

Research Application Summary

Postharvest darkening of seed coat in common bean: Paths towards breeding for better marketability

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Abstract

Postharvest darkening (PHD) of seed coat, resulting from the oxidation of proanthocyanidins, is a critical challenge to the marketability of common bean since this characteristic is associated with long cooking time by consumers, thus lowering market values of darkened seed. Extensive research has been done to elucidate the phenotypic, biochemical, genetic, and genomic bases of PHD in several market class beans from many regions of the world. However, little or no information is available on the PHD status in the sub-Saharan Africa (SSA) bean germplasm under local environmental conditions. Therefore, the current review aims at systematically revisiting the available scientific information regarding the control of seed coat PHD from the reported studies to better guide future breeding efforts in sub-Sahara Africa. We investigated avenues for improving the trait in SSA in the context of a multi-trait and multi-environment paradigm basing our proposed approaches on the state-of-art standards.

Keywords: Color, market classes, *Phaseolus vulgaris*, Proanthocyanidins

Résumé

L'assombrissement post-récolte (PHD) de l'enveloppe de la graine de haricot, résultant de l'oxydation des proanthocyanidines, est un défi critique pour la commercialisation du haricot commun puisque cette caractéristique est associée à un long temps de cuisson par les consommateurs, diminuant ainsi la valeur marchande de la graine assombrie. Des recherches approfondies ont été menées pour élucider les bases phénotypiques, biochimiques, génétiques et génomiques du PHD sur plusieurs classes commerciales d'haricots dans de nombreuses régions du monde. Cependant, peu ou pas d'informations sont disponibles sur le statut du PHD dans le germoplasme de haricot d'Afrique sub-saharienne (ASS) dans les conditions environnementales locales. Par conséquent, le présent travail vise à faire une revue systématique des informations scientifiques disponibles concernant le contrôle du PHD du tégument à partir des études rapportées afin de mieux guider les efforts futurs de sélection en Afrique subsaharienne. Nous avons étudié les possibilités d'amélioration de ce caractère en Afrique subsaharienne dans le contexte d'un paradigme multi-trait et multi-environnemental, en basant les approches proposées sur des normes technologique de pointe.

Mots-clés: Couleur, classes de marché, *Phaseolus vulgaris*, Proanthocyanidines

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes worldwide in terms of production and consumption. Being the most affordable food legume, common bean is consumed by more than 80 million people across Latino America, the Caribbean, and Eastern and Southern Africa. Most of these producers and consumers are mainly smallholder farmers living with less than two U.S. dollars a day (Mukankusi *et al.*, 2019; Oladzad *et al.*, 2019). The crop is cultivated on over 30 million hectares in Africa, where it is a crucial cheap source of diet components such as starch, protein, fiber, iron, zinc, potassium, selenium, molybdenum, vitamins, and folate (BB, 2018 ; Mukankusi *et al.*, 2019). In East Africa, Uganda ranks second producer and consumer. For the people in Uganda, common bean provides 25% and 45% of the daily requirements in calories and protein (Blessing *et al.*, 2016; Katuramu *et al.*, 2020).

Aside from food and nutrition provision, common bean contributes to household income generation (Ojiewo *et al.*, 2019; Letaa *et al.*, 2020). Different market classes were defined for dry beans based on several seed characteristics, notably seed size, shape, and color (Erfatpour *et al.*, 2021). The market value and consumer acceptance of common bean seed are highly dependent on its ability to retain its light color during storage, since darkened seeds are associated with hard to cook and long cooking time by consumers, which leads to loss of commercial value (Konzen and Tsai, 2014; Siqueira *et al.*, 2014; Erfatpour *et al.*, 2018; Islam *et al.*, 2020; Mutungi *et al.*, 2020; Wiesinger *et al.*, 2021). Besides, reduced seed coat darkening is positively associated with iron bioavailability (Wiesinger *et al.*, 2021), hence promoting bean consumers' health. However, seeds of several bean varieties, especially those belonging to the pinto, cranberry, small red, pink, carioca, and kidney bean market classes, tend to darken after prolonged storage (Figure 1), which poses significant challenges to traders since beans that retain their original color are preferred (Kelly and Bornowski, 2018).

Most of the research carried out on postharvest darkening (PHD) of bean seed coat was carried out on pinto bean, cranberry beans, and carioca beans, mostly grown in southern and northern America and parts of Europe (Alvares *et al.*, 2020; Islam *et al.*, 2020). Also, resistance to seed coat darkening has received limited attention from bean breeders, especially in SSA, despite the economic and health impacts of this phenomenon. In SSA, the majority of bean varieties released are prone to seed coat PHD, which hampers their conservation and prolonged marketability, within both local and international markets. However, limited research has been undertaken to identify lines possessing slow or non-darkening lines within the local germplasm, and to understand the biochemical, genetic, and environmental factors that determine bean seed coat PHD traits.

This dearth of knowledge is a challenge for designing of breeding strategies to develop slow seed coat darkening bean varieties expected to increase their storability, hence, the capacity of bean farmers and consumers to reasonably manage their stocks for planned consumption and sale. In this review, we summarize the factors that underpin PHD in bean seed coat and present research and breeding perspectives that could be considered to sustainably improve PHD in beans.

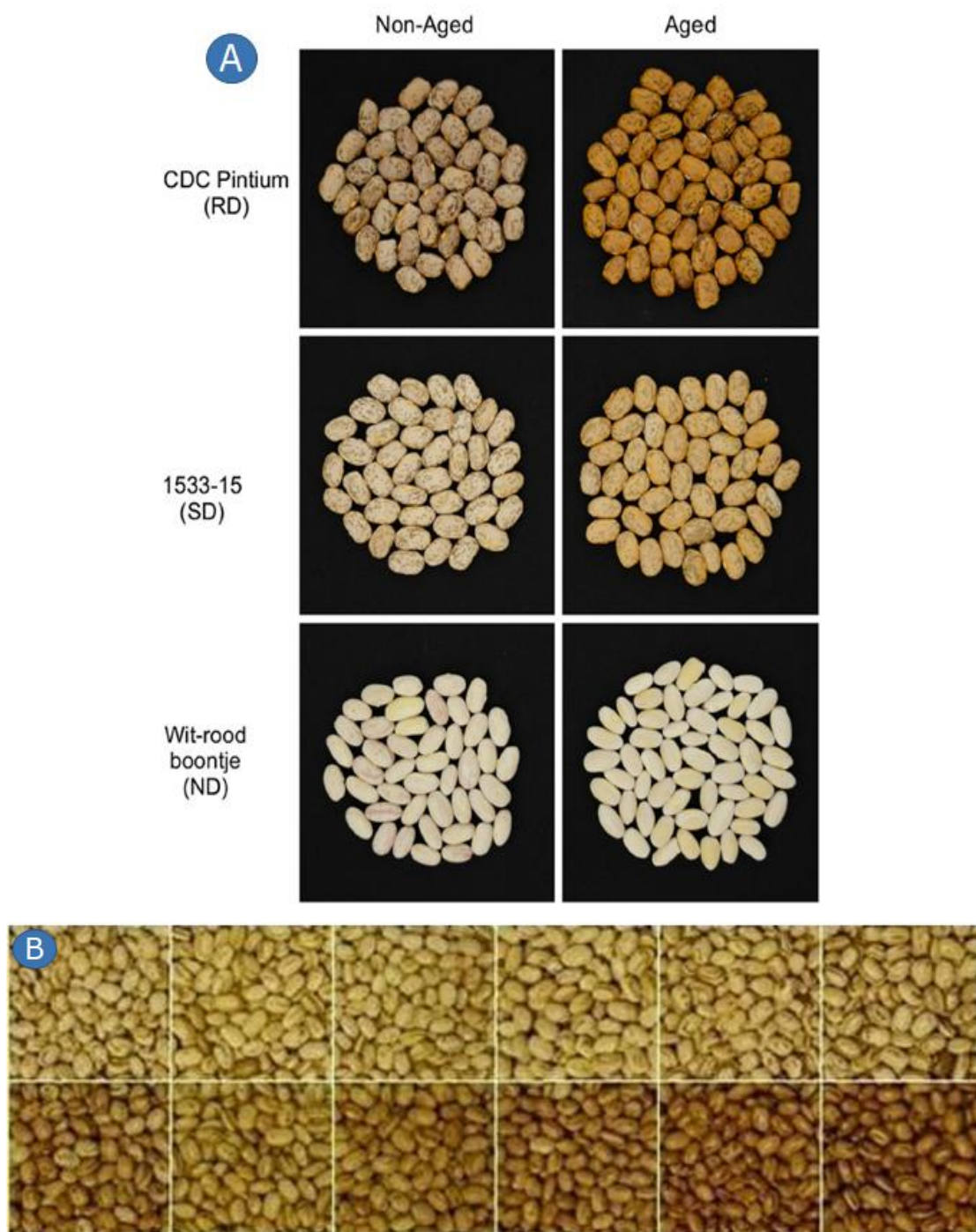


Figure 1. Color changes through PHD during storage of pinto beans (A) (Islam *et al.*, 2020) and carioca beans (B) (Siqueira *et al.*, 2014). In (A), freshly harvested bean seed (Non-aged) are compared with seed stored for six months at room temperature (Aged). In (B), the upper scares represent fleshly harvested seeds while the downer ones consisted of seeds stored for six months at room temperature.

How does bean seed coat PHD take place? Based on PHD, three phenotypic classes were identified for beans, namely non-darkening (ND), slow-darkening (SD), and regular-darkening (RD) (Elsadr *et al.*, 2011). Bean seed coat PHD is controlled by both genetic and environmental factors (Elsadr *et al.*, 2011). Factors such as harvest condition, prolonged storage, levels of heat, humidity, and luminosity while harvesting and storage facilities, contents in some polyphenols such as proanthocyanidins in the seed coat; factors that interact to determine how fast the seed under storage darkens (Alvares *et al.*, 2020; Mutungi *et al.*, 2020).

a. Influence of harvesting time and storage condition on bean seed coat PHD. Bean seed coat PHD can be accelerated by many environmental factors that pertain to the harvesting time and conditions, storage conditions and shelf life duration. Seed coat PHD can be accelerated by high temperature, increased humidity, and high light, the same factor responsible for the bean seed hardening, hence the positive correlation between the two traits, thus the preference of consumers for non-darkened bean seed (Junk-Knievel *et al.*, 2007; Alvares *et al.*, 2020; Islam *et al.*, 2020). Growing season, delayed harvesting, rain during harvest, prevailing temperatures contribute to fast darkening of bean seed (Reis *et al.*, 2021). Silva *et al.* (2014) and Reis *et al.* (2021) reported that the pace of bean seeds PHD depended on the growing season/year and the growing locations which embed environmental specificities surrounding the growing crop. Besides, genotype-by-environment interaction was reported to influence seed darkening (Silva *et al.*, 2014; Alvares *et al.*, 2016). Silva *et al.* (2014) also noted that phenotypic characteristics of the trait may differ from one population to another depending on parental background and a significant genotype-by-environment interaction was reported (Pereira *et al.*, 2012; Silva *et al.*, 2014; Alvares *et al.*, 2016), thus necessitating testing different populations in diverse environments. Duration of seed storage is known to adversely affect color, which is the result of the impacts and interaction between ambient conditions such as temperature, humidity, and light exposure, and biochemical and enzymatic processes that continue in the seed a long way after harvest (Siqueira *et al.*, 2014). Xiong *et al.* (2020) reported that proanthocyanidins content were influenced by temperature and phenolic compounds and seed coat colors were modified by storage duration, with storage temperatures ranging from 20 to 30 °C and storage durations between 120 or 180 days provoking increased seed darkening compared to seeds stored at lower temperatures for shorter durations.

b. Biochemical basis of bean seed coat PHD. The occurrence of seed coat-darkening is associated with the accumulation of polyphenolic compounds in seed coats (Chiorato *et al.*, 2015; Freixas-Coutin, 2017). These compounds interact in the harvested seed while it continues to physiologically and biochemically ripen after harvest, leading in most bean genotypes, to impairing the quality of the seeds, notably their color (Graham and Ranalli, 1997). A product of the flavonoid biosynthesis pathway which is part of the phenylpropanoids pathway, the proanthocyanidin plays a crucial role in the PHD of bean seed (Duwadi *et al.*, 2018; Erfatpour and Pauls, 2020; Islam *et al.*, 2020), and this biosynthetic pathway has been extensively reviewed (see Erfatpour *et al.*, 2018; Erfatpour and Pauls, 2020; Islam *et al.*, 2020) and is illustrated in Figure 2. The biosynthesis of proanthocyanidins starts by the combination of three molecules of malonyl-CoA with one 4-coumaroyl-CoA molecule that produces a chalcone molecule, and through a multi-enzymatic complex, the pathway leads to the synthesis of proanthocyanidins and anthocyanins (Erfatpour and Pauls, 2020). In bean seeds, proanthocyanidins are produced in the endothelium layer and later on, relocate in seed coat during maturation (Sm kal *et al.*, 2014; Erfatpour and Pauls, 2020). It should be noted that the proposed pathways of proanthocyanidins biosynthesis in pinto beans and cranberry beans are slightly different (Freixas-Coutin, 2017; Islam *et al.*, 2020), and the same should be expected in other bean classes depending on genotypic and population-based

characteristics. The oxidation of proanthocyanidins into reactive quinones gives rise to the dark color developed by most bean seeds after harvest and during storage (Freixas-Coutin, 2017; Islam *et al.*, 2020; Erfatpour and Pauls, 2020). Fast darkening bean seeds were reported to accumulate great proportions of proanthocyanidins than their slow or non-darkening counterparts (Freixas-Coutin, 2017; Erfatpour *et al.*, 2018; Erfatpour and Pauls, 2020; Islam *et al.*, 2020; Erfatpour *et al.*, 2021). A metabolic study conducted by Duwadi *et al.* (2018) revealed that most of the most flavonoids accumulated at higher concentrations in regular darkening beans seeds than in the slow-darkening lines. Also, Beninger *et al.* (2005) measured lower kaempferol levels in the regular darkening bean as compared to the slow-darkening bean (Kelly and Bornowski, 2018). Proanthocyanidins and phenolic acids were recorded just ten days post-flowering a regular darkening (at harvest and during storage) bean genotype (Arikara Yellow), whilst CDC sol, a non-darkening genotype, maintained low phenolic compounds in the same conditions during the same period of time (Xiong *et al.*, 2020).

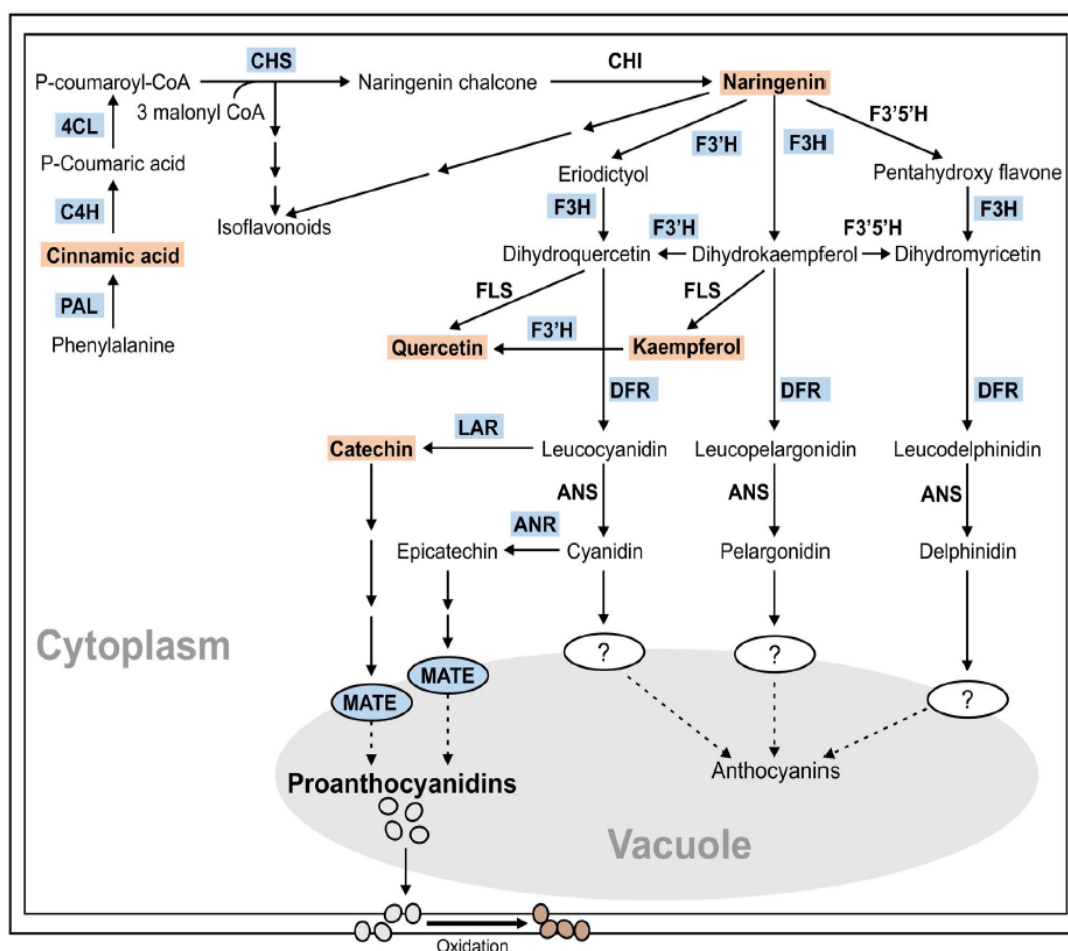


Figure 2. Scheme of proanthocyanidin biosynthesis in pinto bean. The dashed arrows represent speculative steps and multiple arrows indicate multiple steps. The corresponding transcripts (blue highlights) and metabolites (orange highlights) accumulate at a higher level in developing seed coats of CDC Pintium (RD) compared to 1533-15. 4Cl, 4-coumarate-CoA ligase; ANR, anthocyanidin reductase; ANS, anthocyanidin synthase; ANS, anthocyanidin synthase; C4H, cinnamate-4-hydroxylase; CHI, chalcone isomerase; CHR, chalcone reductase; CHS, chalcone synthase; DFR, dihydroflavonol reductase; F3'5'H, flavonoid 3',5'-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3H, flavanone 3-hydroxylase; FLS, flavonol synthase; LAR, leucoanthocyanidin reductase; MATE, multidrug and toxic compound extrusion protein; PAL, phenylalanine ammonia-lyase (Islam *et al.*, 2020).

Genetic and genomic basis of bean seed coat PHD

Genetic variability and inheritance of seed coat PHD. Substantial genetic variability exists among bean germplasm for seed coat PHD, which is a prerequisite for selection after careful crossing and advancement to later generations (Pereira *et al.*, 2008; Araújo *et al.*, 2012; Silva *et al.*, 2014; Alvares *et al.*, 2016; Alvares *et al.*, 2020; Bassett *et al.*, 2021). Seed coat PHD was reported to have high heritability values in carioca, pinto, and cranberry bean types (Junk-Knievel *et al.*, 2008; Araújo *et al.*, 2012; Silva *et al.*, 2014; Erfatpour and Pauls, 2020; Bassett *et al.*, 2021). Also, both genetic diversity (Rodrigues *et al.*, 2019) and heritability (Silva *et al.*, 2014) were reported to increase with storage duration, implying that selection would be more efficient at longer storage periods. However, depending on the objective of a breeding program, as to whether to breed for slow-darkening at short or long storage time, breeders should decide on when to make a selection, since longer storage time may incur delays and more expenses. Nonetheless, these high genetic variabilities and heritability values predict a predominant proportion of additive gene action for bean seed coat PHD, thus potential to achieve quick gain from selection (Pereira *et al.*, 2012; Alvares *et al.*, 2016). However, the maternal control of the traits implies that selection can only be efficiently carried out at F3 (Alvares *et al.*, 2019).

Genes and transcripts controlling PHD. Several studies investigated the genes involved in the control of bean seed coat PHD and their interactions. Junk-Knievel *et al.* (2008) and Silva *et al.* (2008) concluded on the existence of one gene (Sd), controlling the traits with a dominant allele responsible for regular darkening (Rodrigues *et al.*, 2019). This monogenic control was later on confirmed by Alvares *et al.* (2016) on carioca beans, however, Elsadr *et al.* (2011) evaluated the trait in several bean classes and concluded on the existence of two recessively epistatic genes modulating seed coat PHD. Elsadr *et al.* (2011) reported the J gene, mapped on Pv07, controlling the onset of darkening (JJ) or non-darkening (jj), and the Sd gene, located on Pv10, which determined the pace of darkening with the genotype sdsd responsible for slow darkening and the genotype SdSd controlling regular darkening in seeds. Silva *et al.* (2014) reconciled the monogenic and oligogenic control of the trait by concluding from a study that considered carioca beans that seed coat PHD was under the control of one or a few genes. At a transcriptomic level, studies revealed two genes of unknown function and three proanthocyanidin biosynthesis genes, FLAVANONE 3-HYDROXYLASE 1, DIHYDROFLAVONOL 4-REDUCTASE 1, and ANTHOCYANIDIN REDUCTASE 1 (Freixas Coutin *et al.*, 2017) and several phenylpropanoid and transporter genes (Duwadi *et al.*, 2018) differentially expressed between slow-darkening and regular-darkening bean genotypes. Also, it should be noted that the presence of the dominant J locus is the only instance where variability darkening pace will occur since any line carrying the recessive alleles (jj) will be ND (Elsadr *et al.*, 2011).

Markers associated with seed coat PHD. In the quest of a faster way to breed for slow-darkening in beans, especially the use of marker-assisted selection (MAS), which should be an efficient approach for improving seed coat PHD in beans, given the oligogenic nature of the trait, several studies have investigated genomic regions controlling the trait and associated markers. McClean *et al.* (2002) identified a sequence-tagged site (STS) OL4S500 associated with the J locus. In a study that involved, Couto *et al.* (2010) identified three makers linked to a quantitative trait loci (QTL) responsible for delayed seed darkening in a population of 185 F2:3 lines (VC 3 x BRSMG Majestoso). These markers were the PVM02TC116 (co-segregating with the QTL) and its flanking markers PVESTBR 98 (2.00 cM) and PV176 (12.24 cM) (Couto *et al.*, 2010). Also,

Felicetti *et al.* (2012) identified three simple sequence repeats (SSRs) markers, namely Pvsd-1157, Pvsd-1158, and Pvsd-0028 tightly linked to the *sd* locus in F2 and F3 population derived from pinto parents. However, only the Pvsd-0028 could be validated by Erfatpour *et al.* (2018) in a recombinant inbred line (RIL) population generated from crossing a cranberry-like bean line (Wit-rood boontje) and a pinto line characterized by slow-darkening seed. Additionally, Erfatpour *et al.* (2018) identified a major QTL associated with non-darkening between single nucleotide polymorphism (SNP 715646341) and SNP 715646348 on Pv10 containing forty candidate genes majorly involved in the phenylpropanoid pathway from which proanthocyanidins are produced. An additional SNP, PvbHLHp12804 was associated with the *Sd* gene by Alvares *et al.* (2020) in four carioca populations developed from crosses between SD parents and four RD parents in Brazil. Rodrigues *et al.* (2019) established the efficiency of Pvsd-1158 and PvbHLHp12804 in MAS. Besides, they also proved that the same gene (*Sd*) controls seed coat darkening in pinto and carioca beans (Rodrigues *et al.*, 2019).

In a follow-up study, Erfatpour and Pauls (2020) conducted an amplicon sequencing of 21 genes located within the previously identified QTL associated with ND (Erfatpour *et al.*, 2018). They identified a single nucleotide deletion in one of the candidate genes, Phvul.010G130600, in RILs possessing the ND trait, and developed a gene-based marker of the dominant allele of Phvul.010G130600 for use in MAS for the ND trait (Erfatpour and Pauls, 2020). Also, Islam *et al.* (2020) identified the *Psd*, a gene encoding a bHLH transcription factor, of which one of the two transcripts is involved in proanthocyanidins synthesis, as a novel gene controlling seed coat PHD in pinto beans. They further demonstrated that the addition of a glutamate residue in the activation domain, and a single nucleotide substitution (arginine to histidine) in the bHLH domain of the transcript involved in the proanthocyanidin biosynthesis could be the basis of low expression of the allele in the SD lines compared to RD lines, resulting in a slow darkening process the former group. The marker developed from *Psd* gene could be instrumental in MAS for breeding bean varieties possessing the SD trait (Islam *et al.*, 2020). In a QTL mapping within a RIL population of dry yellow beans from a cross between Ervilha (Manteca) and PI527538 (Njano), Bassett *et al.* (2021) identified one QTL for non-darkening which colocalized with other QTLs for water uptake, cooking time, total flavor, beany, starchy, bitter, seed-coat perception, cotyledon texture, and seed color on Pv10, prompting the possibility of combining these traits in bean lines.

Relationship between seed coat PHD and other important bean traits

Although seed coat PHD is an important bean trait with tremendous consumer consideration, it is important to make sure its selection is not achieved at the expense of other important traits such as short cooking time, pest and disease resistance, yield, and related traits. Paulino *et al.* (2022) identified two superior inbred lines combining slow-darkening with multiple disease resistance and good agronomic traits such as yield and 100 seed weight, indicating that selection for ND or SD is possible along with some other important traits in beans. Alvares *et al.* (2020) reported positive but weak genetic correlations between seed coat PHD and cooking time, signifying that long storage time may result in negative status for both traits in beans, which was in line with results reported by Bento *et al.* (2020). However, Reis *et al.* (2021) found no correlation between seed coat PHD and cooking time. Also, hydration capacity, which is a companion trait for cooking time, was found to be weakly correlated with PHD (Erfatpour *et al.*, 2021). In the same vein, Pereira and Batista (2014) indicated that bean seed PHD and hardening did not happen concomitantly in most bean genotypes they evaluated at different storage times, resulting in low correlations

between the two traits. Furthermore, bean seed coat PHD, especially the ND characteristic, was found to be weakly positively correlated with days to maturity and weakly negatively correlated with grain yield (Erfatpour *et al.*, 2021). These reports prove inconclusive as to the relationship between bean seed coat PHD and cooking time, and therefore, required more investigation in diverse genetic and geographical backgrounds to better inform breeding decisions.

Generating pre-breeding knowledge for the development of non or slow seed darkening bean varieties

It is worth noting that the occurrence and pace of seed darkening are influenced by environmental factors, and these parameters are highly variable depending on the geographical locations, besides the genetic control at play in each germplasm (Araújo *et al.*, 2012; Alvares *et al.*, 2020; Islam *et al.*, 2020). Therefore, to be able to set sustainable breeding strategies that would enable incorporate SD or ND traits in local germplasm, it is pivotal to verify the information of seed coat PHD reported in studies carried out elsewhere, bearing in mind the specificities of the local context. This necessity of generating information locally is further supported by the variability of seed coat PHD underpinned by genetic and population parameters (Elsadr *et al.*, 2011). Therefore, in East Africa, especially in Uganda, the available bean germplasm should be evaluated in multi-environment trials to determine the genetic parameters that control the traits, their interaction with the environment, and to identify superior genotypes for targeted or broad adoption.

Furthermore, proanthocyanidins are products of the phenylpropanoids biosynthesis pathway (He *et al.*, 2008; Erfatpour and Pauls, 2020; Islam *et al.*, 2020), which also involves the biosynthesis of several other compounds that play critical roles in plant productivity, biotic and abiotic resistance, the antioxidant capacity of the bean seed, bioavailability of some nutrients such as iron, cooking time, and many other determinant parameters for the quality of the seed (Dwivedi *et al.*, 2016; Shah and Smith, 2020). Therefore, efforts to improve seed coat PHD in beans should take into consideration other traits that could be altered by single-trait selection.

A knowledge-informed multi-trait selection strategy (Figure 3) should help to durably improve bean seed coat while maintaining other agronomically and commercially important traits. Such approaches should firstly make a collection of a genetically and geographically diverse, and regionally adapted bean germplasm, with if required, necessary introductions for seed coat PHD traits. Then, it should adopt a holistic investigation and understanding of the metabolic, biochemical, phenotypic, and genomic factors that control seed coat darkening, its interaction with and the influence of growth, management, and storage environments, and with other consumer-preferred traits, to design breeding strategies and interventions that will lead to packaging seed technologies that will not only be adopted by farmers and consumers, but also provide enough productivity and health guarantees to end-users. Figure 3 summarizes the main steps for a sustainable improvement of bean seed coat PHD in a particular region, moving from germplasm collection to varieties release, with the most relevant stages in the breeding pipeline to optimize and accelerate the release of slow or non-darkening bean varieties that adequately respond to different stakeholder interests.

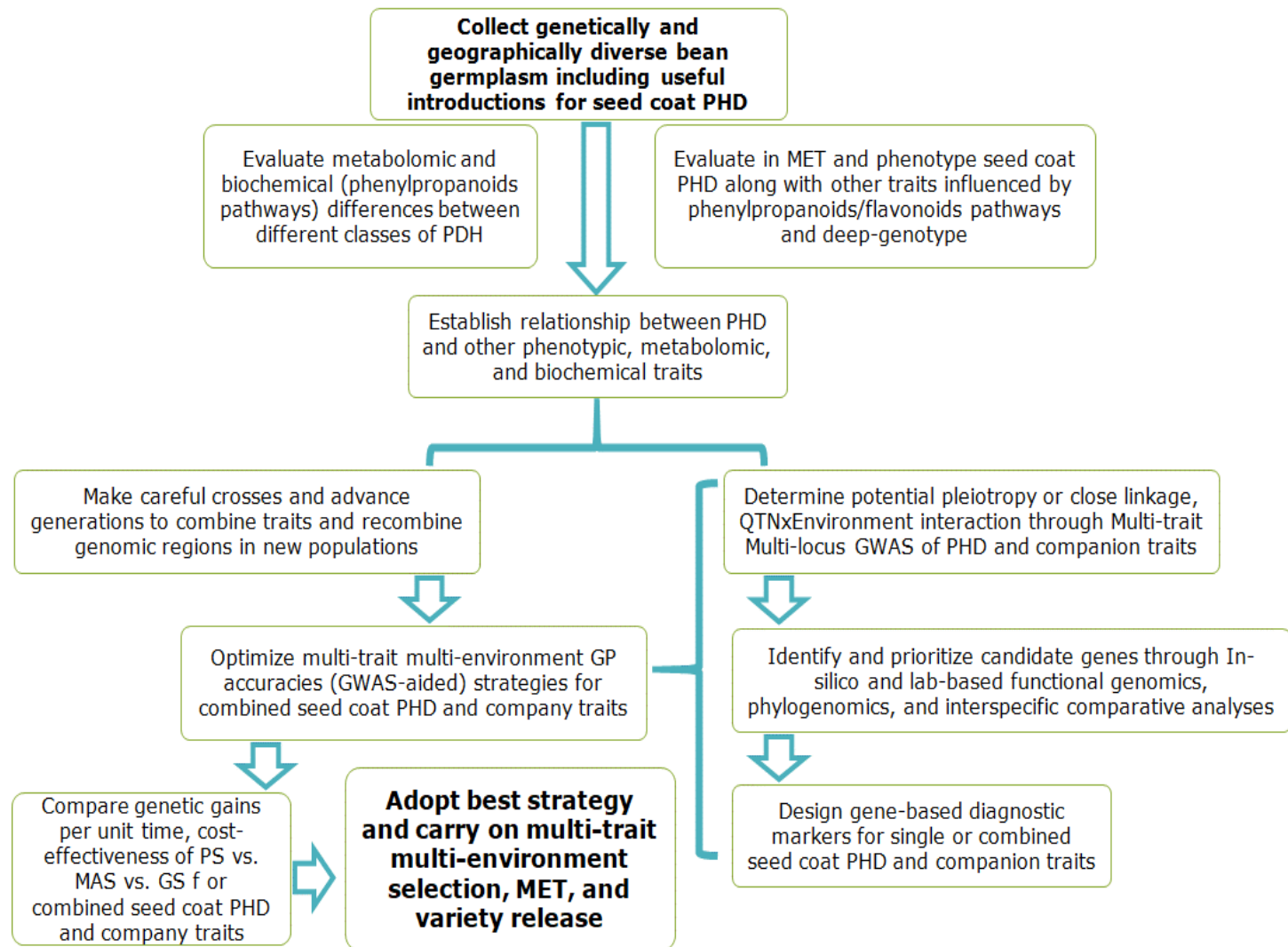


Figure 3. Proposed pre-breeding and breeding scheme to improve seed coat PHD while maintaining other agronomically and commercially important traits.

GP=Genomic prediction; GS=Genomic selection; GWAS=Genome-wide association study; MET=Multi-environment trial, PHD=Postharvest darkening; PS=Phenotype-based selection.

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References

- Alvares, R. C., Silva, F. C., Melo, L. C., Melo, P. G. S. and Pereira, H. S. 2016. Estimation of genetic parameters and selection of high-yielding, upright common bean lines with slow seed-coat darkening. *Genetics and Molecular Research* 15 (4): 1–10. <https://doi.org/10.4238/gmr15049081>
- Alvares, Renata C., Pereira, H. S., Melo, L. C., Miklas, P. N. and Melo, P. G. S. 2020. Induction of seed coat darkening in common beans (*Phaseolus vulgaris* L.) and the association with cooking time after storage. *Australian Journal of Crop Science* 14 (1): 21–27. <https://doi.org/10.21475/ajcs.20.14.01.p1500>
- Alvares, Renata C., Stonehouse, R., Souza, T. L. P. O., Melo, P. G. S., Miklas, P. N., Bett, K. E., Melo, L. C., Rodrigues, L. A., Souza, L. L. and Pereira, H. S. 2019. Generation and validation of genetic markers for the selection of carioca dry bean genotypes with the slow-darkening seed coat trait. *Euphytica* 215:141. <https://doi.org/10.1007/s10681-019-2461-y>
- Araújo, L. C. A. de, Ramalho, M. A. P. and Abreu, Â. de F. B. 2012. Estimates of genetic parameters of late seed-coat darkening of carioca type dry beans. *Ciência e Agrotecnologia* 36 (2): 156–162. <https://doi.org/10.1590/s1413-70542012000200003>
- Bassett, A., Kamfwa, K., Ambachew, D. and Cichy, K. 2021. Genetic variability and genome-wide association analysis of flavor and texture in cooked beans (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 134 (3): 959–978. <https://doi.org/10.1007/s00122-020-03745-3>
- BB, D. 2018. Common Bean Improvement Status (*Phaseolus vulgaris* L.) in Ethiopia. *Advances in Crop Science and Technology*, 06(02). <https://doi.org/10.4172/2329-8863.1000347>
- Beninger, C. W., Gu, L., Prior, R. L., Junk, D. C., Vandenberg, A. and Bett, K. E. 2005. Changes in polyphenols of the seed coat during the after-darkening process in pinto beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry* 53 (20): 7777–7782. <https://doi.org/10.1021/jf0500511>
- Bento, J. A. C., Lanna, A. C., Bassinello, P. Z., Oomah, B. D., Pimenta, M. E. B., Carvalho, R. N. and Moreira, A. S. 2020. Aging indicators for stored carioca beans. *Food Research International* 134: 109249. <https://doi.org/10.1016/j.foodres.2020.109249>
- Blessing, A. O., Stanley, T. N., Clare, M., Pamela, P., Rubaihayo, P., James, K. and James, S. 2016. Prevalence and variability of the common bean rust in Uganda. *African Journal of Agricultural Research* 11 (49): 4990–4999. <https://doi.org/10.5897/ajar2016.11600>
- Chiorato, A. F., Carbonell, S. A. M., Bosetti, F., Sasserón, G. R., Lopes, R. L. T. and Azevedo, C. V. G. 2015. Common bean genotypes for agronomic and market-related traits in VCU trials. *Scientia Agricola* 72 (1): 34–40. <https://doi.org/10.1590/0103-9016-2013-0172>

- Couto, K. R., dos Santos, J. B., Ramalho, M. A. P. and da Silva, G. S. 2010. Identificação de marcadores microssatélites relacionados ao escurecimento de grãos em feijão. *Pesquisa Agropecuária Brasileira* 45 (11): 1268–1274. <https://doi.org/10.1590/S0100-204X2010001100006>
- Czern, L., Gupta, S., Akhatar, J., Kaur, P., Sharma, A., Sharma, P. and Mittal, M. 2019. Genetic analyses of nitrogen assimilation enzymes in Brassica juncea. *Molecular Biology Reports* 46(4), 4235–4244. <https://doi.org/10.1007/s11033-019-04878-5>
- Duwadi, K., Austin, R. S., Mainali, H. R., Bett, K., Marsolais, F. and Dhaubhadel, S. 2018. Slow darkening of pinto bean seed coat is associated with significant metabolite and transcript differences related to proanthocyanidin biosynthesis. *BMC Genomics* 19 (1): 1–13. <https://doi.org/10.1186/s12864-018-4550-z>
- Dwivedi, S. L., Upadhyaya, H. D., Chung, I. M., de Vita, P., García-Lara, S., Guajardo-Flores, D., Gutiérrez-Urbe, J. A., Serna-Saldívar, S. O., Rajakumar, G., Sahrawat, K. L., Kumar, J. and Ortiz, R. 2016. Exploiting phenylpropanoid derivatives to enhance the nutraceutical values of cereals and legumes. *Frontiers in Plant Science* 7: 1–27. <https://doi.org/10.3389/fpls.2016.00763>
- Elsadr, H. T., Wright, L. C., Pauls, K. P. and Bett, K. E. 2011. Characterization of seed coat post harvest darkening in common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 123 (8): 1467–1472. <https://doi.org/10.1007/s00122-011-1683-8>
- Erfatpour, M., Navabi, A. and Pauls, K. P. 2018. Mapping the non-darkening trait from “Wit-rood boontje” in bean (*Phaseolus vulgaris*). *Theoretical and Applied Genetics* 131 (6): 1331–1343. <https://doi.org/10.1007/s00122-018-3081-y>
- Erfatpour, M. and Pauls, K. P. 2020. A R2R3-MYB gene-based marker for the non-darkening seed coat trait in pinto and cranberry beans (*Phaseolus vulgaris* L.) derived from ‘Wit-rood boontje.’ *Theoretical and Applied Genetics* 133 (6): 1977–1994. <https://doi.org/10.1007/s00122-020-03571-7>
- Erfatpour, Mohammad, Duizer, L. and Pauls, K. P. 2021. Investigations of the effects of the non-darkening seed coat trait coded by the recessive jj alleles on agronomic, sensory, and cooking characteristics in pinto beans. *Crop Science* 61 (3): 1843–1863. <https://doi.org/10.1002/csc2.20477>
- Felicetti, E., Song, Q., Jia, Gaofeng., Cregan, Perry., Bett, Kirstin. E. and Miklas, P. N. 2012. Simple Sequence repeats linked with slow darkening trait in pinto bean discovered by single Nucleotide Polymorphism assay and whole genome sequencing. *Crop Science* 52 (4):1600–1608. <https://doi.org/10.2135/cropsci2011.12.0655>
- Freixas Coutin, J. A., Munholland, S., Silva, A., Subedi, S., Lukens, L., Crosby, W. L., Pauls, K. P. and Bozzo, G. G. 2017. Proanthocyanidin accumulation and transcriptional responses in the seed coat of cranberry beans (*Phaseolus vulgaris* L.) with different susceptibility to postharvest darkening. *BMC Plant Biology*,17 (1): 1–23. <https://doi.org/10.1186/s12870-017-1037-z>
- Freixas-Coutin, J. 2017. Transcriptomic and biochemical analyses of proanthocyanidin metabolism in seed coats of cranberry bean (*Phaseolus vulgaris* L .). Doctoral dissertation, University of Guelph.
- Graham, P. H. and Ranalli, P. 1997. Common bean (*Phaseolus vulgaris* L.). *Field Crops Research* 53 (1–3): 131–146.
- He, F., Pan, Q. H., Shi, Y. and Duan, C. Q. 2008. Biosynthesis and genetic regulation of proanthocyanidins in plants. *Molecules* 13 (10): 2674–2703. <https://doi.org/10.3390/>

molecules13102674

- Islam, N. S., Bett, K. E., Pauls, K. P., Marsolais, F. and Dhaubhadel, S. 2020. Postharvest seed coat darkening in pinto bean (*Phaseolus vulgaris*) is regulated by P^{sd}, an allele of the basic helix-loop-helix transcription factor P. *Plants, People, Planet* 2 (6): 663–677. <https://doi.org/10.1002/ppp3.10132>
- Junk-Knievel, D. C., Vandenberg, A. and Bett, K. E. 2007. An accelerated postharvest seed-coat darkening protocol for pinto beans grown across different environments. *Crop Science* 47(2): 694–702. <https://doi.org/10.2135/cropsci2006.05.0325>
- Junk-Knievel, D. C., Vandenberg, A. and Bett, K. E. 2008. Slow darkening in pinto bean (*Phaseolus vulgaris* L.) seed coats is controlled by a single major gene. *Crop Science* 48 (1): 189–193. <https://doi.org/10.2135/cropsci2007.04.0227>
- Katuuramu, D. N., Luyima, G. B., Nkalubo, S. T., Wiesinger, J. A., Kelly, J. D. and Cichy, K. A. 2020. On-farm multi-location evaluation of genotype by environment interactions for seed yield and cooking time in common bean. *Scientific Reports* 10 (1): 1–12. <https://doi.org/10.1038/s41598-020-60087-2>
- Kelly, J. D., and Bornowski, N. 2018. Marker-Assisted Breeding for Economic Traits in Common Bean. In: Gosal, S. S. and S. Wani, H. (Eds.), *Biotechnologies of Crop Improvement* 3: 211–238. Springer International Publishing. https://doi.org/10.1007/978-3-319-94746-4_10
- Konzen, E. R. and Tsai, S. M. 2014. Seed coat shininess in *Phaseolus vulgaris*: Rescuing a neglected trait by its screening on commercial lines and landraces. *Journal of Agricultural Science* 6 (8): 113–130. <https://doi.org/10.5539/jas.v6n8p113>
- Letaa, E., Katungi, E., Kabungo, C. and Ndunguru, A. A. 2020. Impact of improved common bean varieties on household food security on adopters in Tanzania. *Journal of Development Effectiveness* 1–20. <https://doi.org/10.1080/19439342.2020.1748093>
- McClellan, P. E., Lee, R. K., Otto, C., Gepts, P. and Bassett, M. J. 2002. Molecular and Phenotypic pattern and color in. *Journal of Heredity* 148–152.
- Mukankusi, C., Raatz, B., Nkalubo, S., Berhanu, F., Binagwa, P., Kilango, M., Williams, M., Enid, K., Chirwa, R. and Beebe, S. 2019. Genomics, genetics and breeding of common bean in Africa: A review of tropical legume project. *Plant Breeding* 138 (4): 401–414. <https://doi.org/10.1111/pbr.12573>
- Mutungu, C., Chamwilambo, M., Masanja, S., Massam, C., Wayda, P., Tungu, J., Gaspar, A., Bekunda, M. and Abass, A. 2020. Quality and storability of common beans in small-holders farm stores in Northern Tanzania: A multivariate analysis of agro-location, variety, and storage method effects. *Journal of Stored Products Research* 89: 101723. <https://doi.org/10.1016/j.jspr.2020.101723>
- Ojiewo, C., Rubyogo, J. C., Wesonga, J., Bishaw, Z., Abang, M. and Gelalcha, S. 2019. Mainstreaming efficient legume seed systems in eastern Africa: Challenges, opportunities and contributions towards improved livelihoods. Food and Agriculture Organization of the United Nations. <https://doi.org/10.18356/ce824af1-en>
- Oladzad, A., Porch, T., Rosas, J. C., Moghaddam, S. M., Beaver, J., Beebe, S. E., Burrige, J., Jochua, C. N., Miguel, M. A., Miklas, P. N., Ratz, B., White, J. W., Lynch, J. and McClellan, P. E. 2019. Single and multi-trait GWAS identify genetic factors associated with production traits in common bean under abiotic stress environments. *G3: Genes, Genomes, Genetics* 9 (6): 1881–1892. <https://doi.org/10.1534/g3.119.400072>
- Paulino, J. F. de C., de Almeida, C. P., Santos, I. L., Gonçalves, J. G. R., Carbonell, S. A. M., Chiorato, A. F. and Benchimol-Reis, L. L. 2022. Combining disease resistance and postharvest

- quality traits by early marker-assisted backcrossing in carioca beans. *Scientia Agricola* 79 (2): <https://doi.org/10.1590/1678-992x-2020-0233>
- Pereira, H. S., de Almeida, V. M., Melo, L. C., Wendland, A., de Faria, L. C., del Peloso, M. J. and Magaldi, M. C. de S. 2012. Influência do ambiente em cultivares de feijoeiro-comum em cerrado com baixa altitude. *Bragantia* 71 (2): 165–172. <https://doi.org/10.1590/S0006-87052012005000024>
- Pereira, H.S., Santos, J.B.D., Souza, T.P.D. and Lima, I.A. 2008 Seleção fenotípica e assistida por marcadores moleculares de famílias de feijoeiro-comum com alta produtividade. *Pesquisa Agropecuaria Brasileira* 43 (11): 1551–1558. <https://doi.org/10.1590/S0100-204X2008001100014>
- Pereira, W. J. and Batista, K. 2014. Influence of storage on darkening and hardening of slow- and regular-darkening carioca bean (*Phaseolus vulgaris* L.) genotypes. September. <https://doi.org/10.5296/jas.v2i2.5859>
- Reis, R. L. M., Silva, D. A., Gonçalves, J. G. R., Carbonell, S. A. M. and Chiorato, A. F. 2021. High temperature increases cooking time and seed darkening of common beans. *Genetics and Molecular Research* 20 (2): 87-104. <https://doi.org/10.4238/gmr18779>
- Rodrigues, L. L., Rodrigues, L. A., de Souza, T. L. P. O., Melo, L. C. and Pereira, H. S. 2019. Genetic control of seed coat darkening in common bean cultivars from three market classes. *Crop Science* 59 (5): 2046–2054. <https://doi.org/10.2135/cropsci2019.03.0161>
- Shah, A. and Smith, D. L. 2020. Flavonoids in agriculture: Chemistry and roles in, biotic and abiotic stress responses, and microbial associations. *Agronomy* 10 (8):. <https://doi.org/10.3390/agronomy10081209>
- Silva, F. C., Melo, P. G. S., Pereira, H. S. and Melo, L. C. 2014. Genetic control and estimation of genetic parameters for seed-coat darkening of carioca beans. *Genetics and Molecular Research* 13 (3): 6486–6496. <https://doi.org/10.4238/2014.August.25.12>
- Silva, G. S., Ramalho, M. A. P., Abreu, Â. F. B. and Silva, F. B. 2008. Genetic control of early grain darkening of carioca common bean. *Crop Breeding and Applied Biotechnology* 8 (4): 299–304. <https://doi.org/10.12702/1984-7033.v08n04a07>
- Siqueira, B. S., Pereira, W. J., Batista, K. A., Oomah, B. D., Fernandes, K. F. and Bassinello, P. Z. 2014. Influence of storage on darkening and hardening of Slow- and Regular-Darkening Carioca Bean (*Phaseolus vulgaris* L.) genotypes. *Journal of Agricultural Studies* 2 (2):87-104. <https://doi.org/10.5296/jas.v2i2.5859>
- Sm Kal, P., Vernoud, V., Blair, M. W., Soukup, A. and Thompson, R. D. 2014. The role of the testa during development and in establishment of dormancy of the legume seed. *Frontiers in Plant Science* 5: 1–19. <https://doi.org/10.3389/fpls.2014.00351>
- Wiesinger, J. A., Osorno, J. M., McClean, P. E., Hart, J. J. and Glahn, R. P. 2021. Faster cooking times and improved iron bioavailability are associated with the down regulation of procyanidin synthesis in slow-darkening pinto beans (*Phaseolus vulgaris* L.). *Journal of Functional Foods*, 82: 104444. <https://doi.org/https://doi.org/10.1016/j.jff.2021.104444>
- Xiong, M., Zhao, M., Lu, Z. X. and Balasubramanian, P. 2020. Genotypic variation for phenolic compounds in developing and whole seeds, and storage conditions influence visual seed quality of yellow dry bean genotypes. *Canadian Journal of Plant Science* 100 (3): 284–295. <https://doi.org/10.1139/cjps-2019-0153>