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SHORT COMMUNICATION

Chemical composition and microstructure of *Bauhinia* grains

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Abstract *Bauhinia* is a leguminous plant species found in almost every part of the world, including southern Africa. In this study, grain composition and protein body microstructure of two indigenous southern African *Bauhinia* species, *B. galpinii* and *B. petersiana* were determined. Protein (38 g/100 g) and fat (23 g/100 g) were the major constituents of *Bauhinia*. *Bauhinia grains* also contained substantial amounts of zinc (6 mg/100 g) and iron (3 mg/100 g) when compared to FAO/WHO standards. The parenchyma cells of *Bauhinia* showed spherical protein bodies with globoids inclusions and these were surrounded by lipids. However, the protein bodies of *B. petersiana* were smaller in size (7±3 μm) than those of *B. galpinii* (13±4 μm). The microstructure of protein bodies in *Bauhinia* is very similar to that of soya, suggesting that the processing technology developed for soya protein may be adopted for *Bauhinia*.

Keywords Bauhinia · Soya · Composition · Microstructure · Protein

Introduction

Bauhinia is a leguminous plant species found in almost every part of the world, including southern Africa. The genus *Bauhinia* consists of about 300 species (Filho 2009). *Bauhinia* grains are protein-rich oilseeds (Arnold et al. 1985; Anhwange et al. 2005) similar to soya bean (Amonsou et al. 2011) and peanuts (Venkatachalam and Sathe 2006). Up to 33 % protein and 29 % lipid have been reported *for B. monandra* (Anhwange et al. 2005). Some *Bauhinia* species found in southern Africa are moderately to highly drought tolerant (Fanie and Venter 1996; Bosch 2006) and therefore, have some advantages over soya and peanuts as alternative food sources. *B. galpinii* also known as the "*Pride of De Kaap*" is widespread and can be found in many provinces in South Africa. This species is hardy to drought and moderate to frost (Palgrave and Palgrave 2002). *B. pertersiana*, known as the "*wild coffee bean*" or the "*Kalahari white Bauhinia*" is distributed across southern African Countries including Botswana, Zimbabwe, Namibia and part of South Africa. It is highly drought tolerant (Brummitt and Ross 1975). However, in comparison with peanuts and soya, the *Bauhinia* grains are much underutilised and under-researched. Traditionally, *Bauhinia* grains are consumed as roasted nut (Fanie and Venter 1996). Roasted grains are pounded and used to make a pleasant tasting meal (Fanie and Venter 1996). The knowledge of the grain composition may be important for the purposes of nutrition and grain utilisation.

Further, food microstructure is fundamental to the understanding of the functionality of a food material. Knowledge of the grain microstructure may be important in the extraction, processing and utilisation of protein (Aguilera 2005; Parada and Aguilera 2007). The physical localisation of protein bodies relative to either the lipids has been found to influence protein extractability (Shand et al. 2007) and functional properties such as protein digestibility (Aguilera 2005). Grain microstructure also affects grain hardness (Aguilera and Stanley 1999), an important parameter in milling operations and equipment design for processing. Depending on plant species, some variations in the protein body microstructures have been found among these oilseeds (Young et al. 2004; Amonsou et al. 2011). According to Amonsou et al. (2011),

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Fig 1 Species of *Bauhinia* grains

B. petersiana ***B. galpinii***

globoid and druse crystal inclusions found in marama protein bodies appeared to be absent in soya. The microstructure of protein bodies in *Bauhinia grains* is not known.

In the study, the chemical composition and microstructure of protein bodies in grains of *Bauhinia* species were determined.

Materials and methods

Materials

Two indigenous southern African *Bauhinia* species, *B. petersiana* and *B. galpinii*, were used. The grains of these species were gathered in 2012 by the National Botanical Garden in the Lowveld region, Nelspruit (Mpumalanga Province), South Africa. The plant specimens were deposited at the Herbarium at the University of KwaZulu-Natal, Pietermaritzburg, South Africa. Soya bean (*Glycine max*) obtained from PANNAR SEED (Greytown, South Africa) was used as a reference sample.

Physical properties of *Bauhinia* grains

The colour of *Bauhinia* grains were determined by visual observation in day light. The size of randomly selected grains (n=50) was determined in three linear perpendicular dimensions using a micrometer screw gauge reading to an accuracy of 0.01 mm.

Flour preparation

Bauhinia grains were dehulled by crushing the grains in a laboratory grinder and the grain coats removed manually. The cotyledons were then milled and the resulting flours were stored at 4 °C until analysed.

Chemical analysis

Proximate composition

The moisture, fat, crude fibre and ash contents of grain flours were determined using the AOAC Methods no. 934.01, 920.39, 978.10 and 942.05, respectively (AOAC 2000). The protein content (N X 5.71) was determined by the Dumas method of combustion analysis (Method no. 990.03, AOAC 2000). Total carbohydrate was calculated by difference.

Table 1 Proximate composition of *Bauhinia* seed sand soya bean (g/100 g flour) [a]

Samples	Moisture	Protein	fat	Crude fibre	Ash	Carbohydrate[b]
B. petersiana	$5.2^{a}\pm0.1$	$37.7^{a}\pm0.3$	$22.3^{a}\pm0.4$	$1.6^{b}\pm0.2$	$4.1^{a}\pm0.1$	$29.1^{c}\pm0.6$
B. galpinii	$8.0^{b}\pm0.1$	$38.5^{b}\pm0.4$	$24.2^{b}\pm0.3$	$1.4^{a}\pm0.1$	$4.5^{b}\pm0.7$	$23.3^{b}\pm0.4$
Soya	$7.5^{b}\pm0.3$	$42.8^{c}\pm0.1$	$22.8^{a}\pm0.5$	$2.3^{c}\pm0.4$	$5.2^{b}\pm0.2$	$19.8^{a}\pm0.5$

[a] Mean ± SD. Mean values with different superscript letters in columns are significantly different (p<0.05)

[b] Carbohydrate by difference

Table 2 Mineral composition of *Bauhinia* seeds compared to soya bean (mg/100 g flour) [a]

Elements	B. Petersiana	B. Galpinii	soya
K	$629^{a}\pm23$	$907^{b}\pm33$	$1259^{c}\pm23$
P	$775^{b}\pm36$	$743^{b}\pm18$	$600^{a}\pm10$
Mg	$316^{b}\pm17$	$361^{c}\pm19$	$243^{a}\pm14$
Ca	$325^{c}\pm22$	$159^{b}\pm12$	$144^{a}\pm15$
Na	$39^{a}\pm5$	$38^{a}\pm7$	$62^{b}\pm9$
Mn	$1.9^{a}\pm0.1$	$4.2^{b}\pm0.3$	$3.1^{c}\pm0.4$
Cu	$1.8^{b}\pm0.3$	$1.9^{b}\pm0.1$	$1.1^{a}\pm0.2$
Fe	$2.7^{b}\pm0.4$	$2.3^{a}\pm0.3$	$6.2^{c}\pm0.1$
Zn	$7.5^{c}\pm0.1$	$4.7^{a}\pm0.2$	$5.1^{b}\pm0.1$

[a] Mean ± SD is reported on dry basis. Mean with different superscript letters in rows are significantly different ($p<0.05$)

Mineral composition

Grain flours were digested as described in Amonsou et al. (2011) and the mineral content analysed by AOAC (1984)) using the Inductively Coupled Plasma (ICP) spectroscopy.

Microscopy

Sample preparation for Scanning Electron Microscopy, (SEM), Light Microscopy (LM) and Transmission Electron Microscopy (TEM)

Tissue blocks (1 mm^3) cut from the surface of the interior part of the cotyledons were fixed in 0.05 M cacodylate buffer, pH 7.2, containing 3 % (*w*/*v*) glutaraldehyde for 48 h. These were then washed and postfixed in Osmium for 2 h. This was followed by washing with buffer and dehydration in a graded series aqueous ethanol.

For LM and TEM, fixed and dehydrated tissues in ethanol were further dehydrated twice in propylene oxide at 30 min intervals. These were then infiltrated with three different ratios of Epon to propylene oxide and embedded in Epon. The resin was polymerized at 70 °C for 24 h.

Sections (1 μm) were cut for LM using an ultramicrotome. These sections were stained separately with Ladd and Coomassie Brilliant Blue R 250 (Gahan 1984).

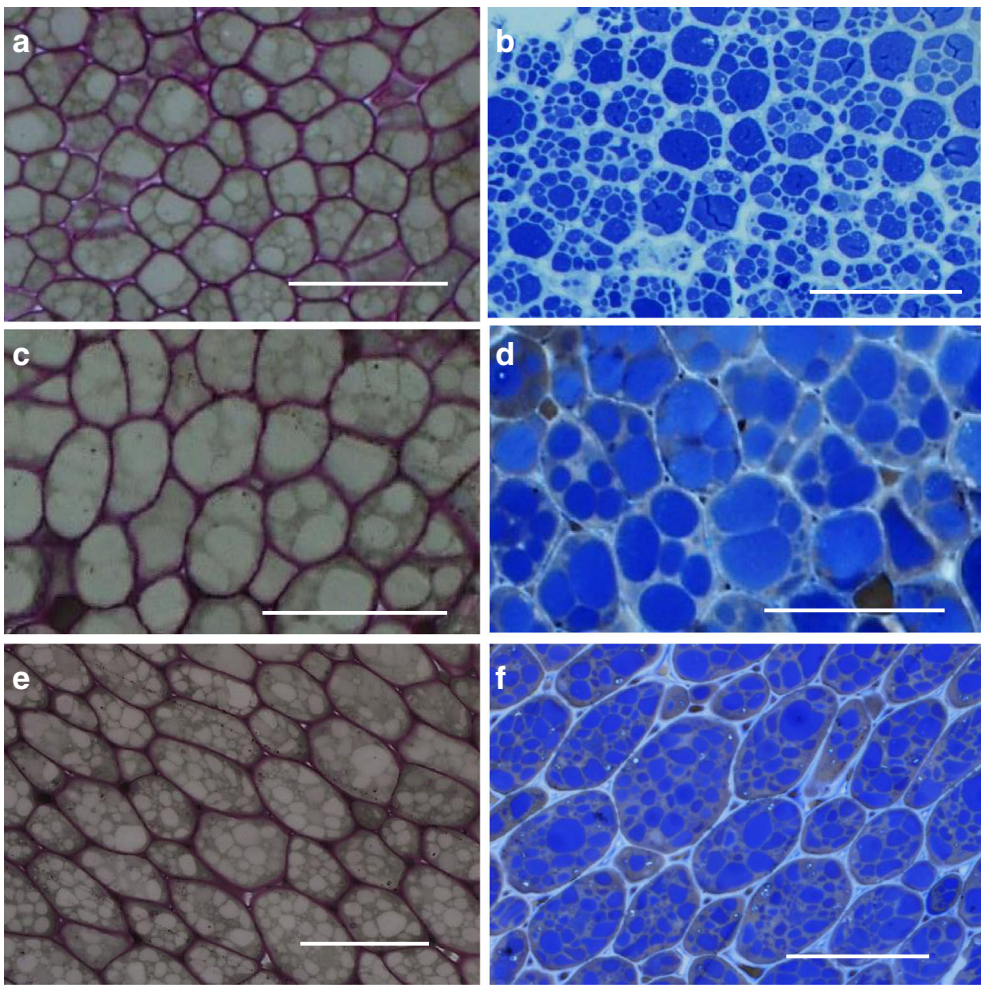

Fig 2 Light microscopy of *Bauhinia* seed and soya bean parenchyma cells stained with Ladd (**a**, **c** & **e**) and Coomassie Brilliant Blue (**b**, **d** & **f**). **a** & **b**: *B.petersiana*, **c** & **d**: *B. galpinii*, **e** & **f**: *Soya bean*. Bar: 50 μm. Cell walls are stained purple with Ladd and protein bodies are stained blue with Coomassie Brilliant Blue

Ultrathin sections were cut for TEM. These sections were placed on copper grids and contrasted in 4 % aqueous uranyl acetate and Reynolds lead acetate, respectively.

Confocal Laser Scanning Microscopy (CLSM)

Fresh tissue sections (2–3 μm) were cut from the cotyledon inner surface. These sections were analysed with a ZEISS 710 confocal laser scanning microscope without any further treatment. The excitation wavelength was 405 nm. Fluorescing protein was detected after passing through a 420 μm long filter, with a pinhole set at 55 μm.

Statistical analysis

Analysis of variance (ANOVA) was done on chemical composition data. Means were compared by using the Fisher Least Significant Difference (LSD) test ($p<0.05$). The mean size of protein bodies and their distribution per cell ($n=50$ cells) were determined.

Results and discussion

Physical properties of *Bauhinia* grains

Bauhinia grains appeared similar in colour and shape, but different in size (Fig. 1). *B. galpinii and B. petersiana* grains appeared brown and elliptical. However, unlike *B. petersiana* grains, *B. galpinii* grains appeared slightly concave on one side and convex on the other. In terms of the size, the thickness of *B. galpinii* grains (2.4±0.4 mm) was about twice that of *B. petersiana* grains. *B. galpinii* grains were also slightly longer (12.3±0.7 mm) and wider (8.9±0.9 mm) than *B. petersiana* grains (Length: 9.3±0.6 mm; width: 6.5± 0.5 mm). The difference in size may influence the chemical composition of the *Bauhinia* species.

Chemical composition of *Bauhinia* grains

The protein contents of *B. petersiana* and *B. galpinii* were only slightly low compared to soya (Table 1). But, *B. galpinii*

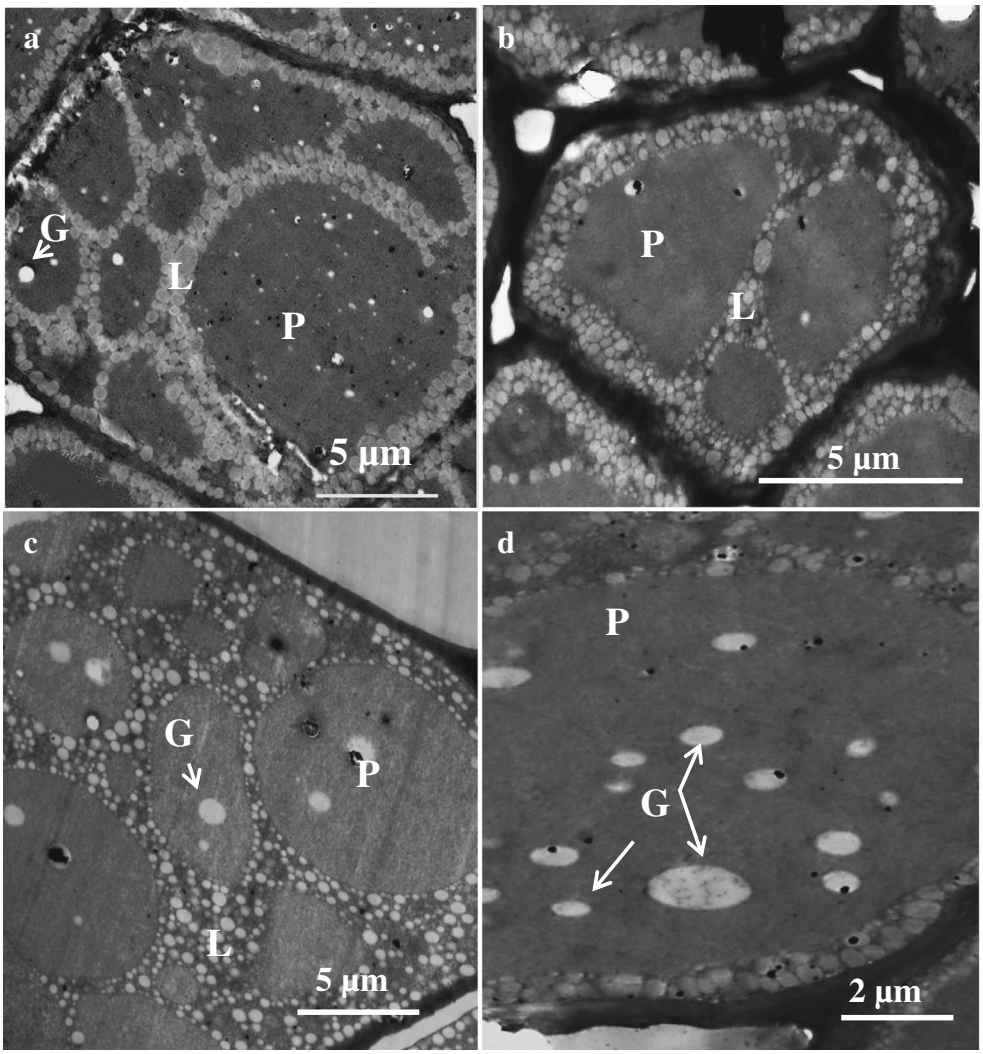

Fig. 3 Transmission electron microscopy of protein bodies in *Bauhinia* seed and soya bean **a**: *B. Petersiana*, **b**: *B. Galpinii*, **c**: Soya bean, **d**: Globoids (*G*) in protein body of *B. galpinii*, *L* lipid bodies, *P* protein bodies

contained slightly high protein compared to *B. petersiana*. This may be attributed to differences in grain size of *B. galpinii* and *B. petersiana* as described above. The two *Bauhinia* species and soya had much similar fat contents. But, the crude fibre contents of *Bauhinia* were low (approx. 1.5 g/100 g flour), about half that of soya.

In comparison with other *Bauhinia* species, the protein and fat contents of *B. galpinii and B. petersiana* appeared higher than those of *B. malabrica* (Vijayakumari et al. 1993). *B. purpurea* (Vijayakumari et al. 1997) and *B. Tomentosa* (Agbede 2007). But, the crude fibre and carbohydrate contents of the two *Bauhinia* species appeared low compared to *B. purpurea* (Vijayakumari et al. 1997). In this study, chemical analyses were done on the cotyledon. The authors who reported on *B. purpurea* did not specify whether the grain coat were removed before milling as this may have accounted for the difference in carbohydrate and/or fibre. Further, grain chemical composition may differ according to species, environmental factors and agricultural practices.

Potassium, phosphorus, magnesium and calcium were the major minerals in *Bauhinia*, similar to soya (Table 2). *Bauhinia* grains also contained substantial amounts of zinc (6 mg/100 g) and iron (3 mg/100 g) when compared to FAO/WHO standards. The mineral composition of *Bauhinia* appeared similar to oilseeds such as marama bean (Amonsou et al. 2011), peanuts (Wu et al. 1997) and other *Bauhinia* species such as the *B. monandra* (Anhwange et al. 2005).

Microstructure of protein in *Bauhinia* grains

With LM, the parenchyma cells of *Bauhinia* and soya showed circular bodies in cross section (Fig. 2). The walls of these cells were stained purple with Ladd leaving the circular bodies unstained (Fig. 2a, c, e). Dwarte and Ashford (1982) reported a purple colour of the parenchymal cell walls in celery when these cells were stained with toluidine blue. The Ladd stain that was used in this study contained toluidine Blue and this may have reacted with cell wall components. Thus, staining with Ladd was useful to differentiate the cell walls in *Bauhinia* and soya parenchyma cells. To determine whether the unstained circular bodies were protein bodies, the tissue sections were stained with Coomassie Blue, a standard dye for protein (De Moreno et al. 1986; Hafiz 2005). Following this treatment, the circular bodies appeared distinct and stained blue within the cells in both *Bauhinia* and soya (Fig. 2b, d, f), suggesting that these were protein bodies. In a similar manner, the protein bodies in marama and soya stained blue when treated in the same way (Amonsou et al. 2011; Mosele et al. 2011).

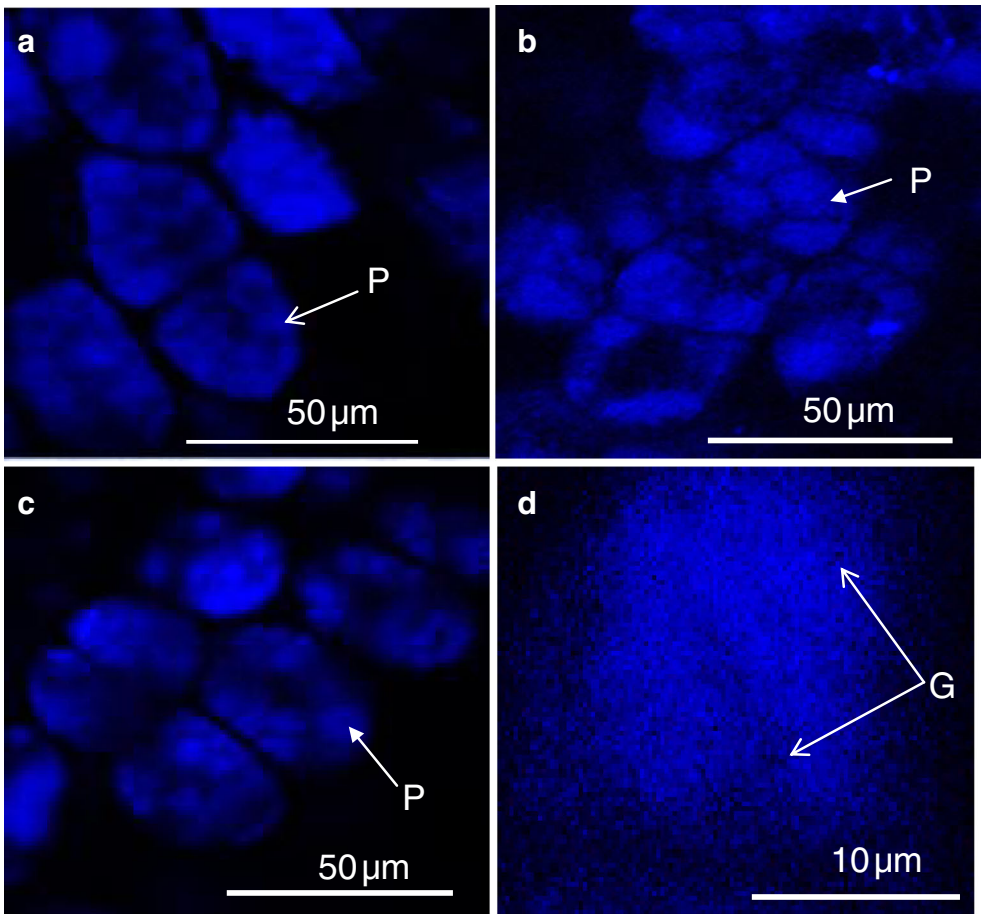

Fig. 4 Confocal laser scanning microscopy of protein bodies in *Bauhinia* seeds and soya bean parenchyma cells **a**: *B. petersiana*, **b**: *B. galpinii*, **c**: Soya bean, **d**: Globoids (*G*) in a single protein bodies of *B. galpinii*

TEM of the *Bauhinia* grains also showed circular, electron dense protein bodies in cross-section surrounded by networks of lipid bodies similar to what was observed in the soya bean (Fig. 3). A similar organisational structure of protein bodies relative to the lipid bodies has been observed in marama bean and soya (Amonsou et al. 2011) and peanuts (Young et al. 2004). However, the distribution and size of these protein bodies in the parenchyma cells of the two *Bauhinia s*pecies were different. *B. petersiana* seemed to contain more smaller protein bodies per cells (approx. 8 per cell, size: 7±3 μm), than did *B. galpinii* (approx. 4 per cell, Size: 13±4 μm). The protein bodies in parenchymal cells of *B. petersiana* also appeared to be of a regular pattern consisting of one big and single protein body occurring together with smaller ones per cell (Fig. 2a, b; Fig. 3a), the pattern which is unique and different from those in the parenchyma cells of *B. galpinii* and soya. The size of protein bodies in *Bauhinia* is within the range reported in the literature for most oilseeds including soya (Lott 1981; Young et al. 2004; Amonsou et al. 2011).

Further, the protein body inclusions were found in *Bauhinia* grains and soya bean (Fig. 3). According to Lott and Buttrose (1978)), the protein bodies in seeds may contain inclusions, namely the globoid crystals, which constitute a storage site for phosphorus deposited as insoluble phytate (Martinez 1979). Protein body inclusions have also been reported in oilseed such as peanuts (Young et al. 2004) and marama bean (Amonsou et al. 2011). Previous research on the elemental analysis of protein body inclusions showed that the inclusions contained mainly phosphorus, potassium, magnesium and calcium (Lott and Spitzer 1980; Lott and Buttrose 1978). These minerals in *Bauhinia* grains (Table 2) may have originated from the globoid sites.

The protein bodies in the *Bauhinia* were further studied with CLSM. Confocal microscopy revealed the presence of autoflorescent protein bodies within the parenchyma cells of *Bauhinia* and soya (Fig. 4a, b, c). These protein bodies appeared in a cluster and are similar to those observed with TEM and LM in terms of their spherical shape. Inclusions that did not fluoresce were also found in protein bodies (Fig. 4 d). These are possibly the globoids inclusions, thus confirming the TEM result (Fig. 3).

Conclusions

B. galpinii and *B. petersiana* contain substantial amounts of protein, fat and micronutrients such as the zinc and iron. The protein bodies in *Bauhinia* grains are very similar to those in soya in terms of their spherical shape and localization within the parenchyma cells. The great similarity in the protein body microstructure between *Bauhinia* grains and soya suggests that the processing technology developed for soya protein may be adopted for *Bauhinia*.

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