HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Comparisons of phaseolin type and α-amylase inhibitor in common bean (*Phaseolus vulgaris* L.) in China

Yang Yao[1], *Yibo Hu*[1], *Yingying Zhu*, *Yue Gao*, *Guixing Ren*[*]

Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China

ARTICLE INFO

Article history:
Received 5 February 2015
Received in revised form
29 September 2015
Accepted 27 November 2015
Available online 3 December 2015

Keywords:
Common bean
Phaseolin
α-Amylase inhibitor
Phenolic acid

ABSTRACT

The objective of this study was to characterize the phaseolin type and α-amylase (αAI) level in common bean (*Phaseolus vulgaris* L.) accessions deposited in the Chinese National Genebank. The 40 accessions sampled were common varieties originating in Asia, North America, South America, Europe, and Africa. No Inca (I-) phaseolin was observed in the accessions. Only four accessions contained Tendergreen (T-) phaseolin and the remaining 36 contained Sanilac (S-) phaseolin. αAI proteins extracted from nine accessions showed higher α-amylase inhibitory activity than the control (Phase 2, IC_{50} = 0.65 μg). These common bean accessions have potential use as nutraceutical ingredients.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume in the world, accounting for half of grain legumes in direct human consumption [1]. Common bean is rich in protein, unsaturated fatty acids, and dietary fiber in addition to vitamins and minerals [2]. Recently, researchers have focused interest on common bean proteins with specific functions, such as in anti-obesity [3], anti-hypersensitivity, and antioxidant [4] and anti-diabetic [5] activities. Phaseolin is the major storage protein in common bean seed, accounting for about 50% of total protein. Phase 2 (Pharmachem Laboratories, Kearny, NJ, USA) is a common bean extract product that can reduce human body weight at a daily dose of 500–3000 mg (D. Brady, N. D. CarbXzyme). Clinical studies also showed that Phase 2 has the potential to induce weight loss and reduce spikes in blood sugar caused by carbohydrates through its α-amylase inhibiting activity [6–8].

A proteinaceous inhibitor of α-amylase (αAI) isolated from common bean has been reported to have great potential to treat obesity and diabetes without side effects such as asthma and dermatitis [9]. Several companies have marketed common bean αAI extracts for controlling appetite and energy intake [10]. To date, information on phaseolin type and αAI

* *Corresponding author.*
E-mail address: renguixing@caas.cn (G. Ren)
Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.cj.2015.09.002

deposited in the Chinese National Genebank, Beijing, China is very limited. To contribute to the knowledge in this area, we investigated the phaseolin types and αAI levels of 40 common bean accessions deposited in the Genebank.

2. Materials and methods

2.1. Materials

Forty numbered common bean accessions (Table 1) were obtained from the Chinese National Genebank. The sampled accessions were the most common varieties originating in Asia, North America, South America, Europe, and Africa. Each sample was milled into fine (60-mesh) powder, cooled immediately, and stored at –20 °C. Porcine pancreatic α-amylase, ammonium sulfate, *tert*-butanol and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals used were of analytical grade. Phase 2 extracted from white kidney bean was used as a reference for comparison.

2.2. Isolation and purification of phaseolin

Phaseolin was purified as described by Carrasco-Castilla et al. [11]. The powder sample was defatted with hexane for 24 h and extracted in NaOH solution (1:10, w/v, pH 9.5), with agitation at 40 °C for 30 min. The supernatant from centrifugation at 5000 ×*g* for 30 min was adjusted to pH 4.5 with 1 mol L^{-1} HCl.

Table 1 – Information, α-amylase inhibitory activities, specific porcine pancreatic α-amylase inhibitory activities, and amounts of αAI necessary for 50% inhibition of α-amylase activity (IC_{50}) in common bean accessions.

No.	Accession code	Origin	Seed color	Total activity (U g^{-1})	Specific activity (U mg^{-1} protein)	IC_{50}
1	F0005800	Colombia	Red	4068.9 ± 245.9	1356.3 ± 117.1	0.62 ± 0.11 l
2	F0005801	Colombia	Red	3300.4 ± 368.5*	1100.1 ± 102.8*	0.77 ± 0.08
3	F0005860	China	Light brown	3603.5 ± 276.9*	1201.1 ± 115.1	0.70 ± 0.07
4	F0005861	China	Black	2501.9 ± 335.2*	834.0 ± 81.7*	1.01 ± 0.12#
5	F0005862	China	White	3970.7 ± 316.0	1323.5 ± 111.6	0.64 ± 0.06
6	F0005863	United States	White	2386.7 ± 206.7*	795.5 ± 96.8*	1.06 ± 0.18#
7	F0005865	Colombia	Red	3150.9 ± 193.7*	1050.3 ± 108.9*	0.81 ± 0.03
8	F0005870	Mexico	Black	2297.0 ± 214.6*	765.6 ± 95.2*	1.10 ± 0.07#
9	F0005873	Peru	White	2049.4 ± 265.2*	683.1 ± 70.2*	1.24 ± 0.02#
10	F0005874	Mexico	Dark brown	3402.8 ± 298.3*	1134.2 ± 116.2*	0.75 ± 0.10
11	F0005875	Brazil	Black	1485.8 ± 305.4*	495.2 ± 31.5*	1.71 ± 0.05
12	F0005876	Mexico	Black	2852.0 ± 328.2*	950.7 ± 91.1*	0.89 ± 0.06
13	F0005877	Venezuela	Black	4559.9 ± 318.3	1519.9 ± 167.8	0.56 ± 0.08
14	F0005879	Colombia	Red	3706.0 ± 286.2*	1235.3 ± 145.8	0.68 ± 0.14
15	F0005881	Mexico	Light brown	3372.9 ± 261.2*	1124.3 ± 189.4*	0.75 ± 0.05
16	F0005882	Portugal	Red	3488.2 ± 285.4*	1162.7 ± 152.2*	0.73 ± 0.08
17	F0005885	Argentina	White	3441.2 ± 249.1*	1147.1 ± 121.8*	0.74 ± 0.12
18	F0005886	Brazil	Dark brown	3727.3 ± 337.5*	1242.4 ± 121.2	0.68 ± 0.08
19	F0005888	Bulgaria	White	3360.1 ± 354.2*	1120.0 ± 168.8*	0.75 ± 0.09
20	F0005889	Bulgaria	Light brown	4308.0 ± 301.5	1436.0 ± 125.6	0.59 ± 0.05
21	F0005891	Haiti	Red	3624.8 ± 192.1*	1208.2 ± 163.7	0.70 ± 0.03
22	F0005892	Peru	White	1912.7 ± 315.4*	637.6 ± 50.0*	1.33 ± 0.26#
23	F0005893	Mexico	Light brown	4286.6 ± 383.5	1428.8 ± 115.5	0.84 ± 0.14
24	F0005896	Mexico	Light brown	2980.1 ± 346.3*	993.3 ± 95.1*	0.85 ± 0.06#
25	F0005897	Mexico	Light brown	4150.0 ± 312.3	1383.3 ± 185.5	0.61 ± 0.07
26	F0005898	Tanzania	Red	3970.7 ± 243.4	1323.5 ± 107.4	0.64 ± 0.09
27	F0005899	Turkey	Brown	3513.8 ± 296.7	1171.2 ± 191.2*	0.72 ± 0.07
28	F0005900	Guatemala	Red	3906.6 ± 256.1	1302.2 ± 155.7	0.65 ± 0.07
29	F0005904	Brazil	Light brown	3219.2 ± 243.6*	1073.0 ± 110.1*	0.79 ± 0.01
30	F0005905	Bolivia	Brown	3227.8 ± 288.2*	1075.9 ± 120.6*	0.79 ± 0.06
31	F0005906	Dominican Republic	Red	3014.3 ± 277.5*	1004.7 ± 106.6*	0.84 ± 0.09
32	F0005907	Ecuador	Black	2694.1 ± 246.3*	898.0 ± 82.4*	0.94 ± 0.07#
33	F0005909	Colombia	Black	3125.3 ± 283.3*	1041.7 ± 117.0*	0.81 ± 0.05
34	F0005910	Colombia	Black	3552.3 ± 283.5	1184.1 ± 101.1*	0.71 ± 0.04
35	F0005911	Haiti	Black	1511.4 ± 330.8*	503.8 ± 53.5*	1.68 ± 0.06#
36	F0005912	Honduras	Red	3488.2 ± 341.1	1162.7 ± 112.3*	0.73 ± 0.05
37	F0005914	Macedonia	White	1840.2 ± 363.6*	613.4 ± 56.1*	1.38 ± 0.09#
38	F0005915	United States	White	5776.7 ± 352.1#	1925.5 ± 104.3#	0.44 ± 0.08*
39	F0005917	United States	Brown	5170.4 ± 297.7#	1723.4 ± 115.8#	0.49 ± 0.05
40	F0005918	United States	Brown	4559.9 ± 286.0	1519.9 ± 116.7	0.56 ± 0.07
41	Phase 2			4367.7 ± 261.3	1455.9 ± 108.2	0.65 ± 0.04

Data are expressed as mean ± standard deviation of triplicate samples.
These accessions showed significantly ($P < 0.01$) higher levels than Phase 2.
* These accessions showed significantly ($P < 0.01$) lower levels than Phase 2.

The protein precipitate was retrieved by centrifugation at 10,000 ×*g* for 30 min and lyophilized. The lyophilized flour was suspended in the NaCl (0.5 mol L^{-1}) and HCl (0.025 mol L^{-1}) mixture (1:20, w/v, pH 2.0) for 1 h and centrifuged at 13,500 ×*g* for 30 min. The supernatant was centrifuged again for 30 min at 4 °C and 13,500 ×*g* after the addition of five volumes of distilled water at 4 °C. The precipitate was washed with distilled water and centrifuged again. The final precipitate was dialyzed against distilled water at 4 °C for 24 h and lyophilized.

2.3. Extraction of α-amylase inhibitors

Alpha-amylase inhibitors were extracted as described by Wang et al. [9]. Each common bean powder sample of 10 g was suspended in 100 mL distilled water (pH 6.50), stirred for 2 h at room temperature, and centrifuged at 12,000 ×*g* for 60 min. The supernatant (pH 5.25) was heated for 15 min at 70 °C to denature heat-labile proteins, which were removed by centrifugation at 12,000 ×*g* for 20 min. The remaining supernatants were subjected to 1 h protein partitioning by addition of 30% ammonium sulfate and *tert*-butanol. The mixture was then centrifuged (2000 ×*g* for 10 min) to facilitate the separation of phases. The lower aqueous layer was collected and desalted using a Sephax G-75 column (GE Healthcare, USA) equilibrated with 10 mmol L^{-1} citrate/phosphate buffer (pH 8.0). The products were then subjected to activity testing and protein measurement.

2.4. SDS-PAGE

The protein products were analyzed by SDS-PAGE [12]. Protein loadings were 0.2 and 0.1 mg per well for pure phaseolin and α-amylase inhibitor, respectively. Molecular weight standards (10.0–250.0 kDa; BioRad, USA) were also loaded in a separate well on each gel. The electrophoresis results were visualized by Coomassie brilliant blue staining.

2.5. Measurement of α-amylase inhibition activity

The α-amylase inhibition activity was determined as previously described [13]. Porcine pancreatic α-amylase (40 U mL^{-1}) was dissolved in a sodium succinate buffer (15 mmol L^{-1} NaOH, 20 mmol L^{-1} $CaCl_2$, and 0.5 mol L^{-1} NaCl, pH 5.6). A mixture composed of 100 μL α-amylase solution and 100 μL of extracted α-amylase inhibitor was first incubated in a water bath at 37 °C for 30 min. Then, 400 μL of 2% (w/v) soluble starch (dissolved in 20 mmol L^{-1} sodium phosphate buffer containing 6.7 mmol L^{-1} NaCl, pH 6.9) was added and the reaction was stopped after 1 min by an addition of 800 μL of 3,5-dinitrosalicylic acid and heating in a boiling water bath for 10 min. The volume of the mixture was finally increased to 6 mL with double-distilled water. A parallel measurement was performed simultaneously, with the same amount of enzyme but without the inhibitor. The results of the two measurements were compared to determine the inhibition activity. One inhibitory unit was defined as the amount of α-amylase inhibitor that completely inhibited the activity of one unit of enzyme [13]. The total activity was expressed as inhibitory units per gram of common bean (dry weight). The specific activity was expressed as inhibitory units per milligram of protein. The IC_{50} was defined as the amount of α-amylase inhibitor that inhibited 50% of enzyme activity.

2.6. Statistical analysis

All results are expressed as mean ± standard deviation (SD). The results were subjected to one-way analysis of variance. Tukey's test was performed using SPSS (Statistics for Social Science) version 17.0. The significance of differences was set to $P < 0.01$.

3. Results and discussion

3.1. Phaseolin patterns of the varieties

According to Montoy et al. [14], phaseolin subunits have a molecular weight ranging from 43.1 to 51.5 kDa and can be divided into three types: Tendergreen (T-) phaseolin with three visible subunits and Sanilac (S-) and Inca (I-) phaseolins with two visible subunits. The largest subunit (52 kDa) is present in S- and T-phaseolins but absent in I-phaseolin [15]. We observed 36S-phaseolin patterns showing two major bands in the molecular-weight range of 43–52 kDa (Fig. 1) and 4T-phaseolin patterns (F0005865, F0005873, F0005879, and F0005888) in the 40 common bean accessions. We found no I-phaseolin. Begbie and Rossone [16] have reported lower ileal digestibility of T-phaseolin-containing than of S-phaseolin-containing beans in pigs. Most accessions in this study give evidence of nutritional improvement in common bean breeding programs, given the finding of 36 accessions of S-phaseolin type.

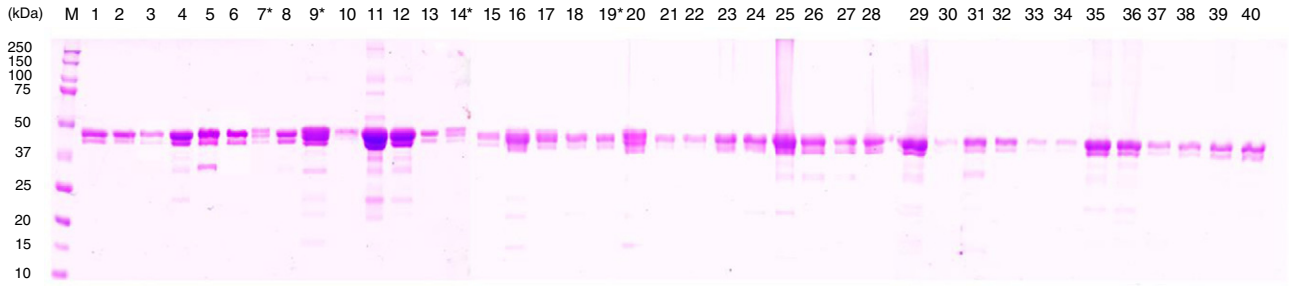

Fig. 1 – SDS-PAGE electrophoresis of phaseolins. The numbers from 1–40 correspond to those in Table 1. *These accessions show T-phaseolin patterns.

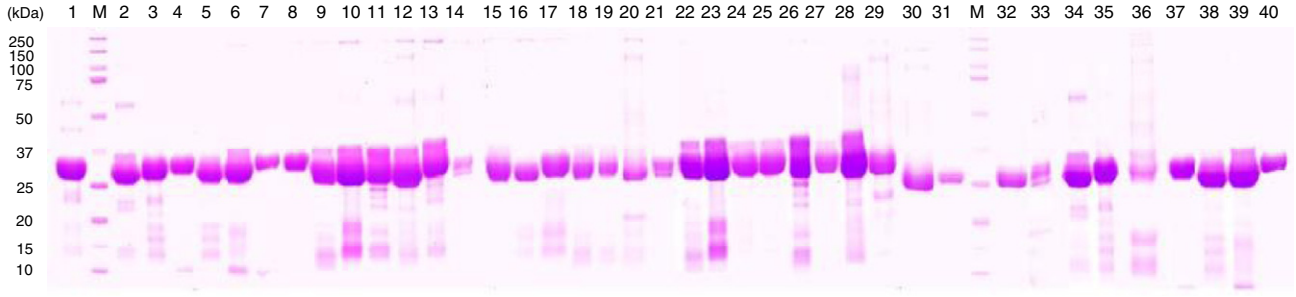

Fig. 2 – Polypeptide pattern of α-amylase inhibitors under SDS-PAGE separation. The numbers from 1–40 correspond to those in Table 1.

3.2. *Alpha-amylase inhibition activity*

The 40 common bean accessions showed different αAI protein banding patterns (Fig. 2). Large variation in total and specific activities of αAI was observed among these accessions (Table 1). The αAI total activity ranged from 1485.83 U g^{-1} dry weight for F0005875 to 5776.75 for F0005915. The αAI specific activity ranged from 613.40 U g^{-1} protein for F0005914 to 1925.58 for F0005915. F0005915 and F0005917 also showed significantly higher levels of total and specific activities, respectively, than the control. Wang et al. [9] observed higher αAI specific activity in black than in white-colored varieties of common bean. However, Frels and Rupnow [17] reported the contrary result that white common bean showed significantly higher αAI specific activity than black common bean. In this study, we found no clear correlation between αAI specific activity and seed color. Such differences may be due to the purification procedure of αAI, in which phenolic compounds were removed.

A low IC_{50} value indicates potent porcine pancreatic α-amylase inhibitory activity of a given protein. Nine αAI proteins extracted from the common bean accessions F0005915, F0005917, F0005918, F0005889, F0005877, F0005891, F0005800, F0005862, and F0005898 had lower IC_{50} than that of the control (IC_{50} = 0.65 μg), with the αAI protein from F0005915 showing the strongest activity (IC_{50} = 0.44 μg). The IC_{50} of the tested accessions ranged from 0.44 to 1.71 μg. This result is similar to that of Wang et al. [9] who reported an IC_{50} interval of 0.40–1.60 μg.

In conclusion, the 40 common bean accessions showed considerable variation in phaseolin type and α-amylase inhibition activity. Most were of the S-phaseolin type, with only four being of the T-phaseolin type. No accessions of the I-phaseolin type were found. Nine accessions had excellent α-amylase inhibitors and show potential use as sources of nutraceutical ingredients.

Acknowledgments

This study was supported by the Program of Science and Technology Cooperation with Hong Kong, Macao, and Taiwan, China (2013DFH30050), the special fund for Agro-scientific Research in the Public Interest (201403063), the earmarked fund for China Agriculture Research System (CYTX-014) and Agricultural Science and Technology Innovation Program.

REFERENCES

[1] P. McClean, J. Kami, P. Gepts, Genomics and genetic diversity in common bean, in: R.F. Wilson, H.T. Stalker, E.C. Brummer (Eds.), Legume Crop Genomics, AOCS Press, Champaign, IL 2004, pp. 60–82.

[2] T. Kutos, T. Golob, M. Kac, A. Plestenjak, Dietary fibre content of dry and processed beans, Food Chem. 80 (2003) 231–235.

[3] X. Wu, X. Xu, J. Shen, N. Perricone, H. Preuss, Enhanced weight loss from a dietary supplement containing standardized *Phaseolus vulgaris* extract in overweight men and women, J. Appl. Res. 10 (2010) 73–79.

[4] K. Sandeep, K.V. Alok, S. Akanksha, R. Ruchi, K. Dinesh, B.H. Giridhar, T. Anurag, P.C. Bhushan, S.K.J. Mukul, D.D. Premendra, Phaseolin: a 47.5 kDa protein of red kidney bean (*Phaseolus vulgaris* L.) plays a pivotal role in hypersensitivity induction, Int. Immunopharmacol. 19 (2014) 78–90.

[5] H.G. Preuss, Bean amylase inhibitor and other carbohydrate absorption blockers: effects on diabesity and general health, J. Am. Coll. Nutr. 28 (2009) 266–276.

[6] M. Barrett, J. Udani, A proprietary alpha-amylase inhibitor from white bean (*Phaseolus vulgaris*): a review of clinical studies on weight loss and glycemic control, J. Nutr. 10 (2011) 1–10.

[7] L. Celleno, N.V. Perricone, H.G. Preuss, Effect of a dietary supplement containing standardized *Phaseolus vulgaris* extract on the body composition of overweight men and women, Int. J. Med. Sci. 4 (2007) 45–52.

[8] J. Udani, M. Hardy, D.C. Madsen, Blocking carbohydrate absorption and weight loss: a clinical trial using Phase 2 brand proprietary fractionated white bean extract, Altern. Med. Rev. 9 (2004) 63–69.

[9] H.H. Wang, C.L. Chen, T.L. Jeng, J.M. Sung, Comparisons of α-amylase inhibitors from seeds of common bean mutants extracted through three phase partitioning, Food Chem. 128 (2011) 1066–1071.

[10] D. Chokshi, Toxicity studies of blackal, a dietary supplement containing phase 2 starch neutralizer (Phase 2), a standardized extract of the common white kidney bean (*Phaseolus vulgaris*), Int. J. Toxicol. 25 (2006) 361–371.

[11] J. Carrasco-Castilla, A.J. Hernandez-Alvarez, C. Jimenez-Martinez, C. Jacinto-Hernandez, M. Alaiz, J. Giron-Calle, J. Vioque, G. Davila-Ortiz, Antioxidant and metal chelating activities of *Phaseolus vulgaris* L. var. Jamapa protein isolates, phaseolin and lectin hydrolysates, Food Chem. 131 (2012) 1157–1164.

[12] P. Salgado, L. Montagne, J.P.B. Freire, R. Ferreira, A. Teixeira, O. Bento, M. Abreu, R. Toullec, J.P. Lalle's, Legume grains

enhance ileal losses of specific endogenous serine-protease proteins in weaned pigs, J. Nutr. 132 (2002) 1913–1920.
[13] J.J. Pueyo, D.C. Hunt, M.J. Chrispeels, Activation of bean (*Phaseolus vulgaris*) α-amylase inhibitor requires proteolytic processing of the proprotein, Plant Physiol. 101 (1993) 1341–1348.
[14] C.A. Montoy, P. Leterme, S. Beebe, W.B. Souffran, D. Molles, J.P. Lalles, Phaseolin type and heat treatment influence the biochemistry of protein digestion in the rat intestine, Brit. J. Nutr. 99 (2008) 531–539.
[15] S. Beebe, J. Rengifo, E. Gaitan, M.C. Duque, J. Tohme, Diversity and origin of Andean Landraces of common bean, Crop Sci. 41 (2001) 854–862.
[16] R. Begbie, A.W. Ross, Resistance of the kidney bean reserve protein, phaseolin, to proteolysis in the porcine digestive tract, J. Sci. Food Agric. 61 (1993) 301–307.
[17] J.M. Frels, J.H. Rupnow, Purification and partial characterization of two a-amylase inhibitors from black bean (*Phaseolus vulgaris*), J. Food Biochem. 8 (1984) 281–301.