











# Stomatal Diversity in Wild *Coffea* Species: Implications for Coffee Breeding in Mozambique

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### Introduction

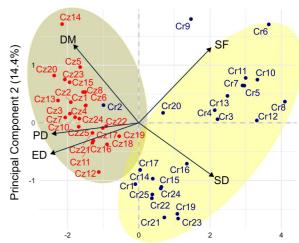
In Mozambique, coffee is cultivated across regions with high edaphoclimatic diversity, including *Coffea arabica*, *C. canephora*, *C. zanguebariae*, and *C. racemosa*. The latter two are native species [1]. Despite the cultivation of native species, there remains the need to identify genotypes with higher productivity and better adaptation to specific microregions. In addition, genetic diversity studies are essential to explore their potential use in breeding programs and to guide strategies to develop new cultivars. This study was based on the hypothesis that *C. racemosa* and *C. zanguebariae* exhibit significant variability in stomatal density and morphology.

# Materials/Methods

The collection of 48 accessions (24 of *C. racemosa* and 24 of *C. zanguebariae*) was carried out in several provinces of Mozambique. Leaves from the middle third of plagiotropic branches were collected for dry mass (DM) evaluation and anatomical analysis. Epidermal impressions were made using colorless gloss, fixed with adhesive tape, and mounted on semi-permanent slides. A total of 580 leaf epidermis images were analyzed under an optical microscope, with 10 images per plant. The images were evaluated using the Anati Quanti2 software to measure stomatal density (SD), polar diameter (PD), equatorial diameter (ED) and stomatal functionality (SF). Based on the means, the five variables were standardized for Principal Component Analysis (PCA)

## **Results/Discussion**

PCA revealed that stomatal density (SD) and stomatal functionality (SF) were the primary traits distinguishing *C. racemosa* (Cr) and *C. zanguebariae* (Cz) accessions, with each species projecting on opposite sides of the PCA biplot. On average, *C. racemosa* accessions exhibited higher SD and SF values than *C. zanguebariae*. Notably, accessions Cr8, Cr6, Cr10, and Cr12 of C. racemosa had the highest SD and SF values. One accession, Cr2, differed from the rest of *C. racemosa*, clustering closer to *C. zanguebariae* due to its lower SD and SF. Subgroup patterns were observed within *C. racemosa* accessions, whereas *C. zanguebariae* accessions displayed a more homogeneous distribution, hindering subgroup differentiation.



Principal Component 1 (71.9%)

**Figure 1:** Analysis of principal components for five leaf traits in 48 accessions of Coffea belonging to two species: *C. racemosa* (Cr) and *C. zanguebariae* (Cz) sampled in Mozambique. DM: dry mass of leaves (g); SD: stomatal density (stomata. mm²); PD: polar diameter of the stomata ( $\mu$ m); ED: equatorial diameter of the stomata ( $\mu$ m); SF: stomatal functionality ( $\mu$ m).

#### **Conclusion/Perspectives**

Intraspecific variability was more pronounced in *C. racemosa* than in *C. zanguebariae*. These findings confirm the study's hypothesis and highlight wild accessions with promising traits for use in coffee breeding programs aimed at improving physiological efficiency and adaptability.