RESEARCH PAPER

Crassulacean acid metabolism in the Basellaceae (Caryophyllales)

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ABSTRACT

- C_4 and crassulacean acid metabolism (CAM) have evolved in the order Caryophyllales many times but neither C_4 nor CAM have been recorded for the Basellaceae, a small family in the CAM-rich sub-order Portulacineae.
- 24 h gas exchange and day–night changes in titratable acidity were measured in leaves of *Anredera baselloides* exposed to wet–dry–wet cycles.
- While net CO_2 uptake was restricted to the light period in well-watered plants, net CO_2 fixation in the dark, accompanied by significant nocturnal increases in leaf acidity, developed in droughted plants. Plants reverted to solely C_3 photosynthesis upon rewatering.
- The reversible induction of nocturnal net CO_2 uptake by drought stress indicates that this species is able to exhibit CAM in a facultative manner. This is the first report of CAM in a member of the Basellaceae.

INTRODUCTION

The photosynthetic physiologies expressed within the 12,500 species of Caryophyllales are diverse (Hernández-Ledesma *et al.* 2015). Seven families contain species with crassulacean acid metabolism (CAM; Smith & Winter 1996), the most water-use efficient form of photosynthesis, and eight families contain C_4 species (Sage 2016). It has been estimated that in the Caryophyllales C_4 has originated at least 23 times and there have been multiple CAM origins (Sage *et al.* 2011; Christin *et al.* 2014, 2015). Only the Asparagales, which includes the speciose Orchidaceae (~28,000 species; Christenhusz & Byng 2016), contains more CAM species than the Caryophyllales, and only the Poales has more C_4 species (Sage *et al.* 2011). Within the Caryophyllales, Aizoaceae and Portulacaceae contain both CAM and C_4 species. The Portulacaceae is particularly noteworthy in that it contains the only known lineage of terrestrial C_4 plants with Kranz anatomy that also uses CAM. At least six species of *Portulaca*, the sole genus in the family, express both C_4 and CAM photosynthesis in their leaves (Koch & Kennedy 1980; Ku *et al.* 1981; Holtum *et al.* 2017a; Winter & Holtum 2017).

In contrast to C_3 and C_4 photosynthesis, which involve net CO_2 uptake in the light, CO_2 assimilation in CAM tissues occurs during the dark. The carbon fixed is stored overnight as malic acid in vacuoles of the chloroplast-containing cells in which it was fixed. During the ensuing light period, stomata close and the carbon is converted to carbohydrate (Holtum & Osmond 1981; Holtum *et al.* 2005). CAM tissues are thus characterised by nocturnal CO_2 assimilation, nocturnal acidification and stomatal conductance that is low during large parts of the day. Low stomatal conductance during the heat of the light period is a major contributor to the low water use of CAM plants (Neales *et al.* 1968; Winter *et al.* 2005).

Mature photosynthetic organs in most terrestrial C_3 and C_4 plants exhibit only one form of photosynthetic carbon metabolism during their lifetime. Their rate of CO_2 uptake may vary in response to environment and/or ontogeny but the pathway by which CO_2 is assimilated does not vary. In contrast, with the exception of possibly some cacti (Nobel 1988), there are no known species in which CAM is the sole contributor to carbon gain in mature photosynthetic tissues of well-watered terrestrial plants. CAM almost always co-exists with C_3 photosynthesis, or, in *Portulaca*, with C_4 photosynthesis. Moreover, in plants that express CAM, the proportional contributions of CAM, C_3 or C_4 photosynthesis to net carbon gain are affected by ontogeny and/or environment.

While in archetypal CAM plants such as cacti and agaves nocturnal CO_2 fixation is the major contributor to carbon gain under well-watered conditions, it has become increasingly clear in recent years that, in a large number of the species in which the CAM cycle is present, nocturnal CO_2 fixation contributes less to carbon gain than does C_3 photosynthesis (Winter *et al.* 2015). For both CAM species with strongly expressed CAM,

and C_3-CAM species with low-level CAM, exposure to stress such as drought generally results in a reduction in the 24-h carbon gain. Since the drought-related decline in CO_2 uptake during the light is disproportionately large, the proportional contribution of dark fixation to the remaining carbon gain usually increases (Winter & Holtum 2014).

In species with constitutive or obligate CAM, CAM is part of a pre-programmed developmental sequence, and CAM gradually develops as photosynthetic organs mature. In these plants, the expression of CAM is not optional, it continues throughout the life of the organ and is observed in well-watered plants. In contrast, facultative CAM is a phenomenon whereby, following water deficit stress (including high soil salinity in some species), nocturnal CO_2 uptake is induced, or increases in magnitude. When the stress is removed, nocturnal CO_2 uptake is reduced, or disappears entirely. Thus, in facultative CAM plants, the expression of CAM is optional, as it depends upon the presence of the stress (Winter & Holtum 2014). The term 'facultative' refers to reversible environmentally mediated increases in the magnitude of CO_2 uptake and acid accumulation at night (Winter & Holtum 2011).

Constitutive and facultative CAM are not mutually exclusive, since a plant with constitutive CAM may exhibit a facultative CAM component that only shows when the plant is stressed (Winter & Holtum 2007). An issue that has led to confusion in the past (Adams et al. 1998; Dodd et al. 2002) is that stress may enhance the expression of CAM by accelerating ontogeny and increasing the physiological age of a tissue. In those cases where CAM is not lost or reduced when the stressor is removed, CAM enhancement would not be considered facultative.

The majority of the species known to have CAM in the Caryophyllales are in the 'portullugo', the clade that includes the sub-order Portulacineae plus Molluginaceae (Ogburn & Edwards 2010; Edwards & Ogburn 2012). CAM is known in six of its nine families (Hernández-Ledesma et al. 2015): Anacampserotaceae (Kluge & Ting 1978), Cactaceae (Richards 1915), Didiereaceae (Kluge & Ting 1978; Winter 1979; Von Willert et al. 1992), Montiaceae (Guralnick & Jackson 2001), Portulacaceae (Koch & Kennedy 1980) and Talinaceae (Martin & Zee 1983) but has not been reported in the Basellaceae, Halophytaceae or Molluginaceae.

The portullugo clade displays the entire spectrum of CAM expression. In the Anacampserotaceae facultative and constitutive CAM species have been reported (Guralnick & Jackson 2001; Guralnick et al. 2008; Winter & Holtum 2017). In Cactaceae, CAM is the predominant pathway of CO_2 fixation, with the exception of, for example, Pereskia, a lineage sister to core Cactaceae, which contains both C_3 and facultative CAM species (Nobel & Hartsock 1986; Edwards & Diaz 2006; Edwards & Donoghue 2006). In the Didiereaceae, CAM is strongly expressed in Alluaudia, Alluaudiopsis, Decarya and Didieria (Winter 1979), whereas in Portulacaria (which now includes Ceraria; Bruyns et al. 2014) CAM is less pronounced and tends to be facultative (Ting & Hanscom 1977; Veste et al. 2001). Facultative and constitutive CAM have been reported in C_3–CAM species of the Montiaceae and Talinaceae (Harris & Martin 1991; Herrera et al. 1991; Guralnick & Jackson 2001; Winter & Holtum 2011; Holtum et al. 2017b). Portulacaceae contains the only known C_4–CAM species, in all of which

CAM appears to be facultative (Koch & Kennedy 1980; Guralnick et al. 2002; Holtum et al. 2017a; Winter & Holtum 2017).

The abundance and multiple occurrences of CAM in the Caryophyllales, particularly in the portullugo clade, prompted us to investigate the Basellaceae, especially since it is closely related to the iconic CAM family Didiereacae (Anton et al. 2014). Here we demonstrate, using both leaf gas exchange and measurements of nocturnal acidification in leaves, that CAM is present in the geophytic vine Anredera basselloides (Kunth) Baill., the first report of CAM in the family Basellaceae.

MATERIAL AND METHODS

Plants were grown from tuber cuttings in either 1-l terracotta pots with an upper diameter of 13 cm (gas exchange) or in 3-l terracotta pots with an upper diameter of 19 cm (titratable acidity). Pots contained potting mix (Miracle-Gro Lawn Products, Marysville, OH, USA).

In three separate experiments, an attached leaf of a plant that was watered daily to field capacity was enclosed inside a circular gas exchange cuvette (PMK 10; Walz, Effeltrich, Germany). The stem, other leaves, roots plus pot remained outside the cuvette. Drought treatments were imposed by withholding irrigation until net CO_2 uptake in the light was reduced to close to zero, after which the plants were rewatered daily.

The gas exchange cuvette was located inside a controlled environment chamber operating under 12-h light (28 °C):12-h dark (22 °C) cycles. Photon flux density was 650 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the level of the cuvette. The cuvette was supplied with air containing 400 ppm CO_2 at a flow rate of 1.26 $l \cdot min^{-1}$. Net CO_2 exchange was measured in a flow-through gas exchange system consisting of Walz components, a LI-6252 CO_2 analyser (Li-Cor, Lincoln, NE, USA) and a CR-1000 data logger (Campbell Scientific, UT, USA; Holtum & Winter 2003).

In an experiment separate to the gas exchange experiment, five plants were grown under generally sunny conditions within a naturally lit rain shelter in 3-l pots as described above under about 70% sunlight (ca. 22 °C minimum temperature, ca. 32 °C maximum temperature, ca. 12-h light period). Plants watered daily to field capacity were deprived of water for 12 days and then rewatered for 8 days. Mature leaves, excised at the end of the day and night from each well-watered, droughted and rewatered plant, were weighed for fresh mass determination and leaf area was measured with a LI-3100 area meter (Li-Cor). Samples were then frozen in liquid nitrogen and freeze-dried. After determination of dry mass, samples were boiled in 80 ml 50% ethanol until the volume had about halved. Water was then added to bring the volume back to 80 ml and the extract was boiled until the volume again decreased by about half. The extracts were brought to the original volume with water, cooled to room temperature and titrated with 5 mM KOH to pH 6.5.

RESULTS

Figure 1 shows the day–night CO_2 exchange pattern of the leaf of an A. basselloides undergoing a wet–dry–wet cycle in a controlled environment chamber. Under well-watered conditions the CO_2 exchange pattern is that of a C_3 plant. Net CO_2 uptake was restricted to the light and CO_2 was lost during the dark. In
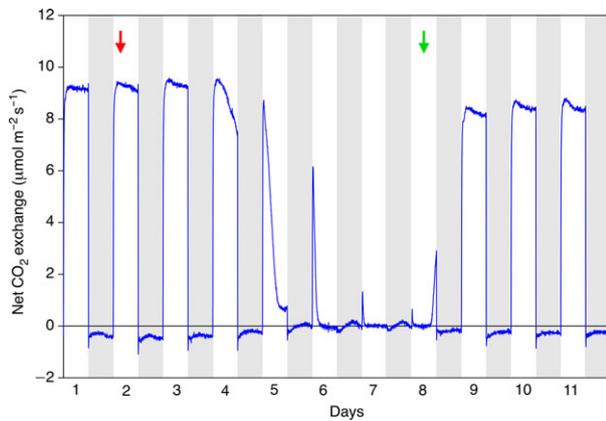
Fig. 1. Eleven days of net CO_2 exchange by a leaf of an *Anredera basel-loides* plant in a pot. Watering was withheld on day 2 (red arrow) and recommenced on day 8 (green arrow). Shaded areas represent the 12-h dark periods. This experiment is representative of three that were performed.

well-watered plants in an associated experiment, there was no difference in the titratable acidity of leaves harvested at the end of the day and at the end of the night, when the data are expressed on fresh mass or leaf area basis (Fig. 2). When expressed on a dry weight basis the acidity level in the tissue harvested at the end of the night was marginally greater than at the beginning of the night. The difference was statistically significant but very small.

Following the cessation of watering, CO_2 uptake during the day declined precipitously until, by day 7, uptake was restricted to a short pulse at the beginning of the light period (Fig. 1). Concomitant with the reduction of net CO_2 uptake during the light, was a reduction of CO_2 loss at night that culminated in a period of CAM-type nocturnal net CO_2 uptake that was initially observed on day 5. In plants that had been droughted in the associated experiment, substantial nocturnal acidification occurred (Fig. 2). In leaves harvested at the end of the night, titratable acidity was 3.5 to 4.0 times higher than in leaves harvested at the end of the day.

Rewatering of droughted plants was accompanied by a rapid recovery in net CO_2 uptake during the light and a loss of net CO_2 uptake during the dark (Fig. 1). Within 24 h of resupplying water, CO_2 uptake in the light and CO_2 loss at night had returned to a pattern similar to that observed prior to the drought treatment. Consistent with the leaf gas exchange, leaves from rewatered plants did not acidify at night (Fig. 2).

DISCUSSION

Anredera baselloides is a plant species capable of employing facultative CAM. The CAM phenotype is clearly present in plants exposed to water deficit stress as they exhibit nocturnal net CO_2 uptake and leaf acidification in the dark. The CAM phenotype is essentially absent in well-watered plants as they show net CO_2 uptake exclusively during the light. When operating in the CAM mode, the maximum rate of observed dark CO_2 uptake, 0.16 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 1, dark period of day 7), was nonetheless small, equivalent to only about 2% of the
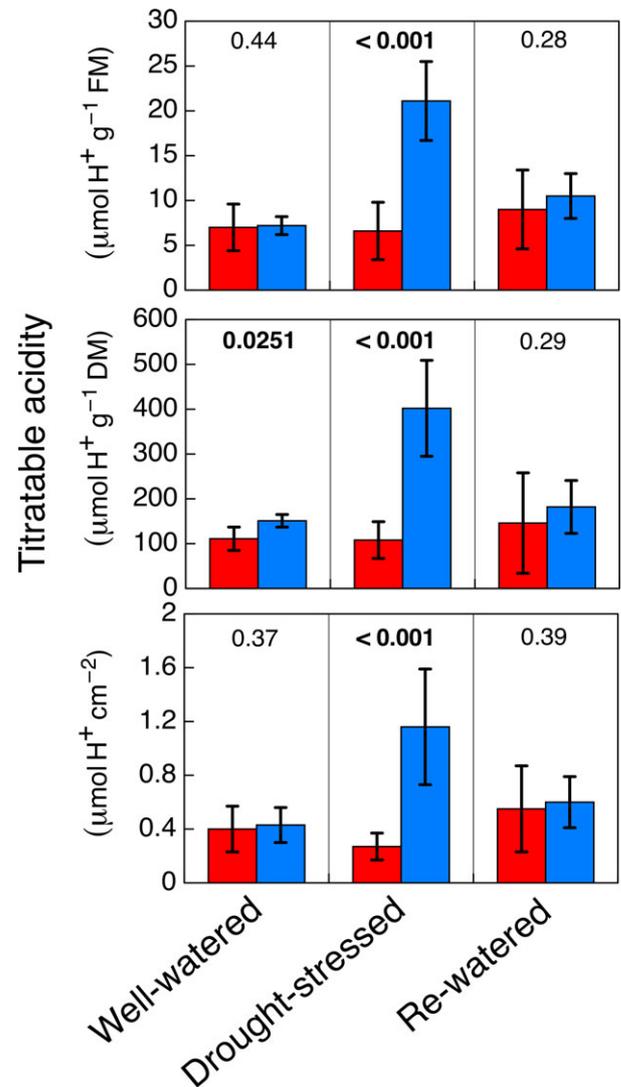

Fig. 2. Titratable acidity in mature fully expanded leaves of *Anredera baselloides* at the end of the day (red) and the end of the night (blue) in well-watered plants (left-hand column), droughted plants (middle column; 12 days without irrigation) and in plants that had been droughted and rewatered (right-hand column; 8 days with irrigation). The data are expressed on a fresh mass basis (upper row), a dry mass basis (middle row) and a leaf area basis (bottom row). Bars are SD (n = 5 leaves; at a given time point each leaf was harvested from a different plant) and numeric values are *P*-values (one-tailed *t*-test).

maximum rate of C_3 photosynthetic CO_2 uptake in the light by well-watered plants.

The overnight accumulation of acid in stressed plants of about 0.9 $\mu mol \cdot cm^{-2}$ (Fig. 2) is equivalent to about 0.1 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ averaged over the night, assuming that the uptake of one CO_2 leads to the accumulation of one malate^{2-} and two H^+. This averaged value is within the range of measured nocturnal net CO_2 exchange (Fig. 1, dark period of day 7). In fact, if one assumes that all values above a constant rate of respiration of -0.07 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$ reflect CO_2 uptake, the resulting average CO_2 uptake rate would essentially be

identical to the value derived from the measurements of nocturnal acidification.

A survey of the literature suggests that species with facultative CAM appear to fall into roughly two groups. In one, the rates of dark CO_2 fixation are up to about 25% of rates in the light under unstressed conditions *e.g. Clusia* spp. (Winter & Holtum 2014), *Mesembryanthemum crystallinum* L. (Winter & Holtum 2007) and *Calandrinia polyandra* Benth. (Winter & Holtum 2011). In a second group, the expression of CAM is much lower and maximum dark CO_2 fixation rates are about 5% or less than light fixation rates for unstressed plants. *Anredera* would belong to this second group, as do some species of *Talinum*, *Portulaca* and *Calandrinia* (Winter & Holtum 2014). Because facultative CAM is expressed in stressed plants, it is not surprising that rates of dark CO_2 fixation are relatively low, but the reasons for this apparent differentiation into two categories defined on the basis of the magnitude of nocturnal CO_2 uptake are not yet clear.

Eight families in the Caryophyllaceae are now known to contain species with CAM. This report further highlights the portullugo clade as a hot-spot of CAM (Edwards & Ogburn 2012),
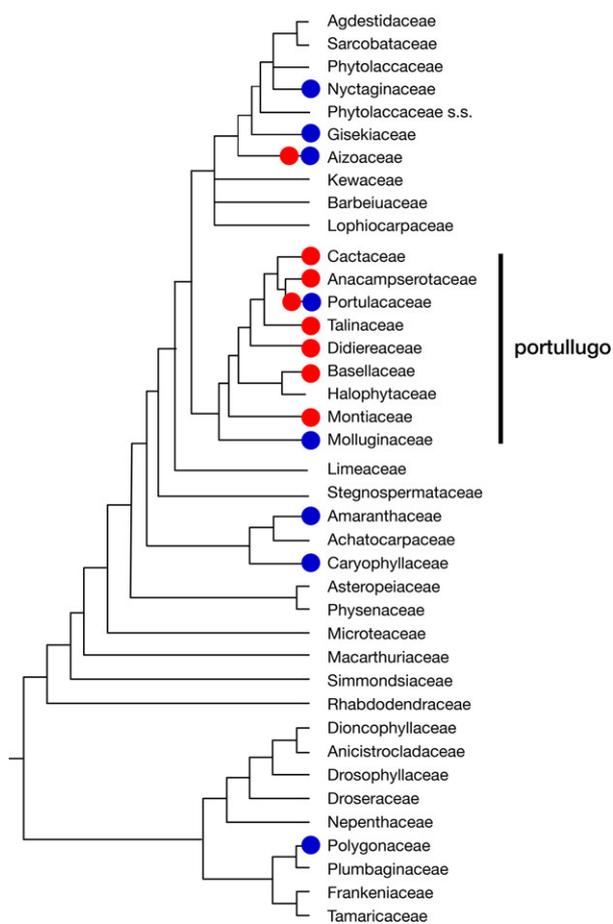

Fig. 3. Phylogenetic relationships of families within the Caryophyllales (adapted from Moore *et al.* 2017) showing the currently known distribution of crassulacean acid metabolism (●) and C_4 (●) photosynthesis among them. The C_4 distribution is from Sage (2016). Portullugo, the clade that includes the subfamily Portulacineae plus the Molluginaceae (Edwards & Ogburn 2012), is indicated with the vertical bar.

with CAM present in seven of its nine families (Fig. 3) and in close to 90% of the genera therein, leaving the Aizoaceae as the only CAM family outside the portullugo in the Caryophyllales. In the two portullugo families with C_4, the Portulacaceae contains CAM species but the Molluginaceae, as far as we know, does not. This pattern may change as more surveys for the presence of CAM are performed.

The Basellaceae, to which *Anredera* belongs, and its sister family, Halophytaceae, are closely related to the CAM-containing Didiereaceae plus Anacampserotaceae, Talinaceae, Portulaceae and Cactaceae (ATPC clade; Moore *et al.* 2017). It has yet to be determined whether CAM is present in *Halophytum ameghinoi* Speg., the only species in the Halophytaceae. However, given a CAM-type $\delta^{13}C$ value of $-18.6‰$ (E. Edwards unpublished), it is likely that *H. ameghinoi* engages in CAM photosynthesis. This $\delta^{13}C$ value along with fleshy leaves, a herbaceous habit and a habitat that includes low rainfall (80–250 mm) arid and semi-arid areas in the Argentine Monte region, further indicates that the CAM cycle may be present within the lineage (Zuloaga & Morrone 1999).

The current centre of diversity of the Basellaceae is north-western South America, although a south-eastern African origin is possible (Anton *et al.* 2014). Of the four genera within the Basellaceae, *Anredera* (12 species), *Tournonia* (one species) and *Ullucus* (one species) are native to the tropics and subtropics of the Americas and the Caribbean, whereas *Basella* (five species) is native to south-eastern Africa, Madagascar and possibly Asia. *Ullucus tuberosus* subsp. *tuberosus* is an important high Andean crop cultivated for its edible, starchy tubers (Sperling & King 1990). All Basellaceae are herbaceous perennial scandent or trailing vines with tuberous rootstocks (Eriksson 2007).

Although the ecology of *A. baselloides* is not well documented, the majority of Basellaceae inhabit open habitats such as scrubs, rocky slopes and sandy areas that are subject to periodic water stress (Eriksson 2007). In common with *Tournonia* and *Ullucus*, most *Anredera* are Andean, generally inhabiting altitudes of *ca.* 1500–3500 m. Exceptions to this trend are *A. floribunda* and *A. vesicaria*, which frequent lowlands, and *A. cordifolia*, *A. ramose* and *A. tucumnanensis*, which have more widespread ranges occurring from sea level to montane habitats. *Anredera vesicaria* extends into the southern United States and *A. cordifolia*, *A. krapovickasii* and *A. tucumanensis* extend to Argentina (Eriksson 2007). *Anredera cordifolia* (Madeira vine), which is very similar to *A. baselloides* in phenology, is a serious invasive weed in subtropical to tropical regions in Australia, Hawaii, New Zealand, South Africa and Sri Lanka (Palmer & Senaratne 2012). It is not known whether *A. cordifolia* exhibits CAM and, if so, whether an ability to switch rapidly between a rapid growth C_3 mode and a low-growth, water-conserving, survival-oriented facultative CAM mode contributes to the extreme weediness of the species.

The recent proliferation of discoveries of species with facultative CAM (cf. Holtum *et al.* 2017a,b; Winter & Holtum 2017) indicates that this form of CO_2 assimilation is more common than hitherto considered, especially in annuals and functionally annual geophytes that inhabit semi-arid regions. Bearing in mind that in large swathes of the tropics and subtropics climate is essentially a sequence of wet and dry periods, in many places it may be an advantage for plants to employ, even to reversibly switch between, a combination of growth

physiologies: a C_3 or C_4 one which is based on not overly water-use efficient rapid growth when water is relatively abundant, and a CAM one which is based on slower but prolonged water-use efficient growth when water is limiting. In effect, the option to exhibit CAM facultatively permits high water use when the resource is available, enabling plants to rapidly expand in size and to create meristems that form reproductive structures. When the water resource depletes, the switch to CAM prolongs carbon gain and cell survival, enabling seed filling in the reproductive structures to extend into the dry period. The adaptive significance of facultative CAM has only been studied in annuals such as *M. crystallinum* (Winter & Ziegler 1992). *In situ* studies are now required to explore the potential benefits of facultative CAM in perennials such as *A. baselloides*.

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REFERENCES

Adams P., Nelson D.E., Shigehiro Y., Chmara W., Jensen R.G., Bohnert H.J., Griffiths H. (1998) Growth and development of *Mesembryanthemum crystallinum* (Aizoaceae). *New Phytologist*, **138**, 171–190.

Anton A.M., Hernandez-Hernandez T., De-Nova J.A., Sosa V. (2014) Evaluating the phylogenetic position of the monotypic family Halophytaceae (Portulacineae, Caryophyllales) based on plastid and nuclear molecular data sets. *Botanical Sciences*, **92**, 351–361.

Bruyns P.V., Oliveira-Neto M., Melo-de-Pinna G.F., Klak C. (2014) Phylogenetic relationships in the Didiereaceae with special reference to subfamily Portulacarioideae. *Taxon*, **63**, 1053–1064.

Christenhusz M.J.M., Byng J.W. (2016) The number of known plant species in the world and its annual increase. *Phytotaxa*, **261**, 201–217.

Christin P.-A., Arakaki M., Osborne C.P., Bräutigam A., Sage R.F., Hibberd J.M., Kelly S., Covshoff S., Wong G.K.-S., Hancock L., Edwards E.J. (2014) Shared origins of a key enzyme during the evolution of C_4 and CAM metabolism. *Journal of Experimental Botany*, **65**, 3609–3621.

Christin P.-A., Arakaki M., Osborne C.P., Edwards E.J. (2015) Genetic enablers underlying the clustered evolutionary origins of C_4 photosynthesis in angiosperms. *Molecular Biology and Evolution*, **32**, 846–858.

Dodd A.N., Borland A.M., Haslam R.P., Griffiths H., Maxwell K. (2002) Crassulacean acid metabolism: plastic, fantastic. *Journal of Experimental Botany*, **53**, 569–580.

Edwards E.J., Diaz M. (2006) Ecological physiology of *Pereskia guamacho*, a cactus with leaves. *Plant, Cell and Environment*, **29**, 1365–3040.

Edwards E.J., Donoghue M.J. (2006) *Pereskia* and the origin of the cactus life-form. *The American Naturalist*, **167**, 777–793.

Edwards E.J., Ogburn R.M. (2012) Angiosperm responses to a low CO_2 world: CAM and C_4 photosynthesis as parallel evolutionary trajectories. *International Journal of Plant Science*, **173**, 724–733.

Eriksson R. (2007) A synopsis of Basellaceae. *Kew Bulletin*, **62**, 297–320.

Guralnick L.J., Jackson M.D. (2001) The occurrence and phylogenetics of crassulacean acid metabolism in the Portulacaceae. *International Journal of Plant Science*, **162**, 257–262.

Guralnick L.J., Edwards G.E., Ku M.S.B., Hockema B., Franceschi V.R. (2002) Photosynthetic and anatomical characteristics in the C_4-crassulacean acid metabolism-cycling plant, *Portulaca grandiflora*. *Functional Plant Biology*, **29**, 763–773.

Guralnick L.J., Cline A., Smith M., Sage R.F. (2008) Evolutionary physiology: the extent of C_4 and CAM photosynthesis in the genera *Anacampseros* and *Grahamia* of the Portulacaceae. *Journal of Experimental Botany*, **59**, 1735–1742.

Harris F.S., Martin C.E. (1991) Correlation between CAM cycling and photosynthetic gas exchange in five species of *Talinum* (Portulacaceae). *Plant Physiology*, **96**, 1118–1124.

Hernández-Ledesma P., Berendsohn W.G., Borsch T., Von Mering S., Akhani H., Arias S., Castañeda-Noa I., Eggli U., Eriksson R., Flores-Olvera H., Fuentes-Bazán S., Kadereit G., Klak C., Korotkova N., Nyffeler R., Ocampo G., Ochoterena H., Oxelman B., Rabeler R.K., Sanchez A., Schlumpberger B.O., Uotila P. (2015) A taxonomic backbone for the global synthesis of species diversity in the angiosperm order Caryophyllales. *Willdenowia*, **45**, 281–383.

Herrera A., Delgado J., Paraguatey I. (1991) Occurrence of crassulacean acid metabolism in *Talinum triangulare* (Portulacaceae). *Journal of Experimental Botany*, **42**, 493–499.

Holtum J.A.M., Osmond C.B. (1981) The gluconeogenic metabolism of pyruvate during deacidification in plants performing crassulacean acid metabolism. *Australian Journal of Plant Physiology*, **8**, 31–44.

Holtum J.A.M., Winter K. (2003) Photosynthetic CO_2 uptake in seedlings of two tropical tree species exposed to oscillating elevated concentrations of CO_2. *Planta*, **218**, 152–158.

Holtum J.A.M., Smith J.A.C., Neuhaus H.E. (2005) Intracellular transport and pathways of carbon flow in plants with crassulacean acid metabolism. *Functional Plant Biology*, **32**, 429–449.

Holtum J.A.M., Hancock L.P., Edwards E.J., Winter K. (2017a) Optional use of CAM photosynthesis in two C_4 species, *Portulaca cyclophylla* and *Portulaca digyna*. *Journal of Plant Physiology*, **214**, 91–96.

Holtum J.A.M., Hancock L.P., Edwards E.J., Winter K. (2017b) Facultative CAM photosynthesis (crassulacean acid metabolism) in four species of *Calandrinia*, ephemeral succulents of arid Australia. *Photosynthesis Research*, **134**, 17–25.

Kluge M., Ting I.P. (1978) *Crassulacean acid metabolism*. Ecological Studies 30. Springer, Berlin, Germany.

Koch K., Kennedy R.A. (1980) Characteristics of crassulacean acid metabolism in the succulent C_4 dicot, *Portulaca oleracea* L. *Plant Physiology*, **65**, 193–197.

Ku S.-B., Shieh Y.-J., Reger B.J., Black C.C. (1981) Photosynthetic characteristics of *Portulaca grandiflora*, a succulent C_4 dicot. Cellular compartmentation of enzymes and acid metabolism. *Plant Physiology*, **68**, 1073–1080.

Martin C.E., Zee A.K. (1983) C_3 photosynthesis and crassulacean acid metabolism in a Kansas rock outcrop succulent, *Talinum calycinum* Engelm. *Plant Physiology*, **73**, 718–723.

Moore A.J., De Vos J.M., Hancock L.P., Goolsby E., Edwards E.J. (2017) Targeted enrichment of large gene families for phylogenetic inference: phylogeny and molecular evolution of photosynthesis genes in the portullugo clade (Caryophyllales). *Systematic Biology* (in press). https://doi.org/10.1093/sysbio/syx078

Neales T.F., Patterson A.A., Hartney V.J. (1968) Physiological adaptation to drought in carbon assimilation and water loss of xerophytes. *Nature*, **219**, 469–472.

Nobel P.S. (1988) *Environmental biology of agaves and cacti*. Cambridge University Press, Cambridge, UK.

Nobel P.S., Hartsock T.L. (1986) Leaf and stem CO_2 uptake in the three subfamilies of the Cactaceae. *Plant Physiology*, **80**, 913–917.

Ogburn M., Edwards E.J. (2010) The ecological water-use strategies of succulent plants. *Advances in Botanical Research*, **55**, 179–225.

Palmer B., Senaratne W. (2012) *Anredera cordifolia* (Ten.) Steenis – Madeira vine. In: Julien M., McFadyen R., Cullen J. (Eds), *Biological control of weeds in Australia*. CSIRO Publishing, Collingwood, Australia, pp 60–64.

Richards H.M. (1915) Acidity and gas exchange in cacti. *Carnegie Institute of Washington Publication*, **208**, 107.

Sage R.F. (2016) A portrait of the C_4 photosynthetic family on the 50th anniversary of its discovery: species number, evolutionary lineages, and Hall of Fame. *Journal of Experimental Botany*, **67**, 4039–4056.

Sage R.F., Christin P.-A., Edwards E.J. (2011) The C_4 plant lineages of planet Earth. *Journal of Experimental Botany*, **62**, 3155–3169.

Smith J.A.C., Winter K. (1996) Taxonomic distribution of crassulacean acid metabolism. In: Winter K., Smith J.A.C. (Eds), *Crassulacean acid metabolism*. Ecological Studies 114. Springer, Berlin, Germany, pp 427–436.

Sperling C.R., King S.R. (1990) Andean tuber crops: worldwide potential. In: Janick J., Simon J. E. (Eds), *Advances in new crops*. Timber Press, Portland, OR, USA, pp 428–435.

Ting I.P., Hanscom Z. (1977) Induction of acid metabolism in *Portulacaria afra*. *Plant Physiology*, **59**, 511–514.

Veste M., Herppich M.B., von Willert D.J. (2001) Variability of CAM in leaf-deciduous succulents from the Succulent Karoo (South Africa). *Basic and Applied Ecology*, **2**, 283–288.

Von Willert D.J., Eller B.N., Werger M.J.A., Brinckmann E., Ihlenfeldt H.-D. (1992) *Life strategies of*

succulents in deserts. With special reference to the Namib desert. Cambridge University Press, Cambridge, UK.

Winter K. (1979) δ^{13}C values of some succulent plants from Madagascar. *Oecologia*, **40**, 103–112.

Winter K., Holtum J.A.M. (2007) Environment or development? Life-time net CO_2 exchange and control of expression of crassulacean acid metabolism in *Mesembryanthemum crystallinum*. *Plant Physiology*, **143**, 98–107.

Winter K., Holtum J.A.M. (2011) Induction and reversal of CAM photosynthesis in *Calandrinia polyandra* Benth: effects of soil moisture and nutrients. *Functional Plant Biology*, **38**, 576–582.

Winter K., Holtum J.A.M. (2014) Facultative crassulacean acid metabolism (CAM) plants: powerful tools for unravelling the functional elements of CAM photosynthesis. *Journal of Experimental Botany*, **65**, 3425–3441.

Winter K., Holtum J.A.M. (2017) CO_2-exchange patterns demonstrate facultative CAM photosynthesis (crassulacean acid metabolism) in four small C_3 and C_4 leaf-succulents. *Australian Journal of Botany*, **65**, 103–108.

Winter K., Ziegler H. (1992) Induction of crassulacean acid metabolism in *Mesembryanthemum crystallinum* increases reproductive success under conditions of drought and salinity stress. *Oecologia*, **92**, 475–479.

Winter K., Aranda J., Holtum J.A.M. (2005) Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism. *Functional Plant Biology*, **32**, 381–388.

Winter K., Holtum J.A.M., Smith J.A.C. (2015) Crassulacean acid metabolism: a continuous or discrete trait? *New Phytologist*, **208**, 73–78.

Zuloaga F.O., Morrone O. (1999) Catálogo de las plantas vasculares de la República Argentina. II. Angiospermae (Dicotyledoneae). *Monographs in Systematic Botany from the Missouri Botanical Garden*, **64**, 1–1269.