

# Contractile roots in succulent monocots: convergence, divergence and adaptation to limited rainfall

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## ABSTRACT

**Contractile roots (CRs) that pull shoots further down in the soil are a possible example of convergent evolution in two monocot families, the Agavaceae and the Asphodelaceae. The association between CRs, water uptake and habitat aridity was investigated for agaves, yuccas and aloes by assessing the occurrence of CRs and the amount of root contraction for glasshouse-grown plants with respect to mean annual rainfall of their native habitats. Structural features of CRs as well as root hydraulic conductance were compared with those of non-contractile roots (NCRs). CRs occurred in 55% of the 73 species examined, including 64% of the agaves and 85% of the yuccas, but in none of the aloes despite the occurrence of CRs in related genera. The phylogenetic distribution of CRs was consistent with multiple acquisitions or losses of the trait. The amount of root contraction showed a highly significant negative relationship with mean annual rainfall, although other environmental factors may also be important. Radial hydraulic conductance of the basal (contractile) zone exceeded that of the midroot zone for CRs; for NCRs, the opposite was true. Thus, CRs in the species examined may provide a mechanism for greater water uptake near the soil surface in regions with limited rainfall.**

*Key-words:* *Agave*; *Aloe*; *Yucca*; convergent evolution; drought adaptation; hydraulic conductance; root anatomy; water uptake.

## INTRODUCTION

Roots are rarely cited as examples of convergent evolution, perhaps because they are generally underground and unseen (but see Pate & Dixon 1996). Nevertheless, a few distinctive root types can be considered convergent in that they represent similar structural and functional adaptations to similar selective pressures despite differences in plant lineage (e.g. Cody & Mooney 1978). Two examples are ageotropic (upward-tending) aerial roots produced by disparate taxa of tropical trees to infiltrate canopy mats (Nadkarni 1981) and proteoid (cluster) roots that occur in several plant families as a convergent response to nutrient-poor soils (Purnell 1960; Skene 1998). Contractile roots

(CRs), with a zone usually near the shoot base that is developmentally and anatomically specialized to contract longitudinally, appear to be likely candidates for convergence. They occur in diverse plant groups such as cycads, ferns, dicotyledons and many monocotyledons, and in diverse life-forms, including arborescent taxa and geophytes (Pütz 2002; Jaffe & Leopold 2007). The habitats for plants with CRs vary from tropical to alpine, mesic to arid, although most have unfavourable seasons or periods characterized by cold temperatures or drought (Pütz 2002). The diversity of the plant groups and their habitats obscures possible similarities in the functional responses of CRs to similar selective pressures that would point towards convergent evolution.

One broad function of CRs, to improve plant anchorage, can be considered common to all plants that possess the trait. Other, more specialized functions have been proposed, such as positioning underground organs at depths where temperatures are less extreme, pulling ramets away from a parent plant (Pütz 2002) and protecting apical buds from fire (Gill & Ingwersen 1976) or herbivory (Koch, Richardson & Lamont 2004). CRs were previously described for a few members of the Agavaceae, including *Agave americana*, *Dasyllirion acrotriche*, *Furcraea gigantea*, *Yucca aloifolia* and *Yucca angustifolia* (Rimbach 1922). Rimbach also noted that two species of *Aloe* lacked CRs, but a later study reported roots of an unidentified *Aloe* as contracting 4 mm, or 10% of their length (Rimbach 1929). Recent investigations of the structure and function of the basal root zone (just below the root–shoot junction) in *Agave deserti* and *Yucca schidigera* have shown this zone to be unexpectedly permeable to water, even under drying conditions (North, Martre & Nobel 2004; North & Baker 2007). In addition, preliminary evidence indicates the basal root zone of both species to be contractile, suggesting a possible link between CRs and water uptake in the Agavaceae.

Plants in the Agavaceae and the genus *Aloe* (Asphodelaceae) have long been considered exemplars of convergent evolution (Silvertown 2005). They have a similar range of shoot morphologies from acaulescent rosettes of more or less succulent leaves to the arborescent forms of Joshua tree (*Yucca brevifolia*, Agavaceae) and quiver tree (*Aloe dichotoma*), and a secondary thickening meristem occurs in both groups (Tomlinson & Zimmermann 1969). In most recent considerations of the order Asparagales, the families Agavaceae and Asphodelaceae are distantly related, the former belonging to the ‘higher’ Asparagales and the latter

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belonging to the 'lower' (Chase *et al.* 2000; Chase 2004). Although distantly related, these plant groups face similar environmental constraints, with limited water availability foremost among them. Their functional responses are also similar, as shown by the ability of plants in the Agavaceae and the genus *Aloe* to take up water rapidly upon the cessation of drought or in response to light rainfall (Ruess, Eller & Ferrari 1988; Ehleringer *et al.* 1991; Patrick *et al.* 2007). In addition, general root anatomy in all members of the group is similar; notably, many monocots including several species in the Asparagales have lignified and/or suberized cell layers on the root periphery and just outside the vascular cylinder that help restrict root water loss and may represent convergent evolution (Kauff, Rudall & Conran 2000).

A relatively high root permeability near the base of the shoot in members of the Agavaceae may be an example of function following form (Woodward 2008) in that improved water uptake may be ancillary to the primary role of CRs, which is to pull plants further down into the soil to protect shoots from unfavourable conditions above-ground. For most species with CRs and for all monocots examined, root contraction is accomplished by the radial expansion and longitudinal shortening of cells in the inner and/or middle cortical layers (Jernstedt 1984; Pütz 2002). Outer cortical cells are crushed as the root contracts, and vascular tissues are compressed. To accommodate such deformation, cell layers such as the endodermis and adjacent cortical cells that typically develop extensively suberized and lignified cell walls in *Agave* and *Yucca* may have thinner, more elastic walls in the contractile zone to permit bending instead of breaking during contraction. Because suberization and lignification may decrease root radial conductivity (North & Peterson 2005), a reduction in these processes in the contractile zone may increase water permeability.

Because of the relatively high hydraulic conductance of the CR zone of *A. deserti* and *Y. schidigera*, we sought to examine the functional consequences of CRs with respect to water uptake for members of the Agavaceae and the genus *Aloe*. To assess CRs as a possible adaptive trait in arid habitats, we addressed the following questions: (1) How are CRs distributed within the Agavaceae (primarily the genera *Agave* and *Yucca*), as compared with the genus *Aloe*? (2) Do CRs and the amount of contraction correlate with habitat aridity? (3) Does the contractile zone of a root have greater hydraulic conductance than the non-contractile zone?

The apparent similarity in the mechanism of root contraction within the monocots may indicate a common genetic basis for the phenomenon, raising the question of whether the evolution of CRs in this group should be considered convergent or parallel; because the genetic programming underlying the trait has not been determined, no distinction will be made between convergence and parallelism (Arendt & Reznick 2007), and multiple independent occurrences of CRs will be interpreted as possible convergent evolution. Evidence for CRs as adaptation would be a positive correlation between root contraction and habitat

aridity, and a greater hydraulic conductance for the contractile than the non-contractile root (NCR) zone.

## MATERIALS AND METHODS

### Plant material

Plants of species native to southern California were grown from seeds collected in the north-western Sonoran Desert at the University of California Philip L. Boyd Deep Canyon Desert Research Center, near Palm Desert CA, 33°38'N, 116°24'W, and 820 m elevation (*A. deserti* and *Y. schidigera*), on a hillside on the Occidental College campus in Los Angeles, CA at 34°7'39"N, 118°12'37"W (*Hesperoyucca whipplei*), and near Joshua Tree National Park, San Bernardino County, CA, 33°53'N, 115°49'W (*Y. brevifolia*). Seeds of other species and larger plants were purchased from sources that provided accession numbers in most cases. Plants were grown in a 1:1:1 mix of commercial cactus potting soil, coarse sand and volcanic pumice in progressively larger containers. Seedlings were grown for 90 d in a growth chamber (Environmental Growth Chambers, Chagrin Falls, OH, USA) with 12 h light (photosynthetic photon flux density of 450  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 24/18 °C. After 90 d, the plants were moved to a glasshouse at Occidental College where they received 80% ambient solar radiation, with daily average maximum/minimum temperatures of 28/16°C and an annual maximum/minimum of 45/5 °C. Depending on seasonal temperatures and plant size, the plants were watered once or twice weekly and fertilized monthly with 0.1-strength Hoagland's solution supplemented with micronutrients.

### Root contraction

When seedlings developed their first true leaves (*ca.* 8 weeks after emergence), fine plastic-coated wires *ca.* 70 mm long were inserted at the base of the shoot just above the root–shoot junction. The soil line was marked on the container with ink, and the height of the wire above the ink mark was measured to the nearest millimetre with a ruler weekly for 8 weeks for four species ( $n = 7\text{--}9$  plants) and monthly or bimonthly for 1 year or more for 24 species; measurements of wire height were corrected for soil settling by the difference between the ink mark and the soil level. Wire displacement caused by stem growth was not observed during measurements. CRs were also observed directly for small plants grown in 40 × 8 × 22 cm containers with one inward-slanted clear plastic side, covered with a removable sheet of opaque plastic (Fig. 1a; Root-View Farm, HSP Nature Toys, Coronado, CA, USA). Larger plants were grown in tapered pots 11 × 11 × 36 cm deep to accommodate longer roots and root contraction. Unless specified otherwise, annual rates of root contraction were measured for 2- to 3-year-old plants ( $n = 4\text{--}5$  plants).

### Root anatomy

To investigate possible anatomical differences between contractile and NCRs, transverse and longitudinal sections



**Figure 1.** (a) Two-year-old plants of *Agave deserti* in Plexiglas chamber, with wires attached to plant base to measure root contraction; roots of (b) *Hesperoyucca whipplei*, a species with contractile roots (CRs) (note that central white root has not yet contracted) and (c) *Agave dasyliroides*, with non-contractile roots (NCRs). Scale bars = 10 mm.

were made by hand with razor blades and with a vibratome, model 1000 (The Vibratome Company, St Louis, MO, USA). Sections were stained with 0.1% (w/w) toluidine blue O in phosphate buffer for general anatomical features, and examined using a Nikon Eclipse ME 600 light microscope (Nikon Instruments, Inc., Melville, NY, USA) at magnifications of 100–1000 $\times$ . To detect suberin in cell walls, sections were stained with 0.1% (w/w) Sudan red 7B in 70% ethanol; for lignin, sections were stained with 0.5% (w/w) phloroglucinol in water followed by 20% HCl (Jensen 1962). Photographic images were made using a Spot RT Color digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI, USA) and Adobe Photoshop Elements 4.0 software (Adobe Systems, Inc., San Jose, CA, USA).

### Root hydraulic conductance

Plants used for measurements of root hydraulic conductance ( $L_P$ ;  $\text{m s}^{-1} \text{MPa}^{-1}$ ) were grown in shallow containers ( $52 \times 26 \times 6$  cm) of 1:1:1 potting soil, coarse sand and vermiculite to allow roots to be easily removed. Two 10-cm-long root zones were investigated, the basal zone immediately below the root–shoot junction and the midroot

zone, ca. 15–25 cm from the shoot base. For both root zones, segments 80 mm long were cut while the root was immersed in distilled water, and tissues external to the vascular cylinder were removed from one end of the segment, leaving a 10 mm length of stele exposed. The stele was sealed into a microcapillary using a compression fitting, and the other end of the root segment was sealed with dental impression material to prevent water entering the xylem directly. Basal segments of NCRs were free of lateral roots; other root segments were selected with as few lateral roots as possible, but when present lateral roots were trimmed to 1 mm and sealed with dental impression material. The root segment was suspended in distilled water, and a partial vacuum was applied to draw water through the root. The volumetric flow rate of water into the root segment ( $Q_V$ ,  $\text{m}^3 \text{s}^{-1}$ ) was measured and used to calculate  $L_P$  (North & Nobel 1991; North *et al.* 2004).

Axial (xylem) hydraulic conductance ( $K_h$ ;  $\text{m}^4 \text{s}^{-1} \text{MPa}^{-1}$ ) was measured on the same root segments as for  $L_P$ , with the distal seal removed and the end retrimmed under water. About 1 mm of the cut end was immersed in water, and  $Q_V$  was measured and used to calculate  $K_h$ :

$$K_h = Q_V / \Delta P / \Delta x \quad (1)$$

where the pressure difference  $\Delta P$  (MPa) was applied across the length  $\Delta x$  (m) of the root segment.

The radial hydraulic conductance ( $L_R$ ;  $\text{m s}^{-1} \text{MPa}^{-1}$ ) along a root was calculated using values of  $L_P$ ,  $K_h$  and the length  $l$  (m) and the radius  $r_{\text{root}}$  (m) of the root segment:

$$L_R = L_P \alpha / \tanh(\alpha l) \quad (2)$$

where  $\alpha$  ( $\text{m}^{-1}$ ) equals  $(2\pi r_{\text{root}} L_R / K_h)^{1/2}$  which represents the length along the xylem where the pressure decreases by half (Landsberg & Fowkes 1978).

### Phylogeny and environmental variables

A pruned phylogenetic tree including Agavaceae and Asphodelaceae was generated based on previously published studies using morphological and molecular data. Published ranges and herbarium listings (Reynolds 1950; Gentry 1982; TROPICOS database, Missouri Botanical Garden, <http://mobot.mobot.org/W3T/Search/vast.html>) were used to determine latitude, longitude and elevation whenever possible for the species investigated, and mean yearly rainfall for the last 50 years was obtained for plants in the USA using climate data collected and summarized by PRISM (<http://prism.oregonstate.edu/>). For species in Mexico and South Africa, climate data were obtained from published sources (Gentry 1982) and from the Global Historical Climate Network, accessed online: <http://iridl.ldeo.columbia.edu/>.

### Statistical analysis

Analysis of variance (ANOVA) and paired *t*-tests were done using SigmaStat (Systat Software, Inc., Richmond, CA,

**Figure 2.** Photomicrographs of fresh sections of roots stained with toluidine blue O. Cross sections of roots of *Hesperoyucca whipplei*, a species with contractile roots (CRs), were made (a) in the contractile zone at 20 mm from the shoot base of a glasshouse-grown plant, showing the exodermis (exo); outer, middle and inner cortex (cor); endodermis (en); and vascular cylinder (vc), and at (b) 30 mm and (c) 300 mm from the shoot base of a root excavated from a ca. 3-year-old plant in the field. Cross sections of a root of *Yucca schidigera*, a species with CRs, were made at (d) 20 mm from the shoot base in the contractile zone, and (e) 120 mm from the shoot base in the non-contractile zone. Radial longitudinal sections showing inner cortical cells of a root of *Yucca flaccida* were made at (f) 20 mm from the shoot base in the contractile zone, and (g) 100 mm from the shoot base in the non-contractile zone; arrows indicate direction of root base and root apex. Cross sections of a root of *Aloe brevifolia*, a species with non-contractile roots (NCRs), were made at (h) 20 mm from the shoot base, and (i) 120 mm from the shoot base. Radial longitudinal sections of roots of *Agave ocahui*, a species with CRs, were made (j) through the outer tissues, showing the epidermis (with root hairs), exodermis (exo) and outer cortex (cor; note that two roots are shown) and (k) through the vascular cylinder, showing compressed xylem (xyl). Scale bars = 50  $\mu$ m.

USA). Mixed-model ANOVA, analysis of covariance (ANCOVA), correlations and regressions were done using BIOMstat (Exeter Software, Setauket, NY, USA). Data were transformed as necessary, and either the Bonferroni or Holm-Sidak method was used to adjust for multiple comparisons.

## RESULTS

### Root morphology and anatomy

Contractile roots were identified morphologically by transverse wrinkling of outer root tissues and radial enlargement of the basal root zone (directly beneath the shoot) relative to the midroot zone, as shown for a 3-year-old plant of *H. whipplei* (Fig. 1b). For most species with CRs, a 7- to 10-cm-long root zone just below the shoot was contractile, although a few species such as *Y. brevifolia* (Joshua tree) had roots that were transversely wrinkled along most of their length. In plants with typical NCRs, such as *Agave dasyliroides* (Fig. 1c), the basal root zones showed neither wrinkling nor radial enlargement. In addition, NCRs usually produced lateral roots within 3 cm of the shoot base (Fig. 1c), whereas this zone of CRs generally lacked lateral roots.

Anatomically, the contractile basal zone of CRs differed from the non-contractile midroot zone (of the same roots) in several ways. A cross section of the basal root zone in *H. whipplei* (Fig. 2a) shows cellular features that were characteristic of all the species examined that possessed CRs. Such features were also observed for roots of *H. whipplei* in the field (Fig. 2b,c). Firstly, cells of the inner and/or middle cortex in basal zones of CRs were elongated radially (Fig. 2a,b,d), whereas cortical cells in midroot (non-contractile) zones of the same roots were either collapsed, as seen in field-grown plants (Fig. 2c) or nearly isodiametric (Fig. 2e). In longitudinal sections, inner cortical cells of the basal zone of CRs (Fig. 2f) were ca. 25–30% shorter and two times wider than cortical cells in non-contractile zones of the same root (Fig. 2g). Secondly, for CRs, cell walls in the endodermis and cortical cells just outside the endodermis were less suberized and/or lignified in basal (Fig. 2d) than in midroot zones (Fig. 2e), and late metaxylem vessels in the basal zone of CRs were not fully lignified. In contrast, for NCRs, basal (Fig. 2h) and midroot zones (Fig. 2i) had isodiametric cortical cells, and cell walls in the endodermis and adjacent cortex were slightly more suberized and lignified in the basal (Fig. 2h) than in the midroot zone (Fig. 2i).

Thirdly, in longitudinal sections of the contracted basal zone, external tissues (epidermis, exodermis and the outer cortex; Fig. 2j) and vascular tissues (primarily protoxylem and early metaxylem vessels; Fig. 2k) showed compression. Only outer cortical cells appeared damaged by compression, whereas the epidermis, exodermis, inner cortical cells and vascular tissues remained intact, and root hairs were often present (Fig. 2j).

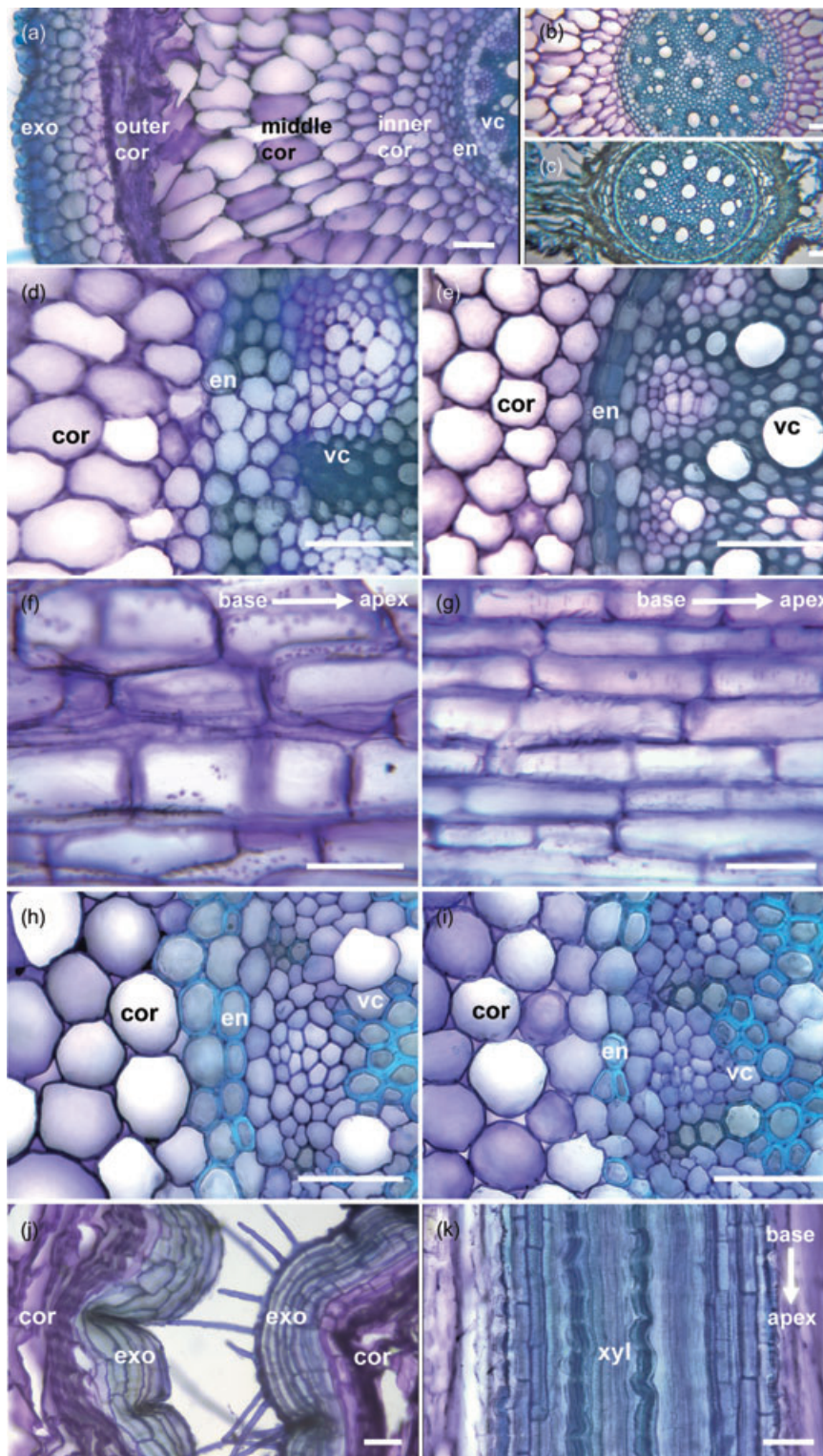
### Phylogenetic distribution of CRs

Based on morphological and anatomical criteria, 40 of the 73 species examined or 55% had roots with contractile zones near the base of the shoot, including 64% of the agaves and 85% of the yuccas (Table 1). For all species examined in the Agavaceae, morphological and anatomical differences between CRs and NCRs were unambiguous. In the Asphodelaceae, the two root types were less easily distinguished, especially for the genus *Aloe*. One species, *Aloe cooperi*, had radially enlarged, succulent roots, but no transverse wrinkling was observed, and there was little difference in endodermal suberization or lignification between basal and midroot zones. The basal root zones of two aloes, *Aloe tomentosa* and *Aloe vacillans*, showed slight transverse wrinkling and compression of external and vascular tissues; however, suberization and lignification in the endodermis and adjacent cortical cells were slightly more extensive in basal than in midroot zones, and cortical cells in both zones were nearly isodiametric. Thus, all species of *Aloe* were designated as having NCRs.

A phylogenetic tree of the taxa examined in the Asphodelaceae and Agavaceae was drawn, including only genera and species whose evolutionary relationships were previously investigated using molecular and other current methods of analysis (Fig. 3). The genus *Agave* is represented by only two taxa because of lack of resolution at the species level. Only genus names (e.g. *Aloe*) are given when all members of the taxon examined had either CRs or NCRs. The distribution of CRs throughout the tree is consistent with multiple independent acquisitions or losses of the trait.

### Root contraction

Seedlings of three of the four species measured were pulled down further into the soil over an 8 week period, as



indicated by the decrease in height above the soil line of wires attached to shoot bases (Fig. 4). *Agave attenuata*, a species with NCRs, showed no significant change over time (repeated measures ANOVA,  $P = 0.07$ ). For the other three species, shoot descent ranged from 9 mm for *Y. schidigera* to 21 mm for *Agave mckelveyana* over 8 weeks.

For larger plants, root contraction as quantified by shoot descent ranged within the genus *Agave* from nearly 60 mm year<sup>-1</sup> for *A. deserti* to no measurable displacement for seven of the 16 species examined (Fig. 5a). Only one species within the genus *Yucca* showed no root contraction over the course of a year, whereas the other six species measured

**Table 1.** Species examined morphologically and anatomically for contractile roots (CRs); presence indicated as CR, absence as NCR

Family	Genus	Species	Root type	Family	Genus	Species	Root type
Agavaceae							
	<i>Agave</i>			<i>Yucca</i>			
		<i>americana</i> L.	CR			<i>angustissima</i> var. <i>kanabensis</i> McKelvey	CR
		<i>angustifolia</i> Haworth	CR			<i>brevifolia</i> Engelm.	CR
		<i>attenuata</i> Salm-Dyck	NCR			<i>elata</i> Engelm.	CR
		<i>bovicornuta</i> Gentry	NCR			<i>elephantipes</i> Regel	NCR
		<i>bracteosa</i> S. Watson ex Engelm.	NCR			<i>filamentosa</i> L.	CR
		<i>celsii</i> J. Hooker	NCR			<i>flaccida</i> Haworth	CR
		<i>chrysantha</i> Peebles	CR			<i>glauca</i> Nuttall	CR
		<i>colimana</i> Gentry	NCR			<i>gloriosa</i> L.	NCR
		<i>dasyliroides</i> Jacobi et Bouche	NCR			<i>grandiflora</i> Gentry	CR
		<i>deserti</i> Engelm.	CR			<i>linearifolia</i> Clary	CR
		<i>desmetiana</i> Jacobi	NCR			<i>rostrata</i> Engelm. ex Trelease	CR
		<i>geminiflora</i> (Tagliabue) Ker-Gawler	CR			<i>schidigera</i> Roezl ex Ortgies	CR
		<i>gypsophila</i> Gentry	NCR	Asphodelaceae		<i>torreyi</i> Shafer	CR
		<i>mckelveyana</i> Gentry	CR		<i>Aloe</i>		
		<i>murpheyi</i> Gibson	CR			<i>aristata</i> Haworth	NCR
		<i>ocahui</i> Gentry	CR			<i>brevifolia</i> Mill.	NCR
		<i>palmeri</i> Engelm.	CR			<i>ciliaris</i> Haworth	NCR
		<i>parryi</i> Engelm.	CR			<i>cooperi</i> Bak.	NCR
		<i>pelona</i> Gentry	CR			<i>dawei</i> Berger	NCR
		<i>schidigera</i> Lemaire	CR			<i>dichotoma</i> Masson	NCR
		<i>shawii</i> Engelm.	CR			<i>ferox</i> Mill.	NCR
		<i>sisalana</i> Perrine	CR			<i>haworthioides</i> Bak.	NCR
		<i>stricta</i> Salm-Dyck	CR			<i>hereroensis</i> Engler	NCR
		<i>toumeyana</i> Trelease	CR			<i>juvenna</i> Brandham & S. Carter	NCR
		<i>triangularis</i> Jacobi	NCR			<i>marlothii</i> Berger	NCR
		<i>utahensis</i> Engelm.	CR			<i>pubescens</i> Reynolds	NCR
		<i>victoriae-reginae</i> Moore	CR			<i>sinkatana</i> Reynolds	NCR
		<i>vilmoriniana</i> Berger	NCR			<i>striata</i> Haworth	NCR
		<i>Beschorneria decosteriana</i> × <i>septentrionalis</i>	CR			<i>tomentosa</i> Defl.	NCR
		<i>Dasyliirion wheeleri</i> S. Watson ex Rothrock	CR			<i>vacillans</i> Forssk.	NCR
		<i>Hesperaloe parviflora</i> (Torr.) Coulter	CR			<i>variegata</i> L.	NCR
		<i>Hesperoyucca whipplei</i> (Torr.) Trelease	CR			<i>venusta</i> Reynolds	NCR
		<i>Hosta plantaginea</i> L.	NCR			<i>vera</i> Mill. non L.	NCR
		<i>Manfreda maculosa</i> (Hook.) Rose	CR			<i>wickensii</i> Pole-Evans	NCR
		<i>Nolina bigelovii</i> S. Watson	CR			<i>Gasteria armstrongii</i> Schonl.	CR
						<i>Haworthia</i>	
						<i>cymbiformis</i> (Haworth) Duvall	CR
						<i>fasciata</i> (Willdenow) Haworth	CR
						<i>retusa</i> (L.) Haworth	CR
						<i>venosa</i> (Lamarck) Haworth	CR

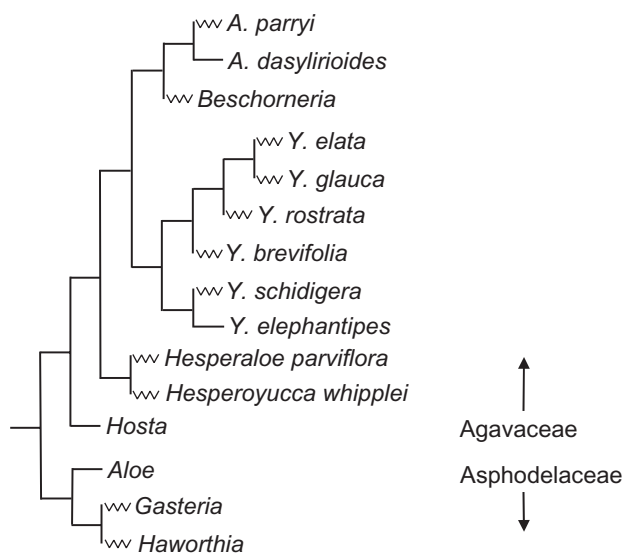
had an average shoot descent of  $34.7 \pm$  mm year<sup>-1</sup> (Fig. 5b). Formerly classified in the genus *Yucca* but now in its own genus, *H. whipplei* descended 40 mm year<sup>-1</sup> (Fig. 5b). Of the four species of *Aloe* examined for root contraction using the wire method, none showed shoot descent (data not shown).

The amount of root contraction per year, as measured for well-watered plants grown in the glasshouse, showed a significant negative relationship with mean annual rainfall in the habitats where the examined species of *Agave* and *Yucca* occur (Fig. 6). For the agaves alone, the regression of root contraction against rainfall yielded values similar to the overall regression ( $y = 50.6 - 0.04x$ ; adjusted  $r^2 = 0.60$ ).

For the yuccas and *Hesperoyucca*, variation in rainfall explained slightly more of the variation in root contraction ( $y = 51.9 - 0.05x$ ; adjusted  $r^2 = 0.69$ ), although the slope did not differ from the overall regression (ANCOVA,  $P = 0.95$ ).

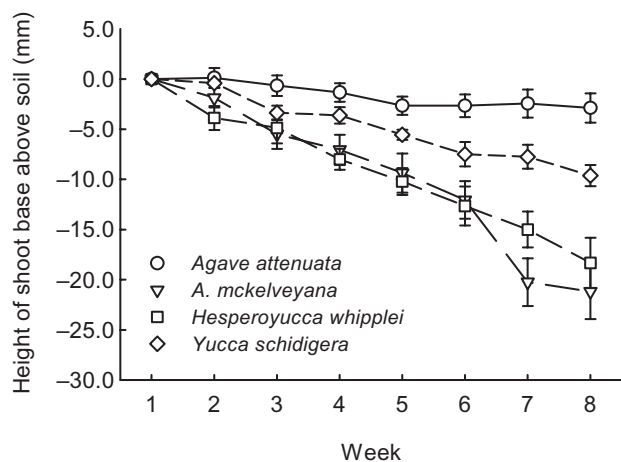
### Root hydraulic conductance

Root hydraulic conductance ( $L_p$ ) and axial conductance ( $K_h$ ) were measured for roots of seven species, three with CRs and four with NCRs (Fig. 7). *Agave attenuata* had significantly higher  $L_p$  than did the other species except for *Aloe brevifolia*, which in turn had higher  $L_p$  than *H.*



**Figure 3.** A pruned phylogenetic tree with representative members of the Agavaceae and Asphodelaceae that were examined for contractile roots (CRs). Relationships among the taxa were determined from previous studies; specifically, Bogler, Pires & Francisco-Ortega (2005) and Good-Avila *et al.* (2006) were used for relationships within *Agave* and the Agavaceae; Adams *et al.* (2000), Chase *et al.* (2000) and Graham *et al.* (2006) were used for relationships within the Asphodelaceae, and between the Asphodelaceae and the Agavaceae; and Pellmyr *et al.* (2007) was used for relationships within *Yucca*. Branches were set to an arbitrary uniform length; taxa with CRs are indicated by a zig-zag line.

*whipplei* and *Y. brevifolia* (two-way ANOVA,  $P < 0.01$ ); other interspecific pair-wise comparisons yielded no significant differences. Within individual roots,  $L_P$  for the basal zone differed significantly from that for the midroot zone for five of the seven species (two-way repeated measures ANOVA,  $P = 0.001 - 0.042$ ; Fig. 7a). For species with CRs,  $L_P$  for the

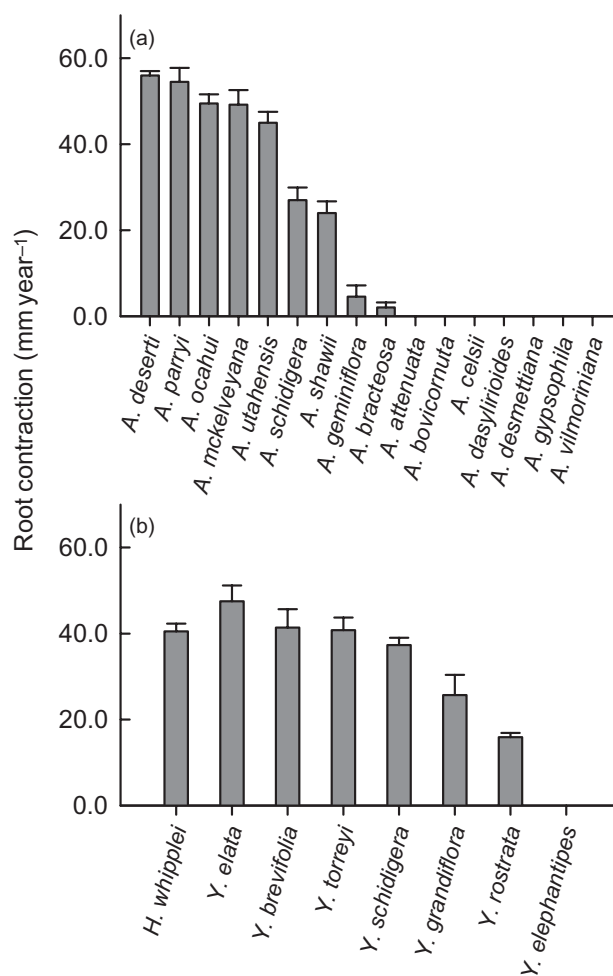


**Figure 4.** Root contraction measured as shoot descent during 8 weeks for seedlings of four species (*Agave attenuata*, *Agave mckelveyana*, *Hesperoyucca whipplei* and *Yucca schidigera*). Data are means  $\pm$  SE for  $n = 7-9$  plants.

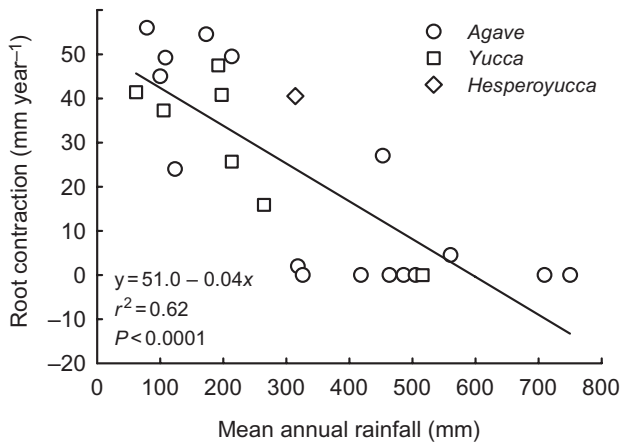
basal root zone was greater than at midroot, with a basal/midroot ratio of  $1.88 \pm 0.19$  ( $n = 23$ ), which differed significantly from the NCR ratio of  $0.71 \pm 0.06$  ( $n = 15$ ; *t*-test,  $P < 0.001$ ).

Axial hydraulic conductance ( $K_h$ ), conductance through the xylem, was more variable than  $L_P$  within species, but again *A. attenuata* had the greatest conductance (Fig. 7b). Pair-wise comparisons indicated that *A. attenuata* had higher  $K_h$  than the other species except for *A. deserti* and *Y. brevifolia*, and both *A. deserti* and *Y. brevifolia* had higher  $K_h$  than the aloes (two-way ANOVA,  $P < 0.01$ ); no other interspecific differences were significant. Within individual roots, basal  $K_h$  was lower than at midroot for *A. deserti* and *H. whipplei* (two-way repeated measures ANOVA,  $P < 0.01$ ), reflecting the trend for  $K_h$  to increase from the basal to the midroot zone for all CRs (Fig. 7b). The ratio of basal/midroot  $K_h$  was  $0.68 \pm 0.11$  ( $n = 23$ ) for CRs, which was significantly lower than the ratio for NCRs,  $2.92 \pm 0.82$  ( $n = 15$ , *t*-test,  $P < 0.001$ ).

Root radial conductance ( $L_R$ ), from the root surface to the root xylem, was calculated using values of  $L_P$  and  $K_h$  in



**Figure 5.** Root contraction measured as shoot descent during 1 year for 2- to 3-year-old plants of (a) 16 species of *Agave* and (b) *Hesperoyucca whipplei* and seven species of *Yucca*. Data are means  $\pm$  SE for  $n = 4-5$  plants.



**Figure 6.** Annual root contraction compared with mean annual rainfall for 24 species in the Agavaceae (as named in Fig. 5). Rainfall data were obtained from publically available meteorological data as described in the text.

Eqn 2. In all cases but one,  $L_R$  exceeded  $L_P$  by less than 10% (data not shown), indicating that the primary hydraulic resistance across root segments was radial rather than axial. For midroot segments of *A. brevifolia*,  $L_R$  exceeded  $L_P$  by 29%, but this difference was not significant (*t*-test,  $P = 0.308$ ).

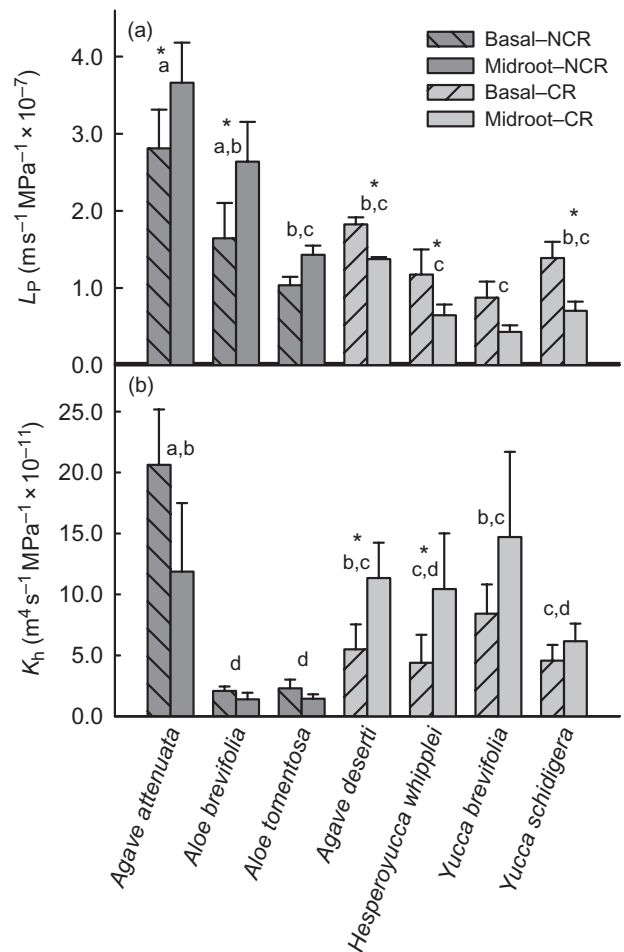
## DISCUSSION

### Phylogenetic distribution

The distribution of CRs among the taxa examined and interspecific differences in the amount of contraction suggest that the trait reflects both phylogenetic constraint and convergent evolution. The lack of CRs in the species of *Aloe* investigated here indicates that aloes diverged with respect to this trait not only from the Agavaceae, but also from two genera that are usually grouped with *Aloe* in the same subfamily of the Asphodelaceae, *Gasteria* and *Haworthia* (Treutlein *et al.* 2003). Taxonomic uncertainties within *Aloe* as well as the large size of the genus (perhaps 400; Adams *et al.* 2000) render claims about the presence or absence of a trait such as CR tentative; in addition, two *Aloe* species examined here showed some, but not all, of the characteristics associated with CRs. Interestingly, both of these species, *A. tomentosa* and *A. vacillans*, occur at locations in Saudi Arabia and Yemen that are geographically and environmentally distinct from the South African sites of most of the other aloes examined.

Within the Agavaceae, the distribution of CRs is consistent with either multiple acquisitions or losses of the trait. Whether CRs or NCRs are ancestral in the family is difficult to determine from trait distribution in extant taxa. *Hosta plantaginea*, whose genus is placed near the base of the family or just outside the Agavaceae *sensu stricto* according to molecular evidence (Bogler *et al.* 2005), lacks CRs and is native to east Asia; however, the closely related genera *Camassia* and *Chlorogalum* have CRs and are native to

California (Rimbach 1929). It is suggestive that *Yucca elephantipes*, one of only two species of *Yucca* found to lack CRs, occupies a basal or nearly basal position within the genus (Pellmyr *et al.* 2007). Despite generally poor taxonomic resolution within the genus *Agave* (Bogler *et al.* 2005; Good-Avila *et al.* 2006), molecular evidence supports a basal position for *A. dasylirioides*, as originally suggested by Gentry on the basis of its relatively non-succulent leaves, iteroparous reproduction and generalized floral traits (Gentry 1982). Like *Y. elephantipes*, *A. dasylirioides* lacks CRs, suggesting that NCRs may be the primitive or ancestral condition in both agaves and yuccas. However, molecular and morphological data link two species possessing CRs, *Agave stricta* and *Agave schidigera*, with *A. dasylirioides* (Bogler *et al.* 2005; Good-Avila *et al.* 2006). Thus, CRs appear to be a trait that is neither definitively ancestral



**Figure 7.** (a) Root hydraulic conductance ( $L_P$ ) and (b) axial hydraulic conductance ( $K_h$ ) for root segments from the basal zone (diagonal bars) and the midroot zone (plain bars) for species with contractile roots (CRs; light grey bars) and species with non-contractile roots (NCRs; dark grey bars). Different letters above bars indicate significant differences between species, and asterisks indicate significant differences between basal and midroot regions within a species ( $P < 0.05$ ). Data are means  $\pm$  SE for  $n = 4-6$  plants.



or derived, yet distributed among the taxa examined in a pattern consistent with independent and possible convergent evolution.

### Root contraction and rainfall

For the genera *Agave*, *Hesperoyucca* and *Yucca*, the relationship between annual root contraction and yearly rainfall was negative and highly significant ( $P < 0.0001$ ), yet not fully explanatory (adjusted  $r^2 = 0.62$ ). As shown in Fig. 6, all species with NCRs are from regions that receive more than 650 mm of rainfall per year. Only two species with CRs, *A. schidigera* and *H. whipplei*, occur in regions with more than 500 mm of rainfall, and both experience dry seasons lasting at least 3 months. If low rainfall and CRs are functionally related, one hypothesis to explain the lack of CRs in the aloes examined is that they tend to occur in regions where rainfall is more evenly distributed throughout the year than it is in the south-western USA and northern Mexico, where most agaves and yuccas with CRs occur (Esler & Rundel 1999). For example, the range of *A. brevifolia* includes Riversdale (Natal, South Africa), where mean annual rainfall for a 10-year period was  $439 \pm 26$  mm, with a low monthly mean of  $20 \pm 3$  mm and a high monthly mean of  $57 \pm 10$  mm. In contrast, a site for *Agave parryi* in Arizona (USA) had an annual rainfall of  $346 \pm 19$  mm, a low monthly mean of  $8 \pm 2$  mm and a high monthly mean of  $67 \pm 10$  mm. However, the ranges of *Haworthia* and *Gasteria*, both groups with CRs, overlap the ranges of several aloes; in addition, some of the aloes examined, including *A. dichotoma*, occur in regions with very low rainfall, including months with no precipitation. An important caveat with respect to the relationship between rainfall and CRs is that other environmental factors such as temperature are likely to covary with rainfall.

Despite its imperfect predictive power, the correlation between low rainfall and CRs in the genera *Agave* and *Yucca* suggests several possible advantages of CRs in arid habitats. Firstly, all plants in the Agavaceae form adventitious roots at the base of the shoot; therefore, positioning the shoot deeper may allow new roots to be initiated in moister soil, where they can take up water and nutrients. Secondly, the more moderate soil temperatures that prevail at greater depths are conducive to root growth, at least for *A. deserti* (Drennan & Nobel 1996). Thirdly, root contraction may help anchor or re-anchor seedlings in dry, shifting and/or sandy soil. In this regard, roots of the arborescent *Y. brevifolia* (Joshua tree) are contractile for most of their length, perhaps reflecting greater anchorage demands associated with the arborescent form. Fourthly, and perhaps most important, certain morphological and anatomical properties of CRs may facilitate water uptake.

### Root contraction and hydraulic conductance

Within individual CR, the basal root zone had higher hydraulic conductance ( $L_P$ ) than did the midroot zone, a pattern opposite not only to what would be expected on the

basis of root ontogeny, but also to what was observed in NCRs. For NCRs, as for typical roots of most dicots and monocots, the basal root zone as the oldest zone had tissues that were more highly differentiated than in younger zones; specifically, both the endodermis and adjacent cortical cells had more lignified and suberized cell walls than did these tissues at midroot. Although the relationship between such tissue differentiation and water permeability is complicated (North & Peterson 2005),  $L_P$  for desert succulents including various cacti and *A. deserti* is reduced in highly lignified/suberized root zones (North & Nobel 1991, 1992). In the CR zone of CRs, the cell walls of the endodermis remained less suberized than at midroot, and unsuberized passage cells were common, even for older roots (>6 months of age) and roots of plants in the field (e.g. *H. whipplei*). The lack of endodermal cell differentiation may help the vascular cylinder and inner cortex of the basal root zone undergo radial expansion and longitudinal compression without tissue shearing or tearing.

Other features of CRs that could improve water uptake in the basal root zone are the radial expansion of cortical cells and their remaining intact. Radial swelling could improve root-soil contact near the soil surface, which can otherwise be reduced by root shrinkage during drought, forming a gap that interferes with water movement (North & Nobel 1997). Unlike midroot zones, the basal zones of CRs were frequently observed to have root-hairs, which would also help maintain root-soil contact and facilitate water absorption. From the root surface to the root xylem, the basal root zone of CRs had a more continuous path for water movement than was evident in the midroot zone, as could be seen most clearly in roots of *H. whipplei* in the field (Fig. 2a versus 2b). Only outer cortical cells collapsed in the basal zone of CRs, in distinction to the disruption of the entire cortex with age at midroot for CRs and in both basal and midroot zones of NCRs. Not only was the radial pathway intact in the basal zone of CRs, but the continued vitality of cortical cells in the contractile zone, as previously observed in the basal root zone for *A. deserti*, is associated with greater aquaporin activity than in the midroot zone (North *et al.* 2004).

In contrast to higher  $L_P$ , axial conductance ( $K_h$ ) was lower for the basal root zone than for the midroot zone of CRs, a pattern that was reversed for NCRs. Presumably, the lower basal  $K_h$  for CRs is caused by immaturity of the late metaxylem. The compression evident in the protoxylem and early metaxylem may have been a factor as well, although no breakage or discontinuities in vessels were observed. The decrease in  $K_h$  and increase in  $L_P$  (as well as  $L_R$ ) in the basal zone of CRs relative to distal root zones can be considered an increase in the 'leakiness' of CRs near the soil surface; in other words, radial water movement would be favoured at the expense of axial movement, thereby decreasing the effective length of the absorptive zone and restricting uptake to near the soil surface (Landsberg & Fowkes 1978; Hsiao & Xu 2000; Zwieniecki, Thompson & Holbrook 2003). Such restriction might be beneficial to succulents with CRs for two reasons. Firstly, a bottleneck in the

xylem of the basal root zone may help to prevent reverse water flow from the succulent shoot to a drier soil (Ewers, North & Nobel 1992). Secondly, for species with CRs in arid habitats, the absorptive basal root zone would be located in the region of the soil that is most likely to be wetted because of limited and infrequent rainfall.

An alternative root adaptation that could lead to improved water uptake near the soil surface, proliferation of lateral roots, did not occur for agaves and yuccas with CRs. Despite reports of desert succulents producing ephemeral 'rain roots' in response to rainfall events, *A. deserti* tends to produce lateral roots in favourable microsites such as under rocks, not near the soil surface (Nobel, Miller & Graham 1992), and abscises them when soil water potential declines below a critical level (North, Huang & Nobel 1993). In contrast, species with NCRs, including *A. attenuata*, *A. dasylirioides*, *Agave desmettiana* and *Y. elephantipes*, produced several lateral roots near the base of the shoot. Even with lateral roots removed, as in the case of *A. attenuata*, the interruption of the suberized layers in the parent root by the emergence of lateral roots can increase hydraulic conductance (Zwieniecki *et al.* 2003). The four mentioned species with NCRs and copious lateral roots are all from regions with mean annual rainfall of 1000 mm or more; for these species, production of lateral roots would be more cost effective than for desert species such as *A. deserti* that lose lateral roots at the onset of drought. For the desert species, an additional advantage of the absorptive zone associated with CRs would be to facilitate water uptake quickly after rainfall without the lag time involved in the production of lateral roots.

## CONCLUSIONS

The evidence that CRs are adaptive for succulent monocots in arid habitats is threefold. Firstly, an evolutionary gain or loss of CRs occurred repeatedly in the Asphodelaceae and Agavaceae in a pattern that is not entirely explained by phylogenetic constraints. Further taxonomic resolution within the genera *Agave* and *Aloe* will be useful in determining the extent to which root contraction is independent of phylogeny. Convergent evolution between agaves, yuccas and aloes did not appear to extend to their root systems, although the absence of CRs in the aloes may be caused by inadequate sampling or to real differences in rainfall patterns between the African and American locations. A second line of evidence is the significant negative relationship between mean annual rainfall and amount of root contraction. To isolate rainfall as a key environmental determinant of CRs, however, will require further investigation of the trait with respect to variables such as temperature, particularly temperature extremes. The third line of evidence is that CRs and NCRs diverged in hydraulic conductance, with the basal zone of CRs having a greater capacity for water uptake than non-contractile midroot zones. A general role for CRs in the plant kingdom is to pull shoots further down into the soil to help them evade unfavourable conditions. A further benefit for succulent

monocots is that CRs can increase water uptake near the soil surface, and thereby improve chances for survival in regions of limited rainfall.

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