

STOMATA COUNTING AUTOMATION FACILITATES *C. canephora* TETRAPLOID SELECTION

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INTRODUCTION

- we've been using anti-mitotics to double a nematode resistant *C. canephora* clone chromosome number in order to facilitate introgression to *C. arabica*;
- chromosome and stomata numbers are inversely related in diploid and tetraploidized *C. canephora* [1];
- counting stomata is time consuming and monotonous.

The objective of this work was to test a software tool to identify and quantify stomata.

RESULTS/DISCUSSION

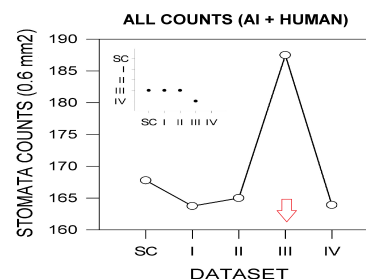
- SC performed better than a human being without previous training (count III, Fig. 1A);
- scores produced by SC did not differ statistically ($P < 0.05$) from three in four datasets produced by human beings (Fig. 1A);
- human and SC stomata identification and counting resulted in the **indication of one same coffee plant among the six** evaluated as **displaying significantly lower stomata number** (Fig. 1B x 1C).

Whether these results remain stable through time, **chromosomes will be counted from one instead of six plants.**

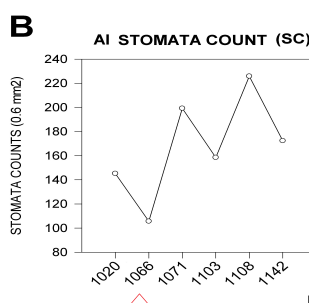
MATERIAL AND METHODS

- nail polishing applied to the surface of size standardized leaves;
- epiderm impressions transferred to glass slides and photographed under 100X magnification [2];
- nine 0.6 mm² digital images taken from 6 plants (54 images);
- stomata in the same set of images identified and counted by human beings and by the Stomata count (SC) algorithm [3];
- ANOVA (SigmaPlot, v.11.2).

A



B



C

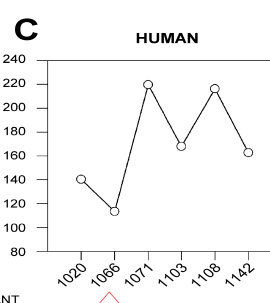


Figure 1. A. number of stomata in nematode resistant *C. canephora* leaves as counted four times by human beings (I to IV) and once by the algorithm Stomata Count. Count III differs ($P < 0.05$) from all the others. B and C. Stomata no. in individual plantas to demonstrate that SC and trained humans can identify the same plant by the reduced stomata number

CONCLUSIONS/PERSPECTIVES

Automation can save time and contribute effectively to identify nematode resistant *C. canephora* with low stomata frequency, which can be tetraploid, among hundreds of plants 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