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

Fig. 1. *Aeonium*

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ABSTRACT

Literature on the occurrence of mycorrhizae in the Crassulaceae is limited. As a result, detailed information regarding colonization status and specific fungal associates in the Crassulaceae is needed. This study examined colonization and the abundance of vesicular arbuscular mycorrhizal (VAM) fungi associated with the roots of *Aeonium* (Crassulaceae) species in the Canary Islands. On a recent trip to the Canary Islands, the roots of *Aeonium* species from 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 demonstrate that VAM fungi colonize more than 80% of the roots of *Aeonium* species on the Canary Islands.

INTRODUCTION

Aeonium (Crassulaceae) compromises 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 (Lems 1960). 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 this aspect had yet to be investigated, an important piece to 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.

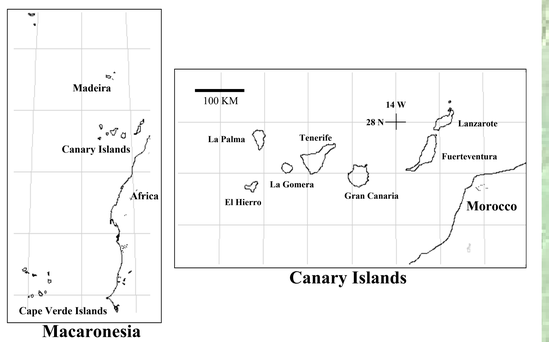

Fig. 2. Maps of Macaronesia and the Canary Islands

MATERIALS AND METHODS

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 (Kormanik and McGraw 1982) for the presence VAM and were considered mycorrhizal if they contained vesicles, arbuscles 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 H_2O_2 for 20 min. The fixed roots were then rinsed thoroughly with three changes of distilled water to remove the H_2O_2. The specimens were soaked in 1% HCl for 3 to 4 minutes before the HCL solution was poured off. The fixed roots were not rinsed but were covered with a 0.01% 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

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

Table I. Distribution of Arbuscles and Vesicles in the roots of *Aeonium* Species from the Canary Islands

Aeonium species	Number of Populations Sampled	Arbuscles	Vesicles
A. aizoin	**1**	**x**	**x**
A. aureum	**2**	**x**	**x**
A. canariense	**1**	**x**	**x**
A. cuneatum	**1**	**x**	**x**
A. dodentralis	**1**	**x**	**x**
A. goochiae	**1**		**x**
A. haworthii	**1**	**x**	**x**
A. lindleyi	**1**		**x**
A. nobile	**2**	**x**	**x**
A. palmense	**1**	**x**	**x**
A. rubrolineatum	**1**		
A. sedifolium	**2**	**x**	**x**
A. smithii	**1**	**x**	**x**
A. spathulatum	**2**	**x**	**x**
A. spathulatum	**1**		**x**
A. urbicum	**1**	**x**	**x**
A. urbicum	**1**	**x**	

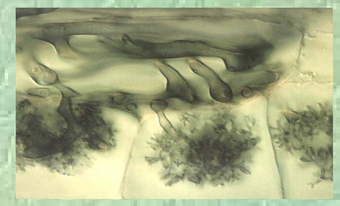

Fig 3. Arbuscles and convoluted hyphae in the inner cortex of a root.

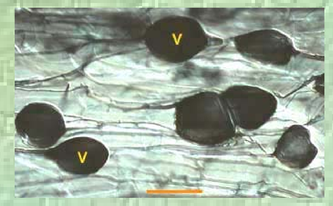

Fig 4. Vesicles and intercellular hyphae in a root

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 *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 to observe and quantify the presence and abundance of VAM fungi is recommended for future studies.

LITERATURE CITED

Kormanik, P. P,, McGraw, A. -C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In, Methods and Principles of Mycorrhizal Research, N.C. Schenk, ed. APS Press, Minneapolis.

Koske, R.E., and J.N. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research*. 92: 486-488.

Lems, K. 1960. Botanical notes from the Canary Islands II. The evolution of plant forms in the islands: *Aeonium*. *Ecology*. 41: 1-17.

Mes, T. H. M. 1995. Origin and evolution of the Macaronesian sempervivoideae. Ph. D. Thesis, Utrecht University, Utrecht, The Netherlands.

Mort, M. E., D. E. Soltis, P. S. Soltis, J. F. Ortega, and A. S. Guerra. 2002. Phylogenetics and evolution of the Macaronesian Clade of Crassulaceae inferred from nuclear and chloroplast sequence data. *Systematic Botany*. 27: 271-288.

Spongberg, S. A. 1978. The genera of Crassulaceae in the Southeastern United States. Journal of Arnold Arboretum. 59: 198-248.

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