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Effect of Cultivar and Growing Location on the Trypsin Inhibitors, Tannins, and Lectins of Common Beans (*Phaseolus vulgaris* L.) Grown in the Semiarid Highlands of Mexico

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Trypsin inhibitors (TI), tannins, and lectins appear to have a role in preventing chronic diseases in humans. The genetic variability of these traits in common bean needs to be ascertained in order to increase levels through breeding. The variability of TI, tannin, and lectins was determined in five bean cultivars grown at five locations in Mexico. TI and tannins contents in colored beans that belong to the Jalisco race were higher (11.1−11.9 trypsin units inhibited (TUI)/mg and 29.0−38.1 mg catechin equivalent (CE)/g, respectively) than cultivars of the Durango race (7.9−8.3 TUI/mg and 16.8−19.9 CE/mg, respectively). Bayo Victoria, a Durango race cultivar, had three times more lectins than levels reported for soybean. Cultivar influenced TI and tannins contents ($p < 0.001$), whereas site affected lectins ($p < 0.001$). An increase in levels of TI and tannins could be enhanced through breeding.

KEYWORDS: Common bean; *Phaseolus vulgaris*; trypsin inhibitors; tannins; lectins; nutraceutical

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important food legume for the people of Latin America and Africa where animal protein is limited and beans are consumed in large quantities. Deshpande (*1*) reported that this crop contributes 22% of the total protein consumed in the world. In Mexico, Castellanos et al. (*2*) estimated an annual per capita bean consumption of 22 kg and indicated that 74% of the population eat beans 5 days a week.

Common bean is a good source of protein, some vitamins and minerals, and complex carbohydrates. In addition to these nutritional components, it also contains some antinutritional factors such as protease inhibitors, polyphenols, lectins, and phytic acid, among others. However, there is increasing evidence that these antinutritional components convey health benefits to humans by virtue of their role in preventing some diseases; levels of those components that define their nutraceutical or antinutritional role have not been yet established. This is an inherent confrontation that needs to be studied in order to evaluate a hopeful balance between health benefits and antinutritional effects. Soybeans are unique among the legumes because they are a concentrated source of protease inhibitors and lectins. However, because of the colored characteristics of bean seeds, beans exhibited higher tannin contents than soybean. On the other hand, given the chemical composition and high consumption of common beans in developing countries of Latin America, it is important that agronomists, chemists, and nutritionists make a concerted effort to learn the nutraceutical potentials of these legume.

From an antinutritional point of view, low protein digestibility is an important nutritional problem of common bean, which is often attributed to the presence of TI, tannins, lectins, and phytic acid (*3*, *4*). In addition, TI also diminish the protein efficiency ratio (*5*), reduce the gain/feed rate (*6*), and are an occupational inhalant allergen (*7*). Tannins are also responsible for decreases in feed intake, growth rate, and mineral bioavailability (*4*, *8*). Pure lectins apparently induce pancreatic growth (*9*) and produce

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Table 1. Geographic Location, Average Temperature during Growing Season, and Soil Type of Growing Sites of Common Bean Cultivars

site	latitude	longitude	altitude	average temp during growing season (C°)	soil type[a]
Sandovales, Aguascalientes (site 1)	22° 11′	102° 20′	2000	18.4	Xerosol
Satevo, Chihuahua (site 2)	27° 57′	106° 06′	1430	22.4	Regosol
Francisco I, Madero (site 3)	22° 20′	104° 20′	1932	20.2	Xerosol
San Luis de la Paz, Guanajuato (site 4)	21° 13′	100° 29′	2188	17.5	Pheozem
Yahualica, Jalisco (site 5)	20° 59′	103° 48′	1800	21.3	Pheozem

[a] Xerosol and Regosol soil types are characteristics of semiarid regions of low fertility and low organic matter. Pheozem is found in template and tropical regions of intermediate fertility and organic moisture content (*40*).

ulceration and necrosis of the intestinal epithelium in rats (*10*). However, cooking procedures partially inactivate TI, lectins, and tannins in soybean and common bean (*3*, *5*).

Despite the negative aspects of antinutritional factors of beans, these and other compounds currently referred to as phytochemicals (*11*) might provide additional health benefits. Scientific evidence continues to accumulate in support of the role of phytochemicals such as TI, tannins, and lectins in the prevention and treatment of human diseases, particularly in soybean (*12*); the potential health benefits of consuming common bean have largely been overlooked. TI confer protection against rotavirus (*13*), inhibit carcinogenesis (*14*), and can be used as chemopreventive agents (*15*). Moreover, TI have not produced detectable clinical or laboratory toxicity when provided at high doses (*15*). Condensed and hydrolyzable tannins have been shown to be effective antioxidants (*4*, *16*), anticarcinogens (*17*), and antimutagens (*18*, *19*). Bean lectins diminish the growth of non-Hodgkin's lymphoma (*20*) and can be used as diagnostic markers in tumors (*21*).

Genetic variation and environmental effects on the levels of TI, tannins, and lectins in common bean have been reported. Barampama and Simard (*22*) reported that these phytochemicals as well as phytic acid were highly influenced by growing location and genotype. Muzquiz et al. (*23*) indicated that antinutritional factors in common bean are influenced by both environmental and genetics factors. Differences in the concentration of TI, tannins, and lectins among cultivars grown in Mexico and the U.S.A. were also reported (*24*−*27*).

Resolution of genetic and environmental factors affecting the accumulation of TI, tannin, and lectins as well as their consequences in foods and health may enable breeders to select for cultivars that have a balance of health-promoting activities with antinutritional effects. Such cultivars could be used for direct consumption or for clinical or laboratory studies.

The objective of this study was to evaluate the level of TI tannin, and lectin contents in seeds of five bean cultivars grown at five locations in the semiarid highlands of Mexico.

MATERIALS AND METHODS

Common Bean Cultivars. Five popular bean cultivars (*P. vulgaris*) were chosen for this study: Flor de Mayo Criollo, Flor de Mayo M-38, Flor de Junio Marcela (three members of the Jalisco race), Bayo Victoria, and Pinto Villa (two members of the Durango race). Bean cultivars were planted during the 1999 growing season under rainfed conditions at five locations in the semiarid highlands of Mexico: Sandovales; Aguascalientes (site 1); Satevó, Chihuahua (site 2); Francisco I, Madero, Durango (site 3); San Luis de la Paz, Guanajuato (site 4); and Yahualíca, Jalisco (site 5). Geographic location, temperature, and soil type of the five sites are shown in **Table 1**.

Cultivars were planted in a randomized complete block design with four replicates. Planting was done after the onset of the rainy season at the end of June to early July in all sites, and the agronomic practices followed those recommended for the crop in each region. When harvested, seed moisture ranged from 12 to 14%. To adjust moisture content, seed samples were air-dried under laboratory conditions at 23 ± 3 °C and relative humidity of 50 ± 5% for 20 days, reducing the seed moisture to 9 ± 0.5%. Damaged seeds were removed, and samples were stored at 5 °C until chemical analyses were performed. For chemical analysis, flour of whole seeds was utilized.

TI. Trypsin inhibitory activity was measured as the residual tryptic enterolytic activity, using BAPNA (N-benzoyl-DL-arginine *p*-nitroanilide) as substrate (*28*). One TUI is defined as the amount of inhibitor that inhibits 1 μg of pure trypsin. Total trypsin inhibitory activity was expressed as TUI per mg sample (TUI/mg) on a dry weight basis.

Tannins. Tannins were determined according to the modified vanillin−HCl method of Price et al. (*29*) using (+)-catechin (Sigma) as a reference. Tannin content was expressed in mg of CE per g of sample (mg CE/g). To correct for interference of natural pigments in dry bean seed coat, a blank sample was prepared by subjecting the original extract to the reaction conditions without the vanillin reagent.

Lectins. The bean extract used to determine lectin activity (LA) was prepared as described previously (*30*). The dried beans were ground into flour with a Wiley mill (40 mesh). To obtain active lectins, flour was extracted overnight at 5 °C with 50 mM phosphate-buffered saline (100 mL/g flour), pH 7.6, and then centrifuged at 12 000*g* for 20 min to yield the crude extracts. The hemagglutinin assay activity of lectins was determined by 2-fold serial dilution as described by Crowley and Goldstein (*31*) using washed human erythrocytes. LA was expressed as the maximal geometric dilution in which visible macroscopic hemagglutination was observed (HAU/g sample).

Protein. Storage protein concentration was measured by the method of Lowry et al. (*32*) using bovine serum albumin as a standard and was reported as percent protein.

Seed Weight. The weights of 100 randomly chosen seeds were determined and reported as g/100 seeds. The seed moisture of all samples was 9 ± 0.5%.

Statistical Analyses. Analyses of variance (ANOVA) on TI, tannins, and LA were performed with cultivars and sites as fixed effects. Means were separated by LSD at the 5% probability level (*33*). The Statgraphics Plus 5.0 program for microcomputers was used for all analyses (*34*). TI, tannins, LA, protein, and seed weight in raw beans were the average of eight determinations.

RESULTS AND DISCUSSION

Bean Characteristics. Flor de Mayo Criollo, Flor de Mayo M-38, and Flor de Junio Marcela are cultivars that belong to the race Jalisco and exhibit a beige color with pink mottles and stripes on the seed coat (**Table 2**). These cultivars are grown in central Mexico in areas with favorable rainfall patterns. Bayo Victoria and Pinto Villa belong to the race Durango and exhibit a solid cream color (Bayo Victoria) and cream with brown mottles (Pinto Villa) on the seed coats. These cultivars are grown in north central Mexico in semiarid areas with less unfavorable rainfall patterns. The highest seed weight was exhibited by Bayo Victoria ($p < 0.05$), and the protein content was similar among cultivars (**Table 2**).

Table 2. Seed Color, Seed Weight, Protein Content, and Race of Five Bean Cultivars Grown at Five Locations in the Semiarid Highland of Mexico

cultivar	seed color	seed weight (g/100 seeds)	protein content (%)	race[a]
Flor de Mayo Criollo	CPM[b]	30	21.4	Jalisco
Flor de Mayo M-38	CPM	28	20.5	Jalisco
Flor de Junio Marcela	CPS[c]	34	20.4	Jalisco
Bayo Victoria	beige	44	19.6	Durango
Pinto Villa	CBM[d]	34	20.2	Durango
LSD[e]		5	1.8	

[a] The Jalisco race includes subhumid highlands adapted full season bean genotypes while the Durango race included semiarid highland adapted early maturing bean genotypes (*41*). [b] Cream with pink mottles. [c] Cream with pink stripes. [d] Cream with brown mottles. [e] LSD ($p < 0.05$). Seed weight and protein content values are the averages from all five growing sites.

Table 3. TI in the Seeds of Five Bean Cultivars Grown at Five Locations in the Semiarid Highlands of Mexico[a]

	TIs (TUI/mg)					
cultivar	site 1	site 2	site 3	site 4	site 5	average
Flor de MayoCriollo	10.2	9.6	12.2	12.4	11.1	11.1
Flor de Mayo M-38	11.9	12.5	10.3	13.6	11.1	11.9
Flor de Junio Marcela	11.6	10.9	11.3	11.4	14.5	11.9
Bayo Victoria	9.2	7.0	7.1	8.4	10.0	8.3
Pinto Villa	6.3	6.7	6.8	8.4	10.8	7.9
average	9.8	9.3	9.5	10.8	11.5	
LSD[b] among cultivars = 1.97						
LSD among sites = 3.01						

[a] Site 1, Sandovales, Aguascalientes; site 2, Satevó, Chihuahua; site 3, Francisco I, Madero, Durango; site 4, San Luis de la Paz, Guanajuato; and site 5, Yahualica, Jalisco. [b] LSD ($p < 0.05$).

TI. On average, the content of TI was higher at site 5 with 11.5 TUI/mg. However, this value was not statistically different from the content found in the rest of the growing sites (**Table 3**). The concentration of the TI in the five cultivars ranged from 6.3 TUI/mg for Pinto Villa at site 1 to 14.5 TUI/mg for Flor de Junio Marcela at site 5. These TI levels are considerably lower than those of crude soybean (70−85 TUI/mg) (*35*). On average, cultivars Bayo Victoria (8.3 TUI/mg) and Pinto Villa (7.9 TUI/mg) exhibited the lowest levels of TI ($p < 0.05$) (**Table 3**). The cultivars with the highest content of TI ($p < 0.05$) were those from the Jalisco race: Flor de Mayo Criollo (11.1 TUI/mg), Flor de Mayo M-38 (11.9 TUI/mg), and Flor de Junio Marcela (11.9 TUI/mg). Our results agree with those of Guzman-Maldonado et al. (*26*) who found that cultivars with colored seeds (red, pink spotted, and yellow) displayed higher levels of TI in comparison to cultivars of a light cream color such as those of Bayo Victoria and Pinto Villa.

Levels of TI in the cultivars investigated in this research were slightly lower than those reported by Barampama and Simard (*22*) and Guzman-Maldonado et al. (*26*) in common bean grown in Burundi and Mexico, respectively. These differences could be attributed to the effects of the genotype, growing site, or different analytical methods used in this study and in previous papers (*22*).

The lower TI contents are suggestive of why most basic studies on these proteins have been conducted on the soybean seeds. Kadam et al. (*36*) reported that TI displayed high stability when exposed to heat; therefore, despite the lower contents in common beans, the importance of TI cannot be underestimated, particularly in developing countries where there is a high consumption of common bean. The anticarcinogenic and antimutagenic effects of TI might contribute to the minor incidence of cancer that occurs in these countries, as compared to the incidence reported in developed nations (*12*).

Table 4. Tannin Content of Five Bean Cultivars Grown at Five Locations in the Semiarid Highlands of Mexico[a]

	tannin content (mg CE/g)					
cultivar	site 1	site 2	site 3	site 4	site 5	average
Flor de MayoCriollo	24.9	38.6	37.7	17.8	26.4	29.0
Flor de Mayo M-38	37.4	35.5	44.2	36.9	37.3	38.1
Flor de Junio Marcela	34.9	38.1	37.3	17.1	36.8	32.9
Bayo Victoria	20.4	19.2	17.5	16.8	10.1	16.8
Pinto Villa	17.7	18.8	27.2	20.0	15.9	19.9
average	26.9	30.1	32.9	21.6	25.3	
LSD[b] among cultivars = 0.85						
LSD among sites = 1.33						

[a] Site 1, Sandovales, Aguascalientes; site 2, Satevó, Chihuahua; site 3, Francisco I, Madero, Durango; site 4, San Luis de la Paz, Guanajuato; site 5, Yahualica, Jalisco. [b] LSD ($p < 0.05$).

In soybean, TI are found in the cotyledon (*35*); therefore, if this is the case in common bean, the apparent relation between the seed coat color and the observed TI content cannot be explained. Moreover, the fact that the cultivars that belong to the Jalisco race contain higher contents of TI than those from the Durango race needs to be further studied in a larger sample of genotypes.

Tannins. The content of tannins in the cultivars ranged from 10.1 mg CE/g in Bayo Victoria grown at site 5 to 44.2 mg CE/g in Flor de Mayo M-38 from site 3 (**Table 4**). These levels were lower than those reported by Guzman-Maldonado et al. (*26*) since they determined tannins in seed coat. Tannins are higher when determined in seed coat than determined in whole seed (*37*). On the other hand, Barampara and Simard (*22*) and Reddy et al. (*27*) reported higher tannin contents than levels found in the present work in bean cultivars grown in Mexico, Burundi, and the U.S.A., respectively. Because most of the tannins are located in the seed coat and smaller seeds usually have more seed coat area by weight than larger seeds, smaller seeds could also have a higher tannin concentration as it can be noted in our results (**Tables 2** and **4**).

Cultivars Flor de Mayo M-38 and Flor de Junio Marcela showed the highest average tannin contents ($p < 0.05$) of 38.1 and 32.9 mg CE/g, respectively. The tannin content of Flor de Mayo Criollo was similar ($p < 0.05$) to that in Flor de Junio Marcela. Cultivar Bayo Victoria had the lowest level of tannins (16.8 mg CE/g) along with Pinto Villa (19.9 mg CE/mg). Large amounts of tannins are located in the seed coat with low or insignificant amounts in the cotyledons (*38*). Beans with light-colored coats show lower tannin contents than the beans with dark pigmented coats such as black beans (*22*). However, this relationship is controversial; Ma and Bliss (*39*) and Guzman-Maldonado et al. (*37*) did not detect a significant relationship between the seed coat color of the beans and the content of tannins. On the other hand, Barampama and Simard (*22*) found that darken-colored coats showed higher tannin content than beans with a cream or white color. Regardless of the seed coat color, our results showed a higher content of tannins in those cultivars from the Jalisco race in comparison to those from the Durango race.

Despite differences in tannin content among cultivars and growing sites, the presence of tannins in raw or cooked beans

Table 5. Lectins of Five Bean Cultivars Grown in Five Locations of the Semiarid Highlands of Mexico[a]

	lectins (HAU/g)					
cultivar	site 1	site 2	site 3	site 4	site 5	average
Flor de Mayo Criollo	2.5	0.8	2.9	2.3	0.4	1.8
Flor de Mayo M-38	0.9	0.7	3.4	2.5	0.4	1.6
Flor de Junio Marcela	2.0	0.8	1.7	2.4	1.0	1.6
Bayo Victoria	1.9	0.5	3.2	5.5	0.5	2.3
Pinto Villa	1.5	0.3	2.7	2.9	0.4	1.5
average	1.6	0.6	2.8	3.1	0.5	
LSD[b] among cultivars = 1.79						
LSD among sites = 0.96						

[a] Site 1, Sandovales, Aguascalientes; site 2, Satevó, Chihuahua; site 3, Francisco I, Madero, Durango; site 4, San Luis de la Paz, Guanajuato; site 5, Yahualica, Jalisco. [b] LSD ($p < 0.05$).

is of interest if beans are to be used as a nutraceutical. Tannins from common bean show antimutagenic activity against aflatoxin B1 and premutagenic (benzo[*a*]pirene) and mutagenic (1-nitropyrene) agents (*18*, *19*).

Lectins. On average, there were no statistical differences among cultivars for LA. In contrast, the average in the LA from the five growing sites displayed significant statistical differences (**Table 5**); on average, sites 3 and 4 showed the highest levels ($p < 0.05$) of LA with 2.8 and 3.1 HAU/g, respectively, while the lowest levels were detected at sites 2 (0.6 HAU/g) and 5 (0.5 HAU/g). At site 4, the cultivar Bayo Victoria displayed the highest LA ($p < 0.05$) with 5.5 HAU/g. The content of LA was similar to those levels reported by Guzman-Maldonado et al. (*26*) and Barampara and Simard (*22*), who found significant differences in the content of LA among bean cultivars.

The levels of LA found in Bayo Victoria make this cultivar of great interest, since it contains 2−3 times the content of LA reported for soybean (*35*). Pure lectins from raw Bayo Victoria beans could be useful for clinical tests for cancer studies (*20*, *21*).

In the present study, no relationship was observed between protein content and any of the antinutritional factors. Our results agree with those of Barampara and Simard (*22*), who found no correlation between antinutritional factors and protein concentration.

Effect of Cultivar and Site on Levels of Trypsin, Tannin, and Lectins. The combined ANOVA for TI, tannins, and LA including bean cultivars and growing sites show that all main effects were significant, with the exception of lectins activity for cultivars (**Table 6**). The site effect was significant ($p < 0.05$) for TI and tannins and was highly significant ($p < 0.001$) for lectins. The cultivar effect was highly significant ($p < 0.001$) for TI and tannins; meanwhile, site × cultivar interaction was significant ($p < 0.05$) for the three traits studied in the five genotypes.

The sum of squares for each phytochemical illustrates the relative contribution of each source to the total sum of squares (**Table 6**). Cultivar contributed substantially more for TI and tannins than site and site × cultivar interaction. Barampama and Simard (*22*) detected a significant effect of cultivar and environment over the accumulation of TI in the common bean. In contrast, site contributed substantially for lectins activity than cultivar and site × cultivar interaction.

The significant effect of cultivar and site on the concentration of TI has an important implication for the breeding of common bean because it suggests a relatively simple genetic control of these traits. Because the contribution of cultivar to TI concentration was three and half times higher than that of growing site, progress in increasing levels of TI could be made by breeding.

Table 6. ANOVA for TI, Tannins, and LA of Five Cultivars of Common Bean Grown at Five Sites in the Semiarid Highlands of Mexico

source	df	mean square	*F* ratio
	TI		
site	4	16.74	3.11[b]
replicate	2	0.54	1.50[a]
cultivar	4	54.95	10.20[c]
site × cultivar	16	5.39	14.90[c]
residual	48	0.36	
total		77.98	
	tannins		
site	4	281.99	3.33[b]
replicate	2	0.10	0.15[a]
cultivar	4	1182.49	13.95[c]
site × cultivar	16	84.76	129.86[c]
residual	48	0.65	
total		1550.00	
	LA		
site	4	2.13 × E7	13.00[c]
replicate	2	2034.01	0.27[a]
cultivar	4	1.65 × E6	1.00[a]
site × cultivar	16	1.64 × E6	216.91[c]
residual	48	7561.78	
total		2.46 × E7	

[a] Nonsignificant. [b] Significant at $p < 0.05$. [c] Significant at $p < 0.001$.

In the case of tannin content, the contribution of cultivar was three and half times higher than the combined contribution of site and site × cultivar interaction. Ma and Bliss (*39*) found that tannin content has a quantitative inheritance pattern; however, homozygous lines differing in tannin content were identified. They also reported that color, which is controlled by dominant genes and modifiers, is usually but not always associated with higher tannin levels in such a way that breeding for tannin content in common bean seed is feasible.

The contribution of site to LA of common bean was 13 times higher than cultivar and interaction of site × cultivar, suggesting that breeding of this trait could be difficult due to lack of genetic variability and the large environmental effect on the trait. However, further studies are necessary in a larger sample of genotypes.

From the point of view of using beans as a nutraceutical, the selection of cultivars with a higher content of TI and tannins looks promising due to relatively simple genetic control of those and similar traits. Flor de Mayo M-38 and Flor de Junio Marcela cultivars could be used as a source of TI and tannins; meanwhile, Bayo Vicotria could be used as an outstanding source of lectins.

ABBREVIATIONS USED

CE, catechin equivalent; LA, lectin activity; TI, trypsin inhibitors; TUI, trypsin units inhibited; HAU, hemagglutinin activity units; LSD, least significant differences.

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