



## Evaluation of *Hevea* Clones Subjected to Water Deficits

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### Abstract in Bahasa Malaysia

Enam klon *Hevea* telah diperlakukan dengan perlakuan-perlakuan penyahhidratan selanjar dan berkitar untuk menilai respon klon-klon ini dari segi fotosintesis, perpeluhan dan rintangan stomata terhadap taraf air tumbuhan. Ujian-jian ini dibuat ke atas pokok-pokok *Hevea* muda lapan puluh lima hari setelah tunggul-tunggul divedung di dalam rumah kaca. Penyahhidratan tanah yang kian meningkat mempengaruhi klon-klon ini secara berbeza-beza dengan mengurangkan potensi air daun, perpeluhan dan fotosintesis; dan meningkatkan rintangan stomata. Dalam setengah-setengah klon, penyahhidratan tanah berkitar menyebabkan pengurangan yang ketara di dalam paras pemulihan kadar-kadar perpeluhan dan fotosintesis bersih masing-masing hingga kepada kitaran kekurangan air yang pertama dan kedua. Bagi semua klon, potensi air daun telah berkurang mengikut kitaran-kitaran kekurangan; dan 48 jam selepas pengairan semula nilai-nilai purata keadaan prategasan telah pulih semula sepenuhnya. Rintangan stomata telah meningkat dengan kitaran kekurangan-kekurangan air. Selepas pengairan semula, pemulihan yang berbeza dalam parameter-ini telah diperolehi. Dengan mempertimbangkan variabel-variabel yang dikaji secara menyeluruh, keadaan kemarau yang diperlakukan memberi kesan yang sedikit ke atas klon-klon IAN 3087 dan IAN 6323, sementara klon FX 3899 adalah lebih peka.

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### Abstract

Six clones of *Hevea* were subjected to both continuous and cyclic dehydration treatments in order to evaluate their response with respect to photosynthesis, transpiration and stomatal resistance, to plant water status. The trials were carried out on young *Hevea* plants at eighty-five days after the stumps were transplanted in a green house. The increasing soil dehydration influenced the clones differently by reducing the leaf water potential, transpiration and photosynthesis; and increasing the stomatal resistance. In some clones the cyclic soil dehydration caused significant reduction in the level of recovery of the transpiration rates and net photosynthesis up to the first and second water deficit

cycle, respectively. For all clones, the leaf water potential decreased with the deficit cycles; and 48 h after re-irrigation the mean values of the pre-stress condition were fully recovered. The stomatal resistance increased with the cyclic water deficits. After re-irrigation a differential recovery of this parameter was observed. Considering the variables studied as a whole, clones IAN 3087 and IAN 6323 were less influenced by the imposed drought conditions while FX 3899 was more sensitive.

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The Amazonian region, which is the natural environment for *Hevea* species is characterised by its tropical wet climate – Koeppen's Afi, Ami, and Awi climatic types<sup>1</sup>. This, associated with the episodic growth of the rubber tree, has favoured the incidence of spreading of the fungus *Microcyclus ulei* P. Henn v. Arx., the causal pathogen for South American Leaf Blight (SALB), which is the main limitation to the development and expansion of *Hevea* cultivation in Brazil<sup>2</sup>. In an attempt to avoid the disease, some workers<sup>2,3</sup> suggested that rubber be grown in areas with a well-defined dry season.

Under drought conditions e.g. the Central Basin of Congo with 1200 mm of rain and six dry months, only Tjir 16 was economically suitable. In India, where available areas for rubber expansion have a well defined dry season, clone G1 1 is the most drought resistant<sup>4</sup>. In Brazil, the more resistant national clones are IAN 3087, IAN 2903 and IAN 3156. They are grown in Acailandia, State of Maranhao, in an area with water deficit<sup>5</sup> of about 355 mm.

Young rubber plants have been shown to have low tolerance to soil-water deficits. Transpiration rates are high<sup>3,6</sup> and water use efficiency varies from clone to clone<sup>6</sup>. Stomata close under moderate water deficits to avoid progressive stress<sup>7</sup> thus affecting the transpiration/photosynthesis ratio<sup>3</sup>.

This paper reports on a comparative study on physiological indices that take into account the energetic level of plant-water and its effects on stomatal opening and closure, water loss by transpiration and photosynthesis of six Brazilian clones. The aim is to establish reliable and consistent indices to help select *Hevea* clones for cultivation in less suitable ecological regions.

## MATERIALS AND METHODS

Budded stumps of clones IAN 717, IAN 873, IAN 2903, IAN 6323 and FX 3899 planted in polybags with a capacity for 8 kg dry substratum were raised under glass-house conditions having irradiance *circa* 50% of the open, maximum and minimum temperatures of 37.4°C + 3°C and 24.4°C + 2°C, respectively and R.H. 73.4% + 5%, at the Rubber and Oil Palm National Research Centre, EMBRAPA/CNPDS, Manaus, Amazonas. Soil used in this experiment was the upper 20 cm of a medium-texture yellow latosol under virgin tropical forest. After planting, soil moisture was maintained at or near field capacity by one- or two-daily water applications. Two experiments were conducted.

### Experiment 1 - Increasing Soil Dehydration

This experiment was carried out when the plants were eighty-five days old. Water supply was withheld for eighteen days.

The plants were transferred to the laboratory and left to acclimatise for 24 h under partially controlled temperature and relative humidity with temperature of 26°C + 2°C and R.H. of 70% + 5%. The experiment lasted till the permanent wilting points of the plants were visible. The following determinations were made.

*Net photosynthesis.* Net photosynthesis ( $PN$ ) was measured using the method described by Zunker and Kreeb<sup>8</sup> and modified by Bergonci<sup>9</sup> under a radiant flux density of 81 mW cm<sup>-2</sup>. The rates of net photosynthesis were measured on mature leaves of the latest whorl at 0700 h and at 1000 h. Parallel measurements of the leaf water potential, stomatal resistance and transpiration rates were made.

*Stomatal resistance.* Stomatal resistance,  $r_s$  was measured on the same leaf immediately after the rate of photosynthesis was determined using an automatic diffusion porometer, model Delta-T Devices Mk II.

*Leaf water potential.* Leaf water potential ( $\psi_l$ ) was estimated using a pressure chamber<sup>10</sup>. This determination was carried out on leaves opposite to those used for  $PN$  measurement.

*Transpiration.* Transpiration rate ( $E$ ) was determined on the same leaves for  $PN$  measurement using the gravimetric method<sup>11</sup>.

A completely randomised design with six treatments and three replications was used. Data were subjected to the analysis of variance and fitted with polynomial equations.

### Experiment 2 - Cyclic Soil Dehydration

Plants were grown under the same conditions described in *Experiment 1*. The cyclic treatment consisted of the depletion of the soil-water content down to -1.5 MPa soil water potential by withholding irrigation. Soil moisture content was gravimetrically monitored as the function of time. The soil water potential ( $\psi_s$ ) was estimated by substituting the value of the moisture percentage ( $P_w$ ) in *Equation 1* that relates both hydric parameters:

$$\psi_s = ae^{-bP_w} \quad \dots 1$$

where  $a$  and  $b$  are constants.

When the soil water potential attained -1.5 MPa water was applied to bring back the soil water content to field capacity (*Figure 1*). This soil moisture level (-0.13 MPa) was maintained for five days, then a new cycle was initiated. During the experimental period, plants were subjected to five successive soil depletion periods.

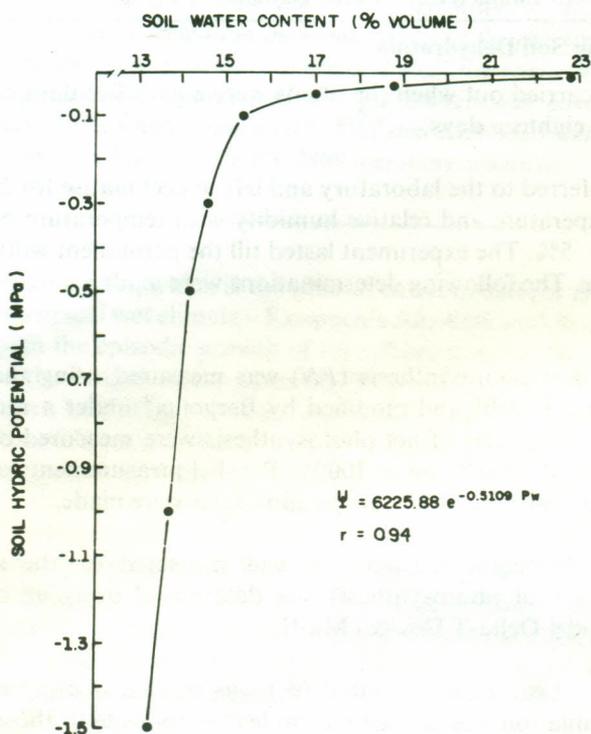


Figure 1. Soil moisture retention curve.

The same parameters determined in *Experiment 1* were measured in *Experiment 2*. Net photosynthesis and transpiration were determined at 48 h after every water application. Likewise, the stomatal resistance and the leaf water potential were determined when the soil-water potential was brought back to approximately -1.5 MPa and also 48 h after re-irrigation within each cycle.

The experimental design was a completely randomised block with twelve treatments, two soil moisture levels, six *Hevea* clones and three replications.

## RESULTS AND DISCUSSION

### Experiment 1

The results of leaf water potential for *Hevea* clones subjected to continuous soil-water depletion are presented in *Table 1*. For all the six clones it was observed that  $\psi_l$  decreased as the soil water potential decreased. A low reduction in  $\psi_l$  was shown by clone IAN 6323; the remaining clones exhibited similar differences both when the leaves were turgid or wilted. The lower leaf water potentials shown by turgid leaves of IAN 717, IAN 3987 and IAN 873 clones compared to the FX 3899 and IAN 6323, could indicate a lower water holding capacity of the latter which was partially confirmed by the values of its stomatal resistance (*Figure 2*) and even clearer by the transpiration data (*Figure 3*). Nevertheless, clone IAN 6323 showed a somewhat

TABLE 1. VARIATIONS OF THE LEAF WATER POTENTIAL DURING EIGHTEEN-DAY WATER DEFICIT PERIOD

Clone	Treatment	Leaf water potential (MPa)								
		2 days	4 days	6 days	8 days	10 days	12 days	14 days	16 days	18 days
IAN 717	C	-1.12	-1.10	-1.17	-1.07	-1.12	-1.07	-1.05	-1.05	-1.08
	WD	-1.07	-1.13	-1.20	-1.30	-1.67	-1.93	-2.32	-2.87	-3.10
IAN 873	C	-0.97	-1.20	-1.07	-1.17	-1.03	-1.05	-0.97	-1.03	-0.95
	WD	-1.05	-1.17	-1.30	-1.55	-1.58	-2.08	-2.47	-3.10	-3.20
IAN 2903	C	-0.82	-1.00	-1.02	-0.92	-1.03	-1.15	-1.08	-0.92	-1.03
	WD	-0.82	-1.13	-1.18	-1.37	-1.45	-1.35	-1.83	-2.20	-2.72
IAN 3087	C	-0.90	-1.17	-1.20	-1.05	-1.03	-1.12	-1.08	-1.03	-1.05
	WD	-1.07	-1.20	-1.57	-1.70	-1.73	-2.30	-2.68	-2.82	-3.03
IAN 6323	C	-1.42	-1.52	-1.43	-1.50	-1.35	-1.40	-1.37	-1.40	-1.45
	WD	-1.50	-1.47	-1.48	-1.60	-1.80	-1.97	-2.25	-2.40	-2.57
FX 3899	C	-0.70	-0.90	-1.00	-1.03	-0.93	-1.00	-0.93	-1.00	-1.00
	WD	-0.78	-0.83	-1.07	-1.35	-1.55	-1.82	-2.10	-2.20	-2.47

C = Control

WD = Continuous soil-water deficit

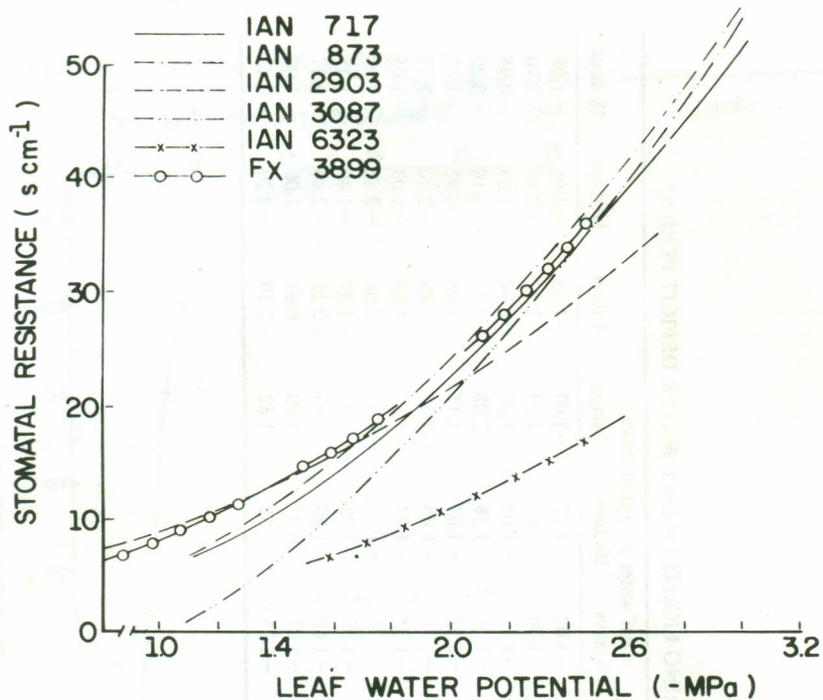


Figure 2. Leaf water potential-stomatal resistance relation for rubber clones subjected to continuous dehydration.

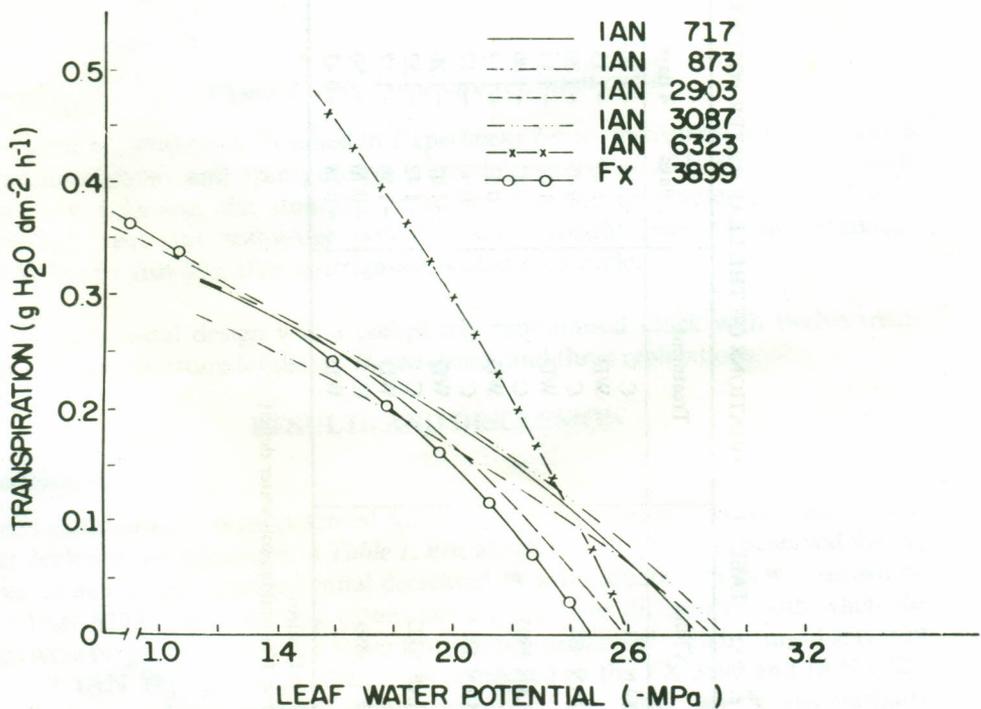


Figure-3. Leaf water potential-transpiration relation for rubber clones subjected to continuous dehydration.

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different behaviour of spending water when the leaves were turgid and retaining it under stress. This was also demonstrated by the  $\psi_t$ ,  $PN$  and  $E$  data. The values of  $\psi_t$  down to the visible permanent wilting point, varied between  $-2.5$  MPa and  $-3.2$  MPa and were relatively lower than those found by Resnik and Mendes<sup>7</sup> for clone FX 2261 subjected to three hydric regimes. As  $\psi_t$  decreased, the stomata of all clones were closed thereby increasing stomatal resistance ( $r_s$ ) to water diffusion (Figure 2).

Stomata closed slowly till  $\psi_s$  reached  $-1.5$  MPa beyond which a marked increase in the  $r_s$  values was demonstrated by all clones except IAN 6323 that showed values of  $r_s$  around half of those obtained for the remaining clones when subjected to the same water levels. The  $r_s$  results for IAN 6323 were coherent with those of  $PN$  (Figure 4). Clone IAN 6323 apart from being the one that showed the highest  $PN$  under increasing deficits, was able to reduce its transpiration to the same level as the other clones with only 50% of the  $r_s$ . This behaviour could be explained by a significant difference in density and stomatal size between clone IAN 6323 and the remaining ones. However, the density and the size of the stomata, generally showed a negative correlation. Therefore, the velocity at which stomata closed was more important in facing dehydration than their difference in size and frequency<sup>12</sup>.

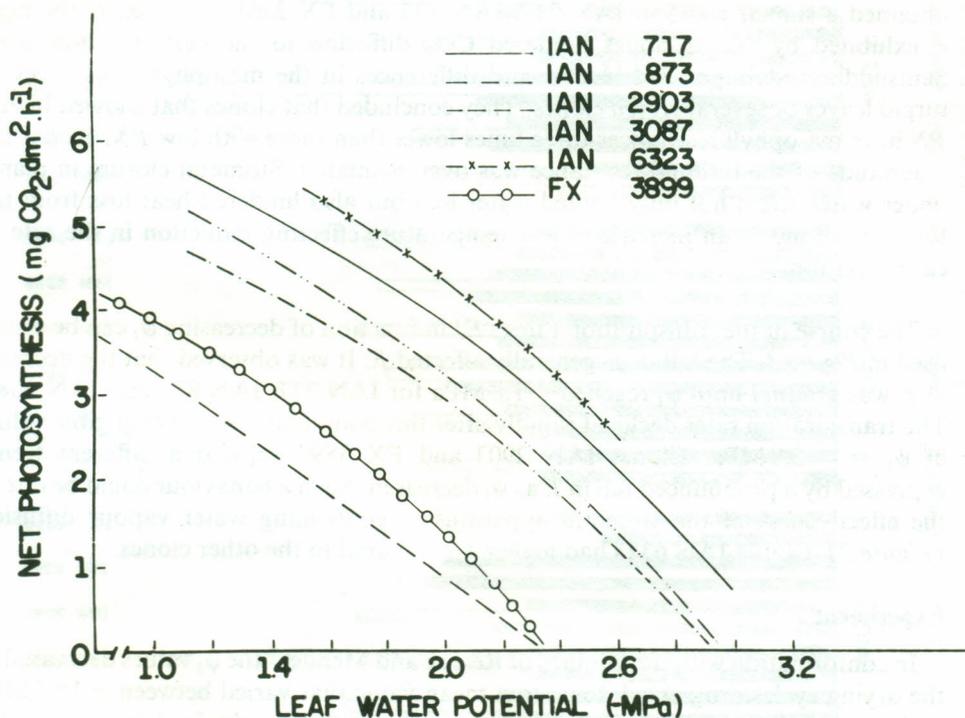


Figure 4. Leaf water potential - net photosynthesis relation for rubber clones submitted to continuous dehydration.

Leaf water potential around  $-1.6$  MPa induced a rapid stomatal closure in IAN 717, IAN 873, IAN 6323, IAN 3087 and FX 3899. Stomatal closure in IAN 6323 was progressive and did not depend on a limiting  $\psi_l$  value to initiate the process. Rocha Neto *et al.*<sup>3</sup> and Bergonci<sup>9</sup> found that clones IAN 717, IAN 873 and FX 2261 started to close their stomata at soil saturation deficit of 25%.

Net photosynthesis ( $PN$ ) detected in turgid leaves were 6.7, 6.2, 5.0, 6.5, 5.4 and 4.9  $\text{mgCO}_2\text{dm}^{-2}\text{h}^{-1}$  for IAN 717, IAN 873, IAN 2903, IAN 3187, IAN 6323 and FX 3899, respectively. These values were slightly lower than those obtained by Samsuddin and Impens<sup>6</sup> for Malaysian clonal seedlings and by Rocha Neto *et al.*<sup>3</sup> for turgid leaves of IAN 717 and IAN 873. Similar results were obtained by Bergonci<sup>9</sup> and Pita<sup>13</sup> for FX 2261 and *Hevea pauciflora*, respectively. Unlike  $r_s$ ,  $PN$  of plants under water stress were different among clones. Differences in  $PN$  among turgid leaves did not change drastically until the hydric compensation point, defined as the leaf water potential at which the  $\text{CO}_2$  uptake equals  $\text{CO}_2$  evolution, was reached. Clones IAN 6323, IAN 717, IAN 3087 and IAN 873 showed lower hydric compensation points and smaller reduction in  $PN$  (Figure 4).

Rocha Neto *et al.*<sup>3</sup> and Bergonci<sup>9</sup> showed that  $PN$  decreased with lower  $\psi_l$  and obtained a similar trend in IAN 717, IAN 873 and FX 2261. Apparently the high  $r_s$  exhibited by *Hevea* clones hindered  $\text{CO}_2$  diffusion to the carboxylation sites. Samsuddin and Impens<sup>6,14,15</sup> observed differences in the mesophyll resistances of turgid leaves of several *Hevea* clones. They concluded that clones that showed higher  $PN$  have mesophyll resistances three times lower than those with low  $PN$ , even if the magnitude of the residual resistance was over-estimated. Stomatal closure in plants under water stress not only limited water loss but also hindered heat loss from the leaves resulting in an increase in leaf temperature effecting reduction in the rate of carboxylation.

The course of the transpiration rates ( $E$ ) in function of decreasing  $\psi_l$  can be visualised in Figure 3. The fall in  $\psi_l$  generally affected  $E$ . It was observed that the decrease in  $E$  was gradual until  $\psi_l$  reached  $-1.8$  MPa for IAN 717, IAN 873 and IAN 3087. The transpiration rates declined rapidly after this point and reached negligible values at  $\psi_l = -2.8$  MPa. Clones IAN 2903 and FX 3899 showed a different trend, expressed by a pronounced fall in  $E$  as  $\psi_l$  decreased. Such a behaviour could be due to the effectiveness of the stomatal apparatus in controlling water vapour diffusion (Figure 2). Clones IAN 6323 had higher  $E$  compared to the other clones.

## Experiment 2

In conformation with the findings of Resnik and Mendes<sup>7</sup> the  $\psi_l$  values decreased as the drying cycles progressed down to a mean value that varied between  $-1.65$  MPa and  $-1.96$  MPa (Figure 5). After irrigation,  $\psi_l$  practically obtained its initial value (Figure 4). This tendency was however, not observed by Resnik and Mendes<sup>7</sup> in clone FX 2261.

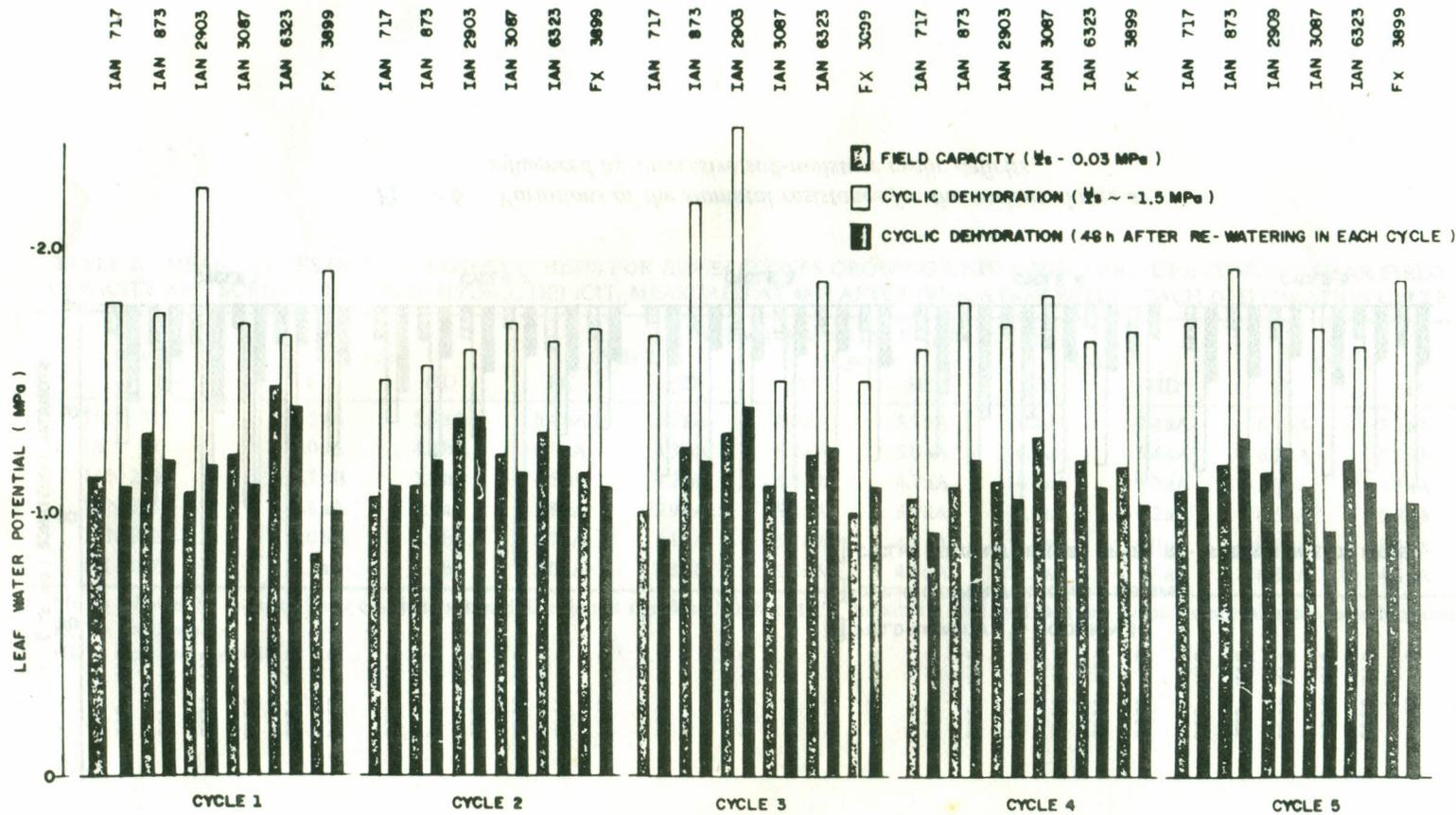


Figure 5. Variations of the leaf water potential for the various clones as influenced by successive soil-moisture cyclic deficits.

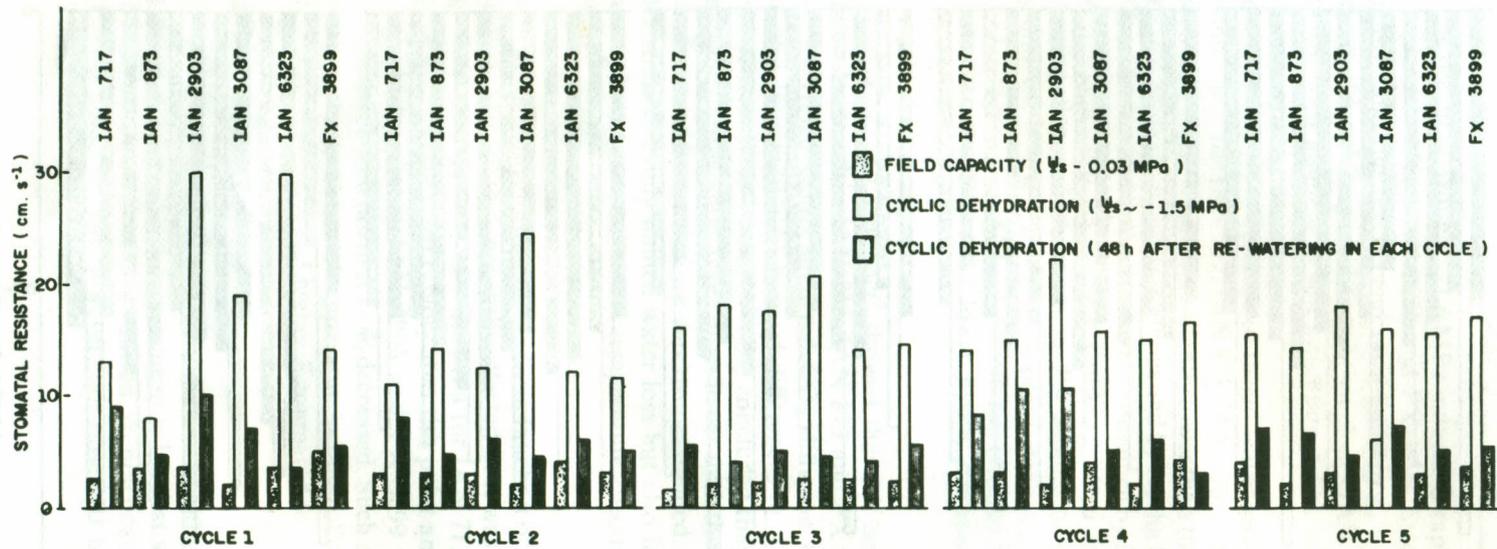


Figure 6. Variations of the stomatal resistance for the various clones as influenced by successive soil-moisture cyclic deficits.

TABLE 2. MEAN VALUES OF NET PHOTOSYNTHESIS FOR *HEVEA* CLONES GROWING UNDER SOIL MOISTURE CONTENT NEAR FIELD CAPACITY AND SUBJECTED TO SOIL HYDRIC DEFICIT, MEASURED AT 48 H AFTER IRRIGATION WITHIN EACH DEHYDRATION CYCLE

Clone	Net photosynthesis (mg CO <sub>2</sub> .dm <sup>-2</sup> .h <sup>-1</sup> )									
	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
	FC	HD	FC	HD	FC	HD	FC	HD	FC	HD
IAN 717	6.8 aA	5.8 aA	6.4 aA	5.3 aA	6.6 aA	5.7 aA	8.2 aA	7.4 aA	6.7 aA	7.5 aA
IAN 873	7.0 aA	4.4 bA	6.1 aA	4.3 bA	6.2 aA	5.0 aA	6.2 aA	5.4 aA	6.2 aA	5.2 aA
IAN 2903	4.7 bB	3.9 bB	4.5 bB	4.2 bB	5.0 aA	4.7 aA	4.6 aA	5.2 aA	5.2 aA	4.3 aA
IAN 3087	6.8 aA	6.5 aA	5.8 aA	5.9 aA	5.8 aA	5.9 aA	6.8 aA	6.2 aA	6.5 aA	5.4 aA
IAN 6323	5.0 bB	4.9 bB	5.7 aA	5.1 aA	5.2 aA	4.4 aA	6.5 aA	6.0 aA	6.8 aA	5.0 aA
FX 3899	5.3 aB	4.6 bB	5.0 aA	4.8 aB	6.8 aA	4.0 aA	5.5 aA	5.5 aA	6.5 aA	4.6 aA

Means followed by the same lower case letters in each line do not significantly differ at 5% probability level by Tukey test. Same for capital letters in each column.

FC = Field capacity

HD = Soil hydric deficit



TABLE 3. MEAN VALUES OF TRANSPIRATION FOR *HEVEA* CLONES GROWING UNDER SOIL MOISTURE CONTENT NEAR FIELD CAPACITY AND SUBJECTED TO SOIL HYDRIC DEFICIT, MEASURED AT 48 H AFTER IRRIGATION WITHIN EACH DEHYDRATION CYCLE

Clone	Transpiration ( $\text{g H}_2\text{O} \cdot \text{dm}^{-2} \cdot \text{h}^{-1}$ )									
	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
	FC	HD	FC	HD	FC	HD	FC	HD	FC	HD
IAN 717	0.53 aA	0.25 bA	0.64 aA	0.52 aA	0.50 aA	0.33 aA	0.53 aB	0.57 aB	0.64 aA	0.46 aA
IAN 873	0.59 aA	0.33 bA	0.71 aA	0.52 aA	0.66 aA	0.57 aA	0.70 aA	0.55 aA	0.73 aA	0.64 aA
IAN 2903	0.53 aA	0.30 bA	0.43 aA	0.49 aA	0.53 aA	0.30 aA	0.50 aB	0.50 aB	0.64 aA	0.49 aA
IAN 3087	0.55 aA	0.27 bA	0.73 aA	0.54 aA	0.50 aA	0.30 aA	0.55 aB	0.51 aB	0.55 aA	0.29 bA
IAN 6323	0.63 aA	0.62 aA	0.53 aA	0.57 aA	0.59 aA	0.57 aA	0.70 aA	0.61 aA	0.55 aA	0.36 aA
FX 3899	0.51 aA	0.47 aA	0.56 aA	0.38 aA	0.68 aA	0.42 aA	0.52 aB	0.39 aB	0.58 aA	0.41 aA

Means followed by the same lower case letters in each line do not significantly differ at 5% probability level by Tukey test. Same for capital letters in each column.

FC = Field capacity

HD = Soil hydric deficit

Figure 6. Variations of the stomatal water loss for the various clones influenced by the soil moisture cyclic deficits.

Figure 6 shows  $r_s$  values for the different clones in function of cyclic water deficits. The mean values for the control plants fluctuated during the whole experimental period from  $1.4 \text{ s cm}^{-1}$  to  $5.0 \text{ s cm}^{-1}$ . Maximum  $r_s$  varied from  $13.5 \text{ s cm}^{-1}$  to  $30.1 \text{ s cm}^{-1}$ , the higher values being exhibited by IAN 2903 followed by IAN 3087 and IAN 6323.

The values of  $r_s$  recovered after every watering. Mean values of plants after being conditioned to the drying cycles, varied between  $4.8 \text{ s cm}^{-1}$  and  $7.6 \text{ s cm}^{-1}$ . Clones IAN 3087, IAN 6323 and FX 3899 had lower values at the end of the experimental period.

Table 2 shows the mean values of  $PN$  for the different clones as well as among moisture levels. Net photosynthesis of IAN 873 was significantly reduced in the first two cycles and those of clone FX 3899 only in the first cycle (Table 2). The variation in the recovery rates of  $PN$  among the clones suggests that water stress affects them differentially. The influence of water deficit was more pronounced in the first two cycles, especially for IAN 873 and FX 3899. However, as the stress cycles continued there was a recovery of the mean  $PN$  that might be due to the adaptation of the clones to the imposed hydric deficit conditions.

Following irrigation,  $PN$  of stressed plants can either recover completely or remain low for a rather long period depending on the severity of the stress and on the plant species<sup>16</sup>. The lower  $PN$  of re-irrigated clones of IAN 873, IAN 2903 and FX 3899 were probably caused by the damage on the photosynthetic apparatus as there was no post-effect on the stomatal resistance.

Mean values of the transpiration rates for the various clones during the stress cycles are shown in Table 3. The transpiration rates of clones IAN 717, IAN 873, IAN 2903 and IAN 3087 were significantly reduced in the first cycle. After this, there was a general recovery in  $E$  in the fifth cycle.

## CONCLUSION

The stress physiological parameters studied here have the potential to characterise clones suitable for dry regions. Considering the variables studied as a whole, clones IAN 3087 and IAN 6323 were less influenced by the imposed drought conditions while FX 3899 was more sensitive.

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