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Physiological diversity in Brazilian common bean (*Phaseolus vulgaris* L.) landraces based on selection index

Diversidade fisiológica em feijão comum brasileiro (Phaseolus vulgaris L.) com base no índice de seleção

Diego Medeiros Gindri[1]* e Cileide Maria Medeiros Coelho[2]

[1] Companhia Integrada de Desenvolvimento Agrícola, Lages, SC, Brasil. *Autor para correspondência: diegogindri@gmail.com.
[2] Universidade do Estado de Santa Catarina, Lages, SC, Brasil.

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ABSTRACT

Common bean landraces represent heterogeneous, local adaptations of domesticated species, and thereby provide genetic resources that meet current and new challenges for farming. These local ecotypes can show variable phenology and low-to-moderate edible yield, however, they are highly nutritious. Therefore, strategies to stimulate the conservation of this genetic diversity are important. The maintenance of seed germination potential is also an important feature in the characterization of genetic diversity to conserve biodiversity. The main objective of this study was to discriminate common bean landrace cultivars available at Santa Catarina State University (UDESC) germplasm bank according to their seed physiological diversity and agronomic parameters (germination rate, viability, seed vigor, and seedling growth) and based on Mulamba and Mock selection index. There is a large physiological diversity in common bean landrace cultivars. BAF 112 and BAF 81, BAF 84, BAF 42, BAF 60, and BAF 75, were identified as more promising for the storage of the seeds under conventional conditions, being able to maintain the viability and vigor during storage. Mulamba and Mock selection index were capable of selecting superior cultivars with high physiological quality.

KEYWORDS: genetic resources, biodiversity, seed storage, seed viability, seed vigour.

RESUMO

As variedades crioulas de feijão representam adaptações locais heterogêneas de espécies domesticadas e, assim, fornecem recursos genéticos que atendem aos desafios atuais e novos da agricultura. Estes ecótipos locais podem mostrar fenologia variável e produtividade, baixa a moderada, mas são altamente nutritivos. Portanto, estratégias para estimular a conservação dessa diversidade genética são importantes. A manutenção do potencial de germinação de sementes também é uma característica relevante na caracterização da diversidade genética, a fim de conservar a biodiversidade. Objetivou-se com este trabalho discriminar cultivares de feijão da Universidade Estadual de Santa Catarina (UDESC) quanto à diversidade fisiológica de sementes e parâmetros agronômicos (taxa de germinação, viabilidade, vigor de sementes e crescimento de plântulas) e com base em Mulamba e Índice de seleção de simulação. Existe uma grande diversidade fisiológica nas cultivares de feijão. BAF 112 e BAF 81, BAF 84, BAF 42, BAF 60 e BAF 75, foram identificados como os mais promissores para o armazenamento de sementes sob condições convencionais, sendo capazes de manter a viabilidade e vigor durante o armazenamento. O índice de seleção de Mulamba e Mock foi capaz de selecionar cultivares superiores com alta qualidade fisiológica.

PALAVRAS-CHAVE: recursos genéticos, biodiversidade, armazenamento de sementes, viabilidade de sementes, vigor de sementes.

Narrowing the diversity of crop species contributing to global food supply has been considered a potential threat to food safety. The current system of industrial agriculture may be the most important threat to biodiversity (DWIVEDI et al. 2016, KHOURY et al. 2004, GEPTS 2006).

The landrace concept is useful for naming or distinguishing among cultivated varieties through simple traits that are locally adapted to traditional farming systems (CAMACHO VILLA et al. 2005). In Latin America, bean landraces contribute 70–90% of the seed planted by farmers for the production of food grain (ARENAS

CALLE et al. 2015), and according to the Asian Pacific Seed Association, the seed saved by farmers accounts for 80–90% of all of the seeds used in Asia (GRAIN 2016). Therefore, strategies to stimulate the conservation of this genetic diversity are important.

Common bean (*Phaseolus vulgaris* L.) is a valuable legume for human consumption worldwide, being an important source of high-quality proteins, carbohydrates, vitamins, minerals, dietary fiber, phytonutrients (flavonoids, lignins, phytosterols), and antioxidants (CARDADOR-MARTÍNEZ et al. 2002, REYNOSO-CAMACHO et al. 2006). Many of these compounds have important beneficial effects on human health, therefore, common bean has considerable potential as a functional food.

Plant landraces represent heterogeneous, local adaptations of domesticated species, and thereby provide genetic resources that meet current and new challenges for farming. These local ecotypes can show variable phenology and low-to-moderate edible yield, however, they are highly nutritious. The main contributions of landraces to plant breeding have been traits for more efficient nutrient uptake and utilization, as well as useful genes for adaptation to stressful environments such as water stress, salinity, and high temperatures (DWIVEDI et al. 2016). The conservation and maintenance of genetic diversity of species are considered environmental services (FEIJÓ et al. 2012), and they are strategic for an agriculture that seeks sustainability (GLIESSMAN 2000, PENTEADO 2010).

The phenotypic and genetic diversity of common bean landraces is typically evaluated through morphological traits, phaseolin seed proteins, allozymes, the biochemical-nutritional traits of the grain and DNA markers, with the local populations that are preserved on-farm as references, to describe the population structure, to understand diversification processes and biogeographic distributions, and to define strategies for conservation and utilization (BLAIR et al. 2012, ANGIOI et al. 2010, ASFAW et al. 2009, ZHANG et al. 2008).

However, the maintenance of seed germination potential is also an important feature in the characterization of genetic diversity to conserve biodiversity. The knowledge of the potential characteristics of landraces accesses, such as tolerance to seed deterioration, can help farmers to regain the habit of multiplying and conserving their seeds.

In this study, previously hypothesized that there is a large physiological diversity in common bean landrace cultivars available at UDESC germplasm bank and the main objectives of the research were to evaluate the physiological quality and agronomic parameters of common bean cultivars and discriminate them using MULAMBA & MOCK (1978) selection index.

Sample selection

16 common beans from UDESC germplasm bank cultivars were selected according to morph-agronomic traits as previously reported by COELHO et al. (2010a) and PEREIRA et al. (2009), technological quality (COELHO et al. 2008, BORDIN et al. 2010, ZÍLIO et al. 2014), nutritional quality (PEREIRA et al. 2011), and physiological seed quality (COELHO et al. 2010b, MICHELS et al. 2014). Table 1 summarizes the sampled cultivars.

Table 1. Identification and origin of the common bean (*Phaseolus vulgaris* L.) accessions used in the experiment.

Accession	Name of origin	Provenance	Color	Accession	Name of origin	Provenance	Color
BAF 13	Taquara	Caxambú do Sul/SC	Black	BAF 81	Preto 70 dias	Lebom Régis/SC	Black
BAF 36	Rasga	São José do Cerrito/SC	Black	BAF 84	Carioca Rosado	Pinheiro Machado/RS	Cream
BAF 42	Vagem Branca	Capão Alto/SC	Black	BAF 102	México 309	Goiânia/GO	Black
BAF 46	Sem nome	Lages/SC	Black	BAF 108	Branco	Recife/PE	White
BAF 50	Carioca Brilhante	Lebom Régis/SC	Cream	BAF 110	Guará	Lages/SC	Cream
BAF 55	Preto	Cunha Porã/SC	Black	BAF 112	Uirapuru	Lages/SC	Black
BAF 60	Preto 60 dias	Lebom Régis/SC	Black	BAF 115	BRS Valente	Lages/SC	Black
BAF 75	Serrano	Formigueiro/RS	Black	BAF 121	IAPAR 81	Lages/SC	Cream

BAF = number at the UDESC active germplasm bank collection, in the Santa Catarina state, Brazil, BAF 110 (SCS 202 Guará), BAF 112 (IPR 88 Uirapuru), BAF 115 (BRS Valente) and BAF 121 (IAPAR 81) are commercial cultivars.

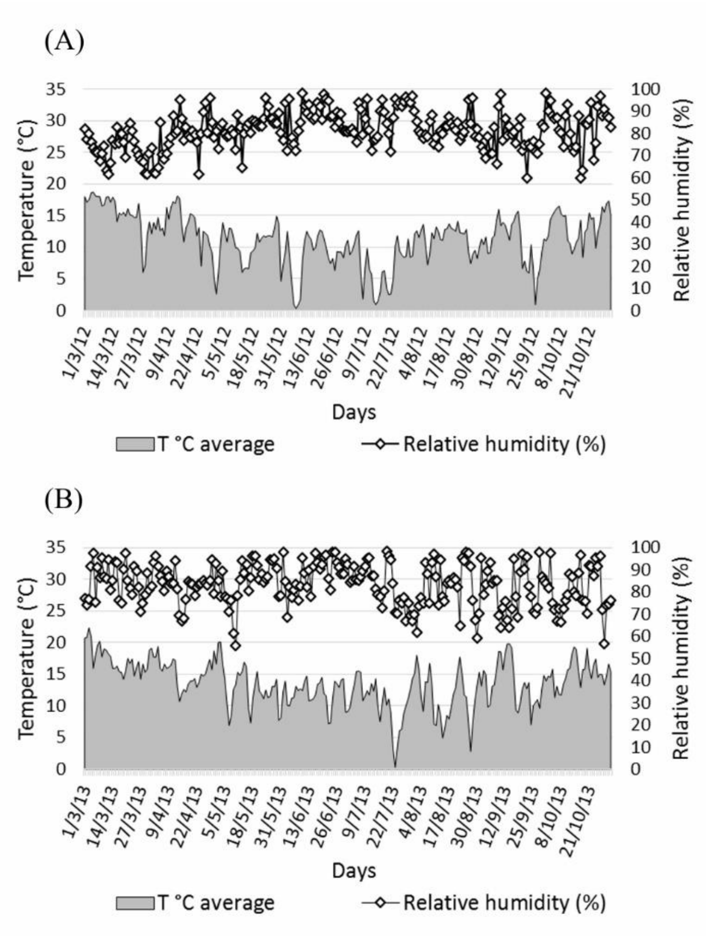
Seed multiplication and experimental design

An experiment following a randomized block design with three repetitions was performed with the main aim of seed multiplication for subsequent physiological and agronomic analyses. Seeds were harvested manually when they acquired 12% moisture and then packed in bags, transported to the laboratory and stored for subsequent analysis. The working sample consisted of 600 g of each cultivar (MAPA 2009).

Seed storage

The average samples were stored in cloth bags and kept in a conventional storage condition (without temperature control and relative humidity) between March and October (Figure 1). Mean air temperature during storage of seeds varied between 18 and 0 °C (March and June respectively) in the first year, and 22 and 0 °C (March and July respectively) in the second year. The relative humidity of the air oscillated between 60 and 100% in the two years of storage, most of the time remaining above 80%.

Figure1. Average temperature and relative air humidity determined from March 2012 to October 2012 (a) and March 2013 to October 2013 (b) in Lages, SC.



Source: own author, compiled by Epagri / Ciram / Inmet (2014).

Seed viability by germination test

The viability test was conducted using four replications of 50 seeds, wrapped in paper roller of type "Germitest" dampened with distilled water at a ratio of 2.5 times the mass of the dry paper, under constant temperature of 25 °C, at germination chamber. The analyses of seed germination were performed six days after the beginning of the test, as described by MAPA (2009).

Seed vigor by accelerated aging test

The accelerated aging test was conducted according to MONDO et al. (2016), using four replications of 50 seeds each. The seeds were distributed in single and uniform layer on stainless steel screen set in the interior of the plastic box type "seed dispersal" containing 40 mL of distilled water. The boxes were capped and kept for 48 h in aging chamber with 100% relative humidity and temperature of 420C/72 hours. After five days, the germination index and seedling length was determined as measures of vigor. For seedling length 20 normal seedlings from each repetition were measured with a ruler. The average was calculated and the results expressed in centimeters.

Data mining and selection index of Mulamba and Mock (1978)

The Genes software (CRUZ 2006) was used for statistical analyses, applying the F-test for analysis of joint variance and the Scott-Knott test at 5% to compare the means. Data of germination were arcsine transformed to follow normal distribution. The sum of ranks index proposed by MULAMBA & MOCK (1978) was used to identify the superior cultivars, based on the simultaneous selection of the traits assessed. This consists of classifying accessions in order, according to the classes of each trait assessed. After classification, the scores of the various traits of each accession were added up, with the lowest value indicating a more favorable combination of physiological seed quality traits and the highest an unfavorable combination with traits exhibiting less than desirable values. The sum of ranks index was associated with the Scott-Knott clustering method to characterize the accessions, in terms of physiological seed quality.

Seed viability, vigor and seedling growth

Table 2 and 3 summarizes the results of physiological parameters evaluated in the study. The results of seed viability evaluated by germination index, before sample storage, highlighted BAF 75, BAF 81, BAF 112, BAF 42, BAF 84, BAF 55, and BAF 13 as the most promising cultivars. Seed vigor results (accelerated aging tests and seedling length) highlighted BAF 84, BAF 81, BAF 55, BAF 75, and BAF 112 (Table 2).

Table 2. Percentage of germination and vigor by accelerated aging after harvesting the seeds of the landraces and commercial accessions of beans grown in the organic system in the 2011/2012 and 2012/2013 agricultural crops.

BAF	G (%)	Cl.[2]	BAF	AA (%)	Cl.[2]	BAF	LSAA (cm)	Cl.[2]
75	93a	1	81	93a	1	84	17,7a	1
81	92a	2	75	89a	2	81	17,1a	2
112	92a	2	13	88	3	55	16,5a	3
42	91a	4	84	87a	4	75	16,3a	4
84	90a	5	36	85a	5	112	16,1a	5
55	90a	5	60	84a	6	110	15,8b	6
13	90a	5	42	83a	7	60	15,5b	7
121	88b	8	115	83a	7	42	15,4b	8
115	87b	9	110	83a	7	115	15,4b	8
110	84b	10	112	82a	10	121	15,3b	10
60	84b	10	55	80a	11	102	14,7b	11
50	83b	12	121	80a	11	108	14,3c	12
36	80b	13	46	79ª	13	36	14,2c	13
102	79c	14	108	77a	14	13	13,7c	14
46	78c	15	50	76b	15	50	13,3c	15
108	77c	16	102	76b	15	46	13,2c	16

[1]Means followed by the same lowercase letter in each column belong to the same class, according to the Scott-Knott test, at a 5% probability level. [2]Classification of the observed variable. G = Germination, AA = Accelerated aging, LSAA = Length of seedlings after accelerated aging. Source: author's own production.

GINDRI et al. (2017) have already demonstrated the genetic diversity in the physiological quality of the seeds, shortly after harvest (before sample storage), in terms of viability and vigor, in the bean landraces accessions. However, seed deterioration begins after the seed reaches physiological maturity and continues to lose its ability to germinate, so that the evaluation of the physiological potential of the seeds soon after harvesting is necessary for the evaluation of their behavior during storage.

The results of seed viability evaluated by germination index, after sample storage, particularly noted were BAF 81, BAF 42, BAF 60, BAF 112, BAF 75, BAF 84, and BAF 115, with germination results above 84%. The seed vigor results, for accelerated aging tests, the BAF 81, BAF 42, BAF 60, BAF 112, BAF 75, BAF 84, and BAF 115 maintained the high percentages of vigor. Using the seedling length, the accesses were grouped into three classes, with emphasis on BAF 84, BAF 81, and BAF 110 (Table 3).

After the storage period, the accessions maintained the high germination percentages obtained shortly after harvesting. However, although the viability of a seed population is generally maintained at a high degree for a relatively long time, most of the cultivated species, when seeds are stored under adequate conditions, signs of deterioration appear as the storage period advances (NEDEL 2003). The reduction in vigor of seedlings growth and the increase in the number of abnormal seedlings are signs of seed deterioration.

MCDONALD (1999) reported that lipid peroxidation has been identified as the main cause of deterioration and, considering that lipids are part of the cell membrane of the seeds, there would be a direct relationship between lipid peroxidation and loss of cell membrane integrity.

Table 3. Percentage of germination and vigor by accelerated aging after the storage of the seeds of the landraces and commercial accesses of beans grown in the organic system in the 2011/2012 and 2012/2013 agricultural crops.

BAF	G (%)	Cl.[2]	BAF	AA (%)	Cl.[2]	BAF	LSAA (cm)	Cl.[2]
13	96a	1	81	88a	1	84	19,3a	1
42	94a	2	42	85a	2	81	18,4a	2
112	91b	3	60	85a	2	110	17,5a	3
75	89b	4	112	85a	2	55	16,6b	4
81	89b	4	75	84a	5	75	16,4b	5
84	89b	4	84	84a	5	60	15,9b	6
60	87c	7	115	84a	5	121	15,8b	7
55	87c	8	102	81b	8	42	15,7b	8
36	86c	9	108	81b	8	36	15,6b	9
110	85c	10	36	80b	10	13	15,6b	9
115	85c	10	50	79b	11	112	15,5b	11
121	84c	12	55	78b	12	115	15,4b	12
50	83c	13	46	77b	13	50	15,0b	13
102	82c	14	110	76b	14	108	14,3c	14
108	81c	15	13	74b	15	102	13,4c	15
46	80c	16	121	73b	16	46	13,3c	16

[1]Means followed by the same lowercase letter in each column belong to the same class, according to the Scott-Knott test, at a 5% probability level. [2]Classification of the observed variable. G =: Germination, AA = Accelerated aging, LSAA = Length of seedlings after accelerated aging. Source: author's own production.

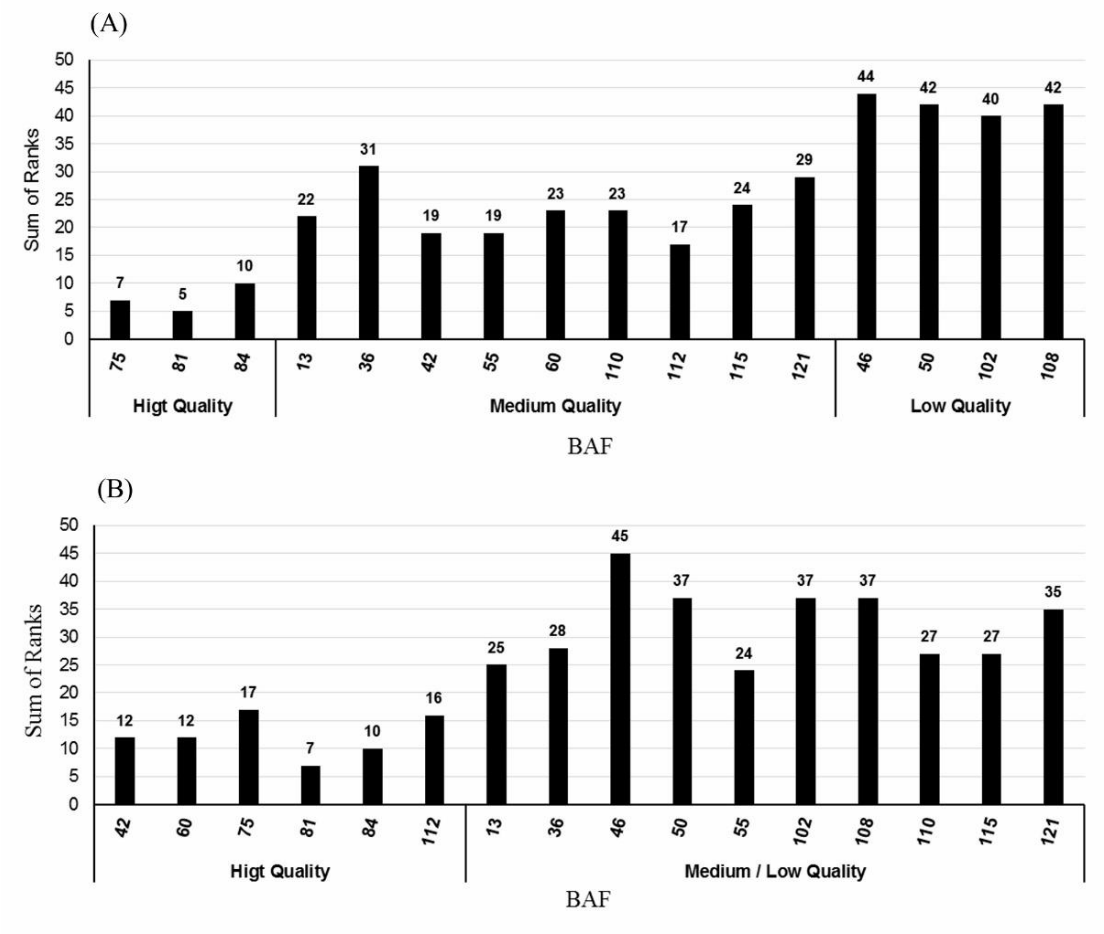
On the other hand, changes in sugars during accelerated seed aging indicate that the decline in vigor is associated with a marked decline of monosaccharides and raffinose (BERNAL-LUGO & LEOPOLD 1992). According to CHÂTELAIN et al. (2013), the methionine sulfoxide reductase repair system plays a decisive role in establishing and preserving the longevity of plant seeds.

As it can be observed, the main causes for the seed deterioration process are not yet fully understood, given the high number of cytological and metabolic alterations involved.

Cultivar discrimination using Mulamba and Mock selection index

The sum of ranks index of MULAMBA & MOCK (1978), before sample storage classified the cultivars into three classes, namely high, medium, and low physiological seed quality (Figure 2A). BAF 75, BAF 81, and BAF 84 were classified as higher physiological quality of seed, reaching germination percentage above 90% and vigor by accelerated aging above 87%. BAF 13, BAF 42, BAF 55, BAF 60, BAF 110, BAF 112, BAF 115, BAF 121, and BAF 36 were classified as intermediate physiological seed quality, with germination percentage above 84% and vigor by accelerated aging above 80%. They were included in the classification lower BAF 102, BAF 108, BAF 50, and BAF 46 showed the percentage of germination and accelerated aging effect for less than 79% (Figure 2A).

The sum of ranks by MULAMBA & MOCK (1978) index after sample storage combined the cultivars in two classes, defined as superior, intermediate/inferior physiological quality of the seeds produced (Figure 2B). BAF 81, BAF 84, BAF 42, BAF 60, BAF 112, and BAF 75 were classified as superior physiological quality of the seeds in the sum of stations, with germination percentage above 88% and vigor by accelerated aging above 84%. BAF 55, BAF 13, BAF 110, BAF 115, BAF 36, BAF 121, BAF 50, BAF 102, BAF 108, and BAF 46 were classified as intermediate/inferior physiological seed quality, with germination percentage above 80% and vigor by accelerated aging above 74% (Figure 2B). The BAF 81 and BAF 84 maintained their superiority in the physiological quality of the seeds. BAF 75 access had a reduced physiological quality during storage, mainly in relation to vigor by accelerated aging, which decreased from 93% to 84%, but remained in the group of higher physiological quality of the seeds after storage. BAF 42, BAF 60, and BAF 112, before storage included in the intermediate group, were included in the upper group after storage due to the maintenance of their physiological qualities.



BAF = number at the UDESC active germplasm bank collection, in the Santa Catarina state, Brazil.

Figure 2. Sum of ranks proposed by Mulamba and Mock (1978) and classified by the Scott-Knott clustering method of 16 landrace and commercial common bean accessions after harvest (A) and after storage (B), considering the variables under study (sum of ranks groups: high quality, medium quality, and low quality).

Results of the genetic factor shown to be highly significant on seed storage potential have also been reported in soybean, maize (LABBÉ 2003), and beans (MAIA et al. 2011), evidencing the possibility of success in selection of genotypes regarding the longevity of the seeds.

The multiplication and distribution of high-quality seeds may be an alternative to stimulate the conservation of genetic diversity. However, no accession should be ruled out for inferior seed quality, since they exhibit other important characteristics. As such, alternative management techniques should be found to achieve a better seed quality, as well as additional studies to improve storage conditions, in order to preserve the quality of seeds produced.

There is a large physiological diversity in common bean landrace cultivars. BAF 112 and BAF 81, BAF 84, BAF 42, BAF 60 and BAF 75, were identified as more promising for the storage of the seeds under conventional conditions, being able to maintain the viability and vigor during storage. MULAMBA & MOCK (1978) selection index were capable of selecting superior cultivars with high physiological quality.

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