Leaf anatomy of an interspecific hybrid of cotton

Sumario. Se produjeron híbridos entre las dos especies diploides de algodón, **Gossypium herbaceum** y **G. arboreum**, y fueron tratados con colchicina para duplicar su número de cromosomas. El tetraploide sintético resultante fue cruzado con **G. hirsutum**. Se estudiaron secciones transversales de las hojas de los diploides, tetraploides sintéticos y su híbrido. Los diploides asiáticos tenían hojas isobilaterales con una empalizada adaxial bien desarrollada y un parénquima en empalizada abaxial menos desarrollado. El tetraploide sintético tenía también una anatomía foliar similar. Su híbrido con **G. hirsutum tenía** una estructura foliar similar a los diploides asiáticos pero con un ancho de hoja foliar más grande. Las hojas de **G. hirsutum** fueron dorsiventrales con una sola empalizada (adaxial). Se discute la significación de estos hallazgos.

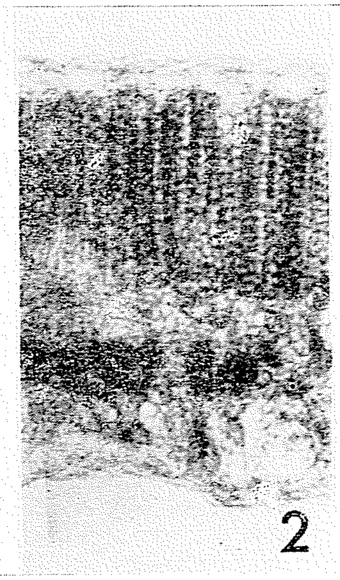
The cultivated cotton comprises of four species, viz., *Gossypium herbaceum* L. (A_1), *G. arboreum* L. (A_2), *G. hirsutum* L. and *G. barbadense* L. (AD). The first two, commonly referred to as Asiatic diploids, are under cultivation in India, Burma and Pakistan. The New World cultivated species are tetraploids. The Asiatic diploids are well adapted to adverse environmental conditions like drought as well as insect pests and diseases. Many attempts had been made to transfer their useful characters to cultivated tetraploids but with limited success. Direct hybridization of cultivated diploids with cultivated tetraploids was made difficult by the embryo-endosperm incompatibility (3).

Earlier report on the foliar anatomy of the cultivated species of *Gossypium* had indicated that the Asiatic diploids possess a leaf structure adapted to withstand drought (2). Since there is ve[illegible] on the inheritance of anatomical characters of leaf in interspecific hybrids involving these species, studies were made on the inheritance of leaf structure in Old World x New World tetraploid hybrid.

Material and Method

G. herbaceum (A_1) cv 'V. 797' from Western India and *G. arboreum* race *cernuum* (A_2) from Eastern India were chosen to represent the Asiatic species. The commercial variety 'MCU. 5' was the *G. hirsutum* representative. Direct crosses were attempted between the diploid species and *G. hirsutum* without success. To overcome the incompatibility *G. herbaceum* was crossed with G. *arboreum* and the hybrid was treated with 0.1% colchicine solution to induce chromosome doubling. The resultant synthetic tetraploid, $2(A_1A_2)$, was crossed with *G. hirsutum*. The latter was used as the pollen parent and good, viable seeds were obtained. Plants raised from these seeds were vigorous.

Mature leaves from the 12th node and above were collected from all the three species, synthetic tetraploid and its hybrids and fixed in formalin acetic alcohol (FAA). Transverse sections of leaf were cut and stained with safranin. Measurements of leaf blade thickness, adaxial and adaxial palisade width were made with ocular micrometer and expressed in microns. The lateral density of palisade layers were expressed as number of palisade cells in three ocular divisions of the micrometer.



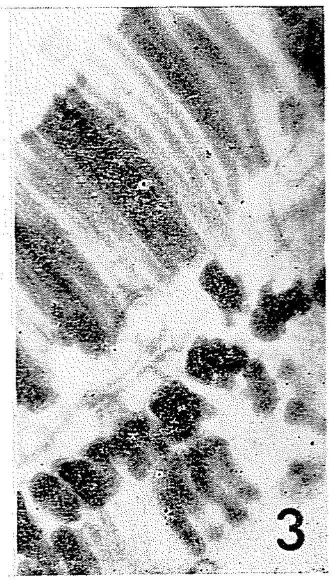


Fig. 1.—*T. S. of leaf of* G. arboreum *showing two layers of palisade.*
Fig. 2.—*T. S. of leaf of* G. hirsutum.

Fig. 3.—*T S. of leaf of the hybrid with adaxial and abaxial palisade (Magnification x 400).*

Table 1 —Leaf blade width, palisade width, palisade ratio and lateral density of palisade cells in species and hybrids of *Gossypium*.

Species, variety and genome constitution	Leaf blade width (in microns)	Width of Palisade layer		Palisade ratio (palisade/blade width)		Lateral density of palisade cells*	
		Adaxial (microns)	Abaxial (microns)	Adaxial	Abaxial	Adaxial	Abaxial
1 *G herbaceum* Var V-797 (A_1)	356	92	48	0 367	0 194	14	8
2 *G arboreum* race *cernuum* (A_2)	246	176	66	0 495	0 185	20	10
3 *G hirsutum* Var MCU 5 (AD)	286	128	—	0 449	—	25	—
4 Synthetic tetraploid 2 (A_1A_2)	389	180	81	0 377	0 208	10	8
5 Hybrid 2 (A_1A_2) x MCU 5	528	235	103	0 444	0 197	13	6

* Number of palidase cells in 33 microns length

Results

The leaf anatomy of the two Asiatic diploids were more or less similar. Their leaves had a well developed adaxial palisade parenchyma and another but less developed abaxial palisade (Fig 1) The leaf type in these species can be termed isobilateral (1) The leaves of *G. hirsutum* were of dorsiventral type with a well developed adaxial palisade layer but there was no abaxial palisade (Fig 2) The synthetic tetraploid had a leaf structure similar to the Asiatic diploids. Its hybrid with *G hirsutum* had leaves with larger leaf blade thickness, well developed adaxial palisade and a layer of abaxial palisade Thus it was an isobilateral type similar to the Asiatic species (Fig 3).

Quantitative data on leaf blade width, palisade width, palisade ratio and lateral density of palisade cells are presented in Table 1 *G. arboreum* had the high qest adaxial palisade ratio and the synthetic tetraploid had the highest abaxial palisade ratio The hybrid involving the synthetic tetraploid and *G hirsutum* had the maximum leaf blade width but its adaxial palisade ratio was comparable to its *G. hirsutum* parent. The abaxial palisade ratio of the hybrid was comparable to the Asiatic diploid species. The lateral density of palisade cells was high in *G hirsutum* and *G arboreum* as compared to the hybrid. This indicated the larger cell size of the palisade tissue in the hybrid.

Discussion

Studies on the comparative anatomy of leaves of different species of cultivated cotton had shown that the Asiatic diploids possess leaf structure of xeromorphic type which help them to withstand moisture stress during periods of drought The presence of two layers of palisade in the mesophyll was considered to be an important adaptation to xerophytic habitat (2). The New World species, *G. hirsutum*, has only one layer of palisade and it is less adapted to moisture stress compared to the diploids. Transfer of the leaf characters of Asiatic diploids to New World cultivars across the species barrier may help in developing genotypes suited to arid areas The nature of genetic control of such anatomical characters and their inheritance was not known Overcoming the species barrier to effect gene transfers make such investigations difficult

In the present study, the two diploids with similar leaf structure were utilized to synthesise a new tetraploid which was crossed with *G. hirsutum*. The resultant hybrid had all the xeromorphic features of the diploid species in addition to the increased leaf blade width which may confer additional advantage in a xerophytic habitat (2) However, it is too early to predict whether the hybrid will be able to transmit its anatomical characters to its progeny without dilution in subsequent generations of backcrossing.

Summary

Hybrids between the two Asiatic diploid species of cotton, *G. herbaceum* and *G. arboreum* were produced and treated with colchicine to double their chromosome number. The resulting synthetic tetraploid was crossed with *G. hirsutum*. Leaves from the diploids, synthetic tetraploid and its hybrid with *G. hirsutum* were killed and fixed in FAA. Tran-verse sections of leaves were studied. The Asiatic diploids had isobilateral leaves with a well developed adaxial palisade and a less developed abaxial palisade parenchyma. The synthetic tetraploid too had a similar leaf anatomy. Its hybrid with *G. hirsutum* had a leaf structure similar to the Asiatic diploids but with larger leaf blade width. *G. hirsutum* leaves were dorsiventral with a single layer of (adaxial) palisade. The significance of these findings had been discussed.

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Estimating leaf water potential of coffee with the pressure bomb

Resumo. Foi utilizada análise de regressão linear para calcular a relação entre potencial da água do xilema e potencial da água de folhas de cafeeiros, determinando-se uma curva de calibração para estimativa, através da bomba de pressão, do potencial da água de folhas adultas de cafeeiros (**Coffea arabica**, L.), cultivar Mundo Novo'.

Introduction

Thermocouple psychrometry has been considered the most accurate method for measuring leaf water potential. Several difficulties encountered in the determinations, such as errors caused by liberation of heat by respiring tissue, adsorption of water vapor on the walls of the psychrometer chamber, and resistance of tissue to vapor transfer (3), have been overcome by suitable procedures (2). However, there remains the inconvenience regarding the time necessary for samples of some species to reach equilibrium with the psychrometer chamber, thus imposing a limitation on the use of the method to estimate plant water status under field conditions. On the other hand, the pressure bomb (10) allows fast measurements of negative hidrostatic sap pressure in the xylem. Boyer (4) suggested that accurate determination of leaf water potential can be made if the pressure chamber measurements are calibrated against the values obtained by the thermocouple psychrometer. Determination of water potential by the pressure bomb has been carried out in citrus (8), forest trees (8), tomato (2, 6), sorghum and corn (5), and pine seedlings (7).

This paper reports the calculation of the relationship between xylem potential and leaf water potential in coffee for estimating plant water status by the pressure bomb.

Materials and methods

Coffee plants (*Coffea arabica* L., cv. 'Mundo Novo'), two years old, growing in the greenhouse, were subjected to different levels of soil moisture. For the determination of xylem potential, mature leaves were detached from the plant and immediately placed in the pressure chamber, "PMS" model 1000. At the time the leaf was excised, one disc was punched out from the blade and placed in a thermocouple psychrometer apparatus, "Wescor" model MJ-55, with chamber model C-51. The effect of leaf hydration on the equilibrium time of tissue with the psychrometer chamber was determined in discs removed from leaves with different water potentials and measurements were made in 10 minute intervals after the sample was placed in the chamber.

Results and discussion

Figure 1 shows the equilibrium times curves for three mature leaves with water potentials of —12.5 bars, —16.5 bars and —25.0 bars. Equilibration of leaf tissue with the psychrometer chamber took from 30 to 90 minutes, depending on the leaf water status. The leaf with high water potential reached equilibrium in approximately 90 minutes while samples with lower water potential attained equilibrium sooner, 50 and 30 minutes at —16.5 and —25.0 bars, respectively. The relatively long time for equilibration imposes some difficulty on the use of the thermocouple psychrometer to determine the daily pattern of leaf water potential of coffee in the field.

Figure 2 presents the calibration curve relating leaf water potential to xylem potential. Linear regression can be expressed by the equation:

$$y = 0.8493 + 0.8346\,x \qquad (r^2 = 0.97)$$

where y indicates leaf water potential and χ measures xylem water potential in bars.

Except when the water potential was high (above —4 bars), xylem pressure was always 1.5 to 5 bars