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The mycorrhizal status of *Welwitschia mirabilis*

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Abstract. This is the first report of the mycorrhizal status of *Welwitschia mirabilis*, a gymnosperm endemic to the Namib Desert. Like all other gymnosperms except the Pinaceae and Gnetaceae, *W. mirabilis* is associated with vesicular-arbuscular mycorrhizal (VAM) fungi. Mycorrhizal colonization of roots and the diversity and abundance of VAM species were determined at seven sites. Six sites received annual rainfall of 0–100 mm, varying widely from year to year. The seventh site experienced more predictable annual rainfall of 150–200 mm. Perennial vegetation was sparse at the six low-rainfall sites. Dry annual grasses from previous rain events were present at only three of these six sites and mean mycorrhizal colonization levels of *W. mirabilis* at these three sites were as high as 18%. *W. mirabilis* was not mycorrhizal at sites where grasses were absent. The seventh site, receiving higher rainfall, supported small trees and annual grasses in addition to *W. mirabilis*. Mycorrhizal colonization levels of *W. mirabilis* at this site were significantly higher than at the other six sites, closely paralleling those of the surrounding annual grasses. The mycorrhizal flora of *W. mirabilis* consisted of four *Glomus* species. These taxa were not unique to *W. mirabilis*, having been found with *Stipagrostis* and *Cladoraphis* grasses throughout the Namib and Kalahari deserts.

Key words: Gnetales – *Welwitschia mirabilis* – Vesicular-arbuscular mycorrhizae – Namib Desert

Introduction

Welwitschia mirabilis Hook F. is a monogeneric, monospecific gymnosperm endemic to the Namib Desert (Foster and Gifford 1959). Various features of this plant and other members of the Gnetales have caused considerable debate regarding their origins and phylogenetic positions within the gymnosperms and angiosperms. In addition to the Welwitschiaceae, the order Gnetales consists of two other families, each containing one genus: Gnetaceae (*Gnetum*, 30 species) and Ephedraceae (*Ephedra*, 40 species) (Willis 1973). Although these genera are more closely related to one another than to any other plants, they differ greatly in their characteristics. *Gnetum* species are tropical trees and vines with large, leathery leaves. Fassi (1957) and St. John (1980) have reported ectomycorrhizal species of *Gnetum*. *Ephedra* species are desert and arid-adapted shrubs with jointed stems, small scale-like leaves and are associated with vesicular arbuscular mycorrhizal (VAM) fungi (Harley and Smith 1983). The mycorrhizal status of *W. mirabilis* has not been previously reported.

W. mirabilis grows in a 150-km wide belt along the coast of Namibia and Angola (Kers 1967) (Fig. 1). Vegetation types include the northern Namib, central Namib, and mopane savanna, as described by Giess (1971). Two to more than 1000 plants grow in geographically isolated populations throughout this range. The estimated life span of *W. mirabilis* is 400–1500 years (Herre 1961). The plant grows throughout the year from two leaves which have monocotyledonous-type meristems at their bases, adjacent to the woody stem (Bornman 1977). Other plants in the Namib grow seasonally, either making use of the erratic rainfall (mostly annual plants), or utilizing fog moisture (mostly perennial plants).

The water needs of *W. mirabilis* are extremely high for a plant which is endemic to the desert. It was originally thought that *W. mirabilis* was a crassulacean and metabolizing plant (Bornman 1972), but subsequent work has shown that it is a C_3 plant (Eller et al. 1983). As a result, *W. mirabilis* maintains a high transpiration rate throughout the day. At midday, when transpirational water losses are highest, the plant replaces 25–32% of its leaf water content per hour. Fog moisture can not replace this loss, even on foggy days, and thus these plants must be dependent on ground water supplies which are accessed by a long (1–3 m) tap root (Eller et al. 1983). This hypothesis is supported by the fact that the plants grow predominantly in dry water courses throughout the driest parts of their range.

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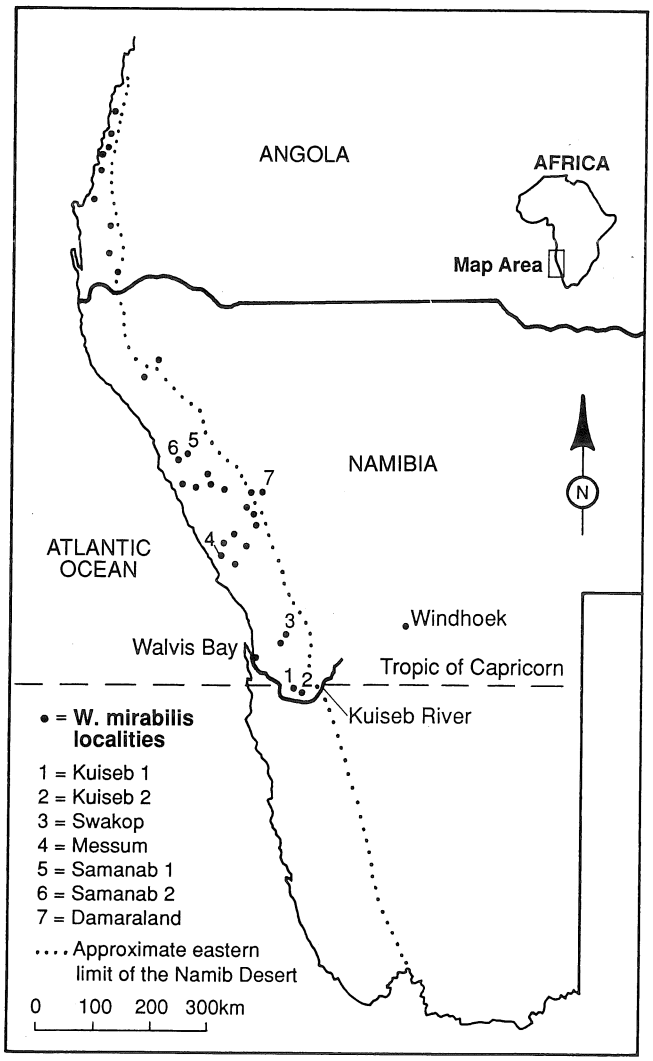

Fig. 1. The range of *Welwitschia mirabilis* and sampling sites used in this study

The objective of this investigation was to determine the mycorrhizal status of *W. mirabilis*. Roots and soil from *W. mirabilis* and surrounding vegetation were sampled at remote sites throughout the range of the plant. This broad sampling strategy facilitated a first approximation of the effects of variable rainfall patterns and surrounding vegetation on the mycorrhizal status of this species.

Materials and methods

Sampling sites

The Kuiseb 1 site (Fig. 1) was located in a narrow (3–20 m) ravine containing a shallow, sandy soil. *W. mirabilis* grew in the ravine, as well as on adjacent rocky ledges. Plants growing on ledges were not sampled, due to the difficulty of excavating roots. Additional vegetation was composed primarily of perennial species: *Adenolobus pechuellii* (Kuntze) Torre & Hillc. (Fabaceae), *Orthanthera albida* Schinz (Asclepiadaceae), *Sutera maxii* Hiern (Scrophulariaceae), and *Euphorbia phylloclada* Boiss (Euphorbiaceae). *W. mirabilis* and the other plant species were separated by distances of 2 m or more. No annual vegetation was present.

Fig. 2. *W. mirabilis* at the Samanab 1 site

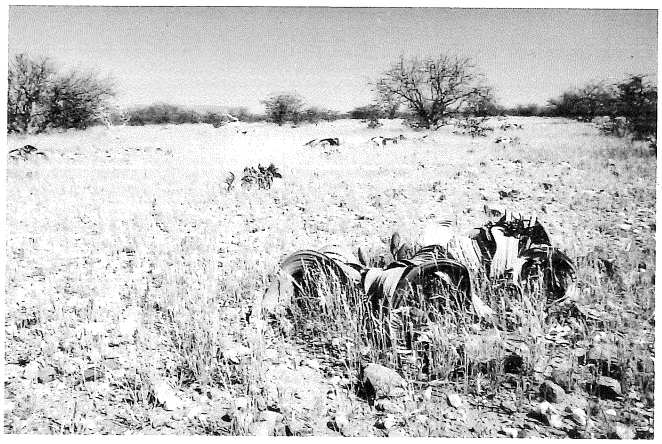

Fig. 3. *W. mirabilis* at the Damaraland site

The Kuiseb 2 site was also located in a shallow ravine where *W. mirabilis* grew in sandy soils. Perennial vegetation was similar to that of the Kuiseb 1 site. In addition, a dry annual grass was present, *Stipagrostis ciliata* (Desf.) De Winter, left from the previous year's rains (2 plants/m^2).

The Swakop site was located on a wide, gravel plain. Hundreds of *W. mirabilis* grew on a hard, gypsum crust in shallow, dry water courses. Other perennial vegetation was sparse, consisting of *Zygophyllum stapffii* Schinz (Zygophyllaceae), *Arthraerua leubnitziae* (Kuntze) Schinz (Amaranthaceae), and *Asclepias buchenaviana* Schinz (Asclepiadaceae). These plants were separated from *W. mirabilis* by at least 5 m. Annual grass (*Stipagrostis* spp.) remnants from a previous rain event were present (1 plant/m^2).

The Messum site was situated in a side channel south of the Messum River. The substrate was a hard, gypsum crust and solid rock was frequently encountered before viable roots could be found. *Z. stapffii* was the only other perennial vegetation present and plants were separated from *W. mirabilis* by 10 m or more. No annual vegetation was present.

At the Samanab 1 site, *W. mirabilis* was sampled within the shallow, rocky channel of the Samanab River and on the adjacent gravel plains (Fig. 2). *Z. stapffii* was the only other perennial vegatation present and plants were separated from *W. mirabilis* by 10 m or more. There was a low uniform cover (10–15 plants/m^2) of dry *Stipagrostis ciliata* from a rain event which had occurred at least 1 year prior to sampling.

The Samanab 2 site was very similar to the Samanab 1 site except that all plants sampled were in the riverbed. Additional perennial vegetation consisted of *Z. stapffii* and large clumps of a

perennial grass, *Stipagrostis damarensis* (Mez) de Winter. All plants were separated by at least 3 m and no annual vegatation was evident.

The Damaraland site was distinctly different from the other sites sampled (Fig. 3). The rocky soil was covered with a dense growth of grass which completely surrounded the numerous *W. mirabilis* plants. In addition, two species of small tree occurred in this habitat: *Colophospermum mopane* (Kirk ex Benth) Kirk ex J. Leonard (Caesalpinioideae) and *Terminalia pruinioides* C. Lawson (Combretaceae). There were six annual grass species representing five genera present in the sampling site: *Stipagrostis*, *Eragrostis* (2), *Enneapogon*, *Rhynchelytrum*, and *Anthephora*.

All of the sites, except Damaraland, occur within the 0–100 mm rainfall isohyet. At Gobabeb, 10 km west of the Kuiseb 1 site, the mean yearly rainfall is 26 mm. It must be understood, however, that an average value is a misleading measure at all of these sites due to the high variability of rainfall in the Namib Desert (Robinson and Seely 1980). The Damaraland site lies within the 150–200 mm rainfall isohyet and receives more predictable annual rains which support the mopane savanna characterizing the region.

Determining percent mycorrhizal colonization

Fine roots of five *W. mirabilis* plants were taken at each of the seven sites and stored in 50% ethanol. Roots were sampled at the Damaraland site in April, 1991 and at other sites in December, 1990 or January, 1991. Fine roots were removed from lateral roots uncovered 10–15 cm underground and within 0.5 m of the plant base. No attempt was made to sample deeper roots due to the extremely rocky substrates in which the plants grew. The unstained samples were initially examined for ectomycorrhizal sheaths with a dissecting microscope at ×40 magnification. Subsequently, roots were stained using the trypan blue method of Koske and Gemma (1989), and cut into 1-cm segments. Stained roots were examined at ×100 and ×400 magnification to differentiate between VAM structures and septate endophytes. Percent mycorrhizal colonization of 40 1-cm root segments from each plant was measured using the systematic slide method discussed by Kormanik and McGraw (1982) and Giovanetti and Mosse (1980).

To determine whether there were between-site differences in *W. mirabilis* VAM colonization levels, a one-way analysis of variance (ANOVA) was performed using the five samples as replicates at each location. Percentage values were transformed using the arc sine transformation, prior to performing the ANOVA procedure (Zar 1984). Due to the high levels of variation in colonization within a site [which could violate the constraints of the ANOVA procedure (Zar 1984)], the non-parametric Kruskal-Wallis test was also performed on the data. When the ANOVA and Kruskal-Wallis tests yielded the same results, Duncan's multiple range test was used to determine which sites exhibited significant differences in percent colonization.

Living roots of other plants found in the *W. mirabilis* rhizospheres were also sampled, stained and examined as above. Pairwise *t*-tests were performed on each of the pairs of *W. mirabilis* and associated plants from the same rhizosphere, to determine whether their mycorrhizal colonization levels were similar.

Determining spore diversity and abundance

Dry soil samples were collected with *W. mirabilis* root samples and sieved through a 500-μm sieve to remove stones and debris. The samples from each of the five plants at a site were combined in one bag. Samples were kept dry in plastic bags at 4° C prior to examination for spores.

Spores were separated from 150 ml of soil via a sucrose gradient (Daniels and Skipper 1982) and characterized according to color, size, wall-layer thickness and composition, and hyphal attachment. These examinations were completed on both whole and squashed spores in water. A photographic record of these characteristics (×80, ×100, and ×400 magnification) was made to aid comparisons of spores from different *W. mirabilis* samples and ongoing studies of grass mycorrhizae from the Namib and Kalahari Deserts. The mycorrhizal species were identified at the generic level using Schenck and Perez (1990).

Results

None of the *W. mirabilis* root samples examined were ectomycorrhizal. However, VAM structures were present in the rootlets of some of the *W. mirabilis*. Arbuscules were not abundant but were seen in all of the samples which were mycorrhizal. Aseptate external and internal hyphae (5–10 μm diameter) and vesicles were the most common indication of colonization by VAM fungi. Brown, nonstaining, septate endophytes were also observed in the roots, necessitating the use of the systematic slide method and careful examination of the hyphae at ×100 magnification for accurate determinations of mycorrhizal colonization.

Of the seven sites sampled, three (Kuiseb 1, Messum, Samanab 2) showed no mycorrhizal structures in the five samples (Table 1). At two sites (Swakop, Samanab 1), some of the plants had no mycorrhizal structures, while others showed levels of colonization of up to 20%. Finally, at two sites (Kuiseb 2, Damaraland), all plants possessed mycorrhizal structures.

The ANOVA and Kruskal-Wallis tests revealed significant differences in mycorrhizal colonization levels of *W. mirabilis* at the seven sites ($\alpha = 0.05$; $P < 0.0001$ for ANOVA). Duncan's multiple range test subsequently showed that significant differences existed between the Damaraland site and all others. No significant differences were detected between the other six sites.

Dry, dead grass roots were infrequently encountered in the *W. mirabilis* rhizospheres at the Kuiseb 2, Swakop and Samanab 1 sites, but not at the Kuiseb 1, Messum and Samanab 2 sites. The Damaraland site was the only sampling location where living grass roots were found within *W. mirabilis* rhizospheres. The paired *t*-tests showed no significant differences between the mycorrhizal colonization levels of *W. mirabilis* roots and grass roots with overlapping rhizospheres at the Damaraland site (Table 2).

Table 1. Percent vesicular-arbuscular mycorrhizal colonization of *Welwitschia mirabilis* at each site

Site	Mean % colonization	SE	Range
Kuiseb 1	0	0.0	0
Kuiseb 2	18	9.9	2–56
Swakop	10	4.8	0–20
Messum	0	0.0	0
Samanab 1	7	4.2	0–19
Samanab 2	0	0.0	0
Damaraland	50	8.5	27–72

Table 2. Comparison of percent colonization of *W. mirabilis* roots and grass roots from the same sampling locations at the Damaraland site using *t*-tests. *P* values less than 0.05 are significant

Sample	*W. mirabilis* roots		Grass roots		*t*-test *P* value
	%	SE	%	SE	
1	72	2.5	56	4.4	0.1815
2	27	3.1	35	2.0	0.2521
3	66	1.9	74	4.6	0.0887
4	47	4.6	47	2.4	0.1529
5	37	1.1	39	1.5	0.3016

Table 3. Spore types and abundance in 150-ml soil samples from sites exhibiting mycorrhizal colonization of *W. mirabilis* roots

Site	*Glomus* 1	*Glomus* 2	*Glomus* 3	*Glomus* 4	Total
Kuiseb 2	—	115 (45%)	52 (21%)	86 (34%)	253
Swakop	138 (79%)	24 (14%)	9 (5%)	3 (2%)	174
Samanab 1	240 (85%)	—	—	42 (15%)	282
Damaraland	—	60 (3%)	2100 (81%)	420 (16%)	2580

Four *Glomus* species were found in the rhizospheres of *W. mirabilis*. All four species were also found in association with various grasses at sites within the Namib Desert where *W. mirabilis* did not occur. Descriptions of these species will be reported in a subsequent taxonomic paper detailing the VAM fungal flora associated with grasses and *W. mirabilis* in the Namib Desert.

No spores were found at sites where *W. mirabilis* was not mycorrhizal: Kuiseb 1, Messum and Samanab 2. Soil samples from the Kuiseb 2, Swakop, and Samanab 1 sites had similar spore densities (174–282 spores/150 ml soil) (Table 3). The Damaraland site, where colonization levels of *W. mirabilis* were significantly higher than at the other sites, exhibited a much higher spore density (2580 spores/150 ml soil).

It was not possible to confirm whether spores occurring in the rhizosphere of *W. mirabilis* were from the species forming mycorrhizae with this plant. In addition, the viability of the spores is unknown, although all spores counted were intact. However, corresponding trends in spore density and percent mycorrhizal colonization were observed for all of the sites. None of the four VAM fungi dominated the rhizosphere at all four sites where *W. mirabilis* was mycorrhizal.

Discussion

Miller et al. (1983) noted that when *Atriplex confertico-la* (Torr. & Frem.) S. Wats. grew alone or with *A. spinescens* (Moq.) Standl. (non-mycorrhizal), no VAM fungal colonization was seen. However, when *A. confertícola* grew in association with various grass species that were mycorrhizal, it showed colonization levels similar to those of the grasses. We observed a similar pattern with the mycorrhizal status of *W. mirabilis* at the seven sites. At sites where insufficient rains in the past several years had prevented grass germination, no grass roots were present in *W. mirabilis* rhizospheres and no mycorrhizal structures or spores were found (Kuiseb 1, Messum, Samanab 2). At sites where sufficient rain had fallen within the past several years, dry grass roots were sporadically present in *W. mirabilis* rhizospheres. In addition, spores were present, and some of the *W. mirabilis* were mycorrhizal (Kuiseb 2, Swakop, Samanab 1). At the Damaraland site, where spore abundance was 10-times greater and percent colonization of *W. mirabilis* roots significantly higher than the other sites, living annual grass roots were abundant in the *W. mirabilis* rhizosphere. Thus, we attribute the variability in the mycorrhizal status of *W. mirabilis* to differences in rainfall between sites and concomitant effects upon grass distribution and abundance.

Given the rapid, seasonal growth of annual grass roots relative to those of *W. mirabilis*, we hypothesize that grasses provide the primary means by which VAM inocula (spores and colonized roots) become associated with *W. mirabilis* roots. Read et al. (1976) suggested that most VAM fungi are spread via root-to-root contact of adjacent plants. In contrast, Warner et al. (1987) suggested that wind-blown spores can function as sources of inoculum for establishment of new mycorrhizal associations. However, given the hard, calcrete-encrusted substrates of the Namib plains where *W. mirabilis* is most abundant, it is unlikely that wind dispersal of spores plays a direct role in inoculation. Rather, the similarities in root colonization levels of *W. mirabilis* and grass roots growing in close proximity, suggest that the plants share mycorrhizal associations. Finally, the fact that the four VAM fungi occurring in the rhizospheres of *W. mirabilis* are also regular associates of *Stipagrostis* and *Cladoraphis* species throughout the region suggests that the primary source of inoculum for *W. mirabilis* is the grass roots within the rhizosphere.

Larger sample sizes and an expanded sampling period might have provided a more precise assessment of mycorrhizal colonization, spore abundance, and diversity. However, numerous practical constraints limited the breadth of this study. Our primary objective was to determine whether *W. mirabilis* was mycorrhizal and to sample as many locations as possible in order to document between - site variability in mycorrhizal status. The rocky substrates at most of the sites made it difficult to find plants from which we could obtain roots. Thus, given the rare and unusual nature of this plant, we sampled in the most nondestructive means possible and minimized the sample size at each site. Despite these constraints, this study conclusively shows that *W. mirabilis* is colonized by VAM fungi and that significant differences in colonization levels exist between plants growing at sites experiencing different rainfall regimes.

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