ORIGINAL ARTICLE

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Betaxanthins as pigments responsible for visible fluorescence in flowers

Abstract Betalains are water-soluble nitrogen-containing pigments present in flowers and fruits of plants of the order Caryophyllales, where they replace anthocyanins. This article describes how flowers containing yellow betaxanthins are fluorescent. Betaxanthins exhibit spectra with excitation maxima between 463 nm and 474 nm and emission maxima between 509 nm and 512 nm. Thus, betaxanthins are able to absorb blue light and emit green light. Relations between fluorescence and the structural properties of the pigments are discussed. For the first time, pictures of flowers naturally emitting light are presented. Yellow flowers of the ornamental plant *Portulaca grandiflora* were chosen as a model for the studies in fluorescence due to the existence of the white phenotype, which was used as a control. Studies were also performed in *Lampranthus productus* flowers, which contain dopaxanthin as a single pigment. The visible fluorescence of betaxanthins inside the petal cells was detected in a confocal microscope after laser excitation.

Keywords Plant pigments · Visible fluorescence · Betalains · Betaxanthins · Caryophyllales

Introduction

Betalains are nitrogenous plant pigments, which are characteristic of the order Caryophyllales, where they exhibit a yellow (480 nm, betaxanthins) or violet (536 nm, betacyanins) coloration (Strack et al. 2003). General structures of the pigments are shown in Fig. 1. Betanidin is the basic structure of betacyanins, and be-

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taxanthins are made up of betalamic acid condensed with different amino acids or amines.

Interest in these molecules has grown since their antioxidant and radical scavenging properties were characterized (Escribano et al. 1998; Kanner et al. 2001) and they are widely used as additives in the food industry on account of their natural colorant properties and the absence of toxicity, even at high concentrations (Schwartz et al. 1983). Betalains are located in different parts of plants, from roots (e.g. beetroot *Beta vulgaris*) (Hempel and Böhm 1997) to fruits (e.g. cactus fruit *Hylocereus*) (Wybraniec and Mizrahi 2002). The presence of these pigments is of particular interest in flowers (e.g. *Bougainvillea, Celosia, Gomphrena, Portulaca, Mirabilis*) (Stintzing and Carle 2004) due to the importance of color as an attractant for some animals which act as pollinator agents.

Anthocyanins and betalains are highly hydrophilic pigments and they are both confined in the vacuole (Stintzing and Carle 2004; Wink 1997). However, betalains have never been found together with anthocyanins in the same plant (Stafford 1994). In some cases, anthocyanins have also been described in the cell cytoplasm (Markham et al. 2001).

Physicochemical properties of betalains have been extensively described in the literature, with special attention to stability and color. Thus, since the earliest works on betalains (Wyler and Dreiding 1957; Piattelli et al. 1964) the absorbance spectra of the different species and the limited effect of diverse substituents on the color have been characterized. The hypsochromic shift that occurs in betacyanins when they are formed through the glycosylation of betanidin is well known, as is the bathochromic effect when esterification with hydroxycinnamic acids is performed (Stintzing and Carle 2004). Despite the numerous studies on betalains and the interest in their colorant properties, fluorescence has never been reported.

A wide number of flower characteristics are known to be factors in attracting pollinator agents. These include the presence of nectar (De la Barrera and Nobel 2004)
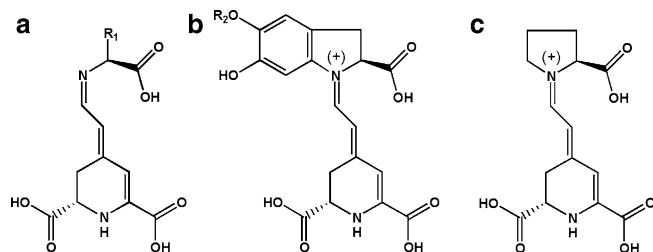
Fig. 1 General structures of betalain pigments: **a** betaxanthins, where the structural unit betalamic acid is conjugated with different amino acids, and **b** betacyanins in which condensation is made with the closed structure *cyclo*-DOPA (betanidin, $R_2 = $ H; betanin, $R_2 = $ glucose). **c** Indicaxanthin

and pollen, the existence of a special color pattern (Heiling et al. 2003) or the production of volatile compounds (Larsson et al. 2003), including pheromones (Ayasse et al. 2003). The existence of light emission by flowers has not, to our knowledge, been considered. The physiological relevance of fluorescence in signaling has recently been described for budgerigars' plumage (Arnold et al. 2002; Parker 2002) in mate choice experiments and has been suspected for a mantis shrimp (Mazel et al. 2004), where fluorescent spots were described. However, in these studies the compounds responsible for fluorescence have not been identified.

In the present work, the fluorescence capacity of betaxanthins is described and its involvement in the fluorescence of the flowers where these pigments are present is demonstrated.

Materials and methods

Chemicals

Amino acids, chemicals, solvents and reagents were purchased from Sigma and Fluka (St. Louis, MO, USA). HPLC-grade acetonitrile was purchased from Labscan Ltd. (Dublin, Ireland). Distilled water was purified using a Milli-Q system (Millipore Corporation, Bedford, MA, USA).

Plant material

Portulaca grandiflora (white, pale yellow, yellow, and violet phenotypes) and yellow *Lampranthus productus* plants were purchased in Murcia (Spain) and grown by the authors under the same conditions. Flower samples were carefully collected and the petals were removed and washed. Pigments were extracted in 10 mM phosphate buffer, pH 6.0, with 10 mM ascorbic acid in a Polytron homogenizer (Kinematica AG, Switzerland) (5 s, 2 pulses). The homogenate was filtered through nylon cloth and centrifuged at 120,000 *g* for 40 min. The supernatant was filtered through Centriplus YM-10 membranes (Millipore) to remove proteins. The whole process was carried out at 4°C. Samples were immediately used for pigment analysis and fluorescence studies.

Preparation of standards

Betaxanthins

Standard semisynthetic betaxanthins for fluorescence assays and HPLC identification analysis and calibration were obtained as immonium condensation products of betalamic acid with the corresponding amino acid (tyrosine, DOPA, glutamine, and proline) or amine (dopamine) (Schliemann et al. 1999).

Betacyanins

Standard betanin was obtained from red beetroot: extraction was performed in 10 mM phosphate buffer, pH 6.0 in a Model 230 Omnimixer (Sorvall Inc., Norwalk, CT) at maximum speed for 10 s. The homogenate was filtered through cheesecloth and centrifuged at 120,000 *g* for 40 min. The supernatant was then filtered using YM-10 membrane (Millipore) to remove proteins. The pigment was then purified by using a Sephadex G-25 gel (Sigma) according to the method previously described by Escribano et al. (1998). Betanidin was obtained enzymatically from purified betanin through β-glucosidase treatment. A betanin solution 4 µM was incubated for 30 min with 14 units ml^{-1} of β-glucosidase (provided by Sigma) in 50 mM acetate buffer, pH 5.0 at 25°C. Transformation was complete, as HPLC demonstrated, and the enzyme was removed by ultrafiltration through YM-10 membranes and the filtrate was used as the standard.

HPLC

A Shimadzu LC-10A apparatus (Shimadzu Corporation, Kyoto, Japan) equipped with a SPD-M10A photodiode array detector was used for analytical HPLC purifications. Reversed phase chromatography was performed with a 250×4.6-mm Kromasil 100 C-18 column packed with 5 µm particles (Teknokroma, Barcelona, Spain). Gradients were formed between two helium degassed solvents. Solvent A was water with 0.05% trifluoroaceticacid (TFA), and solvent B was composed of acetonitrile (AcN) with 0.05% TFA. Linear gradient was performed over 25 min from 0%B to 35%B. The flow rate was 1 ml min^{-1}, operated at 25°C. Injection volume was 20 µl. Sample pigments and standards had the same retention times and superimposable spectra.

Fluorescence spectroscopy

Fluorescence excitation and emission spectra were recorded in water at 25°C in a LS50B spectrofluorometer (PerkinElmer Life and Analytical Sciences, Inc., Boston,

MA, USA). Excitation was measured for emission at the maximum wavelength, and emission was measured for excitation at the corresponding maximum. The final concentration of individual pigments in the quartz cuvette was 6 μM. Absorbance measurements were performed in a Kontron Uvikon 940 spectrophotometer. Pigment concentration was evaluated taking a molar extinction coefficient of $\epsilon = 48{,}000$ M^{-1} cm^{-1} at 480 nm for betaxanthins (Trezzini and Zrÿd 1991b; Schliemann et al. 1999) and $\epsilon = 65{,}000$ M^{-1} cm^{-1} and $\epsilon = 54{,}000$ M^{-1} cm^{-1} at 536 nm for betanin and betanidin, respectively (Schwartz and von Elbe 1980). Natural samples were diluted after extraction in water to give a final total betaxanthin concentration of 6 μM.

Photography

Fluorescence photographs were taken using an excitation filter to limit the flash output between 360 nm and 480 nm. A yellow barrier filter blocked off the reflected blue light under 490 nm, transmitting only the wavelengths emitted by the fluorochromes. Both filters were performed and supplied by Physical Sciences Inc. (Andover, MA, USA). Transmitted light spectra are shown in Fig. 2 for both filters.

Microscopy

Microscope images were obtained in a TCS SP confocal microscope (Leica Microsystems Heidelberg GmbH, Mannheim, Germany), equipped with a Kripto–Argon ion laser. Images of fresh samples were obtained by excitation at 488 nm with the laser beam. Emission wavelengths were recorded at 500–525 nm.
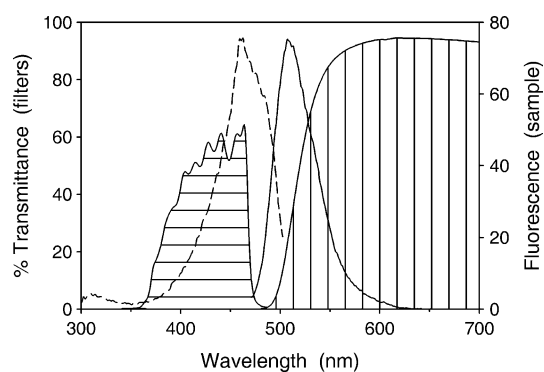

Fig. 2 Photography filters and excitation (*dashed line*) and emission (*continuous line*) spectra for yellow *Portulaca grandiflora* extract. This figure shows how the filters work. The excitation filter (*horizontal pattern*) is limiting the flash output to the area marked of the electromagnetic spectrum, overlapping with the excitation spectrum of betaxanthins (*dashed line*). Hence, excitation occurs only in this area, where betaxanthins absorb. The barrier filter (*vertical pattern*) blocks the reflected light, and only fluorescence (emitted light, *continuous line*) reaches the camera
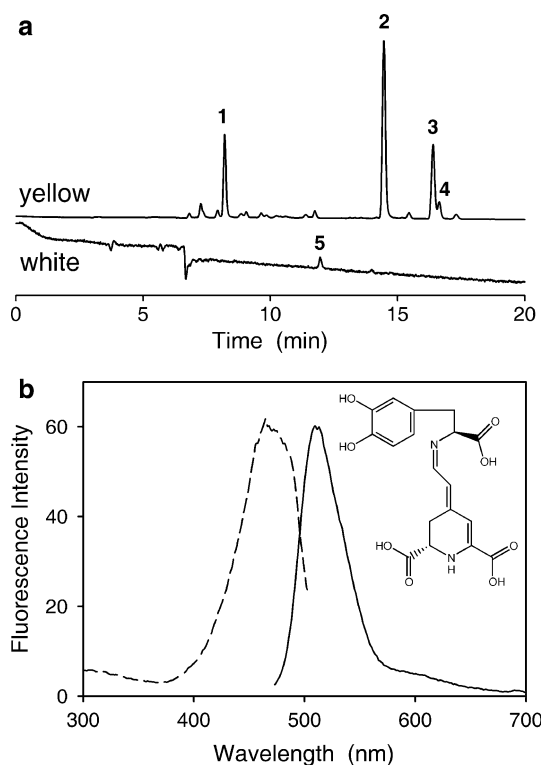

Fig. 3 a HPLC elution profiles for the analysis of the pigment composition of yellow and white flowers of *Portulaca grandiflora*. Full scales are $A_{480} = 0.1$, and $A_{480} = 0.003$ absorbance units for the analysis of yellow and white phenotypes, respectively. Using standards, peaks were identified as vulgaxanthin I (1), dopaxanthin (2), portulacaxanthin II (3), miraxanthin V (4) and betanin (5). Optimum detection of betanin can be carried out at $\lambda = 536$ nm. **b** Excitation (*dashed line*) and emission (*continuous line*) spectra for synthetic dopaxanthin (*inset*) pigment recorded in water

Results and discussion

Pigment analysis by HPLC

Portulaca is a genus of plants native to South America belonging to the family Portulacaceae. The existence of betalains in *Portulaca* has been known since Trezzini and Zrÿd (1991a) characterized its presence in four different *P. grandiflora* phenotypes. Extraction and HPLC analyses of pigments present in flowers of *P. grandiflora* made in our laboratory were in agreement with the previously published results (Trezzini and Zrÿd 1991a), revealing the presence of the following betaxanthins in the yellow flowers (Fig. 3a): dopaxanthin, vulgaxanthin I, portulacaxanthin II and miraxanthin V, as main pigments, with a total pigment content of 0.5 mg g^{-1} of fresh petals (Table 1). HPLC retention times and maximum absorbance wavelengths are shown in Table 1 for the betaxanthins identified. Complex pigment compositions are usual in the distribution of betaxanthins, which are frequently reported as appearing in mixtures (Piattelli et al. 1965; Schliemann et al. 2001).

Table 1 Fluorescence and HPLC data for pigments present in yellow *Portulaca grandiflora* flowers and indicaxanthin

Betaxanthin	Amino acid	Content[a] $(mg\ g^{-1})$	λ_m exc (nm)	λ_m ems (nm)	Rel. fluor. int.[b] (%)	Stokes shift (nm)	HPLC	
							Rt (min)	λ_m (nm)
Dopaxanthin	DOPA	0.27	463	510	100	47	14.46	472
Vulgaxanthin I	Gln	0.12	464	509	127	45	8.02	472
Portulacaxanthin II	Tyr	0.10	474	509	116	35	16.43	472
Miraxanthin V	Dopamine	0.01	465	512	67	47	16.62	456
Indicaxanthin	Pro	–	463	515	108	52	12.65	480

[a]Pigment content is expressed in mg g^{-1} petal fresh weight. Maxima for excitation and emission spectra correspond to synthetic standards
[b]Relative fluorescence intensity values are calculated in relation to dopaxanthin. Spectra were recorded as described in Materials and methods

Portulaca grandiflora was chosen as the model for fluorescence analysis and photographs due to the existence of yellow and violet flowers, containing betaxanthins and betacyanins, respectively, and to the existence of white flowers, without pigments. According to the data reported by Trezzini and Zrÿd (1991a), betanin is the only pigment responsible for the coloration in *P. grandiflora* violet flowers. During our investigations, betanin was found to be the main pigment (0.53 mg g^{-1} petal fresh weight), but its isomer iso-betanin (0.013 mg g^{-1}) and betanidin (0.16 mg g^{-1}) were also detected. Although betanidin has been described in flowers of other species (Piattelli and Impellizzeri 1970), this is the first time that it is reported in flowers of the Portulacaceae. Betanidin is the structural unit and the precursor of most betacyanins, including betanin (Strack et al. 2003). It is known to be a labile molecule (Stintzing et al. 2002) and this probably prevented proper identification in previous studies. Small amounts of betaxanthins were found in violet flowers, mainly portulacaxanthin II (0.014 mg g^{-1}), as previously described (Trezzini and Zrÿd 1991a), but vulgaxanthin I was also detected (3 µg g^{-1}).

White flowers can be considered controls when studying the pigment properties of colored ones. However, HPLC analysis demonstrated that there is a small amount of betanin in the petals (5 µg g^{-1}). As can be seen in Fig. 3a, betaxanthins were not detected.

Simple compositions are less common, but are also possible. Simplicity is desirable for further discussion on the first models of flower fluorescence. *Lampranthus* is a genus of plants that belongs to the Aizoaceae family, native to South Africa. The existence of betalains in *Lampranthus* has been known since Piattelli and Impellizzeri (1969) described the presence of betacyanins in the violet flowers. However, no report exists on the pigments present in the yellow ones. Extraction of pigments and HPLC analyses revealed the presence of a single peak corresponding to the betaxanthin dopaxanthin. Identity was determined by retention time and absorbance spectrum (Table 1) and confirmed by coelution with synthetic dopaxanthin standard. The dopaxanthin content of the flowers was 4 mg g^{-1} of fresh petals. Other authors reported dopaxanthin to be the only pigment in flowers of *Glottiphylum longum* (Im-pellizzeri et al. 1973), which also belongs to the Aizoaceae family.

Fluorescence in betaxanthins

Fluorescence spectroscopy measurements of synthetic betaxanthin standards were performed in water and exhibited a fluorescence that has not previously been characterized. In Fig. 3b are shown the spectra for synthetic dopaxanthin, the pigment responsible for the yellow coloration of *L. productus* and *G. longum* flowers and the main pigment found in *P. grandiflora* yellow flowers. As can be seen, the pigment exhibits an excitation spectrum with a maximum at 463 nm, corresponding to blue light. The emission spectrum shows a maximum centered at 510 nm, which corresponds to green light. Similar results were found for the other betaxanthins standards. Table 1 compiles the excitation and emission maximum wavelengths measured for each of the betaxanthins assayed. All the species show a similar behavior, with excitation maxima between 463 nm and 474 nm and emission maxima between 509 nm and 512 nm. Betaxanthins are able to absorb and emit light within the visible area of the electromagnetic spectrum.

Fluorescence intensities, Stokes shifts (Table 1) and the shapes of the spectra recorded are also very similar in all cases. All betaxanthins (Fig. 1a) can be considered as immonium derivatives of betalamic acid, condensed with different amines or amino acids (Schliemann et al. 1999). The betalamic acid moiety is known to act as a chromophore group in betalains due to the presence of the conjugated double bonds (Stintzing and Carle 2004). Electronic resonance in the betalamic acid moiety may also be responsible for the fluorescent properties of betaxanthins.

Loss of fluorescence in betacyanins

Violet betacyanins are the counter partner of betaxanthins in giving color to flowers and fruits. In betacyanins structure (Fig. 1b) the same betalamic acid moiety is exhibited, but condensation in this case is made with the

closed structure of *cyclo*-DOPA. The electronic resonance of the betalamic acid moiety is thus extended to the diphenolic aromatic ring. The extra conjugation shifts the absorption maximum from 480 nm to 540 nm.

Betanidin is the basic structural unit of most betacyanins and was assayed for fluorescence under the same conditions used for betaxanthins. However, no fluorescence could be detected. Betanin (betanidin-*O*-β-glucoside) is the best-known betacyanin, first described in red beetroot (Wyler and Dreiding 1957). In this case, the same negative results were obtained when fluorescence was investigated. The electronic system supported by the betalamic acid moiety and the imine group derived from the condensation reaction present in betaxanthins implies fluorescence but this is lost when condensation is made with *cyclo*-DOPA. This suggests that the extension of the resonance is responsible for the lack of fluorescence in betacyanins as well as for the shift in the absorbance spectra.

To confirm the implication of the aromatic ring in the lack of fluorescence, the structurally related pigment indicaxanthin (Fig. 1c) was synthesized. It is a special betaxanthin, derived from the condensation of betalamic acid with proline. The pigment contains the closed structure present in betacyanins condensed with betalamic acid, but without the attached aromatic ring. When indicaxanthin was assayed under the same conditions used for the other betalains, fluorescence was detected. Despite the structural differences with betaxanthins that possess open structures, the fluorescent behavior exhibited by indicaxanthin is similar (Table 1). The existence of fluorescence in indicaxanthin is consistent with the implication of the coupling between the aromatic ring of *cyclo*-DOPA and the double bonds of the dihydropyridine moiety in the loss of fluorescence. When both elements are present but the resonances are not connected, the resulting structure corresponds to dopaxanthin, a fluorescent molecule, as demonstrated above.

Based on these data, we conclude that the fluorescent phenomenon in betaxanthins is due to the resonance system contributed to the pigment structure by the dihydropyridine moiety. Thus, all betaxanthins are potentially fluorescent, including those derived from aliphatic amino acids, such as alanine (λ_m exc = 463 nm, λ_m ems = 508 nm) or leucine (λ_m exc = 464 nm, λ_m ems = 509 nm). The presence of extra electronic resonance due to the aromatic ring of the *cyclo*-DOPA moiety in betacyanins prevents the violet pigments from being fluorescent.

Fluorescent plant extracts

When we analyzed yellow *P. grandiflora* flower extracts, a fluorescence not seen previously in flowers by other authors was detected. Excitation and emission spectra of the yellow extracts were recorded, obtaining maximum wavelengths of 463 nm and 509 nm, respectively. These results are near to the data obtained for the synthetic betaxanthins. Shapes of the spectra were also similar, as can be seen in Fig. 2. Similarities are in agreement with the described presence of a betaxanthin mixture in the petals which is responsible for the flower extract fluorescence.

For dopaxanthin containing *L. productus* extracts, the maxima for excitation and emission were 463 nm and 510 nm, respectively, corresponding to those obtained for the synthetic pigment.

The presence of aromatic compounds in the petals does not contribute in any case to the visible fluorescence exhibited by the extracts. Aromatic compounds and amino acids present absorbance spectra situated in the UV range of the electromagnetic spectrum. Some of them are fluorescent and are able to emit light, although always in the UV range (Teale and Weber 1957). Visible fluorescence is due only to betaxanthins. In fact, there is nothing in the flowers able to absorb light at these wavelengths but betaxanthins, as demonstrated by HPLC analysis with photodiode array detection (results not shown). The characteristics of the individual betaxanthins make the yellow flower extracts fluorescent.

Extracts of white flowers of *P. grandiflora* were considered as controls in the fluorescence exhibited by the yellow ones, since betaxanthins were not detected. No visible fluorescence could be measured. Betacyanins from violet flowers of *P. grandiflora* were also extracted and the same negative result was obtained as for the standard pigments.
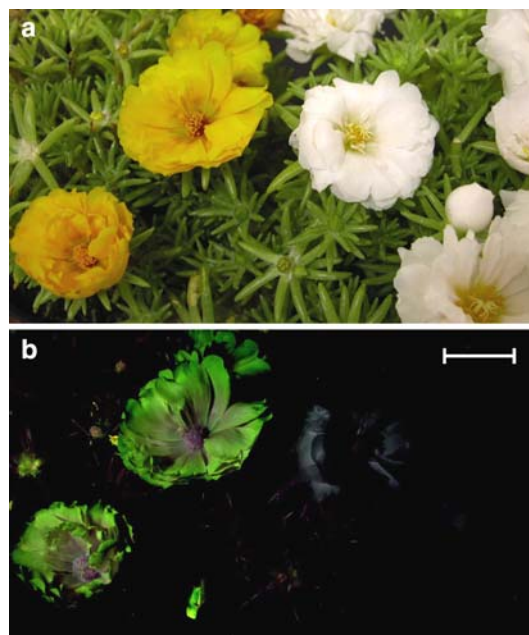

Fig. 4 White and yellow flowers of *P. grandiflora*: **a** illuminated by white light and **b** stimulated by blue light. Fluorescence is exhibited by yellow flowers. Both plants were grown under the same conditions. *Scale bar* 2 cm

Light emitted by fluorescent flowers can be photographed

The establishment of the fluorescent properties of individual pigments and flower extracts raised the question of whether flowers containing betaxanthins could be considered as fluorescent. When illuminated by white light, flowers containing betaxanthins appear yellow, as can be seen in Fig. 4a for two *P. grandiflora* flowers. This is due to the reflectance of the nonabsorbed radiation combined with the light emitted by means of the fluorescence. To observe the fluorescent phenomenon, it was necessary to filter the incident light in order to avoid contamination of the emitted fluorescence. Based on the properties of betaxanthins in aqueous solution, a photography filter system specially designed for green fluorescence visualization was used. Figure 2 shows the spectra of the light transmitted by the filters.

When flowers are illuminated by filtered blue light, they exhibit green fluorescence, as shown in Fig. 4b. This is the first time ever a flower emitting light has been photographed by using natural fluorescence. Only the yellow flowers, containing betaxanthins are fluorescent, while white flowers are not visible.

Violet flowers do not present light emission and they appear dark in fluorescence photographs when the described filter system is used (results not shown), as do white flowers. As in the case of extracts, white and violet phenotypes can be considered as controls in the fluorescence exhibited by the yellow one. Betaxanthins fluorescence, described above in an aqueous environment, is responsible for the brightness photographed. During the excitation process, UV light is filtered and neither UV excitation nor emission is possible.

Fluorescence photographs were also taken of the yellow flowers of *L. productus*, obtaining the results shown in Fig. 5b. As can be seen, the petals are glowing in the same way as the yellow flowers of *P. grandiflora*. In *L. productus* flowers, dopaxanthin gives the yellow coloration to the petals and the fluorescence described of this pigment makes it possible for the entire flower to glow.

Natural fluorescence of betaxanthins gives new insights into the brightness exhibited by ornamental flowers containing them, and should be of horticultural interest and potentially of commercial relevance. In the flower industry, the search for novel classes of colors or intensities is of great importance, and the idea that fluorescence can be used to obtain new shades has led other researchers to express Green Fluorescent Protein in ornamental plants (Mercuri et al. 2001). Fluorescent colors were thus obtained without taking into account that some plants show similar results through betaxanthin fluorescence.

In most cases, flowers seek to catch the attention of animals. Native fluorescence may play the role of an attracting signal, as demonstrated for budgerigars' plumage (Arnold et al. 2002; Parker 2002). At this point it should be considered that pollinators have different spectral sensitivities and perceive the colors in different ways. Recently, a nectar eating and cactus flower pollinator, bat, was found to be able to see light with an optimum wavelength of 510 nm (Winter et al. 2003), which corresponds with the maximum of emission of betaxanthins. The described native fluorescence of these pigments (also present in the family Cactaceae) enhances the brightness of the flowers at this wavelength and may, therefore, enhance their visibility. Bees are common and efficient pollinators of a wide variety of plants and are used as a model study for insect vision and pollination (Gumbert et al. 1999). The existence of a green receptor in bees' vision and the fact that bright targets were detected more reliably than dim ones in behavioral experiments (De Ibarra et al. 2000), suggest that betaxanthin fluorescence may play a physiological role. The influence of pollinators on the evolution of floral traits has been pointed out for *Antirrhinum majus*, where pollination rate and visit duration were factors in the reproduction success (Jones and Reithel 2001). However, other factors, such as self-fertilization may maintain the presence of less attractive forms (Brown and Clegg 1984).

Petal cells visualization through betaxanthin fluorescence

Characterization of betaxanthins fluorescence was made in water because the pigments are highly hydrophilic and because an aqueous environment is the expected physi-

Fig. 5 Fluorescent flowers of *L. productus* under white (**a**) and blue (**b**) light. *Scale bar* 1 cm
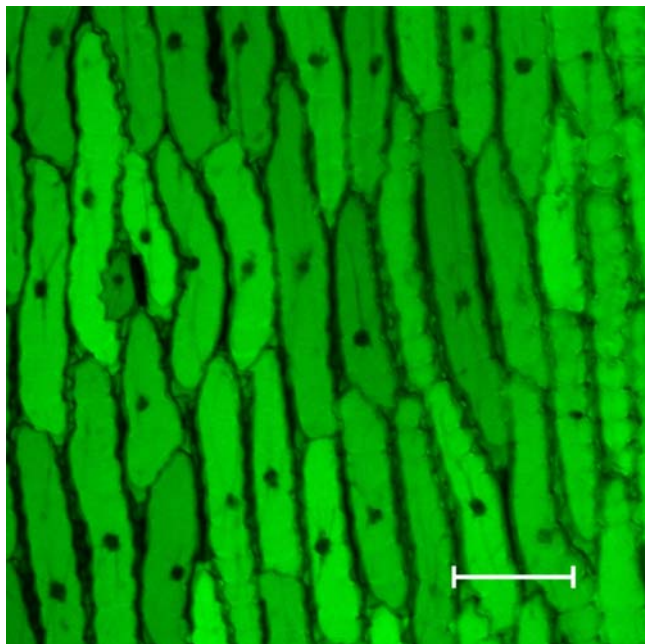
Fig. 6 Confocal microscope image of a yellow *P. grandiflora* petal obtained by exciting with blue light (laser 488 nm). *Scale bar* 80 µm

ological one. The characteristics of the spectra recorded for the excitation process opened up the possibility of exciting betaxanthins with the 488 nm line of an Argon laser. The coherent light produced by the laser differs from ordinary light in that it is made up of waves with the same wavelength. In this case, excitation filters are not necessary and only light of the desired wavelength reaches the sample.

Excitation with the laser was performed in a confocal microscope, using a system similar to the one used for measurements with the Green Fluorescent Protein technique (Ehrhardt 2003). As expected, betaxanthins were excited by the laser. Light emitted by betaxanthins in the fluorescence phenomenon allowed the visualization of the structure of petal cells where the pigments are stored. Fig. 6 shows the image obtained for a section of a *P. grandiflora* yellow flower petal. The big vacuole contains the fluorescent pigments (Wink 1997) and takes up almost the whole petal cell. The nucleus appears as a dark spot, without pigments, situated in the center of every cell. Future studies should be able to shed light on poorly understood topics of betaxanthins metabolism, like the transport of the pigments into the vacuole (Strack et al. 2003).

Controls were performed with sections of white flower petals. Black fields were obtained, confirming that visualization in yellow flowers was a result of the betaxanthins fluorescence. Betacyanin-containing cells of the violet flowers were not visible under this system (data not shown).

Betaxanthins are fluorescent molecules, and light emission is maintained when they are present in the physiological environment, inside the petal cells. The characterization of native fluorescence due to betaxanthins and the demonstration that this phenomenon is maintained in the living plant opens up new possibilities for the flower industry and for the study of signaling between flowers and pollinators. Naturally occurring fluorescence present in yellow *Portulaca* and *Lampranthus* flowers may well be extensive to all betaxanthin-containing flowers and tissues.

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