soil samples were not available for field work, the isolation, characterization, and identification of VAM fungi associated with the roots of Aeonium could not be incorporated into this study. It is imperative that another trip to the Canary Islands be planned in order to collect soil samples for the identification and quantification of VAM fungi with the roots of Aeonium species on the islands.

While the protocol utilized for preserving and staining roots of Aeonium was adequate for this study, it soon became apparent that a more refined technique would be useful for future studies. The protocol described by Koske and Gemma (1989) that utilizes roots fixed in 50% ethanol and stained with 0.05% trypan blue is recommended. Although the presence and abundance of VAM fungi is recommended for future studies.

**LITERATURE CITED**


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