

MICROSTRUCTURE OF PROTEIN BODIES IN MARAMA BEAN SPECIES

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ABSTRACT

Marama bean is an underutilised indigenous legume from Southern Africa. The understanding of the microstructure of marama protein bodies, the organelles of protein storage, is an important step towards the characterisation and utilisation of its protein. The protein body structures of two species of marama bean species (*Tylosema esculentum* and *Tylosema fassoglense*) were determined in comparison with soya bean (*Glycine max*). *T. fassoglense* seemed to have higher protein content than soya. Marama showed clustered spherical protein bodies surrounded by lipid bodies similar to soya bean. *T. esculentum* seemed to contain smaller sized ($4 \pm 2 \mu\text{m}$) protein bodies per cell as compared with *T. fassoglense* ($7 \pm 4 \mu\text{m}$). Marama protein bodies contained spherical globoid and druse crystal inclusions, which were absent in soya. P, K, Mg and Ca were the major minerals in marama, which probably originated mainly from storage protein sites. The protein body structure of marama is similar to soya in terms of spherical shape and localisation within the parenchyma cells.

Key words: marama bean, soya bean, protein bodies, microstructure

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1. INTRODUCTION

Marama bean is an underutilised indigenous legume from Southern Africa. *Tylosema esculentum* and *Tylosema fassoglense* are the two species of marama bean that occur in South Africa and Namibia (Coetzer and Ross, 1976). Marama bean is found to be a good source of protein (Amarteifio and Moholo, 1998) similar to oilseed legumes such as peanuts (Venkatachalam and Sathe, 2006) and soya beans (Garcia, Marina, Laborda and Torre, 1998). However, the use of marama bean remains mainly domestic. Traditionally, marama bean is roasted and consumed as a snack. Matured roasted seeds are also ground or pounded for making porridge or a coco-like beverage (Keegan and Staden, 1981). Marama oil has reportedly been used for cooking (Ketshajwang *et al.*, 1998). Marama bean could become a valuable crop and a potential source of income in production zones if its major component parts like the protein and fat are adequately utilised.

Understanding of the microstructure of protein bodies, the organelles of protein storage is an important step towards the isolation, characterisation and utilisation of food proteins. In particular, the knowledge of the seed microstructure may be important in commercial processing and utilisation of legume protein. When isolating proteins from peas and soya bean, residual lipids in extracted isolates were attributed to not only differences in lipid compositions but also on the physical location of lipid in the seeds (Shand, Pietrasik, and Wasnasundara, 2007). The physical location of storage protein in the seed may affect its protein digestibility (Aguilera, 2005). Furthermore, the microstructure of seeds has been found to influence their physical properties such as seed hardness (Aguilera and Stanley, 1999).

The structure of protein bodies in marama beans is not known. The objective of this study was to determine the protein body structure of the two species of marama bean (*T. esculentum* and *T. fassoglense*) in comparison with soya bean (*Glycine max*).

2. MATERIALS AND METHODS

2.1 Materials

Two species of marama bean *Tylosema esculentum* (Burch) A. Schreib and *Tylosema fassoglense* (Schweinf) Torre and Hille and soya bean (*Glycine max* L. Merr.) were used. *T. esculentum* was harvested from two different locations, Rooidraaitrust, South Africa (voucher specimen deposited at H.G.W.J. SCHWEICKERDT (PRU), Accession number: 113873) and Gantsi, Botswana in 2008 (voucher specimen deposited at PRU, Accession number: 113872). *T. fassoglense* was harvested near Kruger Park, South Africa in 2007 (voucher specimen deposited at PRU, Accession number: 113870). Species were identified based on the seed morphology (Fig. 1) and leaves. Soya bean (*Glycine max* L. Merr.) was obtained from AGRICOL, Pretoria, South Africa.

2.2 Samples preparation

Marama beans were dehulled using a cracking device (WMC Sheet Metal Works, Tzaneen, South Africa). Soya beans were dehulled with a Tangential Abrasive Dehulling Device (TADD), (Shepherd, 1979). Dehulled marama and soya beans were ground into flours using a laboratory attrition mill coupled with 0.5 mm screen sieve (Ika Werke, Staufen, Germany). Processed bean flours were stored at 4°C until analysed.

2.3 Chemical analyses

2.3.1 *Proximate composition*

Bean flours were analysed for moisture content (934.01), fat (920.39), ash (942.05), crude fibre (962.09) and crude protein (N x 5.71) by combustion analysis (968.06) (AOAC, 2000). Total carbohydrate was obtained by difference.

2.3.2 *Mineral composition*

Bean flours (0.5 g) were acid digested with 5 ml nitric/perchloric acid (2:1) mixture. Mineral content was analysed by AOAC method no. 6.1.2 (1984), using Inductively Coupled Plasma (ICP) Spectroscopy.

2.4 *Light Microscopy (LM), Transmission Electron Microscopy (TEM)*

and Confocal Laser Scanning Microscopy (CLSM)

Sample fixation for LM and TEM was by the method of Young, Pattee, Schadel, and Sander (2004) with slight modifications. Modifications included fixing of the tissue blocks (1 mm³) cut from the inner surface of cotyledons in 0.075 M phosphate buffer, pH 7.4 containing 2.5% (w/v) glutaraldehyde for 48 h at room temperature.

For LM, sections of 1 µm were cut after fixation using an ultramicrotome. These sections were stained with Coomassie Brilliant Blue R 250 (Gahan, 1984). Stained specimens were mounted in immersion oil.

For TEM, ultrathin sections were cut using an ultramicrotome. The sections were placed on copper grids and contrasted in 4% aqueous uranyl acetate for 10 min, followed by 2 min in Reynolds lead acetate.

For CLSM, tissue blocks (1 mm) cut from the cotyledon inner surface were fixed in 2.5% (w/v) formaldehyde overnight. Fixed tissues were rinsed three times for 10 min in 0.075 M phosphate buffer and dehydrated at room temperature at 10 min intervals in a graded series of aqueous ethanol (50%, 70%, 90%, 100%, 100%, 100%). Dehydrated samples were infiltrated with 50% (v/v) LR White resin in ethanol for 1 h and 100% LR White resin overnight before polymerisation at 60°C for 39 h. Sections with thickness of 1.5 µm were cut and viewed under a CLSM. Excitation was at 405 nm. Fluorescence protein was detected after passing through a 420 long passing filter, with a pinhole set at 55 µm.

2.4.1 Proteinase K digestion of marama bean and soya bean cotyledons

Tissue blocks (1 mm³) were cut from the inner surface of the cotyledons and digested with Proteinase K (Sigma P 2308) following the procedure of Mifflin and Burgess (1982) with modifications. Sectioned tissue blocks were incubated in Proteinase K (500 µg/ml) in 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM calcium chloride for 45 min at 37°C. Control samples were incubated in the same buffer without Proteinase K. Incubated tissue samples were then fixed in glutaraldehyde as described above and viewed with the TEM.

2.5 Statistical analyses

Chemical analyses were done on duplicate samples. Mean diameter of protein bodies and the distribution of protein bodies per cell (N = 50 cells) were determined. One way analysis of variance was conducted on proximate and mineral data and the means compared using Fishers Least Significant Difference Test.

3. RESULTS AND DISCUSSION

3.2 Chemical composition of marama bean

Marama *T. fassoglense* seemed to have higher protein content compared with marama *T. esculentum* and soya (Table 1). The protein contents of *T. esculentum* are within the range previously reported for this specie (Mmonatau, 2005; Amarteifio and Moholo, 1998). The protein contents of marama bean appeared to be high compared with peanuts (Wu, Lu, Jones, Mortley, Loretan, Bonsi and Hill, 1997) and other oilseeds such as hazelnut and macadamia nut (Venkatachalam and Sathe, 2006). Marama bean also contained almost twice as much fat as soya (Table 1). The fat content of marama beans is in agreement with previous reports (Amarteifio and Moholo, 1998; Ketshajwang, Holmback and Yeboah, 1998). Marama and soya had similar fibre contents. However, the total carbohydrate and ash contents were different among these legumes. The carbohydrates contents of marama from this study are lower than the values previously reported by Amarteifio and Moholo, (1998). Changes in chemical composition may occur in seed as a result of the changes in environmental conditions, type of soil and agricultural practices such as the use of fertiliser in growing areas.

Phosphorus, potassium, calcium, magnesium and sulphur were the major minerals in both species of marama and soya (Table 2). The concentration of the various mineral in marama is also in agreement with previous reports (Amarteifio and Moholo, 1998; Mmonatau, 2005). The mineral composition of marama bean species were also similar to mineral profiles reported for oilseed legumes such as peanut (Wu *et al.*, 1997) and soya bean (Garcia *et al.*, 1998).

Legume storage proteins are found in specialised organelles, the protein bodies (Lott, 1981). The protein bodies also act as compartments for mineral reserves in seed. These mineral reserves occur as phytate, which is stored in globoid inclusions within the protein bodies (Lott and Buttrose, 1978). The structure of protein bodies in marama was determined in comparison with soya using LM and TEM.

3.1 Microscopy of protein bodies in marama bean

Pre-fixed sections of marama and soya beans were stained with Coomassie Brilliant Blue and viewed by LM. The protein bodies appeared as distinct bodies, circular in cross-section and stained blue within the cells while the remaining space of the cytoplasm was not stained (Fig. 2). Coomassie Brilliant Blue dye is frequently used to stain protein as it binds to it via physical adsorption to arginine, aromatic amino acids and histidine (Hafiz, 2005). From LM, the parenchyma cells of marama bean contain circular organelles, protein bodies similar to soya bean.

With TEM, marama bean parenchyma cells showed circular, electron-dense protein bodies surrounded by lipid bodies (Fig. 3 A, B). The protein bodies were similar to those in the soya bean parenchyma cells (Fig. 3 C). Structurally, the protein bodies in legumes, including peanuts, hazelnuts and soya beans consist of homogenous proteinaceous matrix material surrounded by a network of lipid bodies (Lott and Buttrose, 1978; Young *et al.*, 2004). The organisational structure of marama protein bodies is therefore similar to microstructure of protein bodies in most legumes including soya.

The dimensions of marama protein bodies, which varied between 2-12 μm in diameter are similar to sizes reported for oilseed legumes, including soya bean (Martinez, 1979) and peanuts (Young *et al.*, 2004). Differences in protein body size and distribution within the parenchyma cells were observed between the two species of marama bean. The parenchyma cells of *T. esculentum* from both South Africa and Botswana seemed to contain more smaller sized protein bodies (16 ± 9 per cell; $4 \pm 2 \mu\text{m}$) per parenchyma cell compared with *T. fassoglense* (7 ± 4 per cell; protein body size: $7 \pm 4 \mu\text{m}$). Protein bodies of *T. esculentum* and soya bean seemed to be similar in terms of protein body size and distribution per cell. The variations in protein body size and number between *T. esculentum* and *T. fassoglense* may be related to species. The higher protein content of *fassoglense* (Table 1) may be related to the relatively larger size of its protein bodies.

TEM of marama protein bodies also showed the presence of globoid inclusions within its protein body (Fig. 3 D, E). It appeared that spherical globoids and druse crystals (Lott and Buttrose, 1978) were the two types of inclusion in

protein bodies of marama bean. Globoids constitute storage sites for seed phosphorus deposited as insoluble phytate in protein bodies (Martinez, 1979). Spherical globoids are the most common inclusion reported in protein bodies of legumes like peanuts (Young *et al.*, 2004), walnuts and hazelnuts (Lott and Buttrose, 1978). Marama bean is thus similar to most legumes in terms of spherical globoid inclusions in its protein bodies. Elemental composition analysis of spherical globoids from many legumes showed that they are rich sources of P, K, Ca and Mg (Lott and Buttrose, 1978; Lott, 1981). These minerals were found in high concentration in marama beans (Table 3), probably originated mainly from the globoid sites. Most soya protein bodies did not contain globoid inclusions. According to Prattley and Stanley (1982), phytic acid in soya is likely in the form of soluble protein-phytate salt with 10-15% of phytate specifically deposited in globoids in an insoluble form. The lack of globoids in protein bodies of soya may be attributed to the soluble form of its phytic acid. Druse crystals (one per protein body) were observed only in protein bodies from *T. esculentum* (Fig. 3 D). Druse crystals consist of cluster of small crystals in arrangement called a druse or rosette (Lott, 1981). This type of inclusion has been reported in some protein bodies of hazelnuts (Lott and Buttrose, 1978). The norm is that where druse crystal is found, only one occurs per protein body (Lott, 1981). Compared to soya, marama appeared to have a relatively higher calcium content (Table 2), which is probably due to druse crystals in its protein bodies (Fig 3). Druse crystals have been found to be rich in calcium (Lott and Buttrose, 1978). Thus, the mineral composition of marama bean seems to be related to the structure of its protein bodies, the organelle of protein storage.

Marama bean parenchyma cells showed clusters of spherical protein bodies with CLSM (Fig. 4). Small spherical bodies were also observed within the protein bodies that did not fluoresce. These bodies seemed to represent the globoid inclusions that were observed with TEM (Fig 3). CLSM of marama bean thus confirms that its protein bodies contain globoid inclusions.

To elucidate marama bean protein body structure, tissue sections were incubated with Proteinase K prior to fixation with glutaraldehyde. The protein bodies in marama and soya that were incubated in Tris-HCl buffer (pH 8) without Proteinase K maintained their structure and did not show any sign of digestion (Fig. 5 A, B, C), whereas protein bodies treated with Proteinase K (Fig. 5 D, E, F) showed disruption of the cell structure with digested protein bodies within the parenchyma cells. The TEM of the control samples (Fig. 5 A, B, C) seemed to be slightly different from the initial TEM (Fig. 3). This is possibly due to some components being leached out from the parenchyma cells during the incubation of sample in Proteinase K buffer. Proteinase K is a stable serine protease that digests native protein effectively (Ebeling, Hennrich, Klockow, Metz, Orth, Lang, 1974). This enzyme has been used to digest protein bodies in maize, wheat and peas (Miflin and Burgess, 1982). Treatment of marama with Proteinase K confirms that digested organelles are protein bodies. The outer layer of the protein bodies that resisted the digestion with Proteinase K are likely to be the protein body membranes (Fig. 5 D, E, F). Various organelles in the parenchyma cells including the protein bodies are membrane bound (Pusztai, Croy, Stewart, Watt, 1979). Protein body membranes mainly consist of phospholipids and glycosylated proteins (lectins) (Pusztai *et al.*, 1979; Hafiz, 2005). These

membrane proteins are insoluble and disruption of the membrane and extraction with organic solvents is required to solubilise them (Hafiz, 2005). The insoluble nature of protein body membranes reduces the interaction with the enzyme and the membranes will remain indigested within the cell. However, differences were observed in the manner in which protein bodies were digested within the parenchyma cells between marama and soya. Marama protein bodies were clearly digested from the inside to the outer part as compared to those of soya bean, which showed pockets of digestion throughout the cytoplasmic network (Fig 5 F). This difference in the manner of digestion between marama and soya beans may be due to differences in solubility behaviour of their proteins in Proteinase K buffer.

Protein and fat constituted major components of marama bean. The physical location of protein bodies relative to fat has been found to be similar to that of soya bean. These findings suggest that the extraction of marama protein may be done in the same way as soya. The presence of crystalline inclusion in marama bean, suggests that phytic acid in marama may be predominantly in an insoluble protein-phytate complex form. This may influence the protein nutrition by reducing its digestibility when compared with soya in which the phytic acid is likely in the form of soluble protein-phytate (Prattley and Stanley, 1982). It may be necessary to determine the elemental composition of these inclusions in marama protein for further characterisation.

4. CONCLUSIONS

Marama beans are rich in protein and fat. Potassium, phosphorus, calcium magnesium and sulphur are the major minerals in marama beans. The protein body structure of marama is similar to soya in terms of spherical shape and localisation within the parenchyma cells. However, marama bean protein bodies contain spherical globoid and druse crystal inclusions. Due to similarity in seed microstructure, marama protein can be extracted in the same way as soya protein.

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REFERENCES

- *Aguilera, J. M. (2005). Why food microstructure. *Journal of Food Engineering* 67, 3–11.
- Aguilera, J. M., & Stanley, D. W. (1999). Microstructure principles of food processing and engineering (2nd ed.) (pp. 259-310). Gaithersburg, MD: Aspen Publishers, Inc.
- *Amarteifio, J. A., & Moholo, D. (1998). The chemical composition of four legumes consumed in Botswana. *Journal of Food Composition and Analysis*, 11(4), 329-332.
- AOAC (1984). *Official Methods of Analysis* (14th ed.). Arlington, VA: Association of Official Analytical Chemists.

- AOAC (2000). *Official Methods of Analysis* (17th ed.). Rockville, Maryland: Association of Official Analytical Chemists.
- Bidlingmeyer, B. A., Cohen A. S., & Tarvin L. T. (1984). The PICO-TAG method for amino acid determination. *Journal of Chromatography*, 336(1) 93-104.
- Coetzer, L. A., & Ross, J. H. (1976). Tylosema. *Tree in South Africa*, 28, 77-80.
- Ebeling, W., Hennrich, N., Klockow, M., Metz, H., Orth, D. H., & Lang, H. (1974). Proteinase K. from *Tritirachium album* Limber. *European Journal of Biochemistry*, 47(1), 91-97.
- Gahan, B. P. (1984). Qualitative analysis. In B. P. Gahan. *Plant histochemistry and cytochemistry: An introduction*. Experimental botany (pp 201-243). London: Academic Press.
- Garcia, M. C., Marina, L. M., Laborda, F., & Torre, M. (1998). Chemical characterisation of commercial soybean products. *Food Chemistry*, 62(3), 325-331.
- Hafiz, A., (2005). Principle and reaction of protein extraction, purification and characterisation (pp 84-89). Boca Raton: CRC Press.
- Keegan, A. B., & Van, Staden, J. (1981). Morama bean *Tylosema esculentum*, a plant worthy of cultivation. *South African Journal of science*, 77, 387.
- Ketshajwang, K. K., Holmback, J., & Yeboah, S. O. (1998). Quality and compositional studies of some edible leguminosae seed oil in Botswana. *Journal of the American Oil Chemist's Society*, 75(6), 741-743.
- *Lott, J. N. A., & Buttrose, M. S. (1978). Location of reserves of mineral element in seed protein bodies: macadamia, walnut, and hazel nut. *Canadian Journal of Botany*, 56, 2072-2082.

- Lott, J. N. A. (1981). Protein bodies in seeds. *Nordic Journal of Botany*, 1(3), 421-432.
- Martinez, H. W. (1979). The importance of functionality of vegetable protein in food. In H. Wilck, T. D. Hokpin & H. D. Waggle. Soy protein and human nutrition (pp. 53-77). London: Academic Press.
- Mifflin, B., & Burgess, R. S. (1982). Protein bodies from developing seed of barley, maize, wheat and peas. *Journal of Experimental Botany*, 33(133), 251-260.
- Mmonatau, Y. (2005). Flour from the marama bean: composition and sensory properties in Botswana perspective. (MS thesis), Stellenbosch University, Stellenbosch, South Africa.
- *Prattley, C. A., & Stanley, D. W. (1982). Protein-Phytate interactions in soybean. I. Localisation of phytate in protein bodies and globoids. *Journal of Food Biochemistry*, 6(4) 243-253.
- Pusztai, A., Croy, R. R. D., Stewart, J. C., & Watt, W. B. (1979). Protein body membranes of *Phaseolus vulgaris* L. cotyledons: Isolation and preliminary characterization of constituent proteins. *New Phytologist*, 83, 371-378.
- Shand, P. J., Ya, H., Pietrasik, Z., & Wanasundara, P.K.J.P.D. (2007). Physicochemical and textural properties of heat-induced pea protein isolate gel. *Food Chemistry*, 102, 119-130.
- Shepherd, A. D. (1979). Laboratory abrasive decorticating mill for small grains. *Cereal Chemistry*, 56, 545-548.

Venkatachalam, M., & Sathe, S. (2006). Chemical composition of selected edible nut seeds. *Journal of Agricultural and Food Chemistry*, 54(13) 4705-4714.

Wu, W. H., Lu, J. Y., Jones, A. R., Mortley, D. G., Loretan, P. A., Bonsi, C. K., & Hill, W. A. (1997). Proximate composition, amino acid profile, fatty acid composition, and mineral content of peanut seeds hydroponically grown at elevated CO₂ levels. *Journal of Agriculture and Food Chemistry*, 45(10) 3863-3866.

*Young, T. C. T., Pattee, E. H., Schadel, E. W., & Sanders, H. T. (2004). Microstructure of peanut (*Arachis hypogaea* L. cv 'NC 7') cotyledons during development. *Lebensmittel-Wissenschaft und-Technologie*, 37(4) 439-445.

* These are key references that were useful in explaining (1) the importance of seed microstructure in food processing and protein utilisation (2) identifying the knowledge gap with respect to our samples and comparing our experimental results to existing literature. These references were used to elucidate the localisation of protein bodies, storage protein organelles and lipid bodies in marama bean.

Table 1.

Proximate composition of marama bean and soya bean (g/100 g)¹

¹ Mean \pm SD

¹ Mean values with different superscript letters in columns are significantly

Samples	Moisture	Protein	Fat	Crude fibre	Ash	Total ²
						carbohydrates
<i>T. esculentum</i> (SA)	7.0 ^b \pm 0.2	30.6 ^a \pm 0.2	32.9 ^c \pm 0.5	7.9 ^a \pm 0.1	3.3 ^a \pm 0.1	18.3 ^a \pm 0.7
<i>T. esculentum</i> (BW)	5.1 ^a \pm 0.7	30.6 ^a \pm 0.3	36.2 ^d \pm 0.3	8.1 ^a \pm 0.2	3.2 ^a \pm 0.7	16.8 ^a \pm 0.3
<i>T. fassoglense</i> (SA)	7.1 ^b \pm 0.1	37.3 ^c \pm 0.2	29.2 ^b \pm 0.7	8.4 ^a \pm 0.2	3.4 ^a \pm 0.4	14.9 ^a \pm 0.5
Soya bean	7.9 ^b \pm 0.8	32.5 ^b \pm 0.2	14.9 ^a \pm 0.1	8.6 ^a \pm 0.2	4.8 ^b \pm 0.7	31.3 ^b \pm 1.3

different ($p < 0.05$).

SA: South Africa, BW: Botswana.

²Total carbohydrate by difference.

Table 2.

Mineral composition of marama bean and soya bean (mg/100 g flour)¹

Elements	<i>T. esculentum</i>	<i>T. esculentum</i>	<i>T. fassoglense</i>	<i>Soya bean</i>
	(SA)	(BW)	(SA)	
P	1065 ^b ± 108	796 ^a ± 82	1123 ^c ± 100	1199 ^d ± 130
Ca	157 ^b ± 13	200 ^c ± 6	156 ^b ± 19	133 ^a ± 2
K	634 ^b ± 14	500 ^a ± 51	615 ^b ± 16	1159 ^c ± 73
Mg	358 ^b ± 26	319 ^b ± 12	402 ^b ± 29	272 ^a ± 10
S	297 ^a ± 37	270 ^a ± 60	340 ^a ± 45	409 ^b ± 50
Na	6.0 ^c ± 0.5	3.4 ^a ± 1.1	6.9 ^c ± 0.2	4.8 ^b ± 2.1
Cu	1.8 ^a ± 0.2	0.4 ^a ± 0.2	0.9 ^a ± 0.1	0.6 ^a ± 0.2
Fe	1.7 ^a ± 1.6	1.3 ^a ± 1.0	1.4 ± 1.1 ^a	4.2 ^a ± 2.2
Mn	2.9 ^a ± 1.0	2.1 ^a ± 2.0	1.3 ^a ± 0.9	1.7 ^a ± 1.1
Zn	3.7 ^a ± 0.7	3.2 ^a ± 0.5	4.1 ^a ± 1.0	4.4 ^a ± 0.8

¹ Mean ± SD is reported on a dry matter basis.

Mean values with different superscript letters in rows are significantly different ($p < 0.05$).

SA: South Africa, BW: Botswana

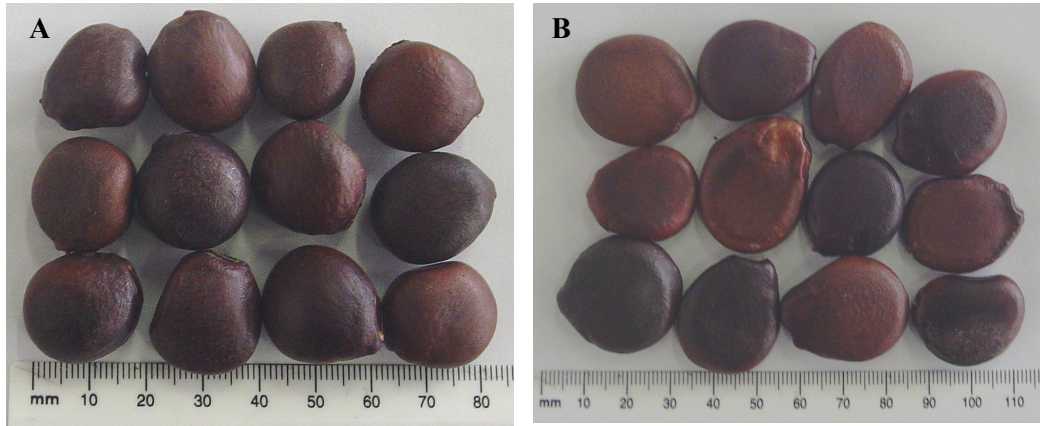


Fig. 1. Marama bean *T. esculentum* (A) and *T. fassoglense* (B)

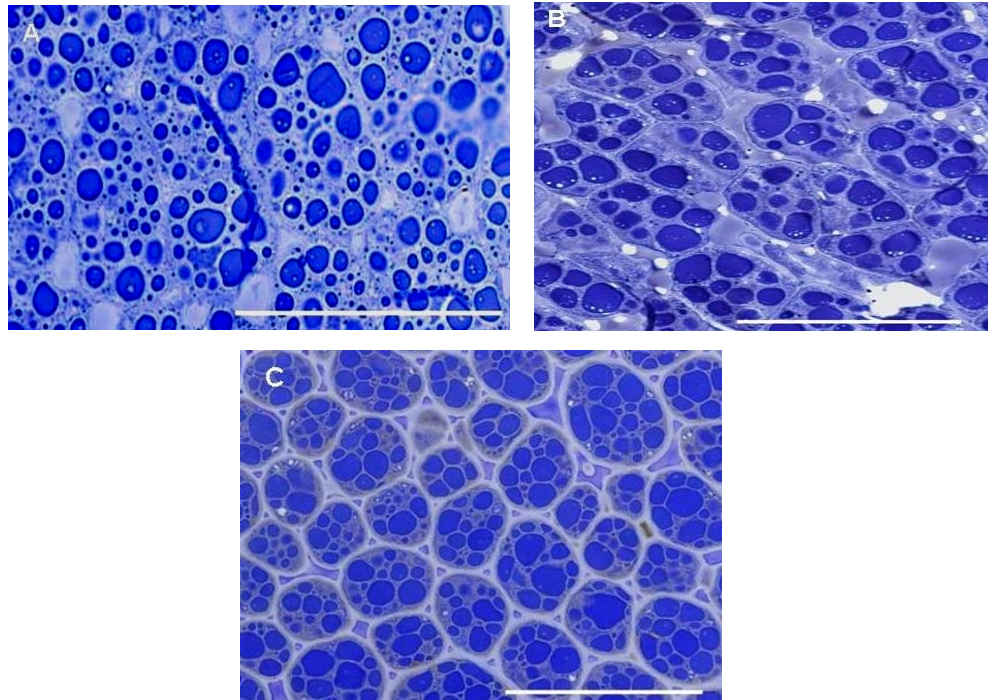


Fig. 2. Light microscopy of marama and soya bean parenchyma cells stained with Coomassie Brilliant Blue R 250. A: Marama bean (*T. esculentum*) from Botswana; B: Marama bean (*T. fassoglense*) from South Africa, and C: Soya bean. Bar: 100 μ m. Protein bodies are stained blue in parenchyma cells.

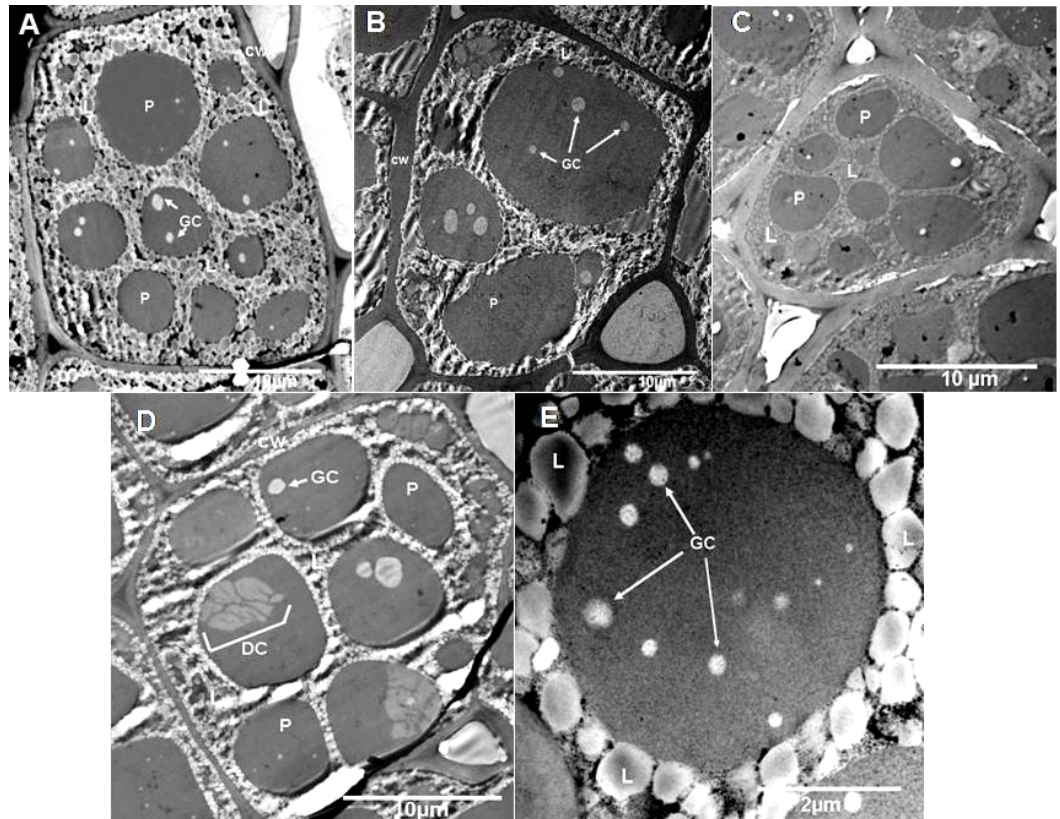


Fig. 3. Transmission electron microscopy of protein bodies in marama and soya bean parenchyma cells. A: Marama bean (*T. esculentum*) from Botswana; B: Marama bean (*T. fassoglense*) from South Africa; C: Soya bean; D: Marama bean (*T. esculentum*) from Botswana with druse crystal (DC) type of inclusion in protein body; E: Protein body of marama bean (*T. esculentum*) from South Africa with spherical globoid crystals (GC); P: Protein bodies; L: Lipid bodies.

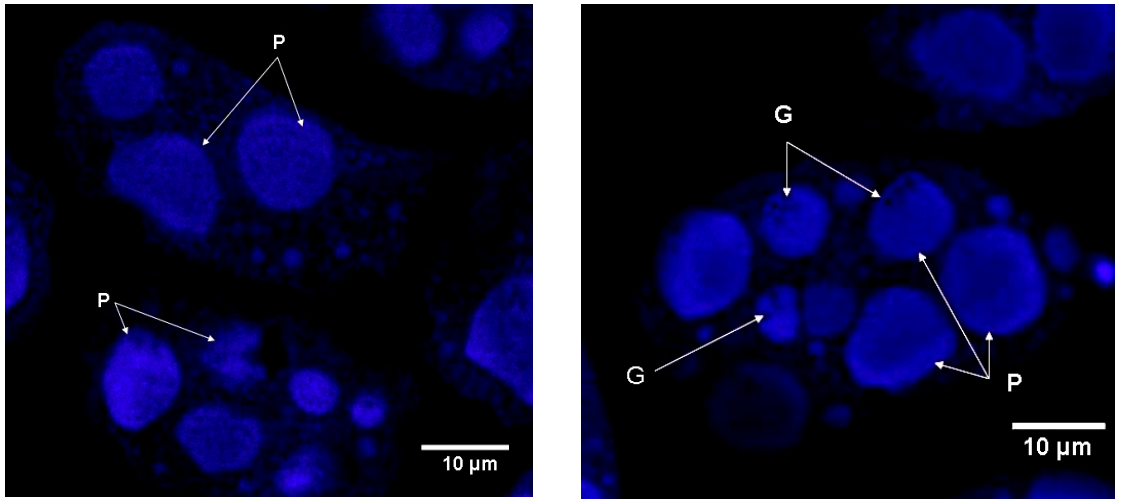


Fig. 4. Confocal laser scanning microscopy of protein bodies in marama bean;

P. protein bodies, G: Globoids.

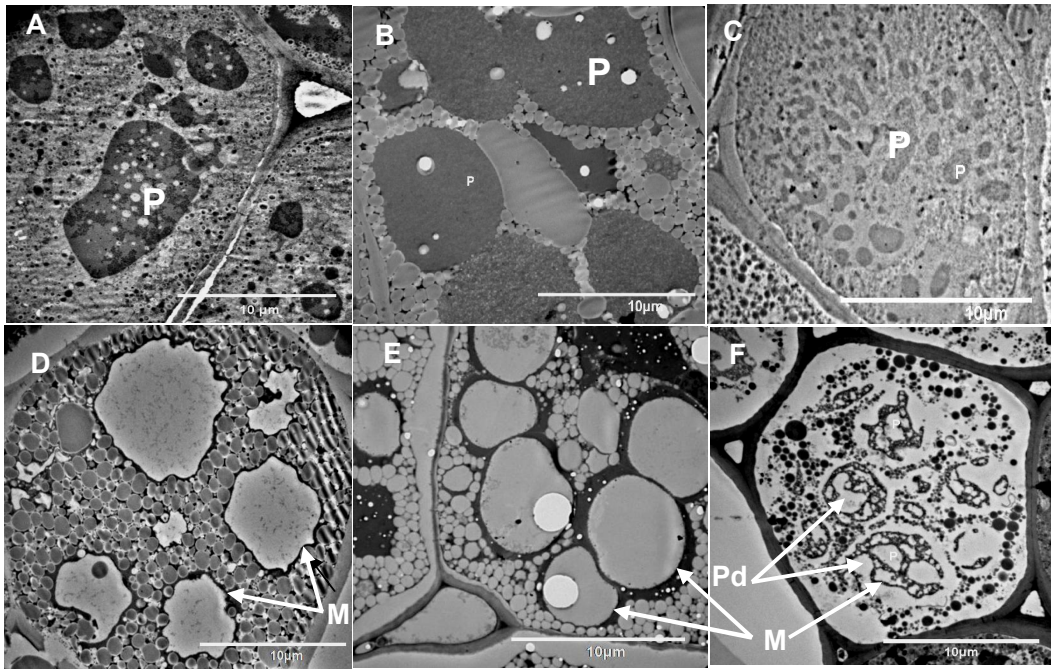


Fig. 5. Transmission electron microscopy of marama and soya beans protein bodies treated with Proteinase K. A & D: Marama bean (*T. esculentum*); B & E: Marama bean (*T. fassoglense*), C & F: Soya bean; P: protein bodies, M: Protein body membranes, Pd: Pockets of digestion; A, B & C: untreated.