Review

Seed Coat Pigmentation Plays a Crucial Role in Partner Selection and N2 Fixation in Legume Root-Microbe Associations in African Soils

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Review

Seed Coat Pigmentation Plays a Crucial Role in Partner Selection and N_2 Fixation in Legume Root-Microbe Associations in African Soils

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Abstract: The legume-rhizobia symbiosis is one of the most important plant-microbe interactions in sustainable agriculture due to its ability to provide much needed N to cropping systems. This interaction is mediated by the mutual recognition of signaling molecules from the two partners, namely legumes and rhizobia. With legumes, these molecules are in the form of flavonoids and anthocyanins, which are responsible for the pigmentation of plants parts, such as seeds, flowers, fruits and even leaves. Seed coat pigmentation in legumes is a dominant factor influencing gene expression relating to N_2 fixation, and may be responsible for the different N_2-fixing abilities observed among legume genotypes under field conditions in African soils. Cowpea, Kersting's bean and Bambara groundnut landraces with black seed coat color are reported to release higher concentrations of *nod*-gene-inducing flavonoids and anthocyanins when compared to the Red and Cream landraces, hence the black seed coat pigmentation is considered a biomarker for enhanced nodulation and N_2 fixation. Cowpea, Bambara groundnut and Kersting's bean with differing seed coat colors are known to attract different native soil rhizobia, confirmable by PCR-RFLP analysis of bacterial DNA from root nodules of these legumes. Even when seeds of the same legume with diverse seed coat colors were planted together in one hole, the nodulating bradyrhizobia clustered differently in the PCR-RFLP dendrograms. In one study, Kersting's groundnut, Bambara groundnut and cowpea with differing seed coat colors were selectively nodulated by different bradyrhizobial species. Multilocus sequence analysis showed that different *Bradyrhizobium* species nodulated the Kersting's bean based on seed coat color. Phylogenetic analysis also placed the bradyrhizobial isolates in close proximity to different *Bradyrhizobium* species such as *B. vignae* 7-2^T, *B. subterraneum* 58 2-1^T, *B. kavangense* 14-3^T, *B. liaoningense* 2281 (USDA 3622)^T, *B. yuanmingense* LMG 21827^T, *B. huanghuaihaiense* CCBAU 23303^T, *B. pachyrhizi* PAC48^T, and a reference type strain of *B. elkanii* according to seed coat color. Using 16S rDNA amplicon sequencing, we also found significant selective influences of seed coat pigmentation on microbial community structure in the rhizosphere of five Kersting's groundnut landraces. For example, the rhizosphere of Belane Mottled landrace was dominated by Proteobacteria, while Bacteroidetes dominated the rhizospheres of the other landraces. With legumes, seed coat pigmentation therefore plays a dominant role in the selection of the bacterial symbiotic partner.

Keywords: flavonoids; anthocyanin; pigmentation; soil microbes; disease resistance; nodulation and nitrogen fixation

1. Introduction

Plants and microorganisms have coexisted for millions of years. At the root-soil interface, plants and microorganisms engage in complex signaling to establish beneficial associations known as symbiosis[1]. These interactions are crucial for nutrient acquisition and fitness improvement, and serve as the foundation for symbiotic relationships, with the most ancient being those formed with nitrogen-fixing bacteria. A key alternative to soil N improvement via fertilization in cropping systems is biological N_2 fixation (BNF) [2], a process that has been recognized as the most smart environmentally-friendly and economically-sustainable alternative for overcoming soil N infertility [3]. BNF contribution to the N economy of terrestrial ecosystems can be symbiotic, associative, or by free-living microorganisms [4]. However, symbiotic N_2 fixation in legumes has contributed the most N to global agriculture due to its ability to fix large amounts of biological N in cropping systems for use by the legume and succeeding crops [5].

Within the broader context of the legume-rhizobia symbiosis, seed coat pigmentation has emerged as a significant factor influencing the establishment of this plant-bacterial association [6]. Though often overlooked, seed coat pigmentation plays a crucial role in mediating partner selection during the early stages of the legume host/strain symbiosis.

Effective nodulation and N_2 fixation depend on the presence of host plants and compatible nodulating soil bacteria in adequate numbers. Once these conditions are met, legumes offer a great opportunity for improving the N economy of nutrient-poor soils through their ability to establish effective N_2-fixing symbiosis with compatible soil bacteria that can contribute symbiotic N to cropping systems [7,8]. While N_2-fixing bacteria can contribute significantly to this BNF process, the interplay between seed coat pigmentation and N_2 fixation remains relatively unexplored and untapped for sustainable development. The aim of this review is to highlight the role of seed coat pigmentation in partnership development and nitrogen fixation in the legume/rhizobia symbiosis.

2. A Historical Perspective on Legume Seed Coat Pigmentation

Grain legumes have played an important role in the development of Neolithic agriculture some 12,000 years ago [9] The evolution of seed coat pigmentation spans millions of years, reflecting both dynamic environmental pressures and intricate adaptations [10]. Diversity in seed coat pigmentation is evident across the plant kingdom, and reflects the unique ecological niches and adaptive strategies adopted by different plant species. A wide range of seed coat colors exist within and among legume species [9] (see Figure 1).

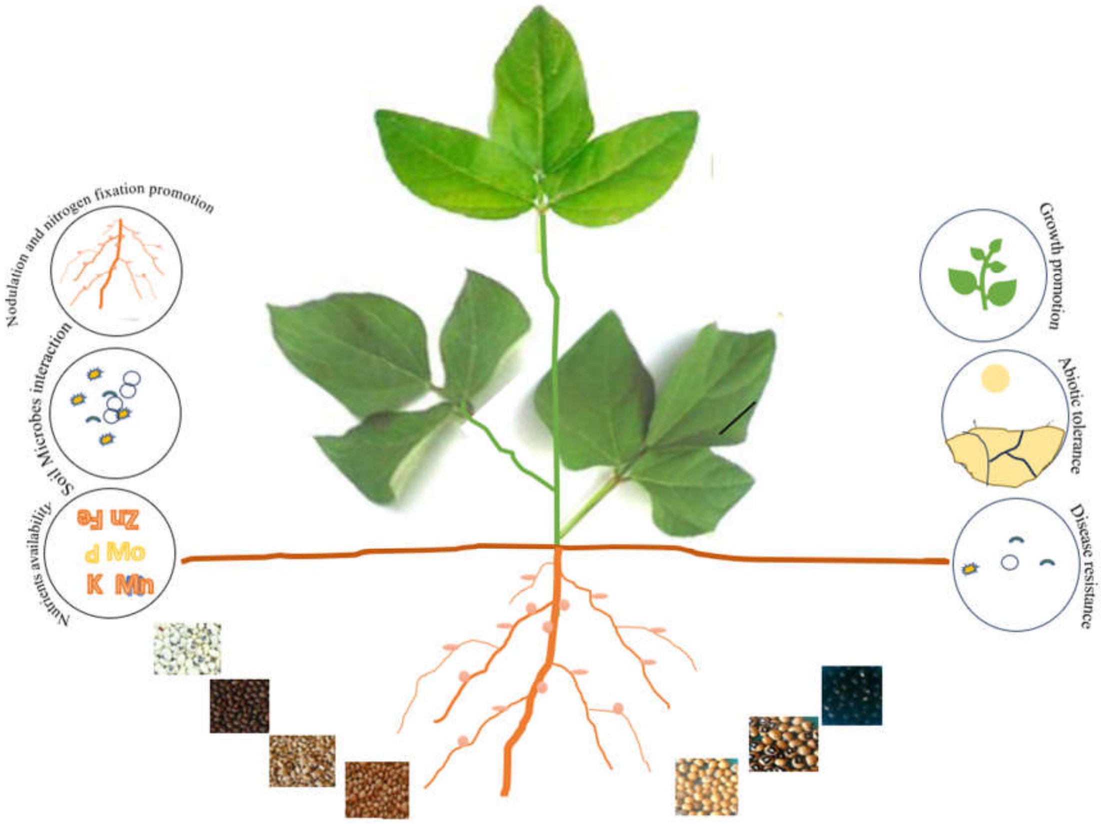

Figure 1. Seed coat colors and their roles in sustainable agriculture.

The color of legume seed coats is a trait that is influenced by polyphenolic substances, and holds significant evolutionary and agricultural implications. However, crop domestication processes have led to the loss of seed coat pigmentation, possibly as a trade-off for protection from foragers [11,12]. Wild chickpea (*Cicer arietinum* L.) progenitors, for example, are known to exhibit seed coat pigmentation that mimicks soil color, thus emphasizing the evolutionary role of seed coat color [13]. Pigmentation loss has however been linked to the level of bitter-tasting compounds, which suggest a potential relationship between seed coat color and metabolite concentration [14].

Legume genotypes have been shown to fix varying amounts of symbiotic N. Although the effect of seed coat color in legume nodulation has been established [6,15–18], little is known about its influence on the choice of rhizobia by legumes for symbiotic partnership, root nodulation and N contribution. The importance of seed coat color in legume nodulation has been properly documented [15,17,19–21], and darker seed coat color is reported to contain greater levels of flavonoids, anthocyanins, and anthocyanidins that promote superior symbiotic functioning [17,22,23]. Hungria and Phillips [17] and dos Santos Sousa [24] have, in fact, shown that common bean (*Phaseolus vulgaris L.*) seedlings generated from a black-seeded genotype formed more root nodules compared to its white isogenic counterpart.

Though known for their aesthetics in Africa, legume seed coat coloration goes beyond mere aesthetics as it serves as an adaptive trait with functional significance. The ability of seed coats to exhibit diverse colors is often linked to ecological adaptations such as UV-B protection, signaling mechanisms, and interactions with symbiotic microorganisms. An understanding of the adaptive significance of seed coat color is likely to unveil its role as a dynamic and strategic trait in the evolutionary arms race between plants and microbes. In the African marketplace, legume accessions are often given local names based on the color of the testa or seed coat [23]. From the primitive seed coat color to the sophisticated pigmentations observed in legumes today seems to suggest a historical perspective on the fascinating journey of seed coat pigmentation.

3. Mechanisms of Partner Selection in Symbiotic Interactions

Partner selection in symbiotic interactions involves intricate genetic and molecular mechanisms that govern the recognition and compatibility between plants and microorganisms. The rhizobia-legume symbiosis is an excellent model system for studying partner selection in mutualistic relationships. Despite the differences in host specificity during symbiotic establishment, commonalities and overlaps do exist in signaling pathways that can provide insights for understanding how seed coat pigmentation contributes to partner recognition and selection during nodulation. Although seed coat pigmentation is a significant determinant in partner recognition during symbiotic establishment, our understanding of these roles is essential for unraveling the broader dynamics of symbiosis.

At the molecular level, the pathways linking seed coat pigmentation to N_2 fixation remain a focal point of investigation. In the absence of effective indigenous soil bacteria, rhizobial strains must be applied as inoculants for nodulation to occur in legumes. The ability of rhizobia to successfully establish a N_2-fixing relationship with a legume depends on the symbiotic signals involved in the molecular conversation between legume and rhizobia during nodule formation [25]. Usually the first signals are produced by the plant in the form of flavonoids which interact with *nod*D proteins of the rhizobia. The *nod*D-flavonoid complex then activates the transcription of *nod* boxes that cause the deformation of root hairs, thus allowing entry of the rhizobia into plant roots [25].

The flavonoids released by legume seed coats and young roots, act as chemo-attractants, cell growth enhancers, and *nod*-gene inducers in symbiotic rhizobia [16,26,27]. The same flavonoid molecule can therefore elicit different responses in different rhizobia [18]. Unraveling these pathways can provide a molecular framework for understanding the functional role of seed coat pigmentation in N_2-fixing symbioses

4. Genetic Determinants of Legume Seed Coat Pigmentation

The genetic control of seed coat color involve can the influence of specific genes that have dysfunctional pathways with potential for causing variations. For instance, the stay-green type seed coat color in chickpea is caused by the loss of function in the 'CaStGR1' gene which affects chlorophyll degradation and retention [28]. In soybean, however, seed pigmentation is tied to the I locus, which affects the expression of chalcone synthase gene and subsequently flavonoid biosynthesis [29]. Similar homologous genes in other legumes such as the P locus in common bean, Mendel's A locus in pea, B locus in chickpea, and Tan gene in lentil, play major roles in anthocyanin and flavonol biosynthesis [30–33]. Faba bean presents an intriguing case with two identified homologous genes [34,35].

Recently, studies of the genetics of seed coat color in common bean using phenotypic segregation of a 'TU' × 'Musica' cross-derived recombinant inbred line population indicated three genes that controlled seed coat color: one for white (mapped on Pv07) and two for black (mapped on Pv06 and Pv08) [36]. The mapped positions were consistent with classical studies on the V gene and C locus. Crossing three selected lines validated these regions, confirming that TU alleles resulted in a black phenotype. Furthermore, two genes involved in anthocyanin biosynthetic pathway were identified as *Phvul.006G018800* encoding a flavonoid 3′5′hydroxylase and Phvul.008G038400 encoding MYB113 transcription factor [36].

Campa et al [37] also examined 308 common bean lines for 10 seed coat color traits, and discovered 31 significant SNP-trait associations (QTNs) that were distributed across 20 chromosome regions. Of these, chromosome Pv08 played a central role, as it influenced phenolic metabolites on chromosomes Pv01, Pv02, Pv04, Pv08, and Pv09 and seed coat color on chromosomes Pv01, Pv02, Pv06, Pv07, and Pv10. Chromosome Pv08 therefore plays a key role in the phenylpropanoid pathway, with significant effect on common bean seed coat pigmentation.

The genetic basis of red seed coat color in cowpea was found to involve two loci, Red-1 (R-1) on chromosome Vu03 and Red-2 (R-2) on chromosome Vu07 [38]. The candidate gene for R-1 was *Vigun03g118700*, encoding dihydroflavonol 4-reductase which is a cyanidin biosynthesis catalyst, while the candidate gene for R-2 was *Vigun07g118500*, with a nucleolar function and elevated expression in developing seeds. The red color was attributed to the accumulation of cyanidin in the seed coat [38].

5. Genetic Determinants of Host Plants in Symbiotic Interactions with Rhizobia

The genetic makeup of host plants can play a pivotal role in shaping the outcomes of symbiotic interactions. From signaling pathways to nutrient exchange, the genetic determinants of host plant can contribute significantly to the success of the symbiosis. As a heritable trait seed coat pigmentation can contribute to determining host plants in symbiotic partnership. As found with common bean, cowpea also exhibits different seed coat colors that can significantly affect the N_2-fixing ability of genotypes [39–41]. The varying seed coat pigmentation of cowpea was due to genetic differences [11,23,38], with seed coat color being a major determinant of the differences in N_2 fixation under field conditions. Many strategies have been used to select and breed for enhanced N_2 fixation in legumes [42]. Despite the known importance of seed coat color in legume nodulation and N_2 fixation, cowpea breeding in West Africa has generally favored genotypes with cream (or white) seed coat color [43]. Recently, Mohammed et al [44] reported a large variation in plant growth, grain yield and symbiotic functioning between and among genetically diverse cowpea accessions with different seed coat colors, which was attributed to the ability of different seed coat colors to attract different native soil rhizobia.

In another study involving Kersting's groundnut (*Macrotyloma geocarpum* Harms), which is a neglected, underutilized, and endangered food and medicinal legume in Africa, analysis of SSR sequences revealed significant genetic diversity among the Kersting's groundnut landraces with diverse seed coat colors [45]. Due to the unavailability of genetic information specific to Kersting's groundnut, Mohammed et al [45] employed SSR markers derived from cowpea. The successful cross-

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genus transferability of SSRs in that study did not only indicate a practical approach for future studies, but also suggested an evolutionary closeness between cowpea and Kersting's groundnut.

According to Puozaa et al. [6], seed coat pigmentation is responsible for the observed differences in symbiotic functioning among legumes with different seed coat colors. Furthermore, it was found that three landraces with many similar traits except for seed coat color performed differently. Despite having similar growth habits, including phenology, flowering and maturity dates, the seed coat color emerged as the dominant factor influencing gene expression relating to N_2 fixation [6]. In fact, studies of seed coat colors (e.g. black, brown and yellow) in soybean found isoflavone malonyl-CoA acyltransferase GmMaT2 to be involved in the nodulation of soybean through modifying the synthesis and secretion of isoflavones by the seed coat [19].

However, the effective cross-nodulation of Kersting's groundnut plants by cowpea-nodulating strain CB756 [6], which was initially isolated from *Macrotyloma africanum* by Blumenthal and Staples [46] suggested potential synteny between cowpea and Kersting's groundnut. A comparison of transcriptomes of contrasting seed coat colors (black and yellow) and RNA-seq analysis of soybean also discovered the presence of 318 differentially expressed genes involved in ethylene, lipid, brassinosteroid, lignin, and sulfur amino acid biosynthesis in cultivars with black seed coat pigmentation.

6. Seed Coat Pigmentation and N_2 Fixation in Legumes

The importance of seed coat pigmentation in the nodulation of legumes has been reported before [6,15,17,21,47,48] (see Figure 1), and apparently the variation in seed coat color may be responsible for the different N_2-fixing abilities observed among legume genotypes under field conditions in African soils [6,45,47]. The efficiency of N_2 fixation in plants could be intricately linked to seed coat pigmentation. In fact, Puozaa et al. [6] recently reported that seed coat color is a biomarker for increased nodulation, N_2 fixation, and plant growth. Studies of focused on Bambara groundnut (*Vigna subterranea* L. Verdc) landraces in Ghana, using ^{15}N natural abundance and ureide analysis of xylem sap and petiole extracts consistently revealed a correlation between ureide levels and shoot $\delta^{15}N$ values, as well as between ureide levels and %N derived from fixation. Where shoot $\delta^{15}N$ values were low and %N derived from fixation was high, ureide concentrations in xylem sap and petiole extracts were also high, indicating a robust precision in the estimation of N_2 fixation in diverse seedcoat-colored Bambara groundnut landraces.

Those studies by Puozaa et al. [6] found that Black Bambara groundnut landraces consistently exhibited greater nodule number and nodule fresh weight per plant across all sites. Although the Black and Red landraces generally displayed lower shoot $\delta^{15}N$ values, derived a higher proportion of their N from fixation, and contributed more symbiotic N, the Black landrace surpassed the Red. This superior symbiotic performance of the Black Bambara groundnut landrace was attributed to its ability to produce and release higher concentrations of *nod*-gene-inducing anthocyanins and flavonoids when compared to the Red and Cream landraces [49–51]. An earlier study by Hungria and Phillips [17] had also demonstrated that a black-seeded common bean genotype that released higher concentrations of nod-gene-inducing flavonoids similarly elicited greater nodulation than its isogenic cream counterpart with reduced flavonoid biosynthesis.

Furthermore, Mbah and Dakora [52] also found that root nodulation, N_2 fixation and shoot micronutrient accumulation differed significantly among different grain legumes (Bambara groundnut, soybean, and Kersting’s groundnut) with diverse seed coat colors in South Africa. However, supplying 5 mM NO_3^- to inoculated seedlings of Bambara groundnut, soybean, and Kersting’s bean led to reduced plant growth, nodule formation, and nodule dry matter across all seed coat colors. Furthermore, seedlings of Bambara groundnut with diverse seed coat colors revealed varied nodulation and plant growth under different field conditions in African soils in Cameroon, Burkina Faso and Nigeria [53–55].

7. Effect of Seed Coat Pigmentation on Microbial Colonization

Besides N_2-fixing efficiency, seed coat pigmentation can also influence root colonization by both rhizobia and plant growth-promoting microbes [56,57] (Figure 1). Puozaa et al [58,59] found that PCR-RFLP analysis of bacterial DNA from root nodules of cowpea and Bambara groundnut landraces (namely, Black, Red, Cream, Red mottled, Black mottled and Blackeye) that were grown in Ghanaian and South African soils revealed differences in nodule occupancy based on seed coat color. In other words, seeds differing in seed coat color attracted different rhizobial strains. Even when planted together in one hole, the nodulating bradyrhizobia clustered differently in the PCR-RFLP dandrograms [58]. This suggested that the different Bambara groundnut seed coat colors (e.g. Black, Black mottled, and Cream) probably contributed to a soil environment that was conducive to rhizobial richness. In fact, Mohammed et al [57](2019), Dlamini et al. [60], and Puozaa et al. [6] found that Kersting's groundnut, Bambara groundnut and cowpea with differing seed coat colors were selectively nodulated by different rhizobial species.

This was further confirmed by multilocus sequence analysis performed on rhizobia nodulaing Kersting's groundnut. The results revealed different *Bradyrhizobium* species responsible for nodulating Kersting's groundnut with differing seed coat colors [57]. Phylogenetic analysis also placed the test rhizobial isolates in close proximity with different *Bradyrhizobium* species such as *B. vignae* 7-2^T, *B. subterraneum* 58 2-1^T, *B. kavangense* 14-3^T, *B. liaoningense* 2281 (USDA 3622)^T, *B. yuanmingense* LMG 21827^T, *B. huanghuaihaiense* CCBAU 23303^T, *B. pachyrhizi* PAC48^T, and the type strain of *B. elkanii* based mostly according to seed coat color [57]. However, in some instances, a significant divergence was found between test isolates and the reference type strains, suggesting those isolates could be novel *Bradyrhizobium* species [57].

The higher genetic diversity observed among the nodule occupants of the dark-seeded Kersting's groundnut landraces in this study could be linked directly or indirectly to the greater phenolic compounds produced by the Black landrace compared to the white-seeded Boli landrace [57,61]. Earlier studies have, however, reported higher concentrations of phenolic compounds exuded by seeds with dark seed coat pigmentation when compared to those with lighter seed coat color [49,50], and these molecules are known to play a role in signal exchange during the legume-rhizobia symbiosis.

The effect of different seedcoat colors on microbial community structure in the rhizosphere of five Kersting's groundnut landraces (namely, Belane Mottled, Boli, Funsi, Puffeun, and Heng Red Mottled) was investigated by Jaiswal et al. [56] using 16S rDNA amplicon sequencing. The results revealed significant selective influences of the landraces on rhizosphere bacteria. The microbial composition and abundance differed significantly among the landraces, with a major landrace effect on some phyla. For example, the rhizosphere of landrace Belane Mottled was dominated by Proteobacteria, while Bacteroidetes dominated the rhizospheres of the other landraces. Furthermore, except for Puffeun (with Black seed coat), whose rhizosphere was dominated by *Mesorhizobium*, only *Bradyrhizobium* and *Rhizobium* species of alpha-Proteobacteria were present in the rhizosphere of all the other landraces, though indole-3-acetic–acid-producing Sphingomonas and cellulose-degrading Fibrobacteres were also abundant in the rhizosphere of all landraces [56].

8. Evolutionary Dynamics of the Legume-Rhizobia Partnership

Symbiotic relationships between plants and microbes are THE outcome of intricate co-evolutionary processes. Most grain legumes, including cowpea, soybean, groundnut, and Bambara groundnut, meet a significant proportion of their N requirements from atmospheric N_2 fixation. Like all grain legumes, seed coat color variation is strong among Bambara groundnut landraces, and can range from black to mottled to lighter cream color. This diversity is primarily attributed to different types of flavonoid compounds and their concentrations present in the seed coat [62]. Seed coat colors are also reported to correlate with distinct N_2-fixing abilities under different agro-ecologies, thus underscoring the role of seed coat compounds in selecting microsymbiont partners [58]. Independent of the cropping system, seed coat pigmentation is reported to significantly influence the diversity of microsymbionts residing in Bambara groundnut root nodules [58]. Even when co-planted in the same hole, landraces with different seed coat colors formed nodules with bacterial partners that exhibited divergent clustering at the 16S-23S rDNA-RFLP level. This suggests a link between seed coat pigmentation and the specificity of the symbiosis associated with each landrace [58].

The flavonoid profile of leguminous seeds is often dictated by seed coat color, which strongly contributes to the selection of rhizobial partners in symbiotic relationships. For example, Bambara groundnut landraces with distinct seed coat pigmentation were found to contain varying levels of flavonoids and anthocyanins in their seed exudates [6,62]. These phenolic compounds are abundantly present in the seed coat, and often act as chemo-attractants for rhizobia, bacterial cell growth promoters, and inducers of *nod*-genes in symbiotic legumes [16,17,25,63].

The presence of a specific legume in the soil can trigger the selection of specific rhizobial groups due to rhizodeposition, consisting mainly of flavonoids released by the host plant roots. During germination, however, legume seeds release these flavonoid compounds abundantly from the seed coat [25,62,64,65].

Kersting's groundnut, is a neglected, underutilized and endangered food and medicinal grain legume native to Africa, and whose symbiosis has remained unexplored [45,56]. The multi-seedcoat colors found in Kersting's groundnut landraces are believed to be genetically influenced rather than environmentally-induced, given the observed differences in nucleotide sequences of simple sequence repeats in their genomic regions [45].

Symbiotic data from Kersting's groundnut landraces revealed marked variations in root nodulation, shoot biomass, δ^{15}N, percent N derived from fixation, amount of N-fixed, and soil N uptake [45].

Furthermore, the Black landrace consistently outperformed others in terms of nodule number, nodule fresh weight, and N contribution from symbiosis. Phylogenetic analysis showed that Kersting's groundnut is nodulated mainly by *Bradyrhizobium* species as they were closely related to known strains and novel groups within the genus [57]. Because of the diverse seed coat colors of Kersting’s groundnut, the species tends to attract different microbial profiles in its rhizosphere.

9. Adapting to Environmental Changes: The Role of Seed Coat Pigmentation

Environmental changes can pose challenges to symbiotic associations that necessitate adaptive responses. Seed coat pigmentation plays a key role in the adaptive strategies employed by plants in response to changing environmental conditions. According to Puozaa et al. [59], the cultivation of cowpea with differing seed coat colors in the low rainfall site of Morwe in South Africa contributed to a higher diversity of cowpea-nodulating rhizobia. Furthermore, when cowpea seeds with Black, Red, and Cream pigmentation were planted together in one hole, they attracted diverse microsymbionts, which exhibited varied PCR-ITS-RFLP profiles and occupied distinct positions in the phylogenetic tree.

Puozaa et al. [6] also reported that the Black Bambara groundnut landrace exhibited greater plant growth (measured as shoot dry matter) at all study sites in Ghana and South Africa compared to the Red and Cream landraces. The variation in plant growth among landraces with different seed coat colors could be linked to differences in solar radiation interception efficiency, genetic variations in photosynthate production, and/or greater resistance to environmental stresses which affect crop productivity. Seedling vigor which is, crucial for early crop establishment, may also be influenced by seedcoat color, as evidenced by the differences in seedling vigor among Bambara groundnut landraces with varying seed coat pigmentation [66].

In other studies, site-specific comparisons showed that the Red landrace consistently produced higher grain yield than the Black and Cream landraces to the extent that in Cameroon and Tanzania, Red seedcoat pigmentation is associated with increased grain yield of Bambara groundnut landraces [67,68].

Although Bambara groundnut is generally considered to be drought-tolerant, little evidence current exists to support that argument, except for the report by Puozaa et al. [6] which found significant differences in shoot δ^{13}C or water-use efficiency among the Bambara groundnut landraces tested across different study sites, with the Black landrace exhibiting higher $\delta^{13}C$ values, and hence greater water-use efficiency than the Red and Cream landraces. The relative drought tolerance of the Black landrace can be attributed to the higher tissue concentration of flavonoids, anthocyanins and anthocyanidins, which are known to protect the photosynthetic apparatus under drought conditions [69]. Furthermore, a significant correlation was found between shoot $\delta^{13}C$ and shoot N content, as well as between shoot $\delta^{13}C$ and soil N uptake [6], suggesting that the enhanced N nutrition from fixation by the Black landrace contributed to the greater water-use efficiency and drought resistance relative to the Red and Cream landraces.

10. Grain legume Seed Coats Are a Natural Source of Nutraceuticals and Anthocyanins

Flavonoids secreted by legume seeds play a major role as signal molecules for attracting compatible rhizobia during symbiotic establishment. As the reproductive unit, seeds carry the genetic material of crop species over time and space, and not only does the seed coat play a vital role in protecting the embryo, and/or determining seed dormancy and germination [70–72], it also contains a host of novel compounds which are released following imbibition of water during germination. These molecules include flavonoids, proteins, peptides, amino acids, alkaloids and terpenoids [62,71]. Flavonoids are phenolic secondary metabolites that confer color on seeds, flowers and fruits [38]. They play many important roles in plant development, including defense against insect pests and diseases, as well as nodulation in symbiotic legumes (Table 1; Figure 1) [62,73–75]. Puozaa et al. [6] also found marked variations in the concentration of phenolic compounds in the seed exudate of Bambara groundnut with differing seed coat colors. In fact, seeds of the Black landrace released higher levels of flavonoids and anthocyanins, followed by the Red, with Cream being the lowest. The Black landrace also elicited significantly higher nodule number and nodule fresh weight per plant, greater shoot biomass, lower shoot $\delta^{15}N$, greater N derived from fixation, higher symbiotic N contribution, and increased water-use efficiency across all study sites. These findings underscore the importance of seed coat color as a biomarker for breeding Bambara groundnut varieties with enhanced nodulation, N_2 fixation, and grain yield. The results of UPLC-qTOF-MS analysis by Tsamo et al. [49] confirmed earlier findings on the presence of elevated levels of phenolic compounds in darker-colored Bambara groundnut seeds, black-seeded azuki bean, and soybean. Ndakidemi and Dakora [62] also reported higher concentrations of anthocyanins and flavonoids in a black-seeded Bambara groundnut accession when compared to its Cream counterpart. Seed coat pigmentation therefore plays a crucial role in determining the profile and concentrations of flavonoids, anthocyanins, and anthocyanidins in seeds of cowpea, Bambara groundnut, and Kersting's groundnut [49–51]. Metabolite profiling by Tsamo et al. [51] showed that black seeds of legumes are a natural source of nutraceuticals for human nutrition/health, and a reservoir of anthocyanins that can be exploited for developing cosmetic, food, and pharmaceutical products.

However, consumer preferences for cowpea and Bambara groundnut consumption in West Africa, where most of the cowpea is produced in Africa, are influenced by seed coat pigmentation. Most consumers in West Africa prefer cowpea and Bambara groundnut with cream or white seed coat color [76]. This contrasts with Brazil, where "black beans" are highly preferred, possibly due to their high levels of total phenolics, flavonoids, anthocyanins, anthocyanidins, and antioxidant activity when compared to their counterparts with cream or white seed coat color [20,49–51] (Table 1). The consumption of these antioxidant compounds has implications for human nutrition and health [77,78].

Table 1. Legume seed coat colors and their impact on plant activities.

Legume	Seedcoat color	Agronomical effect	References
Bambara Groundnut (*Vigna Subterranea* L. Verdc.	Black	Enhanced nodulation and nitrogen fixation	Puozaa et al. (2021)

Winged bean (*Psophocarpus tetragonolobus*)	Brown	Enhanced nodulation,and nitrogen fixation	Adegboyega et al. (2021)
Common bean (*Phaseolus vulgaris* L.)	light red	disease resistance and symbiotic nitrogen fixation	Wilker et al. (2020)
Soybean (*Glycine max* (L.) Merr.)	Black and Brown	Enhanced antioxidant activities and anthocyanins	Lim et al. (2021); Jung et al. (2022)
Soybean (*Glycine max* (L.) Merr.)	Yellow	Higher water absorption	Abati et al (2022)
Adzuki Bean (*Vigna angularis* L.)	Black	Higher accumulation of anthocyanins	Chu et al. (2021); Nagao et al. (2023)
Lentil (*Lens culinaris* Medik.)	Black	Higher nutraceutical values	Mishra et al. (2022)
Peanut (Arachis hypogaea *L.*)	Dark red	Higher polyphenol content	Nayak et al. 2020
Kersting's groundnut (*Macrotyloma geocarpum* Harms)	Black	Higher nitrogen fixation	Mohammed et al. (2018)

11. Conclusions

Rhizobia play a pivotal role in agroecosystems, contributing significantly to enhancing overall soil health through their symbiotic relationship with legumes. Numerous reports have indicated that legumes with varied seedcoat colors exhibit diverse responses to N_2 fixation and sustainable agriculture in African soils. Understanding the molecular and genetic aspects of seed coat pigmentation is crucial for unraveling symbiotic interactions. Evolutionary dynamics, illustrated by diverse seed coat colors in legumes like Bambara groundnut and Kersting's groundnut, showcase the intricate co-evolutionary processes influencing N_2-fixing partnerships. The connection between seed coat pigmentation, flavonoid profiles, and rhizobial selection suggests potential applications in optimizing microsymbiont associations. Further research on neglected legumes, such as Kersting's groundnut, and the impact of seed coat colors on soybean seed quality opens avenues for improved crop productivity and sustainability.

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