Understanding the biochemical basis of flower colour in Australian native *Ptilotus* and *Gomphrena*

Final Research Report
Prepared for the Australian Flora Foundation

By

Dr Dion Harrison
Dr Jitka Kochanek
Professor Daryl Joyce

Centre for Native Floriculture
School of Land, Crop and Food Sciences
The University of Queensland, Gatton Campus, Gatton QLD 4343.

October 2010

Abstract
Originating largely from the semi-arid regions of Australia, many native species of *Ptilotus* and *Gomphrena* (Family Amaranthaceae) exhibit high ornamental horticultural potential with their showy colourful flowers. However, to fully realise this potential requires the development of elite cultivars with a range of vibrant flower colours. Interestingly, flower colour in plants belonging to the Order Caryophyllales, such as *Ptilotus* and *Gomphrena*, result from the rarely studied betalain pigments rather than the more common and well-researched anthocyanin pigments that are found in most flowering plants. This project used HPLC and LCMS methods to investigate the biochemical basis of flower colour for seven Australian *Ptilotus* and three Australian *Gomphrena* accessions. In total, ten major and three minor betalain pigments were identified, including five unknown betalain pigments. This knowledge provides the first steps needed to facilitate the development of new Australian *Ptilotus* and *Gomphrena* cultivars with novel flower colours for ornamental horticulture. Given that betalain research is still in its relative infancy, this project also makes an important contribution to our understanding of betalain pigments in previously unstudied Australian native plant species.

Keywords
Amaranthaceae, betalains, flower colour, *Gomphrena*, plant pigments, *Ptilotus*.

Introduction

The global market for ornamental plants, including cut flowers, potted colour, bedding and garden plant production, is worth over US$70 billion annually and growing (Grotewold, 2006). Novelty is the key driver in this competitive market, and new species and new flower colours remain a key priority (Grotewold, 2006). Native Australian species of *Ptilotus* and *Gomphrena* (Family Amaranthaceae) have great ornamental potential. These genera also contain wide genetic diversity with over 100 *Ptilotus* (Lee *et al*., 2007) and 30 *Gomphrena* (Palmer, 1998) species endemic to Australia. For example, *Ptilotus nobilis* and *P. exaltatus* have large showy cream and pink inflorescences, and *Gomphrena leontopodioides* and *G. flaccida* have numerous small spherical to globular pink/purple inflorescences. However, the limited flower colour range (pink to purple-red) and often pastel nature of the flower colours within these species currently limits their ornamental potential. Smaller, less showy species with vibrant coloured flowers such as *P. spicatus* (deep red) and *P. gaudichaudi* (bright yellow) do not readily cross hybridise with other *Ptilotus* species (D. Harrison, unpublished data). In order to devise the most appropriate breeding and selection strategies for manipulating flower colour, an understanding of the biochemistry of the pigments involved is first required. While flower colour in most plants results from anthocyanin pigments, plants of 13 families in the Order Caryophyllales, including Family Amaranthaceae, have betalain pigments instead (Clement and Mabry, 1996). There are two basic types of betalains, the red-purple betacyanins and the yellow-orange betaxanthins. Base understanding of the major betalain pigments in Australian *Ptilotus* and *Gomphrena* is lacking. In fact, there are very few studies on betalain pigments in Australian plant species. Studies on other exotic betalain-containing species (e.g. *Beta vulgaris*, *G. globosa*, and *Bougainvillea*) indicate that colour variation within a species mostly results from variation in the relative proportion of betaxanthins versus betacyanins, whereas different species tend to have a different suite of betalain pigments (Kugler *et al*., 2004; 2007). This report describes the major betalain pigment compositions for 10 Australian *Ptilotus* and *Gomphrena* accessions and relates the findings to the diversity of flower colours among the accessions studied.

Research methodology

Plant material

The plant species and accessions sampled (Fig. 1) were sourced from greenhouse grown plants in the Centre for Native Floriculture germplasm collection at The University of Queensland, Gatton Campus. For sampling, only new fully-open florets were selected and any adhering pollen was

carefully removed. All samples were replicated three to six times. Collected samples were transported in paper bags, on ice, and stored at -80°C. Samples were subsequently freeze dried for about one week until completely dry. Dried samples were stored at 4°C in the dark in airtight plastic bags containing silica gel.

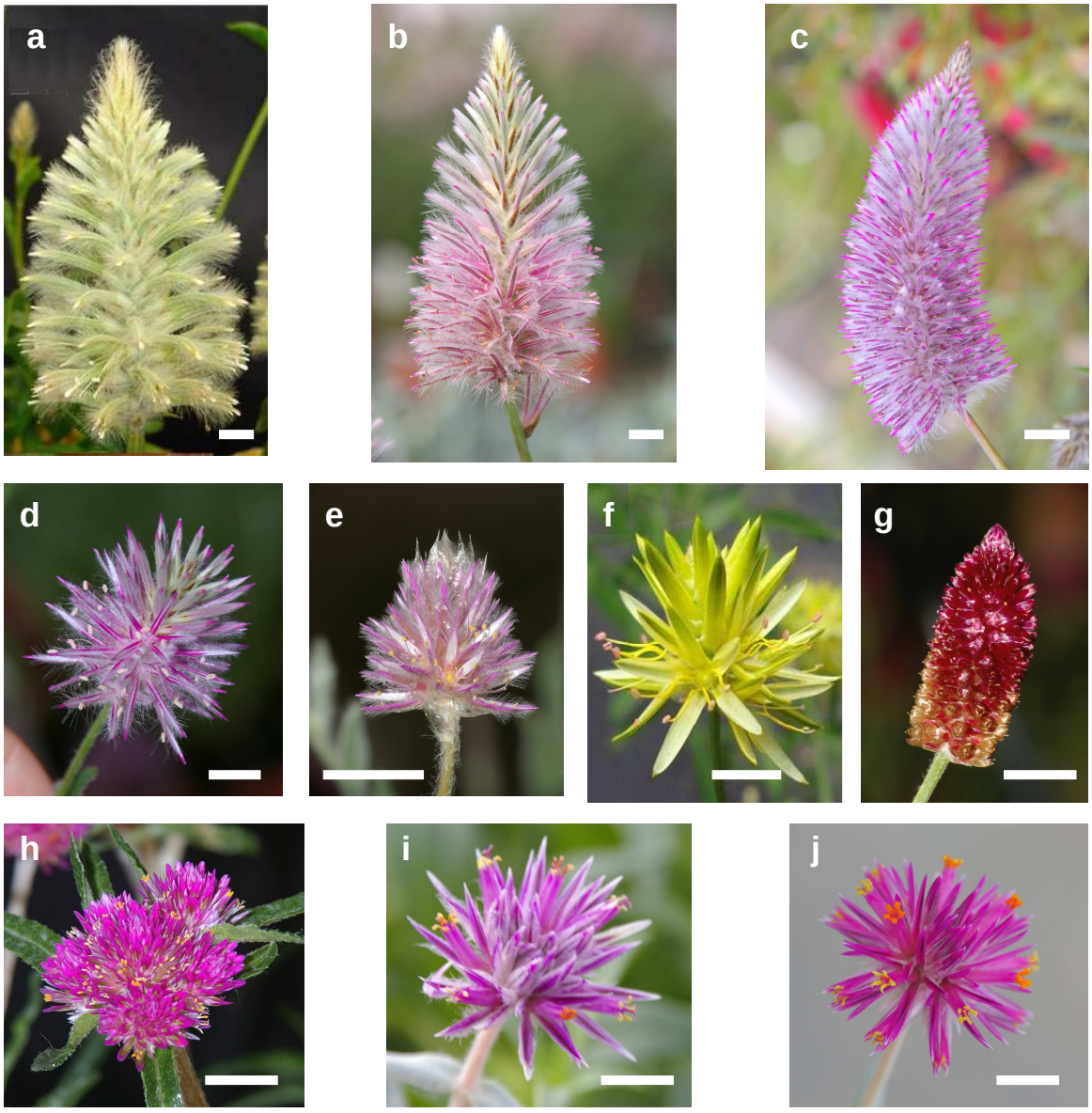

Figure 1. Flowers of species and accessions used in this study.
a) *Ptilotus nobilis* cv. Purity, (b) *P. nobilis* cv. Passion, (c) *P. exaltatus* cv. Joey, (d) *P. exaltatus* var. *semilanatus*, (e) *P. helipteroides*, (f) *P. gaudichaudi*, (g) *P. spicatus*, (h) *Gomphrena flaccida*, (i) *G. leontopodioides* cv. Empress, and (j) *G. leontopodioides* (dark purple flower).

Colour measurements

The colour parameters of lightness, chroma and hue-angle (L, C, *h*°) were measured with a Konica Minolta CR-400 Chroma Meter standardised on a white tile supplied with the instrument and a D65 light source for illumination. Lightness represents the proportion of total incident light that is reflected, chroma is a measure of colour intensity in relative intensity units, and hue angle relates colour to a position on a colour circle or wheel with red-purple at 0/360°, yellow at 90°, blue-green at 180°, and blue at 270° (Lewis *et al*., 1998). Flower colour measurements were performed in triplicate on fresh tepals excised from the inflorescence and heaped onto a white tile (see Fig. 2). For colour measurements on pigment extracts, betalain pigments were extracted from 0.1 g of freeze-dried tepal tissue in 4 mL of extraction buffer (80% aqueous methanol and 50 mM sodium ascorbate) according to Schliemann *et al*. (1999) and Vogt *et al.* (1999). Colour measurements were performed on cuvettes (path length 1 cm; UVette®, Eppendorf, Hamburg Germany) containing 1 mL of pigment extract using a white tile as the background.

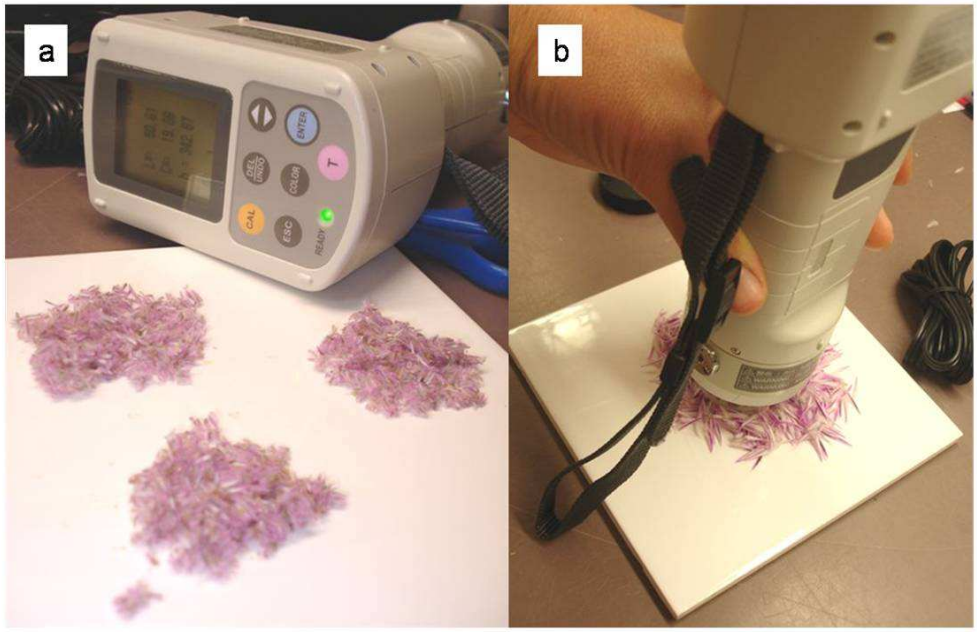

Figure 2. Flower colour measurement technique.
A Konica Minolta CR-400 Chroma Meter was used to make colour measurements of lightness, chroma and hue-angle (L, C, h°). (a) fresh tepals were placed onto a white tile background in replicates of three for each sample as shown for *P. helipteroides*; (b) colour measurements being taken for *G. leontopodioides* tepals.

Betalain pigment content and composition

Betalain pigments were extracted in 80% aqueous methanol containing 50 mM sodium ascorbate as per Schliemann *et al*. (1999) with the method modified to incorporate freeze drying and subsequent resuspension in deionised water as per Svenson *et al*. (2008). Betalain quantification was determined using a Pharmacia LKB-UltrospecIII UV/Vis spectrophotometer (Pharmacia LKB Biochrom Ltd, Cambridge, UK). The aqueous pigment extracts were diluted with McIlvaine buffer (pH 6.0, citrate-phosphate) to obtain absorption values around 1.0 at absorption maxima. The betalain content (*BC*) was calculated after Kugler *et al.* (2007): BC (mg/kg) = $(A - A_{650}) \times DF \times MW \times V_e \times 1000 / (\varepsilon \times L \times W_d)$; where, A is absorbance value at the absorption maximum corrected by subtraction for absorbance at 650 nm; DF is the dilution factor; L is the path length of the cuvette (1 cm); V_e is total extract volume (mL); and, W_d is dry weight of extracted material. The

molecular weight (*MW*) and molar extinction coefficient (ε) of betanin [*MW* = 550 g/mol; ε = 60,000 L/(mol cm) in H_2O; λmax = 538 nm; (Kugler *et al.*, 2007)] was used for quantification of betacyanins. Betaxanthins were quantified using the molecular weight of vulgaxanthin I (*MW* = 339 g/mol) and molar extinction coefficient [ε = 48,000 L/(mol cm) in H_2O] at λmax = 470 nm (Kugler *et al.*, 2004).

High performance liquid chromatography (HPLC) was used to separate and identify individual pigments in each pigment extract. HPLC analysis was carried out using a Dionex Ultimate 3000 solvent delivery system (Dionex Corporation, Sunnyvale, CA, USA) with a Phenomenex Luna (5µm, 150 x 4.6 mm) RP-18(2) end capped HPLC column (column temperature 25ºC) and a Dionex 3000 PDA detector. A 20 µL injection volume was used per sample. Elution was performed with a mobile phase consisting of solvent A (1% formic acid v/v) and solvent B (acetonitrile:water 80:20 v/v). Separate elution gradients were used to separate betaxanthins and betacyanins as per Kugler *et al.* (2004) and Svenson *et al.* (2007). Betacyanins were monitored at 538 nm and betaxanthins at 470 nm. Individual pigments were identified using HPLC retention times published for previous betalain pigment studies and using beetroot (*Beta vulgaris*) and *Gomphrena globosa* extracts as reference samples (Trezzini and Zyrd, 1991; Kugler *et al*., 2004; Cai *et al*., 2005; Kugler *et a.l*, 2007; Svenson *et al*., 2008).

Liquid chromatography-multistage mass-spectroscopy (LCMS) analyses provided the basic chemical structure to confirm the identity of pigments determined by HPLC. Only the selected accessions *P. spicatus*, *P. gaudichaudi*, *P. nobilis* cv. Passion, *P. exaltatus* cv. Joey and *G. leontopodioides* cv. Empress were analysed with LCMS because they collectively represented all the major HPLC peaks found among the accessions sampled. The LCMS system consisted of a Thermo Electron Corporation (San Jose, CA, USA) Finnigan Surveyor MS pump, Finnigan MicroAS auto-sampler, Finnigan Surveyor PDA detector and a ThermaSphere TS-130 column heater (Phenomenex, Torrance, CA, USA). The same mobile phase and elution gradients as with HPLC were applied. A 2 µL aliquot extract was separated by reverse phase chromatography using a 4 x 2 mm, 10 µm Aqua guard cartridge and a 250 x 2.1 mm, 4 µm Synergi Hydro-RP C18 (Phenomenex, Torrance, CA, USA) maintained at 25°C with a flow rate of 200 µL/min. The eluent was scanned by PDA at 200 to 700 nm and analysed by API-MS (LTQ, 2D linear ion-trap, Thermo-Finnigan, San Jose, CA, USA) with electrospray ionisation (ESI, positive). Data were acquired for parent masses from m/z 250 to 1500 amu with MS^4 fragmentation. Data were processed with the aid of Xcalibar® 2.05 (Thermo Electron Corporation).

Results and discussion

Flower colour and betalain content

The colour properties of fresh *Ptilotus* and *Gomphrena* tepals and their betalain extracts are presented in Table 1. Generally, flower colour (based on *h*° measurements) was similar to the colour of the betalain extracts, although purple-red flowers tended to produce a more red coloured extract (Table 2). Betalain pigments were found in tepals of all *Ptilotus* and *Gomphrena* accessions sampled (Table 3). In general, flowers in the purple-red colour spectrum contained only betacyanins, while those with a yellow-green colour contained only betaxanthins (Table 3). The only exception was *P. nobilis* cv. Passion (purple-red colour) which contained a mix of both betacyanins and betaxanthins (Table 3). The highest total betalain content was in the dark red tepals of *P. spicatus* (4.2 $mg.g^{-1}$DW) and the lowest in pale yellow-green coloured tepals of *P. nobilis* cv. Purity (0.09 $mg.g^{-1}$DW). For accessions containing only betaxanthins (i.e. *P. nobilis* cv. Purity and *P. gaudichaudi*), chroma values were proportional to the total betaxanthin content (Table 3). This trend was not observed for accessions containing only betacyanins, where chroma values were probably influenced by a combination of the total betalain content and the specific composition of the various different betacyanin pigments present (see below). No betaxanthins were detected in any of the three Australian *Gomphrena* accessions studied (Table 3).

Table 1. Colour characteristics (L^* = lightness, C^* = chroma, $h°$ = hue angle*) of tepals from various *Gomphrena* and *Ptilotus* species (mean ± SE, *n* = sample number).

	Colour characteristics of fresh flower parts				Colour characteristics of pigment extracts			
Accession	L^*	C^*	$h°$	*n*	L^*	C^*	$h°$	*n*
P. spicatus	25.2 ± 0.5	36.0 ± 0.7	1.1 ± 0.9	6	30.0 ± 0.5	28.2 ± 1.0	4.3 ± 0.9	3
P. gaudichaudi	68.9 ± 1.5	52.1 ± 2.9	101.9 ± 1.1	4	56.1 ± 0.6	41.4 ± 3.9	97.2 ± 0.4	3
P. helipteroides	55.8 ± 1.2	15.4 ± 0.8	0.1 ± 4.1	3	46.3 ± 0.2	21.4 ± 0.3	31.8 ± 7.4	3
P. nobilis cv. Purity	63.0 ± 0.2	18.8 ± 0.5	109.6 ± 0.5	3	55.8 ± 0.2	30.2 ± 0.4	102.5 ± 0.3	3
P. nobilis cv. Passion	52.3 ± 0.6	16.4 ± 0.5	353.5 ± 1.0	3	35.1 ± 0.3	27.0 ± 0.6	1.1 ± 1.2	3
P. exaltatus cv. Joey - entire tepals	55.4 ± 1.5	16.0 ± 0.6	355.7 ± 0.1	3	34.4 ± 2.0	33.8 ± 0.0	9.3 ± 1.4	2
P. exaltatus cv. Joey – tips of tepals	55.1 ± 1.2	21.7 ± 0.7	352.4 ± 0.1	3	40.5 ± 0.2	32.4 ± 1.2	8.3 ± 4.1	2
P. exaltatus var. *semilanatus*	59.0 ± 1.1	20.8 ± 0.9	336.2 ± 1.2	4	33.9 ± 0.7	30.5 ± 2.4	358.7 ± 1.7	3
Gomphrena flaccida	52.0 ± 2.3	28.9 ± 2.5	351.1 ± 3.4	3	42.2 ± 1.0	27.4 ± 3.8	348.8 ± 2.9	3
G. leontopodioides cv. Empress	60.1 ± 2.9	17.7 ± 1.2	350.3 ± 2.1	3	37.4	29	357.5	1
G. leontopodioides (dark purple flower)	49.4 ± 1.2	36.1 ± 1.5	341.6 ± 0.7	6	36.0 ± 0.7	35.1 ± 1.6	347.9 ± 0.9	3

*On the colour wheel 0°/360° = red-purple, 90° = yellow, 180° = green, 270° = blue; 327° would appear as purple petals, 52° as orange.

Table 2. The visual appearance of tepals from various *Gomphrena* and *Ptilotus* species and cultivars according to their hue angle measurements (Table 1).

Accession	**Flower parts**	**Pigment extracts**
Ptilotus spicatus	Red-purple	Red
P. gaudichaudi	Yellow-green	Yellow
P. helipteroides	Red-purple	Red-orange
P. nobilis cv. Purity	Yellow-green	Yellow-green
P. nobilis cv. Passion	Purple-red	Red-purple
P. exaltatus cv. Joey - entire tepals	Purple-red	Red
P. exaltatus cv. Joey – tips of tepals	Purple-red	Red
P. exaltatus var. *semilanatus*	Purple	Purple-red
Gomphrena flaccida	Purple-red	Purple-red
G. leontopodioides cv. Empress	Purple-red	Purple-red
G. leontopodioides (dark purple flower)	Purple-red	Purple-red

Table 3. Betalain pigment content (dry weight basis) in tepal tissue of various *Gomphrena* and *Ptilotus* species and cultivars (mean ± SE, n = 3).

	Betalain content (mg.g^{-1} DW)		
Accession	**Betaxanthins**	**Betacyanins**	**Total betalains**
P. spicatus	0	4.20 ± 0.18	4.20 ± 0.18
P. gaudichaudi	0.25 ± 0.06	0	0.25 ± 0.06
P. helipteroides	0	0.57 ± 0.06	0.57 ± 0.06
P. nobilis cv. Purity	0.09 ± 0.01	0	0.09 ± 0.01
P. nobilis cv. Passion	0.02 ± 0.00	2.01 ± 0.08	2.03 ± 0.08
P. exaltatus cv. Joey – entire tepals	0	2.00 ± 0.08	2.00 ± 0.08
P. exaltatus cv. Joey – tips of tepals	0	0.80	0.80
P. exaltatus var. *semilanatus*	0	1.27 ± 0.04	1.27 ± 0.04
Gomphrena flaccida	0	0.36 ± 0.03	0.36 ± 0.03
G. leontopodioides cv. Empress	0	0.70 ± 0.10	0.70 ± 0.10
G. leontopodioides (dark purple flower)	0	0.74 ± 0.07	0.74 ± 0.07

Betalain pigment composition

Studies on exotic betalain-containing species (e.g. *Beta vulgaris*, *G. globosa* and *Bougainvillea*) indicate that different species tend to have a different suite of betalain pigments (Kugler *et al.*, 2004; 2007). In this study, we found nine major betacyanins (including two unknown pigments), one major betaxanthin pigment and three unknown betaxanthin pigments present in trace amounts in the Australian *Ptilotus* and *Gomphrena* accessions studied (Table 4). Very few betaxanthins were detected in this study. These were only found in *P. gaudichaudi*, *P. nobilis* cv. Purity and *P. nobilis* cv. Passion. Proline-betaxanthin or indicaxanthin, one of the first betaxanthins ever isolated (Piattelli *et al.*, 1964), was the only major betaxanthin detected and was found in *P. gaudichaudi*. HPLC detected trace levels of unknown betaxanthins in *P. nobilis* cvs. Purity and Passion. However, they were not detected by LCMS. Their retention times and spectral maxima differ from proline-betaxanthin found in *P. gaudichaudi*.

The betacyanin pigments identified in this study generally corresponded to betalains found in other Amaranthaceae species studied previously (Cai *et al.*, 2001). However, the relative pigment compositions in the accessions of this study were not typical for most species in Amaranthaceae. Betacyanins can be classified into five pigment types according to the location and number of glucose and acyl molecules attached to the basic betanidin molecule: amaranthin-, gomphrenin-, betanin- and bougainvillein-type and the aglycones which are neither acylated nor glycosylated (Strack *et al.*, 1993). Of the seven known betacyanin pigments detected in this study, there were five amaranthin-type (amaranthin, isoamaranthin, celosianin I, celosianin II and isocelosianin II) and two betanin-type (betanin and isobetanin) betacyanins. While both non-acylated and acylated betacyanins were detected, six out of eight *Ptilotus* and *Gomphrena* accessions in this study contained acylated betacyanins (i.e. celosianin I, II and/or isocelosianin II) as major pigments (>50% of total betacyanins), with some accessions containing as much as 93 to 100% acylated betacyanins (Table 4). Interestingly, the acylated celosianins are either absent or present as minor pigments for most members of the Amaranthaceae family studied to date, which predominantly contain simple non-acylated betacyanins such as amaranthin / isoamaranthin (Kugler *et al.*, 2007; Cai *et al.*, 2001). Indeed, for thirty seven species from eight genera in Amaranthaceae, 81% of the total peak area (HPLC) is dominated by amaranthin (Cai *et al.*, 2005b). *Celosia cristata* (violet) is one exception for which celosianin II composed 28.8% of the betacyanin profile (Cai *et al.*, 2001). Other species containing predominantly acylated betacyanins include *Iresine herbstii* (76.9% as iresinin I / isoiresinin I) and *G. globosa* (68.4% as gomphrenins) (Cai *et al.*, 2001).

The presence of predominantly acylated betacyanins in this study correlated with accessions that were generally more purple in colour (e.g. *Gomphrena* accessions, *P. helipteroides* and *P. exaltatus* var. *semilanatus*) (Table 4), while those with non-acylated betacyanins were more red coloured (e.g. *P. spicatus*, *P. exaltatus* cv. Joey). This observation concurs with betalain pigment studies on exotic *G. globosa*. For example, amaranthin, a non-acylated betacyanin, is a major pigment in red and orange flowers but only a minor pigment in purple coloured flowers of *G. globosa* which accumulate mostly acylated betacyanins (gomphreninins) (Kugler *et al.*, 2007). This is thought to be due to a bathochromatic shift following acylation of the basic betacyanin structure (Kugler *et al.*, 2007). Interestingly, no gomphrenin-type betacyanins were found in any of the Australian *Ptilotus* and *Gomphrena* accessions in this study, which contained amaranthin-type acylated celosianins instead.

Implications for breeding and future directions

Based on the results of this study and comparison with studies conducted on exotic betalain containing species, it seems likely that more vibrant flower colours and hence novel cultivars of *Ptilotus* and *Gomphrena* may be achieved though conventional breeding and selection for increased betalain pigment concentrations. Indeed, cultivated *Amaranthus* species contain much higher betacyanin concentrations than those from the wild (Cai *et al*., 1998). Similarly, betalain pigment concentrations in beetroot were increased 3-fold over a series of breeding cycles (*Beta vulgaris* L. ssp. *vulgaris*) (Gaertner and Goldman, 2005). For *Ptilotus* species with showy large flowers, such

Table 4. Summary of betalain pigments identified by HPLC / LCMS within tepal tissues of various *Gomphrena* and *Ptilotus* species and cultivars.

Accession	Pigment trivial name [a]	% of peak area [b]	Type [c]	Acylation [d]
Ptilotus spicatus	Amaranthin	59	A	-
	Isoamaranthin	20	A	-
	Celosianin I	6	A	*p*-coumaroyl
	Celosianin II	10	A	feruloyl
	IsoCelosianin II	5	A	feruloyl
P. gaudichaudi	Indicaxanthin	100	BX	
	Unknown BX1	Trace	BX	
	Unknown BX2	Trace	BX	
P. helipteroides	Amaranthin	4	A	-
	Celosianin I	36	A	*p*-coumaroyl
	Celosianin II	39	A	feruloyl
	IsoCelosianin II	21	A	feruloyl
P. nobilis cv. Purity	Unknown BX3	Trace	BX	
P. nobilis cv. Passion	Amaranthin	22	A	-
	Isoamaranthin	6	A	-
	Betanin	5	B	-
	Isobetanin	1	B	-
	Unknown BC1	3	Unknown	-
	Celosianin I	Trace	A	*p*-coumaroyl
	Celosianin II	48	A	feruloyl
	IsoCelosianin II	13	A	feruloyl
	Unknown BC2	2	Unknown	-
	Unknown BX3	Trace	BX	
P. exaltatus cv. Joey – entire tepals	Amaranthin	71	A	-
	Isoamaranthin	21	A	-
	Betanin	6	B	-
	Isobetanin	2	B	-
P. exaltatus cv. Joey – tepal tips	Amaranthin	73	A	-
	Isoamaranthin	23	A	-
	Celosianin II	4	A	feruloyl
P. exaltatus var. *semilanatus*	Amaranthin	7	A	-
	Isoamaranthin	Trace	A	-
	Celosianin I	16	A	*p*-coumaroyl
	Celosianin II	61	A	feruloyl
	IsoCelosianin II	16	A	feruloyl
Gomphrena flaccida	Amaranthin	7	A	-
	Celosianin I	15	A	*p*-coumaroyl
	Celosianin II	55	A	feruloyl
	IsoCelosianin II	23	A	feruloyl
G. leontopodioides cv. Empress	Celosianin I	10	A	*p*-coumaroyl
	Celosianin II	72	A	feruloyl
	IsoCelosianin II	18	A	feruloyl
G. leontopodioides, (dark purple flower)	Celosianin I	15	A	*p*-coumaroyl
	Celosianin II	67	A	feruloyl
	IsoCelosianin II	18	A	feruloyl

[a] BX = betaxanthin, BC = betacyanin. [b] Relative amount that individual betalains contribute to the HPLC profile (% value). Trace refers to pigments detected by either LCMS or HPLC in trace amounts. [c] Pigment type: A = amaranthin-type betacyanin, B = betanin-type betacyanin, BX = betaxanthin (Strack *et al.*, 1993), [d] (-) indicates that the betacyanin pigment is not acylated.

as *P. nobilis* and *P. exaltatus,* novel cultivars with large vibrant deep red flowers similar in colour to that of *P. spicatus* may be achieved by increasing the amaranthin content. *Ptilotus nobilis* cv. Passion had the greatest pigment diversity of the accessions studied providing scope for breeding a range of novel flower colours, particularly if betaxanthin content can be elevated. This provides scope for developing a series including gold, red, orange and magenta coloured flowers.

However, perceived flower colour is not solely the result of the pigment composition and concentration. Visual appearance is also influenced by flower texture. For example, petal surface striations (Mudalige *et al*., 2003), the volume and location of air spaces (Zhang *et al.*, 2008), cuticle thickness (Mudalige *et al*., 2003), epidermal cell shape and the packing of pigments within cells (Grotewold, 2006) all influence how light is reflected and thus the flower colour attributes. Flower colour in *Ptilotus* and *Gomphrena* may also be affected by the presence of other pigments, such as carotenoids or chlorophyll, which were not analysed in this study. For many of the accessions in this study (i.e. *P. helipteroides*, *P. nobilis* cv. Purity, *P. nobilis* cv. Passion, *P. exaltatus* cv. Joey, *P. exaltatus* var. *semilanatus*, *G. leontopodioides* cv. Empress), the extracted pigment colour was about twice as vibrant as the fresh flower parts (Table 2). This suggests that other factors like co-pigmentation and cell structure do affect flower colour in these species. By contrast, in *P. gaudichaudi* and *P. spicatus,* the colour vibrancy of fresh flower parts was greater than their pigment extracts. This may be due to the fact these species have less hairs on the tepals, which in turn, may make the tepal colours appear more vibrant. Thus, structural features of flower parts should ideally be considered in conjunction with pigment composition for future selection and breeding programs targeting novel flower colours.

Conclusions

This report describes the biochemical basis of flower colour in seven Australian *Ptilotus* and three Australian *Gomphrena* accessions. Flowers of all the accessions studied contained betalain pigments. Overall, ten major and three minor betalain pigments were identified including five unknown betalain pigments. While the identified pigments corresponded to betalains found in previously studied Amaranthaceae species, most of the accessions in this study contained acylated celosianins as the major betacyanin pigments, which is not typical for plants in the Family Amaranthaceae or the genus *Gomphrena*. The results also indicate that, through conventional breeding and selection, an increase in the various different betacyanin and betaxanthin pigments present in *P. nobilis* and *P. exaltatus* could facilitate the development of new cultivars of Australian *Ptilotus* with a range of large, showy, vibrant coloured flowers for use in ornamental horticulture.

Acknowledgements

We gratefully acknowledge the Australian Flora Foundation for funding this project, and the Queensland State Government and The University of Queensland for providing operational funds for the Centre for Native Floriculture. We also gratefully acknowledge Dr David Lewis, Plant & Food Research, New Zealand, for assistance with HPLC and LCMS analyses of betalain pigments and interpretation of the results.

References

Cai, Y. Z., Sun, M., Wu, H. X., Huang, R. H. and Corke, H. (1998) Characterization and quantification of betacyanin pigments from diverse *Amaranthus* species. *Journal of Agricultural and Food Chemistry,* **46,** 2063-2070.

Cai, Y. Z., Sun, M. and Corke, H. (2001) Identification and distribution of simple and acylated betacyanins in the Amaranthaceae. *Journal of Agricultural and Food Chemistry,* **49,** 1971-1978.

Cai, Y. Z., Sun, M. and Corke, H. (2005) Characterization and application of betalain pigments from plants of the Amaranthaceae. *Trends in Food Science and Technology,* **16,** 370-376.

Cai, Y. Z., Sun, M. and Corke, H. (2005b) HPLC characterization of betalains from plants in the Amaranthaceae. *Journal of Chromatographic Science,* **43,** 454-460.

Clement, J.S. and Marby, T.J. (1996) Pigment evolution in the Caryophyllales: A systematic overview. *Botanica Acta,* **109**: 360-367.

Grotewold, E. (2006) The genetics and biochemistry of floral pigments. *Annual Review of Plant Biology,* **57,** 761-780.Gaertner, V. L. and Goldman, I. L. (2005) Pigment distribution and total dissolved solids of selected cycles of table beet from a recurrent selection program for increased pigment. *Journal of the American Society for Horticultural Science,* **130,** 424-433.

Kugler, F., Stintzing, F. C. and Carle, R. (2004) Identification of betalains from petioles of differently coloured swiss chard (*Beta vulgaris* L. ssp *cicla* [L.] Alef. cv. Bright Lights) by high-performance liquid chromatography-electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry,* **52,** 2975-2981.

Kugler, F., Stintzing, F. C. and Carle, R. (2007) Characterisation of betalain patterns of differently coloured inflorescences from *Gomphrena globosa* L. and *Bougainvillea sp.* by HPLC-DAD-ESI-MS^n. *Analytical and Bioanalytical Chemistry,* **387,** 637-648.

Lee, K. K., Harrison, D. K., Johnston, M. E. and Williams, R. R. (2007) Molecular taxonomic clarification of *Ptilotus exaltatus* and *Ptilotus nobilis* (Amaranthaceae). *Australian Systematic Botany,* **20,** 72-81.

Lewis, D. H., Bloor, S.J and Schwinn, K.E. (1998) Flavanoid and carotenoid pigments in flower tissue of *Sandersonia aurantiaca* (Hook.). *Scientia Horticulturae*. **72**, 179-192.

Mudalige, R. G., Kuehnle, A. R. and Amore, T. D. (2003) Pigment distribution and epidermal cell shape in *Dendrobium* species and hybrids. *Hort Science,* **38,** 573-577.

Palmer, J. (1998) A taxonomic revision of *Gomphrena* (Amaranthaceae) in Australia. *Australian Systematic Botany,* **11,** 73-151.

Piattelli, M., Minale, L. and Prota, G. (1964) Isolation, structure and absolute configuration of indicaxanthin. *Tetrahedron,* **20,** 2325.

Schliemann, W., Kobayashi, N. and Strack, D. (1999) The decisive step in betaxanthin biosynthesis is a spontaneous reaction. *Plant Physiology,* **119,** 1217-1232.

Strack, D., Steglich, W. and Wray, V. (1993) Betalains. *Methods in Plant Biochemistry: Alkaloids and sulphur compounds* (eds P. M. Dey and H. J.B.), pp. 421-450. Academic Press, London.

Strack, D., Vogt, T. and Schliemann, W. (2003) Recent advances in betalain research. *Phytochemistry*, **62,** 247-269.

Svenson, J., Smallfield, B. M., Joyce, N. I., Sanson, C. E. and Perry, N. B. (2008) Betalains in red and yellow varieties of the Andean tuber crop ulluco (*Ullucus tuberosus*). *Journal of Agricultural and Food Chemistry*, **56,** 7730-7737.

Trezzini, G. F. and Zryd, J. P. (1991) Characterization of some natural and semisynthetic betaxanthins. *Phytochemistry,* **30,** 1901-1903.

Vogt, T., Ibdah, M., Schmidt, J., Wray, V., Nimtz, M. and Strack, D. (1999) Light-induced betacyanin and flavonol accumulation in bladder cells of *Mesembryanthemum crystallinum*. *Phytochemistry*, **52,** 583-592.

Zhang, Y., Hayashi, T., Inoue, M., Oyama, Y., Hosokawa, M. and Yazawa, S. (2008) Flower color diversity and its optical mechanism. *Acta Horticulturae,* **766,** 469-475.