

## Stomata counting automation facilitates *C. canephora* tetraploid selection

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### Rationale:

Chromosome doubling can enhance reproductive compatibility between genotypes with different ploidy levels. We've been using anti-mitotics to double a *C. canephora* clone chromosome number in order to facilitate nematode resistance introgression to *C. arabica* cultivars. Tetraploid plants will be homozygous, and will be potentially able to contribute complete resistance gene sets for interspecific hybrid progenies. Once treated with anti-mitotics, the plants had the stomata counted as the initial screening for tetraploids. The chromosome number is inversely correlated to the stomata number in diploid and tetraploid *C. canephora* plants [1]. Stomata counting is, nevertheless, time consuming and monotonous. The objective of this work was to test a software tool to identify and quantify stomata and compare scores with human scores produced by different people.

### Material & Methods:

Nail polishing was applied to the surface of size/developmental stage standardized leaves to produce epidermic impressions, which were transferred to glass slides and photographed under 100X magnification [2]. Nine digital images with approximately 0.6 mm<sup>2</sup> were produced from epidermic impressions of six different plants maintained in the same environment and treated simultaneously with anti-mitotics, making a set of 54 images. Stomata in the same set of images were identified and counted by four human beings and by the 'Stomata Count' (SC) algorithm, improved by a heuristic overlap filter and by noise reduction techniques [3] The five datasets, reached the impressive number of 45787 itens counted, and were compared by ANOVA (SigmaPlot, v.11.2).

### Results:

SC performed better than a human being without previous training to identify coffee stomata, which can be found in different sizes and sometimes in immature forms. So, despite slight differences, the scores produced by SC did not differ statistically ( $P < 0.05$ ) from three in four datasets produced by human beings. Analyses of scores generated by both methods - human or machine stomata identification and counting - resulted in the indication of a same coffee plant among the six evaluated as displaying significantly lower stomata number. Whether this characteristic remains stable in future evaluations, chromosomes will be counted from one instead of six plants.

### Conclusions & Perspectives:

Automation can save time and contribute effectively to identify nematode resistant *C. canephora* plants with low stomata frequency, which can be tetraploids, among hundreds treated with anti-mitotics.

### References:

1. Mishra. Annals of Botany 80: 689–692, 1997.
2. van Duren et al. Euphytica 88: 25-34, 1996.
3. GitHub - celso-vitor/StomataCount.