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# Genetic Variation of Landraces of Common Bean Varying for Seed Coat Glossiness and Disease Resistance: Valuable Resources for Conservation and Breeding

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Enéas Ricardo Konzen and Siu Mui Tsai

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## Abstract

In this chapter, we outline the significance of landraces of common bean (*Phaseolus vulgaris* L.) for unraveling novel morphological, biochemical and genetic variation that could be integrated to breeding programs, related to seed coat color and glossiness and disease resistance. Moreover, we emphasize how important the conservation of such genetic resources is in small-farming areas, the prevailing system for bean cultivation. A particular Brazilian landrace referred as Serro Azul by local farmers is highlighted to show new evidences of the genetic control of seed glossiness in common bean and how it implicates in the seed protection against diseases and insects. Moreover, new findings presented here give insights into a remarkable anthracnose resistance of one of the variants of Serro Azul, which also presents seed coat glossiness. The potential benefits for human health after consuming beans with glossy seed coat are also discussed. This is one among the various landraces that need better understanding for strengthening the knowledge of the genetic diversity of common bean. Such knowledge is important for conducting conservation actions and performing new crosses for providing genetic materials with desirable combinations for farmers, breeders and consumers.

**Keywords:** Serro Azul, anthracnose resistance, asper gene, proanthocyanidins, crop genetic resources

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## 1. Introduction

The conservation of crop genetic resources is a fundamental step for further breeding of traits of interest. Common bean (*Phaseolus vulgaris* L.) is the major legume for human consumption

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throughout the world [1, 2]. It is naturally distributed from northern Mexico to northern Argentina, with a marked genetic structure. From the classic to the most recent reports, two major gene pools have been recognized for the species, the Mesoamerican and the Andean [3, 4]. Domestication has been independently performed within each gene pool [5, 6], selecting specific genes for growth habit, seed size, color and yield, a phenomenon described as the “domestication syndrome” [7]. Therefore, an enormous panel of diversity can be observed from wild to domesticated genotypes of common bean, making it as a model for understanding crop evolution [8].

The introduction of common bean to other areas than its natural habitat, such as in Brazil [9], led to new combinations of seed and flower colors, shapes and sizes, growth habits, cycle, photoperiod and yield [10]. It has also shaped the interaction between beans and the environment, leading to new diseases and pests.

In Brazil, common bean is a staple food among most citizens along with rice. Usually, its cultivation is performed in small farming systems along with other crops and animal production systems, providing self-sufficiency for farmers in various regions of the country. Several cycles of selection by local farmers in specific environments have generated new landraces, which are not yet known and available from core collections such as the ones from Centro Internacional de Agricultura Tropical (CIAT, Colombia), United States Department of Agriculture (USDA, USA) and Empresa Nacional de Pesquisa Agropecuária (EMBRAPA, Brazil). Landraces have singular aspects that might assist breeding programs for disease resistance, abiotic stress tolerance, improvement of nutrition facts, among several other desirable aspects of common bean grains.

A particular landrace has been discovered with local farmers from the municipality of Cunha, Sao Paulo state, Southeast Brazil. The farmers referred this landrace as “Serro Azul” and have been cultivating it along with other varieties [11]. Serro Azul shows considerable morphological variability, revealing different types of seeds. It shows high genetic variation for seed colors and patterns [12], disease and insect resistance and nodulation ability [11, 13], which are among the main aspect studies in breeding programs of common bean.

We drive the topic of this chapter highlighting Serro Azul as a case study of how the conservation of landraces might be important for further breeding strategies. First, we describe the importance of landraces of common bean in the discovery of new allele combinations for seed color and pattern genes, using the example of Serro Azul. Then, we briefly discuss the implications of the research of common bean landraces for nutrition and health. Moreover, we outline a significant number of original findings about our landrace in focus, which serve as examples of disease resistance as well as indications of insect resistance. Finally, we guide the reader through other perspectives of the importance of a better knowledge of landraces, their collection and conservation for future endeavors in breeding programs.

## **2. The Serro Azul landrace: parental genotypes and segregating populations**

Serro Azul is a landrace that was so named by local farmers living in the countryside of the small municipality of Cunha, in São Paulo state, Brazil. From the search along the distinct

farms at that area, De Oliveira et al. [11] were able to collect seeds with three main patterns of colors such as gray background with black strips (Serro Azul Malhado—SAM), light brown with glossy seed coat (Serro Azul Brilhante—SAB) and dark gray with dull seed coat (Serro Azul Fosco—SAF). Interestingly, at one of the farms, from a fresh sample of SAF seeds collected (approximately 1 kg), only 10 seeds presented the SAB pattern (**Figure 1A** shows the phenotypes of SAB and SAF). Seeds with the SAB pattern were crossed to the SAF pattern and the reciprocal as well, and populations were advanced to  $F_4$  generation, revealing consistent patterns of segregation for seed glossiness and color [12].

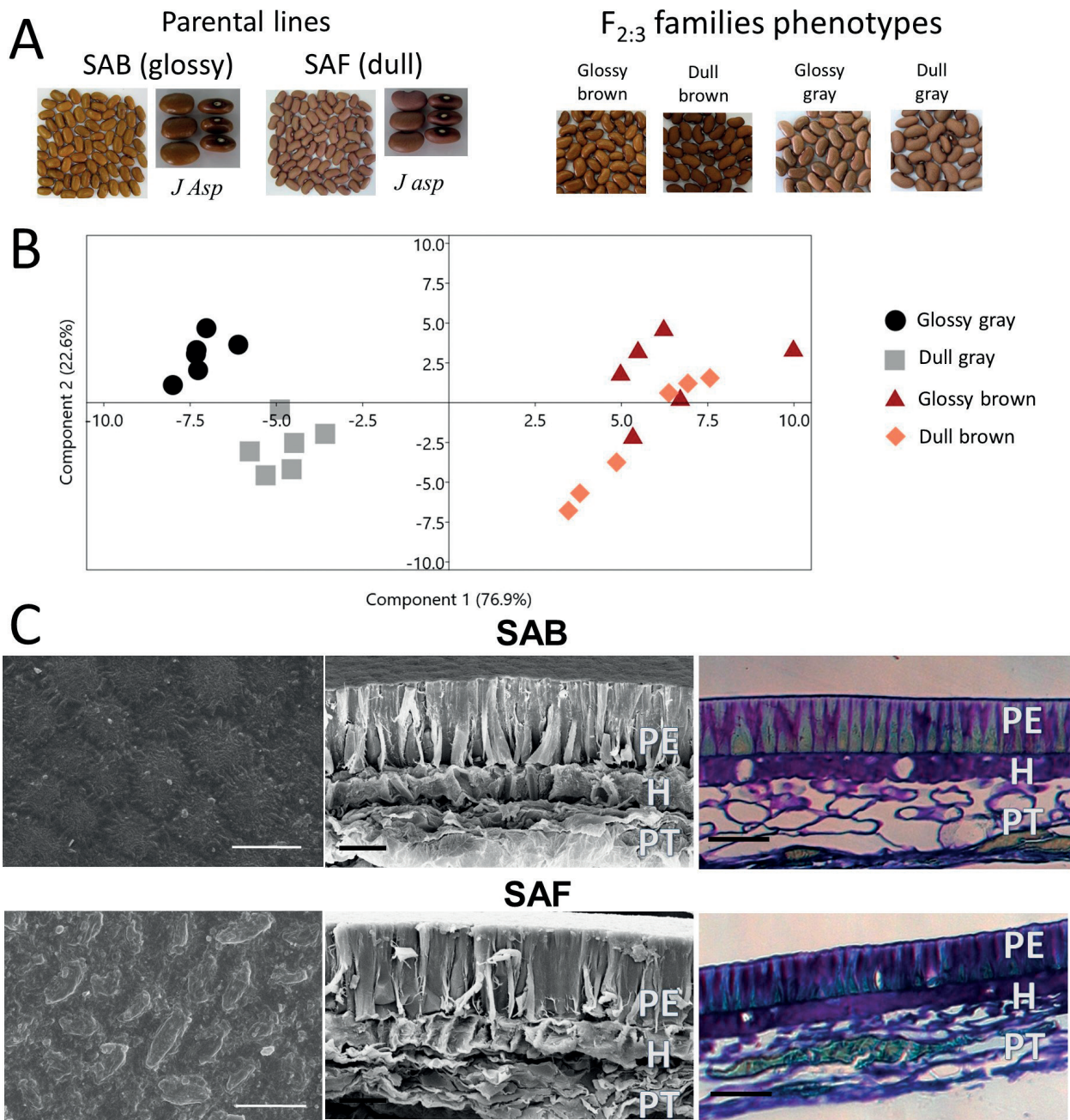
Serro Azul has been the object of a few studies, considering its importance to local farmers and the need of improving its productivity. It has been subjected to many diseases, such as anthracnose, and pests. De Oliveira et al. [11] and De Oliveira and Tsai [13] showed low yield from this variety from experiments performed at different farms in Cunha, SP, when no fertilizer was applied. This treatment represented the traditional way that farmers cultivated this landrace at their farms. However, when a sort of fertilizer treatments were applied in soil cultivated with this variety, along with one commercial standard at the time (IAC-Carioca 80SH), the yield was significantly improved from around 930 kg ha<sup>-1</sup> (without fertilizer) to 1360 kg ha<sup>-1</sup> (fertilizer application along with lime and foliar spray of molybdenum) [13].

### 3. Unraveling the genetic control of seed glossiness and its importance taking Serro Azul as an example

#### 3.1. Genetic control of seed glossiness

The genetic control of seed coat color, pattern and glossiness in common bean has been a major issue for scientists in decades. Frequently, the gene nomenclature for loci related to such traits was confusing, leading to a series of meetings for establishing standard nomenclatures (Bean Improvement Cooperative meetings). As regards seed glossiness, Bassett [14] has clarified the differences between glossy and opaque seed coats through a series of genetic crosses, providing genetic stocks (pure lines for specific alleles of seed coat loci) that have been used since then for unraveling the genetic and biochemical aspects of the trait.

Landraces might be the source of additional alleles or allele combinations for studying genes related to color, pattern and glossiness of the seed coat. Moreover, these new sources might be interesting for being added to the breeding programs concerned with such traits. The study of Konzen and Tsai [12] examined the particular aspect of Serro Azul of segregating for seed glossiness and color patterning. The population developed from the cross SAF × SAB was analyzed from the parental genotypes to the  $F_4$  generation for the segregation of these traits. The variation of glossiness was attributed to two alleles at the *Asp* locus, usually referred as the gene controlling glossiness in common bean [14]. SAB carries the dominant allele *Asp*, which confers the intense glossy aspect of the seed coat, while SAF has a dull seed coat [12] (**Figure 1A**). This gene is known to be located in chromosome 7 [15], where genes associated with anthracnose and angular leaf spot resistance [16, 17], common bacterial blight [17] and nodulation [18] are also located.



**Figure 1.** Morphological and anatomical aspects of seeds of the common bean landrace Serro Azul. (A) Parental genotypes (Serro Azul Brillhante—SAB and Serro Azul Fosco—SAF) and the four phenotypes of  $F_{2:3}$  families of the cross SAF  $\times$  SAB. The parent SAB has a glossy brown seed coat, while the SAF seed coat shows a dull gray phenotype. The  $F_{2:3}$  families segregate for both color and glossiness. (B) Principal component analysis with the  $L^*a^*b^*$  variables, obtained with a colorimeter for  $F_{2:3}$  families of the cross SAF  $\times$  SAB.  $a^*$ : the amount of green or red;  $b^*$ : the quantity of blue or yellow;  $L^*$ : the quantity of brightness. (C) Scanning electron profiles of the seed coat surface (left image) and their transversal profile (central image) and histological sections (image on the right) of transversal sections of the seed coat of both SAB and SAF. PE: palisade epidermis; H: hypodermis; PT: parenchymatous tissue. The scale bars indicate 10  $\mu$ m (left image), 20  $\mu$ m (central image) and 100  $\mu$ m (right image) (adapted from [12]).

However, the glossiness of the seed coat in Serro Azul is also related to the expression another gene located in chromosome 10 and referred as the *Joker* (*J*) locus [19]. The dominant allele *J* is responsible for an even distribution of the color shown by each seed, while *jj* genotypes exhibit irregular coloring, especially around the hilum ring [14]. Another peculiar aspect

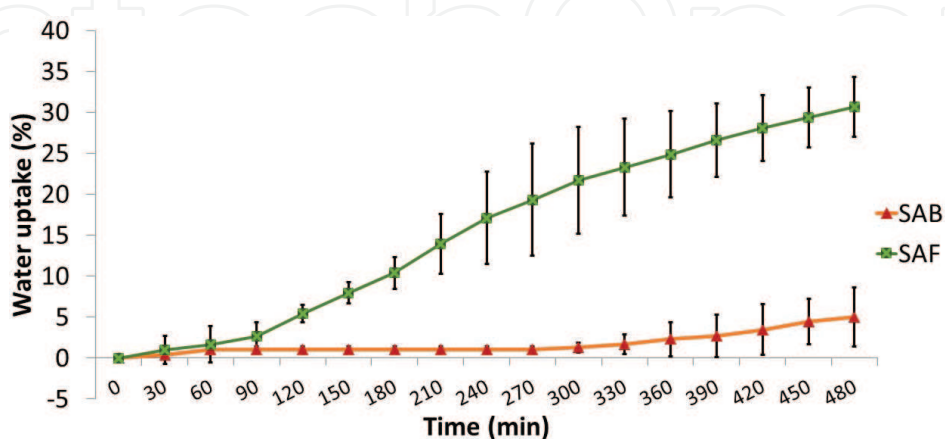
related to the *J* allele is that it intensifies the glossy aspect of the seed, as seen in both SAB and SAF (**Figure 1A**). Therefore, SAF presents a slight shiny aspect on the seed coat due to the expression of *J* [12].

The segregation of the seed coat glossiness along with the color pattern is clearly observed in  $F_{2,3}$  lines (**Figure 1A**) of the cross SAF  $\times$  SAB and is only due to the *Asp* locus. *J* only contributed to intensify the shiny aspect and is present as the dominant allele in both SAB and SAF. The segregation of glossiness is according to the expected Mendelian proportions (3,1) in  $F_2$  and  $F_3$  generations of the cross between SAF (*asp asp*) and SAB (*Asp Asp*), being attributed to *Asp*. Measurements performed with a colorimeter allowed to validate the categories established (glossy and dull) (**Figure 1B**), based on the  $L^*a^*b^*$  color system, in which  $L^*$  is the main variable associated with glossiness, while  $a^*$  and  $b^*$  are measures of distinct colors [12]. In this study, in general, glossy seeds presented higher  $L^*$  values than seeds with dull seed coat. However, there was some extent of interaction between the color and the brightness, especially with brown-colored seeds (**Figure 1B**).

Another important aspect of the seed coat glossiness of SAB was shown from microscopy analyses. It followed and confirmed previous findings of classic studies of the glossiness of other common bean genotypes. In general, dull genotypes (*asp asp*) have a rough textured surface of the seed coat, while glossy seed coats have an even surface, as shown from scanning electron micrographs [20] (**Figure 1C**, left image). Moreover, glossy seeds show a thicker palisade epidermis from the seed coat than the dull seed coats (**Figure 1C**, central and right figures) [12].

### 3.2. How important might seed glossiness be?

The seed glossiness has been frequently neglected in breeding programs due to consumer preferences. This is explained by the fact that glossy seeds tend to require higher cooking times than seeds with an opaque seed coat [21]. At first, it seems that since glossiness retards water absorption by the seeds [12, 22] (check **Figure 2**), they take longer to be cooked. However, some line of evidence showed no significant correlation between the cooking time



**Figure 2.** Water uptake on the course of 480 min of the variants Serro Azul Brillhante (SAB, with glossy seed coat) and Serro Azul Fosco (SAF, with dull seed coat). Three replicates of seeds were embedded in distilled water and paper dried every 30 min for weighing and determining the weight change (adapted from [12]).

and the water absorption rate [23]. Further examination of the genes involved in cooking time is necessary, though.

It is well known that the seed coat is the structure that protects the seeds from pathogens and insects, and the glossiness seems to have an important role in such protection. Moreover, seeds with glossiness might have enhanced antioxidant properties due to a higher concentration of specific secondary metabolites in the seed coat, therefore, having an impact in human health [12].

Usually, in the case of landraces, where local selection has been performed, it is more frequent to find common bean accessions that show glossy seed coat [24] (checking the list of genotypes) than in breeding programs. In the case of the landrace Serro Azul, both variants Serro Azul Brillhante (glossy) and Serro Azul Fosco (opaque) have been cultivated [11, 13]. Morphological and biochemical findings are hereafter discussed to show advantages of the seed glossiness for aspects related to human health.

### 3.3. Biochemical nature of seed coat glossiness and its implications for human health

The seed coat glossiness has been studied to be mainly conditioned by the *Asp* gene but also influenced by the *J* locus, especially with the dominant allele [14]. A number of studies have also been conducted to better understand the biochemical implications of the expression of such genes on the seed coat.

Classical work has suggested that *J* is essential for the synthesis of proanthocyanidins or condensed tannins [14]. Therefore, the recessive *jj* genotypes have been found to be absent in condensed tannins [25], contrary to the *J*<sub>-</sub> genotypes, that are able to synthesize such compounds. Based on the genetic maps that identified the RAPD marker as linked to *J*, a recent study has shown that *J* is linked to a region containing *MYB123* [26], similar to TT2 in *Arabidopsis thaliana* (AT5G35550.1) [27] and *Glycine max* [26], which acts as a key determinant in the proanthocyanidin accumulation of a developing seed [27].

Proanthocyanidins are oligomers or polymers formed by the condensations of flavan-3-ols units such as catechins and epicatechins [28, 29]. In common bean, condensed tannins are mainly composed of catechin monomers [30]. As secondary metabolites, they play important roles as antioxidants, anticarcinogenic and anti-inflammatory [28, 31, 32].

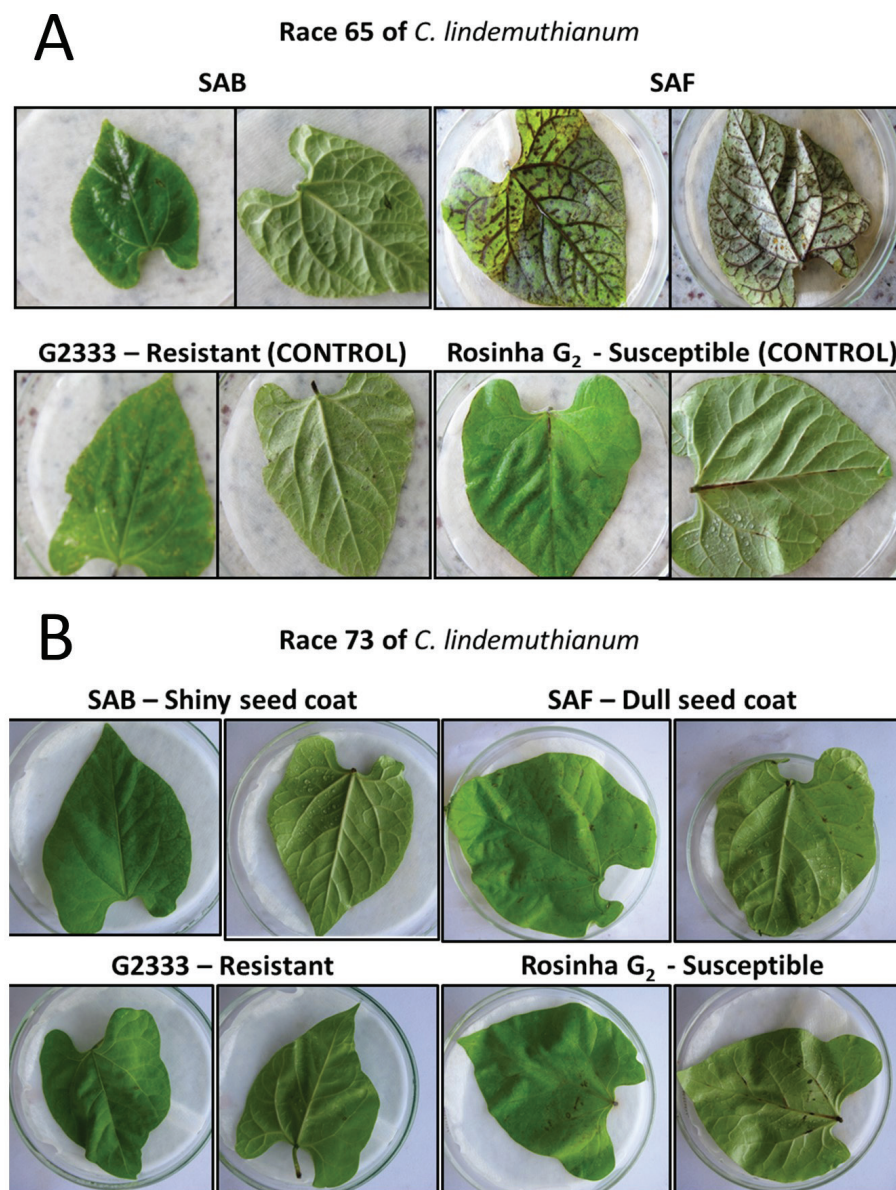
On the other hand, the *Asp* locus is said to be the main gene involved in seed glossiness. Some line of evidence has shown that *Asp* affects the accumulation of anthocyanins due to a structural change that it promotes on the seed coat. Therefore, genotypes with glossy seed coat (*Asp*<sub>-</sub>) accumulate more anthocyanins than dull seed coats (*asp asp*) [20]. Anthocyanins have been investigated for their roles in humans such as in anti-inflammatory, lipid peroxidation and membrane strengthening processes [33, 34], as well as in preventing cancer [35].

Therefore, although glossiness has been generally neglected by consumers and, as a result, by selection programs, it might have positive implication for human health. Moreover, the indication that glossiness is not necessarily associated with higher cooking time (since there is a lack of correlation between water absorption and cooking time) as shown by Garcia et al.

[23] needs to be further explored by the researchers. Landraces such as Serro Azul are one of the sources for rescuing the value of seed glossiness.

#### 4. Serro Azul as a source of disease resistance

One of the most important aspects of a breeding program is to find genotypes that are tolerant or even resistant to diseases. The cultivation of common bean is majorly affected by diseases such as common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli*, the angular leaf spot caused by the fungus *Pseudocercospora griseola* and anthracnose by the fungus



**Figure 3.** Screening for anthracnose resistance with races 65 and 73 (*Colletotrichum lindemuthianum*) on SAB and SAF plants. Controls: Rosinha G<sub>2</sub> (susceptible) and G2333 (resistant).



*Colletotrichum lindemuthianum*. The genetic architecture and mechanisms of resistance to such diseases have been studied at genomic scales, identifying specific genes, pathways and QTL associated to each one (see [16, 36, 37]). As landraces are usually genetically structured and locally adapted, they might be the source of new alleles for disease resistance, which could be added to the disease resistance breeding programs.

Here, we present new findings obtained with experiments conducted with Serro Azul Brillhante and Serro Azul Fosco, as regards their variation for anthracnose resistance. The resistance degree to *C. lindemuthianum* was evaluated using the method of detached leaves [38], with modifications. Seeds of each parental line (SAB and SAF) and control standards from the literature (cultivars Rosinha G2 and G2333 are highly susceptible and resistant, respectively) were germinated and transferred to pots with substrate (Plantmax) in a greenhouse, and irrigated properly until the establishment of the first trifoliolate (around 21 days). One leaf was collected from each plant and immediately placed in a suspension of  $1.2 \times 10^6$  conidia mL<sup>-1</sup> of *C. lindemuthianum* races 65 and 73 [39]. After 1 min, the leaves were placed in Petri dishes containing two layers of moistened filter paper. The plates were then incubated in a BOD type chamber and maintained in a photoperiod of 12 h at 21°C ( $\pm 2^\circ\text{C}$ ) for 7 days. For analysis, plant resistance was inferred according to the standards proposed by CIAT, using a scale from 1 (resistant—no symptoms) to 9 (susceptible—evident necrosis).

The results revealed an interesting difference between the parents SAF and SAB, used to constitute the segregating populations. The detached leaf method clearly showed that SAB was highly resistant to both races studied (65 and 73), while SAF showed high susceptibility to the *Colletotrichum* races, especially race 65 (**Figure 3**). The scores for disease resistance were significantly different among the two parents (SAB and SAF) for the race 65, but the average score with the race 73 also suggested higher degree susceptibility in SAF (**Table 1**). It is interesting to notice that the score for SAF was higher than the standard, which is used as a control for anthracnose susceptibility (Rosinha G2). In a similar manner, SAB was even more resistant than the standard resistant line G2333 (**Table 1**).

Genotype	Mean score	
	Race 65	Race 73
G2333 (resistant control)	2 $\pm$ 0.8	1 $\pm$ 0.5
Rosinha G2 (susceptible control)	5 $\pm$ 0.4	4 $\pm$ 0.2
SAB	1 $\pm$ 0.0	3 $\pm$ 0.6
SAF	7 $\pm$ 3.0	5 $\pm$ 1.1
Mean comparisons—Tukey's test		
G2333 $\times$ Rosinha G2	p = 0.012*	p = 0.001*
SAB $\times$ SAF	p = 0.0001**	p = 0.081 ns

\*Significant at  $P < 0.05$ , \*\*Significant at  $P < 0.01$ .

**Table 1.** Average scores of *Colletotrichum lindemuthianum* infection in leaves of the variants Serro Azul Brillhante (SAB) and Serro Azul Fosco (SAF), compared to G2333 (resistant control) and Rosinha G2 (susceptible control).

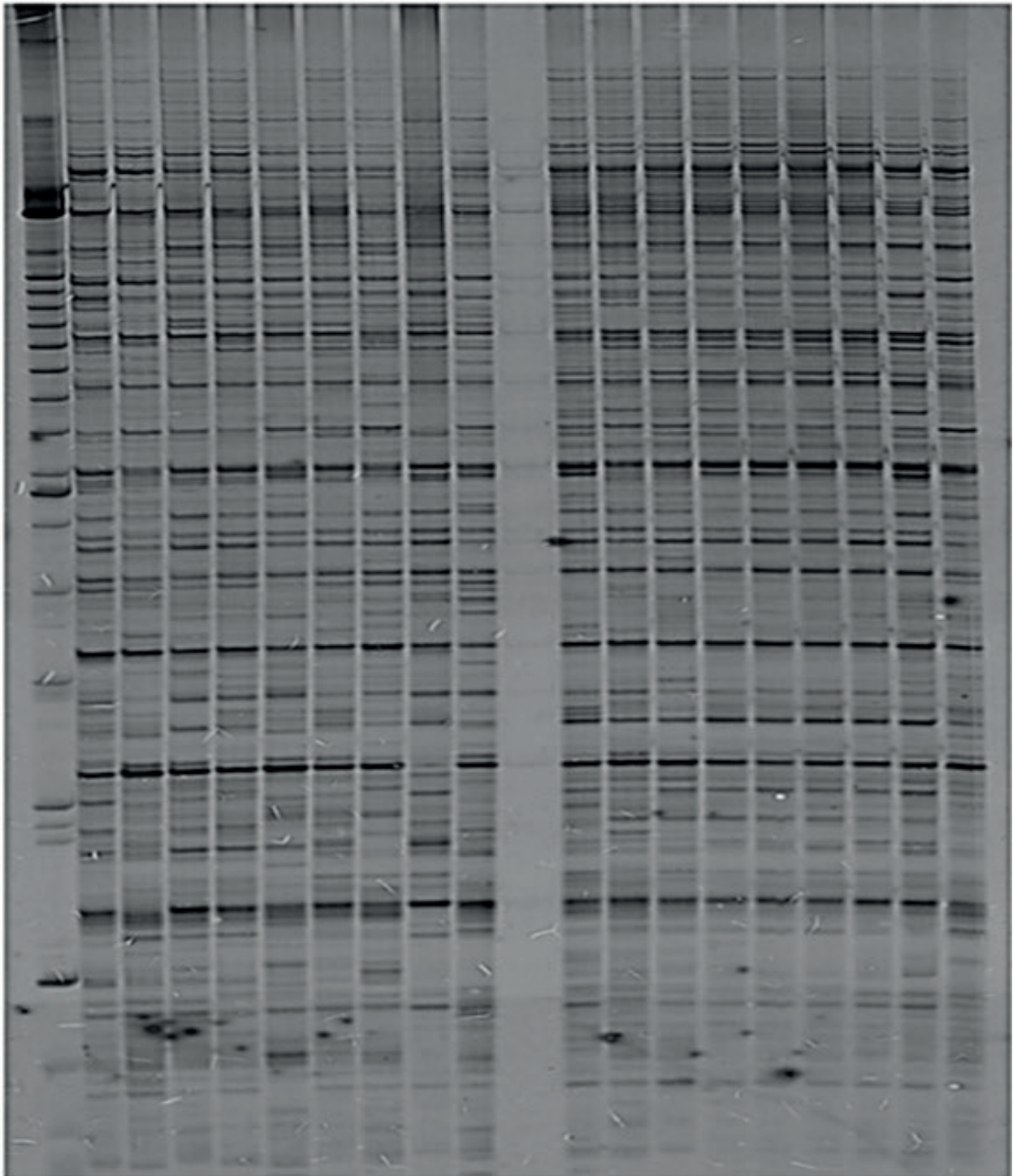
The evident difference between SAF and SAB needs further examination. It raises questions such as if the anthracnose resistance is somehow influenced by the glossiness of SAB. After all, genes related to anthracnose resistance are also located in chromosome 7 [40], but a specific study linking *Asp* to such genes is not yet available. However, it could be simply a new source of resistance originated from a mutation or to a combination of specific alleles conferring resistance. These are only speculations that need experimental clarification. The available genomic technology and the bioinformatic tools for constructing genetic maps with high resolution might be helpful in answering those questions.

## 5. Analysis and conservation of the genetic diversity of landraces

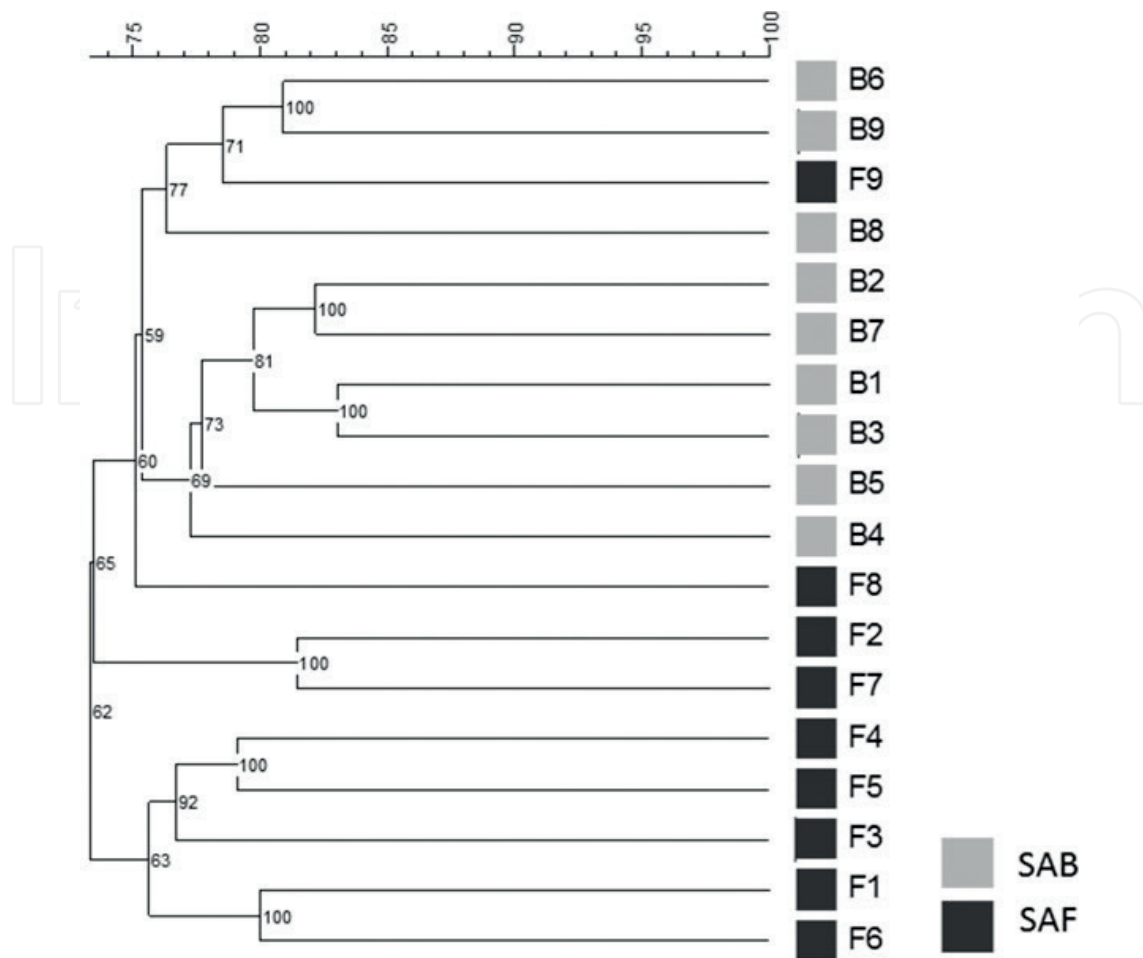
The genetic diversity of common bean landraces, cultivars and wild accessions has been investigated in multiple studies, mainly based on morphological and molecular markers in the last two decades (from 1995 to 2017). In the 1990s, studies have shown high genetic diversity based on morphological, enzyme and DNA-based markers (RAPD and microsatellites) [41–43]. After 2000, several studies involving amplified fragment length polymorphic (AFLP) [44, 45] and microsatellite [24, 46, 47] markers have been conducted. After 2010, with the advances of the sequencing technology, numerous papers have addressed the molecular diversity of common bean based on SNP markers [4, 48–51]. In general, most of the studies revealed that common beans are divided into two main gene pools, the Mesoamerican and Andean. In the case of Brazil, where common beans were introduced and have been cultivated mainly in small farming systems, varieties of both pools have been encountered. However, Burle et al. [24] investigated the genetic diversity of almost 300 landraces cultivated in Brazil and demonstrated that almost 80% of the genotypes have a Mesoamerican background, based on a population structure analysis with microsatellite polymorphisms.

In the case of Serro Azul, we also examined the molecular diversity of a sample of plants from both the variants (Serro Azul Brilhante and Serro Azul Fosco) by using the AFLP markers. Selective amplifications were done with four primer combinations (*EcoRI-A/MseI-CC*; *EcoRI-AC/MseI-CC*; *EcoRI-AT/MseI-CA*; *EcoRI-T/MseI-CA*). The AFLP gels revealed a considerable variation within and among SAB and SAF plants (**Figure 4**). An UPGMA tree was designed using Bionumerics software (**Figure 5**). Our data revealed a good separation among SAB and SAF samples, although some mixture was detected. In general, the SAB subgroup presented 75% of similarity with the SAF subgroup. Two plants of the SAF, however, were grouped within the SAB subgroup. From the analysis of the UPGMA tree, it is possible to infer that SAB plants were derived from SAF, which is consistent with the prior observation that SAB seeds were observed within 1 kg of SAF seeds harvested at a farm in Cunha, SP. Hereby, the high variability at the morphological levels can be verified at the molecular level, revealing a landrace with considerable genetic diversity to be explored. The development of the SAF × SAB population, for instance, revealed a high variation for seed size, color and water uptake [12]. Moreover, we showed that the parental lines have noticeable differences for disease resistance.

M SAB (glossy) SAF (dull)



**Figure 4.** AFLP profile (primer combination *EcoRI-A/MseI-CC*) of individual plants from SAB (Serro Azul Brillhante) and SAF (Serro Azul Fosco). M stands for the ladder DNA of 100 bp.



**Figure 5.** UPGMA tree based on Jaccard similarity analysis and AFLP profiles of the two variants of the landrace Serro Azul.

## 6. Perspectives of the conservation and use of bean landraces

The findings about Serro Azul provide interesting insights of the use and application of landraces in common bean breeding. A distinguishable morphologic diversity is noticeable within the landrace, which can be further explored to investigate genes responsible for color and glossiness [12]. SAB and SAF are consistently different at the molecular level as well, as revealed by the AFLP profiles. An examination of AFLP polymorphisms among  $F_4$  lines of the cross SAF  $\times$  SAB revealed a potential discrimination of color classes in the population by the molecular approach [12]. Furthermore, Serro Azul and the population developed might be used to investigate the genetic control for such incredible difference in anthracnose resistance between SAB and SAF. Another interesting observation comes from field observations where the SAF  $\times$  SAB population was being tested. Usually, lines with similar features to the SAB parental line, especially the seed glossiness, presented very low incidence of bruchid attacks. On the other hand, SAF-derived lines were usually susceptible to the insects, leading to damages to the seeds.

The remarkable variability of Serro Azul and the interesting association with glossy seeds (SAB) with resistance to anthracnose and bruchids raises further research questions and opportunities for new crosses. As previously suggested, seed coat glossiness might after all have an important role in protecting seeds against biotic stresses, as SAB has shown. Conversely, we have not demonstrated that the disease resistance of SAB plants has association with glossiness, which needs more experiments. Either way, this is an important feature which might be explored in depth with the population derived from this landrace to appropriately answer this question. Landraces such as Serro Azul hold particularities that should not be disregarded, after all, local communities need those seeds for their supply, and they have traits of high interest to be explored by breeders, especially concerning the threat of anthracnose and insects to common bean cultivation.

From this example to a wider set of landraces, several traits of interest might be improved with the use of distinct allele combinations if not new alleles provided by such genetic materials. Burle et al. [24] analyzed 279 landraces of common bean from Brazil and discovered considerable genetic diversity among all the accessions evaluated. Those genotypes are distributed from colder to warmer and from wetter to drier areas in the country. The local adaptation implicated in such genotypes has implicated in potential sources of disease and insect-resistant accessions. Moreover, the climatic diversity provides the potential for adapting to distinct abiotic stresses. New sources for tolerance to drought, soil salinity, high and low temperatures are to be investigated from these collections. In fact, Burle et al. [10] continued the previous work and integrated phenotypic evaluations to the genetic analysis of the same 279 landraces. The authors screened these accessions based on 22 morphological traits, including resistance to rust and common bacterial blight, yield, flowering time, determinacy and growth habit, seed coat color and brilliancy, among others. The study provided valorous information for supporting initiatives toward conservation and management of the accessions. It also allowed to detect the particularities of landraces and how they can be explored in controlled crosses for designing new populations and cultivars.

## 7. Conclusions

In this chapter, we described the importance of the conservation and study of landraces of common bean. As an example, to go through the extent of morphological, biochemical and genetic aspects of landraces, we outlined the previous results as well as the new findings about the Brazilian landrace Serro Azul. This landrace has been produced by local farmers from Sao Paulo state and has remarkable features for exploring variation of seed and yield traits in common bean, constituting an additional and valuable genetic resource for germplasm collections. We also showed a genetic diversity analysis of Serro Azul by examining the molecular variability within subsamples of SAB and SAF, based on amplified fragment length polymorphic (AFLP) markers. Furthermore, a detached leaf assay for screening anthracnose resistance was employed for both variants within this landrace, revealing remarkable differences between the highly resistant Serro Azul Brilhante (glossy seed coat) and the susceptible Serro Azul Fosco (opaque seed coat). Together, these results demonstrated the importance of

studying genetic aspects related to traits such as color, glossiness, disease resistance and yield components. This is necessary to conserve such valuable resources as the ones maintained by small farming systems. It may as well be applicable to other landraces of common bean, in order to provide better understanding of the genetic resources available and how they can be explored in favor of the farmers and breeders.

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## Conflict of interest

The authors declare no conflicts of interest.

## Author details

Enéas Ricardo Konzen<sup>1,2\*</sup> and Siu Mui Tsai<sup>2</sup>

\*Address all correspondence to: [erkonzen@gmail.com](mailto:erkonzen@gmail.com)

1 State University of Santa Catarina, Lages, SC, Brazil

2 Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil

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