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Seed Protein and Starch Qualities of Drought Tolerant Pigeonpea and Native Tepary Beans

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Abstract

Tepary bean and pigeonpea were highly drought tolerant food legumes and were profitable in arid regions with sustainable productivity. Protein and starch, major constituents of legume grain, were used in various forms for human and animal consumption. Therefore, present investigation was carried out with an objective to evaluate protein content and various factors influencing qualities of starch in pigeonpea and tepary bean. Significant differences ($p < 0.05$) were observed for protein and starch qualities among the two crops and between the drought responsive cultivars of these crops. The protein content of pigeon pea (22.5-26.2 mg/100 mg) was high compared to tepary bean (17 to 26 mg/100 mg) seed. The resistant starch content of pure starch extracted from drought responsive cultivars varied from 1.09 (TB #7) to 8.75 (TB #31) in teparybean and 14.68% (G2) - 21.35 % (W1) in pigeonpea. Amylose content of pigeonpea varied from 7.76 to 12.31% and the degree of crystallinity were 13.42 - 19.30% with X-ray diffraction pattern of the C-type. The pigeonpea starch granules were large, oval to ellipsoidal shape with a smooth surface and mean granule size of 47.6 µm in length X 36.4 µm in width. The transition temperatures and enthalpy of gelatinization ranged from 67.25-68.29 °C (T_o); 71.4-72.49 °C (T_p) and 77.04-78.51 °C (T_c) and 4.80-6.43 J/g respectively. Amylose content of tepary starch varied from 8.08 to 12.38% and the degree of crystallinity were 13.52-18.29% with X-ray diffraction pattern of the C-type. The tepary bean starch granules were round to oval shape with a smooth surface and mean granule size of 39.26 µm in length X30.36 µm in width. The transition temperatures and enthalpy of gelatinization ranged from 73.12-74.65 °C (T_o); 77.71-78.85 °C (T_p); 82.65-84.29 °C (T_c) and 2.21-5.27 J/g respectively. Pigeon pea starches were with low molecular weights of amylopectin (5.26×10^8 Da) and high molecular weights of amylose (4.63×10^5 Da) compared to those in tepary bean (13.6×10^8; 1.35×10^5) on an average. In both crops, the gelatinization showed significant correlation with amylose content, crystallinity and granule size. Pigeonpea grain was superior with quality starch and protein with adequate amounts of digestible fiber without any reduction in quality during extraction of starch compared to tepary bean. Black seed coated tepary had lowest gelatinization energy compared to brown and white. The drought tolerant cultivars identified in teparybean (TB #24) and Pigeonpea (W1) with high resistant starch and protein content will be useful in selection for crop production, developing homozygous line for industrial use in southern USA.

Keywords: cultivars, resistant starch, amylose, amylopectin, crystallinity, granule size and shape, physiochemical properties

1. Introduction

Drought is the major constraint for agricultural production in USA. The severe impact of drought lead to reduced food grain quality, production and consumption. Food legumes like pigeonpea (4000 kg/ha) and tepary bean (2239 kg/ha) are ancient drought tolerant globally known resources for plant protein and carbohydrates (Singh et al., 2004). Pigeonpea was in cultivation in Asia and Africa for food, feed, forage and fuel (Kassa et al., 2012) and tepary bean, native to southwestern USA (Federici et al., 1990) was used as food (hummus),feed and forage (Bhardwaj et al., 2013).

Food legume grain is an alternative supplement for patients suffering from cancer, diabetes, obesity and bone related health disorders and was proved to be the best choice for human consumption with high amylose content compared to amylopectin (FAO, 1998; Sajilata et al., 2006). Nutritional potential of tepary bean (Gonzalez de mejia et al., 1998) was revealed with high amount of protein (24.5%), minerals (Bhardwaj & Hamama, 2004), resistant starch (Abbas & Berry, 1986; Abbas et al., 1987), high levels of antioxidant enzymes (Turkan et al., 2004), and anti-cancerous properties (Valadez-vega et al., 2011).

Pigeonpea cultivars were observed with good quality resistant starch (Narina et al., 2012), protein rich in sulfur containing amino acids (Singh & Eggum, 1984; Pathak, 1993), cajanol (Luo et al., 2010), several minerals and with quality forage (Cantrell et al., 2001) with medicinal (Pal et al., 2011) and antimicrobial properties for healthy ruminant nutrition (Mahala et al., 2012) globally and in USA (Sloan, 2012). Pigeonpea seed has 36.5% protein (Pathak et al., 1993) with excellent water retention (250.3 ml/100g), fat absorption (130 ml/100g), emulsification (120%) and foaming (130%) capacities (Eltayeb et al., 2012).

These two crops gaining pride for cultivation in southeastern USA (Pathak et al., 1993, Bhardwaj et al., 2002) due to their adaptability, and production capabilities and were potential alternative crop resources (Bhardwaj et al., 1999) for tobacco farmers and drought prone areas of USA (Narina et al., 2013) and as forage crops for animal consumption (Bhardwaj, 2013). Further, these legumes were also potential for industrial use in production of biodiesel, forage, biodegradable substances like plastics, oils, gums, and medicines besides bread, chips, tortillas, yogurt, and flavor (Graham & Vance, 2003). Resistant starch, an indigestible component of starch, improves the fiber content, crispiness, expansion in snacks and various food products. Reports of Ho et al. (2011) stated that a low carbohydrate and high protein diet reduce the tumor growth and prevent cancer initiation. Therefore, legume nutrition is a natural resource for combating several health ailments besides securing food to poor families in the tropics.

The main objective of the present study is to identify the drought tolerant cultivars with high protein content and superior quality seed starch with high amylose/amylopectin ratio which is positively correlated with high resistant starch. Neither potential cultivars nor the information on starch qualities of high yielding and drought tolerant cultivars of pigeonpea and tepary bean were available to date for crop improvement purposes. Drought responsive cultivars selected from core germplasm of pigeonpea and tepary bean during 2011-13 at VSU were evaluated for protein content, proximate composition, physiochemical and functional qualities of starch to select the potential cultivars in these two crops to use in ongoing breeding trials for nutritional quality.

2. Materials and Methods

2.1 Source of Seed and Chemicals Used

The pigeonpea and tepary bean seed were grown at Randolph Farm of Virginia State University (VSU) and the seed was harvested during summer 2012. Chemicals used in the present investigation were purchased from Fisher Scientific (Pittsburgh, PA, USA) and Sigma Aldrich (St. Louis, MO, USA).

2.2 Protein Extraction

Total proteins were extracted by adding 100mg of freeze dried whole ground seed powder in 1 ml of 50mMTris-Hcl (pH 7.5) and 0.1N NaoH (pH 12.8) at 4 ^{o}C for 60 minutes followed by centrifugation at 10,000 g for 15 minutes (Miller et al., 1972). Proteins were determined by Bradford's method (Bradford, 1976).

2.3 Starch Extraction

The seed was ground to fine flour for analysis of resistant starch (RS) in both tepary bean and four pigeon pea cultivars to select the cultivars for pure starch extraction (Narina et al., 2012). The ground seed samples with low, medium and high RS including their drought tolerance were selected for pure starch extraction in tepary bean. The seed samples of four selected cultivars with drought tolerance were used in pigeonpea. A total of 20 seed samples were processed for isolation of starch using the protocol standardized for food legume crops (Xu et al., 2012, 2013) with slight modification during initial soaking step. The seeds with white/light color seed coat were soaked overnight (12 hr) in warm water at room temperature to soften seed coat with a 3:1 (v/v) water to seed ratio. The cultivars with colored seed coat required an additional 12 hours soaking time in water containing 0.2% (w/v) SO_2 at 50 ^{o}C. The peeled seeds (without seed coats) were ground to fine paste using a ratio of 1:1:3 (v/v) seed to ice to water in a blender for 1 minute at high speed. The starch slurry was sieved through nylon mesh and cheese cloth. The filtrate was allowed to settle for 8 hours to collect the starch particles gravimetrically. The isolated pure starch was dried in oven at 40 ^{o}C and ground to pass through 40 mm sieve to analyze the nutritional composition.

2.4 Quality of Starch

A total of 20 pure starch samples in triplicates were analyzed for moisture%, crude protein, oil and ash using the standard methods in core laboratory as described by Association of Official Analytical chemists (AOAC, 2000). Crude protein was calculated by multiplying the nitrogen concentration with a factor of 6.25 and the concentration of nitrogen was measured by combustion method (AOAC, 2000).

2.5 Physicochemical properties

After confirming the good quality, pure starch was assayed for following properties.

1) *Resistant Starch* (%) was analyzed in terms of glucose using assay procedure as described by Megazyme's K-STAR kit (Megazyme International, Ireland Ltd.,) using Amylo-glycosidase and α-amylase enzymes. The sample absorbance was measured at 510 nm for glucose concentration.

2) *Amylose content* (%) was determined as per the procedure described by Xu et al., 2013 by mixing 20 mg starch and 8ml DMSO (Dimethyl sulfoxide) on vertex for 5 minutes followed by incubation in hot water bath at 85 ^{o}C for 15 minutes. The heated starch solution was allowed to cool to room temperature, and the final volume was made up to 100 ml using volumetric flasks with distilled water. An Aliquot (1.0 ml) of starch solution pipetted into a new 50 ml volumetric flask and 5 ml of iodine solution was added and made the volume to 50 ml. The absorbance was measured at 600 nm.

3) *Water binding capacity* (WBC) was measured (Sandhu & Singh, 2007; Xu et al., 2013) for 5 gm starch (dry weight) in 75 ml water. The starch solution was agitated for one hour using orbital shaker followed by centrifugation for 3000 g for 10 minutes. The free water was removed and the wet starch was drained for 10 min and weighed. The WBC is calculated as% = {(weight of wet starch - weight of dry starch) / weight of dry starch} × 100.

4) *Functional properties*

A) Thermal Properties were studied using TA 2000 differential scanning calorimeter (DSC: TA instruments, New Castle, DE, USA) following previously standardized protocols for legume starch analysis (Singh et al., 2004 and Xu et al., 2013). The onset (T_o), peak (T_o) and conclusion (T_p) temperatures and gelatinization ranges (ΔH) and enthalpy (ΔT) for the gelatinization were determined for hermetically sealed pans containing starch suspensions with 70% moisture by subjecting to 30-100 ^{o}C at 10 ^{o}C.

B) Morphological structure of the starch sample was analyzed placing it on a double sided pelco Tabs (12 mm OD) mounted on aluminum specimen studs followed by gold palladium coating for 90 seconds to a thickness of 30 nm. The granule size of the coated samples was determined at an accelerating potential of 5kv using scanning electron microscope (SU-70, Hitachi, Tokyo, Japan) following the standard procedures for starch granule size and shape determinations (Li et al., 2011, Xu et al., 2013).

C) Crystallinity (%) was analyzed by X - Ray diffractometer (Panalytical B.V., Almelo, and the Netherlands) following standard method described for chickpea (Xu et al., 2013) and quantitatively estimated (Nara & Komiy, 1983) by VCU core services for morphological structure and crystallinity.

D) Molecular weight distribution of the selected lines from drought tolerance evaluations were measured for amylose and amylopectin (Ratanayake & Jackson, 2009). Briefly, the dispersed (10ml, 90% DMSO) starch samples were kept on a multi-tube rotator (Model: 4632Q, Thermo scientific, Madison, WI, USA) to shake at 30 rpm at room temperature. The dispersed sample was filtered through and was injected to HPSEC system with size exclusion columns and degassed distilled water was used as mobile phase at 1ml / min flow rate. Pullulan standards were used to create standard curve. The molecular weights of samples were calculated (R^2 = 0.9983) using the equation MW = 10 - 0.2905RT + 14.759, where RT is retention time.

All the data was analyzed statistically using Excel 2010 and SAS 9.3 version statistical analysis software (SAS Institute Inc., Cary, NC) for ANOVA, correlations and DUNCAN multiple range test. The R^2 values were ranging from 0.96 - 0.99 for all the traits measured for data analyzed.

3. Results and Discussion

3.1 Starch Properties

Starch quality was observed pure with very low contents of crude protein, fiber, oil and ash (Table 1). Significant differences were observed for all the physiochemical properties and protein content studied between two crops and among cultivars with in each crop (R^2 = 0.97 - 0.99; 0.05 > probability < 0.01).

Table 1. Quality of starches isolated from tepary bean and pigeonpea for proximate composition (% Dry weight basis)

Cultivar	Moisture (%)	Crude Protein (%) (NX6.25)	Crude oil (%)	Ash (%)
Tepary bean	9.01±0.12	0.64±0.17	0.09±0.04	0.00±0.00
Pigeonpea	9.59±0.15	1.04±0.52	0.12±0.01	0.07±0.08

Data was mean ± standard deviation (n = 3) from drought responsive cultivars of tepary bean and pigeonpea.

3.2 Variability Among Crops

The protein content, total starch (TS) and resistant starch (RS) contents of pigeonpea were significantly ($p < 0.05$) superior compared to tepary bean (Table 2). The RS content was relatively stable in whole ground seed and pure starch extracted from pigeon pea. The RS content was reduced four times in tepary bean pure starch (Table 3) from that of whole ground seed (Table 4). The reason could be attributed to removal of seed coat for starch extraction from all the cultivars in two crops. The reason for severe reduction in mean RS content of tepary bean could be attributed to the incubation step (at 50 ^{o}C overnight) during starch extraction procedure followed for cultivars with colored seed coat. It was supported by previous observations (Abbas et al., 1987) as the RS content decreases at increased temperatures during processing (> 37 ^{o}C). Tepary starch was sensitive to temperature which explains the relatively low amylose/amylopectin values in tepary and high content of hydrolysable starch (Tables 2, 3).

Table 2. Comparison of extracted starch qualities and protein content in seed obtained from drought tolerant food legume crops, pigeonpea and tepary bean

Cultivar	Protein (%)	Resistant Starch (%)	Total Starch (%)	Particle length (μm)	Particle width (μm)	Molecular Weight (Da)		Crystallinity (%)
						Amylopectin ($\times10^8$)	Amylose ($\times10^5$)	
Tepary bean	22.17±0.27	4.64 ±0.55	60.±0.08	39.26±5.70	30.36±3.54	13.6±1.34	1.66±0.22	16.16±5.35
Pigeon pea	24.74±0.02	13.76±3.2	67.99±3.5	47.6±5.4	36.4±4.7	5.26±0.72	4.63±1.07	16.19±6.05

Data was mean ± standard deviation (n = 3) from drought responsive cultivars of tepary bean and pigeonpea.

Table 3. Cultivar variability in two food legumes for protein content and pure starch qualities

Cultivar	Protein (%)	Resistant Starch (%)	Total Starch (%)	Amylose (%)	WBC (%)
TB1	18.76± 0.24	8.05±0.00	68.73±0.13	8.48±0.01	82.7±10.6
TB2	21.61±0.27	6.60±0.61	69.02±0.18	11.28±0.00	97.3±1.5
TB3	22.89±0.39	2.30±0.24	91.36±0.02	12.38±0.01	74.7±13.5
TB4	25.35±0.19	3.13±0.47	64.53±0.05	9.46±0.00	68.0±25.9
TB7	21.20±0.13	1.09±0.47	60.94±0.09	10.45±0.01	69.7±19.1
TB15	18.93±0.38	2.45±0.71	57.18±0.09	11.20±0.01	74.3±19.1
TB18	25.27±0.19	2.99±0.47	64.19±0.40	9.62±0.00	71.0±13.5
TB23	21.18±0.29	6.76±0.02	48.42±0.13	8.08±0.01	67.3±10.3
TB24	26.26±0.32	4.79±2.37	52.09±0.11	9.53±0.00	67.3±1.30
TB29	24.81±0.63	5.03±0.02	33.43±0.06	9.54±0.01	71.5±13.0
TB30	22.55±0.19	3.70±0.71	66.36±0.09	9.60±0.00	72.7±12.7
TB31	17.19±0.06	8.74±0.47	50.91±0.91	9.07±0.00	80.7±16.2
G1	26.26±0.02	18.17±4.5	70.15±1.79	7.76±0.01	65.83±0.24
G2	24.81±0.32	10.80±1.33	71.12±1.08	10.95±0.01	65.33±0.00
W1	22.55±0.82	14.47±3.73	67.13±2.93	11.06±0.02	66.33±0.00
W3	25.35±0.78	11.62±3.39	63.55±8.14	12.31±0.02	77.17±0.71

The values were the averages ± standard deviation from three replications. The alphabet a & b indicates significance difference at probability < or = to 0.0. Same letter indicate no difference and different letter indicate significant difference between the varieties; Column 1: TB1 to TB 31 were teparybean cultivars, G1, G2, W1, W3 were pigeonpea cultivars.

Size of the starch granule (both length and width) was larger in pigeonpea compared to tepary bean (Table 2, Figures 1a-1d and 2a-2d). The tepary bean starch granules were round to oval shape with a smooth surface (Figures 1a-1d) and mean granule size of 39.26 µm in length X30.36 µm in width. The transition temperatures and enthalpy of gelatinization ranged from 73.12-74.65 °C (T_o); 77.71-78.85 °C (T_p); 82.65-84.29 °C (T_c) and 2.21-5.27 J/g respectively. The pigeonpea starch granules were large, oval to ellipsoidal shape with a smooth surface and mean granule size of 47.6 µm in length X 36.4 µm in width. The transition temperatures and enthalpy of gelatinization ranged from 67.25 - 68.29 °C (T_o); 71.4-72.49 °C (T_p) and 77.04-78.51 °C (T_c) and 4.80-6.43 J/g respectively.

Pigeon pea starches were with low molecular weights of amylopectin (5.26×10^8 Da) and high molecular weights of amylose (4.63×10^5 Da) compared to those in tepary bean (13.6×10^8; 1.35×10^5) on an average. There was non-significant ($p > 0.05$) difference between pigeon pea and tepary bean for crystallinity with high values in pigeon pea. The amylopectin content was high in tepary bean compared to pigeon pea while amylose was high in pigeon pea compared to tepary bean which indicates that pigeonpea starch was qualitative for the use in production of RS for food preparations and for diabetic purposes.

3.3 Cultivar Variation for Resistant Starch

The data on starch quality for RS, TS and amylose contents of extracted pure starch (Table 3) and whole ground seed powder (Table 4) for teparybean and pigeonpea was discussed below.

3.3.1 Tepary Bean

The per cent RS in ground seed powder varied from 12.81 (TB #2) to 21.01 (TB #16). The per cent TS in whole ground seed was ranging from 24.27 (TB #15) to 45.01 (TB #6). Out of 31 native cultivars tested initially for RS from ground seed powder, we selected only 2, 3, 4, 10 as low (20-30% RS), 7, 18, 21, 24, 27 as medium (30-40 % RS) and 1, 26, 30, 31 and 15 as high (> 40% RS) based on the per cent contribution of RS from TS (Table 4) and were also drought responsive. It was also observed that most of the brown, tan or black seed coated cultivars with relatively high per cent RS compared to white seed coated cultivars with few exceptions in cultivars TB #13 and TB #17. The reason for high RS content in colored seed coat cultivars was due to the high amount of enzyme inhibitors like polyphenols as reported previously in black bean (Moron et al., 1989). Colored teparies were observed with high tannins and other polyphenolic compounds (Blair et al., 2010; Narina et al., 2013 unpublished).

Table 4. Total and resistant starch contents from whole ground seed powder of native teparybean and pigeonpea Cultivars

Cultivar	Seed coat color	Resistant Starch (%)	Total Starch (%)
TB1	White	15.85±0.09	43.29±0.02
TB2	White	12.81±0.47	38.15±0.45
TB3	White	13.92±0.63	36.03±0.64
TB4	Brown	13.91±0.04	29.69±0.04
TB5	Brown	14.34±0.78	33.55±0.76
TB6	Brown	22.07±1.5	45.01±1.4
TB7	Speckled tan	15.51±0.80	38.67±0.82
TB8	Brown	16.31±0.17	34.91±0.18
TB9	Brown Speckled	16.32±0.06	35.54±0.03
TB10	Brown	17.59±0.39	32.84±0.39
TB11	Brown	15.33±0.52	32.06±0.52
TB12	Black	19.61±0.39	38.64±0.37
TB13	White	19.95±0.24	38.56±0.25
TB14	Coffee Brown	19.02±0.09	34.22±0.10
TB15	Brown	15.35±0.68	24.27±0.69
TB16	Tan	21.01±0.42	35.63±0.35
TB17	White	19.14±0.16	38.78±0.15
TB18	Black	16.87±0.07	35.93±0.05

TB19	White	14.09±0.29	32.85±0.29
TB20	Black	14.81±0.30	33.38±0.28
TB21	White	15.49±0.88	34.48±0.89
TB22	White	14.35±0.73	35.62±0.75
TB23	White	19.32±0.03	40.26±0.02
TB24	Brown	19.32±0.0	35.63±0.04
TB25	White	14.73±0.46	31.71±0.47
TB26	White	18.20±0.74	40.71±0.71
TB27	White	16.63±0.08	38.88±0.02
TB28	Brown	18.24±0.01	33.44±0.09
TB29	Brown	18.27±0.02	34.44±0.02
TB30	White	20.38±0.43	39.62±0.43
TB31	White	17.37±0.26	38.28±0.38
S6	White	1.31±0.06	6.48±0.05
G1	Red	17.91±0.12	23.11±0.10
G2	Red	14.68±0.13	19.83±0.01
W1	White	21.35±0.34	27.95±0.30
W3	White	15.03±0.60	22.15±0.23

Column 1: TB1-TB31 indicates Tepary bean cultivars, G1, G2, W1, W3 indicate Pigeonpea cultivars, S6 is Control Soybean Cv Carter; The values were the averages ± standard deviation from three replications at significance difference p < or = to 0.05.

The values of RS and amylose contents were highly influenced by cultivar and crop, though the extraction procedure slightly changed the values of pure starch in color seed coated cultivars (Table 3). It was also observed that the RS content of whole ground seed powder from drought tolerant cultivar of soybean (S6) was less than pigeon pea and tepary bean (Table 4).

The RS content of pure starch extracted from drought responsive cultivars varied from 1.09 (TB #7) to 8.75 (TB #31).The amylose content of pure starch was ranging from 8.08% (TB #23) to 12.38% (TB #3) in tepary bean. The RS values of pure starches were low compared to those in whole ground seed powder and were not in line with the reported valuesof increased resistant starch content in starch obtained from peeled chickpea by Xu et al. (2012). The present observations of reduced values of RS were supported by similar observation in teparybean (Abbas et al., 1987) and were due to differences in granule structure, crystallinity and amylose content and due to increased enzyme availability.

3.3.2 Pigeonpea

The TS and RS contents of whole ground seed powder was high in W1 (27.95%; 21.35%) and low in G2 (19.83%; 14.68%) respectively (Table 4). The pure starch of pigeonpea was observed (Table 3) with significantly high RS in cultivar G1 (18.17%) followed by W1 (14.47%) with high TS contents (70-71%) in G1 and G2 compared to W1 and W2 (63-67%). The amylose content was high in W1 and W2 (11-12.3%) compared to G1 and G2 (7.8-11%) in pigeonpea. The values of ground seed powder were close to the previous results obtained in food legumes (Narina et al., 2012; Xu et al., 2012). The starches observed in pigeonpea and tepary bean were C-type and were entirely resistant to digestion by pancreatic amylases with resistant starch type III (RS3) as explained by Asp and Bjorck (1992).

3.4 Cultivar Variation for Protein Content

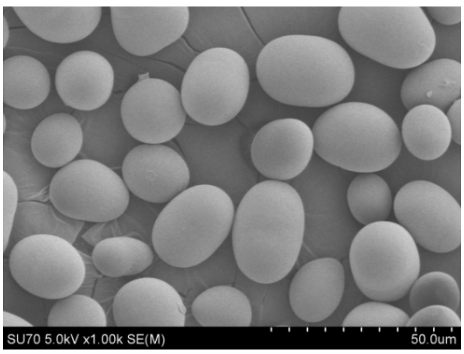
Twelve drought responsive tepary bean lines were evaluated for protein based on dry weight basis using Bradford assay (Table 3). The protein concentration was high in tan and black seed coated teparies compared to white seed coated cultivars. The protein concentration was ranging from 17 to 26 mg/100 mg of seed was supported by Bardwaj and Hamama, 2004. The protein concentration was positively influenced by drought tolerance with a significant difference ($p < 0.05$) between various cultivars studied (Table 3). The protein content was high in drought tolerant cultivars 4, 18, 24 and 29. The optimal contents of 17-21% were observed in low and moderately drought tolerant cultivars. This could also be due to the size of seed as the white seed coated cultivars have small sized seed compared to black and brown seed coat cultivars. The protein content of G1 and

G2 (24.8-26.2%) was significantly high compared to W1 and W2 (22.5-25.3%). The protein content was low in pigeonpea compared to values reported previously (Pathak et al., 1996; Sloan, 2012). Amount of photosynthetic assimilates produced by drought tolerant cultivar, TB #24 with high protein content (26%) might be high compared to the drought tolerant cultivar, TB #29 with less protein (24%) in the seed and same reason for G1 and G2 compared to W1 and W3 cultivars in pigeonpea. Future experiments with nitrogen requirement, amount of assimilates produced by drought tolerant cultivars will help us to reveal the potential cultivars with high protein content which can be evaluated to measure the amino acid contents for quality.

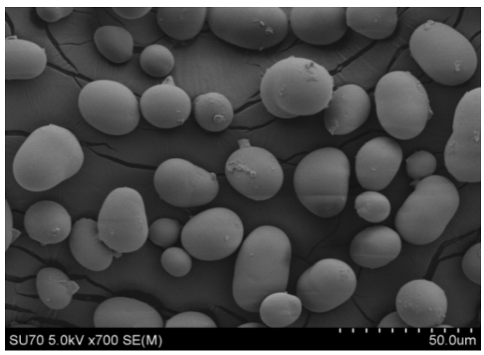
The cultivars with high protein content were with low RS (Table 3) and it was observed in both crops and cultivars as supported by Escarpa et al. (1997). It was revealed from our results as presented in Table 3 and 4 that, extracted pure starch values were the best to measure other attributes contributing quality of starch in cultivar, but not the TS or RS values of the ground seed powder for cultivar selection for industrial use and TS content from ground seed was the best measure for crop breeding and production use.

3.5 Cultivar Variation for Morphological Properties and Size Distribution of Starch Granules

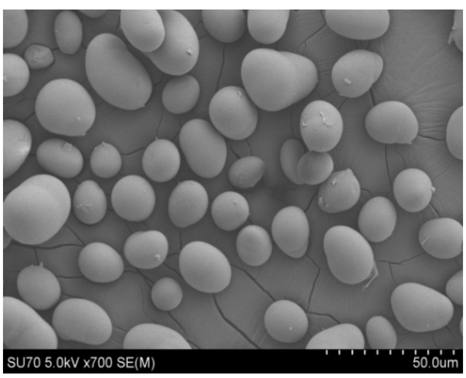
The morphological structures of starch granules observed, in both crops, were significantly different in shape (Figures 1a-d and 2a-d) and size (Table 5). The pigeonpea starch granules appeared as oval or elliptical in shape (Figures 2a-2d) and tepary starch granules were mostly round with a smooth surface (Figures 1a-1d). Larger starch granules were observed in cultivars W1 and W2 (52 µm X 39 µm) of pigeonpea and TB #4 (53 µm X 41 µm) of tepary bean. Except cultivar TB #23, most of the brown and black seed coat types had larger size starch granule in tepary bean. The size of the granule was ranging from 27.32 µm × 23.54 µm (TB #1) to 52.81 (TB #4) in tepray bean and was from 39.7 µm X 31 µm to 52.3 µm X 38.9 µm in pigeonpea. The size of the starch granules and shape were similar to the previously reported observations in pigeonpea (Lawal, 2008) and teparybeans (Abbas and Berry, 1986). The larger size granule increases its surface area as observed in pigeonpea and some of the colored teparies, and might reduce the hydrolysis of the starch increasing the resistance of the starch to the quantity of enzyme absorbed onto the surface (Xu et al., 2013; Sajilatha et al., 2006).



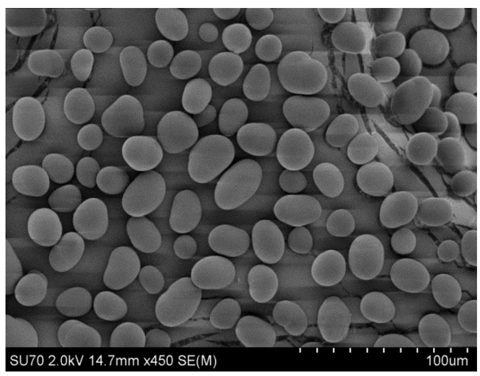
1a. Cultivar TB 4 (Brown Seed coat)



1b. Cultivar TB 29

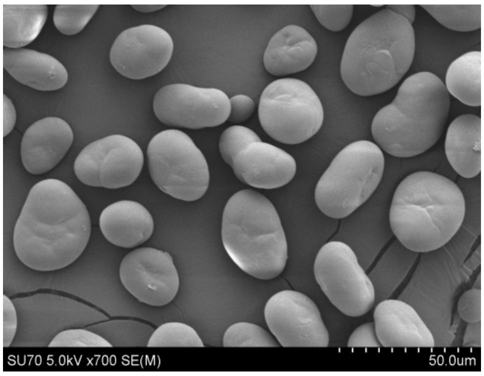


1c. Cultivar TB 18 (Black seed coat)

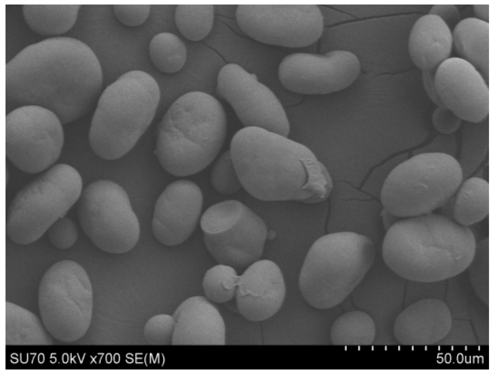


1d. Cultivar TB 1 (White seed coat)

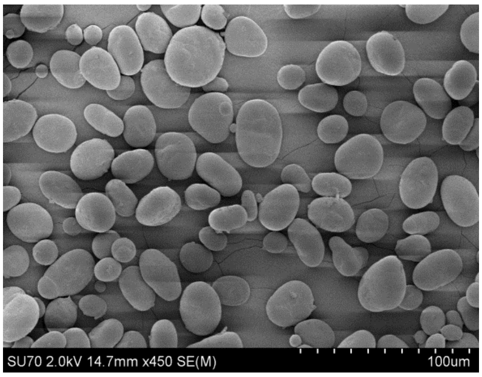
Figure 1(a-d). Starch granules of tepary bean cultivars



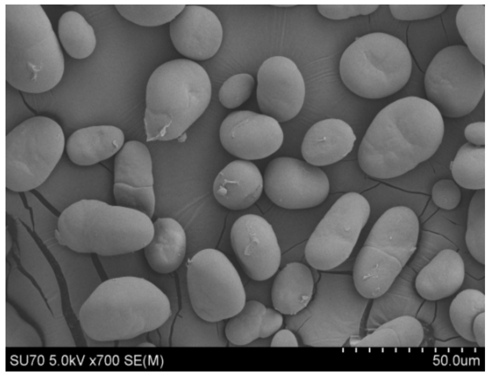
2a. Cultivar W1



2b. Cultivar W3



2c. Cultivar G1



2d. Cultivar G2

Figure 2 (a-d). Starch granules of pigeonpea cultivars

Table 5. Cultivar variability for size, crystallinity and molecular weight of starch particles in pigeonpea and tepary bean

Cultivar Code	Particle length (μm)	Particle width (μm)	Crystallinity (%)	Molecular Weight (Da)	
				Amylopectin (×10^8)	Amylose (×10^5)
TB1	27.32±0.72	23.54±4.13	14.79±4.48	10.3±0.85	1.35±0.04
TB2	38.95±2.01	29.32±3.14	14.79±4.48	12.2±2.53	2.72±0.27
TB3	42.38±5.34	27.84±3.14	18.17±6.33	6.41±1.44	2.29±0.17
TB4	52.81±16.01	40.78±5.84	16.74±4.97	24.6±0.73	1.17±0.02
TB7	40.78±5.00	30.01±3.67	16.27±6.39	10.1 ± 1.13	2.26±0.45
TB10	29.09±5.21	22.91±2.48	13.52±4.95	10.8±0.97	1.07±0.04
TB15	37.57±7.93	26.80±4.29	15.38±4.63	13.7 ± 1.24	1.62±0.04
TB18	33.33±5.67	26.23±5.16	15.56±4.22	7.54 ± 0.30	1.95±0.11
TB23	49.83±4.81	41.58±4.29	15.25±4.12	20.3±1.23	1.82±0.79
TB24	36.19±3.57	25.54±1.30	18.29±8.74	7.72 ± 0.64	2.05±0.11
TB27	43.99±3.78	35.74±2.40	15.27±2.87	20.4±0.46	1.67±0.70
TB29	45.59±4.66	41.00±2.88	18.97±6.47	16.1 ± 0.86	1.13±0.11
TB30	34.36±7.45	26.69±5.00	14.68±5.79	17.9 ± 2.43	0.93±0.71
TB31	37.46±7.59	27.03±1.69	17.21±5.62	12.6 ± 3.86	1.24±0.17
G1	39.7±2.5	31.0±3.7	19.30 ±0.0	5.43±0.39	5.46±0.52
G2	46.3±6.1	36.0±5.8	13.94±0.74	5.36±0.23	4.80±0.24
W1	52.3±8.2	38.9±4.7	17.39±0.09	5.35±0.79	4.11±1.59
W3	52.0±4.9	39.7±4.4	15.70±3.21	4.89±0.15	4.16±1.93

Column 1: TB- indicates Tepary bean, G1, G2, W1, W3 indicate Pigeonpea cultivars; The values were the averages ± standard deviation from three replications. The alphabet a & b indicates significance difference at probability < or = to 0.05. Same letter indicate no difference and different letter indicate significant difference between the cultivars.

X-ray diffraction studies revealed high per cent crystallinity in G1 (19.30), TB #29 (18.97) and low per cent in G2 (13.94) and TB #10 (13.52) cultivars respectively in pigeonpea and tepary bean. The diffraction patterns followed the expected C-type common to legume starches. Prominent peaks were centered between 15 and 23 with a mixture of A and B type polymorphs. It has been reported that starches with amylopectin of short chain length with A type crystallinity while those longer chain length show the type pattern (Hizukuri, 1986) and results are in agreement with the previous reports in pigeonpea (Lawal, 2008), teparybean (Abbas & Berry, 1986) and other food legumes (Xu et al., 2012, 2013).

Amylose by amylopectin ratio was high for pigeonpea compared to tepary bean and differences were significant ($p < 0.05$, $R^2 = 0.98$). The amylopectin content was high (> 75%) in teparybean cultivars (6.41-24.6) compared to pigeon pea cultivars (4.89-5.43). The amylose content was high in pigeon pea cultivars (4.11-5.46) compared to those of tepary bean (0.93-2.72). Over all, the high amylopectin were positively associated with high percent crystallinity in teparey bean and pigeon pea and were supported (Abbas & Berry, 1986, Xu et al., 2012, 2013). The cultivars G1 in pigeon pea was with high amylose with high crystallinity per cent unlike the previous results reported in chickpea and mungbean (Xu et al., 2012, 2013). The reason could be due to non-significant differences observed for amylose and amylopectin contents in pigeon pea. The extraction process in brown colored tepary (TB #4) might have influenced increased amylopectin due to reduced crystallinity but the crystallinity was unaffected by extraction process in TB #29 another brown colored variety (Table 4 & 5). In pigeonpea and tepary bean cultivars, the high amylose and amylopectin contents, were responsible for high crystallinity and high gelatinization temperature and enthalpy of gelatinization as explained by high proportion of short and long side chains of amylopectin (Singh et al., 2008).

3.6 Cultivar and Crop Variability for Thermal Properties of Starch

The WBC was high in TB #2 (97.3%) and low in TB #23 and #24 (67.3%) in tepary bean while it was high in W3 (77.17%) and low in G2 (65.33%) in pigeonpea and the differences were significant at 5% among these two crops (Table 6). The WBC was high in tepary compared to pigeonpea suggesting that main starch type C is more close towards A type in pigeon pea and towards B type in tepary bean and the differences observed mainly due to extent of solubility of starches in water which were associated with leaching of amylose from amorphous regions of starch due to disruption of inter and intra molecular hydrogen bonds (Lawal, 2008).

Table 6. Cultivar variability for thermal properties of starch extracted from pigeonpea and tepary bean

Cultivar Code	Thermal Properties				
	T_o (°C)	T_p (°C)	T_c (°C)	ΔT (T_c-T_o °C)	ΔH (J/g)
TB1	74.21±0.69	78.03±0.32	83.38±0.24	9.18±0.56	4.68±0.27
TB2	74.53±1.44	78.57±0.91	82.94±0.44	8.41±0.99	2.21±0.75
TB3	73.99±0.42	78.59±0.18	83.64±0.91	9.65±1.33	4.28±0.77
TB7	73.12±0.12	77.71±0.07	82.65±0.58	9.54±0.70	4.42±0.65
TB10	74.34±0.39	78.65±0.19	83.5±0.17	9.17±0.22	5.27±0.44
TB15	74.31±0.42	78.64±0.33	84.29±0.94	9.98±1.37	4.55±1.51
TB18	74.65±0.11	78.85±0.37	83.98±1.74	9.34±1.85	3.83±0.99
TB24	73.31±0.35	78.64±0.33	82.78±0.32	9.47±0.67	4.69±0.49
TB30	74.03±0.63	78.85±0.16	83.66±0.39	9.63±1.02	3.98±1.02
TB31	74.00±0.46	78.53±0.14	82.78±0.4	8.78±0.42	3.59±0.77
G1	67.74±0.83	71.73±0.09	78.51±2.62	10.76±3.45	6.43±2.88
G2	67.25±1.18	71.49±0.30	77.48±1.09	10.24±2.28	5.21±1.58
W1	68.29±0.33	72.49±0.18	78.06±0.74	9.78±1.07	6.15±0.99
W3	67.58±0.48	71.4±0.43	77.04±1.20	9.46±1.68	4.80±1.69

Column 1: TB 1- TB 31 indicates Tepary bean, G1, G2, W1, W3 indicate Pigeonpea cultivars; The values were the averages ± standard deviation from three replications. The alphabet a & b indicates significance difference at probability < or = to 0.0. Same letter indicate no difference and different letter indicate significant difference between the varieties.

Significant difference ($p < 0.05$) among the two crops and non-significant diferences ($p > 0.05$) among the cultivars with in each crop were observed for starch gelatinization transition temperatures T_o, onset; T_p, peak and T_c, conclusion (Table 6). The T_o, T_p, and T_c values were high in tepary bean (74.05, 78.45 and 83.36 ^oC) and low in pigeon pea (67.71, 71.67 and 77.77 ^oC). The mean values of T_o of starch were ranging from 73.12 ^oC (TB #7) to 74.65 ^oC (TB #18) in tepary bean and 67.25 ^oC (G2) to 68.29 ^oC (W1) in pigeon pea. The values of T_p were ranging from 77.71 ^oC (TB #7) to 78.85 ^oC (TB #18). The values of T_c were ranging from 82.65 ^oC (TB #7) to 84.29 ^oC (TB #15).These results were supported by the previous observations in tepary bean (Abbas & Berry, 1986) and mungbean starches (Xu et al., 2013). Tepary starch values from our study were in agreement with the values obtained by Abbas and Berry (1986) for amylose content of 30.7% and gelatinization temperature range of 70.5-84 ^oC with mean diameter of 33.5 microns of spherical to oval granules. The WBC trait and other thermal properties of pigeonpea were supported by Eltayeb et al. (2012), Singh et al. (2008) and Acevedo et al. (2013) with two endothermic peaks at 80-89 ^oC and 96-100 ^oC. The black tepary bean has lowest gelatinization energy compared to white and brown varieties with an exception white cultivar TB #2 with very low gelatinization energy. The cultivar TB #2 was observed with high amylose content and amylose/amylopectin ratio with small granule size which was supported by the factors influencing the gelatinization temperatures and dissociation of starch chains due to interaction with water (Lawal, 2008; Kaur et al., 2010). All the pigeon pea and rest of the brown and white teparies were observed with high enthalpy of gelatinization suggesting a high degree of association between double helices of starch due to strong hydrogen bonds requiring more energy to disrupt the bonds and were positively associated with resistant starch in all native drought tolerant tepary and pigeon culitvars.

4. Conclusions

The identified drought tolerant pigeonpea and tepary bean lines with high protein content and superior starch qualities will be useful for producers, breeders and food processing industries. It might also be helpful to study the starch qualities by using different extraction procedures in color seed coated cultivars to enhance the quality of starch extracted besides efforts to breed a cultivar with high amylose contents in a seed without reducing the quality of protein. These are our preliminary efforts to identify nutritionally qualitative new food legumes and its specific seed quality traits influencing as they were mostly consumed as seed and were rich in protein with essential amino acids, antioxidants, less fat content, more percent of resistant starch and minerals like Ca, Mn, Zn and Fe. The research on whole ground seed was in progress to study protein in-vitro digestibility and our current results revealed that brown seed coat cultivars with more quality protein with high protein content compared to white seed coat. Agronomic studies for total nitrogen requirement for quality seed harvest to evaluate nitrogen content of high protein seed will be useful to assess the amount of assimilates produced during drought by these drought tolerant cultivars in pigeonpea and teparybean.

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