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Stomatal Density in Six Genotypes of Cassava

Oliveira E.C., Miglioranza E.

Abstract*— Stomatal parameter frequency of distribution was studied in six genotypes of cassava (IAC 12, IAC 13, IAC 14, IAC 15, IAC 90 and IAC 576-70. The genotypes were grown under uniform agro-climatic condition. Results indicated that the stomata were classified as paracytic for all the genotypes, being distributed the abaxial surface of the leaves. The means observed for density stomata varied in the apical portion of the leaf between 67-100 stomata per mm^2. In this part of the leaf, the following genotypes IAC 15, IAC 576-70 and IAC 9 were those who had submitted the greatest number of stomata. The other genotypes IAC 12, IAC 13 and IAC 14 were placed in a smallest group. Estimates of stomatal frequency between lower leaf surfaces revealed her that may be utilized as drought tolerant genotypes.

***Index Terms*—Abaxial, Manihot esculenta Crantz, paracytic.**

I. INTRODUCTION

Brazil is the third largest producer of cassava in the world, behind only Nigeria and Thailand [1]. Its estimated production for 2013 is approximately 21.178.686 tons of roots, covering an area of 2.149.403 ha, with an average yield of 14 t ha^{-1} [2].

The anatomical aspects of cassava have been little studied, more is necessary, due to the plasticity in leaf anatomy in different genotypes, which would cause differences in the photosynthetic rate, affecting CO_2 diffusion [3]. Cassava leaves are simple and composed of palmately lobed leaf blade. The number of lobes in a leaf is variable, but it is usually an odd number ranging from three to nine. The cassava leaves as hypostomatic, with a defined palisade and spongy parenchyma, bundle sheath cells developed with radially distributed chloroplasts. The distribution of stomata on leaves of cassava in the abaxial epidermis and restricted only to the proximity of the largest vascular bundles [4].

The reduction in the amount of water lost from the plants occurs by changes in the mechanism, size and distribution of stomata [5]. Stomatal density is an important ecophysiological parameter that also affects gas exchanges and photosynthesis, stomatal density may vary in leaves of plants of the same species [6]. Other possible sources of variation in stomatal density are linked to different genotypes and atmospheric CO_2 [7], moisture [8], intensity and quality of light [8] and solar radiation [9].

The different genotypes of the same species can contain variations in the structure of the leaves, which may represent as an excellent tool in the selection process of cassava plants. Assuming these variations can be verified in cassava plants, this study aimed to establish the anatomical characteristics, as well as determine the density and distribution of stomata in the abaxial surface of leaves of different cassava genotypes.

II. MATERIALS AND METHODS

For the study of stomatal density, were collected of portions of plants, portions of fully expanded leaves of genotypes: IAC 12, IAC 13, IAC 14, IAC 15, IAC 90 and IAC 576-70. The observation of the density and the distribution of stomata were performed using the North replica method as in [10], where an impression of the leaf surface was obtained with cellulose acetate film adhesive tape over an area of approximately 1.0cm^2. Sections of the acetate film formed were removed and mounted on slides for microscope assessment. An optical microscope eyepiece lens 15x and objective lens 40x was used, which provided 600x magnification and a field of view of 0.39 mm^2. The images used in the analysis were captured by a capture system (Carl Zeiss, Germany) composed of an AxioPhot I microscope equipped with AxioCam ICc3 camera and Bel View software. Image processing and analysis was performed with public domain software Image J 1.43a, version 64, with the measurement of five fields replicated for each analyzed leaf. Stomatal density DE – number of stomata per unit of area) was calculated according to [11]. For the purposes of statistical analysis, stomatal density on the abaxial surface and the frequency of stomata in the apical portions of leaves were studied.

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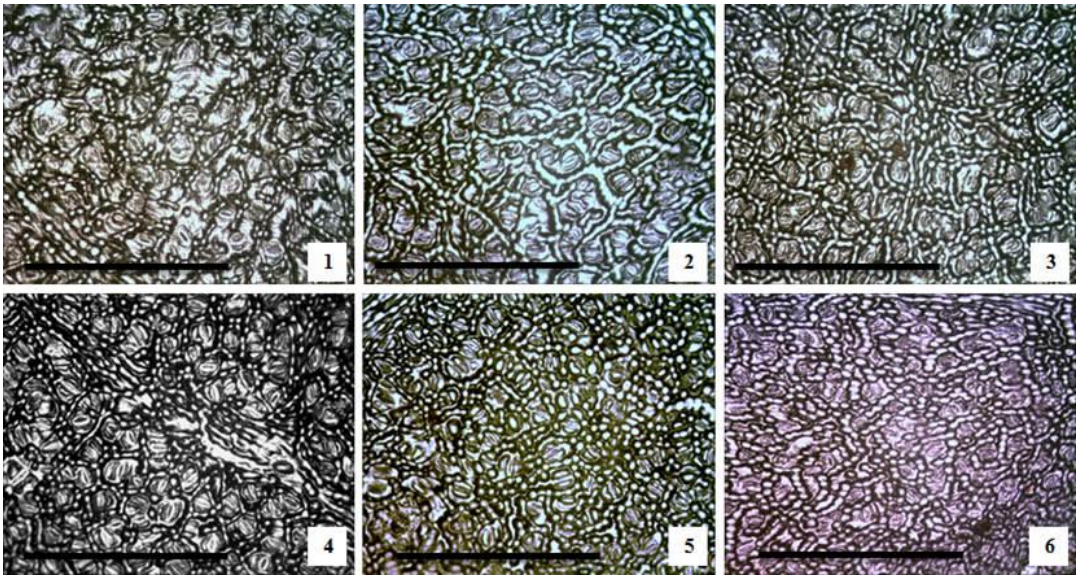
The data were subjected to analysis of variance (ANOVA), the parameters measured and the averages were grouped according to the criteria of Scott-Knott ($P<0.05$). As the data were normally distributed (Shapiro-Wilk) no transformation was used before analysis of variance.

III. RESULTS AND DISCUSSION

The stomata were classified as paracytic for all the genotypes, being distributed in a waveform on the abaxial surface of the leaves (Figure 1).



Figs: 1-6. Paracytic stomata of cassava plants.
1) IAC 12, 2) IAC 13, 3) IAC 14, 4) IAC 15, 5) IAC 90, 6) IAC 576-70. Scale bar: 100 µ.

The means observed for DE varied in the apical portion of the leaf between 67-100 stomata per mm^2, combining cassava genotypes in two groups, according to Scott-Knott. In this part of the leaf, the following genotypes deserve mention: IAC 15 (97), IAC 576-70 (92) and IAC 90 (100). The other genotypes IAC 12, IAC 13 and IAC 14 were placed in a smallest group, with 75, 78, and 79 stomata per mm2, respectively (Figure 2).

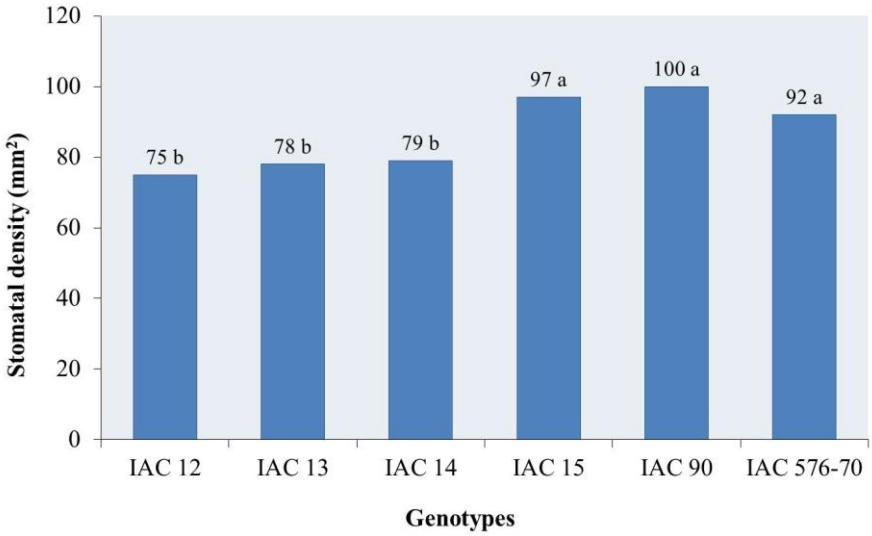


Fig 2. Stomatal density (stomata per mm^2) portions of the abaxial surface of leaf in different cassava genotypes.

DE changes observed in different parts and on the surface of cassava leaves are consistent with those found in several other plants [12]. Similar results were obtained in leaves of *Zebrina purpusii* Bruckn, which revealed a

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stomatal density of 62.5 stomata per mm^2, arranged in rows parallel to the main rib at the apex of the leaf [13]. Observations in stomatal density on the adaxial surface of leaves of Sorghum halapense L. varied between 25 to 106 stomata per mm^2 and on the abaxial surface stomatal density varied between 76 - 129 stomata per mm^2, both distributed on the apex of the leaf [14].

The effect of water scarcity on leaf anatomy of cassava genotypes, found high plasticity in DE for genotype UFLA E, under dry conditions, and the level of plasticity was different for the different genotypes studied [15]. Thus, a higher DE may allow stomatal opening in a shorter period of time, with proper capture of CO2 and reducing the period of time that these stomata are open, and, therefore, reducing transpiration, allowing better adaptation of cassava genotype to conditions of low availability of water [14]-[15].

For some authors, there is a direct response of stomatal density to increased shading [16]. This finding in their analysis of the growth of *Spondias purpurea* L. (*Anacardiaceae*) in response to different sunlight levels, reporting that stomatal density decreased with decrease in light levels [17]. Another factor that can influence the number of stomata in the leaves is their architecture, and in studies with *Xylopia brasiliensis* Sprengel, smaller plants with more branches showed lower stomatal density values. Also, a low positive correlation was observed between stomatal density and average CO_2 assimilation in the plants of this study. These results are directly related with the cassava crop, since many genotypes usually have trichotomous stems (with three branches) [18]. The cassava is a compact plant where competition for light is intense, which can lead to reduced availability of solar radiation to the leaves located in the lower third of the plant.

Estimates of stomatal frequency between lower leaf surfaces revealed her that may be utilized as drought tolerant genotypes. However further research will be undertaken to confirm or refute this statement.

IV. CONCLUSIONS

The stomata were classified as paracytic for all the genotypes, being distributed the abaxial surface of the leaves. The cassava genotypes were divided into two groups: being IAC 15, IAC 576-70 and IAC 90 were those who had submitted the greatest number of stomata and genotypes IAC 12, IAC 13 and IAC 14 were grouped with the lowest number of stomata in area leaf.

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AUTHOR BIOGRAPHY

Bachelor's at Agronomia from Faculdade Integrado (2007), masters at Agronomy from Universidade Estadual de Maringá (2010) and doctorate in progress from Universidade Estadual de Londrina. Has experience in Agronomy, focusing on Agronomy, acting on the following subjects: biorreguladores, weeds, cassava crop, nutrition of plants and statistical analysis.

Agronomy and Crop Science Master in the (ESALQ - USP) School of Agriculture Luiz de Queiroz, University of São Paulo, completed a doctorate in Plant Science (Plant Production) from the Federal University of Viçosa (UFV) in 1992. Has postdoc in Rennes- France, Wisconsin - Madison (USA) and California - Davis (USA) universities. Is Associate Professor C (AC-C) , State University of Londrina (UEL) was Dean of Research and Graduate Studies and Research Director, State University of Londrina (UEL) . President and Local Coordinator of Institutional Program for Scientific Initiation Scholarships (PIBIC), PIBIC - Af, the PIBITI, the Affirmative Action Program for Social Inclusion (Araucaria Foundation), participated in the creation and maintenance of Proarte - UEL, was member of the Editorial Board of Journals Semina . Was Program Coordinator of Graduate Studies in Agronomy at UEL for four years (two terms), when the PhD Course was created in Agronomy. He has supervised 13 dissertations, 07 doctoral dissertations, 18 papers undergraduates and 35 Completion of course work in the areas of Agriculture and Forestry and Forestry Resources. Ongoing guidance has six these two Working Completion of course. Received six awards and / or honors. Between 1990 and 2005 five coordinated research projects. Currently coordinates three research projects. Operates in Agronomy and Physiology of Production, with an emphasis on Cultural practices. In their professional activities interacted with 160 employees in co -authorship of scientific papers. In his Lattes the most frequent terms in the context of scientific , technological and artistic- cultural production are: *Phaseolus vulgaris* (L.) seedlings, germination, *Phaseolus vulgaris*, *Jacaratia spinosa*, nursery, coffee, *Coffea arabica* L , Seed and *Glycine max* (L.) Merrill.