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Functional significance of betalain biosynthesis in leaves of *Disphyma australe* under salinity stress

Gagandeep Jain, Kevin S. Gould*

School of Biological sciences, Victoria University of Wellington, PO Box 600, Wellington 6140, New Zealand

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ABSTRACT

Shoots of *Disphyma australe*, a coastal succulent plant native to New Zealand, vary in colour from entirely red to entirely green. We hypothesised that the red pigmentation develops in response to salinity stress, and that these betalain pigments contribute to salt tolerance. Effects of salinity on betalain content, CO_2 assimilation, stomatal conductance, chlorophyll content and chlorophyll fluorescence were measured in leaves from red and green-leafed morphs. Newly formed leaves of both morphs were entirely green when grown under control conditions in a glasshouse. NaCl treatment increased betalain concentration 10-fold in leaves of the red, but not of the green morphs. The red leaves held six betacyanins (betanin, isobetanin, betanidin, isobetanidin, lampranthin-II, isolampranthin) but no betaxanthins; in the green morphs, neither betacyanin nor betaxanthin was present. In contrast, betalains were present in the petals of both morphs. Photosynthetic CO_2 assimilation and water use efficiency were greater, and stomatal conductance was lower, in leaves of the red than of the green morphs following NaCl treatment. Photosystem II quantum yields and photochemical quenching were both greater in red than in green NaCl treated leaves under white actinic light. The data indicate that betalain accumulation in red morphs is a direct response to salinity, but that the green morphs, although possessing the genetic potential to biosynthesise betalains, lack the mechanism for the induction of betalain in response to salinity stress. Foliar betalains appear to ameliorate responses to salinity stress in *D. australe*.

1. Introduction

Disphyma australe (W. T. Aiton), a succulent plant common on coastal cliffs and dunes throughout New Zealand, shows marked variation in shoot colour (Allan, 1961; Chinnock, 1971). The prostrate stems and erect, fleshy leaves are, in some plants, entirely green; in others, the vegetative shoot is partially or entirely red (Fig. 1). The red and green *D. australe* morphs often co-occur at coastal locations. However, nothing is known of the possible genetic or environmental basis for this colour polymorphism.

As with other members of the Aizoaceae, the red colouration in *D. australe* results from the production of betalains (Chinnock, 1971). Betalains are water-soluble nitrogen-containing pigments synthesized from tyrosine, and there are two structural groups: the red/violet betacyanins, and the yellow/orange betaxanthins (Azeredo, 2009; Strack et al., 2003). Unlike the anthocyanins, which are by far the most common class of red pigment (Gould, 2004), the functional role of both groups of betalains remains poorly understood (Ibdah et al., 2002; Stintzing and Carle, 2004). Anthocyanins and betalains do not co-occur naturally in the same plant (Stafford, 1994), although the simultaneous biosynthesis of both is theoretically possible as shown using transgenic *Arabidopsis* (Harris et al., 2012). As with the anthocyanins, it has been hypothesized that betalain accumulation in leaves may be an ameliorative response to abiotic stressors such as high UV irradiance, strong light, low temperature and salinity (Bothe, 1976; Hayakawa and Agarie, 2010; Ibdah et al., 2002; Wang and Liu, 2007; Wang et al., 2006). Indeed, in the halophyte *Sueda salsa*, red colouration in the vegetative shoot is more pronounced when plants are growing in the intertidal zone than on higher land, possibly indicating the involvement of salinity in betalain accumulation (Wang et al., 2006). Furthermore, those *S. salsa* plants that held the higher levels of betacyanin showed increased tonoplast H^+-ATPase activity; the presence of betalains apparently correlated with an improved removal of Na^+ from cytoplasm to vacuole (Wang et al., 2007).

It is possible therefore, that variation in shoot colour among *D. australe* individuals reflects spatial variation in the severity of salt stress they experience, and that the development of the red colour serves to mitigate the stress. Alternatively, the green morphs may simply lack the genetic capability to synthesise betalain pigments.

* Corresponding author. Tel.: +64 4 4636649; fax: +64 4 4635331.
E-mail address: kevin.gould@vuw.ac.nz (K.S. Gould).

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Fig. 1. Vegetative shoots of *Disphyma australe* (A), showing contiguous red (R) and green (G) morphs at Te Kopahou Reserve, Wellington. Flowers of green (B) and red (C) *D. australe* morphs. Bar = 1 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Here, we compare the pigment composition and physiological responses to salinity of green and red *D. australe*. We hypothesise that *D. australe* accumulate betalain under saline conditions, and that betalainic plants are physiologically more tolerant of salinity stress. We report the effects of NaCl on betalain levels, CO_2 assimilation rate and stomatal conductance in leaves from red and green-leafed morphs, and also quantify the effects of salinity on the photosynthetic yield of PSII in red and green leaves.

2. Material and methods

2.1. Plant material

A healthy shoot was taken from each of 10 red and 10 green *D. australe* plants, randomly collected from south-facing dunes and rocky outcrops along the coast at Te Kopahou reserve, Wellington, New Zealand (41°21′01″S, 174°43′55″E). Shoot cuttings with two leaves attached were rooted in trays containing a 2:1 mix of potting compost and sand for 5 wk, and then transferred one plant per pot to 800 mL pots with the same substrate and grown in an unheated glasshouse at Victoria University of Wellington.

2.2. Salinity treatments

Five individuals of each colour morph were watered with 15 mL of 200 mM NaCl every third day for 2 wk. Control plants were irrigated with distilled water. The youngest fully expanded leaves were harvested from each plant after 2 wk of treatment.

2.3. Pigment extraction and quantification

Exactly 1 g of fresh leaf was flash frozen in liquid nitrogen, ground to a powder and extracted in 10 mL 100% methanol for 1 h at 4 °C. The extracts were centrifuged for 5 min at 10,000 × *g*, the supernatants discarded, and the pellets re-suspended in 10 mL distilled water at pH 5, adjusted using HCl (Wang et al., 2006). Betalain content was estimated spectrophotometrically using a Shimadzu (Kyoto, Japan) 2550 UV–vis spectrophotometer. Betalain content was estimated as $A_{538} - 0.33\,A_{662}$, where $A_{538} = A_{\lambda\max}$. The subtraction of 0.33 A_{662} compensated for the small overlap in absorption by extracted chlorophyll. To estimate foliar chlorophyll and carotenoid content, frozen leaves were extracted in 80% acetone, and absorbances at 470, 647 and 663 nm were measured in a Shimadzu spectrophotometer. Pigment concentrations were calculated using the equations by Lichtenthaler (1987).

Individual betalains were quantified using an Agilent 1100 Series HPLC (Waldbronn, Germany) with a Phenomenex C_{18} reversed phase column (5 μM, 250 × 4.6 mm). The injection volume was 50 μL and column temperature was 25 °C. HPLC gradients were (A) formic acid: H_2O (1:99, v:v), and (B) acetonitrile: H_2O (80:20, v:v). Betaxanthins were separated isocratically with 100% A, followed by a linear gradient from 0% to 20% B in 60 min, and then 20% to 100% B in 5 min. Betacyanin separation was done beginning with 2% B in A and increasing to 33% B in A over 60 min. Betacyanins were detected at 538 nm and betaxanthins at 470 nm (Kugler et al., 2007). Retention times of betalain peaks were compared to those from published records of authentic samples (Kugler et al., 2007, 2004; Svenson et al., 2008) and to previous work on *D. australe* (D. Lewis; pers. comm.).

2.4. Gas exchange

An LI-6400 gas exchange system (LiCor, Lincoln, NE, USA) equipped with a red LED light source and leaf chamber LI-6400-08 was used to calculate maximum net CO_2 assimilation rate (A_{max}) and stomatal conductance (g_s) for one leaf per plant. The