Food Research International 43 (2010) 595–601

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Concentrations of condensed tannins and anthocyanins in common bean seed coats

Aura M. Díaz, Gina V. Caldas, Mathew W. Blair *

International Center for Tropical Agriculture (CIAT), AA 6713, Cali, Colombia

ARTICLE INFO

Article history:
Received 25 January 2009
Accepted 23 July 2009

Keywords:
Phaseolus vulgaris
Condensed tannins
Anthocyanins
Tannin monomer units

ABSTRACT

Seed coat colour in common bean *(Phaseolus vulgaris* L.*)* is determined by activity of the flavonoid biosynthetic pathway resulting in the presence or absence of specific anthocyanins, tannins and glycosidic flavanols. These secondary metabolites have anti-oxidant properties in the case of anthocyanins and glycosidic flavonols and strongly influence dietary mineral bioavailability in the case of tannins. The modification of tannin content is a goal of biofortification breeding programs, while almost all bean improvement considers seed colour in selection priorities as this affects consumer preference and food quality. In the present study, we analyzed condensed tannins, tannin monomers and anthocyanin levels in an intergenepool population derived from the cross DOR364 × G19833 using HPLC and spectrophotometric methods. The overall average for condensed tannins expressed as percentage in seed coats was 20.04%. The ranges were between 8.0% and 27.9% for soluble tannins (ST), 1.5% and 5.4% for insoluble tannins (IT), and 10.7% and 30.9% for total tannins (TT). Anthocyanins in seed coats averaged 0.08% (0.013–0.21% range) expressed as delphinidin-3-glucoside equivalents for the population with the distribution biased towards low content. All traits had large variability between genotypes and showed transgressive segregation, indicating quantitative inheritance for tannin content and oligogenic control of anthocyanins. Condensed tannins in the genotypes were mainly composed of catechin (60.3%), gallocatechin (25%), and afzelechin (14.7%) as monomeric units.

1. Introduction

Grain legumes include common bean (*Phaseolus vulgaris* L.) which is the most important pulse for direct human consumption (Broughton et al., 2003). In addition to having a broad distribution as a crop worldwide, common beans are indispensable to the diet of many countries, particularly those of Central and South America, and Eastern and Southern Africa. As well as being an important protein source, common beans have high levels of essential minerals such as iron, zinc, phosphorus, and calcium (Bazel & Anderson, 1994) and therefore have the potential to address iron deficiency anemia and other diseases associated with deficiencies of micronutrients which affect large numbers of people throughout the world (Bouis, 2003). Biofortification of common beans is underway with the justification that high mineral beans will increase the supply and availability of non-heme iron in various human populations (Graham, Senadhira, Beebe, Iglesias, & Monasterio, 1999). Such nutritional improvement focuses both on increasing nutrient content and reducing the plant's contents of "anti-nutritional" factors such as oxalates, phytates and tannins that together affect the bioavailability of these nutrients to consumers (Welch, 2002).

Tannins are polymeric flavonoids that comprise a small part of the broad and diverse group of phenolic compounds produced by plants as secondary metabolites (Winkel-Shirley, 2001). Tannins can precipitate proteins and complex with iron in the gastrointestinal lumen, reducing the absorption, digestibility and availability of these nutrients (Brune, Rossander, & Halberg, 1989). Meanwhile, anthocyanins are also generated by the flavonoid biochemical pathway but do not reduce digestibility and therefore are not considered anti-nutritional factors (Andersen & Jordheim, 2006). In fact, anthocyanins have been reported as anti-inflammatory, vasotonic, and anti-oxidant compounds, playing an important role in the prevention of degenerative illnesses such as cancer, Alzheimer's disease or cardiovascular illnesses (Macz-Pop, Rivas-Gonzalo, Pérez-Alonso, & González-Paramás, 2006; Markham & Bloor, 1998). Tannins and anthocyanins are of simultaneous interest from a plant breeding perspective because together they influence the colour of plant organs. In bean seed, these metabolites are located mainly in the seed coat and determine the overall colour, hue and intensity of the seed (Beninger & Hosfield, 1998).

Recent studies have demonstrated a quantitative heredity pattern for tannin content (Guzmán-Maldonado, Martinez, Acosta, Guevara, & Paredes, 2003) that is associated with seed colour inheritance (Caldas & Blair, 2009) suggesting the possibility of obtaining genotypes with low tannin content through selection programs (Caldas, Blair, & Restrepo, 2007). Variability in the

* Corresponding author. Tel.: +57 2 4450 000; fax: +57 2 4450 073.
E-mail address: m.blair@cgiar.org (M.W. Blair).

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doi:10.1016/j.foodres.2009.07.014

monomers of condensed tannins is thought to be associated with different seed colours (Beninger, Hosfield, & Bassett, 1999), and the type of flavonoid structure is known to govern chelate formation and reduction of metallic ions (Mira et al., 2002). Hence, the quantification of total tannin and monomer types may provide valuable information for breeding anti-nutrients out of bean seed coats with the added advantage of reducing seed darkening associated with high tannin content (Junk-Knievel, Vanderberg, & Bett, 2007). However, to balance the seed coat colour components, it is important to consider the anthocyanins and glycosidic flavonols that also influence seed coat colour (Choung, Choi, An, Chu, & Cho, 2003; Takeoka et al., 1997). Tannins and anthocyanins are important to nutritional quality because of their status as anti-oxidants and potential anti-carcinogens (Rice-Evans, Miller, & Paganga, 1997) and breeding of these constituents must take into account the balance of anti-nutritional and health promoting aspects of these compounds.

In this research, we use a well-studied recombinant inbred line population to evaluate the content of condensed tannins and anthocyanins in the seed coat of common bean. Our specific objectives were to: (a) evaluate condensed tannin content using a butanol–HCl method; (b) determine tannin monomers with liquid chromatography; and (c) implement a spectrophotometric technique for the fast and reliable evaluation of anthocyanin. The evaluation of these traits in a recombinant inbred line population allowed us to determine the pattern of segregation of tannin and anthocyanin contents, as well as the relationship to seed colour traits.

2. Materials and methods

2.1. Plant materials

The analysis was carried out with 87 ($F_{9:11}$) RIL lines derived from a Mesoamerican by Andean cross (DOR364 × G19833) of common beans. This set of genotypes along with the two parents of the population were planted in a randomized complete block design experiment with three field replications in Darién, Colombia in the second semester of 2006. Seed was harvested from each plot and used fresh for analysis to avoid seed darkening which results from tannin oxidation over time during storage. The seed coats of 30 seeds from each genotype were peeled manually from the cotyledons and ground finely in a Retsch MM2 mixer mill (Retsch, Haan, Germany).

2.2. Phenotypic evaluation

2.2.1. Tannin extraction and quantification

The tannins present in the seed coats were extracted and quantified using the method described by Caldas and Blair (2009). Briefly, 10-mg samples of bean seed coats for each recombinant inbred line from the study population were weighed in triplicate and treated twice with 2.5 mL of an aqueous solution consisting of 70% acetone and 0.1% ascorbic acid followed by one treatment of 2.5 mL diethyl ether. The supernatant was removed by vacuum suction and dried for 1 h in a sample concentrator. A total of 5 mL of distilled water was added to each sample and the samples were centrifuged for 15 min at 3500 rpm. The supernatant was separated from the pellet and analyzed for soluble tannins (ST), while the pellet was analyzed for insoluble tannins (IT).

For soluble tannins, a 300-μL sample was taken and 1.8 mL of butanol-HCl (5%) was added, whereas for insoluble tannins, 0.7 mL of water and 4.2 mL of butanol-HCl (5%) were added to the pellet. The samples were then kept at 95 °C in a water bath for 75 min and were read in a UV-1601 spectrophotometer (Shimadzu Corporation, Columbia, MD) at 550 nm against blanks treated with a 5% butanol-H_2O solution. Total tannins in the bean seed coats were quantified using a calibration curve generated by purified tannins from the population's parents as standards as described in Caldas and Blair (2009). The content of tannins was calculated as milligram of purified tannin per millilitre of solution and then expressed as percentage of seed coat. The values obtained are translatable to whole seed tannin concentration expressed as catechin equivalents (CE), where 1 CE is equivalent to 1 mg tannins per gram of seeds.

2.2.2. Determination of tannin monomers with high performance liquid chromatography

In preparation for high performance liquid chromatography (HPLC) analysis, the extracts from the samples treated with butanol-HCl were evaporated until dry in a sample concentrator and then quickly re-dissolved in 1 mL of 1% HCl in methanol and vortexed. The concentration of delphinidin, cyanidin, and pelargonidin were determined by injecting 20 μL of the sample into a CL-10A liquid chromatographic system, equipped with a UV–VIS detector SPD-10A (both from Shimadzu Corporation, Tokyo, Japan). The anthocyanidins were separated, using a Nova-Pack C18 column (8 × 100 mm) (Waters Corporation, Milford, MA), with a 4-μm particle size maintained at room temperature. Each anthocynidin was identified by comparison to standards for delphinidin, cyanidin and pelargonidin which revealed the presence and quantity of each type of the tannin monomers. Thus, delphinidin indicated the presence of gallocatechin, cyanidin indicated the presence of catechin and pelargonidin indicated the presence of afzelechin.

Before injection, all samples were filtered through a 0.45-μm syringe filter (Pall Corporation, East Hills, NY) and all solvents (HPLC grade) filtered through a 0.45-μm membrane (Millipore Corporation, Billerica, MA). The analysis was performed with A) 100% methanol and B) 5% acetic $acid_{ac}$ as solvents. The elution protocol used was as follows: 60% A for 1 min, 60–70% A for 1 min, 70–60% A for 2 min, and 60–40% A for 2 min at 1.2 mL/min flow rate. Anthocyanidins were detected at 525 nm and data analysis was carried out through Class VP software, v.4.0 (Shimadzu Corporation). Peaks were identified in the chromatograms by comparing retention times with commercial standards of delphinidin, pelargonidin, and cyanidin (ChromaDex Inc., Irvine, CA).

2.2.3. Anthocyanin extraction, purification, and quantification

Anthocyanins were quantified, using modified methods of Takeoka et al. (1997) and Macz-Pop et al. (2006). The modifications mostly reduced the extraction time of the compounds. Ground seed coat (100 mg) was extracted with acidified methanol (30 mL 70% MEOH with 0.5% HCl) in an ultrasound water bath for 10 min then mechanically shaken for 3 h. The samples were kept overnight at 4 °C under darkness, then centrifuged at 3500 rpm and filtered.

To purify the extracts obtained, excess solvent were evaporated for 3 h in a sample concentrator, and the extracts were re-suspended to a final volume of 7.5 mL with HPLC grade water and washed with 5 mL hexane with elimination of the organic phase. After this process, the samples were again dried in a concentrator and anthocyanins were purified by solid-phase extraction with 500-mg C18 columns (Varian Inc, Walnut Creek, CA). The columns were conditioned with 2 mL of methanol, and the samples eluted with 4 mL methanol containing 0.01% HCl. Purified anthocyanins were detected at 510 nm in a UV-1601 spectrophotometer (Shimadzu Corporation). The calibration curve was generated, using a commercial standard of delphinidin-3-O-glucoside chloride (Chromadex Inc., Irvine, CA). The anthocyanins were calculated as mg of delphinidin-3-glucoside per mL of solution and expressed as percentage of delphinidin-3- glucoside equivalents in seed coat of

common bean. Delphinidin-3-glucoside has been reported as one of the main anthocyanins in common bean (Choung et al., 2003; Takeoka et al., 1997; Tsuda, Osawa, Oshima, & Kawakishi, 1994).

2.3. Data analysis

Analyses of variance, variance component, and means separation tests based on the Ryan Einot Gabriel Welsch (REGW) procedure were carried out with SAS, v.9.1.3 (SAS Institute Inc., Cary, NC). For analysis of variance, the genotype, replicate and sub-sampling effects were considered random and the parental genotypes were compared with an orthogonal contrast with one degree of freedom. Descriptive statistics and correlation analyses for contents of soluble, insoluble, and total tannins were determined with STATISTIX program, v.8.0 (Analytical Software, Tallahassee, FL, USA). Simple correlations were calculated based on Pearson's coefficient using this same software program. An additional analysis of variance was carried out to evaluate the association of tannins and anthocyanins with seed colour classes as defined by CIAT (1987), using Levene's test for variance homogeneity in SAS.

3. Results and discussion

3.1. Condensed tannin content and distribution

Table 1 presents the values for condensed tannin content in seed coats found in the population's parents and the average and range of values for the RIL lines. The results, expressed as percentage of condensed tannins in seed coats, ranged between 8.0% and 27.9% for soluble tannins (ST), 1.5 and 5.4% for insoluble tannins (IT), and 10.7% and 30.9% for total tannins (TT). The overall average of the 267 bean samples analyzed for the three field replications was 20.04%, a value in agreement with previous reports (de Mejía et al., 2003; Guzmán, Castellanos, & Gonzalez, 1996; Iniestra, Ibarra, Medrano, Rocha, & Gallegos, 2001; Ma & Bliss, 1978) but were lower than the 24.3% average reported from our previous study (Caldas & Blair, 2009) perhaps because of a shorter seed storage period employed here. Seed storage is known to cause seed coat darkening, as tannin content increases from phenol oxidation catalyzed by polyphenol oxidase (Misnawi, Jamilah, & Nazamid, 2002). The low standard deviations of the means for the parental genotypes and each recombinant inbred line show that tannin contents were highly heritable and did not vary across replicates. In addition, the correlation in tannin content for DOR364 and G19833 in this study and by Caldas and Blair (2009) confirms that the methodology is highly consistent.

Table 1 also shows that differences between the recombinant inbred lines were significant ($p < 0.01$) for all condensed tannin traits, but differences among parents were only significant when DOR364 IT values were comparatively higher than G19833. The large range in ST and TT values suggests transgressive segregation for the trait and made the evaluation of the population valuable even if the parents did not differ for ST and TT. Caldas and Blair (2009) found similar evidence for transgressive segregation for one additional recombinant inbred line population (G2333 × G19839) with large variability in derived lines despite the lack of significant differences in condensed tannin content among the parents.

Condensed tannin traits presented normal distributions in the DOR364 × G19833 population (Fig. 1), and inheritance of the three traits is likely quantitative as suggested by Caldas and Blair (2009). Guzmán-Maldonado et al. (2003) also found quantitative inheritance for tannin content in a wild × cultivated cross of common beans, therefore inheritance of tannin content appears similar within the cultivated species or when combining wild, small-seeded with large-seeded cultivated types. Significant positive correlations were found between IT and TT content ($r = 0.47$, $P < 0.01$) as well as between ST and TT contents ($r = 0.96$, $P < 0.01$) (Table 2). The higher correlation between ST and TT was expected, as 83.2% of TT was derived from soluble condensed tannins. Correlations between ST and TT were also observed by Caldas and Blair (2009), although IT in that study was not correlated with TT.

The minimum amount and maximum limit of condensed tannins needed for a health benefit in terms of mineral absorption has not been reported. However, according to Gu et al., 2004 the mean daily intake of proanthocyanidins or condensed tannins (PAs) was estimated to be 57.7 mg/person in the U.S. population (>2 y old). Therefore, a 25 g serving of common beans with 10% seed coat and approximately 1–3% total tannin based on our results, may affect intake levels substantially highlighting the importance of considering bean tannin levels in breeding or food processing. Apart from bean consumption, Hurrell, Reddy, and Cook (1999), suggested that any beverage providing 20–50 mg total polyphenols would reduce Fe absorption from a bread meal by 50–70%, whereas beverages containing 100–400 mg total polyphenols would reduce Fe absorption by 60–90%. These reports demonstrate the impact of higher polyphenols in mineral bioavailability, as well as the importance of determining the amount and type of polyphenols present in staple foods. Likewise, it is important to determine the effect of the different food processing methods on anti-nutrients such as condensed tannins in different food products.

3.2. Variability in tannin monomers

The chromatographic HPLC analysis of the samples treated with butanol-HCl showed the presence of three major anthocyanidins: delphinidin, cyanidin, and pelargonidin indicating the presence of three flavan-3-ols or monomers: gallocatechin, catechin, and afzelechin. The retention times for delphinidin, cyanidin, and pelarg-

Table 1
Average percentage condensed tannin and anthocyanin in seed coats and relative percentage tannin monomers of parents and recombinant inbred lines (RILs) in the DOR364 × G19833 population of common bean.

Trait	DOR364 (P1)	G19833 (P2)	RILs (average)	Range
Soluble tannin	17.82 ± 1.36[a]	19.03 ± 0.96	16.99 ± 1.11*	8.0–27.9
Insoluble tannin	5.11 ± 1.37**	2.42 ± 0.67**	3.42 ± 0.76*	1.5–5.4
Total tannin	22.94 ± 1.14	21.45 ± 1.52	20.04 ± 1.29*	10.7–30.9
Galocatechin monomer	14.86 ± 1.82[b]	10.65 ± 1.56	24.99 ± 1.68*	14.9–49.1
Catechin monomer	56.50 ± 5.23	79.08 ± 5.41	60.34 ± 3.21*	22.5–79.1
Afzelechin monomer	28.64 ± 2.32	10.28 ± 1.26	14.67 ± 1.13*	1.51–32.8
Anthocyanins	0.208 ± 0.021**	0.013 ± 0.004**	0.080 ± 0.012*	0.01–0.21

[a] Mean ± standard deviation.
[b] Relative percentage of each monomer according to the identified anthocyanidin.
* Significant differences between RILs at $P < 0.01$.
** Significant differences between the two parents at $P < 0.01$.

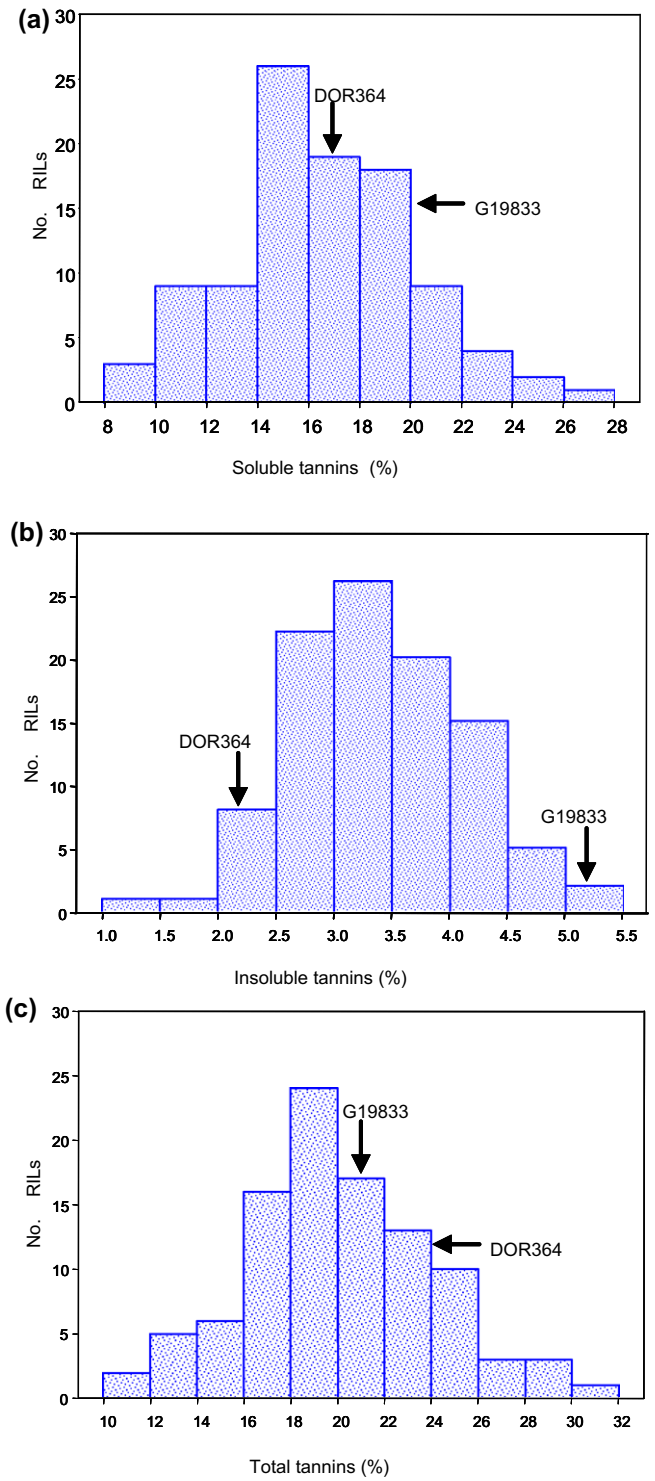

Fig. 1. Population distribution of soluble, insoluble and total condensed tannin contents among the DOR364 × G19833 recombinant inbred lines of common bean.

Table 2
Pearson's simple correlation for soluble, insoluble and total tannins versus anthocyanins in the DOR364 × G19833 population of common bean.

	Total tannins	Soluble tannins	Insoluble tannins
Soluble tannins	0.96**		
Insoluble tannins	–	0.19**	
Total tannins	–	–	0.47**
Anthocyanins	−0.08	−0.05	0.29**

** significant value at $P < 0.01$.

onidin were 2.2, 2.9, and 3.6 min, respectively (Fig. 2). Cyanidin was the principal anthocyanidin, suggesting that the tannins of these genotypes were formed mainly by the catechin monomer. Delphinidin, derived from the gallocatechin monomer was the second most important anthocyanidin. Meanwhile, pelargonidin was significant in only some cases. The average relative percentages of each of the tannin monomers among the genotypes analyzed were 25.0, 60.3, and 14.7% for gallocatechin, catechin, and afzelechin, respectively. The range was greatest in absolute terms for catechin but only showed a 3-fold difference between highest and lowest values within the RIL population. Meanwhile, afzelechin showed a 15-fold difference between those values. Most samples followed a similar pattern with catechin and afzelechin as the predominant and least common tannin monomer, respectively .

The type of monomer and the size of the polymer (condensed tannin) are important nutritional factors of beans and other grain crops. Previous studies have shown that flavonoids that complex iron to a higher degree are those possessing 3′, 4′ or 4′-5′-hydroxyl groups in the B-ring (Beninger & Hosfield, 2003; Mira et al., 2002). Hence, the presence of monomers such as catechin and gallocatechin in the condensed tannins of beans versus other constituents is critical to determining the effect of these tannins in human nutrition.

Tannin monomers vary greatly between different crops and different plant tissues. For example, grape seed tannins consist of a complex mixture of oligomers and polymers composed of the monomeric flavan-3-ols (+)-catechin, (−)-epicatechin and (−)-epicatechin-3-gallate (Nuñez, Gomez-Cordoves, Bartolome, Hong, & Mitchell, 2006). In cashew fruit, tannins contained high percentages of (-)-epigallocatechin and (-)-epigallocatechin-*O*-gallate, followed by lower quantities of (-)-epicatechin and (-)-epicatechin-3-*O*-gallate (Michodjehoun-Mestresa et al., 2009). In lentils, a legume similar to beans, the seed coat tannins consisted mainly of catechin and gallocatechin units, with the polymer fraction the most abundant proanthocyanidin (65–75%) compared to monomers and oligomer fractions (Dueñas, Sun, Hernández, Estrella, & Spranger, 2003).

In addition, for legumes with varying seed types, the tannin monomers can also vary between commercial market classes. For example, in common beans proanthocyanidin oligomers of (epi)gallocatechin is present in black-seeded types, but absent in other coloured beans (Aparicio-Fernandez, Yousef, Loarca-Pina, de Mejia, & Lila, 2005). In contrast, white beans are usually considered to have very low tannins (Welch, House, Beebe, & Cheng, 2000). In future studies, it would be necessary to determine the conformational structure of the monomers in various seed classes to define the chelating capacity of condensed tannins on common bean and their effect on iron absorption.

3.3. Anthocyanin contents and distribution

Anthocyanins of seed coats expressed as delphinidin-3-glucoside equivalents ranged from 0.013 to 0.21%. Although this range is apparently wide, most individuals (about 66%) did not possess more than 0.1% anthocyanins in seed coats. The average for the population was 0.08% and the distribution was biased towards low content (Fig. 3). Compared to the RIL genotypes, the parent G19833 presented the lowest percentage of anthocyanins of all the genotypes analyzed (0.013%) while the parent DOR364 had one of the highest anthocyanin content (0.208%). The skewed distribution of the population suggests that low versus high anthocyanin content is an oligogenic trait where only certain alleles contribute to anthocyanin accumulation. In other words, few genotypes showed high anthocyanin content, and the trait is probably caused by the effect of a small number of epistatic genes. Among the parents, DOR364 had higher value than G19833 suggesting

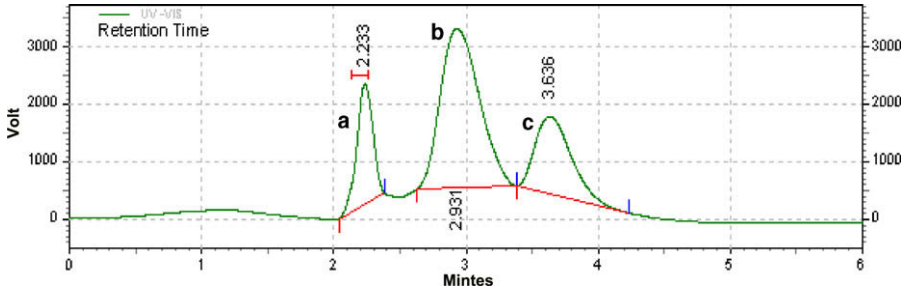

Fig. 2. HPLC chromatogram of a common bean seed coat sample from the DOR364 × G19833 recombinant inbred line population with detection at 525 nm. Anthocyanidins are: (a) delphinidin; (b) cianidin; (c) pelargonidin.

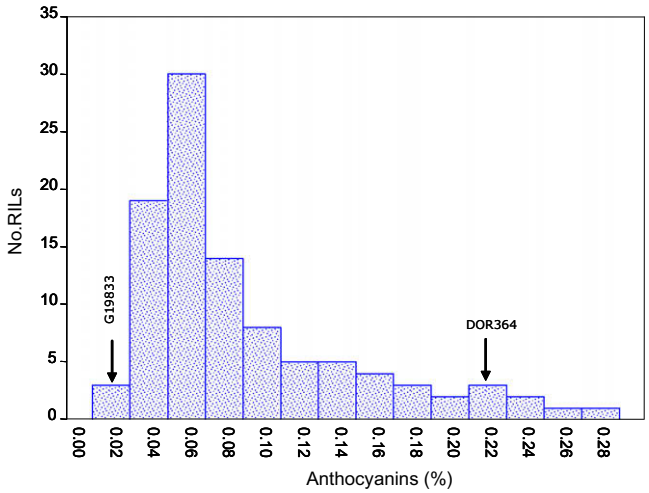

Fig. 3. Population distribution of anthocyanin content among the DOR364 × G19833 recombinant inbred lines of common bean.

that DOR364 with dark red seed coats provides the alleles inducing the manifestation of the trait to a greater extent.

Significant, but low ($r = 0.29$, $P < 0.01$) positive correlation was found for anthocyanins and insoluble tannins but not between anthocyanins and soluble or total tannins (Table 2). In terms of experimental methods, the levels of anthocyanin content in bean seed coat found in this study are comparable to values reported by Takeoka et al. (1997) and Macz-Pop et al. (2006), using extensive methodologies. Our method was comparatively shorter with few steps, as our principal objective was to observe differences between large number of individuals and replicates of the study population, which totalled 267 samples.

Previous studies in black genotypes of common bean identified three types of anthocyanins: delphinidin 3-glucoside (56%), petunidin 3-glucoside (26%), and malvidin 3-glucoside (18%) (Takeoka et al., 1997). Beninger and Hosfield (2003) also found these three anthocyanins in seed coats of other common bean genotypes and concluded that these were the major and most active anthocyanins in terms of anti-oxidant activity in this crop. In addition, the characterization of Korean and Japanese bean cultivars by Choung et al. (2003) and Tsuda et al. (1994), respectively, also suggested the prevalence of pelargonidin 3-glucoside and delphinidin 3-glucoside in red and black genotypes. In general, variation in total anthocyanins content is best expressed as anthocyanidin–glucosides, therefore, we decided to report total anthocyanin content in terms of delphinidin 3-glucoside, probably the most common anthocyanin in bean.

Despite the absence of correlation between anthocyanins and condensed tannins, they are known to be related through a common biochemical pathway with a range of well characterized key enzymes and some regulatory factors. The biosynthesis of anthocyanins is largely regulated at the transcriptional level, and MYB and basic helix–loop–helix (bHLH) proteins are among the regulatory factors which have been characterized (Mol, Cornish, Mason, & Koes, 1999; Springob, Nakajima, Yamazaki, & Saito, 2003). In Arabidopsis, some mutations have caused reductions in proanthocyanin content, while others have shown reduction in both anthocyanins and proanthocyanins (Shirley et al., 1995). In common bean, the *P* locus is a major gene for lack of seed colouration (Prakken, 1970) and has been postulated to regulate the biosynthetic pathways of flavan-3-ols and anthocyanins (McClean, Lee, Otto, Gepts, & Bassett, 2002). However, the recessive allele *p* produces white seed coats with no condensed tannins or anthocyanins (Erdmann, Lee, Bassett, & McClean, 2002). Therefore, other alleles at this locus or independent genes must control anthocyanin accumulation in other coloured beans. Caldas and Blair (2009) showed that different seed colour or pattern genes, notably *Bip, C* and *Z* as well as a new allele of *P* control tannin accumulation in bean seed coats depending on the genetic background analyzed. For this reason we decided to analyze the relationship between the accumulation of condensed tannins or anthocyanins and seed coat colour as part of this research as described below.

3.4. Associations with seed colour

To determine the association of condensed tannins and anthocyanins with seed colour, we performed an analysis of variance using the primary commercial seed colour class as the source of variation for these traits and means separation tests (Table 3). For the three condensed tannin traits, differences were not significant among the colours of the lines analyzed ($p > 0.01$), whereas, for anthocyanins, significant differences were found ($p < 0.01$). In the means separation test for each colour class we found the following results: (a) for soluble condensed tannins, significant differences existed for genotypes among certain colour classes, for example, among purple and brown-coloured genotypes; (b) for insoluble tannins, no significant differences appeared for seed coat colour classes; (c) for total tannins, as for soluble tannins, there were also significant differences that were correlated with those of soluble tannins; and (d) for anthocyanins, significant differences were found when comparing red-seeded genotypes to all other genotypes. These analyses did not take into account the secondary seed colours of each genotype but rather concentrated on the primary seed colour class. Our hypothesis was that primary seed colour determines the majority of seed coat biochemistry while secondary seed colour is limited to small sectors such as stripes or mottles on the seed coat.

Classification by primary colour, therefore, may have influenced the results of the analysis for tannin content among commercial classes. However, the differences in anthocyanin content between different colour classes was highly significant indicated by the high

Table 3
Mean separation by commercial seed colour class of seed coat condensed tannin and anthocyanin content among genotypes from the DOR364 × G19833 population of common bean.

Colour	Genotypes[b]	TS[c]	TI[c]	TT[c]	AN[c]
Cream (2)	15	18.28 B	3.751 A	22.03 B	0.0553 B
Yellow (3)	32	16.86 C	3.141 A	20.00 C	0.0461 B
Brown (4)	11	16.07 C	3.014 A	19.08 C	0.0484 B
Pink (5)	3	16.79 C	3.611 A	20.40 C	0.0620 B
Red (6)	25	16.72 C	3.767 A	20.49 C	0.1617 A
Purple (7)[a]	3	18.89 A	3.583 A	22.47 A	0.0491 B
F value	–	1.24	3.58	1.55	14.16*

TS = soluble tannins, TI = insoluble tannins, TT = total tannins, AN = anthocyanins.
[a] CIAT scale number for seed coat colour of common bean.
[b] Número of genotypes by seed coat colour class.
[c] Values in the same column followed with the same letter are not significantly different with Ryan Einot Gabriel Welsch test.
* Significant differences between colour classes at $P < 0.01$.

F value ($F = 14.16$; $P < 0.01$) in the analysis of variance. Anthocyanin content of commercial seed colour classes (Table 4) showed that most individuals (44.94%) with a low content of anthocyanins (0–0.05%) have seeds that are yellow or yellow in combination with other colours. In contrast, individuals with high anthocyanin content (0.15–0.21%) possessed red seed coats, as found in the parent DOR364. This range was higher than the 0.07% for red beans and 0.21% black bean anthocyanins reported by Choung et al. (2003).

In other species, such as cowpea, chickpea and sorghum, a relationship between tannin content and colour has been described. For example, brown coloured seeds of cowpeas contain more tannins than cream coloured seeds (Tibe, Amarteifio, & Njogu, 2007) while brown-seeded sorghum varieties contained more tannins than white-seeded varieties (Amarteifio, Aganga, & Gabosekwe, 2003). Therefore, it is not surprising that dark purple beans had more tannins than yellow and other light coloured beans in our study. It was unexpected, however, that cream-coloured beans had high tannin content which may be specific to this cross. Ma and Bliss (1978) and Cabrera and Martin (1989) also studied the accumulation of condensed tannins at population level in common beans and chickpea, respectively. In contrast to our study, these authors used some parents with white seed coats, and other parents with dark seed coats to generate populations that were variable for tannin content. In both cases, single genes were found to control white seed coats and the levels of condensed tannins were highly reduced in those genotypes with white seed. Therefore, while condensed tannins do not cause the differences in seed colour they are correlated with seed colour classes, are especially low in white seed and in addition affect seed darkening.

Meanwhile, anthocyanins are known to intervene directly in the colour of bean seed coats and this is especially the case for red and black seed coat colours according to Yoshida et al. (1996), or Takeoka et al. (1997), and in some striped beans according to Choung et al. (2003). This trait is important to consumers who depending on the region of production (for example Central America) often prefer a dark red or purple broth that results from the water soluble nature of the anthocyanins in the cooking pot. In contrast, for white and pale coloured seed coats Choung et al. (2003) found little to no anthocyanins and these results were confirmed in this study.

Table 4
Frequency of anthocyanin level by commercial seed colour class of seed coat condensed tannin and anthocyanin content among genotypes from the DOR364 × G19833 population of common bean.

Colour	Range (%)			
	0–0.05	0.05–0.1	0.1–0.15	0.15–0.21
Cream (2)[a]	8 (53.3%)	7 (46.7%)	–	–
Yellow (3)	22 (68.8%)	9 (28.1%)	1 (3.13%)	–
Brown (4)	7 (63.6%)	4 (36.4%)	–	–
Pink (5)	2 (66.7%)	1 (33.3%)	–	–
Red (6)	–	3 (12%)	10 (40%)	12 (48%)
Purple (7)	1 (33.3%)	2 (66.7%)	–	–

[a] CIAT scale for seed coat colour of common bean.

4. Conclusions

This study found variability for condensed tannin and anthocyanin content and some relationship between these and seed colour. In this regard, the results agree with those of Beninger and Hosfield (1998) and Takeoka et al. (1997) where both components were important to seed coat colour in common beans. This study also found that the inheritance of tannins and anthocyanins in coloured beans is predominantly quantitative with many genes involved although anthocyanin may be controlled by fewer genes related to seed colour pattern or intensity. In addition, we were able to determine which monomer is prevalent in the condensed tannin structure present in common bean. From this information, geneticists can design strategies for selecting varieties that have a positive balance between anti-oxidant activity and anti-nutritional effects of tannins and anthocyanins, thus permitting an increase in the nutritional potential of the seed.

The study of condensed tannins and anthocyanins takes on more importance when considering the impact of these compounds on nutrition. Condensed tannins from beans are known to strongly affect iron bioavailability *in vitro* (Ariza-Nieto, Blair, Welch, & Glahn, 2007) and anthocyanins or polyphenolics are strong anti-oxidants that are thought to contribute to the health properties of beans (Choung et al., 2003). However, further investigations need to be carried out to ascertain whether the condensed tannin content in common bean cultivars affect *in vivo* bioavailability of minerals or proteins in human diets.

Acknowledgements

We wish to thank Agobardo Hoyos and Yercil Viera for field management, Myriam Duque for statistical help, Steve Beebe for germplasm and Patricia Avila, Michael Peters and Carlos Lascano for help with HPLC analysis. This research was supported by the Canadian International Development Agency and by the Harvest Plus Challenge Program.

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