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Posted Date: 29 July 2026

doi: 10.20944/preprints202607.2179.v1

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Agronomic Performance, Bioactive Profile, and Nutritional Quality of Cowpea [*Vigna unguiculata* (L.) Walp] Under Aluminum Edaphic Stress Conditions in the Peruvian Amazon

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Abstract

Cowpea [*Vigna unguiculata* (L.) Walp] is a strategic legume for food security in the Peruvian Amazon owing to its resilience in marginal soils. The aim of this study was to evaluate the agronomic performance, bioactive profile, and nutritional quality of eight cowpea accessions grown under high aluminum saturation (69.01%) and extreme acidity (pH 3.50) in Zungarococha (Loreto, Peru). Phenological and yield traits were assessed under a randomized complete block design, together with total phenolic content and antioxidant capacity (DPPH and ABTS), and the proximate and mineral composition of the grain. Analysis of variance revealed highly significant differences among accessions. Accession PER1005852 was identified as a dual-purpose ideotype, combining the highest grain yield (1.42 t ha^{-1}) with a strong antioxidant profile. Protein content ranged from 21.87% to 24.97% (highest in PER1005851), with a high phosphorus content (616.70 mg/100 g in CP0112) and an iron content consistent with the range reported for the species (7.60 mg/100 g in CP0063). Multivariate analysis confirmed that dark seed-coat pigmentation is a phenotypic indicator of greater nutraceutical potential. It is concluded that Amazonian cowpea is a functional food with a dense nutritional profile, of interest for breeding and biofortification programs in the face of climate change.

Keywords: *Vigna unguiculata*; functional food; antioxidant capacity; phenolic compounds; biofortification; aluminum stress; plant breeding; Peruvian Amazon

1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] has become established as a strategic crop for food security in tropical regions, owing to its remarkable climatic resilience and superior nutritional profile [1,2]. This legume is internationally recognized as a "superfood" for its high plant-protein content, ranging from 20.5% to 27.0%, in addition to providing essential minerals and low-glycemic-index

 doi:10.20944/preprints202607.2179.v1

carbohydrates [3,4]. Beyond this basic contribution, cowpea stands out for its high concentration of phenolic compounds and flavonoids, which exert potent biological activity capable of mitigating oxidative stress and preventing chronic diseases [5,6].

In Peru, the importance of cowpea has extended beyond subsistence. National production is estimated at 17,588.0 tonnes per year, with a harvested area exceeding 12,700.0 hectares [1]. Notably, this crop has recorded a 131.9% increase in production in recent periods, driven by demand in the regional markets of the Peruvian lowland Amazon, where it reaches local yields of 1.2 to 2.5 t/ha, representing a crucial economic base for Amazonian family farming [1,7].

However, productive success in the Peruvian Amazon faces critical edaphic challenges. Much of the upland (terra firme) soil in regions such as Loreto is characterized by extreme acidity and high metal toxicity, with pH values that can drop to 3.50 and aluminum saturation levels exceeding 69% [7,8]. Although cowpea is valued for its phenotypic plasticity, prolonged exposure to these conditions of high aluminum saturation drastically limits root development and nutrient uptake, exerting selective pressure on the biosynthesis of bioactive compounds and on final grain yield [1,5]. Despite this issue, the use of local biodiversity is constrained by a gap in technical information. Current research emphasizes that genetic variability among accessions determines not only the efficiency of phenological cycles but also the plant's ability to mobilize polyphenols as a defense mechanism against abiotic stress [3,4]. Previous studies report that total phenolic content can range from 0.026 to 0.883 g GAE/100 g depending on the genotype [3]. Nonetheless, the characterization of accessions grown in the Amazon has historically overlooked the integrated analysis of nutraceutical potential under the specific stress of aluminum and acidity [7].

Antioxidant capacity, assessed through the DPPH and ABTS assays, is a key parameter for determining the functional value of genetic resources under stress. Free-radical-scavenging activity has been shown to be highly dependent on the chemical composition of the seed coat, where more pigmented varieties tend to exhibit greater metabolic resilience [4,7]. In a context of climate change, identifying "dual-purpose" accessions that combine tolerance to aluminum toxicity, high yields, and robust antioxidant capacity is a priority for genetic improvement and the functional-food industry [1,6]. Unlike previous characterizations of Amazonian cowpea germplasm, which focused mainly on physiological and agronomic performance [7], the present study integrates, in a single panel of accessions grown under extreme acidity and high aluminum saturation, the joint evaluation of agronomic performance, bioactive profile, and nutritional quality, and incorporates seed-coat color as a phenotypic selection criterion. The objective of this research was to evaluate the agronomic performance, bioactive profile, and nutritional quality (proximate and mineral) of eight cowpea accessions grown under aluminum stress and extreme acidity in the Peruvian Amazon, in order to identify the accessions with the best integral performance—combining productivity, nutritional quality, and antioxidant capacity—under these edaphic conditions.

2. Materials and Methods

1.1. Study Site

The study was conducted at the experimental field and laboratories of the Faculty of Agronomy and Food Industries, located on the university campus of the Universidad Nacional de la Amazonía Peruana (UNAP), Iquitos, Peru — representative place of firm ground or non-floodable soils [7,8] and classified as Tropical Humid Forest, characterized by high temperatures and humidity that affect the synthesis of phenolic compounds in cowpea as a defense mechanism against environmental stress [1,7] (Supplementary Figure S1).

1.2. Soil Characterization

A composite soil sample from the upper 20 cm layer was collected before the experiment for physicochemical analysis at the Soil Laboratory, Pucallpa Agricultural Experimental Station, National Institute for Agricultural Innovation (INIA). This included standardized methodologies

 doi:10.20944/preprints202607.2179.v1

such as EPA 9045D for pH [9], ISO 10694 for total carbon and nitrogen [10], Bray and Kurtz [11] for available phosphorus, Bouyoucos [12] for texture, NOM-021-RECNAT-2000 [13] for exchangeable acidity and aluminum, and methods based on ammonium acetate [14] and ICP-OES [15] for the remaining minerals; the results are presented in Supplementary Table S1.

1.3. Plant Material

The plant material consisted of eight cowpea accessions [*Vigna unguiculata* (L.) Walp] (Figure 1), obtained from the Tropical Legume Collection of the Germplasm Bank of the Instituto Nacional de Innovación Agraria (INIA), propagated at the San Ramón Agricultural Experimental Station. The accessions were identified by their respective collection codes (PER) and classified according to their geographic origin. Four accessions were national materials, identified as PER1005851, PER1005852, PER1005853, and PER1005854. Finally, four accessions had the collection codes CP0063, CP0061, CP0062, and CP0112 (Figure 1).

Figure 1. Phenotypic variability of seeds from eight cowpea accessions [*Vigna unguiculata* (L.) Walp]. The seeds are described as follows: (a) PER1005851; (b) PER1005852; (c) PER1005853; (d) PER1005854; (e) CP0061; (f) CP0062; (g) CP0063; and (h) CP0112.

1.4. Experimental Design and Crop Establishment

The experiment was established in August 2024 under a randomized complete block design (RCBD), with eight treatments corresponding to eight cowpea accessions and four blocks, totaling 32 experimental units. Each experimental unit or plot measured 8 m long by 4 m wide, with an area of 32 m^2. Each plot consisted of four rows of plants, with a spacing of 1.0 m between rows and 1.0 m between plants, for a total of 32 plants per plot. Each row comprised eight plants, maintaining a uniform distribution within the experimental unit. The total experimental field area was 1080 m^2, with dimensions of 30 m long by 36 m wide.

1.5. Crop Management and Agronomic Evaluations

Agronomic tasks and phytosanitary control were carried out according to the commercial management of the crop, with four weedings during crop development as needed, as well as the application of Cupravit OB 21® (Bayer, Germany), an agricultural fungicide, plus a cypermethrin-based pyrethroid insecticide, at a rate of 1 mL per liter of water plus an adherent, owing to the presence of *Rhizoctonia solani*, whose occurrence was localized and not generalized.

Data collection was carried out in the field following standard methodologies for legume crop evaluation. Days to flowering were recorded, defined as the number of days elapsed from sowing to

 doi:10.20944/preprints202607.2179.v1

its onset. Likewise, days to the first mature pods were determined for each accession; the number of pods per plant was assessed by counting a sample of 10 randomly selected plants per accession in each replication; pod length (cm) was measured on 20 pods per accession, taken from five randomly selected plants considering four pods per plant. Seed-related variables included length, width, and thickness, determined on a sample of 50 seeds per accession previously selected from harvested pods, using precision measuring instruments. Finally, grain yield was expressed in tonnes per hectare (t ha^{-1}) by converting the weight obtained per plot as a function of the corresponding area.

Pod length in centimeters, as well as seed width, length, and thickness, were measured with a digital caliper with a resolution of 0.01 mm, and grain weight, initially per plant and per plot, was determined with an analytical balance.

1.6. Bioactive Profile

Dry seeds were kept in airtight glass jars until analysis. Polyphenol extraction followed the methodology of Caprioli et al. [16] with modifications. Ten grams of seeds per accession were crushed in a mortar and ground, sieving the resulting flour through a 316 stainless-steel sieve (ISO 3310-1; 500 µm; ASTM-35; Electrolab). One gram of the flour was weighed and subjected to hydroethanolic extraction with acid hydrolysis: 10 mL of aqueous ethanol (70:30 v/v) was added, adjusted to pH 2 with 2 N HCl, and sonicated (Branson 5510R-MT; 40 kHz) in a water bath at 20 °C for 2 h. The extract was centrifuged at 4000 rpm (Hettich Universal 320) for 30 min, and the supernatant was recovered by filtration; the residue was subjected to a second extraction, and both supernatants were combined for the analysis of total phenolic content (TPC) and antioxidant capacity (ABTS and DPPH).

TPC content was determined by the spectrophotometric Folin-Ciocalteu method [17] with modifications. 200 µL of extract (suitably diluted) was mixed with 1500 µL of distilled water and 100 µL of Folin-Ciocalteu reagent; after 30 min of rest, 200 µL of 20% sodium carbonate was added, and the mixture was incubated in darkness (60 min). Absorbance was read at 765 nm on a UV-Vis spectrophotometer (Thermo Scientific Genesys 180, USA). Quantification was performed using a gallic acid calibration curve, and results were expressed as mg gallic acid equivalents per gram of dry matter (mg GAE/g DM).

Two free-radical-scavenging assays were applied. In the ABTS assay [18,19], 2 mL of ABTS•+ radical solution was added to 20 µL of extract and, after 6 min of rest, absorbance was measured at 734 nm. In the DPPH assay [20], 250 µL of diluted extract was mixed with 150 µL of DPPH solution; after 45 min of rest, 1500 µL of solvent was added, and absorbance was measured at 515 nm. In both cases, calibration curves were constructed with Trolox, and results were expressed as µmol Trolox equivalents per gram of dry matter (µmol TE/g DM).

1.7. Proximate and Mineral Analysis

The proximate characterization was performed on a composite sample per accession, combining the grains from the three field replications to obtain a representative reading of the nutritional profile per genotype.

The proximate composition was determined at the CERTILAB laboratory following the Peruvian Technical Standards (NTP): moisture [21], fat [22], protein by the Kjeldahl method with a conversion factor of 6.25 [23], ash [24], and crude fiber [25]; carbohydrates were obtained by difference, and the energy content (kcal/100 g) and the percentage distribution of energy were calculated according to the Peruvian Ministry of Health's Peruvian Food Composition Tables [26], excluding crude fiber from carbohydrates. The mineral profile was quantified by atomic absorption spectrophotometry—calcium and potassium [27], magnesium and manganese [28], iron [29], and copper and zinc [30]—while phosphorus was determined by spectrophotometry [31]; results were expressed in g/100 g (proximate composition) and mg/100 g (minerals).

1.8. Statistical Analysis

A comprehensive statistical analysis was performed to assess the variability of the cowpea accessions in agronomic, grain-yield, phenolic-compound, and antioxidant-capacity traits. In the univariate analysis, after testing for normality, an analysis of variance (ANOVA) was applied under a randomized complete block design (RCBD) for the phenological variables, yield components, and grain yield (field phase), and a completely randomized design (CRD) for total phenolic compounds and antioxidant capacity (laboratory phase). Tukey's multiple mean comparison test ($p \leq 0.05$) was then applied. In addition, descriptive statistics and coefficients of variation (CV) were calculated. In the bivariate analysis, Spearman correlation coefficients were estimated among the eleven quantitative variables to identify relationships among agronomic, yield, and functional traits. Subsequently, a multivariate analysis was performed by principal component analysis (PCA). Complementarily, a heatmap with hierarchical clustering was used to visualize similarity patterns among accessions and variables and their relationship with grain color. All statistical analyses were performed using R software (R Core Team) with the RStudio graphical environment, version 4.5.2.

3. Results and Discussion

3.1. The Experimental Environment

During the crop cycle (June to December 2024), records from the Puerto Almendras meteorological station (SENAMHI) in Iquitos, Peru, revealed marked climatic fluctuations, characteristic of the tropical humid forest ecosystem, which defined the abiotic-stress environment of the trial. Regarding the thermal regime, a progressive increase in temperature was observed from July onward. The maximum temperature reached its most critical peaks during September and October, with values above 34.0 °C, while the mean monthly temperature ranged from a minimum of 27.3 °C in July to a maximum of 29.5 °C in September.

This warming coincided with a sharp drop in relative air humidity. At the beginning of the experiment (June and July), humidity remained above 92%; however, it dropped abruptly to its lowest point in September (82.1%), remaining below 86% for the rest of the evaluation cycle. The convergence of high maximum temperatures and lower relative humidity during the central phase of the crop represents a key environmental-stress factor that, together with the high aluminum saturation of the soil, strongly conditions the physiological responses and the biosynthesis of phenolic compounds in the evaluated germplasm.

In turn, the water and light regimes showed a markedly seasonal pattern. Rainfall trended downward during the first third of the trial, reaching its most critical scarcity in September, before undergoing a sustained increase that culminated in the maximum rainfall peak in December. In contrast, solar radiation (measured as sunshine hours) reached its maximum in August, exceeding 6 hours per day at the height of the low-rainfall period, and then gradually declined to its minimum value (approximately 3 hours) at the end of the experimental period.

The experimental environment of Zungarococha imposed critical selective pressure on the evaluated germplasm. A pH of 3.50 and an aluminum saturation of 69.01% represent conditions of severe toxicity that typically inhibit root growth in most legumes. Nevertheless, all eight accessions completed their productive cycle, demonstrating the ability of cowpea to grow and yield in acidic upland (terra firme) Amazonian soils [1,7]. Accession PER1005852 sustained the highest yield under these constraints; this behavior is consistent with the tolerance mechanisms described for the species—modulation of root architecture and mitigation of aluminum-induced oxidative stress—although its verification would require specific physiological studies [7]. In addition, temperatures above 34 °C may have contributed to the accumulation of secondary metabolites in the national accessions, in line with the adaptive responses to abiotic stress previously described [7,8].

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3.2. Agronomic Performance and Bioactive Profile

In Supplementary Table S2, the mean squares from the analysis of variance of the studied variables showed that the accession factor had a highly significant effect ($P < 0.01$) on all evaluated variables—agronomic, productive, and bioactive—indicating the existence of genetic variability among cowpea accessions. For agronomic traits evaluated under a randomized complete block design, such as days to flowering, days to physiological maturity, pod length, and number of pods per plant, the mean squares associated with accession were considerably higher than those of the experimental error. This pattern confirms that the differences observed among accessions are mainly due to genetic effects rather than environmental variability.

On the other hand, the replication effect showed relatively low mean-square values, suggesting adequate control of the heterogeneity of the experimental field. A similar pattern was observed for yield and seed traits, with highly significant differences among accessions for grain yield and seed dimensions (length, width, and thickness). Coefficients of variation (CV) ranged from 2.91% for days to physiological maturity to 17.30% for number of pods per plant, indicating overall adequate experimental precision. For functional variables evaluated under a completely randomized design, such as total phenolic content (TPC) and antioxidant capacity (DPPH and ABTS), highly significant differences among accessions were also detected, with mean-square values far exceeding the error term. This evidences marked differentiation among accessions not only in their agronomic performance but also in their biochemical and functional profile. Together, these results demonstrate wide phenotypic variability in agronomic, productive, and bioactive traits.

In Supplementary Table S3, Tukey's multiple comparison test showed significant differences among accessions for all evaluated traits, clearly discriminating the agronomic, productive, and functional behavior of the cowpea genotypes. For phenological traits, accessions CP0112 and PER1005854 were placed in the upper group for days to flowering, showing later behavior, while the remaining accessions grouped as earlier-maturing materials. Regarding pod length, CP0063 showed the highest value, differing significantly from accessions such as PER1005853, which showed the lowest values. The number of pods per plant showed high variability, with PER1005851 and PER1005853 standing out in the upper group, while CP0061 showed the lowest values. Regarding seed traits, consistent differences were observed among accessions. CP0112 and PER1005854 stood out in seed length, while accessions such as PER1005851 and PER1005853 showed significantly lower values. In terms of seed width and thickness, accessions such as CP0061 and PER1005852 were placed in the upper groups, indicating larger seeds, in contrast to PER1005851, which showed the lowest values. These differences reflect variability in seed morphology, a key trait in germplasm characterization. In terms of grain yield, accession PER1005852 was placed in the upper group (1.423 t ha^{-1}), followed by PER1005853 and PER1005854, which also showed high values, although in partially shared groups.

In contrast, CP0061 showed the lowest yield, placing it in the lower group. Regarding the functional variables, marked differences were observed among accessions. PER1005852 showed the highest total phenolic content, while accessions such as CP0062, CP0112, and PER1005854 showed intermediate-to-high values. For antioxidant capacity, PER1005852 and CP0112 stood out in DPPH, while CP0062, CP0112, and PER1005852 were placed in the upper group for ABTS. In contrast, PER1005851 and PER1005853 showed the lowest values for these variables. Together, these results demonstrate clear differentiation among accessions, identifying genotypes with high productive potential, such as PER1005852, as well as accessions with a high functional profile, such as CP0112 and CP0062, suggesting the existence of materials with potential for use in breeding programs aimed at both yield and functional quality. A particularly relevant aspect was the behavior of accession PER1005852, which showed consistently outstanding performance across multiple evaluated dimensions. This accession was placed within the group of early-maturing materials, as evidenced by its low values for days to flowering, representing an important agronomic advantage in production systems that seek to shorten the crop cycle. Despite this earliness, PER1005852 showed the highest grain yield (1.423 t ha^{-1}), which is remarkable, since a trade-off between short cycle and productivity commonly exists. Additionally, PER1005852 stood out in the functional variables, recording the highest total phenolic content and placing in the upper group for antioxidant capacity in both DPPH and ABTS. This behavior suggests that this accession not only shows good productive potential but also a high nutraceutical value. The combination of earliness, high yield, and high antioxidant capacity positions PER1005852 as a genotype of high interest, showing that a negative relationship between productivity and functional quality does not necessarily exist in the evaluated germplasm.

3.3. Proximate Characterization and Mineral Profile of the Grain

The proximate analysis (Supplementary Table S4) revealed a dense nutritional profile characteristic of cowpea, with important variations among the evaluated accessions. Grain moisture content ranged from 10.65 g/100 g (PER1005852) to 14.08 g/100 g (CP0062), remaining within adequate margins to ensure postharvest stability and safe seed storage. Ash content, an indirect indicator of overall mineral richness, ranged from 3.51 to 4.65 g/100 g, with accession CP0062 showing the highest value.

Regarding the macronutrient fraction, the accessions confirmed their high value as a source of plant protein. Protein content ranged from 21.87 to 24.97 g/100 g, with the national accessions PER1005851 (24.97 g/100 g) and PER1005852 (24.75 g/100 g) exceptionally standing out with the highest levels in the panel. In contrast, fat content was consistently low across all samples, ranging from 0.88 g/100 g (CP0112) to a maximum of 1.70 g/100 g (PER1005851). This combination of high protein and low lipid content establishes these genotypes as highly healthy, lean ingredients. Crude fiber content was minimal in all samples (≤ 0.14 g/100 g).

Carbohydrates represented the largest fraction of the grain, with values ranging from 56.76 g/100 g (CP0062) to 80.07 g/100 g (CP0112). Consequently, the total energy contribution was homogeneous among treatments, averaging close to 340 kcal/100 g (range: 330.08 to 347.34 kcal/100 g). The caloric distribution showed that the largest proportion of energy comes from carbohydrates (66.84% to 94.29%), followed by a significant contribution from protein (25.70% to 29.07% in most accessions), while the energy contribution from fat was marginal (less than 4.5% in all cases).

The heatmap (Supplementary Figure S2) revealed marked nutritional variability among cowpea accessions, allowing the identification of contrasting profiles in proximate composition, energy distribution, and mineral content. Overall, accessions showed important differences in carbohydrates, protein, energy content, and essential minerals, suggesting broad potential for nutritional use within the evaluated germplasm. Accessions CP0112, PER1005852, and CP0063 grouped into the cluster associated with higher values of several nutritional and mineral components. In particular, CP0112 stood out for showing the highest carbohydrate content (80.07 g/100 g) and phosphorus content (616.70 mg/100 g), together with a high proportion of energy derived from carbohydrates (94.29%), suggesting a predominantly energy-oriented profile. Likewise, CP0063 showed the highest values of iron (7.60 mg/100 g) and manganese (2.42 mg/100 g), indicating important mineral potential. However, calcium, zinc, and potassium values were below the ranges typically reported for the species, possibly due to interference in their analytical determination, and should therefore be interpreted with caution. For its part, PER1005852 showed one of the highest protein contents (24.75 g/100 g)—surpassed only by PER1005851 (24.97 g/100 g)—together with relatively high phosphorus and potassium values, evidencing a balanced nutritional profile.

In contrast, accessions PER1005853 and PER1005854 showed lower relative concentrations of several minerals and energy components, grouping into blue-colored zones on the heatmap. However, PER1005853 showed a relatively high fat content (1.54 g/100 g) and a higher proportion of energy derived from carbohydrates, indicating differentiated nutritional patterns among accessions. The analysis also revealed interesting associations among nutritional variables. The minerals calcium, iron, manganese, and zinc tended to concentrate jointly in certain accessions, suggesting possible shared mechanisms of mineral accumulation. Similarly, the variables related to energy and carbohydrates showed consistent clustering patterns, while protein and ash showed a more

 doi:10.20944/preprints202607.2179.v1

independent distribution among accessions. Together, these results show that the evaluated accessions possess contrasting and complementary nutritional profiles, highlighting the potential of cowpea as a food resource with functional and nutritional value. In particular, accessions such as PER1005852, CP0112, and CP0063 emerge as promising materials for breeding programs aimed at nutritional quality and food security.

3.4. Correlation Analysis

In Supplementary Figure S3, Spearman's correlation analysis showed clear patterns of association among phenological, morphological, productive, and functional variables in the cowpea accessions. A high positive correlation was observed between days to flowering (DF) and days to physiological maturity (DPVM) ($r = 0.75$), indicating a direct relationship between crop earliness and the duration of the phenological cycle. Likewise, seed-size-related variables, such as length (LS), width (AS), and thickness (GS), showed moderate-to-high positive correlations with one another ($r =$ 0.53–0.87), evidencing morphological consistency in grain structure. In contrast, the number of pods per plant (VP) showed moderate-to-high negative correlations with seed-size variables (AS: $r = -0.64$; GS: $r = -0.73$), suggesting a possible inverse relationship between the number of reproductive structures and seed size. This pattern indicates that accessions with a greater number of pods tend to produce smaller seeds. Regarding grain yield, weak-to-moderate correlations were observed with phenological and functional variables, with a moderate positive relationship with DF ($r = 0.47$) and low associations with seed-size variables. This suggests that yield does not depend exclusively on a single component but on the interaction of multiple traits. On the other hand, the functional variables showed a highly consistent pattern, evidenced by very high correlations among total phenolic content (TPC), DPPH, and ABTS ($r =$ 0.87–0.92), confirming the strong interrelation between phenolic compounds and antioxidant capacity. Additionally, seed-size variables (LS, AS, and GS) showed moderate positive correlations with TPC and antioxidant capacities ($r \approx$ 0.51–0.72), suggesting that larger seeds tend to accumulate higher concentrations of bioactive compounds. Together, these results demonstrate the existence of complex relationships between agronomic and functional components, highlighting the possibility of selecting genotypes that combine productive traits with high nutraceutical value.

3.5. Principal Component Analysis and Clustering

In Figure 2, principal component analysis showed that the first two components explain 71.9% of the total variability (PC1 = 55.2% and PC2 = 16.7%). PC1 is mainly associated with variables related to seed size (LS, AS, GS), yield (RG), and functional variables (TPC, DPPH, and ABTS), all with vectors oriented toward the positive side of the axis. This suggests that this component represents a gradient of productive performance and functional quality. In contrast, PC2 mainly discriminates phenological and structural variables, highlighting the influence of the number of pods per plant (VP) on the positive axis and pod length (LV) on the negative axis. In terms of relationships among variables, a clear positive association is observed among TPC, DPPH, and ABTS, whose vectors show a similar orientation, confirming their close functional relationship.

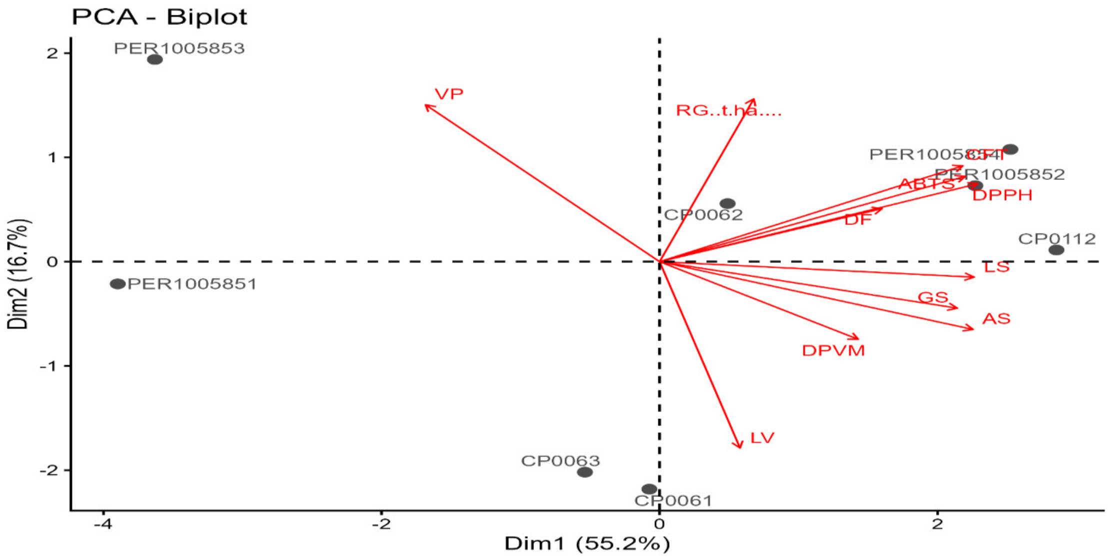

Figure 2. Principal component analysis (PCA) biplot illustrating the relationships among agronomic, yield-related, and functional traits, and their association with *Vigna unguiculata* accessions.

Likewise, seed-size variables (LS, AS, and GS) also show a high positive correlation with one another and with the functional variables, indicating that accessions with larger seeds tend to show higher levels of bioactive compounds. On the other hand, the VP vector is oriented in the opposite direction to the seed-size variables, evidencing the inverse relationship between the number of pods and grain size, in agreement with the correlation analysis results. Regarding the distribution of accessions, a clear differentiation is observed in the multivariate space. Accessions PER1005852 and PER1005854 are located in the positive quadrant of PC1, close to the grain-yield and functional-variable vectors, indicating a strong association with high yield and high antioxidant capacity. In particular, PER1005852 is consistently positioned in the direction of the TPC, DPPH, and ABTS vectors, confirming its profile as a genotype of high functional and productive value. In contrast, accessions such as PER1005851 and PER1005853 are located toward the negative side of PC1, away from these variables, suggesting lower performance in both yield and functional quality. CP0112, for its part, is positioned close to the LS vector, indicating its association with larger seed size, while accessions such as CP0061 and CP0063 group in the region associated with LV and DPVM, reflecting differentiated phenological and structural traits. Overall, the PCA confirms the existence of consistent multivariate patterns integrating agronomic, productive, and functional variables, allowing the identification of accessions with contrasting profiles. PER1005852 stands out in particular, combining proximity to the yield and antioxidant-capacity vectors, consolidating itself as a genotype with dual-purpose potential by integrating productive efficiency and nutraceutical quality.

In Supplementary Figure S4, hierarchical cluster analysis showed a clear differentiation among *Vigna unguiculata* accessions based on their agronomic, productive, and functional traits. Two main groups were identified: the first comprising accessions with predominantly low values for yield and functional-compound variables, and the second comprising accessions with superior performance in these traits. The lower-performing group includes PER1005851 (white) and PER1005853 (small white), characterized by low values (blue tones) for grain yield, seed size, and functional variables (TPC, DPPH, and ABTS). However, PER1005853 shows relatively high values for the number of pods per plant (VP), reinforcing the inverse relationship between the number of pods and seed size/quality previously evidenced in the correlation analysis. This group is associated with accessions with little or no seed pigmentation, suggesting lower accumulation of bioactive compounds. In contrast, the second group includes accessions such as PER1005852 (light brown), PER1005854 (red), CP0112 (brown), and CP0062 (black), which show high values (red tones) for variables related to seed size, yield, and especially functional compounds. Within this group, PER1005852 stands out, showing a consistent pattern of high values in TPC, DPPH, and ABTS, in addition to good yield performance,

 doi:10.20944/preprints202607.2179.v1

confirming its profile as a dual-purpose genotype. Likewise, CP0112 and PER1005854 show similar patterns, although with slight differences in specific variables, suggesting differentiated productive strategies. The presence of seed tones including light brown, brown, red, and black in this group indicates that the presence of pigmentation, more than its intensity, is associated with a higher content of bioactive compounds. Additionally, the functional variables (TPC, DPPH, and ABTS) are observed to cluster in the column dendrogram, showing highly correlated behavior, in agreement with the Spearman correlation analysis. These variables are also associated with seed dimensions (AS, GS), reinforcing the hypothesis that larger seeds tend to accumulate higher levels of bioactive compounds. Overall, the side annotation of seed color shows a consistent pattern, where accessions with pigmentation (PER1005852, PER1005854, CP0112, and CP0062) tend to concentrate in the higher-functional-performance group, while those with light-colored seeds (PER1005851 and PER1005853) group at the lower-performance end. These results suggest that seed color may act as an indirect phenotypic indicator of functional quality, although this relationship should be interpreted with caution and together with the genetic variability inherent to each accession.

Cluster analysis showed that seed pigmentation (brown, red, and black) is a phenotypic trait closely linked to metabolic resilience. Pigmented accessions (PER1005852, PER1005854, CP0112, and CP0063) showed higher levels of total phenolics and antioxidant activity. This finding supports the notion that flavonoids accumulated in the seed coat play a protective role against oxidative damage [4,32]. The superiority of PER1005852 suggests exceptional efficiency in its polyphenolic profile; according to the literature, cultivation in acidic soils can induce the phenylpropanoid pathway and increase the concentration of bioactive compounds as a defense strategy, offering a plausible—though unverified in this study—explanation for the observed levels [5]. In contrast, light-colored accessions (PER1005851 and PER1005853) showed significantly lower functional performance, confirming that the absence of pigments limits the nutraceutical potential of the crop under stress [6,32].

Despite the low cation availability in the soil, the protein profile of the accessions (21.87%–24.97%) remained within the high nutritional-density standards reported for the species [3,4]. Accession PER1005851 (24.97% protein) emerged as a genetic material of high biological value for regional food security [33]. A relevant finding was the high iron (up to 7.60 mg/100 g in CP0063) and phosphorus (616.70 mg/100 g in CP0112) content, values that fall within the high range reported for the species [34]. This ability to mobilize phosphorus and micronutrients to the grain in the presence of aluminum toxicity indicates nutrient-use efficiency mechanisms relevant to biofortification strategies in Amazonian soils [7,34]. The low lipid content (<1.70%) further reaffirms cowpea as a healthy food aligned with current market demands [2].

The use of multivariate analysis made it possible to identify accession PER1005852 as the dual-purpose "ideotype" [35]. Unlike other genotypes that showed a negative correlation between yield and quality, this material managed to integrate high productivity with a superior density of antioxidants and minerals [36]. Principal Component Analysis confirmed that yield and antioxidant-capacity variables are not mutually exclusive, allowing the selection of materials that simultaneously meet agronomic criteria and human-health benefits [1,3]. This multivariate stability suggests superior photosynthetic and metabolic efficiency, positioning these accessions as sustainable productive options in the face of climate change in the Amazon [1].

3.6. Study Limitations

Despite the significant findings regarding the resilience and bioactive profile of the evaluated accessions, the following limitations must be acknowledged for the correct interpretation of the results: agronomic performance and secondary-metabolite synthesis in cowpea are processes highly dependent on the environment. Previous research shows that environmental factors such as temperature and rainfall patterns drastically influence the concentration of phenolic compounds and flavonoids [37]. Since this study was conducted in a single cycle and location, the existence of significant genotype-by-environment interactions could influence the actual expression of the traits,

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requiring multi-year validations to ensure the stability of the recommendations across other Amazonian microclimates [34,37]. The methods used are standard tools for rapid germplasm screening; however, they have documented technical limitations. These in-solution assays fail to replicate the dynamic physiological conditions of living organisms and do not account for the bioavailability of the compounds after ingestion [6,37]. Therefore, the reported antioxidant-capacity values should be considered indicators of chemical potential rather than a direct measure of in vivo biological efficacy [4,6]. Although the eight evaluated accessions showed notable variability in phenotypic traits and seed-coat coloration, this sample represents only a small fraction of the species' overall genetic diversity [7,38]. Breeding programs currently face the challenge of integrating nutritional-quality traits that have historically been undervalued in favor of raw yield, suggesting the need to broaden the evaluation panel in future stages [38,39]. The proximate and mineral characterization was performed on a composite sample per accession, without analytical replicates; therefore, the differences in proximate and mineral composition are descriptive in nature and were not subjected to statistical testing. Although this work identifies genotypes with high iron and phosphorus density, negative correlations between total phenolic content and the concentration of certain minerals have been reported in cowpea [34]. In addition, the presence of secondary metabolites with antinutritional effects could limit the biological utilization of nutrients if not comprehensively analyzed in future bioavailability studies [3,39]. Although this work identifies materials capable of thriving under conditions of high acidity and aluminum saturation, it did not examine in depth the specific physiological mechanisms—such as the secretion of root organic acids—underlying this tolerance. Additionally, since all accessions were evaluated on the same acidic soil without an aluminum-free control treatment, the results reflect comparative performance under these conditions rather than differential tolerance to aluminum in the strict sense [1,7]. The lack of this analysis makes it impossible to determine whether the resilience of accession PER1005852 is due to a metal-exclusion mechanism or to internal tissue tolerance [1,7].

4. Conclusions

The eight cowpea accessions [*Vigna unguiculata* (L.) Walp] completed their productive cycle in a soil with 69.01% aluminum saturation and pH 3.50. This adaptive capacity, which did not compromise grain quality, positions cowpea as a strategic crop for food security in firm ground ecosystems. The proximate and mineral characterization revealed that aluminum stress does not limit the nutritional density of the species. Materials with a superior protein profile were identified, notably accession PER1005851 (24.97%), along with genotypes with an exceptional capacity for mineral translocation, such as CP0063 (7.60 mg/100 g Fe) and CP0112 (616.70 mg/100 g P). These levels suggest that cowpea possesses efficient nutrient-mobilization mechanisms in restrictive soils. Accession PER1005852 was consolidated as the ideotype of greatest overall potential, showing an optimal balance among grain yield, total phenolic compound concentration, and antioxidant capacity. Seed-coat pigmentation was validated as a reliable indirect phenotypic indicator for the selection of functional quality, with dark seeds (brown, red, and black) showing significantly higher nutraceutical potential than light-colored seeds. Finally, multivariate analysis determined that easily measured physical traits, such as seed size, are linked to the accumulation of bioactive compounds. These results demonstrate that cowpea grown under conditions of aluminum toxicity in the Amazon is not only a source of macronutrients but a functional food with a remarkable nutritional density and commercial potential based on its nutritional and antioxidant profile.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org., Table S1: Chemical and physical characteristics of the experimental soil; Table S2: Mean squares of the analysis of variance for agronomic, yield, and bioactive variables; Table S3: Tukey's multiple mean comparison test for the evaluated variables; Table S4: Proximate and mineral composition of the eight cowpea accessions; Figure S1: Geographic location map of the experimental site; Figure S2: Heatmap with hierarchical clustering of nutritional and mineral variables; Figure S3: Spearman correlation matrix among agronomic, morphological, productive, and functional variables; Figure S4: Hierarchical cluster dendrogram of accessions based on agronomic, productive, and functional traits.

Author Contributions: Conceptualization, A.J.B.F. and J.R.-C.; methodology, R.S.V.-P., C.B.M. and F.T.C.; formal analysis, R.S.V.-P. and C.B.M.; investigation, J.R.-C., C.B.M. and J.C.C.C.; resources, A.J.B.F. and J.C.C.C.; data curation, R.S.V.-P.; writing—original draft preparation, R.S.V.-P. and C.B.M.; writing—review and editing, L.L.-M., A.J.B.F. and J.R.-C.; visualization, R.S.V.-P.; supervision, A.J.B.F.; project administration, A.J.B.F.; funding acquisition, A.J.B.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Universidad Nacional de la Amazonía Peruana (UNAP) through the Vice-Rectorate for Research (VRINV), approved under Rectoral Resolution No. 0449-2024-UNAP.

Data Availability Statement: Data is contained within the article or supplementary material.

Acknowledgments: The authors thank the Instituto Nacional de Innovación Agraria (INIA) for providing cowpea accession grains, and the CERTILAB laboratory for the proximate and mineral analyses.

Conflicts of Interest: The authors declare no conflicts of interest.

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