# Diversity in common bean landraces from south Brazil

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Phaseolin is the major protein in legume seeds and has provided evidence for protein diversity studies, particularly for subdividing *Phaseolus vulgaris* in two major gene pools: the Central American (S-type) and the Andean (T-type) groups. In the work reported here, a total of 73 representative landrace genotypes from Santa Catarina State, Brazil, were evaluated according to their phaseolin patterns using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The seed traits analyzed were: a) the 100-seed weight (P100); b) seed shape degree and seed flattening as determined by J and H coefficients; c) soluble and total protein contents; and d) seed colours. The data indicated that landrace genotypes of common bean collected in Southern Brazil were from both gene pools (Central America and Andes) with both »S« (53.42%) and »T« (42.46%) phaseolin types. The P100 was the main character that grouped these gene pools. The landrace genotypes of the common bean showed a wide range of seed size associated with seed colour. The grouping used by comparison of means, allowed efficiently identify promising genotypes to compose valuable source of genetic diversity that would be highly useful for future studies of representative genotypes from each group. The accession numbers 11, 25, 26, 46, 48 and 74 are of interest for breeding purpose, for they showed higher productivity associated with high protein content.

Keywords: bean, *Phaseolus*, genetic variability, phaseolin, SDS-PAGE, protein, polymorphism

# Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most important legumes in the world. Brazil is the major producer of this crop with approximately 3 million ton/year and an annual per capita consumption average of around 17.5 kg (BROUGHTON et al. 2003). In southern Brazil, mainly Santa Catarina State, in the year of 2006/2007 there was a production of around 217.000 tons, which constituted 5.8% of Brazilian production, which is characterized by low-input and medium-input sustainable farming systems (IBGE 2007).

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Beans provide essential proteins (20 to 25%) in the human diet, complementing other food sources like maize and rice (BROUGHTON et al. 2003). Phaseolin is the major protein in the bean seed and has provided evidence for studies into protein diversity.

Common bean cultivars are products from multiple domestications in the American Continent (VAN SCHOONHOVEN and VOYSEST 1991). Archaeological, morphological, biochemical and molecular evidence has suggested two well-defined Andean and Mesoamerican centers of common bean origins, which were confirmed through morphological markers (GEPTs et al. 1986, SINGH et al. 1991, CHACÓN et al. 2005, DE LA CRUZ et al. 2005), isozyme markers (KOENIG and GEPTS 1989, SANTALLA et al. 2004, CHACÓN et al. 2005), phaseolin types by eletrophoretic profiles (GEPTs et al. 1986, PEREIRA and SOUZA 1992, MACIEL et al. 1999, SOLANO 2005), and molecular markers of RFLP (CHACÓN et al. 2005), AFLP (MACIEL et al. 2003), RAPD (BEEBE et al. 2000) and ISSR (DE LA CRUZ et al. 2005).

The primary center of the Central American type is characterized by cultivars with predominantly the S (sphaseolin type and smaller seeds (< 25 g/100 seeds). The other primary center, the Andean, is characterized by cultivars with the T (sphaseolin type and larger seeds (> 40 g/100 seeds) (SINGH et al. 1991). The expression of the phaseolin phenotype is not influenced by environment (BROWN et al. 1981), or by human selection, or any other type of selection (GEPTs et al. 1986). In addition, the heritability of seed may reach values of up to 0.8–0.9 (VAN SCHOONHOVEN and VOYSEST 1991), showing that the phaseolin type can be used as a potential tool to indicate genetic diversity and gene flow between wild and domesticated beans.

In a previous study with 192 landrace beans (PEREIRA and SOUZA 1992) from the CNPAF (Centro Nacional de Pesquisa de arroz e feijão – EMBRAPA, Brazil) germplasm bank 80.6% and 19.4% were established for the »S« and »T« phaseolin types, respectively. Amongst all 192 genotypes only 1 was related with the »T« type from Santa Catarina State. On the other hand, GEPTs et al. (1988) found predominantly the »T« type only in the Minas Gerais and Santa Catarina regions from the 72 Brazilian genotypes studied. But these studies did not include a representative number of landraces from Santa Catarina State.

The genetic diversity available for the common bean in Brazil has been reduced drastically by rigorous consumer preference by seed size, shape and colour (CONAFE 2005). This reduction has limited the source of genetic variability for breeding and conservation programs. Particularly in Santa Catarina State currently in use are landraces of *Phaseolus vulgaris* that display a wide range of seed and colour patterns, maintained for generations by farmers, contributing to the genetic resource (NASS and PATERNIANI 2000).

The preservation and study of landraces are a challenge for the future, as landraces represent a source of variation that can be useful in crop science or breeding programs to increase seed quality. Moreover, these genotypes can contribute important characteristics like resistance to biotic and abiotic stresses, diseases, and seed technology quality, to reduce the vulnerability of improved genotypes (GEPTS et al. 1986).

The traditional bean cropping in Santa Catarina state is based on small to medium properties. This activity is basically an income earner for the farmers. However, it has not been sufficient for the maintenance of the families, leading the farmer to look for new economic alternatives, like the grain differential yield. Moreover, governmental incentives and scientific support concerning the qualities of the landraces that they are producing exist. Based on the above considerations, the objective of the present study was to characterise the diversity among landraces of the common bean from southern Brazil through phaseolin pattern and other traits such seed shape, colour patterns and soluble and total protein, to provide information to the farmer about the cultivation and preservation of these landraces.

# Materials and methods

# **Plant material**

A collection of 73 common bean genotypes were used in the study for comparison against 5 controls of phaseolin type (Active Bank Common Bean- BAFs: 113-»S«, 116-»H«, 117-»T«, 118-»B« and 119-»C«) that were supplied by CNPAF (Embrapa, Centro Nacional de Pesquisa de Arroz e Feijão, Brazil). The 5 commercial cultivars (BAFs: 110, 111, 112, 115 and 121) and 63 landraces of common bean were randomly chosen from the different regions of the State of Santa Catarina, and the seeds were obtained from the Active Bank Bean (BAF), from Santa Catarina State University (UDESC, Brazil). The landraces are defined as locally adapted or domesticated unimproved.

A field experiment was conducted in the 2005/06 season in a randomized complete block design, with three replications, located at Lages, Santa Catarina State, South-Brazil (27  $^{\circ}$  52' South, 50  $^{\circ}$  18' East, 930 m above sea level), characterized by mild summers and regularly distributed rains (EPAGRI 2006). The objective of the experiment was to obtain seeds from the same field at the same time. The unit plot consisted of 4 rows, each 4 m long, with 0.5 m between rows; 1 m between plots and 208 plants per plot.

The soil fertility and moisture conditions were adequate for bean cultures in southern Brazil as described by Chemical and Fertility Committee for Santa Catarina and Rio Grande do Sul states, Brazil (CQFS-RS/SC 2004). Insect and pest control were implemented as needed. At harvest, seeds from the central plants, from each plot from each genotype, was bulked and dried in a forced-air oven (30 °C) until the moisture content of the seeds reached 12%, which was monitored with moisture-testing equipment (Dole Model 500) (GEHAK).

### Seed traits assessed

After harvest, the moisture content of the seeds was standardized at 12%, and samples were stored at 10 °C and 40% relative humidity. The characteristics of the seeds were evaluated in 100-seed weight (P100), seed shape degree and seed flattening (determined by J coefficient and H coefficient), soluble and total protein content, and seed colour. The phaseolin data were characterized by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

The J coefficient was estimated by the ratio between the seed length and width and the H by the ratio between the seed thickness and width (IPGRI 2001). The seeds were grouped in market classes: manteigão, white, mulatinho, black, coloureds, yellow, red and carioca (IPGRI 2001).

#### **Total protein quantification**

The total protein was determined by total N content of samples, where % of protein is equal to N content (%)  $\times$  6.25, according to Kjeldahl (AOAC 1995).

### Soluble protein extraction and quantification

The dry seeds without coat were ground with pestle and mortar and the fine powder produced (100 mg) was added to 1 mL of 0.5 M NaCl buffer (pH 2.4), for 30 minutes with constant stirring at room temperature on a rotator shaker. The samples were centrifuged at 10.000 g for 20 minutes to clarify the supernatant and finally stored at -20 °C. The supernatant was used for quantification and electrophoretic profiles.

The colorimetric protein assay was used according to Bradford. Protein solution was added to 5 ml of the Bradford reagent and the absorbance was measured at 595 nm. A bovine serum albumin (BSA) dilution curve was used as standard (BRADFORD 1976).

#### Phaseolin analysis by electrophoretic profiles (SDS-PAGE)

The supernatant (10  $\mu$ g) obtained by extraction was mixed with an equal volume of cracking buffer containing 0.25 M NaCl, pH 2.4; 0.625 M Tris-HCl, pH 6.8; 0.5 mM EDTA, pH 6.8; 1% (w/v) SDS; 8% glycerol (v/v); 0.5%  $\beta$ -mercaptoethanol (w/v); 0.01% bromophenol blue marker dye (GEPTs et al. 1986). The mixture was heat-treated at 100 °C for 5 min and centrifuged at 10000 g and used for electrophoresis.

Electrophoretic profiles of the polypeptides were obtained by separation using 13% polyacrilamide gels in the presence of SDS under alkaline conditions (LAEMMLI 1970). After the quantification of soluble protein, 10  $\mu$ g was applied in the gel. A high molecular weight calibration Kit (45–200 kDa, Bio Rad) provided standard of known molecular weight for the polypeptides profiles. PAGE was performed in the presence of the running buffer (50 mM Tris-HCl, 0.384 M Glicina, 5mM EDTA and 0.25% SDS) at a constant 35 mA/gel at 8 °C until the bromophenol blue dye reached the bottom of the gels.

The gels were rinsed three times with water, Ultrapure, (Millipore System) for 5 min each and fixed overnight in 400 mL  $L^{-1}$  methanol and 100 mL  $L^{-1}$  acetic acid. Subsequently they were silver stained according to Blum (BLUM et al. 1987). After staining, the phaseolin type from each genotype was scored by comparing the patterns to those of reference genotypes (Table 1, BAFs: 113, 116, 117, 118 and 119). The phaseolin polypeptides were identified from the other seed proteins by their molecular weight (45–51 kDa) (BROWN et al. 1981).

#### Data analysis

The univariate analysis of variance to design model and multivariate analysis were performed on the quantitative characters (weight of the seeds, total protein, soluble protein, seed size). The multivariate analysis was conducted by using canonical variable technique (RAO 1952). The contribution of the different character to the total variation was determined by using Singh's methodology (SINGH 1981). The SCOTT and KNOTT (1974) and the Tukey (STEEL and TORRIE 1980) methods were used in the mean separation procedure. The statistical analysis was performed using the Genes program (CRUZ and CARNEIRO 2003).

BAF	Origin of collection	Phaseolin type	Weight 100 seeds	Total protein (%)	Soluble protein (µg mL <sup>-1</sup> )
01	Ponte Serrada	S	36.64	25.70	8406.49
03	Palmitos	З Т	29.14	28.98	8947.77
03	Lages	T	40.81	29.53	9839.25
04	Lages	S	40.81	29.53	8847.53
10	Palmitos	S	19.80	29.33	7482.43
10	Lages	З Т	39.93	26.80	8367.97
13	Caxambú do Sul	S	22.23	30.63	9186.15
17	Palmitos	S	17.36	24.61	8228.26
19	Palmitos	S	20.97	27.89	9863.99
20	Palmitos	Б Т	21.34	27.89	9149.19
20	Palmitos	S	22.99	27.89	9690.78
21	Palmitos	S	20.20	24.06	8514.25
22	Chapecó	S	20.20	25.16	9011.99
23	São José do Cerrito	S	42.73	25.16	9044.88
25	Palmitos	T	19.66	27.90	9801.67
26	Palmitos	S	21.95	26.80	9472.45
28	Saudades	T	27.94	23.52	9089.36
29	Ituporanga	T	36.37	26.25	8942.77
32	Coronel Freitas	T	34.02	24.61	9667.28
33	Concórdia	T	42.82	26.25	10237.07
36	São José do Cerrito	S	25.98	28.98	8636.42
39	Bom Jardim da Serra	T	37.43	25.70	9946.38
40	Capão Alto	S	20.56	26.80	8601.02
41	Bom Jardim da Serra	S	21.40	28.98	10431.27
42	Capão Alto	S	17.32	26.81	9273.23
43	Capão Alto	T	43.71	24.61	7781.27
44	Capão Alto	S	19.78	26.25	8983.80
45	Capão Alto	S	23.50	24.06	8863.82
46	Lages	Ť	39.19	21.88	9849.60
47	Piratuba	S	38.85	27.90	8068.19
48	Capão Alto	Т	42.57	27.90	9204.00
49	Oeste de SC	Т	33.91	32.02	8272.43
50	Lebon Régis	S	20.45	24.61	9094.99
51	Cunha Porá	Т	35.25	25.16	9270.72
52	Cunha Porá	S	17.96	25.70	7965.45
53	Cunha Porá	Ť	47.73	30.62	8242.98
54	Cunha Porá	Т	33.59	26.80	8994.76
55	Cunha Porá	S	18.43	25.16	8154.02
56	Cunha Porá	S	18.89	26.25	7939.14

 Tab. 1. Identification, origin of collection, phaseolin type, seed weight, total and soluble protein of common bean genotypes from Santa Catarina, Brazil.

BAF	Origin of collection	Phaseolin type	Weight 100 seeds	Total protein (%)	Soluble protein $(\mu g m L^{-1})$
57	Cunha Porá	Т	36.01	20.23	8328.50
58	Cunha Porá	Т	36.93	27.34	8893.27
59	Bom Jardim da Serra	S	15.86	26.25	8193.49
60	Lebon Régis	S	18.78	31.02	9403.54
61	Painel	Т	35.50	23.51	8497.33
62	São Joaquim	S	20.55	28.44	8679.64
63	São Joaquim	Т	36.59	19.14	8851.61
64	Campo Belo do Sul	Т	33.00	24.06	8320.98
65	Lebon Régis	S	22.85	26.25	8641.11
69	Bocaína do Sul	S	51.51	26.80	9437.68
74	Irineópolis	Т	18.01	29.53	8772.67
78	Lebon Régis	S	17.32	25.70	9255.37
80	Fraiburgo	S	19.58	26.80	8409.63
81	Lebon Régis	S	17.04	25.16	9146.99
87	Fraiburgo	S	50.12	26.25	8249.25
88	Curitibanos	Т	41.45	21.87	7479.30
89	Curitibanos	Т	47.75	23.52	8834.69
90	Lebon Régis	Т	31.05	21.87	8244.86
91	Fraiburgo	Т	33.00	25.16	8893.89
92	Fraiburgo	S	16.21	26.25	8004.92
93	Bom Retiro	Т	39.21	28.98	8663.66
110	Lages	S	25.25	27.89	12927.80
111	Lages	Т	21.07	30.81	9849.28
112	Lages	S	21.90	27.90	9183.33
113	Goiáis-CNF5057	$S^{a}$	19.50	31.00	10512.72
114	Capão Alto	S	19.56	24.61	8217.09
115	Lages	S	15.21	29.53	9612.47
116	Goiáis-CNF5493	$\mathrm{H}^{\mathrm{b}}$	17.03	26.80	9520.37
117	Goiáis-CNF6551	T <sup>c</sup>	27.67	26.80	10218.27
118	Goiáis-CNF11513	$\mathbf{B}^{d}$	27.47	26.80	11880.32
119	Goiáis-CNF1446	C <sup>e</sup>	50.85	30.62	9521.01
120	Lages	Т	64.68	23.52	7986.75
121	Lages	S	24.72	28.99	8544.09
SH80	Lages	S	25.50	22.97	8207.90
Means	_	_	28.94	26.60	8996.89
CV (%)	_	-	5.85	8.10	6.68

Tab. 1. - continued

BAF = number of collection bank of germplasm from UDESC, Santa Catarina, Brazil.

 $S^{a}$  = Sanilac,  $H^{b}$  = Pampa,  $T^{c}$  = Tendergreen,  $B^{d}$  = Boyaca and  $C^{e}$  = Contender.

BAFs: 110 = Guará, 111 = Pérola, 112 = Uirapuru, 115 = Valente, 121 = Iapar 81.

# Results

The phaseolin type profiles of the seed proteins from 73 common bean genotypes were analyzed by SDS polyacrylamide gels, showing protein subunit variations. Two different patterns of phaseolin were found among the 73 genotypes analyzed (Tab. 1).

The most common was the »S« type, found in 39 genotypes (53.42%), and »T« type was the second, found in 31 genotypes (42.46%). However, the types H, C and B, contributed only 4.12% (controls), because these types were not found in the landraces from Santa Catarina state. In additional, the commercial cultivars (BAFs: 110, 111, 112, 115 and 121) at the moment used in South-Brazil exhibited a typical »S« phaseolin type (Tab. 1).

The diversity was evidenced by the weight of 100 seeds, where genotypes that presented the T phaseolin type showed a higher mean seed weight (36 g/100 seeds), while the S type, a low mean seed weight (23 g/100 seeds) (Tab. 1). In terms of total and soluble protein, the values ranged from 19 (BAF 63) to 32% (BAF 49) and 7.5 (BAF 10) to 10.4 mg/ml (BAF 41) respectively (Tab. 1).

The quantitative traits (P100, total and soluble protein) showed significant difference (P < 0.05), which indicated a contribution from each character to genetic diversity (Tab. 2). The P100 had higher relative contribution to genetic divergence (91.67%) in overall genotypes, while other traits (total and soluble protein content) showed a smaller contribution (~ 8%) (Tab. 2).

Variables	S.j.	Value in %	
Weight of 100 seeds	237791.996	91.67	
Soluble Protein	11628.383	4.48	
Total Protein	9976.991	3.85	

**Tab. 2.** Relative importance of characters analyzed in 73 genotypes of bean. S.j. – contribution of each character to total distances between the genotypes according to SINGH (1981).

The grouping based in P100, showed a separation in eleven groups within germplasm bank samples from southern Brazil (Fig. 1, Tab. 3). In the association between P100 and phaseolin types two major groups were found (Fig. 1). The first group (A, B, C, D, E, F, G and H) showed P100 higher than the second group (mean of 39 g), with most of the land-races classified as »T« type phaseolin (77%). The second group (I, J and K) was classified as »S« type (87%), and had P100 value lower than the first group (~19 g).

The two groups analyzed showed diversity also with respect to seed colour as well as in the degree of seed flattening. In relation to seed colour, in the Andean group we found seeds of the market classes yellow (11.7%), manteigão (17.6%), mulatinho (2.9%), coloured (29.5%), red (23.54%), black (14.7%); and in the Mesoamerican group, we encountered the types carioca (15.4%), white (7.6%), red (15.4%), black (48.7%) and coloured (12.9%) (Tab. 3, Fig. 1).

The degree of seed flattening assessed by the J and H coefficient proved to be similar to the group of the P100. The Andean group showed seeds with the J coefficient ranging from 1.41–2.25, and the H coefficient from 0.7–0.9. In the Mesoamerican group the J coefficient



Fig. 1. Representative bean market classes included in each group (A, B, C, D, E, F, G, H, I, J, K) from Santa Catarina State/Brazil.

ranged from 1.52–1.91 and H coefficient from 0.74–0.76 (Tab. 3). Indeed, significant differences were observed between phaseolin types with regard to seed length, height, width, and H, and J coefficients. Multiple comparisons of the means (P < 0.05) revealed that the Andean group had a higher size seed (1.25 cm length; 0.74 cm height; 0.58 cm width) than the Mesoamerican group (Tab. 4).

Groups	Genotypes (BAF)	Variation of 100 seeds weight (g)	Phaseolin type	Market classes	*J Coefficient	*H Coefficient
А	120	64.68	Т	manteigão	2.25	0.74
В	69, 119, 87	51.51-50.12	S,C	manteigão, red	2.17	0.82
С	89, 53	47.75-47.73	Т	red, yellow	1.98	0.79
D	43, 33, 24, 48, 88	43.71-41.75	S,T	coloured, black	1.60	0.76
Е	4, 11, 93, 46, 47	40.81-38.85	T,S	red, manteigão, yellow, black	1.64	0.70
F	39, 58, 01, 63, 29, 57, 61,51	37.43-35.25	T, S	manteigão, black, red, coloured	1.46	0.76
G	32, 49, 54, 91, 64,90	34.02-31.05	Т	manteigão, coloured, yellow, mulatinho	1.41	0.90
Н	3, 28, 117, 118,36	29.14–25.98	T,S,B	coloured, red, black	1.74	0.73
Ι	SH80, 110, 121, 45, 21, 65, 23, 13	25.50-22.23	S	carioca, red, coloured, black,	1.91	0.74
J	26, 112, 41, 20, 111, 19, 40, 62, 50, 22, 10, 44, 25, 80, 114, 113, 56, 60	21.90-18.80	S,T	black, red, carioca, coloured, white	1.60	0.76
K	55, 74, 52, 7, 17, 42, 78, 81, 116, 92, 59,115	18.40-15.20	S,T,H	white, black, coloured	1.52	0.76

**Tab. 3.** Groups from common bean genotypes based on 100 seeds weight, market classes, phaseolin type, J and H coefficient

\* The results were based in 20 seeds average each sample and grouped by Scoot Knott (P < 0.05).

 
 Tab. 4. Seed size differences of bean genotypes and relationships with groups Andean and Mesoamerican.

C	Mean seed sizes (cm)*					
Groups	Length (cm)	Height (cm)	Width (cm)	J Coefficient	H Coefficient	
Andean	1.25 <sup>a</sup>	$0.74^{a}$	0.58 <sup>a</sup>	1.69 <sup>a</sup>	0.79 <sup>a</sup>	
Mesoamerican	1.04 <sup>b</sup>	0.63 <sup>b</sup>	0.47 <sup>b</sup>	1.58 <sup>b</sup>	0.75 <sup>b</sup>	
CV (%)	15.35	7.18	11.41	13.87	9.8	

\* The values in the column followed by same letter are not significantly different, Tukey (P < 0.05).

# Discussion

The estimation of the relationship among landraces of the common bean is of great interest to the management of the germplasm bank. The Active Bank Bean from Santa Catarina showed the existence of two patterns of phaseolin, 53.42% »S« type and 42.46% »T« (Tab. 1). In a study executed by GEPTS et al. (1988) with 72 Brazilian genotypes, the »T« type was predominant in Minas Gerais and Santa Catarina states. Therefore, in 192 landrace beans from the CNPAF (Centro Nacional de Pesquisa de arroz e feijão – EMBRAPA, Brazil) germplasm bank 80.6% and 19.4% were found for the »S« and »T« phaseolin types respectively. From 20 genotypes of Santa Catarina state, only one was related to the »T« type (PEREIRA and SOUZA 1992), although this study did not include a representative number of genotypes from Santa Catarina state.

The landraces of the common bean analyzed from Rio Grande do Sul state showed T, S, H, A, and C phaseolin types, with the »T« type accounting for 77.27% (MACIEL et al. 1999). In contrast, our results showed only »T« and »S« types, both around 50%. Most of the data corroborate the hypothesis that in Brazil there are two origins, from the Andean region and the Mesoamerican (GEPTs et al. 1988).

Regarding the concentration of total protein in the grains found in table 1, a variation from 19–31% was encountered, which was similar to the values obtained by Santalla (SANTALLA et al. 2004) in common bean species, with values of 26.5–31%. In Brazilian commercial cultivars values around 20–27% of total protein were found (ANTUNES et al. 1995, SANTALLA et al. 1999, DALLA CORTE et al. 2003). Particularly, the landraces BAF 11, 25, 26, 42, 46, 48, and 74 showed high values of total protein (27%), and the soluble protein was around 9000  $\mu$ g/ $\mu$ l (Tab. 1). The genotypes mentioned above were associated with high productivity (3500–4000 Kg ha<sup>-1</sup>) and also with other important agronomic characters like determinate growth (BAFs: 48 and 74) and indeterminate growth (BAFs: 11, 25, 26 and 46), number of seeds per pod (~4,2 in the BAFs 11, 46 and 48; and 5,7 in the BAFs 25, 26 and 74), number of pods per plant (~13.5 in the BAFs 11, 25, 46, 48, 74 and 19.5 for genotype BAF 26).

Averages of P100 and phaseolin type classes were very similar to those already observed in the Andes and Central America, with »T« phaseolin type with large seeds (>40 g per100 seeds) and cultivars with predominantly »S« phaseolin type, with smaller seeds (<25 g per 100 seeds) (SINGH et al. 1991). These results are in agreement with those of other authors who studied different gene pools (MACIEL et al. 1999, DE LA CRUZ et al. 2005, LOGOZZO et al. 2006).

The Singh methodology showed P100 had a high relative importance for genetic divergence (Tab. 2). This higher contribution found with P100 was observed by other works (BENIN et al. 2002, CHIORATO et al. 2005, COELHO et al. 2007a).

There is a wide diversity of color of the tegument (Tab. 3 e, Fig. 1). In the Mesomerican group, the black seed colour was predominant and in the Andean both the coloured and the red seeds. In the Mesoamerican group, our results were in agreement with another study (LOGOZZO et al. 2006). This work indicated that the proportion observed in the black colour seeds (47.2%) was mostly similar with the darker colour seeds in the American »S« phaseolin type.

Those variations show a wide range of seed size associated with seed colour, which might be an important alternative for the consumer of southern Brazil, where their preference is for black beans. The commercial cultivars showed a high similarity with the »S« phaseolin type, of Mesoamerican origin. Traits like seed size, weight and colour found in the landraces studied represent a source of variation that can useful in the crop science or breeding programs, particularly to reduce the vulnerability of improved genotypes.

These ranges of variation of seed coefficient (J and H) estimated in the present study, found in the Andean group were similar in cultivars representing Andean dry bean and snap bean marked classes (SANTALLA et al. 2004). This similarity was observed by GEPTS et al. (1986) where »T« phaseolin (Andes) showed 1.36 cm (length), 0.82 cm (height) and 0.63 cm (width). Means of seed size by J (1.69) and H (0.79) coefficients (Andean) were similar to those obtained by SANTALLA et al. (2004), which were 1.9 and 0.76 in Andean dry bean and snap bean marked classes respectively. The data proved to be similar to the Brazilian commercial cultivars, in which values around 1.68 for J coefficient and 0.79 for H coefficient were encountered (DALLA CORTE et al. 2003).

This work indicate that landraces of the common bean collected in southern Brazil came from two gene pools, that of Central America (small seeds, »S« type phaseolin) and Andean (higher size seeds and phaseolin type »T«) gene pool, which had 53.42 and 42.46% respectively. The P100 was the principal character that grouped these two gene pools.

The grouping used by comparison of means allowed for the efficient identification of promising genotypes to compose a valuable source of genetic diversity highly useful for future breeding programs and in crop science. The genotypes suggested are the BAFs 11, 25, 26, 46, 48 and 74, which had a higher productivity level and high protein content.

It would be very interesting to further investigate the genetic diversity of protein and micronutrient concentrations associated with phytate concentration in dry bean and other legumes. Regarding the nutrient level in grain, there appears to be little research at the molecular and biochemical level. Recent studies have identified in rice grain significant correlation between phytate and inorganic phosphorus, Fe, Zn, Cu, and Mn, but those compounds were not located on the same chromosomal regions, suggesting that they were genetically different (STANGOULIS et al. 2007). Additionally, the understanding the genetic and control mechanisms of phytate accumulation in seeds is interesting for breeding programs and to improve phosphorus nutrition in bean-eating populations. Results have been published for common bean seed development with evidence that phytate synthesis has regulation points with MIPS (myo-inositol-3-phosphate synthase) enzyme. It is also attractive to investigate further the genetic variability of MIPS activity and gene expression associated with phytate concentration during seed development in the common bean (COELHO et al. 2007b).

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# References

- ANTUNES, P. L., BILHALVA, A. B., ELIAS, M. C., SOARES, G. J. D., 1995: Valor nutricional do feijão (*Phaseolus vulgaris* L.) cultivares Rico 23, Carioca, Piratã-1 e Rosinha-G2. Revista Brasileira Agrociência 1, 12–18.
- AOAC, 1995: Official methods of analysis. Association of Official Analytical Chemists, Washington.
- BEEBE, S., SKROCH, P. W., DUQUE, M. C., PEDRAZA, F., NIENHUIS, J., 2000: Structure of genetic diversity among common bean landraces of CentralAmerican origin based on correspondence analysis of RAPD. Crop Science 40, 262–272.
- BENIN, G., CARVALHO, F. I. F., ASSMANN, I. C., CIGOLINI, J., CRUZ, P. J., LORENCETTI, C., SILVA, J.A.G., 2002: Identificação da dissimilaridade genética entre genótipos de feijoeiro comum (*Phaseolus vulgaris* L.) do grupo preto. Revista Brasiliera Agrociência 3, 179–184.
- BLUM, H., BEIER, H., CROSS, H. J., 1987: Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis 8, 93–99.
- BRADFORD, M. M., 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye banding. Analytical Biochemistry 72, 248–259.
- BROUGHTON, W. G., HERNÁNDEZ, G., BLAIR, M., BEEBE, S., GEPTS, P., VANDERLEYDEN, J., 2003: Beans (*Phaseolus* spp.) model food legumes. Plant Soil 252, 55–128.
- BROWN, J. W. S., MA, Y., BLISS, F. A., HALL, T. C., 1981: Genetic variation in the subunits of globulin-1 storage protein of french bean. Theoretical and Applied Genetics 59, 83–88.
- CHACÓN, M. I., PICKERSGILL, S. B., DEBOUCK, D. G., 2005: Domestication patterns in common bean (*Phaseolus vulgaris* L.) and the origin of the Mesoamerican and Andean cultivated races. Theoretical and Applied Genetics 110, 432–444.
- CHIORATO, A. F., CHIAVEGATO, M. B., CARBONELL, S. A. M., MOURA, R. R., COLOMBO, C.A., 2005: Diversidade genética de feijoeiro por meio de dados morfoagronômicos e moleculares. Anais-VIII-Congresso Nacional de Feijão 2, 413–416.
- COELHO, C. M. M., COIMBRA, J. L. M., SOUZA, C. A., BOGO, A., GUIDOLIN, A. F., 2007a: Diversidade genética em acessos de feijão (*Phaseolus vulgaris* L.). Ciência Rural 37, 1241–1247.
- COELHO, C. M. M., BENEDITO, V. A., FIGUEIRA, A., VITORELLO, V. A., AZEVEDO, R. A., 2007b: Variation in the enzyme activity and gene expression of myo-inositol-3-phosphate synthase (MIPS) and phytate accumulation during seed development in common bean (*Phaseolus vulgaris* L.). Acta Physiologiae Plantarum 29, 265–271.
- COELHO, C. M. M., TSAI, S. M., VITORELLO, V. A., 2005: Dynamics of inositol phosphate pools (tris-, tetrakis- and pentakisphosphate) in relation to the rate of phytate synthesis during seed development in common bean (*Phaseolus vulgaris* L.). Journal of Plant Physiology 162, 1–9.
- CONAFE, 2005: Resumos do VIII Congresso Nacional de Feijão 8.:2005. Santo Antônio de Goiás / Embrapa Arroz e Feijão, Goiânia, GO.

- CQFS-RS/SC, 2004: Manual de adubação e de calagem para os estados do Rio Grande do Sul e Santa Catarina. SBCS Núcleo Regional Sul/UFRGS, Porto Alegre.
- CRUZ, C. D., CARNEIRO, P. C. S., 2003: Modelos biométricos aplicados ao melhoramento genético. UFV, Viçosa MG.
- DALLA CORTE, A., MODA-CIRINO, V., SCHOLZ, M. B. S., DESTRO, D., 2003: Environment effect on grain quality in early common bean cultivars and lines. Crop Breeding and Applied Biotechnology 3, 193–202.
- DE LA CRUZ, E. P., GEPTS, P., MARÍN, P. C. G., VILLAREAL, D. Z., 2005: Spatial distribution of genetic diversity in wild populations of *Phaseolus vulgaris* L. from Guanajuato and Michoacán, México. Genetic Resources and Crop Evoution 52, 589–599.
- EPAGRI, 2006: Atlas climatológico do Estado de Santa Catarina (http://ciran.epagri. rct-sc.br).
- GEPTS, P., KMIECIK, K., PEREIRA, P., BLISS, F. A., 1988: Dissemination pathways of the common beans (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability. I. The Americas. Economic Botany 42, 73–85.
- GEPTS, P., OSBORN, T. C., RASHKA, K., BLISS, F. A., 1986: Phaseolin-protein variability in wild forms and landraces of the common beans (*Phaseolus vulgaris*): Evidence for multiple centers of domestication. Economic Botany 40, 451–468.
- IBGE, 2007: Instituto Brasileiro de Geografia e Estatística. (http://www1.ibge.gov.br/ home/estatistica/indicadores/agropecuaria/).
- IPGRI, 2001: Descritores para *Phaseolus vulgaris*. International Plant Genetic Resources Institute, Rome.
- KOENIG, R., GEPTS, P., 1989: Segregation and linkage of genes for seed proteins, isozymes, and morphological traits in common bean (*Phaseolus vulgaris*). Journal of Heredity 80, 455–459.
- LAEMMLI, U. K., 1970: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.
- LOGOZZO, G., DONNOLI, R., MACALUSO, L., PAPA, R., KNUPFFER, H., ZEULI, P. S., 2006: Analysis of the contribution of Mesoamerican and Andean gene pools to European common bean (*Phaseolus vulgaris* L.) germplasm and strategies to establish a care collection. Genetic Resources and Crop Evoution 54, 1763–1779.
- MACIEL, F. L., ECHEVERRIGARAY, S., GERALD, L. T. S., GRAZZIOTIN, F. G., 2003: Genetic relationships and diversity among Brazilian cultivars and landraces of common beans (*Phaseolus vulgaris* L.) revealed by AFLP markers. Genetic Resources and Crop Evolution 50, 887–893.
- MACIEL, F. L., GERALD, L. T. S., ECHEVERRIGARAY, S., 1999: Variation of phaseolin and other soluble proteins among cultivars and landraces of common beans of south-Brazil. Journal of Genetics and Breeding 53, 149–154.
- NASS, L. L., PATERNIANI, E., 2000: Pre-breding: A link between genetic resources and maize breeding. Scientia Agricola 57, 581–587.
- PEREIRA, P. A. A., SOUZA, C. R. B., 1992: Tipos de faseolina em raças crioulas de feijão no Brasil. Pesquisa Agropecuaria Brasileira 27, 1219–1221.

PEREIRA T., COELHO C. M. M., BOGO A., GUIDOLIN A. F., MIQUELLUTI D. J.

- RAO, R. C., 1952: Advanced statistical methods in biometrics research. Jonh Wiley and Sons, New York.
- SANTALLA, M., RODIÑO, A. P., RON, A. M. D., 1999: Breeding for culinary and nutritional quality of common bean (*Phaseolus vulgaris* L.) in intercropping systems with maize (*Zea mays* L.). Biotechnology Agronomy Society and Environment 3, 225–229.
- SANTALLA, M., SEVILLANO, M. C. M., MONTEAGUDO, A. B., DE RON, A. M., 2004: Genetic diversity of Argentinean common bean and its evolution during domestication. Euphytica 135, 75–87.
- SCOTT, A. J., KNOTT, M., 1974: A cluster analysis methods for grouping means in the analysis of variants. Biometrics 30, 507–512.
- SINGH, D., 1981: The relative importance of characters affecting genetic divergence. Indian Journal of Genetics and Plant Breeding 41, 237–245.
- SINGH, S. P., GEPTS, P., DEBOUCK, D. G., 1991: Races of common bean (*Phaseolus vulgaris*, Fabaceae). Economic Botany 45, 379–396.
- SOLANO, J. P. L., 2005: Patterns of phaseolins and RAPD analysis in domesticated species of *Phaseolus*. Revista Fitotecnia Mexicana 28, 195–202.
- STANGOULIS, J. C. R., HUYNH, B. L., WELCH, R. M., CHOI, E. Y., GRAHAM, R. D., 2007: Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. Euphytica 154, 289–294.
- STEEL, R. G. D., TORRIE, J. H., 1980: Principles and procedures of statistics-a biometrical approach. Megraic Hill, New York.
- VAN SCHOONHOVEN, A., VOYSEST, O., 1991: Common beans: research for crop improvement. CIAT, Cali, Colômbia.