

Associations among agro-morphological, physiological, and leaf and pod biochemical traits in *Corchorusolitorius* L.

Abstract

Understanding relationships among agro-morphological, physiological, and biochemical traits is important for *Corchorusolitorius* L. characterization and improvement. This study investigated trait associations among 13 genotypes, focusing on leaf and pod biochemical characteristics. Thirteen genotypes were evaluated in a randomized complete block design with two replications during the 2025–2026 dry season in Lomé, Togo. Agro-morphological traits, relative leaf chlorophyll content, and leaf and pod biochemical characteristics were assessed. Spearman's correlations were calculated using genotype means, with Benjamini–Hochberg correction at a 5% false discovery rate. Relative leaf chlorophyll content was negatively correlated with leaf width ($\rho = -0.791$, adjusted $P = 0.019$). Leaf moisture was positively associated with ash content ($\rho = 0.835$, adjusted $P = 0.004$). Pod moisture was negatively correlated with high heating value ($\rho = -0.811$, adjusted $P = 0.008$), whereas pod crude fibre showed a positive association ($\rho = 0.804$, adjusted $P = 0.008$). No cross-group correlations remained significant after correction. Significant associations were mainly observed within individual trait groups, highlighting distinct patterns of morphological, physiological, and biochemical variation. These findings reveal significant associations within morphological, physiological, and organ-specific biochemical traits, while highlighting the limited evidence for relationships between morphological and biochemical characteristics.

Keywords: *Corchorusolitorius*; agro-morphological traits; biochemical composition; chlorophyll content; Spearman correlation.

Introduction

Corchorusolitorius L. (*C. olitorius*), from the Malvaceae family, is a leafy vegetable cultivated in tropical and subtropical regions for its nutritional and multiple-use properties. Its leaves contain dietary fibre, minerals, vitamins, lipids, and bioactive compounds (Abdel-Razek *et al.*, 2022; Bonnot *et al.*, 2025). Beyond its use as a vegetable, the species provides fibre and other plant materials with potential industrial applications (Satya *et al.*, 2014; Islam *et al.*, 2017). Understanding the relationships among agronomic, physiological, and biochemical characteristics is therefore relevant to its characterization and improvement.

Previous studies have documented considerable agro-morphological diversity in *C. olitorius*. Adebo *et al.* (2015) identified variation in plant height, branching, leaf dimensions, and flowering characteristics among cultivars from Benin. Youssef *et al.* (2019) reported morphophysiological and molecular diversity among Egyptian landraces. Similarly, Adeyemo *et al.* (2021) demonstrated variation in morphological traits among African cultivars, while AlJuhani and Aljohani (2024) documented differences in vegetative and reproductive characteristics among germplasm from different geographical origins. Other

studies have reported variation in growth and yield-related traits and their associations in *Corchorus* germplasm (Adenijiet *al.*, 2022; Satya *et al.*, 2014; Dubeet *al.*, 2019). These studies provide useful information for germplasm characterization, although they primarily emphasize morphological and genetic diversity.

Biochemical investigations have also contributed to understanding the composition of *C. olitorius*. Abdel-Razek *et al.* (2022) reviewed its nutritional constituents and phytochemical diversity, while Bonnotet *al.* (2025) characterized the chemical and nutritional composition of its leaves. Other investigations have documented variation in proximate composition and bioactive constituents (Ndlovu and Afolayan, 2008; Choudhary *et al.*, 2013). Moisture, ash, crude fibre, and crude lipid contents provide complementary information on plant composition, while high heating value reflects the energy released during combustion. Evaluating these characteristics in both leaves and pods may reveal relationships within and between plant organs.

Correlation analysis provides a means of examining the direction and strength of relationships among quantitative traits. In crop improvement, these associations can help identify traits that vary together and generate hypotheses about their biological relationships (Falconer and Mackay, 1996; Dewey and Lu, 1959). Studies on *Corchorus* have also demonstrated the usefulness of trait associations for understanding morphological variation and yield-related characteristics (Dubeet *al.*, 2019; Adenijiet *al.*, 2022). However, correlations do not necessarily indicate causal relationships or establish reliable criteria for indirect selection. Their interpretation requires consideration of experimental design and statistical uncertainty.

Although previous investigations have characterized morphological diversity and leaf biochemical composition in *C. olitorius*, integrated information on relationships among agro-morphological, physiological, and leaf and pod biochemical traits remains limited. In particular, associations between plant architecture, relative chlorophyll content, and biochemical composition across plant organs require further investigation. Therefore, this study examined trait associations in 13 *C. olitorius* genotypes. Specifically, it aimed to assess relationships (i) among agro-morphological and physiological traits, (ii) among biochemical characteristics within leaves and pods, (iii) between agro-morphological and biochemical traits, and (iv) between leaf and pod biochemical characteristics. The findings provide an exploratory basis for understanding trait relationships and informing future crop improvement research.

Material and Methods

- Study site, plant materials, and experimental design

The experiment was conducted at the Station d'Expérimentation Agricole de Lomé (SEAL-UL), University of Lomé, Togo. The trial was carried out during the 2026 dry season, from December 2025 to March 2026. Thirteen genotypes of *C. olitorius* were evaluated in a randomized complete block design (RCBD) with three replications. Each experimental plot consisted of 15 plants. Five plants per plot were randomly selected for data collection.

- Agro-morphological and physiological measurements

Six agro-morphological and physiological traits were evaluated: plant height, stem diameter, number of primary branches, leaf length, leaf width, and relative leaf chlorophyll content. Measurements followed standard procedures for morphological characterization (Adeboet *et al.*, 2015; Youssef *et al.*, 2019; Adeyemo *et al.*, 2021).

Plant height was measured from the soil surface to the apex of the main stem, Stem diameter was measured at the base of the stem using a digital calliper, Primary branches were counted directly, Leaf length was measured from the base to the tip of the lamina, and leaf width was recorded at its widest point. Relative leaf chlorophyll content was estimated using a portable chlorophyll meter (SPAD-502 Plus, Konica Minolta, Japan). Measurements were taken on healthy, fully expanded leaves and expressed as SPAD index values (Uddling *et al.*, 2007).

- Biochemical analysis

Five biochemical characteristics were evaluated separately in leaves and pods, including moisture content, ash content, crude fibre, crude lipid, and high heating value (HHV). Leaf and pod samples were collected from five representative plants per genotype in each replication. Samples from the five plants within each plot were combined to obtain one composite sample per experimental unit. Fresh leaf and pod samples were first subsampled for moisture determination (3 g per sample). The remaining material was dried at 60 °C until a constant mass was obtained. Dried samples were subsequently milled, sieved through a 1-mm mesh, and kept in sealed containers under dry conditions until biochemical evaluation. Leaves were harvested at the vegetative stage, while pods were collected 10 days after flowering.

Biochemical constituents were quantified using established AOAC methods (AOAC International, 2019). Moisture was assessed through mass loss during drying (AOAC 930.04), while ash was quantified following furnace incineration (AOAC 930.05). Crude fibre was determined by successive acid and alkaline treatments (AOAC 930.10), and crude lipid was extracted using an organic solvent (AOAC 930.09). Except for moisture, which was expressed on a fresh-weight basis, biochemical results were reported relative to dry matter. The gross energy content of leaf and pod materials was assessed by oxygen bomb calorimetry in accordance with ISO 18125:2017. Approximately 1 g of dried sample was subjected to complete combustion in a pressurized oxygen chamber. The resulting temperature increase was used to determine high heating value (HHV), expressed in MJ kg⁻¹ of dry matter.

- Statistical analysis

Agro-morphological, physiological, and biochemical data were used to investigate trait associations. Mean values were calculated for each genotype before correlation analysis. Spearman's rank correlation coefficients (ρ) were computed to assess relationships among traits.

Correlations were examined within and between agro-morphological and physiological traits, leaf biochemical traits, and pod biochemical traits. Statistical significance was evaluated using

the Benjamini–Hochberg procedure to control the false discovery rate at 5% (Benjamini and Hochberg, 1995).

Five correlation heatmaps were generated to illustrate the direction and magnitude of trait associations: (i) among agro-morphological and physiological traits, (ii) among leaf biochemical traits, (iii) among pod biochemical traits, (iv) between agro-morphological and biochemical traits, and (v) between leaf and pod biochemical traits. Positive and negative correlations were distinguished using a colour gradient. Statistically significant associations were identified using adjusted P-values below 0.05. The Benjamini–Hochberg correction was applied separately within the five predefined groups of correlation tests corresponding to the five heatmaps. All statistical analyses were performed using R version 4.5.3.

Results

- Correlations among agro-morphological and physiological traits

Spearman's correlation analysis revealed both positive and negative associations among agro-morphological and physiological traits (Figure 1). A strong negative correlation was observed between relative leaf chlorophyll content and leaf width ($\rho = -0.791$, adjusted $P = 0.019$). This was the only association that remained statistically significant after Benjamini–Hochberg correction. Relative chlorophyll content was also negatively correlated with the number of primary branches ($\rho = -0.592$), although this association was not significant after correction (adjusted $P = 0.165$). The remaining correlations were not statistically significant (adjusted $P > 0.05$).

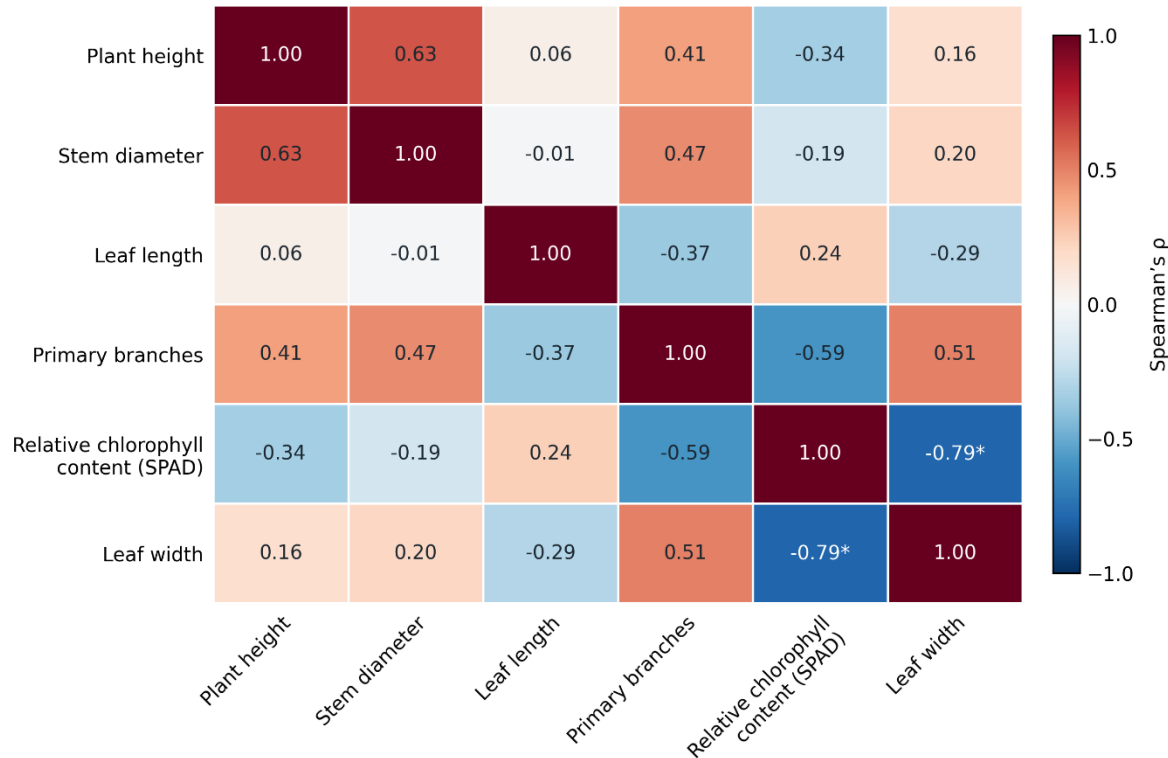


Figure1. Spearman correlation heatmap of agro-morphological and physiological traits in *Corchorus olitorius* genotypes. ρ : correlation coefficients ranging from -1 to $+1$; *: indicate

associations significant after Benjamini–Hochberg (BH) correction (adjusted $P < 0.05$).

- Correlations among leaf and pod biochemical traits

Correlation patterns differed between leaf and pod biochemical characteristics (Figures 2 and 3). In leaves, moisture content showed a strong positive correlation with ash content ($\rho = 0.835$, adjusted $P = 0.004$). This was the only significant association among the five leaf biochemical traits.

In pods, two significant correlations were identified. Moisture content was strongly and negatively correlated with high heating value ($\rho = -0.811$, adjusted $P = 0.008$). Conversely, crude fibre content was positively correlated with high heating value ($\rho = 0.804$, adjusted $P = 0.008$). No other associations among pod biochemical traits remained significant after correction.

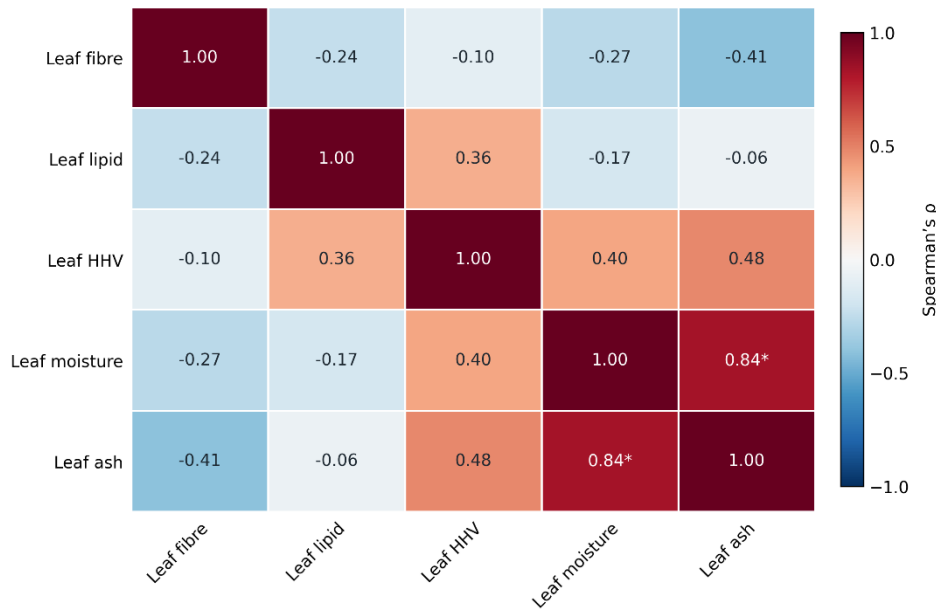


Figure2. Spearman correlation heatmap of leaf biochemical traits in 13 *Corchorusolitorius* genotypes. ρ : correlation coefficients ranging from -1 to $+1$; *: indicate associations significant after Benjamini–Hochberg (BH) correction (adjusted $P < 0.05$).

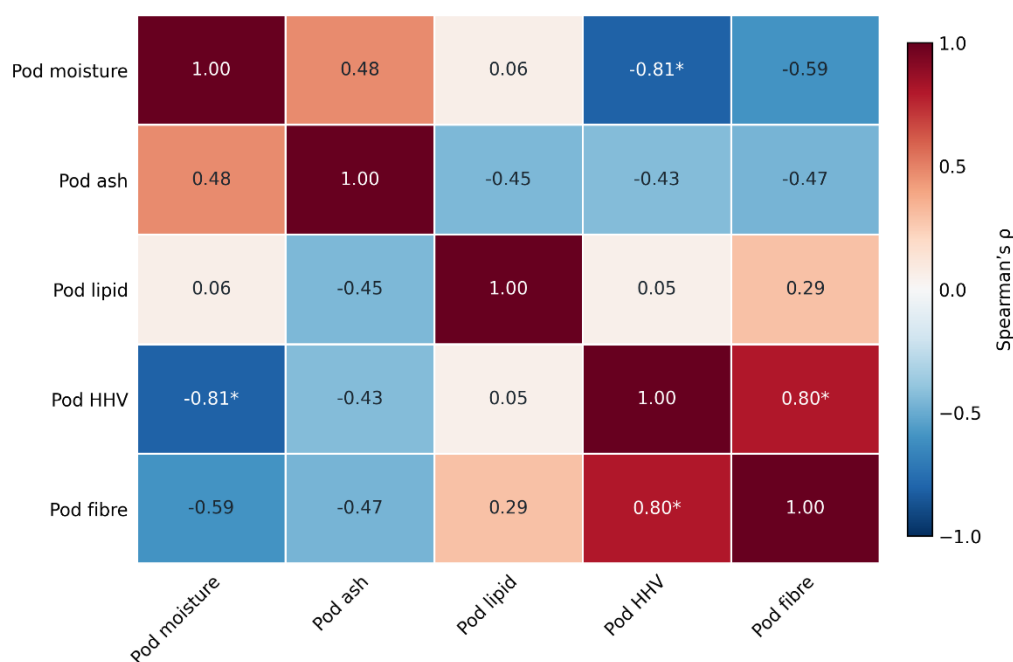


Figure 3. Spearman correlation heatmap of pod biochemical traits in 12 *Corchorusolitorius* genotypes. ρ : correlation coefficients ranging from -1 to $+1$; *: indicate associations significant after Benjamini–Hochberg (BH) correction (adjusted $P < 0.05$).

- Correlations between agro-morphological, physiological and biochemical traits

Correlations between agro-morphological, physiological and biochemical traits varied in direction and magnitude (Figure 4). Leaf width exhibited a strong negative correlation with pod moisture content ($\rho = -0.811$, adjusted $P = 0.082$) and a positive correlation with pod high heating value ($\rho = 0.706$, adjusted $P = 0.230$). Leaf length was negatively correlated with leaf ash content ($\rho = -0.670$, adjusted $P = 0.230$). However, none of the 60 cross-group correlations remained statistically significant after Benjamini–Hochberg correction (adjusted $P > 0.05$).

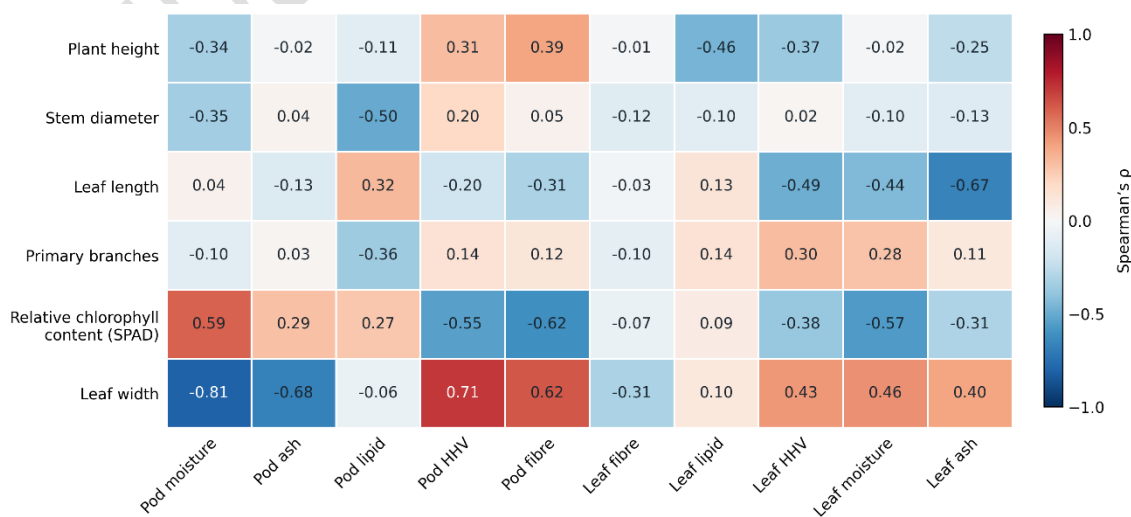


Figure 4. Spearman correlations between agro-morphological, physiological and biochemical traits in *Corchorusolitorius* genotypes. ρ : correlation coefficients ranging from -1 to $+1$; *: indicate associations significant after Benjamini–Hochberg (BH) correction (adjusted $P < 0.05$).

- Correlations between leaf and pod biochemical traits

Correlations between leaf and pod biochemical characteristics are presented in Figure 5. The strongest observed association was a negative correlation between leaf crude lipid and pod crude fibre content ($\rho = -0.683$). However, this association was not statistically significant after correction (adjusted $P = 0.359$). None of the 25 correlations between leaf and pod biochemical traits reached the adjusted significance threshold of 0.05.

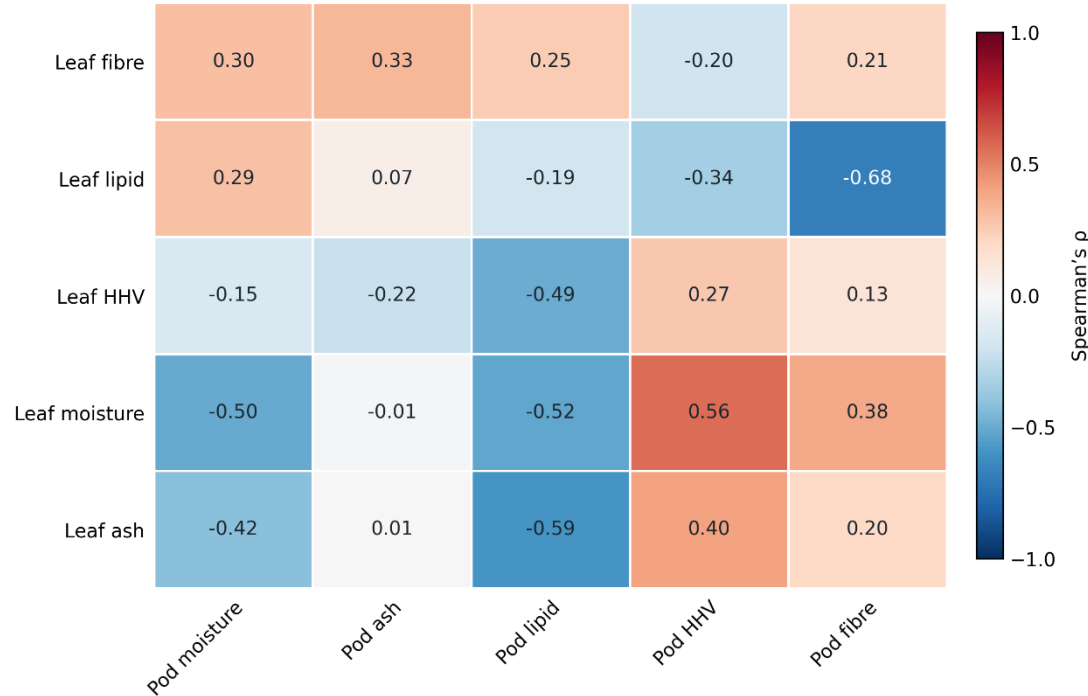


Figure 5. Spearman correlations between leaf and pod biochemical traits in *Corchorusolitorius* genotypes. ρ : correlation coefficients ranging from -1 to $+1$; *: indicate associations significant after Benjamini–Hochberg (BH) correction (adjusted $P < 0.05$).

Discussion

The present study identified distinct patterns of association among agro-morphological, physiological, and biochemical traits in *C. olitorius*, with significant relationships primarily occurring within individual trait groups. Previous studies have documented considerable morphological and physiological diversity in *C. olitorius* germplasm (Youssef *et al.*, 2019; Adeyemo *et al.*, 2021; AlJuhani and Aljohani, 2024). The present findings extend this

knowledge by examining relationships among morphological characteristics, relative chlorophyll content, and leaf and pod biochemical composition. The strong negative correlation between relative leaf chlorophyll content and leaf width ($\rho = -0.791$) indicates that genotypes with wider leaves generally exhibited lower SPAD readings. This association may reflect differences in leaf anatomy, development, or chlorophyll distribution. Uddling *et al.* (2007) demonstrated that SPAD readings are related to leaf chlorophyll concentration but are influenced by leaf structural characteristics. The observed relationship therefore highlights the importance of considering leaf morphology when interpreting chlorophyll-related variation among genotypes. Leaf moisture and ash content were strongly positively correlated ($\rho = 0.835$), indicating that genotypes with higher leaf moisture tended to exhibit greater inorganic residue concentrations on a dry-matter basis. This relationship may reflect differences in mineral accumulation and plant water relations, although individual mineral concentrations and osmotic regulation were not directly assessed (Marschner, 2012). Previous studies have highlighted the mineral richness of *C. olitorius* leaves (Abdel-Razek *et al.*, 2022; Bonnot *et al.*, 2025). The present finding provides additional insight into the relationship between leaf water content and inorganic constituents across genotypes. Pod moisture was negatively correlated with high heating value ($\rho = -0.811$), whereas crude fibre showed a positive association with HHV ($\rho = 0.804$). Since HHV was expressed on a dry-matter basis, these relationships may reflect differences in pod chemical composition rather than the direct influence of water during combustion. Biomass heating value depends on structural carbohydrates, lignin, extractives, and inorganic constituents (Demirbaş, 2001; Vassilev *et al.*, 2010; Esteves *et al.*, 2023). These associations suggest that variation in pod biochemical composition may accompany differences in gross energy content, although, crude fibre alone does not fully characterize the structural components influencing biomass heating value. No correlations between agro-morphological and biochemical traits remained significant after multiple-testing correction, although a relatively strong negative association was observed between leaf width and pod moisture ($\rho = -0.811$). Similarly, leaf crude lipid and pod crude fibre showed a negative correlation ($\rho = -0.683$), but this association was not statistically significant after correction.

These relationships should be regarded as exploratory and require confirmation in larger genotype collections. Such patterns may reflect differences in tissue function, developmental stage, and resource allocation (Taiz *et al.*, 2015). From a crop improvement perspective, the identified relationships provide useful information for trait characterization in *C. olitorius*. The associations involving chlorophyll content, leaf biochemical composition, and pod energy characteristics may guide further evaluation of germplasm for nutritional and biomass-related applications. However, the absence of significant cross-group correlations indicates that morphological characteristics alone may not adequately represent biochemical quality. Although the study involved 13 genotypes evaluated during one growing season, it provides an initial understanding of trait associations in *C. olitorius*. The limited number of genotypes may have reduced statistical power, particularly for detecting associations between trait groups after correction for multiple comparisons. Further investigations involving larger germplasm collections and multiple environments would help establish the consistency of these relationships. Overall, the findings highlight the importance of integrating agro-

morphological, physiological, and leaf and pod biochemical characteristics in germplasm characterization and future crop improvement research.

Conclusion

This study identified significant relationships among agro-morphological, physiological, and biochemical traits in *C. olitorius*. Relative leaf chlorophyll content was negatively associated with leaf width, while leaf moisture showed a positive association with ash content. In pods, moisture was negatively correlated with high heating value, whereas crude fibre exhibited a positive association. These findings highlight distinct patterns of trait association and the relevance of pod biochemical characteristics for evaluating biomass properties. Although several relationships between morphological and biochemical traits were observed, none remained significant after multiple-testing correction. The results therefore emphasize the importance of combining morphological, physiological, and biochemical assessments for comprehensive germplasm characterization. Further evaluation across larger genotype collections and different environments will help establish the consistency of these associations and their potential applications in *C. olitorius* improvement programmes.

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