

Water Potential Gradients between Old and Developing Leaves in *Lithops* (Aizoaceae)

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ABSTRACT

Lithops plants consist of a pair of opposite succulent leaves inserted on a short stem. The apical meristem produces a new pair of leaves within the old one every growing season, recycling water from the old leaves. Since there are no data on water relations between the two pairs of leaves, we measured leaf water potential at different stages of development with a pressure chamber. Osmotic potential of cell sap was measured with a cryoscopic osmometer and turgor pressure was calculated indirectly. Leaf water potentials were never very low even though plants were not irrigated. In old leaves water potential ranged between -0.5 and -0.28 MPa. In young leaves water potential increased with size from -1.05 to -0.5 MPa and was always lower than in the corresponding old leaves. The water potential gradient between old and new leaves was steeper in the early stages of development (0.6 MPa) and gradually decreased (0.15 MPa) when young leaves had almost completed their expansion. Our data show that in *Lithops* water movement from old to young leaves occurs according to a water potential gradient. The maintenance of this gradient may be ascribed to differences in turgor pressure, due to the more elastic and plastic walls of cells of young leaves. The possibility to perform a complete life cycle without external water supply is an extreme adaptation to the arid environment where *Lithops* grows.

Keywords: Aizoaceae, arid environment, *Lithops hallii*, succulence, succulent plants, water potential, water stress

INTRODUCTION

The genus *Lithops* (Aizoaceae) includes 37 species, several subspecies and various cultivars (Cole and Cole 2005). It is distributed in western and central areas of southern Africa, mainly Namibia and South Africa, and only marginally in the south-eastern border of Botswana. *Lithops* species grow in open rocky soils, with annual rainfall ranging between 100 and 500 mm. *Lithops* are succulent plants in which stems are virtually absent and leaves and roots are the only evident organs. Succulence evolved in several families as an adaptation to an arid environment and water storage tissues may be present in different organs, such as stems (e.g. Cactaceae), roots (e.g. Apocynaceae and Cactaceae) or

leaves (e.g. Crassulaceae and Aizoaceae) (Gibson and Nobel 1986; Sajeва and Costanzo 1994, 2000; Albers and Meve 2002; Egli 2003). In *Lithops* leaves are the water storage organs, and plants consist of a pair of opposite, partly fused, succulent leaves inserted on an extremely short stem, forming a body with the shape of an inverted cone where the base is the upper surface and the apex tapers into the junction with the root (Fig. 1). The plants grow embedded in the soil with only the leaf tops exposed to the air; this peculiar growth form permits the survival in very extreme environments: though embedding does not thermally insulate the plants from the surrounding soil (Eller and Nipkow 1983), it buffers changes in daily temperature with the thermal mass of the soil (Vogel 2005), reduces the amount of direct light and protects the plants from water loss (Eller and Ruess 1982; Turner and Picker 1993). Furthermore the apical meristem is placed very low in the plant body, protected both by the soil and by the leaves. The apical meristem of the reduced stem produces a new pair of leaves and a flower bud each growing season. The new pair of leaves develops inside the mature ones, at a 90° angle, and during the first stages of development the young leaves use the water of the old ones, so a complete growth cycle can be performed even if there is no water supply from the soil (von Willert *et al.* 1992).

In succulent plants movement of water between old and young leaves or stems has sometimes, but not always, been observed (e.g. Tüffers *et al.* 1995). When water movement has been observed, it either occurs according to (Herrera *et al.* 2000) or against (Wang *et al.* 1997; Nerd and Neumann 2004) a water potential gradient. Though there are several phytogeographic and taxonomic papers (e.g. Sprechman 1970; Wallace 1990; Hartmann 2001a, 2001b) and some physiological and ecological ones (e.g. Eller and Ruess 1982; Eller and Nipkow 1983; Albanese *et al.* 1989), to our knowledge there are practically no investigations in *Lithops* on the driving forces that cause the movement of water



Fig. 1 A *Lithops* plant in which one old leaf was removed, showing the other old one, the new developing leaves and the flower bud. The short stem connects the leaves to the root system.

from the old, mature leaves to the young developing ones, probably because the study of water relations in *Lithops* is complicated by the peculiar morphology of this plant.

Here we report on the water potential gradients existing between old and developing leaves in *Lithops*.

MATERIALS AND METHODS

Eighty plants of four year old, double-headed *Lithops hallii* de Boer were purchased from Vivai Giammanco (Bagheria, Sicily) and cultivated in the Botanical Garden of Palermo, in 50 ml plastic pots, in the open under a polycarbonate cover to avoid rain. Plants were not watered from October 2005 to June 2006. The water regime adopted was that suggested for cultivation of this genus (Cole and Cole 2005), withholding water until the old leaves were completely withered. Regular irrigation throughout the year leads to abnormal growth of both young and old leaves and plants are subject to rot.

At the onset of a new vegetative cycle, around November, a pair of young leaves begins to develop within the old pair. The plants used for this study were all of the same age, however growth was not synchronized, so plants harvested at the same time did not always have the young leaf pairs of similar size. For this reason, in order to compare homogeneous specimens, for data analysis we grouped the results obtained according to the diameter of the young leaf pair, assigning them to four class sizes: 4-6 mm (class 1), 7-10 mm (class 2), 11-14 mm (class 3), 15-17 mm (class 4). In this way we considered the diameter of the young leaf pair as the parameter representative of the degree of development of the plant. When the young leaves reached a considerable size (10 mm and up) the fissure of the old leaves started to widen and the top surface of the young leaves became visible. When the young leaves reached a diameter of 18 mm and over, the old leaves were almost completely dried up and it was no longer possible to take measurements with the pressure chamber.

Every two weeks from December 2005 to May 2006, three to five plants were collected at around 11:00 a.m. The collected plants were weighed before and after removal of the root system. Diameters of the body were measured with a caliper. After taking the morphometrical measurements, water potential (Ψ) of both the old and young leaves was measured with a pressure chamber (SKPM 1400, Skye Instruments) equipped with a steel cover that fits a wide rubber seal. A hole was modelled through the rubber seal where the tip of the cone of the *Lithops* leaf could fit snugly (Fig. 2), with the cut surface visible at the level or slightly above the rubber seal. This connection was made air tight with a small "collar" of Blu Tack[®] adhesive. The leaf pair was further held in place with a strip of adhesive paper tape, so that the leaf did not fall out of place when the cover was fastened to the steel chamber of the pressure bomb. The cut surface was illuminated with a beam of light (Schott KL 1500 electronic) and observed through the hole in the steel cover, using a stereoscopic microscope. As soon as water appeared on 50% of the cut surface the balancing pressure was recorded as the bulk water potential of the old pair of leaves. After slowly reducing the pressure in the chamber to zero, the sample was removed and cut open with a scalpel to remove the developing leaves and proceed with the morphometrical and water potential measurements of the young leaf pair as described above. A series of preliminary measurements showed that water potential

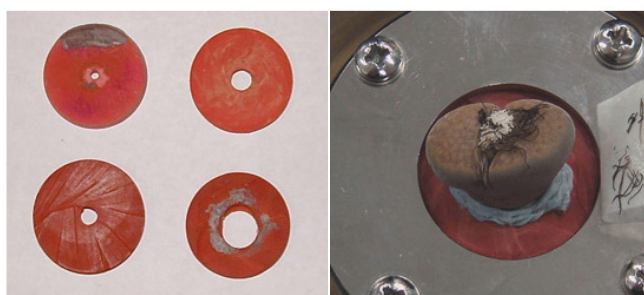


Fig. 2 (Left) Rubber seals modeled to fit the tip of the cone of *Lithops*; **(Right)** The leaf pair sealed with a small collar of Blu Tack[®] and positioned in the steel cover of the pressure chamber.

Table 1 Values of water potential in the four classes of *Lithops* developing and old leaves.

Size class	Old leaves	Young leaves
1	-0.46 ± 0.18 a	-1.05 ± 0.36 b
2	-0.27 ± 0.04 a	-0.74 ± 0.29 b
3	-0.31 ± 0.08 a	-0.61 ± 0.22 b
4	-0.35 ± 0.04 a	-0.51 ± 0.09 b

Values are the mean of at least 8 measurements and are expressed in MPa ± s.d. Values followed by different letters denote significant differences within rows (Student's *t*-test $P < 0.05$).

Table 2 Values of osmotic potential in the four classes of *Lithops* developing and old leaves.

Size class	Old leaves	Young leaves
1	1.90 ± 0.06 a	2.10 ± 0.06 b
2	1.84 ± 0.23 a	1.71 ± 0.14 a
3	1.93 ± 0.27 a	1.91 ± 0.25 a
4	2.26 ± 0.34 a	1.86 ± 0.17 b

Values are the mean of at least 8 measurements and are expressed in MPa ± s.d. Values followed by different letters denote significant differences within rows (Student's *t*-test $P < 0.05$).

Table 3 Values of turgor pressure in the four classes of *Lithops* developing and old leaves.

Size class	Old leaves	Young leaves
1	1.44 ± 0.20 a	1.05 ± 0.37 b
2	1.56 ± 0.23 a	0.97 ± 0.38 b
3	1.62 ± 0.26 a	1.30 ± 0.42 b
4	1.90 ± 0.30 a	1.35 ± 0.13 b

Values are the mean of at least 8 measurements and are expressed in MPa ± s.d. Values followed by different letters denote significant differences within rows (Student's *t*-test $P < 0.05$).

measurements of the old leaves were not influenced by the presence of the young leaves.

After measuring water potential, a pair of both young and old leaves was frozen in liquid nitrogen. After thawing, the leaves were placed in a plastic syringe and cell sap was collected in an eppendorf tube and centrifuged to eliminate cell debris. The osmotic potential of expressed sap was measured with a cryoscopic osmometer (Osmomat 030, Gonotec). Bulk turgor pressure was calculated from the difference between water potential and osmotic potential.

Results are presented as means ± standard deviation. Significance of the comparisons between means was assessed with the Student's *t*-test.

RESULTS

Values of water potential, osmotic potential and turgor pressure for old and young leaves are reported in **Tables 1-3**. In old leaves, water potential ranged from about -0.5 to -0.28 MPa, but variations between the different size classes were not significant. In young leaves, water potential varied from -1.05 to -0.5 MPa, increasing together with the increase in diameter, so leaves at the initial stage of development showed more negative values of water potential. The water potential gradient between old and new leaves decreased with size class (**Fig. 3**), with a difference in water potential of about 0.6 MPa when young leaves were at the initial stage of development and a difference in water potential of about 0.15 MPa when young leaves reached a diameter of 15-17 mm.

The osmotic potential of young and old leaves differed at the initial stage of development (class 1), when osmotic potential of young leaves was significantly higher, and in class 4, when osmotic potential was higher in old leaves, that at this stage were strongly dehydrated. The values calculated for turgor pressure in young leaves ranged between 1 and 1.5 MPa, always significantly lower than those of the old leaves, which varied between 1.5 and 2 MPa.

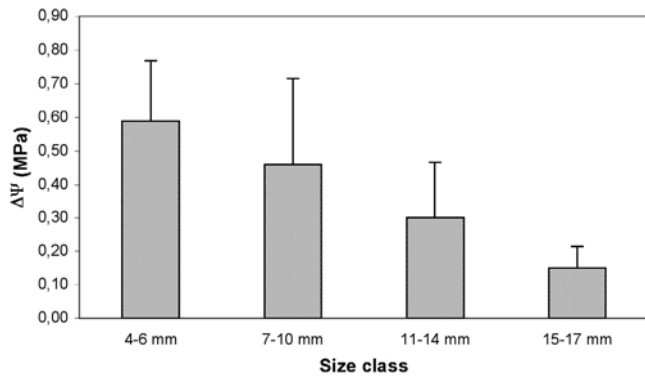


Fig. 3 Water potential gradient between old and new leaves ($\Delta\Psi$ = water potential of old leaves minus water potential of new leaves). Vertical bars are SD of the means.

DISCUSSION

The water potential values measured were in the same range of those reported as an average in the family (von Willert *et al.* 1992), and in other succulents (Nobel 1977, 2006). In some succulent species (von Willert *et al.* 1992) the decrease in water availability leads to a decrease in water potential of leaves, while in *Lithops* the water potential of old leaves remained constant though there was no external water supply, and the changes in water potential of young leaves were related to developmental stage. Leaf bulk water potentials of old and young leaves did not reach very negative values. Developing leaves avoided stress and maintained growth by removing water from the older pair, gradually leading to their dehydration and death.

There is contrasting evidence in the literature with regard to water movement between mature and developing organs. Xylem is generally considered the main pathway for water transport in growing and transpiring organs, and the flow of water occurs under tension and according to water potential gradients. Phloem also may act as an effective supplier of water to developing fruits and stems (Wang *et al.* 1997; Nerd and Neumann 2004), and in this case the flow is driven by a pressure gradient maintained by the loading and unloading of solutes in source and sink tissues respectively. Tüffers *et al.* (1995) investigated two Aizoaceae species, finding that in one, *Prenia sladeniana*, a water shift from older to younger leaves occurred and was most likely a major feature of drought adaptation, while this was not the case in the other species, *Delosperma tradescantioides*. Rabas and Martin (2003) investigated water movement in three plants with succulent leaves (including *Carpobrotus* of the Aizoaceae) and found no evidence of water movement from old leaves to new ones. When movement of water between mature and developing organs occurs, it could take place either through the xylem, according to water potential (Wang *et al.* 1997, 1998), or through the phloem, according to sink strength and against water potential gradient (Nerd and Neumann 2004). In *Lithops* the movement of water from old to young leaves is driven by the water potential gradient. Gradients in water potential may be maintained both by osmotic adjustment and by the properties of the cell wall (Nobel 2006). Osmotic potential of old leaves remained rather constant until they had lost most of their cellular water content. In young leaves osmotic potential did not vary with development, while water content increased (data not shown). Therefore, in *Lithops* a major role in the maintenance of the water potential gradient may be ascribed to differences in the turgor pressure component of water potential, with the cells of young

leaves having more elastic and plastic walls that exert low positive pressures.

The recycling of water from the old leaves is an advantage for the development of the young tissues that will carry on photosynthesis during the new growing season and allow the plant to reach flowering. In our non irrigated plants flowering occurred in May, when the young leaves were fully developed. The possibility to perform a complete growing season without an external supply of water, relying on the older leaves of the plant, is an example of extreme adaptation to the arid environment where *Lithops* grows in habitat.

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