

Agro-morphological characterization of robusta genotypes from the INERA Yangambi coffee collection, DRC

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ABSTRACT

Meeting rising quality standards while at the same time addressing climate challenges will make the commercial cultivation of Robusta coffee increasingly difficult. Whereas breeding new varieties may be an important part of the solution, such efforts for Robusta lag behind, with much of its genetic diversity still unexplored. By screening existing field genebanks to identify accessions with desirable traits, breeding programs can be significantly facilitated. This study quantifies the morphological diversity and agronomic potential of 70 genotypes from the INERA Coffee Collection in Yangambi, Democratic Republic of the Congo. We measured 29 traits, comprising vegetative, reproductive, tree architecture, and yield traits. Classification models were applied to establish whether these traits could accurately classify genotypes based on their background. Furthermore, the agronomic potential and green bean quality of the genotypes were studied. Although significant variation in morphological traits was observed, no combination of traits could yet accurately predict genetic background based on a subsample set of the studied genotypes. Genotypes with promising traits for green beans were identified in both 'Lula' and 'Lula' – Wild hybrids, while promising yield traits were found in 'Lula' – Congolese subgroup A hybrids. Additionally, 'Lula' – Wild hybrids G0023 and G0067 showed low specific leaf area and stomatal density, two traits with potential fitness advantages in dry environments, warranting further study. Our findings highlight the agronomic potential of underexplored Robusta coffee genotypes from the Democratic Republic of the Congo and indicate the need for further screening to maximize their value.

Key words: *Coffea canephora*; germplasm; phenotyping; coffee quality.

1 INTRODUCTION

Coffea canephora, commonly known as Robusta coffee, contributes to more than 40% of the world's coffee production (International Coffee Organization - ICO, 2023). However, climate model predictions indicate that climate change implications may be detrimental to coffee cultivation, with the Robusta-producing countries expected to be strongly affected (Bunn et al., 2015). The cultivation of Robusta coffee under these climate challenges while at the same time meeting rising quality standards will become increasingly difficult. Yet, Robusta research and breeding is in many respects still in its infancy, with a lot of unknown variation in its gene pool and vast unexplored genetic resources for breeders (World Coffee Research - WCR, 2023). In this context, breeding a new generation of climate-smart cultivars (Ferrão, Riva-Souza, et al., 2024a) could capitalize on the extensive genetic diversity of *C. canephora*. In general, there has been no long-term and sustained effort to improve the species in more than 30, and countries with active breeding programs face limited access to agronomic and genetic diversity (Prakash, 2018). Past efforts were further constrained by inadequate genetic resources outside the native range of *C. canephora* and by

political instability within some countries in its zone of origin (Montagnon; Leroy; Eskes, 1998; Stoffelen et al., 2019).

Whereas recent advances in molecular genetics have shed light on the genetic diversity within *C. canephora*, revealing distinct genotypes that could hold the key to improving coffee production and quality (Anagbogu et al., 2019; Kiwuka et al., 2021; Loo Solórzano et al., 2017; Ngugi; Aluka, 2019; Verleysen et al., 2023), the morphological variability and agronomic potential of these genetic resources are understudied. Morphological screening of genetic resources and identifying accessions with desirable morphological traits can facilitate the genetic variation available for breeding programs (Anthony et al., 2011; Kiwuka et al., 2021; Paredes-Espinosa et al., 2023). Furthermore, Robusta germplasm derived from controlled or natural hybridization events between wild or related semi-wild genotypes can be of exceptional value for selection, as they conserve part of the original variation of the wild population while also introducing new trait variation (Campuzano-Duque; Blair, 2022).

By systematically characterizing morphological traits associated with *C. canephora*'s genotypes, new phenotypes that may possess unique attributes, such as increased green bean size and production (Ngugi; Aluka, 2019), climate resilience

(Ferrão, Riva-Souza, et al., 2024a) or enhanced cup quality (Bollen et al., 2024) can be discovered and incorporated into breeding pipelines. The desirable morphological traits of coffee trees can range from plant architectural to microscopical traits. For example, the number of nodes per branch is a morphological indicator of higher yield potential (Cilas et al., 2006; Spinelli et al., 2018). Leaf morpho-anatomical characteristics like the specific leaf area (SLA) and stomatal density of the coffee tree directly reflect water use efficiency and the ability to adapt to different environmental conditions (Dubberstein et al., 2021). These traits have also been reported to correlate with the productivity of the coffee tree (Gagliardi et al., 2015). SLA and stomatal density are highly sensitive to local environmental conditions, but their plasticity does have a genetic basis (De Kort et al., 2020). These leaf traits are valuable to consider when selecting genotypes in a rapidly changing climate (Bertolino; Caine; Gray, 2019). In addition to morphological traits of the coffee tree, green bean morphological traits such as screen size distribution, bean weight, and peaberry rate, which refers to the occurrence of rounded coffee seeds resulting from a single developed seed (Cilas; Bouharmont, 2005; Ngugi; Aluka, 2019). These attributes are particularly important to green coffee importers and significantly influence market pricing. Larger coffee bean sizes, uniform bean weight, and lower peaberry rates are often associated with higher prices (Coffee Quality Institute, 2019; Lingle; Menon, 2017).

Traditionally, *C. canephora* is divided into two genetic groups: the Guinean group, which originates from the rainforests of West Africa, located west of the Dahomey Gap, and the Congolese group, originating from the rainforests of Central Africa (Berthaud; Charrier, 1988). In the early 20th century, the genetic resources of Central Africa played a key role in Robusta coffee's breakthrough as a commercial crop (Leplae, 1936; Van Der Vossen, 1985). Between 1930 and 1960, l'Institut National pour l'Etude Agronomique du Congo Belge (INEAC) was the leading breeding institute for Robusta coffee, with research stations in Lula, Luki and Yangambi. By the 1950s, Yangambi had become the leading research station for Robusta coffee. In later breeding programs, such as those in West Africa (Montagnon; Leroy; Eskes, 1998) and Vietnam (Vi et al., 2023), genetic resources from the Congolese group again played a key role thanks to their valuable agronomic traits and contributions to cup quality (Moschetto et al., 1996). This genetic diversity of *C. canephora* is of utmost importance to the climate adaptation of Robusta coffee (Bramel et al., 2017) but has been overlooked and insufficiently studied (Stoffelen et al., 2019). Natural *C. canephora* populations tend to hold high genetic variation, likely due to its self-incompatibility (Moraes et al., 2018; Verleysen et al., 2023). Conservation of existing Robusta populations is essential for leveraging this diversity to characterize the wide range of variability for quality traits (Prakash, 2018).

The National Agricultural Study and Research Institute (INERA), formerly INEAC, manages the *ex-situ* coffee collection in Yangambi, DRC. Regional instability, weak governance, and lack of resources resulted in the decimation of the INERA Coffee Collection in Yangambi, and much documentation was lost (Stoffelen et al., 2019). In collaboration with Meise Botanic Garden (Belgium), this collection has been rehabilitated, screened, and enriched with new genetic material from the wild and local home gardens (Stoffelen et al., 2019; Vanden Abeele et al., 2021). A comprehensive genetic analysis of the INERA Coffee Collection revealed a relatively broad genetic diversity and genetic material from the Congolese subgroup A (likely originating from the INERA Research Station in Luki) and subgroup BE (hybrid between subgroups B and E; likely corresponding to 'Wild' genotypes from the Yangambi rainforest) were discovered. However, the most abundant materials in the INERA Coffee Collection in Yangambi are known as 'Lula' cultivars. These 'Lula' cultivars are currently of unknown geographical origin (Verleysen et al., 2023). The INERA Coffee Collection also contains individuals with an admixed genotypic background, likely derived from hybridization between 'Lula' and subgroup BE (local 'Wild') or between 'Lula' and subgroup A material. Previous research differentiated eight *C. canephora* genetic groups corresponding to different geographic origins across West and Central Africa (Merot-L'anthoene et al., 2019). The geographical regions of Congolese subgroups A, B, E, and R overlap with the west, north, central, and southeast of DRC, respectively. The INERA Coffee Collection has not been screened for morphological traits and agronomic performance since 1960 (Coste, 1955; Montagnon; Leroy; Eskes, 1998). The wild genetic resources introduced from the nearby Yangambi rainforest and the spontaneous, new hybrids found in the collection have never been studied. However, the sensory quality of 70 genotypes, which represented the genetic diversity of the INERA Coffee Collection, has recently been reported (Bollen et al., 2024).

This study aims to provide an assessment of the morphological diversity and agronomic potential of the same 70 genotypes from the INERA Coffee Collection in Yangambi. We first explore the variation in morphological traits and evaluate if traits can be used to discriminate the genotypes based on genetic background. Secondly, we assess the agronomic potential of the genotypes based on two consecutive harvest years and their green bean traits. The latter in combination with their previously reported sensory quality scores. We hypothesize that the presence of three genetic subpopulations, and their numerous admixed genotypes, will result in a substantial variation in morphological and agronomy-related traits. The extent of hybridization will prevent identifying reliable morphological markers that differentiate the three subpopulations. Overall, we aimed to contribute to the detailed evaluation and baseline phenotyping

of underexplored *C. canephora* genetic resources, laying the groundwork for developing new and improved coffee varieties that are better adapted to the challenges of the coffee market.

2 MATERIAL AND METHODS

2.1 Site description and genetic material

The INERA Coffee Collection in Yangambi is located in the Tshopo province, DRC (Lat: 0°50'59.60"N Long: 24°27'50.85"E, 485m altitude). The Yangambi region is classified as Köppen Af (rainforest) with a dry season, average annual precipitation of 1837 mm, and a mean annual temperature of 25.1°C (Kasongo Yakusu et al., 2023). The soil of the Yangambi region is classified as Ferralsol, a strongly weathered soil with low nutrient-holding capacity (Jones et al., 2013). The coffee collection is maintained as an unshaded monoculture system with coffee trees spaced 2.5x2.5 meters apart. Agricultural management practices include mulching, weeding, pruning, and minimal pesticide application without irrigation or fertilization.

Genetic analysis of the INERA Coffee Collection by Verleysen et al. (2023) delineated three subpopulations: firstly, 'Lula' cultivars that originate from the Lula breeding station of the DRC which currently have an unknown geographic origin; secondly, the INERA accession belonging to Congolese subgroup A originating from another INERA breeding station in Luki; thirdly, the collection contains local wild accessions introduced from the nearby Yangambi rainforest. Additionally, admixed genotypes were identified, likely 'Lula' – Wild hybrids and 'Lula' – Congolese subgroup A hybrids (Figure 1).

Recently, 70 genotypes from the INERA Coffee Collection were selected and studied for their sensory quality. The selected genotypes were subdivided into five classes: Wild (n = 3), 'Lula' – Wild hybrids (n = 14), 'Lula' (n = 39), 'Lula' – Congolese subgroup A hybrids (n = 13), and Congolese subgroup A (n = 1) (Bollen et al., 2024). The same genotypes and clonal material were selected for our study. One to three

clones per genotype, based on the genetic fingerprinting of Verleysen et al. (2023), were used in this study.

2.2 Morphological traits

A set of 29 morphological traits (Table 1) was measured on 118 individual *C. canephora* coffee trees comprising 70 genotypes. The traits were measured from November 2021 to February 2022. A subset of morphological traits was measured again from November to December 2022 (Section 2.3).

2.2.1 Foliar traits

Three healthy plagiotropic branches were sampled from the middle portion of each tree's main stem. Herbarium vouchers were made following Degreef et al. (2004) and transported to Meise Botanic Garden. The stipule and petiole length were measured on the vouchers with an electronic caliper. The leaf length, width, area, and apex were measured from digital scans of the leaves using the ImageJ software (v15.3) (Schneider; Rasband; Eliceiri, 2012). The plant vouchers were dried at 60°C for 24 hours to achieve homogeneous dried plant material. The specific leaf area (SLA) and stomatal density (stomata) were determined from five dried leaf samples. SLA was calculated from the individual leaf area and weight. The stomatal density was determined using the workflow of Meeus et al. (2020). Two imprints of the abaxial side of each leaf were made using the transparent varnish method. The imprints were made in the middle of the leaf, one on each side of the main nerve. Three photomicrographs of 1.600 × 1.200 pixels were taken per leaf print (dimensions = 344×258 µm; area view field = 0.09 mm²) using a Keyence VH-5000 digital microscope (v1.5.1.1) (Keyence International, Mechelen) with full coaxial lightning and default factory settings for shutter speed at ×1.000 lens magnification (VH-Z250R). Detection of stomata on the photomicrographs was achieved using a fine-tuned deep-learning model. We started from an open-source YOLOv8 deep learning detection model developed by Ultralytics (<https://github.com/ultralytics/ultralytics>).

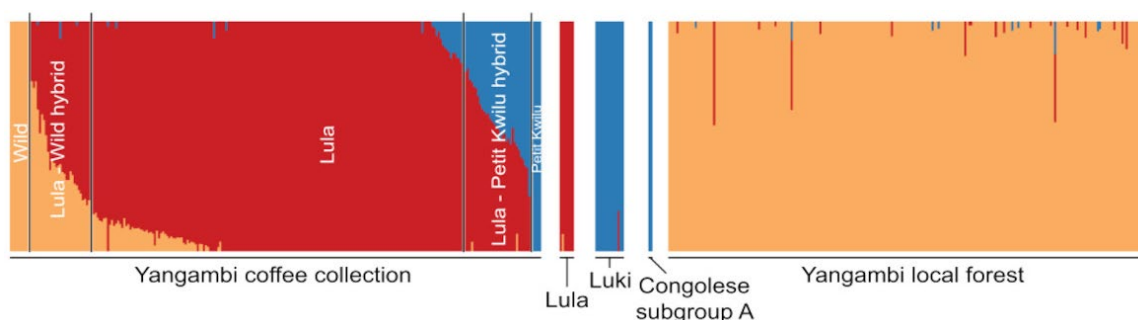


Figure 1: Population genetic structure of the Yangambi Coffee Collection, INERA Luki reference material, Congolese subgroup A reference material, and the Yangambi local forest material. FastSTRUCTURE bar plot representing three clusters ($K = 3$). Colors define subpopulations: orange (Wild), blue (Congolese subgroup A), and red ('Lula' cultivars) (Verleysen et al., 2023).

This model was pre-trained with the COCO dataset (Lin et al., 2014) and then fine-tuned on 14 photomicrographs of *C. canephora* for a total of 392 stomata. This is in line with previous recommendations of Meeus et al. (2020) who recommend training on at least 250 stomata to achieve accurate detection results. Standard data augmentation techniques, such as rotation, flipping, and mosaic augmentation were applied to the training photomicrographs. This species-specific YOLOv8 object detection model was accurate, with a precision of 0.99 and a recall value of 0.98 across 100 photomicrographs of a test set. The stomata count was converted to the number of stomata per square millimeter to obtain the stomatal density.

2.2.2 Reproductive traits

During the main harvest season of November 2021, fresh, developed coffee cherries were handpicked per individual coffee tree. A picture of five coffee cherries was taken for each coffee tree, including a standard ruler (mm). Secondly, after pulping the coffee cherries, a second picture of the ten fresh seeds was taken. Lastly, a transverse cut was made on five coffee cherries for a third picture. During the main flowering period of February 2022, five fully developed flowers were harvested per individual tree. A photo was taken of the lateral and apical view of the flower (Da Silva et al., 2021). An Olympus TG-6 camera was used for all pictures (Olympus Corporation, Tokyo). The reproductive traits were

Table 1: List of morphological traits measured in 70 *C. canephora* genotypes from the INERA Coffee Collection, DRC.

Foliar	Method of evaluation
Leaf length (mm)	Mean length of five leaves from the petiole end to the apex
Leaf width (mm)	Mean width of five leaves at the widest section
Leaf apex (mm)	Mean length of the apex to the convex transition of the leaf margin for five leaves
Stipule length (mm)	Mean length of ten stipules measured from the basis to the tip
Petiole length (mm)	Mean length of ten petioles measured from the basis to the insertion with the blade
SLA (mm ² /g)	Mean specific leaf area of five adult leaves
Stomatal density (number/mm ²)	Mean stomatal density in an area of one mm ² from fifteen leaf prints
Reproductive	
Cherry length (mm)	Mean length of five fully-developed coffee cherries
Cherry width (mm)	Mean width of five fully-developed coffee cherries
Seed length (mm)	Mean length of ten fully-developed coffee seeds
Seed width (mm)	Mean width of ten fully-developed coffee seeds
Cherry thickness (mm)	Mean thickness of five fully-developed coffee cherries
Seed thickness (mm)	Mean thickness of ten fully-developed coffee seeds
Pulp thickness (mm)	Mean thickness of the mesocarp from five fully-developed coffee cherries
Petal length (mm)	Mean length of five fully-developed coffee flower petals
Petal width (mm)	Mean width of five fully-developed coffee flower petals
Anther length (mm)	Mean length of five fully-developed coffee flower anthers
Corolla tube length (mm)	Mean length of five fully-developed coffee flower corolla tubes
Architecture and yield	
Branch length (cm)	The mean length of four adult plagiotropic branches in the middle of the tree
Internode length (cm)	Plagiotropic branch length divided by the nodes averaged over four branches
Plagiotropic branches	Amount of plagiotropic branches on the main stem
Tree height (cm)	Height of the coffee tree
Tree width (cm)	Width of the coffee tree at the widest section
Fruct. nodes	Average amount of fructifying nodes across levels 5, 15, and 25
Cherry per node	Average amount of coffee cherries per fructifying node across levels 5, 15, and 25
Fruct. node ratio	Ratio of fructifying nodes to total nodes across levels 5, 15 and 25
Max. node	Largest amount of coffee cherries recorded at a node
Fruct. branch ratio	Ratio of fructifying plagiotropic branches to total branches on the main stem
Total cherries	Amount of total cherries across the three levels

measured on the resulting images (4000×3000 pixels) using the ImageJ software (v15.3) (Schneider; Rasband; Eliceiri, 2012).

2.2.3 Architectural and yield traits

The architecture and yield of the coffee trees were measured on-site. The coffee cherry yield was estimated using the method proposed by Cilas et al. (2006). Starting from the top of the tree's main stem, two fructifying plagiotropic branches were selected at orthotropic nodes 5, 15, and 25, representing three levels of coffee tree production. For each of the six fructifying branches, the branch length, number of nodes, number of fructifying nodes, and number of coffee cherries per individual node were counted, resulting in an estimation of the tree's coffee cherry production.

2.3 Agronomic potential of the genotypes

To estimate the genotypes' agronomic potential, the following morphological traits were measured and averaged over two harvest years (November-December 2021 and 2022) to account for biennial bearing (Cilas; Montagnon; Bar-Hen, 2011): fructifying nodes, cherry per node, fructifying branches, maximum node, and total cherries. When applicable, an average was made across the genotype's clonal trees. Additionally, the following green bean traits were measured at the genotype level, sourced from green bean samples used in the sensory quality study by Bollen et al. (2024): Screen 15+ (%): Dimension distribution using a perforated screen plate with a 15/64-inch diameter screen. The green beans retained by screen size 15 were weighed and recorded as a percentage; Screen 17+ (%): Dimension distribution using a perforated screen plate with a 17/64-inch diameter screen. The green beans retained by screen size 17 were weighed and recorded as a percentage; 100 bean weight (BW) (g): The weight of 100 dried green beans; Peaberry: The number of rounded coffee beans (peaberries) was counted in 50 g of green beans.

The sensory quality of the selected genotypes was previously evaluated through coffee cupping with the Fine Robusta Standards and Protocols (Coffee Quality Institute, 2019), resulting in a total score on a 100-point scale (Bollen et al., 2024). The reported total cupping score was included as metadata in our study.

2.4 Data analysis

Descriptive statistics and correlations between traits were calculated across the 118 individual coffee trees. Spearman rank correlation was employed as not all traits exhibited a normal distribution. The significance of the Spearman correlations was Bonferroni corrected for multiple testing. Principal component analysis (PCA) of the traits was performed on the genotype level, i.e., based on the average

of the clonal trees when applicable. Data was scaled before multivariate analysis. A linear discriminant analysis (LDA) and a random forest model were then applied to evaluate if the genotypes could be discriminated by their genetic background based on morphological traits. Because the 'Lula' genotypes were overrepresented in this study, a subset of genotypes was created based on the subpopulations described by Verleysen et al. (2023): Wild, 'Lula', and Congolese subgroup A (Figure 1). Based on their fastSTRUCTURE values, the genotypes with the strongest 'Lula' identity ($n = 8$), Wild identity ($n = 6$), and Congolese subgroup A identity ($n = 8$) were selected for this classification study (Bollen et al., 2025). The classification accuracy of the LDA model was evaluated with a 10-fold cross-validation. The random forest classification model was trained with 500 trees, and four variables were tested at each split. The correlation matrix was constructed using the *corrplot* package (v0.84). PCA was applied using the *factoextra* package (v1.0.7), LDA using the *MASS* package (v7.3.60), and the Random Forest using the *randomForest* package (v4.7.1) in R (v4.2.3).

3 RESULTS

3.1 Variation and correlation of morphological traits at the individual tree level

There was considerable variation in the morphological traits across the 118 measured trees (Table 2). The coffee cherry and seed dimensions exhibited the least variation, with seed width having the lowest coefficient of variation of the studied traits. Most variation was observed in the yield traits, with the total cherries having the highest coefficient of variation in the traits studied.

Significant Spearman correlations were observed between the morphological traits studied (Figure 2). Leaf length correlated with SLA ($r = 0.46$, $p < 0.001$) and with stomatal density ($r = -0.47$, $p < 0.001$). Similarly, leaf width correlated significantly with SLA ($r = 0.54$, $p < 0.001$) and with stomatal density ($r = -0.48$, $p < 0.001$). SLA and stomatal density were negatively correlated ($r = -0.52$, $p < 0.001$). Larger coffee cherry dimensions resulted in larger coffee seed dimensions. Flower petal length correlated significantly with petal width ($r = 0.49$, $p < 0.001$) and anther length ($r = 0.67$, $p < 0.001$). Anther length correlated significantly with the corolla tube length ($r = 0.44$, $p = 0.001$). The number of plagiotropic branches on the main stem correlated significantly with the branch length ($r = 0.47$, $p < 0.001$) and tree width ($r = 0.44$, $p < 0.001$). The number of fructifying nodes correlated with cherries per node ($r = 0.46$, $p < 0.001$). The maximum node correlated with the number of cherries per node ($r = 0.77$, $p < 0.001$).

Table 2: Mean $\pm$ standard deviation (SD) and coefficient of variation (CV) of the morphological traits of the 118 individual coffee trees comprising 70 Robusta genotypes from the INERA Coffee Collection, DRC.

Trait	Mean $\pm$ SD	CV
Leaf length (mm)	180.95 $\pm$ 21.42	11.84
Leaf width (mm)	73.88 $\pm$ 9.62	13.02
Leaf apex (mm)	14.46 $\pm$ 3.84	26.53
Stipule length (mm)	6.42 $\pm$ 0.76	11.90
Petiole length (mm)	12.86 $\pm$ 2.16	16.79
SLA (mm ² /g)	8167 $\pm$ 1138	13.94
Stomatal density (number/mm ²)	347.39 $\pm$ 60.27	17.35
Cherry length (mm)	13.84 $\pm$ 1.18	8.50
Cherry width (mm)	13.53 $\pm$ 1.04	7.69
Seed length (mm)	11.31 $\pm$ 0.97	8.58
Seed width (mm)	8.78 $\pm$ 0.64	7.33
Cherry thickness (mm)	11.37 $\pm$ 0.98	8.66
Seed thickness (mm)	5.06 $\pm$ 0.47	9.37
Pulp thickness (mm)	1.21 $\pm$ 0.24	19.38
Petal length (mm)	10.19 $\pm$ 2.13	20.89
Petal width (mm)	2.75 $\pm$ 0.49	17.90
Anther length (mm)	7.56 $\pm$ 1.17	15.42
Corolla tube length (mm)	8.92 $\pm$ 1.71	19.14
Branch length (cm)	85.21 $\pm$ 17.87	20.97
Internode length (cm)	4.81 $\pm$ 0.66	13.79
Plagiotropic branches	45.95 $\pm$ 10.84	23.58
Tree height (cm)	277.24 $\pm$ 59.56	21.48
Tree width (cm)	169.76 $\pm$ 32.71	19.27
Fruct. nodes	36.61 $\pm$ 10.99	30.01
Cherry per node	9.91 $\pm$ 3.50	35.30
Fruct. node ratio	0.41 $\pm$ 0.11	26.07
Max. node	25.19 $\pm$ 9.04	35.89
Fruct. branch ratio	0.62 $\pm$ 0.13	20.20
Total cherries	379.12 $\pm$ 206.01	54.34

To reduce the multicollinearity when analyzing the variation in morphological traits of the genotypes the leaf length and width variables were combined into a leaf length-to-width ratio variable; the cherry length, width, and thickness variables were omitted, given their strong collinearity with the seed dimension variables; The width of the coffee tree was omitted as it correlated strongly with branch length. The maximum node and total cherries variables were omitted as they correlated strongly with the fructifying nodes and cherry per node variables.

The first two principal components explained 28.13% of the variation in data. No clear separation of the genotypes

based on their genetic background was observed (Figure 3). In the first dimension, the samples were grouped based on productive traits (fructifying nodes, cherry per node, and branch length) and floral traits (petal, anther, and corolla tube dimensions). The separation in the second dimension was explained mainly by the coffee seed dimensions. The majority of the ‘Lula’ – subgroup A hybrid genotypes were positioned more along the positive axis of the first dimensions, exhibiting a higher number of cherries per node and fructifying node values.

3.2 Discrimination of the genetic background based on morphological traits

Discrimination of the genotypes by genetic background based on their morphological traits was evaluated through an LDA and random forest classifier on a subset of genotypes. The LDA separated the genotypes with the strongest Wild identity from genotypes with the strongest ‘Lula’ and Congolese subgroup A identity by the first linear discriminant (LD1) (Figure 4). The anther length, petal length, seed thickness, SLA, and stomatal density were the most important traits of the first LD. The ‘Lula’ genotypes were separated from the genotypes with the strongest Congolese subgroup A identity by LD2. The seed thickness, SLA, seed length, and stomatal density were the most important traits of the second LD. The classification accuracy of the LDA model after 10-fold cross-validation was 43.33%. The random forest model reported a prediction error of 59.09% for the classification of the genotypes. The class error rates were 0.50, 0.50, and 0.83 for the ‘Lula’, Congolese subgroup A, and Wild identity groups, respectively. The most important traits in the random forest classifier were, in decreasing order, the seed width, number of fructifying nodes, petiole length, stipule length, and anther length.

3.3 Agronomic potential of the genotypes

The agronomic potential of the genotypes was assessed based on the morphological yield traits over two consecutive harvest years, the green bean traits, and the tree architecture, with additional attention to the sensory quality of the coffee (Figure 5). Screen size distribution ranged from 8% retained by screen 15 to 92% retained by screen 17. The weight of 100 green beans ranged from 8.70 g to 19.77 g. The highest and widest genotypes recorded were 425 and 255 cm, respectively. The number of fructifying branches on the main stem ranged from 11 to 63. The number of fructifying nodes across the three measured coffee tree levels ranged from 14 to 70. The average amount of cherries per node across the three levels ranged from 4 to 17. The highest amount of coffee cherries recorded at a single node was 52. The total amount of coffee cherries counted across the three levels of the coffee tree ranged from 70 to 816.

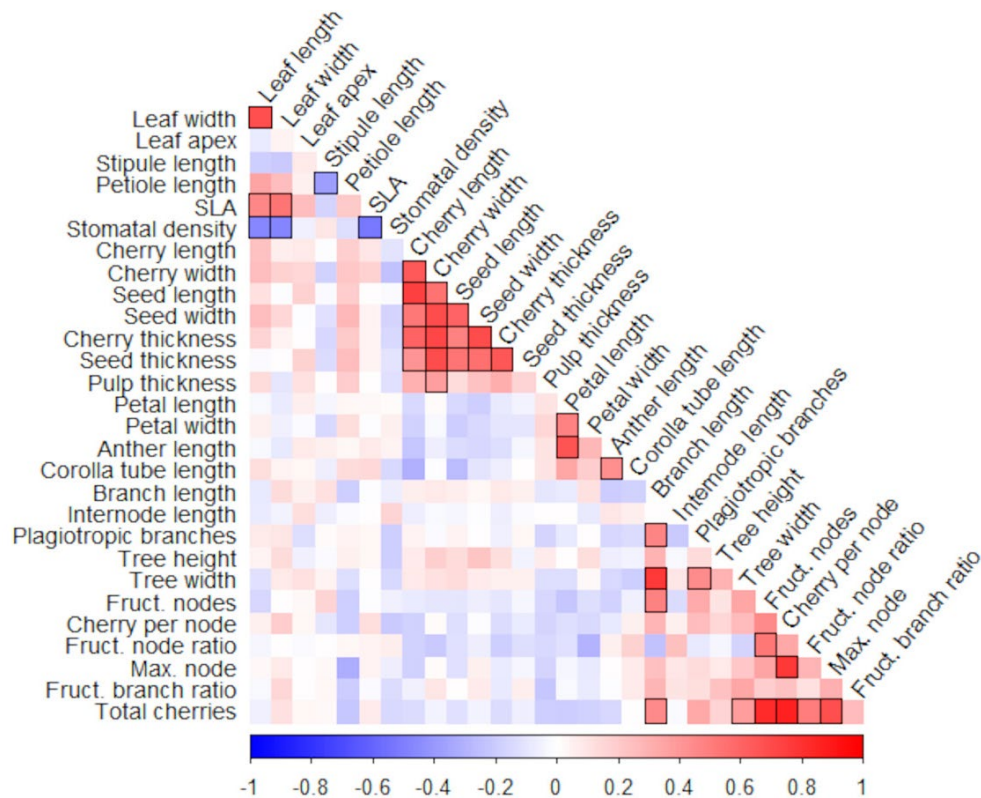


Figure 2: Spearman correlation matrix of the morphological traits of the 118 individual coffee trees comprising 70 Robusta genotypes from the INERA Coffee Collection, DRC. Significant Spearman correlations after Bonferroni correction are indicated by boxes with black edges.

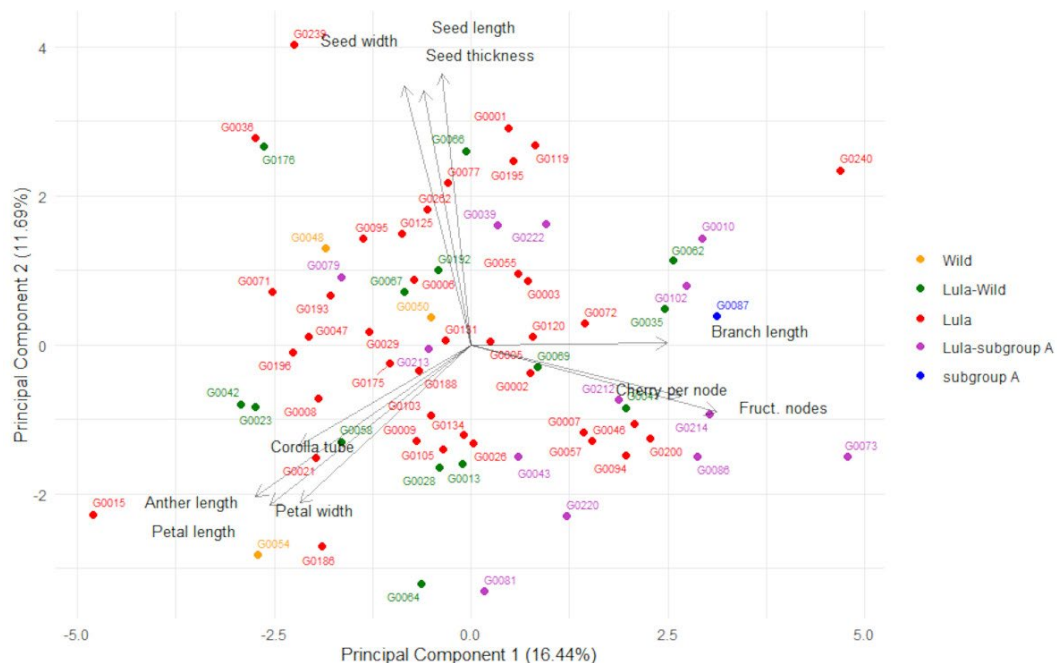


Figure 3: PCA biplot of PC1 and PC2 for the morphological traits of the 70 Robusta genotypes from the INERA Coffee Collection, DRC. The ten trait variables that contributed most to the variation captured by the first two principal components are shown. Genetic classes of the genotypes are Wild, 'Lula' – Wild hybrid, 'Lula', 'Lula' – Congolese subgroup A hybrid, and Congolese subgroup A.

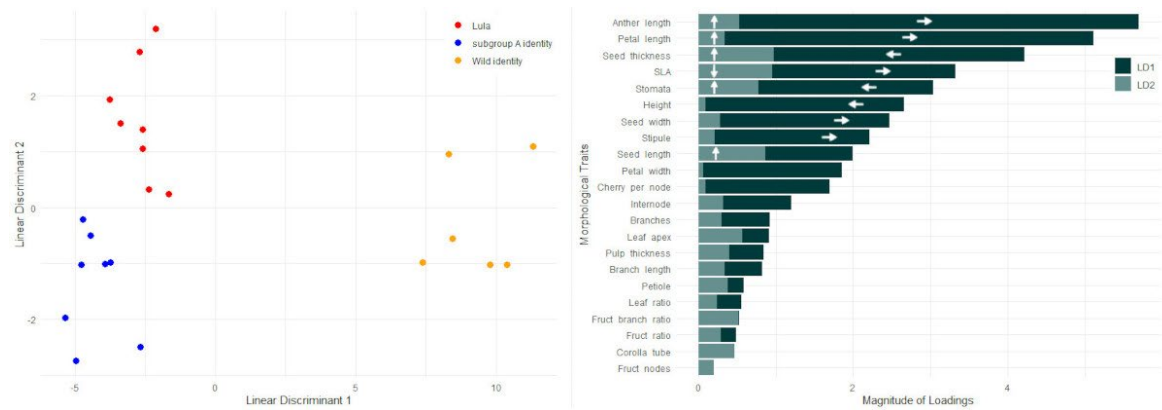


Figure 4: LDA conducted on the morphological traits achieving a classification accuracy of 43.33%. A plot of LD1 and LD2 separates the three genetic backgrounds (left) with the morphological traits most important to LD1 and LD2 (right). Arrows indicate the direction of the positive correlation of the most important traits with LD1 and LD2. Genotypes with the highest ‘Lula’ genetic identity ($n = 8$), Wild genetic identity ($n = 6$), and Congolese subgroup A genetic identity ($n = 8$) were selected for classification based on the population genetic structure analysis of Verleysen et al. (2023).

Genetic class		Genotype	Genetic structure	Total	Screen 15+	Screen 17+	100 BW	Peaberry	Tree height	Tree width	Fruct. branches	Fruct. nodes	Cherry per node	Max. node	Total cherries
Wild		G0048		78.75	0.75	0.29	17.30	44	264	181	26	27	6	32	179
		G0050		78.25	0.74	0.14	12.71	36	195	181	22	32	8	34	237
		G0054		77.50	0.08	0.00	8.70	114	234	129	19	30	7	22	199
		G0028		83.25	0.55	0.20	11.80	45	178	129	26	34	9	32	303
Lula - Wild		G0042		76.50	0.89	0.40	14.24	48	248	142	27	30	5	21	141
		G0058		82.50	0.68	0.08	12.00	92	224	159	28	64	8	29	542
		G0041		79.25	0.80	0.29	13.85	19	318	224	33	26	14	45	392
		G0062		80.50	0.79	0.25	12.66	72	298	162	29	35	12	27	413
		G0064		83.75	0.58	0.12	13.26	33	334	207	46	62	9	21	542
		G0023		83.75	0.59	0.07	10.80	23	300	130	17	17	11	19	185
		G0176		81.25	0.86	0.59	15.10	18	234	141	24	23	7	23	186
		G0069		80.25	0.94	0.71	17.20	23	275	193	46	42	9	28	395
		G0067		83.00	0.93	0.56	14.53	27	301	182	32	39	6	17	240
		G0066		80.50	0.82	0.40	15.00	32	291	214	39	48	11	31	516
		G0035		83.25	0.93	0.52	16.17	39	325	209	34	42	6	22	275
		G0192		82.50	0.83	0.35	16.21	28	236	182	23	43	8	24	334
		G0013		81.25	0.54	0.09	12.93	16	268	163	27	35	7	29	257
		G0119		83.75	0.83	0.33	12.77	28	280	179	36	42	9	35	395
		G0036		79.75	0.84	0.46	18.95	37	227	138	25	37	6	18	225
Lula		G0005		81.75	0.85	0.42	16.16	24	271	164	27	36	10	40	362
		G0026		83.25	0.72	0.11	11.90	33	274	146	34	38	8	22	278
		G0057		81.50	0.89	0.34	13.47	20	312	179	18	45	12	42	557
		G0131		79.50	0.65	0.08	11.81	33	364	219	31	40	10	32	402
		G0021		81.25	0.68	0.11	12.40	19	425	192	17	19	8	32	159
		G0103		84.25	0.89	0.32	9.90	25	257	141	29	42	9	28	323
		G0262		84.00	0.81	0.25	17.74	54	244	167	28	34	7	20	229
		G0200		80.50	0.30	0.04	14.32	46	207	173	26	32	11	31	391
		G0071		75.75	0.74	0.23	13.35	20	273	178	19	26	7	21	182
		G0077		83.25	0.94	0.60	17.08	75	360	160	16	7	22	192	402
		G0094		82.00	0.61	0.20	16.20	10	280	184	35	42	12	41	494
		G0066		83.50	0.90	0.43	14.99	52	267	177	25	32	7	30	233
		G0193		79.00	0.85	0.32	13.87	40	267	149	29	34	8	27	249
		G0002		83.25	0.87	0.38	12.94	16	412	149	32	32	8	24	241
		G0125		81.75	0.86	0.32	13.88	58	271	179	35	29	8	30	206
		G0175		81.75	0.66	0.15	15.98	30	179	111	19	34	6	32	214
		G0120		82.50	0.92	0.63	16.55	37	288	146	21	43	10	47	440
		G0195		81.25	0.96	0.77	11.60	16	245	181	15	31	7	36	318
		G0186		81.00	0.23	0.00	9.35	48	301	148	26	27	9	26	244
		G0007		79.50	0.80	0.37	11.08	20	263	166	31	39	11	35	425
		G0015		80.50	0.68	0.30	13.70	10	421	152	15	29	8	25	238
		G0196		81.50	0.78	0.26	13.67	46	210	115	25	32	4	30	291
		G0009		82.00	0.78	0.33	13.25	23	267	198	31	40	6	27	241
		G0029		80.75	0.75	0.32	13.85	29	209	163	16	14	5	16	70
		G0188		82.00	0.86	0.33	14.54	27	277	139	21	35	5	17	190
		G0001		82.50	0.94	0.69	17.33	29	275	110	20	32	7	30	241
		G0055		82.00	0.68	0.28	13.99	53	366	162	16	43	11	37	484
		G0240		81.00	0.99	0.92	13.77	12	265	255	35	53	7	26	675
		G0008		79.75	0.68	0.24	12.53	23	310	159	23	41	8	23	296
		G0105		80.75	0.87	0.59	16.92	22	186	154	22	38	8	25	297
		G0239		77.75	0.98	0.71	18.82	9	223	146	11	25	8	29	196
		G0072		81.00	0.90	0.59	19.33	15	274	231	27	42	7	23	301
		G0003		82.00	0.80	0.48	19.77	75	270	180	39	45	11	36	497
		G0047		81.00	0.96	0.63	15.97	26	286	153	25	29	7	28	187
		G0095		81.75	0.91	0.66	14.80	36	229	141	16	45	5	13	219
		G0134		81.00	0.66	0.15	12.80	18	282	165	28	41	13	51	528
		G0046		82.50	0.78	0.55	15.10	65	245	174	38	53	14	40	736
Lula - subgroup A		G0079		83.50	0.91	0.49	12.04	128	297	154	25	33	8	32	271
		G0039		81.75	0.65	0.11	12.88	25	333	171	22	37	11	42	404
		G0010		82.75	0.77	0.35	15.80	21	329	193	30	42	17	52	738
		G0220		81.50	0.46	0.32	16.33	16	278	169	35	49	8	37	411
		G0102		79.00	0.57	0.06	14.91	24	293	237	46	70	9	29	647
		G0214		80.00	0.70	0.19	16.58	24	264	165	38	42	10	22	438
		G0086		81.25	0.84	0.23	14.40	71	229	227	54	64	13	32	816
		G0222		84.00	0.78	0.37	14.11	37	358	253	46	56	7	22	409
		G0073		84.75	0.24	0.04	10.40	135	210	164	45	45	12	34	530
		G0213		77.75	0.77	0.18	19.10	97	286	135	20	25	4	17	191
subgroup A		G0212		80.50	0.68	0.19	12.52	100	277	223	63	53	14	29	734
		G0043		79.25	0.53	0.04	13.20	61	276	172	35	43	12	47	539
		G0081		83.75	0.34	0.10	15.30	20	222	175	37	45	12	28	537
		G0087		78.25	0.57	0.08	13.73	109	201	189	23	47	9	24	404

Figure 5: Agronomic and quality-relevant traits of 70 genotypes from the INERA Coffee Collection, DRC: Total cupping score (Bollen et al., 2024); fraction retained by screen 15 and 17; 100 green beans weight (BW); number of peaberries; coffee tree height and width (cm), number of fructifying branches, number of fructifying nodes, average cherries per node, maximum cherries per node and total cherries from six measured branches. Genetic structure showing three defined subpopulations: orange (Wild), blue (Congolese subgroup A), and red (‘Lula’ cultivars) (Verleysen et al., 2023).

4 DISCUSSION

Phenotyping the genetic diversity of *C. canephora* germplasm collections, especially in the geographical origins of the species, is an important first step in discovering promising genetic material for developing new coffee varieties. Our exploration of the morphological diversity and agronomic potential of the understudied *C. canephora* genetic resources from the DRC revealed a substantial variation in morphological traits, as hypothesized. The yield traits exhibited the most variation, while the coffee cherry and seed dimensions exhibited the least variation. The variation in morphological traits was not consistent with the genetic background, and no morphological traits were found to classify and predict the genetic background of the genotype accurately. However, a substantial portion of morphological variation was not captured by the PCA model, and the low classification accuracy of our models limits our statistical inferences. The variation in agronomic and quality-relevant traits was substantial, with the ‘Lula’, ‘Lula’ – Wild hybrid, and ‘Lula’ – subgroup A hybrid material containing promising genotypes for further evaluation.

Regarding the morphological diversity of the genotypes, substantial variation in all traits was found, especially in the yield traits. Akperterey et al. (2019) studied 71 genotypes from germplasm in Ghana and reported similar coefficients of variation for the leaf and coffee cherry dimensions. Similarly, they reported a higher variation in tree architecture and branch traits compared to the coffee leaf and cherry traits. Previous research on Conilon varieties (Congolese subgroup A) reported less variation for anther and corolla tube length compared to our study but similar positive correlations between petal, anther, and corolla tube lengths (Da Silva et al., 2021). We found a substantial range in SLA and stomatal density across the studied genotypes but this variation was not consistent with the genetic background of the respective genotypes. Dubberstein et al. (2021) also reported a wide morphological diversity in stomatal density for Conilon genotypes. In our study, no significant correlation was found between SLA and coffee cherry production or between stomatal density and coffee cherry production. In comparison, previous research reported a correlation between specific leaf area and coffee plant yield (Gagliardi et al., 2015). In line with Pompelli et al. (2010) on *C. arabica* and also previously reported in other plant species (Sun et al., 2014; Xu; Zhou, 2008), we observed a negative correlation between SLA and stomatal density. The environmental plasticity of these two functional leaf traits likely has a genetic basis (De Kort et al., 2020) and plays an important role in the climate adaptation of food crops (Bertolino; Caine; Gray, 2019). Genotypes with a low SLA and stomatal density could have a fitness advantage under dry environmental conditions (Bertolino; Caine; Gray, 2019;

Poorter et al., 2009). This trait combination was found in the ‘Lula’ – Wild hybrids G0023 and G0067 (Bollen et al., 2025), which both, interestingly, also scored a high sensory quality, with G0067 exhibiting a large screen size (Figure 5). Clonal material of these genotypes should be evaluated in stress tolerance trials to evaluate the relationship between their leaf functional traits and drought tolerance.

Accurate prediction of the genetic background of the genotypes based on their morphological traits was not achieved. Cross-validation of the LDA model revealed a low prediction accuracy (43.33%) for the constructed linear discriminants. Both the LDA and random forest models exhibited high classification errors, and the sets of morphological traits identified as important predictors differed between the two models. These inconsistencies indicate a lack of robustness in trait-based classification of genetic background, likely due to a combination of methodological limitations and hybridization levels of the samples. Sampling was imbalanced across subpopulations, and genotypes showed varying degrees of hybridization, with no heterosis testing performed. The absence of environmental replicates further reduced the reliability of the results, while the non-normal distribution of trait variables impacted the LDA model. As a result, inferences about morphological predictors of genetic background must be made with caution. Future research should establish a more balanced and representative sampling of the subpopulations, supported by genetic identification, and include environmental replicates to undertake a more robust and comprehensive evaluation of morphological descriptors. Additionally, controlled crosses and heterosis testing could be undertaken to better understand the genetic basis of morphological variation and disentangle hybrid effects from intergroup differences. Lastly, evaluating pure, clonal material from the subpopulations in multi-environment trials would allow assessment of phenotypic plasticity of potentially identified morphological descriptors. Akperterey et al. (2019) reported high coefficients of variations for most studied traits in Robusta genotypes, with origins in Togo, Ghana, Cameroon, and the Ivory Coast and the grouping of the genotypes based on morphological traits was inconsistent with the genetic background. Similarly, Robusta genotypes from Uganda exhibited morphological variation but this was not predictive for their genetic background (Ngugi; Aluka, 2019). Advancements in molecular research have increased the efficiency and cost-effectiveness of genotyping plant material, making it easier to analyze the genetic composition of Robusta coffee’s genetic resources. However, classifying Robusta genetic material based on morphological traits still requires further evaluation, as it could provide a practical method for screening genetic resources for desirable traits. Additionally, access to molecular research is often limited in the regions where Robusta’s genetic resources are found.

A substantial variation in traits linked to agronomic performance was observed across the genotypes. If the green bean traits and sensory quality would be prioritized, promising material was found within the ‘Lula’ – Wild hybrid and ‘Lula’ material. Genotypes G0067 and G0035 from the ‘Lula’ – Wild and genotype G0077 from the ‘Lula’ class had a total sensory quality score of 83 points or higher and were characterized by large coffee beans (>50% retained by screen 17), a high 100 bean weight, and a moderate peaberry rate. The relatively large coffee bean size of the ‘Lula’ – Wild material, combined with their high sensory quality scores, indicates the potential of this hybrid material for further breeding efforts. These genotypes exhibited a tree height above three meters, an agronomic trait that is less desired but can be managed through adapting cultivation practices. As a comparison, screen sizes of Ugandan Robusta accessions averaged around screen 15, but with a high level of morphological and bean size variability (Ngugi; Aluka, 2019). Also genotype G0240 from the ‘Lula’ class is noteworthy, as it had the largest screen size distribution in the studied material, with good coffee production and sensory quality.

If agronomic production would be prioritized, promising material was found within the ‘Lula’ – subgroup A class. The PCA indicated that the ‘Lula’ – subgroup A hybrids were associated with higher coffee cherry production. More specifically, genotypes G0086 and G0010 had the highest total cherry production across two harvest years, with G0010 recording a maximum node of 52 coffee cherries. Genotype G0102 recorded the most fructifying nodes, which correlates with cumulative coffee production over time (Cilas et al., 2006). The relatively high number of fructifying nodes in these ‘Lula’ – subgroup hybrids is an interesting characteristic. Congolese subgroup A material has its geographical origin in Gabon, the Republic of the Congo, and western DRC, from which the ‘Petit-Kwilu’ variety and the Conilon material were derived. These coffee varieties exhibit high coffee production but small coffee bean dimensions, whereas the ‘Lula’ material is known for its larger green bean size. Genotype G0087 (subgroup A) originated from the Luki region of western DRC and was labeled as ‘Petit-Kwilu’ material, exhibiting a small coffee bean size. Hybridization between the Congolese subgroup A and ‘Lula’ material could result in coffees with a high productivity and larger bean size, as was suggested by genotypes G0222, G0220, and G0010. On the other hand, genotype G0073 from this hybrid class scored exceptionally high in Total score and performed well in agronomic production but lagged in coffee bean size. These results indicate a promising starting point for future breeding activities between these Congolese subgroup A and ‘Lula’ material. Inter-varietal hybridization between Congolese subgroup A (‘Petit-Kwilu’) and subgroup BE (‘Lula’) for the development of drought-resistant varieties with large beans was initiated at INERA (Montagnon et al., 1998)

but it was never completed. Our ‘Lula’ – subgroup A hybrids are possibly the descendants of these initial inter-varietal crossing experiments. The direct parents of these hybrids are not known, so the level of heterosis could not be evaluated. Our results indicate that specific genotypes from ‘Lula’ – subgroup A class exhibited large bean sizes and performed well on sensory quality. It would be interesting to evaluate their performance in dry environments as a considerable range in SLA and stomatal density was reported in this hybrid class (Bollen et al., 2025). The discovered genotypes with promising morphological traits could be used as parent plants in breeding programs. Promising genotypes should next be propagated clonally and tested in multi-location field trials to validate our findings and reassess their agronomic potential.

In this context, no hybrid crossings between the Wild and Congolese subgroup A were found in the INERA Coffee Collection (Verleysen et al., 2023). Future research could undertake targeted crossings between these two genetic classes, as the genetic distance of the parental material correlates with phenotypic performance (Ferrão, da Fonseca, et al., 2024b). Previous research on hybrid crossings between Conilon (Congolese subgroup A) and a Robusta variety (Congolese subgroup E) reported better agronomic and yield performance, with a high expression of heterosis in the hybrid material (Alkimim et al., 2021; Carvalho et al., 2019; Teixeira et al., 2017). Similar hybrid vigor observations were made in the hybrid material of Ghanaian accessions (Akpertey et al., 2022) and Conilon varieties of Brazil (Ferrão, da Fonseca, et al., 2024b). These breeding experiments mainly focused on the already established genetic resources from the same origin groups. The ongoing introduction of local wild materials from the Yangambi rainforest into the INERA Coffee Collection will result in newly captured genetic diversity available for crossing experiments. These hybrids could potentially exhibit a desirable combination of traits.

The multiplication of accessions at the INERA Coffee Collection through seedlings and open pollination resulted in the hybridization of the initial genetic material into many new and unique genetic fingerprints (Verleysen et al., 2023). This resulted in a substantial variation in sensory quality, and promising sensory profiles within the different genetic classes of the collection were discovered (Bollen et al., 2024). Similar observations were made in terms of agronomic potential. Promising material was found within the ‘Lula’ – Wild hybrid, ‘Lula’, and ‘Lula’ – subgroup A hybrid material. Ngugi and Aluka (2019) reported similar observations in agronomic traits from crossings between cultivars and local wild material in Ugandan germplasm. The Wild and Congolese subgroup A material did not perform well, but their sample sizes were too small to draw solid conclusions. A more comprehensive screening of the INERA Coffee Collection is advised in this context, as only 70 of the 263 unique genetic identities reported

in Verleysen et al. (2023) were studied. Furthermore, the initial genetic analysis included only a fraction of the collection's total genetic resources, leaving many opportunities for further exploration. Additionally, the continuous introduction of accessions from plantations and wild populations expands the available genetic resources for phenotypic screening (Verleysen et al., 2024).

Future research should prioritize the design of comprehensive field trials to further evaluate the promising Robusta coffee material identified in this study. To better capture genotype–environment interactions, these trials should be implemented across multiple locations with varying environmental conditions. Our findings underscore the agronomic potential of underutilized Robusta coffee genetic resources from the Democratic Republic of the Congo and reinforce the need for their conservation and systematic evaluation.

5 CONCLUSION

Significant variation in morphological traits was found, with yield traits showing the most and seed dimensions the least variation. Given the sampling limitations, no trait combination accurately predicted genetic background. Desirable green bean traits were identified in both 'Lula' and 'Lula'–Wild hybrids, while promising yield traits were observed in 'Lula'–Congolesse subgroup A hybrids. Genotypes with low SLA and stomatal density, potentially advantageous in dry environments, were found in the 'Lula'– Wild class, warranting further investigation.

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7 AUTHOR CONTRIBUTIONS

Conceptual idea: Bollen, R.; Wyffels, F.; Vandeloos, F.; Stoffelen, P. Methodology design: Bollen, R.; Wyffels, F.; Vandeloos, F.; Honnay, O.; Stoffelen, P. Data collection:

Bollen, R.; Kambale, J.-L.; Tas, A.-S.; Katshela, B.N.; Tshimi, E.A. Data analysis and interpretation: Bollen, R.; Kambale, J.-L.; Tas, A.-S.; Katshela, B.N.; Wyffels, F.; Vandeloos, F.; Honnay, O.; Stoffelen, P. Writing and editing: Bollen, R.; Tas, A.-S.; Wyffels, F.; Vandeloos, F.; Honnay, O.; Stoffelen, P.

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