INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-5, Issue-1, Jan-Mar-2015 **Coden: IJPAJX-USA, Copyrights@2015** **ISSN-2231-4490**

Received: 11th Oct-2014 ***Revised: 26th Oct-2014*** ***Accepted: 02nd Nov-2014***

Research article

ANTI-NUTRIENTS AND BIOACTIVE COMPOUNDS IN DROUGHT RESPONSIVE NATIVE TEPARY BEANS

Satya S Narina[1*], Harbans L Bhardwaj[1], John J Burke[2], and John W Parry[1]

[1]Virginia State University, Petersburg, VA, USA
[2]USDA-ARS, Lubbock, Texas, USA
[1*]Corresponding author: Email: snarina@hotmail.com.

ABSTRACT: Phytonutrients are gaining importance due to their contribution towards healthy nutrition to eliminate the risk of cancer and heart diseases. Food legumes were one of the richest sources of these compounds. Tepary bean is drought tolerant food legume native to USA preferred by various native and international communities and not explored to date for its seed quality traits. Twelve drought responsive cultivars were evaluated to know the content of anti-nutrients and antioxidants and their interaction with seed coat color (cultivar). The cultivars with brown and black colored seed coats showing high antioxidant activity compared to those with white color. The cultivars showed significant ($p<0.0001$) variation for total phenolic content (0-6.59 mg of tannic acid equivalent/g), condensed tannins (0-12.54 % leucocyanad in equivalent), total tannins (0-6.48 mg of tannic acid equivalent/g) and antioxidant activity (0.56-19.72µmol/Trolox Equivalent/ g).Significant variation among the seed components and the cultivars was observed with high contents in seed coat followed by whole ground soaked seed, bean without seed coat and whole ground dry seed. The cultivars TB 4,7,18, 24, 29 were observed with high contents of phenols, tannins and antioxidant activity. The cultivars with high phenolic content are positively associated with high antioxidant activity and drought tolerance. The contents were high in seed coat compared to bean and whole seed due to accumulation of pigments and phenolic compounds in the seed coat and testa. The significant variation observed among cultivars indicated the genetic control of the trait to utilize in breeding new tepary varieties for these traits to produce healthy food grain by studying the nature of inheritance and heritability.
Key words: Nutrients, bioactive compounds, Tepary beans

INTRODUCTION

Drought tolerant food legume, native to USA and suitable for cultivation in southern Virginia is dire need. Tepary is one among these [10] with an average yield of 2239 kg/ha [5]. Limited studies were conducted on seed quality traits of teparybeans [6,7]. The tepary bean seeds are of various size and color which might influence the quality of seed used for both animal and human consumption [3]. Phenols and antioxidants are the bioactive compounds and Condensed tannins are anti-nutrients contributing extra nutritional value to human health protecting from cancers, hyper tensions and cardiovascular diseases. Legumes were historically known for their high antioxidant activity and phenolic contents [11]. Nutritional and anti-nutritional activity of tannins can be reduced or balanced by various processing techniques like soaking, de-hulling, cooking and germination [17]. Soaking significantly reduced the bioavailability of tannins [14], total phenols and DPPH radical scavenging activity in dry common bean [2], eclipse blackbean [18]. Tannins are one of the anti-nutritional compounds biologically available in seed coat or testa ranging from 0-2% depending on the species and seed coat color of various beans [14]. Genetic selection of the cultivars with low tannin content high antioxidant activity would help the plant breeders, growers, food scientists to develop, grow and use the selections respectively.
Therefore, we analyzed the tepary seed quality of various cultivars for anti-nutrient and bioactive compounds to study the variation among the various seed components like seed coat (vary incolor), bean and whole seed dry and soaked overnight. We used twelve drought responsive native tepary cultivars to screen desirable seed quality traits for crop improvement and identify QTLs for the stability of these compounds for enhanced seed quality.

MATERIALS AND METHODS

Seed material: We analyzed the seed from twelve cultivars selected based on drought tolerance evaluations for further evaluation of seed quality traits. The whole seed was ground and sieved to pass through #40 mesh and used for analysis. Besides dried whole ground seed, we also analyzed seed coats, bean and whole seed soaked overnight to study the variability for composition of condensed tannins, phenolics and antioxidants. The seed coat was separated manually after soaking seeds over night before freeze drying [4]. The bean, whole seed (dry and soaked), and seed coat were freeze dried, ground to extract bioactive compounds, tannins and for their quantification.

Extraction Procedure: 250 mg of ground powder of each sample type, 80% acetone and diethyl ether were used for extractions [7] and the supernatants of the extractions after centrifugation were stored at -20^{0}C. The condensed tannins were measured by butanol-HCl method [13]. The total and simple phenols were measured by Folin - Ciocalteu reagent [16]. Calibration curves used for data transformation from absorbance to actual quantities in percentages was on dry matter basis as tannic acid equivalent (TAE) for total and simple phenols and total tannins, leucocyanidin equivalent (LE) for condensed tannins as percent in dry matter content (mg/100ml of extraction (from 2000mg dry matter)).

ABTS radical assay was done as per the previously discussed method [15] by measuring the absorbance at 734 nm at half minute of the reaction and percent inhibition as trolox (6-hydroxy-2,5,7,8-tetramethylchromam-2-carboxylic acid)equivalent was calculated for triplicate samples twice using the standard curve [9]. DPPH radical scavenging capacity was measured as absorbance at 517nm for 1ml of fresh extract with 1ml of DPPH for 10minute reaction for EC50 values following the method described by Parry and Lu [12]. All the chemicals used in the present investigation were purchased from Fisher scientific (Pittsburg, PA, USA) while DPPH (2, 2-diphenyl-1-picrlhydrazyl) from Sigma Aldrich (St. Louis, MO, USA) and ABTS (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) from Wako chemicals USA (Richmond,VA) and all the data was analyzed statistically using SAS 9.3 version.

RESULTS

Phenolic Compounds

Total phenolic content was analyzed for four extractions made (seedcoat, bean without seed coat, whole ground seed dry and soaked) from twelve cultivars (Table1). Three compounds analyzed (total and simple phenols, total tannins were expressed as percentage of tannic acid equivalent on a dry matter basis. The highest contents in seed coat were observed in TB15 (14.25 mg/g) and lowest were in TB 18 (0.77mg/g). The meanphenolic contents of whole seed soaked was ranging from <0 (TB 3) to 6.59 mg/g (TB29). The mean contents in bean (cotyledons without seed coat) were high in TB15 (5.56mg/g) and were very low in TB (0.68mg/g). The whole ground seed dry seed had lowest values in TB1 (0.50) and highest in TB15 (1.88mg/g) and Tb3 (1.87mg/g). Similar pattern was observed for simple phenols (Table 2) and total tannins (Table 3) which were analyzed after removing the bind tannins in extracts using PVPP (polyvinyl polypyrrolidone). Total tannins were measured by subtracting simple phenols from total phenols. The phenolic compounds and tannins were high in colored seed coat types and low or no content in white seed coat types. All the variations observed were highly significant (p<0.0001).

Condensed tannins

Significant variation observed among the twelve cultivars for the condensed tannins in seed coats (p<0.0001). Condensed tannins were high in black (TB18, 19.72%LE) and brown (TB 24, 14.74%LE) seed coated cultivars and were significantly low or no tannins in white seed coated cultivars.

Antioxidants

Significant differences observed for antioxidant activity (ABTS and DPPH radical assays) except for antioxidant content of whole ground dry seed (Table 5) and measured antioxidant activity was expressed as µmol / Trolox Equivalent/ g. The results of DPPH assay and ABTS were observed to be similar and hence we reported only results obtained from ABTS assay. The ABTS values were high in seed coats followed by whole ground seed (soaked) followed by beans and whole ground seed (dry) with significant differences (p<0.0001) among cultivars. The highest contents in seed coat were observed in TB4 (113.79µmol) and TB29 (86.85µmol) while no antioxidant activity was observed in all white seed coated types except TB 2 and 3. The mean antioxidant activity of whole ground seed (soaked) was ranging from <0.56 (TB 2) to 16.2µmol (TB7).The Mean contents in bean (cotyledons without seed coat) were high in TB 7 (14.51µmol) and no content in TB23 and TB4. The whole ground seed (dry) has very undetectable quantities in all the cultivars ranging from 0.0 (TB2) to 0.042 (TB3 and TB 23). The cultivars with color seed coat types had high antioxidant activity compared to speckled or light or white color types.

Table 1: Total phenols (mg/g) in various components of seed from 12 droughts responsive Native tepary bean cultivars

Cultivar	Color	Whole seed		Seed coat	Bean
		Dry	Soaked		
TB1	**White**	0.50±0.22c	1.08±0.11j	2.88±0.04e	5.74±0.09a
TB2	**White**	0.60±0.26bc	0.01±0.03k	5.25±0.41d	3.88±3.31abc
TB3	**White**	1.87±0.39a	-0.26±0.02l	8.05±0.13c	0.68±0.25
TB4	**Brown**	0.95±0.23bc	2.14±0.01h	10.24±0.15b	5.39±0.46ab
TB7	**Speckled tan**	0.85±0.11bc	3.16±0.02f	5.21±0.03d	3.60±3.43abcd
TB15	**Brown**	1.88±1.31a	3.97±0.18de	14.25±2.45a	5.56±0.62a
TB18	**Black**	0.88±0.08bc	2.66±0.10g	0.77±0.02f	1.00±0.13e
TB23	**White**	1.03±0.61bc	3.50±0.08	5.92±0.11d	3.30±2.48bcd
TB24	**Brown**	0.82±0.43bc	5.77±0.03b	2.55±0.02e	2.17±0.25cde
TB29	**Brown**	1.29±0.64ab	6.59±0.36a	10.02±0.04b	3.35±0.36bcd
TB30	**White**	0.94±0.43bc	4.6±0.01c	2.75±0.04e	1.55±0.99de
TB31	**White**	0.38±0.27c	1.34±0.15i	1.46±0.11f	1.88±1.50cde

The means (±sd**) of different alphabet (a, b, c) indicates significance (p<0.0001) with in the same column between the varieties.**

Table2: Simple phenols in various components of seed from 12 drought responsive native tepary bean cultivars

Cultivar	Color	Whole seed		Seed coat	Bean
		Dry	Soaked		
TB1	**White**	0.05±0.0h	0.04±0.00d	0.49±0.03e	0.07±0.00f
TB2	**White**	0.06±0.0f	0.003±0.00f	0.42±0.10fg	0.04±0.02g
TB3	**White**	0.07±0.0e	-0.01±0.00g	0.22±0.03h	0.21±0.00c
TB4	**Brown**	0.07±0.0e	0.06±0.00c	2.28±0.02a	0.28±0.00ab
TB7	**Speckled tan**	0.10±0.0c	0.06±0.00c	1.51±0.02b	0.07±0.00f
TB15	**Brown**	0.22±0.0a	0.001±0.00f	1.27±0.12c	0.17±0.03d
TB18	**Black**	0.09±0.0d	0.99±0.00a	0.47±0.00ef	0.28±0.01ab
TB23	**White**	0.06±0.0f	0.002±0.00e	-0.06±0.01j	0.29±0.00a
TB24	**Brown**	0.06±0.0f	0.11±0.00b	0.60±0.01d	0.01±0.00h
TB29	**Brown**	0.09±0.0d	0.11±0.00b	1.45±0.04b	0.12±0.00e
TB30	**White**	0.05±0.0g	0.03±0.00e	0.36±0.04g	0.11±0.00e
TB31	**White**	0.14±0.0b	0.12±0.01b	0.08±0.0i	0.27±0.00b

The means (±sd**) of different alphabet (a, b, c) indicates significance (p<0.0001) with in the same column between the varieties.**

Table 3: Total tannins (mg/g) in various components of seed from 12 drought responsive nativetepary bean cultivars

Cultivar	Color	Whole seed		Seed coat	Bean
		Dry	Soaked		
TB1	**White**	0.45±0.23cd	1.05±0.10j	2.39±0.04f	5.67±0.09a
TB2	**White**	0.55±0.26bcd	0.004±0.04k	4.84±0.33d	3.84±3.31abcd
TB3	**White**	1.80±0.39a	-0.24±0.03l	7.83±0.14b	0.48±0.26f
TB4	**Brown**	0.88±0.23bcd	2.07±0.01g	7.97±0.13b	5.12±0.46abc
TB7	**Speckled tan**	0.75±0.10bcd	3.09±0.02f	3.70±0.03e	3.53±3.43abcde
TB15	**Brown**	1.67±1.30a	3.97±0.19d	12.98±2.42a	5.39±0.63ab
TB18	**Black**	0.79±0.09bcd	1.66±0.10h	0.30±0.02h	0.72±0.14f
TB23	**White**	0.97±0.61bc	3.48±0.09e	5.99±0.12c	3.01±2.48cde
TB24	**Brown**	0.76±0.43bcd	5.66±0.03b	1.95±0.01fg	2.17±0.25def
TB29	**Brown**	1.19±0.64ab	6.48±0.36a	8.57±0.03b	3.23±0.36bcde
TB30	**White**	0.89±0.44bcd	4.58±0.02c	2.39±0.04f	1.44±0.99ef
TB31	**White**	0.24±0.27d	1.23±0.16i	1.38±0.13g	1.60±1.49ef

The means (±sd**) of different alphabet (a, b, c) indicates significance (p<0.0001) with in the same column between the varieties.**

Table 4: Total condensed tannins (%) as leucocyanadin equivalent from seed coat of 12 drought responsive native tepary bean cultivars

Cultivar	Color	Condensed Tannins (%)
TB1	**White**	0.03±0.02c
TB2	**White**	0.04±0.00c
TB3	**White**	0.35±0.00c
TB4	**Brown**	11.90±0.13b
TB7	**Speckled tan**	12.54±0.07b
TB15	**Brown**	8.50±8.62b
TB18	**Black**	19.72±9.74a
TB23	**White**	0.0±0.0c
TB24	**Brown**	14.74±4.08ab
TB29	**Brown**	11.83±2.75b
TB30	**White**	0.34±0.10c
TB31	**White**	0.57±0.16c

The means (±sd**) of different alphabet (a, b, c) indicates significance ($p<0.0001$) with in the same column between the varieties.**

Table 5: ABTS values (µmol / Trolox Equivalent/ g) in various components of seed from 12 drought responsive native tepary bean cultivars

Cultivar	Color	Whole seed		Seed coat	Bean
		Dry	Soaked		
TB1	**White**	0.005±0.0a	10.36±0.14d	2.78±0.22ef	3.95±1.03c
TB2	**White**	0.00±0.16a	0.56±0.82g	0.65±0.67ef	2.11±0.41e
TB3	**White**	0.04±0.02a	1.23±0.79f	-0.05±0.13ef	0.38±0.33f
TB4	**Brown**	0.003±0.00a	15.65±1.52ab	113.79±5.64a	-0.05±0.01f
TB7	**Speckled tan**	0.002±0.00a	16.2±0.06a	64.15±31.11c	14.51±0.55a
TB15	**Brown**	0.00±0.00a	10.88±0.50d	12.37±8.91e	4.6±0.44c
TB18	**Black**	0.002±0.00a	13.85±0.32c	4±13.16ef	6.11±0.51b
TB23	**White**	0.042±0.01a	1.29±0.39f	-0.88±0.63ef	-0.23±1.68f
TB24	**Brown**	0.00±0.00a	15.46±0.12bc	33.49±3.43d	6.38±0.45b
TB29	**Brown**	0.002±0.00a	16.02±0.08ab	86.85±3.62b	4.23±0.24c
TB30	**White**	0.02±0.00a	3.59±0.64e	-0.88±1.55ef	3.03±0.49d
TB31	**White**	0.002±0.00a	3.72±0.68e	-5.53±0.70f	4.32±0.29c

The means (±sd**) of different alphabet (a, b, c) indicates significance ($p<0.0001$) with in the same column between the varieties.**

DISCUSSION

The variability observed for condensed tannin content was similar to the first attempt made by Blair *et. al.*, [8] supporting the low and undetectable levels of condensed tannins in white seed coated cultivars of tepary bean and high levels in cultivars with colored seed coats. High qunatities of phenolics were positively associated with high contents of antioxidants. The cultivar TB 18 with black color seed coat was observed with high contents in whole seed soaked compared to seed coat (Table1). The reason could be due to loss of some phenolic compounds (pigments) during soaking time and absorption of the same by bean (cotyledons) which resulted in high values in bean. The black color seed coat types were tolerant to drought based on chlorophyll florescence studies in greenhouse (Narina et. al., 2013, article under review for publication) followed by brown and white seed coat types. The total tannins were within the range (0-12mg/g seed coat sample) of tepary bean values observed by Blair et.al., [8]. The amount of seed coat obtained from 100 g seed was 8.78g on dry weight basis.

Therefore 100 g seed will provide optimal quantities, approximately 40-50mg to protect human and animal from health disorders. The condensed tannin content was high in black seed coated type followed by brown seed coat types and were positively associated with phenolic content though genetically controlled as we observed some white seed coated types (TB 3) with very low values of condensed tannins. Reduction in phenolic and condensed tannin contents was observed in soaked white seed from dry white seed types compared to those values in brown seed types (Tables 1-3). Condensed tannins are relatively high in cultivar TB18 (Table 4), as these were anti-nutrients if consumed in high quantities will prevent the absorption of iron, a non-nutritive trait which negatively effects health (Blair et.al, 2013).

It was first time to report antioxidant activity of tepary bean seed and it was observed that the antioxidant activity in brown seed coat types was superior and comparable to those values (17.09-36.96%) in common bean [3]. Our observations were supported by previous studies in *phaseolus vulgaris* [1] that the genotypes with high phenolics produced high antioxidant activity due to presence of anthocyanins and flavonoids in black (colored) seed coat types. TB4 has high values (113.79) of ABTS followed by TB29 (86.85),7(64.15),24(33.49) which can be used for improving other cultivated high yielding cultivars of tepary crop to transfer this trait contributing anti-cancerous activity. The anti-nutrient and antioxidant levels were undetectable in drought susceptible cultivars (23, 30, 31) compared to drought tolerant cultivars (4, 7, 18, 29). The moderately tolerant cultivar TB 15 has optimal resistant (14%) starch compared to tolerant types TB 29 (22%), and TB 4 (14%) (unpublished data), but all these cultivars had equally comparable antioxidant activities which indicate that the starch contents are independent of anti-nutrient or antioxidant contents of a cultivar, but dependent on genotype and its interaction with environment during seed development. Through the present investigation, plant breeders has the choice to select the level of anti-nutrient content required for breeding bio-fortified tepary bean with high quality nutrition. Though the cultivar TB 18 was drought tolerant, but has reduced contents of phenolic compounds due to soaking and increased contents of condensed tannins which helps us to understand the changes occurring within the seed due to soaking and lay the foundation for furture studies to identify the stable genotypes with high low contents by exposing to different processing techniques for use in food industry.

The leaching losses observed in black color and mottled seed coat types would need to be studied and quantified. The QTL identified for seed coat and cotyledonary traits for iron in common bean by Blair *et.al*. [8] Would benefit the tepary crop to identify the cultivars with this trait and correlate with drought tolerance and other mineral (Fe and Zn) and nutritional traits. Further, the size of the white tepary seed is small compared to brown, mottled and black seed coated cultivars. As the size also one of the important factors controlling the quality and quantity of protein, it would be helpful if we can transfer the size and quality traits from dark seeded cultivars into widely cultivated white seed coat cultivars for balanced nutrition and health. There is need to analyze other compounds like phytates, folic acid, minerals of these cultivars to select cultivars with good quality bean besides seed coat traits for phenolic compounds and drought tolerance.

ACKNOWLEDGEMENTS

Authors like to acknowledge the funding agency USDA-NIFA to support the project through CBG funding program and Agricultural Research Station at Virginia State University for providing the facilities. The publication was the contribution of Virginia State University, Agrciutlural Research Station Journal Article Series Number--. We like to thank Dr.Xu in our food science lab for providing her vertex machine and pH meter during the analysis.

REFERENCES

[1] Aparicio-Fernadez,X., Manzo-Bonilla,L., and G.Loarca-Pina 2005. Comparision of antimutagenic activity of phenolic compounds in newly harvested and stored common beans Phaseoulus vulgaris against aflatoxinB1. J.Food.Sci.70:73-78.

[2] Akillioglu, H.G. and S. Karakaya, 2010. Changes in total phenols, total flavonoids, and antioxidant activities of common beans and pinto beans after soaking, cooking and in-vitro digestion process. Food Science and Biotechnology, 19 (3):633-639.

[3] Akond, S.M.G.M.,Khandaker,L.,Berthold,J.,Gates,L.,Peters,K.,Delong,H., and Hossain,K 2011. Anthocyanin, totalphenols and antioxidant activity of common bean. American Journal of Food Technology,6:385-394,doi:10.3923/aift.2011.385.394.

[4] Anton, A.A., Ross, K.A., Beta, T.R., Fulcher,G., and S.D. Arntfield, 2008. Effect of pre-dehulling treatments on some nutritional and physical properties of navy and pinto beans (Phaseolus vulgaris L.), Swiss Society of Food Science and Technology, 771-778.

[5] Bhardwaj, H. L., Rangappa, M. and Hamama, A.A. 2002. Planting date and genotype effects on tepary bean productivity. Hort Science 37 (2): 317-318.

[6] Bhardwaj, H.L. and Hamama, A.A. 2004. Protein and mineral composition of tepary bean seed. Hort Sci., 39(6):1363-1365.

[7] Blair,M.W., Caldas,G.V., Munoz,C. and K. Bett,2010.Evaluation of Condensed Tannins in Teparybean genotypes. pp:130-131.,Ref: naldc.nal.usda.gov/download/IND44060984/PDF.

[8] Blair, M.W., Izquierdo, P., Astudillo, C. and M. A. Grusak, 2013. A legume biofortification quandary: variability and genetic control of seed coat micronutrient accumulation in common beans. Frontiers in plant science: Plant Physiology., 4:1-14, doi:10.3389/fpls.2013.00275;

[9] Decker, E. A., Warner, K., Richards, M.P. and F. Shahidi, 2005. Measuring antioxidant effectiveness in food. Journal of Food Chemistry, 53:4303-4310.

[10] Federici, C.T., Edhaie, B. and J.G. Waines, 1990. Domesticated and wild tepary bean: Field performance with and without drought stress. Agronomy Journal 82, 896-900.

[11] Lee, J.H., Jeon, J.K., Kim, S.G., Kim, S.H., Chun, T. and J.Y. Imm, 2011. Comparative analysis of total phenols, flavonoids, saponins and antioxidant activity in yellow soy beans and mung beans. International journal of food science and technology, 46 (12):2513-2519, doi: 10.1111/j.1365-2621.2011.02775.x;

[12] Parry, J. and L. Yu, 2004. Fatty acid content and antioxidant capacity of cold –pressed black raspberry seed oil and meal. J.Food Sci., 69(3):189-193, doi:10.1111/j.1365-2621.2004.tb13356.x;

[13] Porter, L.J., Hrsrich, L.N. and B.G. Chan, 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry, 1:223-230.

[14] Reddy, N.R., Pierson,M.D., Sathe,S.K. and D.K. Salunkhe, 1985. Dry bean tannins: A review of nutritional implications. Journal of the American Oil Chemists Society. 62(3):541-549.

[15] Rice-Evans, C.A.,Miller,N.J., Bolwell,P.G.,Bramely,P.M. and J.B. Pridham 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic.Res.,22:375-383.

[16] Sanchez-Rangel,J.C.,Benavides,J., Heredia,J.B.,Cisneros-Zevallos, L. and D.A. Jacobo-Velazquez, 2013. The Folin-Ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination. Analytical Methods. DOI 10.1039/C3AY41125G;

[17] Tinsley, A.M., Scheerens, J.C., Alegbejo,J.O., Adan,F.H., Krumhar,K.C., Butler,L.E., and M.J.Kopplin, 1985. Tepary beans (*Phaseolus acutifolius var. latifolius*): a potential food source for African and middle eastern cultures. Qual Plant Plant foods Hum Nutr 35:87-101.

[18] Xu,B.J. and S.K.Chang, 2008. Total phenolic content and antioxidant properties of eclipse black beans (*Phaseolus vulgaris* L.) as affected by processing methods. J. Food Sci., 73 (2): H19-27, doi:10.10.1111/j.1750-3841.2007.00625.x