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# Phenolics, Phytic Acid, and Phytase in Canadian-Grown Low-Tannin Faba Bean (*Vicia faba* L.) Genotypes

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**ABSTRACT:** Thirteen low-tannin faba bean genotypes grown at two locations in north central Alberta in 2009 were evaluated to investigate the variation in seed characteristics, phenolic and phytate contents, and phytase and antioxidant activities and to elucidate the relationship of these components as a breeding strategy. Seed characteristics including color were predominantly genotype dependent. The faba bean genotypes with total phenolic content ranging from 5.5 to 41.8 mg of catechin equiv/g of sample was linearly related to tannin content and the best predictor of antioxidant activity. Phytic acid content and phytase activity varied significantly among genotypes and between locations, ranging from 5.9 to 15.1 g/kg and from 1606 to 2154 FTU/kg sample, respectively. Multivariate data analysis performed on 19 components analyzed in this study using principal component analysis (PCA) and cluster analysis demonstrate that differences in seed characteristics, phenolic components, phytic acid, and phytase are major factors in segregating faba bean genotypes. The relatively low phytic acid content and high phytase activity of these low-tannin faba bean genotypes are beneficial/essential traits for their use in human and animal nutrition.

**KEYWORDS:** phytic acid, phytase, antioxidant activity, phenolics, tannin, zero-tannin, faba beans, genotypes, *Vicia faba* L., seed characteristics, color

## INTRODUCTION

Traditional faba beans have been grown worldwide and adapted successfully in Canada, with the first commercial production in 1972. Since then, Canadian production has been extremely variable during the past decade, ranging from 6000 to 17000 tonnes per year, but trending upward. During that time, considerable progress in plant breeding resulted in the development of tannin-free faba bean genotypes,[1] thereby creating the potential for a new crop that could extend the geographical production area in western Canada, particularly in north central Alberta.[2] This dry land area with sufficient rainfall is important for tannin-free faba bean cultivation because it benefits growers in that region through high yield and the high protein preferred by hog producers. Zero-tannin or tannin-free faba beans contain about 1% tannin, compared to 8−9% tannins in traditional or regular faba beans that are bitter to hogs, thereby restricting feed intake.[3] Since 2002, tannin-free faba bean production has increased due to its high yield, protein, and energy, a reduction in antinutritional factors (e.g., tannin) and similar production costs, higher nitrogen fixing capacity, and increased convenience and flexibility at harvest relative to field pea (*Pisum sativum* L.), the traditional pulse crop of the Canadian prairies and the dryland areas of the Peace region.[4]

Tannin-free faba beans are superior to tannin-containing faba beans as a protein source for monogastric animals as they do not contain antinutritional factors.[2] In Europe, where most of the breeding occurred, faba bean ranks presently second among legume crops in area and production after pea.[5] Interest in tannin-free faba bean as a nitrogen-fixing high-protein feed grain, especially for hogs and poultry, is growing to such an extent that it is expected (forecasted) to comprise as much as 500 000 acres by 2025, becoming the second most important pulse crop in the Canadian prairies.[6]

Faba bean is known for its large (wide) genetic variability and high yield instability, depending on the environment.[5] This has led to the development of desired tannin-free phenotypes suitable for each region (ideotypes), particularly in Europe. Some of these phenotypes along (together/in addition) with those developed through the Canadian breeding programs have been evaluated for agronomic performance in the faba bean cooperative trials in western Canada since 2006. [3,7,8] These studies indicate significant environmental effects on variability in yield performance of the faba bean genotypes. Our investigation focused on determinants of variability in seed characteristics and selected secondary metabolites of these tannin-free faba beans in two selected north central Alberta locations (Barrhead and Namao) because the production of the ideotypes could serve the protein feed supplement markets and possibly human consumption markets.

## MATERIALS AND METHODS

Seeds for this study obtained from Alberta Agriculture and Rural Development were from the Faba bean Coop Regional Yield Trial

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Table 1. Physical Characteristics of Faba Bean Genotypes[a]

| | dimensions | | | | | |
|---|---|---|---|---|---|---|
| | length (mm) | width (mm) | thickness (mm) | sphericity (%) | Tsw[b] (g) | HC[c] (%) |
| genotype[d] | | | | | | |
| AO1155 [W,F] | 10.40d | 8.30 h | 6.71f | 89.56bc | 416.6de | 123.08c |
| AZ10 [W] | 10.17d | 8.63fg | 6.69f | 92.32a | 429.8d | 70.13ef |
| Disco [W,F] | 11.35c | 9.64bc | 7.60a | 92.41a | 623.8b | 69.68ef |
| Divine [C,F] | 11.89b | 9.50cd | 7.38bc | 89.71bc | 602.4b | 74.68e |
| Fatima [C] | 11.41c | 9.33de | 7.28cd | 90.62b | 550.0c | 103.08d |
| FB25-56 [W] | 10.03d | 7.82i | 6.46g | 88.46cd | 379.2ef | 97.20d |
| Florent [C] | 12.86a | 9.87ab | 7.51ab | 87.62d | 693.8a | 149.83a |
| Imposa [W] | 12.19b | 10.04a | 7.35bc | 90.85b | 644.3b | 140.43b |
| Melodie [C,F] | 11.94b | 8.42gh | 7.06e | 84.01e | 507.6c | 97.80d |
| NPZ4-754 [W] | 11.41c | 9.14e | 6.81f | 89.65bc | 504.8c | 95.33d |
| Snowbird [W] | 11.41c | 9.26de | 7.08de | 90.17b | 518.4c | 103.25d |
| SSNS-1[C] | 9.20e | 7.49j | 6.70f | 90.33b | 357.2f | 64.53f |
| Taboar [C] | 11.07c | 8.86f | 7.18cde | 89.67bc | 527.0c | 50.68g |
| locations | | | | | | |
| Barrhead | 11.47x | 9.10x | 7.13x | 89.23y | 544.9x | 87.69y |
| Namao | 10.89y | 8.79y | 7.00y | 90.06x | 494.3y | 103.02x |
| overall mean | 11.18 | 8.94 | 7.06 | 89.64 | 519.0 | 95.36 |
| CV[e] (%) | 9.89 | 7.89 | 6.95 | 4.07 | 20.39 | 5.99 |

[a] Means in a column with different letters are significantly different ($p < 0.05$). [b] Thousand seed weight. [c] Hydration capacity. [d] W, white flowered; C, colored flower; F, Fevita trademark types. [e] Coefficient of variation.

grown in a replicated randomized complete block design at two locations (Barrhead, 54° 6′ N, 114° 17′ W; and Namao, 53° 4′ N, 113° 29′ W) in the Parkland semiarid region of north central Alberta, Canada, in 2009. The 13 faba bean (*Vicia faba* L.) genotypes (Table 1) used in this study included AO1155, AZ10, Disco, Divine, CDC Fatima, FB25-56, Florent (NPZ3-7080), Imposa (CEB 04928), Melodie, NPZ4-7540, Snowbird, CDC SSNS-1, and Taboar. Divine, Fatima, Florent, Melodie, SSNS-1, and Taboar are colored flower genotypes. AO1155, Disco, Divine, and Melodie are low-tannin and vicine−convicine genotypes known as “double zero” with the Fevita trademark registration. The name of the genotypes is used hereafter without the prefix. All seed samples were ground in a hammer mill (IKA-Werke, S1, GmbH & Co., KG, Staufen, Germany) at 6000 rpm to pass a 1 mm screen. Moisture was determined according to the AOAC vacuum oven method.[9]

**Seed Characteristics.** Seed dimensions were determined from 25 randomly drawn seed samples as described previously.[10] The length, width, and thickness of 25 randomly selected seeds for each sample were measured to 0.05 mm using a Digimatic caliper (Mitutoyo Canada Inc., Mississauga, ON, Canada). Kernel or seed weights were determined with an analytical balance, reading weights in grams to four decimal places with an accuracy of ±0.2 mg and expressed as thousand seed weight (Tsw). Sphericity was estimated on the basis of length and width as sphericity (%) = $[(b/a)^{1/2}] \times 100$, where $a$ and $b$ are the seed length and width (mm), respectively.[11]

Water hydration capacity was determined by soaking 50 seeds in deionized water at a 1:4 (sample/water, w/w) ratio at room temperature for 16 h in accordance with method AACC 56-35.[12] After the water was drained, the soaked seeds were blotted dry with a paper towel and weighed. Hydration capacity was expressed on weight percent basis in accordance with the AACC method.[12] The assay was duplicated for each sample.

A Chromameter tristimulus color analyzer (model CR-200, Minolta, Osaka, Japan) was used to measure the color of seed and ground samples in terms of Cielab scale parameters $L$, $a$, and $b$. $L$ represents whiteness, whereas $a$ and $b$ indicate red−green and yellow−blue, respectively. Chroma $C$ ($C = \sqrt{a^2 + b^2}$) and $\Delta E$ $[(\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}]$, representing total chromaticity and total color difference, respectively, between the standard and sample were calculated for the comparison of color changes between samples. Prior to the analysis, the chromameter was calibrated against a standard white reference tile ($L$ = 92.87, $a$ = 0.3178, $b$ = 0.3346). Eight replicates of color measurements were performed for each sample.

**Extraction and Analysis of Phenolics.** Ground samples (200 mg) were extracted with aqueous ethanol 80% (v/v) (8 mL), using a Reacti-Therm heating and stirring module (N 18970, Pierce, Rockford, IL) at 22 ± 1 °C for 2 h and filtered (Acrodisc 0.45 μm, VWR International, Mississauga, ON, Canada). The recovered supernatant was stored at −20 °C in the dark until analysis. All extractions were carried out in duplicate.

The phenolic content of extracts was determined according to the procedure described previously.[13,14] Briefly, the method consisted of adding 100 μL of sample extract with 150 μL of a solution of 2% HCl in 80% ethanol in a 96-well ultraviolet flat-bottom plate (Greiner Bio-One Inc., Longwood, FL). The absorbance of the solution was monitored at 280, 320, 360, and 520 nm after mixing for 3.5 min with a spectrophotometer (Spectramax Plus 384, Molecular Devices Corp., Sunnyvale, CA) using (+)-catechin (0−244 μg/mL), caffeic acid (0−37 μg/mL), quercetin (0−30 μg/mL), and cyanidin-3-glucoside (0−80 μg/mL) as standards for total phenolics, tartaric esters, flavonols, and anthocyanins, respectively. Standards were prepared in aqueous ethanol 80% (v/v). The absorbance was also read at 710 nm for turbidity correction, and the results were expressed in milligrams of (+)-catechin, caffeic acid, quercetin, or cyanidin-3-glucoside equivalents per gram of sample.

Tannin content was determined as described previously[15] using insoluble polyvinylpolypyrrolidone (PVPP; Sigma-Aldrich Canada Ltd., Oakville, ON, Canada). Briefly, 80% ethanol extract (1 mL) was added to 50 mg of PVPP, stirred for 20 min at 22 °C, and then microfuged (14000 rpm, 5 min, Mini spin plus, Eppendorf, Hamburg,

Germany) prior to determination of total phenolics. Tannin content was calculated as the difference between total phenolics and non-tannin phenolics.

**Antioxidant Assay.** Antioxidant activity was measured using the oxygen radical absorbance capacity ($ORAC_{-FL}$) described previously,[16] according to established procedure.[17] A SpectraMax GeminiEM microplate fluorescence reader (Molecular Devices Co.) was used with excitation and emission wavelengths at 485 and 530 nm, respectively. Sample extracts and Trolox standards were diluted with 75 mM phosphate buffer (pH 7.4) prior to transfer to a 96-well microplate (Fluotrac 200, Greiner Bio-One Inc.). A peroxyl radical was generated by 2,2′-azobis(2-methylpropionamide) dichloride (AAPH; Sigma Aldrich, St. Louis, MO) during measurement, and fluorescein was used as the substrate. Measurements were taken every 2 min for 120 min upon addition of AAPH. Final ORAC values were calculated using a regression equation between the Trolox concentration (0−4 μg/mL) and the net area under the curve (AUC in μM Trolox equivalents (TE)/g sample) and converted to milligrams per gram of sample.

**Assay of Phytic Acid.** Phytic acid content was determined on the basis of the modified procedure of Latta and Eskin,[18] using poly prep prefilled chromatographic columns (Bio-Rad Laboratories, Richmond, CA) containing an AG-1-X8 anion exchange resin (100−200 mesh chloride form 0.8 × 4 cm) allowing isolation of phytate from bean extract. Briefly, ground bean sample (1 g) was extracted with 2% HCl (20 mL) by constant magnetic stirring (RT15 IKA-Werke GmbH & Co., Germany) for 1 h at room temperature. After centrifugation (18000*g*, 20 min; Sorvall RC5B, Dupont Co., Wilmington, DE), the supernatant was diluted 25 times with distilled water, and 10 mL of the diluted supernatant was added to the column. Interfering compounds and inorganic phosphorus were removed by washing with distilled water (10 mL) followed by 0.1 M NaCl (15 mL). Bound phytate was eluted with 0.7 M NaCl (3 × 10 mL), and an aliquot of the eluate (1.5 mL) was vortexed with 0.5 mL of Wade reagent (0.03% (w/v) iron(III) chloride, 0.3% (w/v) sulfosalicylic acid). Absorbance of the salicylate−Fe(III) complex was monitored at 500 nm using a microplate reader (SPECTRAmax Plus$^{384}$, Molecular Devices Corp.). The concentration of phytic acid and IP6 equivalent was calculated from a similarly prepared standard curve obtained with sodium phytate (0−50 μg/mL, Sigma-Aldrich Canada Ltd.) and $IP_6$ standard solution (0−40 μg/mL, D-myoinositol, 1312450-1, Calbiochem, Germany), respectively.

**Phytase Assay.** The assay for phytase activity described previously[16,19] was modified for both sample preparation and phytic acid digestion. Briefly, ground bean sample (1 g) was stirred for 10 min in 10 mL of 0.2 M citrate buffer (pH 5.5) and centrifuged (15000*g*, 20 min, 4 °C), and a portion of the supernatant (0.2 mL) was incubated in a water bath (37 °C) for 5 min. Sodium phytate solution (0.2 mL of 9 mM, Sigma-Aldrich Canada Ltd.) in 0.2 M citrate buffer was added to the supernatant to start enzymatic phytate hydrolysis. Incubation was continued for 15 min, and the reaction was stopped by adding 0.4 mL of 15% trichloroacetic acid. The mixture was microfuged (14000 rpm, 10 min, Mini spin plus, Eppendorf) prior to detection of free inorganic phosphate at 640 nm using a colorimetric detection kit (SensoLyte Malachite Green Phosphate Assay kit, AnaSpec, San Jose, CA). A calibration curve was prepared from known phytase activity standard solutions (0.01−0.25 FTU/2 mL, from *Aspergillus niger*, BASF, Ludwisgshafen, Germany). Data expressed in phosphate were converted to phytase activity (FTU/kg sample). One phytase activity unit (FTU) is defined as the quantity of enzyme required to liberate 1 μM/min inorganic phosphate from sodium phytate at 37 °C.

**Statistical Analysis.** At least three determinations were made for all assays, except for antioxidant activity, which was determined in duplicate. The main effects of genotype and location and their interaction were analyzed independently and considered as fixed effects. Analysis of variance by the general linear models (GLM) procedure, means comparison by Duncan's test, Pearson correlation, and principal component analysis (PCA) were performed according to Statistical Analysis System, SAS 9.1 for Windows.[20] Cluster analysis was performed using SYSTAT 12 version 12.02 for Windows,[21] using hierarchical clustering with Ward or average (linkage) and Euclidean (distance).

## RESULTS AND DISCUSSION

Faba bean genotypes differed significantly in seed characteristics evaluated in this study (Table 1). Florent and SSNS-1 represented faba bean genotypes with the highest and lowest length, width, thickness, thousand seed weight, and hydration capacity, respectively (Table 1). Seed length was greatest (>11.8 mm) for Divine, Florent, Imposa, and Melodie; intermediate (11.0−11.5 mm) for Disco, Fatima, NPZ4-7540, Snowbird, and Taboar; and least (<10.5 mm) for AO1155, AZ10, FB25-26, and SSNS-1. Thickness was highest and lowest for Disco and FB25-56 (7.6 and 6.4 mm), respectively. Overall variability in sphericity was low (4.07 coefficient of variation (CV), $p < 0.0001$, $F$ value = 17.3) with Disco and AZ10 at extremely high and Melodie and Florent at low sphericity.

Faba beans were generally grouped into large-seeded (>600 mg; Disco, Divine, Imposa, and Florent) medium-sized seed (500−550 mg; Fatima, Melodie, NPZ4-7540, Snowbird, and Taboar), and small seeded (<500 mg; AO1155, AZ10, FB25-56, and SSNS-1) genotypes, with a similar grouping obtained by average Euclidean cluster analysis based on the physical characteristics (length, width, thickness, sphericity, and thousand seed weight). The thousand seed weights of Fatima, Florent, and Taboar were within the range, whereas those of Imposa and Snowbird were less than those reported previously.[22] However, thousand seed weight of Snowbird was similar to that grown over three site years.[23] Seed weight of faba bean genotypes (Table 1) was on average 6% higher except AZ10 and NPZ4-754 (8 and 10% lower) than those reported earlier.[7,8] All genotypes except SSNS-1 grown at Barrhead had considerably higher seed weight (1.8−16.5%) than those grown at Namao. This contrast with the consistently higher (34 and 19%) yield performance of these genotypes in Namao compared to those at Barrhead observed in the 2008 and 2009 Faba bean Co-operative trials in western Canada.[7,8] Seed weight was therefore not associated with yield because seed size is a highly variable yield component differing with environmental conditions and genotypes.[2] Seed weight of Florent, Fatima, Imposa, and Snowbird in Namao and AZ10, NPZ4-754, and Imposa grown at Barrhead was similar to those reported previously.[7,22] Seed size of the genotypes was similar to those described as medium to relatively small round seeds (0.4−0.8 g), grown mainly for dry seeds used for animal feed or human food.[24] The highest hydration capacity of Florent was 3 times that of the lowest genotype Taboar and twice those of Divine, AZ10, Disco, and SSNS-1. Mean hydration capacity (95%) was only two-thirds (66%) of the large-seeded Sicilian landrace reported earlier.[25]

Analysis of variance (data not shown) showed that the physical parameters of faba bean from two locations were dependent on genotypes and location. The main effects were highly significant ($p < 0.0001$) for length, width, thickness, and thousand seed weight. The variance was predominantly associated with genotype and genotype × location interaction contributing to 51 and 92% of the total variation in width and hydration capacity, respectively. Location had negligible effect on the variation of physical characteristics because its variance was

Table 2. Color Characteristics of Faba Bean Genotypes[a]

| | color | | | | |
|---|---|---|---|---|---|
| | $L$ | $a$ | $b$ | $C^b$ | $\Delta E^c$ |
| genotype | | | | | |
| AO1155 | 24.38cd | 0.367ef | 0.380ef | 0.528ef | 68.49ab |
| AZ10 | 34.35a | 0.361f | 0.378f | 0.522g | 58.53d |
| Disco | 22.23cd | 0.376d | 0.389bc | 0.541d | 70.59ab |
| Divine | 23.44cd | 0.392bc | 0.387bcd | 0.551ab | 69.43ab |
| Fatima | 17.71d | 0.401a | 0.384cde | 0.556a | 75.16a |
| FB25-56 | 32.09ab | 0.365ef | 0.378f | 0.526fg | 60.78cd |
| Florent | 34.86a | 0.388c | 0.389bc | 0.549bc | 58.01d |
| Imposa | 31.13ab | 0.378d | 0.395a | 0.545cd | 61.74cd |
| Melodie | 22.14cd | 0.395ab | 0.391ab | 0.556a | 70.73ab |
| NPZ4-7540 | 24.08cd | 0.370e | 0.383def | 0.533e | 68.79ab |
| Snowbird | 24.79c | 0.366ef | 0.381ef | 0.531ef | 68.08b |
| SSNS-1 | 21.12cd | 0.396ab | 0.388bc | 0.555a | 71.75ab |
| Taboar | 26.94bc | 0.398ab | 0.390ab | 0.557a | 65.93bc |
| overall mean | 26.09 | 0.38 | 0.39 | 0.54 | 66.78 |
| CV | 31.74 | 2.22 | 1.68 | 1.40 | 12.40 |
| locations | | | | | |
| Barrhead | 32.47x | 0.373y | 0.381y | 0.533y | 60.40y |
| Namao | 19.50y | 0.389x | 0.390x | 0.551x | 73.37x |

[a] Means in a column with different letters are significantly different ($p < 0.05$). [b] Chroma. [c] Total color difference.

smaller than that of the experimental error. Our results contrast with the study of the Sicilian large-seeded faba bean landrace,[25] for which significant differences in genotypes were observed only in length, width, and weight, but not in thickness and hydration capacity of the seed.

The color parameters of faba bean differed significantly ($p < 0.0001$) among genotypes and between locations covering the spectrum from brown to beige with mean $L$ (lightness/darkness), $a$ (greenness/redness), and $b$ (yellowness/blueness) values of 26.1, 0.38, and 0.39, respectively (Table 2). Genotypes Florent and Fatima had extreme $L$ and $\Delta E$ values, whereas AZ10 consistently had the lowest $a$, $b$, and $C$ values in combination with a high $L$ value, resulting in the lowest total color difference ($\Delta E$). Faba bean grown at Namao had significantly higher, $a$, $b$, and $\Delta E$ and lower $L$ seed values, indicating a considerably darker seed color than those from Barrhead. Principal seed color varied in segregating ratio, particularly for the colored flower genotypes Fatima, Florent, Melodie, SSNS-1, and Divine (6, 5, 4, 3, 3 brown, respectively; 1 beige), Snowbird (4 pale green; 1 green), and Taboar (2 brown; 1 pale green), indicating strong allelic interactions or epistatic effects reported previously.[26] Seed color of the white-flowered genotypes AO1155, AZ10, FB25-56, Disco, Imposa, and NPZ4-7540 was monomorphic and dominantly green. Cluster analysis segregated the genotypes into three distinct groups primarily based on $L$ value: dark, Disco, Fatima, Melodie, and SSNS-1 ($L < 23$); medium, AO1155, Divine, NPZ4-7540, Snowbird, and Taboar ($L = 23-27$); and light, AZ10, FB25-56, Florent, and Imposa ($L > 30$). Analysis of variance for color of faba bean seed grown at two locations showed that genotype accounted for 51 and 39% of the total variation in $a$ and $C$ values, respectively. Location contributed mainly to 44, 28, 40, and 44% of the total variation in $L$, $a$, $C$, and $\Delta E$ values, respectively.

Phenolic content of faba bean differed significantly ($p < 0.0001$) among genotypes (Table 3) and between locations with FB25-56 and Disco expressing the highest and lowest total phenolics and tannins, respectively. The white-flowered faba bean "double zero" genotypes Disco, Divine, and Melodie, homozygous for the recessive genes *zt1* and *zt2*, had the least phenolic and tannin contents. Total phenolic content of faba bean genotypes (except Disco, Divine, and Snowbird) grown at Barrhead significantly exceeded those from Namao. A change in genotypic ranking occurred in total phenolics between the two locations for genotypes AO1155 and Florent, Divine, and Melodie, Snowbird, and SSNS-1 as a consequence of genotype × location (G × L) interactions. Tannin represented on average about 13%, with Disco an order of magnitude lower than that of the total phenolic content. Differences in tannin content were not significant among the high-phenolic (5.0 ± 0.15 mg/g catechin equiv) Florent, Imposa, Snowbird, SSNS-1, and Taboar and low-phenolic (0.67 ± 0.15 mg/g catechin equiv) faba bean genotypes Melodie, Divine, and Disco. Similar tannin contents were reported previously[27] for white−green Albatross and VT-16 and brown Alfred and Herz Freya genotypes (0.88, 0.81, 4.6, and 5.7 mg/g catechin equiv, respectively). The environmental effect and the G × L interactions for tannin were similar to those observed for total phenolic with tannin content of faba bean genotypes (except Divine and Snowbird) significantly higher at Barrhead than for those from Namao. Genotypes Divine, Disco, and Melodie with the lowest total phenolic contents also expressed the lowest tannin content. Conversely, high-tannin genotypes reflected the presence of high total phenolic content, resulting in a strong linear relationship ($y = 6.7816x + 2.3032$, $r^2 = 0.952$, $p < 0.0001$) and confirming a similar recent report between total polyphenols and condensed tannins in faba beans.[28] The tannin content of genotype Divine (0.76 mg/g catechin equiv) was only a third of that reported for that genotype.[29] The simpler method of phenolic determination used in this study is evidently appropriate for screening tannin content.

Variations in total phenolics (5.5−41.8 mg/g catechin equiv) of faba bean genotypes were 7-fold those of tannins (0.46−5.84 mg/g catechin equiv) and minimal for tartaric esters and flavonols. Interestingly, Disco, unlike the other "double-zero" genotypes, had the highest tartaric ester and flavonol contents and the lowest anthocyanin concentration. The genetic variability in tannin content confirms previous studies in Sicilian faba bean landrace[25] and in Canadian-grown faba bean.[30] Previous studies showed no significant location effect on phenolic content of faba bean genotypes grown in Italy,[31] and only small differences in condensed tannin concentrations (5.16, range = 4.8−5.64 g/kg) among different genotypes grown in replicated plots in six different locations in Manitoba and Saskatchewan in 1972.[30]

Antioxidant activity was genotype dependent, ranging from 37.9 to 76.3 mg Trolox equiv (TE)/g dry matter), but unaffected by location. This indicates that the genotypes had discrete physiological adaptive mechanisms to regulate their levels of oxidative stress in terms of their antioxidant activities. Low-phenolic and -tannin faba bean genotypes exhibited the lowest antioxidant activity (37.9−40.2 mg TE/g dry matter) and may therefore offer only limited protection from increases in reactive oxygen species due to abiotic stress. Values for antioxidant activity were lower than those reported for a white faba bean genotype[28] and most dry beans.[14]

Table 3. Phenolic Content and Antioxidant Activity of Faba Bean Genotypes

| | concentration[a] | | | | | |
|---|---|---|---|---|---|---|
| | total phenolics | tannins | tartaric esters | flavonols | anthocyanins | AA[b] |
| genotypes | | | | | | |
| AO1155 | 30.94g | 4.35e | 0.155c | 0.148a | 14.04abc | 55.73c |
| AZ10 | 34.99e | 4.63cde | 0.135e | 0.128c | 13.78abcd | 62.78b |
| Disco | 5.59i | 0.50f | 0.176a | 0.153a | 8.13f | 39.98e |
| Divine | 6.18h | 0.76f | 0.123f | 0.095e | 11.57de | 37.88e |
| Fatima | 31.77f | 4.33e | 0.127f | 0.126c | 12.47bcde | 46.09d |
| FB25-56 | 40.63a | 5.42a | 0.180a | 0.140b | 12.47bcde | 46.54d |
| Florent | 30.92g | 4.91bcd | 0.101g | 0.103d | 10.62e | 44.70d |
| Imposa | 37.78b | 4.98abcd | 0.135e | 0.107d | 11.62de | 76.30a |
| Melodie | 6.17h | 0.76f | 0.147d | 0.104d | 14.07ab | 40.15e |
| NPZ4-7540 | 35.33e | 4.47de | 0.162b | 0.136b | 11.83bcde | 62.79b |
| Snowbird | 37.76b | 4.86bcd | 0.125e | 0.125c | 11.70cde | 53.20c |
| SSNS-1 | 36.74c | 5.06abc | 0.150cd | 0.135b | 15.46a | 55.95c |
| Taboar | 36.06d | 5.23ab | 0.133e | 0.128c | 13.24abcd | 60.33b |
| locations | | | | | | |
| Barrhead | 29.35x | 4.14x | 0.143x | 0.125x | 13.39x | 52.33x |
| Namao | 27.62y | 3.59y | 0.141x | 0.125x | 11.27y | 52.73x |
| overall mean | 28.48 | 3.86 | 0.14 | 0.13 | 12.33 | 52.54 |
| CV[c] (%) | 1.96 | 16.99 | 5.39 | 6.91 | 22.24 | 6.82 |

[a] Means in a column with different letters are significantly different ($p < 0.05$). Concentrations of phenolic compounds are expressed as milligram equivalents of (+)-catechin, caffeic acid, or quercetin or as microgram equivalents of cyanidin-3-glucoside per gram of sample for total phenolics, tartaric esters, flavonols, and anthocyanins, respectively. [b] Antioxidant activity expressed as milligrams of Trolox equivalent per gram of sample. [c] Coefficient of variation.

Analysis of variance for phenolic compounds of faba bean grown at two locations demonstrated that the main effects, genotypes and location and their interaction, were highly significant ($p < 0.0001$). Genotype effect was dominant, whereas location had significant effects on total phenolics, tannins, and anthocyanins and negligible effects on tartaric ester, and flavonol contents. Furthermore, from the percentage variance components, genotype accounted for 98, 81, 86, and 80% of the total variation in total phenolics, tannins, tartaric esters, and flavonols, respectively. However, location had almost no effect on the variation in phenolic compounds because their variances (<3%) were smaller than experimental error (0.2−19%). The overall variability of anthocyanins in faba beans was due to the larger relative contribution (50%) of the error.

Cluster analysis stratified genotypes for phenolic components into three groups: low (Disco, Divine, and Melodie), moderate (AO1155, Fatima, and Florent), and high (FB2556, Imposa, Snowbird, NPZ4-7540, Taboar, AZ10, and SSNS-1). Antioxidant activity grouped the genotypes into four clusters: low (double-zero genotypes Disco, Divine, and Melodie, ≤40 mg TE/g dry matter) analogous to those defined by phenolic components, (Fatima, FB25-56, and Florent, 41−50 mg TE/g dry matter); (AO1155, Snowbird, and SSNS-1, 51−59 mg TE/g dry matter); and high (AZ10, NPZ4-7540, Taboar, and Imposa, >60 mg TE/g dry matter).

The phytic acid content of faba bean differed significantly ($p < 0.0001$) among genotypes and between locations (Table 4). Phytic acid levels ranged from 5.9 to 15.1 g/kg for Imposa and SSNS-1, respectively, representing a 61% difference in phytate content between these two extreme genotypes. The difference (77%) between these genotypes was magnified in Barrhead.

Table 4. Phytic Acid and Phytase Contents of Faba Bean Genotypes[a]

| | phytate (g/kg db) | | | phytase (FTU/kg) | | |
|---|---|---|---|---|---|---|
| | mean | Barrhead | Namao | mean | Barrhead | Namao |
| genotypes | | | | | | |
| AO1155 | 9.50e | 11.64de | 7.35ef | 2128a | 2113bcd | 2144ab |
| AZ10 | 6.94h | 5.09i | 8.80d | 1788c | 2143bc | 1434d |
| Disco | 11.12cd | 12.50d | 9.74c | 1810bc | 1450f | 2279a |
| Divine | 8.65fg | 10.34ef | 6.97f | 1737cd | 1836e | 1631cd |
| Fatima | 13.81b | 14.73c | 12.88a | 1839bc | 1384f | 2363a |
| FB25-56 | 11.80c | 15.87c | 7.74ef | 2154a | 2532a | 1714cd |
| Florent | 13.10b | 17.47b | 8.72d | 1983ab | 1858e | 2151ab |
| Imposa | 5.89i | 4.93i | 7.32ef | 2097a | 2249b | 1899bc |
| Melodie | 8.06g | 7.99h | 8.12de | 1783c | 2017cde | 1445d |
| NPZ4-7540 | 10.61d | 9.79gf | 11.44b | 2052a | 1880e | 2395a |
| Snowbird | 8.90ef | 8.75gh | 9.05cd | 1844bc | 1559f | 2130ab |
| SSNS-1 | 15.06a | 21.38a | 8.74d | 1606d | 1171g | 2150ab |
| Taboar | 9.10ef | 10.56ef | 7.65ef | 1864bc | 1902de | 1806c |
| overall mean | 10.11 | 11.37x | 8.81y | 1897 | 1845y | 1960x |
| CV[b] (%) | 5.11 | 5.35 | 4.47 | 15.69 | 15.05 | 16.36 |

[a] Means in a column with different letters are significantly different ($p < 0.05$). [b] Coefficient of variation.

These values are comparable to the phytic acid content of faba bean (5.1−17.7 g/kg) reported recently,[32] but a wider range than reported for older Canadian faba bean genotypes (8−11 g/kg) grown in Manitoba.[33] Overall mean phytate content of

Table 5. Correlation Coefficients for Phenolics, Antioxidant Activity, and Seed Size of Faba Beans[a]

| | TN | TE | FL | length | W | T | Tsw[b] | AA[c] |
|---|---|---|---|---|---|---|---|---|
| total phenolics | 0.976* | ns | ns | ns | ns | −0.476*** | ns | 0.678* |
| tannins (TN) | | ns | ns | ns | ns | −0.405*** | ns | 0.613** |
| tartaric esters (TE) | | | 0.736* | −0.490*** | −0.454*** | −0.437*** | −0.467*** | ns |
| flavonols (FL) | | | | −0.623** | −0.408*** | −0.415*** | −0.504*** | ns |
| anthocyanins (AN) | | | | ns | −0.426*** | ns | −0.393*** | ns |
| length | | | | | 0.861* | 0.770* | 0.915* | ns |
| width (W) | | | | | | 0.830* | 0.942* | ns |
| thickness (T) | | | | | | | 0.919* | ns |
| *a* | | | −0.392*** | | | | | |
| *C* | | | −0.397*** | | | | | |

[a] *, $p < 0.0001$; **, $p < 0.001$); ***, $p < 0.005$. [b] Thousand seed weight [c] Antioxidant activity evaluated by fluorescence ORAC assay.

genotypes was higher than those reported earlier for raw bean[34] or whole bean flour.[35] Melodie and Snowbird were the most stable genotypes, with phytic acid contents not significantly different between locations, and SSNS-1 was the least stable in the two environments.

Genotype, location, and their interaction contributed significantly ($p < 0.0001$) to variation in phytic acid of faba bean accounting for 9.5, 19.2, and 69.7% of the total variability, respectively. The high variance of the genotype × location interaction regarding phytic acid (>3 and 7 times larger than that due to location and genotype, respectively) indicates that genotype responded differently to each location. This contrasts with earlier investigation,[31] where phytates were not significantly different among five faba bean genotypes grown at two locations and two seasons in Italy. Cluster analysis based on phytic acid segregated the genotypes into four distinct groups: low, Imposa and AZ10 (≤7 g/kg); average, AO1155, Divine, Melodie, Snowbird, and Taboar (8−10 g/kg); above-average, FB25-56, Disco, and NPZ4-7540 (10−12 g/kg); and high, Fatima, Florent, and SSNS-1 (>12 g/kg).

Phytase activity of faba bean differed significantly among genotypes and between locations, with only a 34% difference between genotypes with the highest and lowest activity (FEB25-56 and SSNS-1) (Table 4). AO1155 and Taboar were the most stable genotypes in regard to phytase activity, whereas SSNS-1 exhibited the highest variability between the two locations. The variation in phytase activity of faba bean was due only to the genotype × location interaction contributing 68% to the total variability. Genotypes maybe segregated on the basis of cluster analysis into high (>2000 FTU/kg; AO1155, FB25-56, Imposa, and NPZ4-7540), moderate (1800−2000 FTU/kg; Disco, Fatima, Florent, Snowbird, and Taboar), and low (<1800 FTU/kg; AZ10, Divine, Melodie, and SSNS-1) endogenous phytase content. The overall mean phytase activity of faba bean genotypes was 7-fold those of dry bean cultivars evaluated by using the same method.[14]

The faba bean genotypes Imposa and SSNS-1 with the lowest and highest phytate contents conversely expressed the highest and lowest phytase activity, respectively. However, phytic acid and phytase activity in faba beans were poorly correlated ($r = -0.228$, $p = 0.26$). The phytate/phytase ratio varied widely from 107 to 356:1, suggesting that faba beans may enable high phosphorus bioavailability by phytase depletion (dephytinization), especially when fed to livestock. Over half (54%) of the genotypes had phytate content of <10 g/kg (8.15 ± 1.39 g/kg), whereas 31% had phytase content >2000 FTU/kg. On the basis of phytate and phytase levels, faba bean genotypes may be segregated into those with high phytic acid and moderate phytase levels (SSN1, Fatima, Florent, and Disco; phytate/phytase ratio 107−170:1); low phytate and high phytase (AZ10 and Imposa; phytate/phytase ratio >250:1), and moderate phytate and phytase levels (phytate/phytase ratio 183−224:1). Seven faba bean genotypes with moderate phytic acid levels (8.1−11.8 g/kg) had moderate to high phytase levels (1737−2154 FTU/kg).

Total phenolic content showed significant positive correlation with tannin content ($r = 0.98$, $p < 0.0001$), indicating that selection for low levels of tannin will result in genotypes with reduced total phenolic content (Table 5). Statistically significant correlation ($r = 0.74$; $p < 0.0001$) existed between tartaric acid esters and flavonols, and both were inversely correlated ($r = -0.41$ to $-0.62$; $p < 0.05$) with seed parameters (length, width, thickness, and weight). All phenolic components were indeed inversely related ($r = -0.42$ to $-0.48$) to thickness such that low-phenolic and -tannin genotypes could be distinguished by their plumpness. This corresponds to previous observation[36] of fine-sized seed varieties of broad bean containing approximately twice as little total phenolic compounds than large-sized seed varieties and the lower seed size of tannin-free near-isogenic lines.[37] High correlations ($r = 0.77-0.94$, $p < 0.0001$) among seed length, width, thickness, and weight were similar to those reported for dry beans.[10] Flavonol content, among all phenolic components, showed a weak inverse correlation ($r = -0.39$, $p < 0.05$) with the color parameter $a$ and $C$ values of the seed suggesting the general premise that seed coat color is a reflection of the dominant flavonoids present in legume seeds.[38] However, seed color was not associated with tannin content contrary to an earlier report of a relationship between these two parameters.[24] Total phenolic and tannin contents were the best indicators of antioxidant activity, but showed no association with hydration capacity, contrary to an earlier report.[39] Total phenolic content provided a good estimate and can therefore be used as the extremely powerful predictor of both tannin content and antioxidant activity based on its significant correlation with these parameters.

PCA was performed on the 19 constituents analyzed in this study to explore their underlying complex interrelationships. The PCA generated six factors with eigenvalues exceeding 1.0 (Kaiser's rule) that accounted for 93% of the total variance. The first component (PC1), accounting for 34% of total variance, had large, approximately equal positive loadings for physical seed

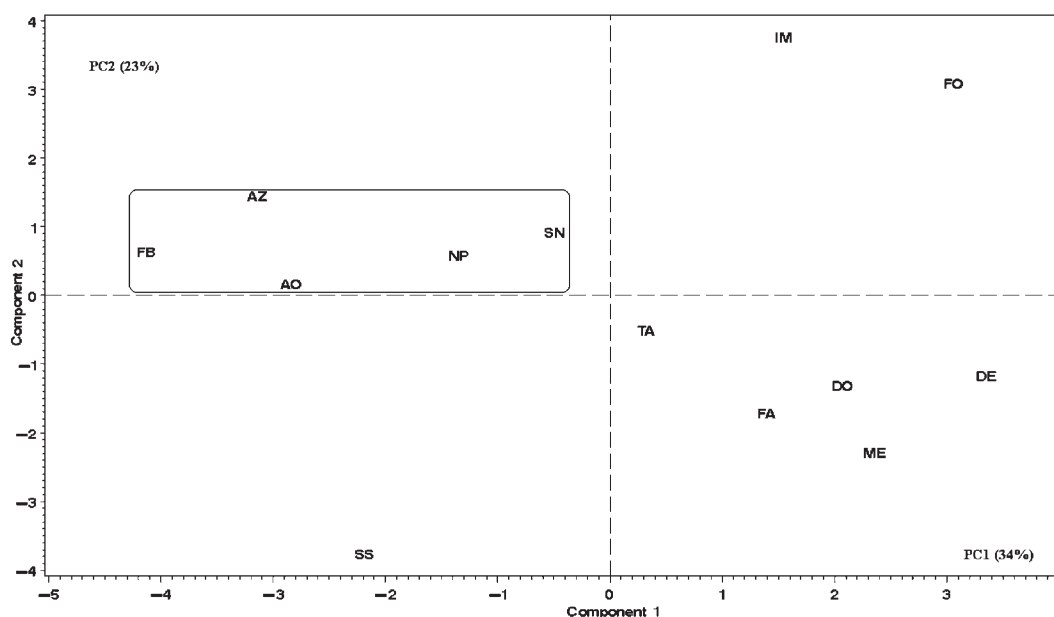


**Figure 1.** Classification of faba bean genotypes grouped according to principal components 1 and 2. Genotypes AO1155, AZ10, Disco, Divine, CDC Fatima, FB25-56, Florent (NPZ3-7080), Imposa (CEB 04928), Melodie, NPZ4-7540, Snowbird, CDC SSNS-1, and Taboar are denoted, respectively, AO, AZ, DO, DE, FA, FB, FO, IM, ME, NP, SN, SS, and TA.

characteristics; thickness (0.366), thousand seed weight (0.347), and length (0.333). The second component (PC2, 23.3%) was primarily influenced by positive loadings of lightness $L$ value (0.381), phytase (0.321), and hydration capacity (0.312) and a negative loading for $\Delta E$ (−0.381) (of the same magnitude as $L$). Although smaller variation was assigned to PC3 (13.2%), it was influenced by phenolic components, tannins (0.332), anthocyanins (0.428), tartaric esters (−0.34), and flavonols (−0.31), and color indicators, $a$ values (0.331) and chromaticity (0.307).

The score plot of the first two principal components accounting for 57% of the total variance (Figure 1) revealed strong differences in seed size and flower color types in faba bean genotypes. Thus, the large-seeded Florent and Imposa genotypes with high positive loadings on both PC1 and PC2 grouped on the upper right quadrant (positive) of the plot diagonally opposite the smallest-seeded SSN-1, whereas Snowbird and Taboar relied heavily on their phytase content and light color. The PCA plot grouped most of the colored-flower genotypes (Divine, Fatima, Florent, Melodie, and Taboar) (lower right, except SSNS-1) based primarily on the first component, whereas the white-flowered genotypes were relegated to the upper left quadrant.

Addition of the third component in the PCA plot resulting in the high cumulative variance (70%) regrouped the genotypes by flower color and segregated the low-tannin Disco, Divine, and Melodie due to the high loadings for anthocyanins (0.428), tannins (0.332), $a$ value (0.331), and chromaticity (0.307). Although smaller variations were assigned to PC4 [9.1%; sphericity (0.601) and antioxidant activity (0.323)], PC5 [7.6%; phytate (0.592), phytase (0.370), and hydration capacity (0.425)], and PC6 [5.7%; antioxidant activity (0.455), tartaric esters (0.387), $b$ value (0.39), phytate (−0.359), and phytase (0.345)], they were nevertheless important delineating factors for faba bean genotypes. The recurrence of phytase in components (PC2, PC5, and PC6) and phytate (PC5 and PC6) with high loadings reveals the importance of these factors in the selection of genotypes.

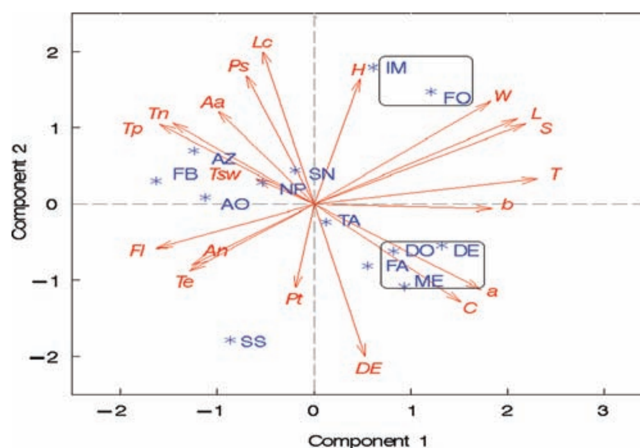


**Figure 2.** Biplot of faba bean genotypes grouped according to principal components 1 and 2. Genotypes AO1155, AZ10, Disco, Divine, CDC Fatima, FB25-56, Florent (NPZ3-7080), Imposa (CEB 04928), Melodie, NPZ4-7540, Snowbird, CDC SSNS-1, and Taboar are denoted, respectively, AO, AZ, DO, DE, FA, FB, FO, IM, ME, NP, SN, SS, and TA. The variables H, W, L, S, T, b, a, C, DE, Pt, An, Te, Fl, Tsw, Tp, Tn, Aa, Ps, and LC represent hydration capacity, width, length, sphericity, thickness, b and a (color values), chromaticity, $\Delta E$, phytic acid, anthocyanin, tartaric esters, flavonols, thousand seed weight, total phenolics, antioxidant activity, phytase, and lightness ($L$ color value), respectively.

The biplot (Figure 2) projects each genotype grown at both locations by a point, and each of the 19 variables (factors) by a vector that accounts for most of the variance. The variable vectors emanate from the origin of the space and go through a point with coordinates that are the coefficients of the variables on the first two components. In the biplot SSNS-1 was positioned away from all other genotypes on the left of the space because of

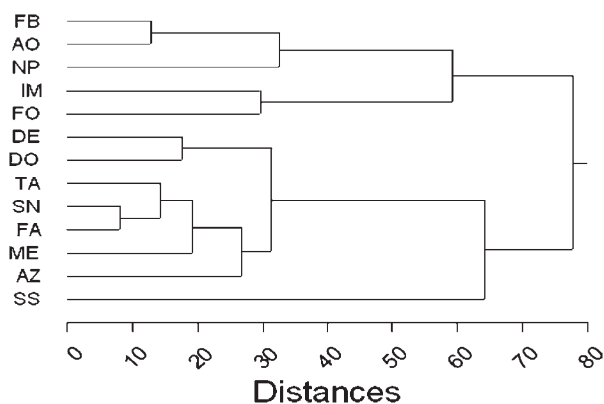


**Figure 3.** Dendogram of cluster analysis performed on 19 constituents of faba bean genotypes FB25-56 (FB), AO1155 (AO), NPZ4-7540 (NP), Imposa (IM), Florent (FO), Divine (DE), Disco (DO), Taboar (TA), Snowbird (SN), CDC Fatima (FA), Melodie (ME), AZ10 (AZ), and SSNS-1 (SS).

high phytate content and poor hydration capacity, whereas Disco, Divine, Fatima, Melodie. and Taboar (vectors between 4 and 5 o'clock) cluster on the right. Imposa and Florent (between 12 and 2 o'clock) form a small cluster at the top of the space, primarily due to their high hydration capacity in addition to their large seed size. The phenolic attributes (total phenolics, tannins, and anthocyanins, Tp, Tn, and Aa) negatively associated with color appearance ($a$, $C$, and $L$) were responsible for clustering AO1155, AZ10, FB25-26, NPZ4-7540, and Snowbird (between 8 and 12 o'clock). However, phenolics were correlated with lightness such that white-flowered genotypes AO1155, AZ10, FB25-26, NPZ4-7540, and Snowbird also had the highest $L$ lightness.

The dendogram (Figure 3) obtained from cluster analysis based on the same 19 variables displayed three major discrete clusters. The white-flowered AO1155, FB25-26, and NPZ4-7540 segregated from the other genotypes with the highest average distance based on color ($a$ and $b$ values, chromaticity) and high phytase. Imposa and Florent were the two most similar genotypes on the basis of their minimal distance because of their seed attributes (high length, width, weight, and hydration capacity) and phytase content. The low-tannin Divine and Disco were also equidistant with similar color attributes ($L$ and $\Delta E$ values), low total phenolics, and low antioxidant activity. The three genotypes Taboar, Snowbird, and Fatima grouped together with Melodie and AZ10, yielding a distinct profile. SSNS-1 with the lowest seed characteristics (length, width, thickness, and weight), phytase, and highest phytate content was the most distant from all genotypes.

The choice of Fatima and Snowbird as check (standard) genotypes for faba bean field trials[7] appears to be based on their similar physical seed attributes and tannin contents. However, the contents of their phytochemical components (phenolics, phytic acid, and phytase) are highly variable. The other standard genotype, SSNS-1, was the most distant of all genotypes, but is preferred for breeding because of its small seed size to help reduce seed cost. Conversely, the large-seeded genotypes Florent and Imposa with high hydration capacity, low phytate, and high phytase bestow attributes highly desirable for the human food market. This study fingerprints phytochemicals in faba bean genotypes that can be grown sustainably under local conditions and provide an understanding of the response of these genotypes to different environments.

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## NOTE ADDED AFTER ASAP PUBLICATION

The original online posting of March 10, 2011, has been corrected to reflect the spelling "faba" bean. The corrections are incorporated in the posting of March 15, 2011.