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Research Article

Effect of Cultivar and Planting Time on Resistant Starch Accumulation in Pigeonpea Grown in Virginia

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Recent recognition of indigestible starch component named as "Resistant Starch" in the human small intestine raised our interest to execute the current study to identify the best cultivar to produce high-quality pigeonpea seed to incorporate in ongoing pigeonpea breeding program. Though pigeonpea was identified as one of the food legumes with high RS, there were no published reports for pigeonpea resistant starch accumulation as influenced by planting time. The experiment was conducted twice in replicated block design with four varieties and two planting times. The resistant and non resistant (hydrolysable) starch components of ground seed powder of four pigeonpea varieties were analyzed to identify the best planting time and best cultivar for high-resistance starch accumulation. Planting time and varieties showed significant influence on resistant starch (RS), total starch (TS), and hydrolysable starch (HS) accumulation. The pigeonpea variety W1 was significantly superior from other three varieties and has highest RS value (21.4 g/100 g) with 70 per cent RS out of its total starch (28 g/100 g). The planting time 2 (June 11) produced seed with highest values for RS (18.7 g/100 g), HS (6.5 g/100 g), and TS (25.2 g/100 g). The cultivar W1 is the better one followed by GA1 for use in further crop improvement.

1. Introduction

The resistant starch, a valuable portion of total starch which is not hydrolyzed in the human small intestine, is considered as nutritious dietary fiber and functional fiber [1]. The resistant starch helps to protect colon tissue by producing healthy compounds like short-chain fatty acid that provides energy to colon bacteria to help maintain the healthy colon epithelium [2]. The FAO recommends consuming foods with low glycemic index (GI) which is based on the ratio of amylose and amylopectin content [3, 4]. Legumes have low GI as they have more amylose content than content compared to cereals [5].

Crops that provide high-quality nutrition are important for human health. Pigeonpea is one of the adapted new legume crops under investigation at the Virginia State University. It is drought tolerant, suitable for low input areas to feed the poor people. It will add alternative income to Virginia farmers beside supplementing with healthy nutrition to lower costs. The pigeonpea seeds are rich in proteins with many essential amino acids, carbohydrates, starch, vitamins, and minerals [6].

Approximately 20 g/person/day is recommended for beneficial health from RS (http://www.criticalbench.com/knowledge_resistant_starch.htm). India (10–18 g/day) is leading the highest intake of RS followed by Australia (5–7 g/day). The energy value of RS was approximately 2 kcal/g and was 50% less than completely digestible starch 4.2 kcal/g [7].

Legume seed starch has high percentage of resistant starch compared to cereal starch which might contribute to lower or stable blood glucose levels and maintain stable weight of human body [2]. Slowly digestible starch, readily digestible starch and resistant starch contents of pigeonpea cultivars (AL-15 and AL-201) in India were observed to be 31.0, 8.1, and 60.9% and 29.6, 5.2, and 65.2%, respectively [8]. Pigeon pea starch showed the highest values for molecular weight of amylopectin ($389 \cdot 106$ g/moL) and amylose

(3.64 · 106 g/moL) and the resistant starch content (78.9%) [9]. Resistant starch (RS) contents of various varieties of raw, decorticated, and processed pigeonpea revealed that RS content differed with variety and was higher in decorticated and pressure-cooked than raw whole seed [10].

Steam-heated legumes are good source of resistant starch [11] while high-pressure cooking will reduce the level of resistant starch. The raw tepary starch was more resistant to hydrolysis (by α-amylase) than maize starch due to differences in granules structure and amylose content [12]. The starch from cooked tepary bean (*Phaseolus acutifolius* A. Gray) was more hydrolyzed compared to its raw starch [12]. Therefore, based on the dependence of variable "resistant starch" on genotype and temperature, we planned to perform RS analysis of the ground pigeonpea seed powders of four cultivars from two planting times during third and second week of May and June, respectively.

Literature review established that the pigeonpea starches were highly resistant to human digestion having high amount of amylose which indicates the possibility of having high RS component in the total starch [8]. However, there were no published reports for pigeonpea resistant starch accumulation as influenced by planting time. The current investigation was undertaken to test the quality of ground seed starch in four pigeonpea varieties and to analyze and compare the percentage of resistant and non-resistant (hydrolysable) starch components of ground seed powder of four pigeonpea varieties as well as with chickpea and commercial starch samples, to identify the best cultivar and planting time for high-resistance starch accumulation for future breeding programs.

2. Materials and Methods

The seed was purchased from a private company for all four pigeonpea cultivars (W1, W2, GA1, and GA2) used in the current investigation. These four cultivars were planted twice on two planting times during summer 2011. The planting time one (PT1) was during third week of May (May 21) and planting time two (PT2) was during second week of June (June 11).

2.1. Seed Material. Seeds were harvested from four pigeonpea cultivars of two plantings times in three replications during 2011. The 20 g seed from all the 24 samples was freeze-dried and ground. Two g of freeze-dried ground whole-seed powder per sample was taken for current resistant starch (RS) assay studies.

2.2. Resistant Starch Assay. The resistant and nonresistant (hydrolysable) starch was quantified following the protocol as outlined in Megazyme resistant starch assay kit, K-RSTAR05/2008 (AOAC method 2002.02 and AACC method 32–40) [13]. The current protocol was slightly modified from the original by scaling down the reagent volumes (fourfold reduction) and sample size (twofold) without changing the output quality.

Whole-ground powdered seed samples (50 mg) from each treatment group (planting time × variety) were hydrolyzed using pancreatic α-amylase (10 mg/mL) and amyloglucosidase (3 U/mL) while incubating in a shaking water bath for 16 h (37°C, 200 strokes/min). The resistant starch was precipitated with 99% ethanol followed by centrifugation at 1500 g for 20 min and washing twice with 50% ethanol and centrifugation after each wash. The supernatant solutions retrieved after precipitation and washing steps were collected separately in 50 ml polypropylene tubes to determine hydrolyzed starch component of the sample. The residual pellet with RS was slowly dissolved on ice water bath with 2 M potassium hydroxide solution using magnetic stir bar. Then sodium acetate buffer (1.2 M, pH 3.8) and amyloglucosidase (0.250 mL, 3300 U/mL) were added and incubated for 30 min at 50°C followed by centrifugation at 1500 g for 20 minutes. Then an aliquot of 0.1 mL from supernatant of each sample was taken in glass test tube to which 3 mL of glucose-oxidase-peroxidase-aminoantipyrine (GOPOD) was added, and the mixture was incubated at 50°C for 20 min to read the final absorbance at 510 nm.

The ethanol wash collected after hydrolysis of digestible starch and was made up to 10 mL volume using 100 mM sodium acetate buffer (pH 4.5). Then 0.1 mL aliquot from supernatant of each sample was taken in a glass test tube to which dilute amyloglucosidase (2.5 μL, 300 U/mL) was added and incubated at 50°C for 20 min followed by addition of 3 ml of GOPOD with incubation at 50°C for further 20 min to read the final absorbance at 510 nm.

The absorbance at 510 nm (by measuring D-glucose content) was measured for both RS and non-RS for all the biological replicates for four genotypes using Bio-RAD's Smart Spec Plus spectrophotometer against the reagent blank. The analysis was done in duplicates. The total starch was estimated by adding the resistant starch (RS) and hydrolyzed starch (HS) components.

The data was statistically analyzed using SAS version 9.2 software (PROC GLM). The resistant starch data of pigeonpea was compared with that of other food legume chickpea and commercial corn starch to determine the potential of pigeonpea seed starch as a natural source for resistant starch.

3. Results and Discussion

Planting time had significant influence on resistant starch (RS), total starch (TS), and hydrolysable starch (HS) as observed from the analysis of variance (Table 1) and had no influence on percent RS. The varieties were significantly different for all the starch components. There was significant interaction between varieties and planting time for all the starch components analyzed except for HS. The mean square values of the varieties were greater than those of planting time versus variety except for per cent RS.

The pigeonpea variety W1, significantly superior from other three varieties (Table 2), has highest RS value (21.4 g/100 g) with 70 per cent RS out of its total starch (28 g/100 g). Among the four varieties, W3 has the highest

Table 1: Analysis of variance for RS, HS, TS (g/100 g dry weight basis) and %RS (percent RS out of TS) from seeds of four pigeonpea cultivars produced during two planting times in 2011.

Dependant variable	Planting time (PT)	Variety (V)	PT*V	R2 (%)	CV (%)	Mean
	Mean squares					
Resistant starch (RS)	189.8**	230.3*	166.7**	26.3	38.4	17.24
Hydrolyzed starch (HS)	23.4*	23.4*	0.414 NS	34.6	23.7	6.02
Total starch (TS)	346.4*	840.1*	169.4**	27.9	30.4	23.3
% RS	137.4 NS	721.9**	920.1**	19.3	22.1	70.4

Means: over two planting times, three replications and two experiments,
**, *Significance at 5% (<0.05) and 1% (<0.01) levels, respectively. NS: nonsignificant.

Table 2: The RS, HS, and TS values (g/100 g dry weight basis) of four pigeonpea cultivars (GA1, GA2, W1, and W2) and two planting times (PT1 and PT2) during 2011.

Cultivar/planting time	Resistant starch*	Hydrolyzed starch*	Total starch*	% RS*
GA1	17.91ab	5.21b	23.11b	76.87
GA2	14.68b	5.16b	19.83b	71.35ab
W1	21.35a	6.6a	27.95a	69.86ab
W3	15.03b	7.11a	22.14b	63.53b
PT1	15.83b	5.52b	21.36b	71.6a
PT2	18.65a	6.51a	25.16a	69.2a

*Means of three replications and two experiments; means with the same letter (a or b) are not significantly different.

Table 3: The resistant starch component of pigeonpea as compared to that of chickpea and corn.

Crop/variable	Pigeonpea	Chickpea*	Corn starch*
RS	17.24	4.35	12.75
HS	6.02	42.07	21.04
TS	23.3	46.42	33.79
%RS	70.4	9.38	37.74

*The values of RS were taken from Xu et al. 2012 [14].

value for HS (6.6 g/100 g). The variety GA1 has the highest percentage of RS (71.4 per cent) out of its total starch (23.1 g/100 g). Although the percent starch was highest in variety GA1, the variety W1 was considered as the best variety identified from the current study followed by GA1 due to its highest values for RS and TS.

Out of the two planting times studied, planting time 2 (second week of June) is better based on the quality of seed starch observed from the current data. The planting time 2 produced seed with highest values for RS (18.7 g/100 g), HS (6.5 g/100 g), and TS (25.2 g/100 g) as compared to planting time 1 (Table 2). It could be due reduced enzyme susceptibility to pancreatic α-amylase and amyloglucosidase [15] under varying temperatures. The weather condition might have created repeated heat/moisture treatment to accumulating seed starch and was associated with a decrease in the hydrolysis limit of pancreatic α-amylase and could increase the formation of RS [16] in pigeonpea during June planting.

Relative comparisons of pigeonpea starch components with those of chickpea and commercial starch [14] were shown in Table 3. Pigeonpea RS value (17.24 g/100 g) and RS percentage (70.4%) were comparatively higher than those of other legumes, chickpea (4.35 g/100 g), and commercial corn starch (12.75 g/100 g). In contrast the chickpea has the highest value (42.07 g/100 g) for readily digestible (hydrolysable) starch. Therefore, the pigeonpea cultivar W1, with highest RS (21.4 g/100 g) value, identified from current study could be utilized in further breeding program to yield good-quality seed for human health.

4. Conclusions

From the current investigation, it was identified that second week of June was the better planting time in Virginia for pigeonpea for improved starch quality with highest RS value and the cultivar W1 was the best followed by GA1 for use in further crop improvement for high-quality starch. Based on the positive results from current study for planting time in June month in Virginia and literature reviews on influence of temperature and moisture on RS formation, further studies on harvesting time could give better understanding of RS formation. Studies on RS accumulation under various maturity stages of seed harvest from August to September could benefit to identify the better cultivar with highest percent RS in seed for Virginia. The current nutrition studies on pigeonpea will enhance the knowledge and understanding the value of this new crop and its production capabilities in Virginia for increased farm income.

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References

[1] J. Higdon, *An Evidence-Based Approach to Dietary Phytochemicals*, Thieme Medical Publishers, New York, NY, USA, 2007.

[2] I. R. Abbas and J. W. Berry, "Tepary bean starch. Part 1. Physico-chemical properties," *Starch*, vol. 38, no. 6, pp. 195–199, 1986.

[3] FAO/WHO (Food and Agriculture Organization/World Health Organization), "Carbohydrates in human nutrition," Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper 66, Rome, Italy, 1998.

[4] J. A. Monro and M. Shaw, "Glycemic impact, glycemic glucose equivalents, glycemic index, and glycemic load: definitions, distinctions, and implications," *American Journal of Clinical Nutrition*, vol. 87, no. 1, pp. 237S–243S, 2008.

[5] M. G. Sajilata, R. S. Singhal, and P. R. Kulkarni, "Resistant starch—a review," *Comprehensive Reviews in Food Science and Food Safety*, vol. 5, no. 1, pp. 1–17, 2006.

[6] K. Saxena, R. Kumar, and R. Sultana, "Quality nutrition through pigeonpea—a review," *Health*, vol. 2, pp. 1335–1344, 2010.

[7] G. Liversey, "Energy value of resistant starch," in *Proceedings of the Concluding Plenary Meeting of Euresta*, G. Asp, J. M. M. Van Amelsvoort, and J. G. A. J. Hautvast, Eds., pp. 56–62, Wageningen, Wageningen, The Netherlands, 1994.

[8] M. Kaur and K. S. Sandhu, "*In vitro* digestibility, structural and functional properties of starch from pigeon pea (*Cajanus cajan*) cultivars grown in India," *Food Research International*, vol. 43, no. 1, pp. 263–268, 2010.

[9] K. S. Sandhu and S. T. Lim, "Digestibility of legume starches as influenced by their physical and structural properties," *Carbohydrate Polymers*, vol. 71, no. 2, pp. 245–252, 2008.

[10] D. Katyal, P. S. Ghugre, and S. A. Udipi, "Resistant starch in selected raw and processed legumes," *Journal of Food Science and Technology*, vol. 42, no. 6, pp. 506–510, 2005.

[11] M. J. Periago, G. Ros, and J. L. Casas, "Non-starch polysaccharides and *in vitro* starch digestibility of raw and cooked chick peas," *Journal of Food Science*, vol. 62, no. 1, pp. 93–96, 1997.

[12] I. R. Abbas, J. C. Scheerens, and J. W. Berry, "Tepary bean starch Part III: *in vitro* digestibility," *Starch*, vol. 39, no. 8, pp. 280–284, 1987.

[13] W. Horwitz and Association of Official Analytical Chemists, *Official Methods of Analysis*, AOAC International, Gaithersburg, Md, USA, 17th edition, 2000.

[14] Y. Xu, E. N. Sismour, S. S. Narina, D. Dean, and H. L. Bhardwaj, "Compositions and physicochemical properties of seeds and starches from Virginia-grown chickpea(*Cicer arietinum* L.) cultivars," *International Journal of Food Science and Technology*. In press.

[15] R. C. Eerlingen, I. Van den Broeck, J. A. Delcour, and H. Levine, "Enzyme resistant starch. VI. Influence of sugars on resistant starch formation," *Cereal Chemistry*, vol. 70, article 345, 1994.

[16] D. Sievert and Y. Pomeranz, "Enzyme-resistant starch II. Characterization and evaluation by enzymatic, thermoanalytical and microscopic methods," *Cereal Chemistry*, vol. 66, no. 4, pp. 342–347, 1989.