

Phenotypic Characterization of *Mucuna pruriens* L. Ecotypes Cultivated in the Tshilenge Territory (Kasai-Oriental, DRC)

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Abstract

The *Mucuna* genus is noted for its significant agronomic advantages, particularly regarding nitrogen fixation capabilities, substantial biomass generation, and its role as a cover crop. In the Democratic Republic of Congo (DRC), various ecotypes of *Mucuna pruriens* are traditionally cultivated; however, their phenotypic diversity has not been systematically studied. *This research seeks to detail the morphological and phenological variations among six ecotypes collected from different agroecological regions within the country and grown at the Ntanda site in Tshilenge Territory.* A randomized complete block design with three replicates was implemented in this study. Findings indicated highly significant variances ($p < 0.001$) among the ecotypes examined. Notably, ecotypes E3 and E4 were characterized by their height, excellent ground coverage, and early maturity. Conversely, ecotype E6 displayed poor performance and a stinging trait. Analyses through principal component analysis and hierarchical clustering revealed three distinct group structures.

Keywords: DRC, Ecotypes, Morphological Diversity, *Mucuna Pruriens*, Phenotypic Characterization, Tshilenge.

I. INTRODUCTION

The *Mucuna* genus (Fabaceae) includes approximately 100 to 150 species that thrive in tropical and subtropical climates. It is recognized for its ecological adaptability and capacity to fix atmospheric nitrogen symbiotically with *Rhizobium* bacteria (Pullaiah et al., 2021; Cherni, 2019; Peoples et al., 2021). Stefan (2002) recorded nitrogen fixation rates between 80 to 150 kg N/ha/year across five varieties of *Mucuna pruriens* in Cameroon—this translates to an equivalent of 200–300 kg urea per hectare (INERA, 2015).

Beyond nitrogen fixation benefits, *Mucuna pruriens* yields considerable biomass (25–35 t/ha fresh weight), enhancing both soil organic matter and structure (Ibouraïman et al., 2022; Koutika et al., 2001; Muyayabantu, 2019). Its rapid growth coupled with a creeping tendency enables it to achieve ground coverage rates between 80% to 95% within just 60–75 days post-sowing while suppressing weeds without herbicide application (Dissanayaka et al., 2024; Carsky et al., 2003; Caronni et al., 2024). Within the DRC context specifically concerning local varieties of *Mucuna pruriens*, systematic characterization has previously been absent—this study aims to bridge that gap by examining the phenotypic variability among six ecotypes collected from Tshilenge.

II. MATERIALS AND METHODS

➤ Study Area

The research was conducted at Ntanda site located in Tshilenge Territory within Kasai Oriental Province at an altitude of approximately 601 m on sandy-loamy ferrallitic soil under a humid tropical climate AW₃ (average annual rainfall: 1492 mm).

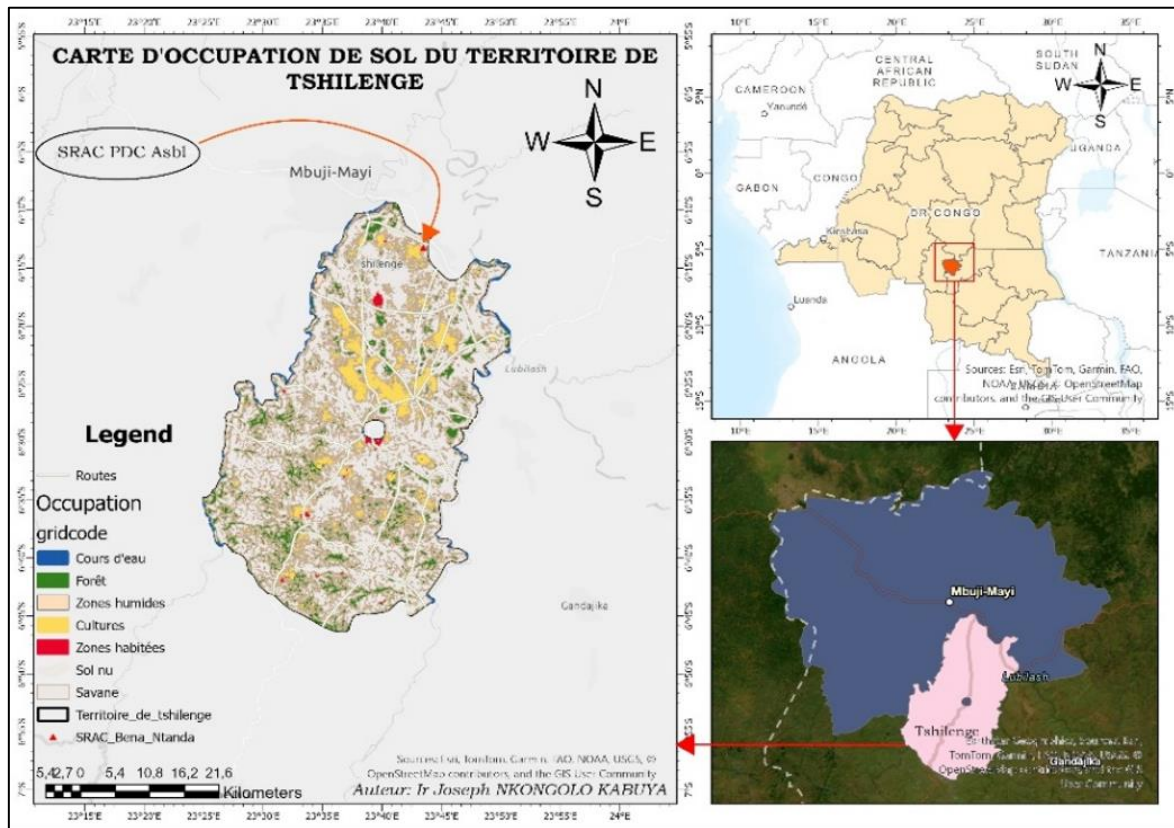


Fig 1 Study Area

➤ Plant Material and Measuring Instruments

Six ecotypes labeled E1 through E6 were harvested from regions including Tshilenge, Lomami, and Bas-Congo. Measurements taken included vine height using a measuring tape over a span of five meters; collar diameter measured with digital calipers accurate to ± 0.01 mm; canopy coverage assessed via point method on standardized quadrats sized at one square meter each; leaf area dimensions recorded with rulers; weight measurements conducted using electronic scales.

➤ Experimental Setup

Utilizing a completely randomized block design with three repetitions resulted in plots measuring twelve square meters each (4 m $\times$ 3 m) with plant spacing set at intervals of half a meter by one meter resulting in a total planting density of forty-eight plants per plot.

• Diagram of the Device

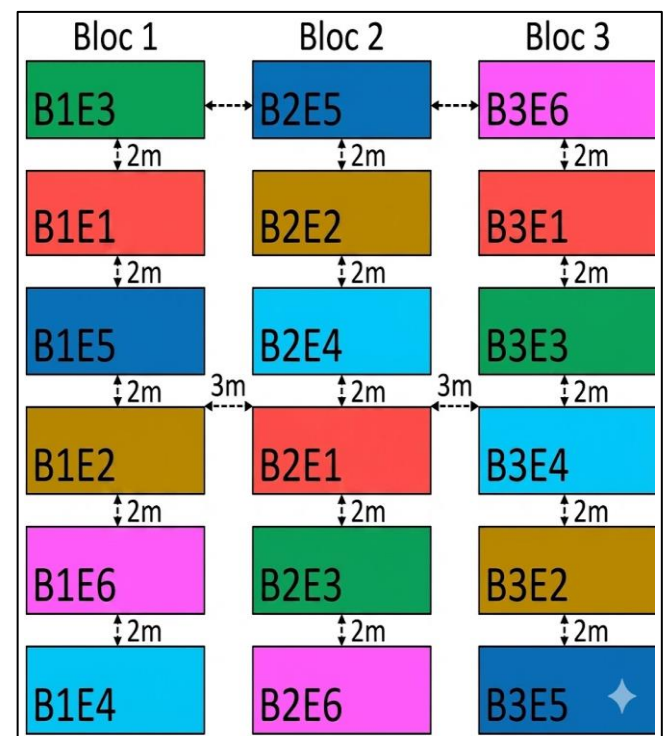


Fig 2 Diagram of the Device

➤ Measured Parameters

Key observations included vine height (m), stem diameter at base (mm), canopy cover (%) assessed at intervals after thirty days post-sowing through ninety days post-sowing (DAS); additional phenological aspects such as emergence rate timing alongside flowering initiation were monitored until reaching fifty percent flowering stage; leaf area calculated using $k = 0.70$ along with qualitative traits including flower color variations pod hairness specifics seed coloration features etc.

➤ Statistical Analyses

Statistical evaluations were performed utilizing R version 4.5.2 employing two-way ANOVA alongside Tukey's test set at $\alpha = 0.05$ thresholds wherein coefficients variation were calculated subsequently PCA hierarchical clustering methods along Pearson correlation matrix analyses were carried out.

III. RESULTS

➤ Quantitative Characteristics

Table 1 presents the means of quantitative traits by ecotype and the coefficients of variation. The differences between ecotypes are highly significant ($p < 0.001$).

Table 1 Effect of Ecotypes on Growth and Phenology

Ecotype	Height 60 JAS (m)	Diameter 90 JAS (mm)	Recovery 60 JAS (%)	Flowering start (JAS)	Lifting rate (%)	Leaf area (cm ²)
E1	0.96 c	12.6 ab	70.0 bc	125 c	90.0	441
E2	1.15 bc	14.0 ab	88.3 ab	121 b	93.3	671
E3	1.66 a	11.9 ab	93.3 a	110 a	96.7	368
E4	1.61 ab	15.3 a	86.7 ab	107 a	95.0	539
E5	1.79 a	12.1 ab	75.0 bc	115 b	91.7	401
E6	1.63 ab	7.09 b	50.0 c	169 d	76.7	169
CV (%)	31.0	31.4	24.8	18.0	12.7	36.7
p-value	< 0.001	< 0.001	< 0.001	< 0.001	—	0.002

In the column, means followed by the same letter do not differ significantly at the 5% threshold according to Tukey. CV = coefficient of variation; DAS = days after sowing.

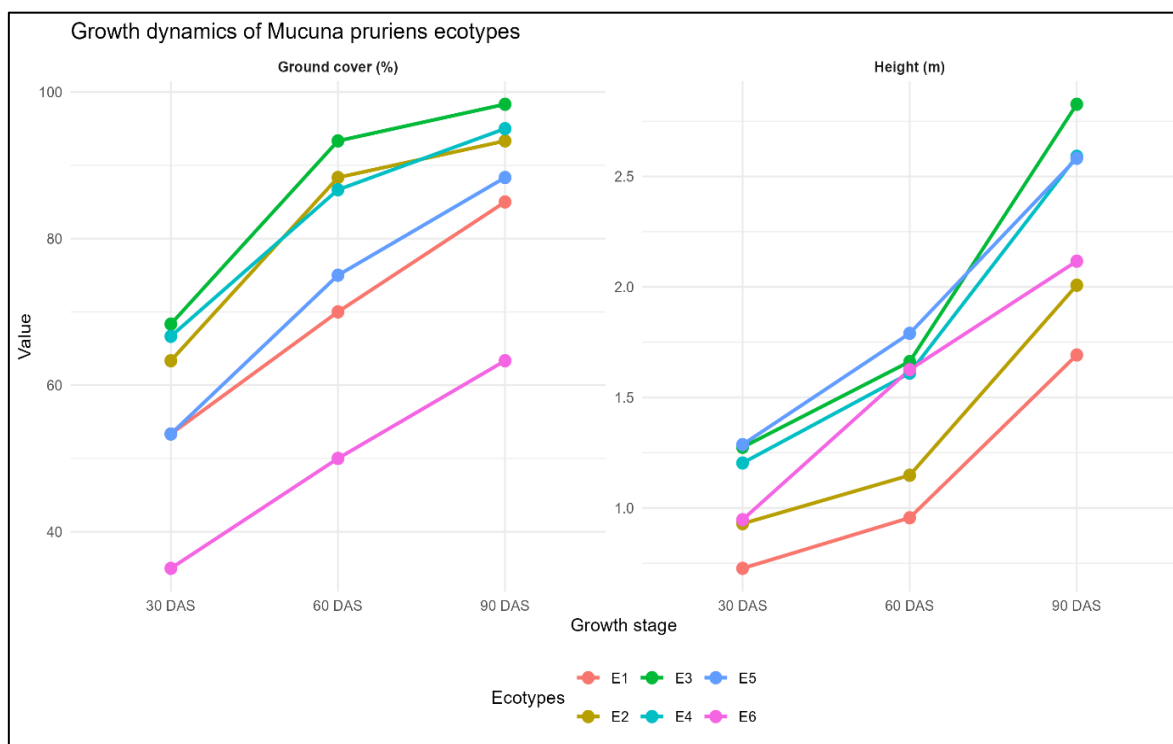


Fig 3 Growth Dynamics of Mucuna Pruriens Ecotypes

Figure 3 shows that the height increases rapidly between 30 and 60 JAS and then slows down. E3 and E5 exceed 2.50 m at 90 JAS. Coverage progresses steadily; E3 reaches 98% at 90 JAS, compared to 63% for E6.

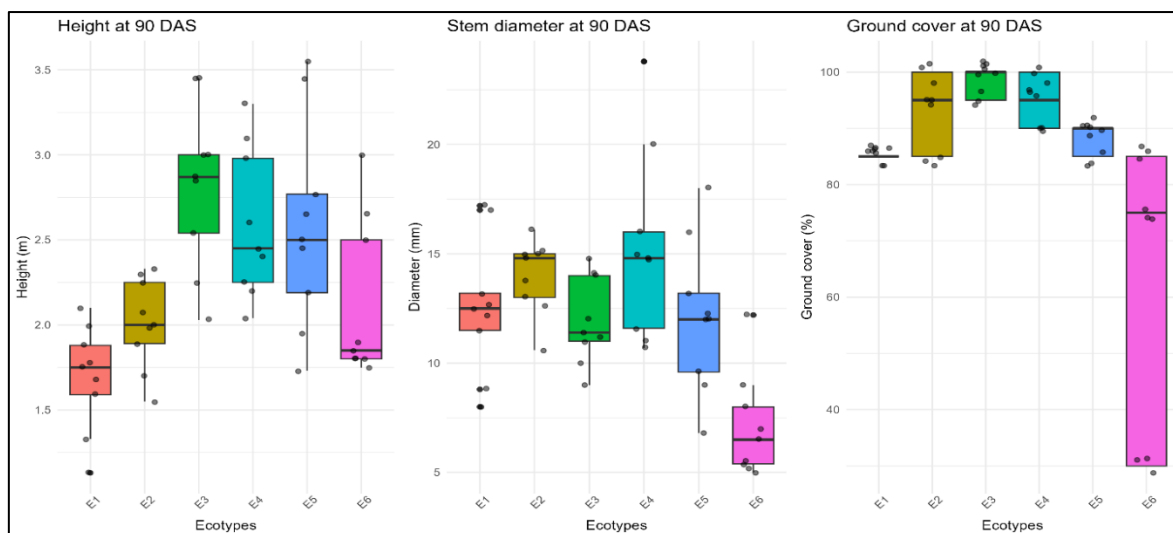


Fig 4 Boxplots at 90 DAS (Height, Stem diameter, Ground Cover)

Figure 2 shows that ecotypes E3 and E5 have the highest median heights. Ecotype E4 is distinguished by a median diameter of approximately 15 mm. Ecotype E6

exhibits the lowest values, with a diameter close to 7 mm. The median cover of ecotype E3 is close to 100%.

➤ Qualitative Characteristics

Table 2 Qualitative Characteristics of Ecotypes

Ecotype	Vigor	Flower color	Pod hair	Seed color	Eye color
E1	Average	Purple	Not very hairy	Black	Beige
E2	Average	Purple	Not very hairy	Speckled grey	Gray
E3	Good	Purple	Not very hairy	Light gray	Cream
E4	Good	White	Not very hairy	Marbled Grey	Cream
E5	Good	Purple	Not very hairy	Marbled brown	Brownish-grey
E6	Weak	Purple	Very hairy (stinging)	Dark brown	Gray

Table 2 demonstrates varying qualitative traits across evaluated samples noting predominant purple flowers appearing predominantly throughout five cases while uniquely distinguishing case four having white flowers along other specific characteristics like hairy stings

featured primarily within case six thereby rendering it less favorable agriculturally speaking overall assessment reveals moderate vigor levels too.

➤ Multivariate Analyses

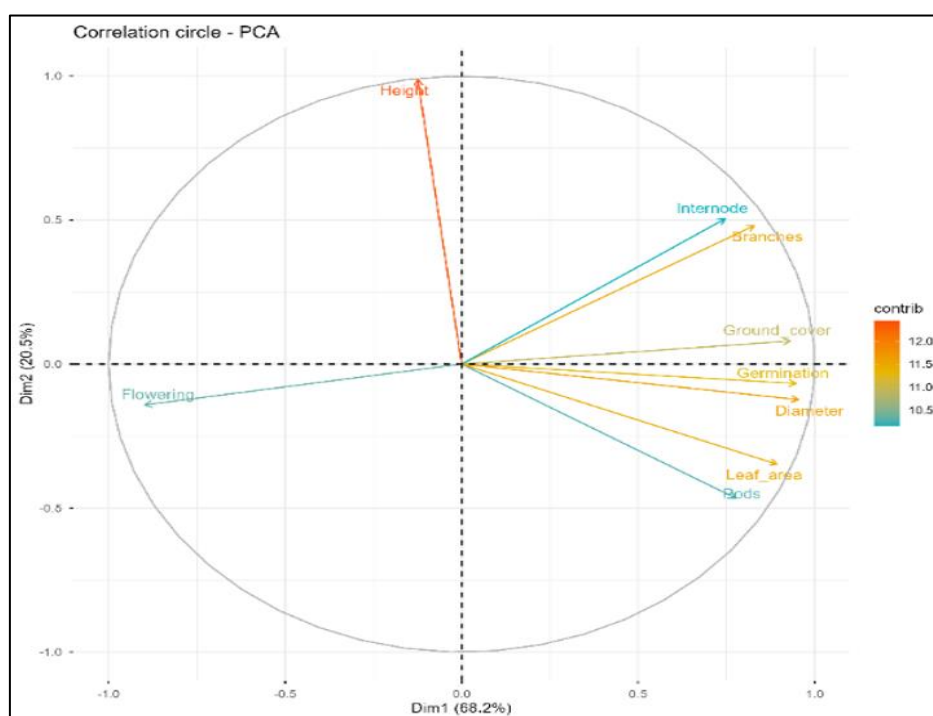


Fig 5 Correlation Circle, PCA

From Figure 5, height, canopy cover, leaf area, and number of branches are positively correlated. Earliness is the inverse: the earlier an ecotype matures, the better its height and canopy cover.

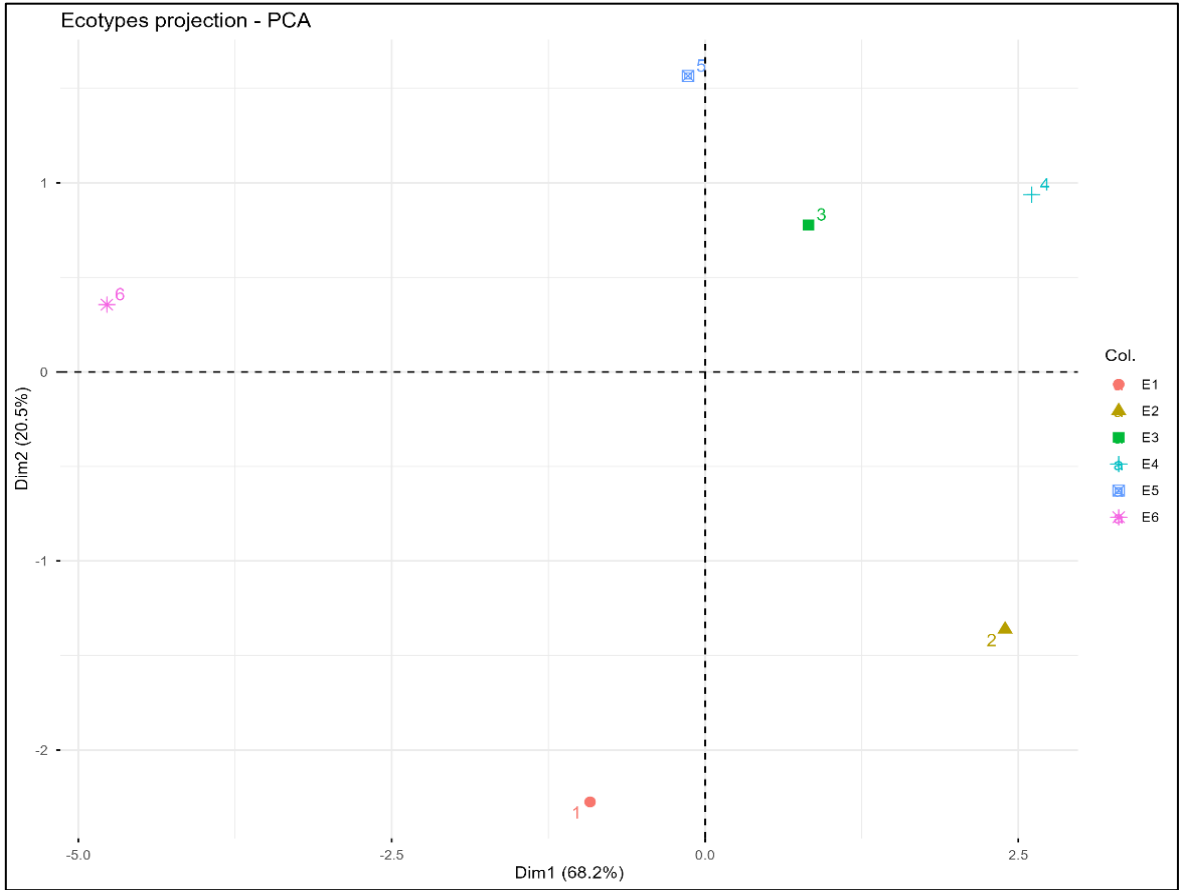


Fig 6 Ecotypes Projection, PCA

E3 and E4 are projected towards the high values. E6 is isolated on the opposite side. E1, E2 and E5 are intermediate.

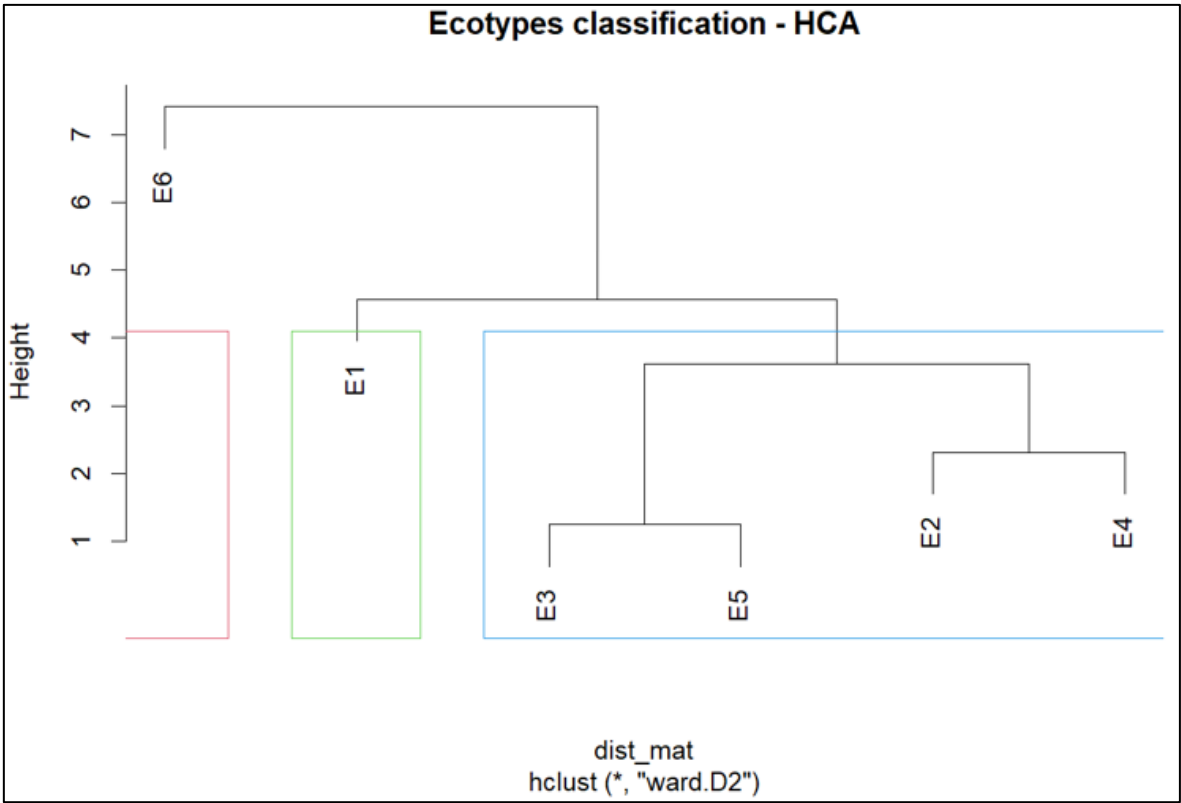


Fig 7 Ecotype Classification – HCA (Dendrogram)

Three groups are distinguished: G1 high-performance (E3, E5), G2 intermediate (E1, E2, E4), and G3 low-performance (E6). E4 is in the intermediate group despite its good diameter because its coverage is less effective than E3.

➤ Correlation Matrix

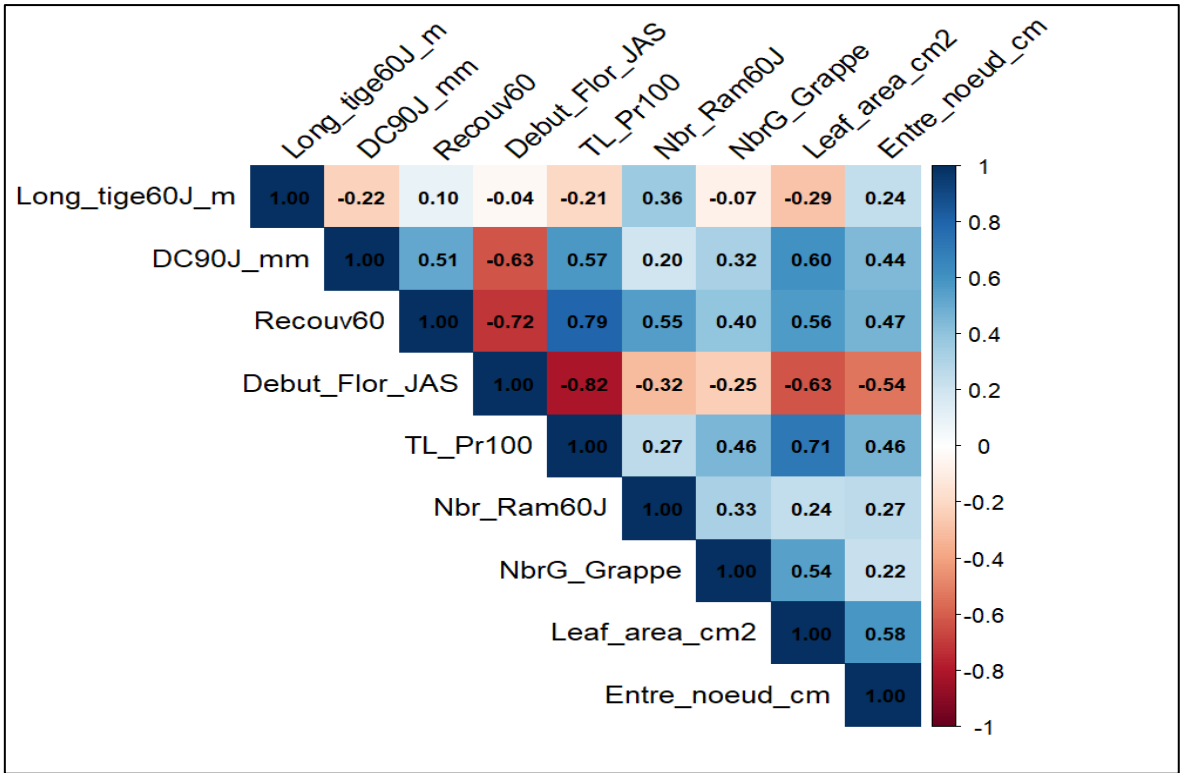


Fig 8 Correlation Matrix

Strong positive correlation between canopy cover and branches ($r = +0.82$). Strong negative correlation between earliness and canopy cover ($r = -0.79$) and between earliness and height ($r = -0.71$). Moderate positive correlation between leaf area and number of pods per bunch ($r = +0.58$).

IV. DISCUSSION

The results show significant phenotypic diversity among the six ecotypes. The differences are highly significant for all quantitative traits ($p < 0.001$). This confirms substantial variability between ecotypes from different agroecological zones.

Sathyanarayana et al. (2016) reported a diverse gene pool in India. Leelambika et al. (2010) observed wide variability in inflorescence, pod, and seed traits ($P < 0.0001$). Variability was lower for foliar traits among 18 Indian accessions of *Mucuna*. The authors reported an average similarity of 50.6 % between accessions of *M. pruriens* (Jaccard index). Diversity was greater for var. *pruriens* (SI 54 %) than for var. *utilis* (SI 71 %).

Lepcha et al. (2024) characterized 70 accessions over two years. They found significant phenotypic differences ($P < 0.05$). They also noted genetic diversity with a Shannon index of 0.62. Fifteen marker-trait associations (MTAs) were identified for nine agronomic traits. These include pod length, number of pods per plant, inflorescence length, and L-DOPA content.

Patel et al. (2025) evaluated 59 accessions. L-DOPA content ranged from 1.79% to 6.05%. A negative correlation existed between 100-seed weight and L-DOPA ($r = -0.614$). Sathyanarayana et al. (2012) analyzed 30 traits on 40 Indian accessions (31 wild, 9 cultivated). The first two principal components explained 65.18 % of the total variation. Morphological diversity was higher between wild and cultivated accessions (63.05 %) than within cultivated accessions (36.95 %).

These results corroborate our observations. The differences between our six ecotypes are also highly significant. The structure of diversity is clear.

Ecotypes E3 and E4 are distinguished by their tall stature. They also exhibit excellent ground cover. These values are comparable to those reported by Dissanayaka et al. (2024), Amani et al. (2025), and Balogoun et al. (2022). This rapid ground cover makes them ideal candidates. They can control invasive weeds such as *Imperata cylindrica*, a species prevalent in Kasai-Oriental (Muyayabantu, 2017). Their use could reduce the need for herbicides and manual weeding. Blomme et al. (2022) also demonstrated the potential of *Mucuna pruriens* in banana-based systems in eastern DRC.

The earliness of ecotypes E4 and E3 is a major advantage. Rainfall is limited in the region. This earliness allows *Mucuna* to be integrated into tight crop rotations. Amani et al. (2025) highlighted this advantage over two consecutive growing seasons. According to Dissanayaka

et al. (2024), the *Mucuna* development cycle varies from 120 to 330 days. Our early ecotypes fall within the lower end of this range. This makes them more likely to be adopted by farmers. The FAO (2022) recognizes conservation agriculture with cover crops like *Mucuna* as a sustainable practice for tropical regions.

The correlation matrix (Figure 6) reveals some interesting relationships. A strong positive correlation exists between ground cover and the number of branches ($r = +0.82$). Branched ecotypes therefore provide better ground cover. The negative correlation between earliness and ground cover ($r = -0.79$) is also informative. Early-maturing ecotypes are not less vigorous. On the contrary, they are taller and provide better ground cover.

Lepcha *et al.* (2024) identified marker-trait associations (MTAs). These MTAs relate to petiole length, number of pods per plant, inflorescence length, and number of days at 50% flowering. These traits are linked and can be jointly improved. Patel *et al.* (2025) observed significant correlations between seed traits. In particular, they found a negative correlation between 100-seed weight and L-DOPA ($r = -0.614$). In our study, the favorable association between earliness and vigor is rare in legumes. It opens up avenues for varietal selection.

Principal component analysis (Figures 4 and 5) structures the diversity into three groups. Hierarchical clustering (Figure 6) confirms this structure. The first dimension of the PCA (71.2% of the variance) is associated with vegetative vigor. The second dimension (17.4%) is related to diameter and branching.

Sathyanarayana *et al.* (2012) showed that pod- and flowering-related traits strongly contributed to accession differentiation. Leelambika *et al.* (2010) separated accessions of var. *pruriens* and var. *hirsuta* (cluster I) from accessions of var. *utilis* (cluster II). Their UPGMA dendrogram was based on morphometric data.

In our study, hierarchical cluster analysis (HCA) placed E4 in the intermediate group (along with E1 and E2). Principal component analysis (PCA) placed it closer to the high performers. This difference is explained by its particular profile. E4 has a good diameter but less overlap than E3. Intharuksa *et al.* (2023) obtained similar results. They used chemical and molecular approaches on twelve *Mucuna* species in Thailand.

Ecotype E6 has low performance and a stinging effect. It is poorly suited for agricultural use. Balogoun *et al.* (2022) have already observed this phenomenon in wild forms in Benin.

Our study has several limitations. The number of ecotypes evaluated is limited (six). The characterization is exclusively phenotypic. We did not measure nitrogen fixation. We also did not measure grain yield or L-DOPA content. Molecular characterization would be necessary. DNA markers (ITS2, matK, rbcL) could confirm the phylogenetic relationships between ecotypes. Recent

advances in molecular characterization have been reviewed by Intharuksa *et al.* (2023), who successfully discriminated twelve *Mucuna* species using DNA barcoding.

V. CONCLUSION

This investigation successfully delineated six distinct ecotypes belonging to *Mucuna pruriens* within the confines surrounding Kasai Oriental Province's Tshilenge territory. It yielded substantial insights into their respective phenotypical distinctions. It emphasized notable diversity across measured attributes. It highlighted promising candidates based upon vigor grounds, covering capacities, and earliness productivity potential. It also signaled necessity for further explorative inquiries extending beyond mere phenotype evaluations. These should encompass molecular characterizations alongside assessments relating directly back towards nitrogen fixation capabilities.

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