

## CHAPTER IV

SOME ASPECTS OF TISSUE WATER RELATIONS IN CASSAVA  
PLANT (Manihot esculenta CRANTZ) AND THEIR  
ECOLOGICAL INTERPRETATIONJ. A. Palta<sup>1)</sup>

## INTRODUCTION

To understand how plants respond to drought stress it is necessary to know the relationship between tissue water content and tissue water potential and its osmotic and turgor components (Weatherley, 1970; Wiebe, 1972), especially since it allows the estimation of some additional water relations characteristics, such as the cell wall water content and its elasticity (Tyree et al 1973). Cheung and Tyree (1975) found out that the osmotic pressure at full turgor, as well as at incipient plasmolysis, and cell water elasticity are all closely related to the point where the leaf may osmoregulate or conserve water within a defined range of changes in the environmental water potential, and therefore, may have an adaptative value. Tyree and Hammel (1972) found out recently that these parameters may be determined by the use of the pressure chamber technique developed by Scholander et al (1964-1965). This technique may be used in elaborating pressure-volume curves, relating the osmotic pressure, turgor pressure and water potential of the

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leaves or branches, with the relative water content.

Effects and differences in response to drought stress in two varieties of cassava (Manihot esculenta Crantz), have been suggested and correlated with low capacity water retention soils (CIAT, Annual Report, 1979).

The physiological basis of these intra and inter-varietal differences needs to be established in order to explain selection for drought resistance. Comparative studies of tissue water relations of woody species (Jarvis and Jarvis, 1963), Eucalyptus (Ladiges, 1978B) and in pastures (Wilson et al, 1979) have provided useful data.

More recently, attention has been focussed on the contribution that water contained in the tissues may have on the response to drought stress and the quantification of such reserves as an important part on the correlation of water stress effects.

Two experiments were conducted: 1) To examine the application and other implications of the pressure/volume technique in the study of water stress in cassava plant. 2) To determine the relation of the water content in the tissue and its water potential.

#### MATERIALS AND METHODS

Branches of cassava plant, (Manihot esculenta Crantz), varieties M Col 22 and M Mex 59 were sampled from irrigated plots and also from plots with plastic covered the soil to prevent rain entering the soil during the growing period. Water relations in the field and laboratory were estimated for each variety.

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The pressure-volume technique was used in the determination of characteristics of water relations, based on the use of the pressure chamber (P/V) of Tyree and Hammel (1972); Tyree, Dainty and Beins (1973).

The branches were collected between 07:30 and 8:30 in the morning, placed in water, recut whilst immersed in water and again hydrated for two further hours, but some branches taken to the laboratory were hydrated for 15 hours. Immediately after cutting, the leaf was covered with a plastic bag and inserted in the pressure chamber, leaving only a 1 cm length of the petiole outside. Gas pressure in the chamber was initially increased until the balancing pressure of the water in the xylem was obtained. Later, it was increased and kept at 5 bars for 20 minutes. Water exuded from this cut was easily collected using a vial connected to a glass-tube (5 - 6 cm. long), and the changes in the weight of the tube-bottle gave the measurement of water expressed at that balancing pressure. This procedure was repeated several times and each time the pressure was increased a further 5 bares until pressures of 35-40 bares were reached during 4 hours.

Immediately after removing the leaf from the pressure chamber, the final fresh weight of the leaf was obtained (FWL). Fresh weight of the leaf at a given balancing pressure was estimated by the addition of the weight of exudated water below that pressure, with the final fresh weight of the leaf. The turgid weight of each leaf (TW) was estimated by Ladiges (1975) method, extrapolating the linear relation of balancing pressure (below 20 bars) with the fresh weight of the leaf, to obtain therefore the fresh weight of the leaf at zero pressure (Fig. 1) and the dry weight (DWL) by drying it for 24 hours. Relative water contents (RWC) corresponding to each balancing pres-

sure (variable values of total water potential) were calculated by the following formula:

$$RWC = \frac{FWL - DWL}{TW - DWL}$$

Parameters of osmotic potential at full turgor ( $\psi_{\pi}^{100}$ ), water potential ( $\psi^{\circ}$ ), and water fraction on the cellular wall ( $\theta$  wall) may be derived from the inverse plot of the balancing pressure ( $1/\psi$ ) against the relative water content (RWC), by the extrapolation of the straight line. Other values of  $\psi_{\pi}$  and  $\psi_p$  were calculated from the relations:

$$\psi_{\pi} = \frac{\psi_{\pi}^{100}}{RWC} ; \psi \text{ total} = \psi_p + \psi_{\pi}$$

The module of the elasticity of leaf tissue ( $\epsilon$ ), was determined as follows:

$$\psi_p = \epsilon \left( \frac{RWC - RWC^{\circ}}{RWC^{\circ}} \right)^n$$

Where n is the slope of the linear relation between  $\log. \psi_p$  and the  $\frac{RWC - RWC^{\circ}}{RWC^{\circ}}$ .

## RESULTS AND DISCUSSION

### a) Application of the pressure/volume technique.

Almost all the pressure volume curves have been made in studies of woody species. They usually present a certain curvilinear followed by a linear relationship, but in 10% of the determinations, deflections from the linear lines have been observed at balancing pressures higher than 30 bars.

with relative contents of water lower than 60-55% (Wilson et al, 1979).

It is also possible to observe the sharp decline in balancing pressure in cassava, associated with small changes in the relative water content (Fig. 1); reported by Tyree et al (1973); Ladiges (1975) and Wilson et al (1979). This effect was accepted initially, as an indicator of death of the tissue when over 50% of relative water content is extracted (Tyree et al, 1975). However, the effect on cassava was present only on stressed leaves of two varieties (M Col 22 and M Mex 59), which may also be the result of the sensitivity of the torn part of petiole to the contraction of the rubber plug in the pressure chamber. Stressed leaves of M Col 22 (Fig. 1-A) showed a more severe decrease, making the effect more outstanding than the one presented on stressed leaves of M Mex 59 (Fig. 2-B), which may indicate a higher number of dead cells in stressed leaves of M Col 22.

Some unclear and unconsistant differences were observed among data and preliminary estimations obtained in the laboratory. Such differences may only be associated with the lenght of time of the day during which the leaves were collected, and with the lenght of rehydration period. Godde (1970); Powell and Blanchard (1976) have noticed the same effect in the technique for apple tree leaves sampled in the afternoon. Wilson et al (1979) reported no deficiency in the estimations of the relations of tissue water relations on pastures during hydration periods shorter than 8 hours. However, it is important to consider the effect of latex in cassava, which flows when the branch is cut, due to the positive pressure on the vessles (Esau, 1975); (Ike et al 1978) and thus solidifying the cut surface, covering the xylem vassels, and avoiding rehydration.

## WATER RELATIONS ON UN-STRESSED LEAVES

The curves that relate the balancing pressure with the relative water content in the tissue and their deflections on un-stressed leaves are presented on figures 3A and 3B, for M Col 22 and M Mex 59 respectively.

The two curves present an inflection point in the region of  $0.04 \text{ bars}^{-1}$  where the linear part of the relation begins. By the extrapolation of this linear relationship to the ordinate,  $\psi_{\pi}^{100}$  at full turgor is obtained, while the extrapolation of the abscissa gives the water fraction of the cell wall ( $\theta$  wall). Calculated values from these two estimations in the field and the laboratory are shown on Table 1.

On direct determination in the field, the osmotic potentials of the cells at full turgor ( $\psi^{100}$ ), showed a difference of 1.7 bars between the two varieties, the lower values of -12.4 corresponding to M Col 22. On the other hand, estimated values for water fraction on the cellular wall and the dry weight relations and turgid weight for the leaves (DWL/DTL) are also very similar.

Relative water contents at incipient plasmolysis ( $\theta_o$ ) were the same for both varieties ( $\psi = -25 \text{ bars}$ ), but they are also relatively high if they are compared with the values reported by Gardner and Ehlig (1965) for other species ( $\theta_o = 0.60$ ). Data indicates that excluding the original osmotic potential, estimations in the field do not show large differences among the two un-stressed varieties caused by drought, even though there exists a tendency of lower values from M Col 22. Although the two varieties grew under the same conditions, the tendency of M Col 22 to present a lower original osmotic

potential, reflects a genotypic difference. It is expected, theoretically, that the cellular elongation should not persist under water stress conditions, (Cleland, 1971) on cells with under  $\psi_{\pi}^{100}$ .

Laboratory determined values shown on Table 1 were not taken because in their estimation, the pressure-volume technique was substantially modified with the time of collecting day and the length of hydration period.

#### WATER RELATIONS ON STRESSED LEAVES

Curves relating the balancing pressure with the relative water content in the tissue of drought stressed leaves in both M Col 22 and M Mex 59, are shown on figures 4A and 4B respectively. The shape and trends of the curves are similar to those shown before on un-stressed leaves. Osmotic potentials of the cells at full turgor  $\psi_{\pi}^{100}$ , are similar for both stressed varieties, in a range of -14.2 and -14.6 bars, but were 3.9 and 1.8 bars lower than the osmotic potentials  $\psi_{\pi}^{100}$  of the un-stressed leaves of M Mex 59 and M Col 22. The values for the water fraction on the wall cell. ( $\theta_{wall}$ ), for stressed leaves, raised 5% for M Mex 59 and 1.0% for M Col 22, with respect to un-stressed leaves. The same occurred in the values of the relation between dry weight and turgid weight (DWT/TW). On stressed leaves the increment for both varieties was 0.01. At incipient plasmolysis the water fractions on the cellular wall ( $\theta_{wall}$ ) were equal for stressed and un-stressed leaves of M Mex 59, however the incipient plasmolysis took place at higher water contents in the wall ( $\theta_o$ ) for M Col 22.

Stressed leaves of M Mex 59 and M Col 22 had a tendency to reach a lower osmotic potential  $\psi_{\pi}^{100}$  with a lower reduction of relative water content,

than for un-stressed leaves. This tendency is more remarkable on M Mex 59, and suggests that when the soil dries it establishes a potential gradient between soil water in the leaf. This ability seems to confer certain adaptive value to M Mex 59 grown under water stress conditions over M Col 22 during the growing period.

The minimum increase of water content in the cellular wall ( $\theta$  wall) for stressed M Col 22 leaves correlates well with the blight and drought sensitivity observed in the leaves during the later weeks of stress. Gaff and Carr (1961), suggest that low values of water content on the cell wall ( $\theta$  wall) during adverse conditions, indicate a drying up of the wall and subsequent water absorption from the most available site. If the water content on the cell wall ( $\theta$  wall) works as a regulator of the water loss from the protoplast it may be suggested that the maximum estimated value of the stressed leaves of M Mex 59 (70% approx.) function in the same way during drought stress.

The lack of considerable differences between the values of the relation between dry weight and turgid weight for the stressed and un-stressed leaves of M Mex 59 and M Col 22, suggest that the amount of water in the cell walls is closely related to the different proportions of the macromolecules (hemicelluloses and pectic substances), rather than the thickness of cell wall (Ladiges, 1975).

The differences  $\psi_{\pi}^{100} - \psi_p$ , shows that M Mex 59 present lower values than M Col 22 under normal water conditions, which indicate a higher rigidity in the cellular walls of M Mex 59. However, in water stress conditions, M Mex 59 showed higher values, which indicate a higher flexibility of its walls, more than M Col 22. This condition of keeping an adequate wall flexi-

bility at higher water deficits seems to have certain adaptative value for species which grow well under dry conditions (Cheung et al 1975). Additional estimations conducted 15 days after the end of the dry period (Fig. 5a and 5b) showed that the osmotic potentials of the cells at full turgor returned relatively fast to the levels of un-stressed leaves, in a recovery range of 3.3 and 3.9 bars for M Mex 59 and M Col 22. The tendency of M Col 22 to reach osmotic potentials with less increase of the relative water content suggests a better ability to recover compared to M Mex 59 plants. As a contrast to the estimates of osmotic potential  $\psi_{\pi}^{100}$ , M Mex 59 reached lower levels of the water fraction in the cell wall ( $\theta$  wall) faster than M Col 22.

The lowest and slowest decreases of water content in the cell wall were in M Col 22 (3.5%) rather than M Mex 59 (30%), suggesting that the regulator capacity of the cell wall in M Col 22 may be an indicative factor of toughness or acclimatization to drought, which is due to the higher increase in the hemicellulose and pectic substances production (Gaff and Carl, 1961). This acclimatization factor correlates well with the low values of the  $\psi_{\pi}^{100} - \psi_p$  estimated for M Col 22, during both stress and recovery cycle, which indicate more cell wall rigidity.

The values of the elastic module on the cellular wall ( $\epsilon$ ) which were obtained for each leaf by using the  $\psi_p = \epsilon \left( \frac{RWC - RWC^0}{RWC^0} \right)$  equation (Ladiges 1975), showed that the values are higher in M Col 22 than in M Mex 59, for stressed as well as for un-stressed leaves. However, there are no significant differences between the values for stressed and un-stressed leaves of the two varieties. The values calculated during the recovery period did not show any differences for M Mex 59, but the values were significantly lower

for M Col 22. The data indicates a higher intra-varietal variation rather than a variation between the two varieties, although M Col 22 leaves tended towards higher  $\epsilon$  levels. The elastic module of the cell wall may be a limited characteristic in limited intra-varietal studies, due to a variation between the leaves or branches used. The precise values of  $\epsilon$  and  $n$  may be affected by error sources such as the equilibrium pressure which could only be read around  $\pm 5$  bars and the RWC percentage which makes an exact determination difficult.

Estimations of osmotic potential at full turgor  $\psi_{\pi}^{100}$ , fraction of water in the cell wall ( $\theta$  wall) and relative water content, RWC at 40 bars in stressed leaves, non-stressed leaves, and leaves in recovery from drought stress, indicate that the ability of the leaves in both of the varieties studied is more associated with the recovery of foliar area diminished under stress conditions.

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Table 1.- Values of the osmotic potential ( $\psi_{\pi}^{100}$ ) of cells at or near full turgor, fraction of the total water content residing in the wall ( $\theta$  wall) and the bulk modulus of elasticity ( $c$ ) of stressed, unstressed and stressed recovery leaves of cassava.

Table 1. Values of osmotic potential ( $\psi_{\pi}^{100}$ ) of cells at or near full turgor, fraction of the total water content residing in the wall ( $\theta$  wall) and the bulk modulus elasticity ( $\epsilon$ ) of stressed, un-stressed and stressed-recover leaves of cassava.

		$\psi_{\pi}^{100}$	$\theta$ wall	$\theta$ o	$\psi_p$	$\psi$ total	DWL/TWL	RWC%	$\psi_{\pi}^{100} - \psi_p$	$\epsilon$
M. MEX 59	Lab.	- 9.3	.53	.70	-13.1	-22.4	.28	64	3.8	-----
CONTROL	Field	-10.7	.65	.82	-12.9	-23.6	.30	75	2.2	$2.08 \times 10^3$
	Recov.	-11.2	.36	.66	-16.9	-28.1	.22	53.6	5.7	$1.98 \times 10^3$
M. MEX 59	Lab.	-10.7	.52	.60	-17.8	-28.5	.25	58.3	7.0	-----
STRESSED	Field.	-14.6	.70	.82	-17.8	-32.4	.31	80.5	3.2	$2.88 \times 10^3$
	Recov.	-11.3	.41	.70	-16.1	-27.4	.22	56.7	4.8	$1.94 \times 10^3$
M. COL 22	Lab.	-10.8	.80	.88	-12.2	-23.0	.26	86.0	1.4	-----
CONTROL	Field	-12.4	.64	.82	-15.0	-27.4	.27	76.0	2.6	$4.06 \times 10^3$
	Recov.	-10.2	.66	.80	-12.8	-23.0	.27	75.0	2.5	$2.06 \times 10^3$
M. COL 22	Lab.	- 9.7	.35	.65	-14.9	-24.6	.26	50.4	5.2	-----
STRESSED	Field	-14.2	.65	.85	-16.7	-30.9	.28	78.0	2.5	$4.89 \times 10^3$
	Recov.	-10.3	.61	.79	-13.1	-23.4	.27	70.0	2.7	$1.99 \times 10^3$

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- Fig. 3A - Relation between inverse balancing pressure and tissue relative water content for leaves of (A) M COL 22 and (B) M MEX 59.
- Fig. 4A - Relation between inverse balancing pressure and tissue relative water content for stressed cassava leaves of (A) M COL 22 and (B) M MEX 59.
- Fig. 5A - Relation between inverse balancing pressure and tissue relative water content for stressed-recovery cassava leaves of (A) M COL 22 and (B) M MEX 59.

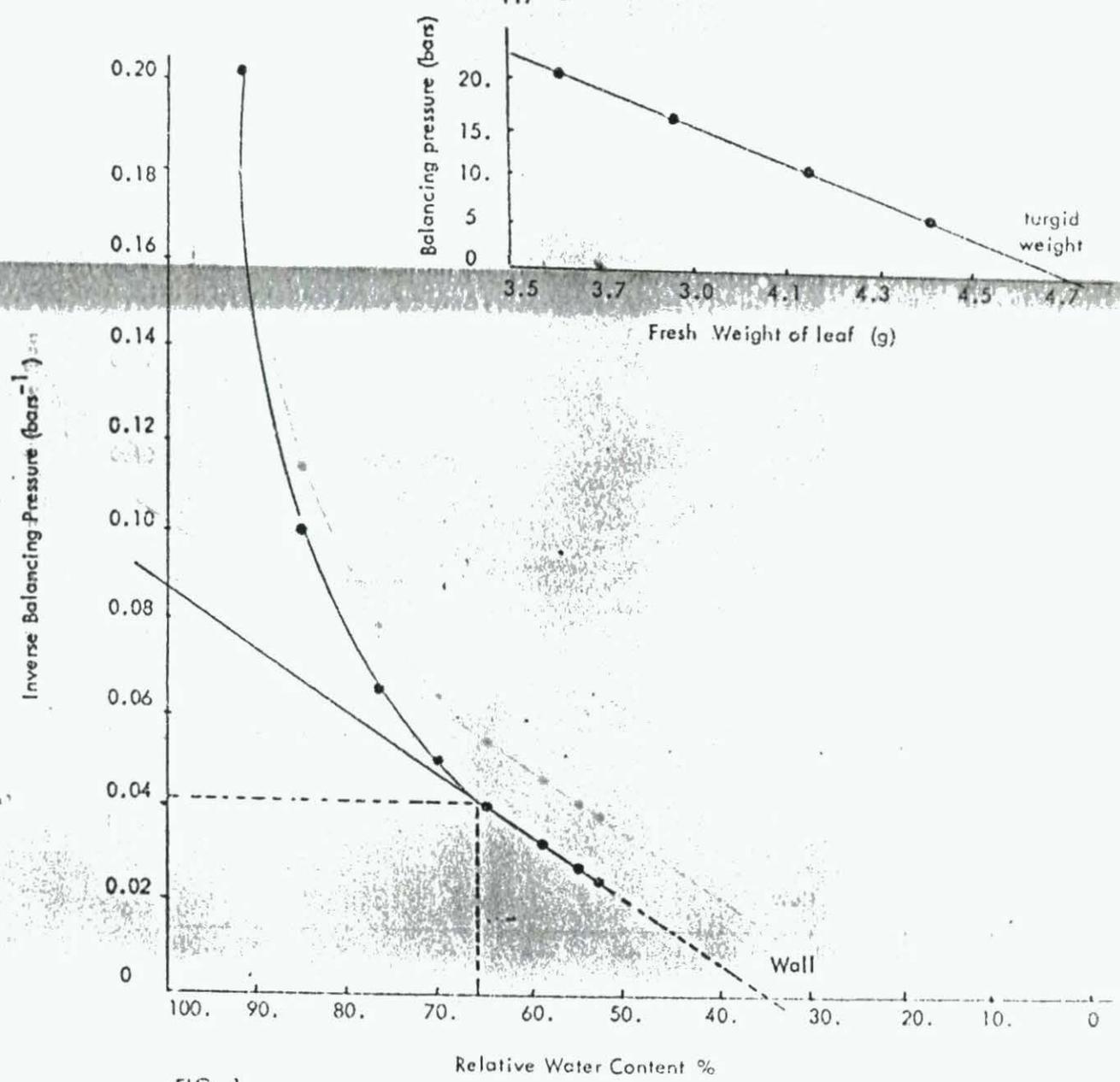
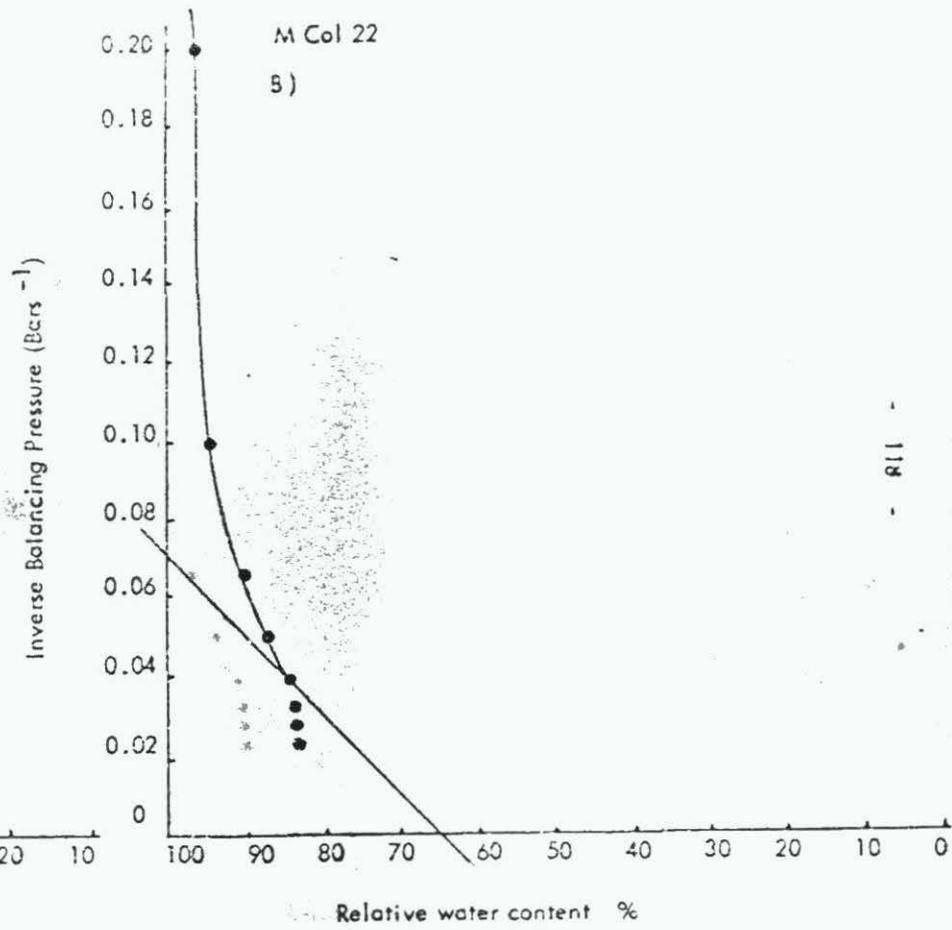
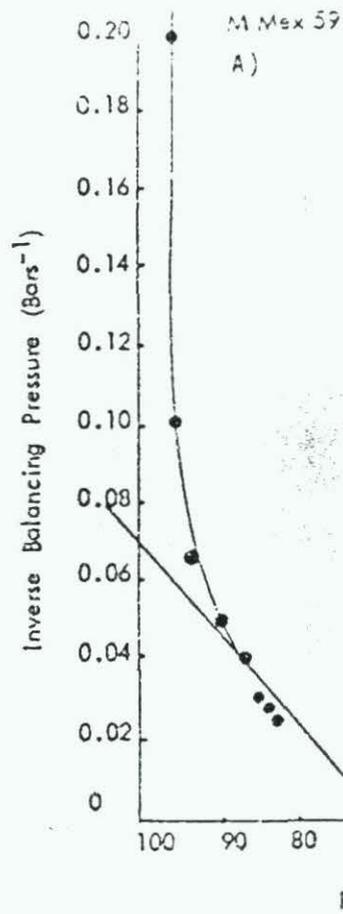


FIG. 1  
Derivación de los parámetros físicos de la relación contenido relativo de agua (CRA) recíproco del potencial de agua total ( $1/\psi$ ) y potencial de presión ( $\psi/p$ ) en yuca.



FIGS. 2A - 2B. Degeneración de la porción lineal de la curva de presión/volumen en hojas de dos variedades en stress por sequía.

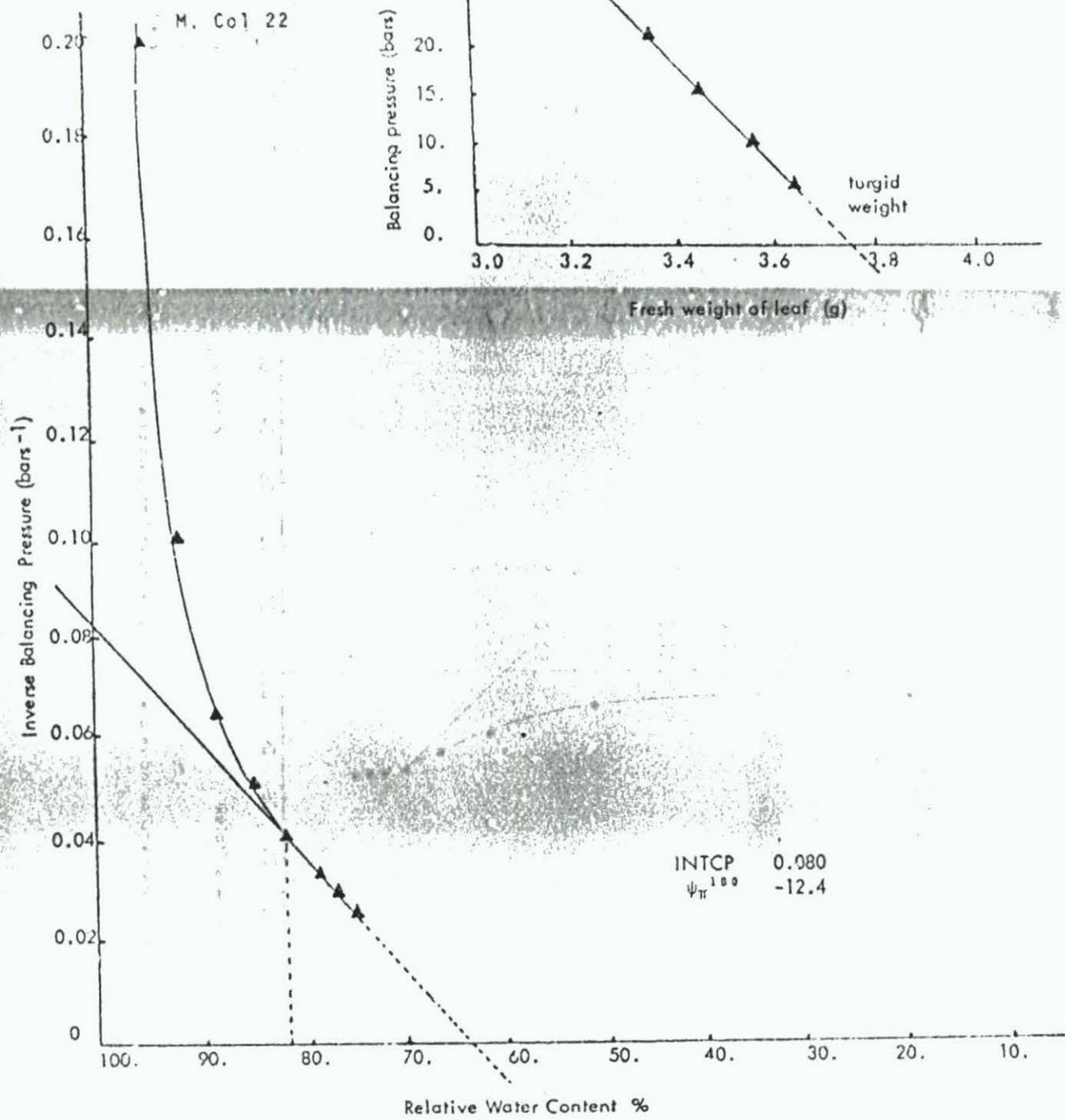


FIG. 3A.  
 Relación de la presión de balance y contenido relativo de agua en el tejido de hojas de yuca, variedad M Col 22 a regímenes normales de agua.

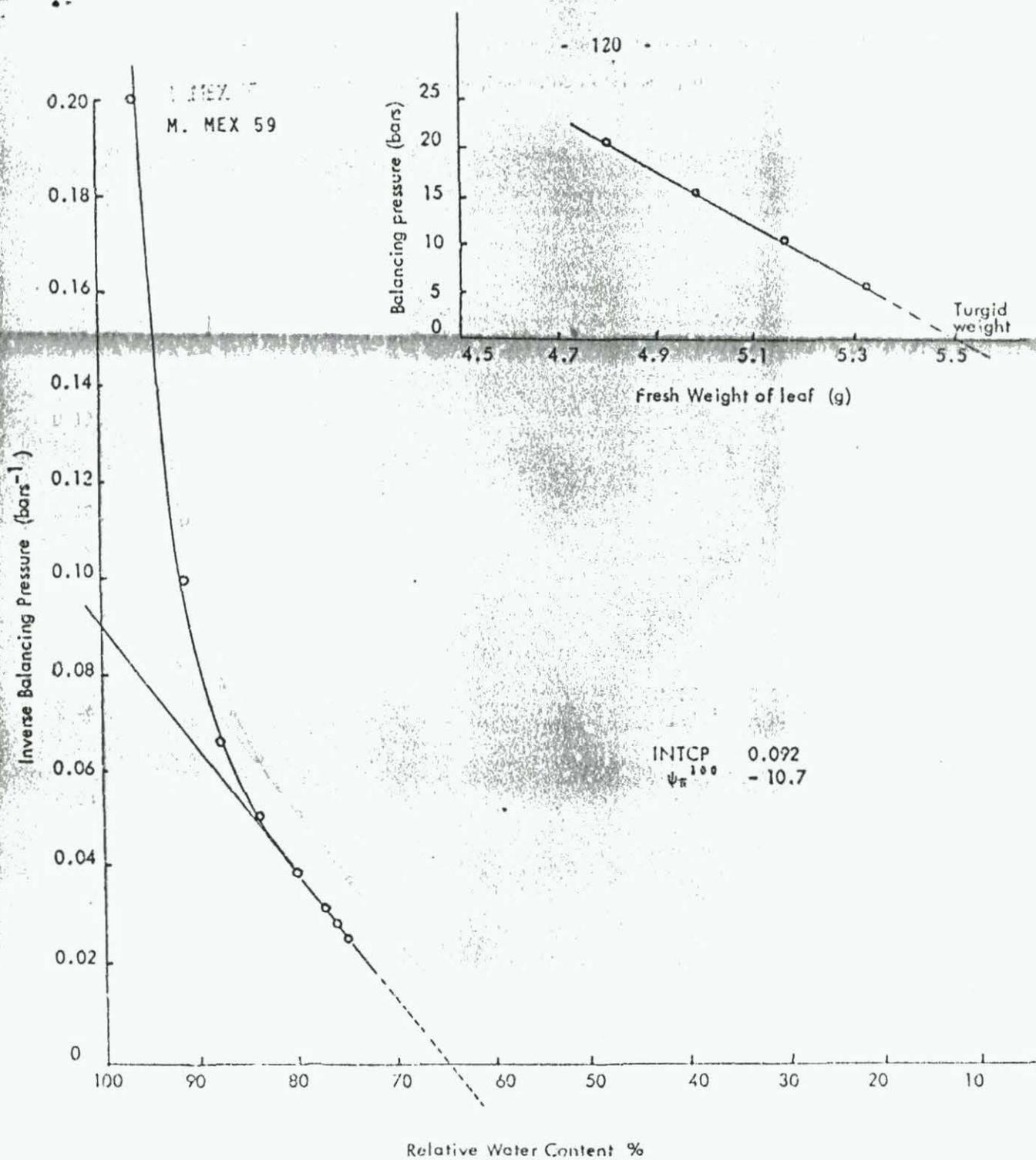


FIG. 3B. Relación de la presión de balance y contenido relativo de agua en el tejido de las hojas de yuca, variedad M Mex 59 a regímenes normales de agua.

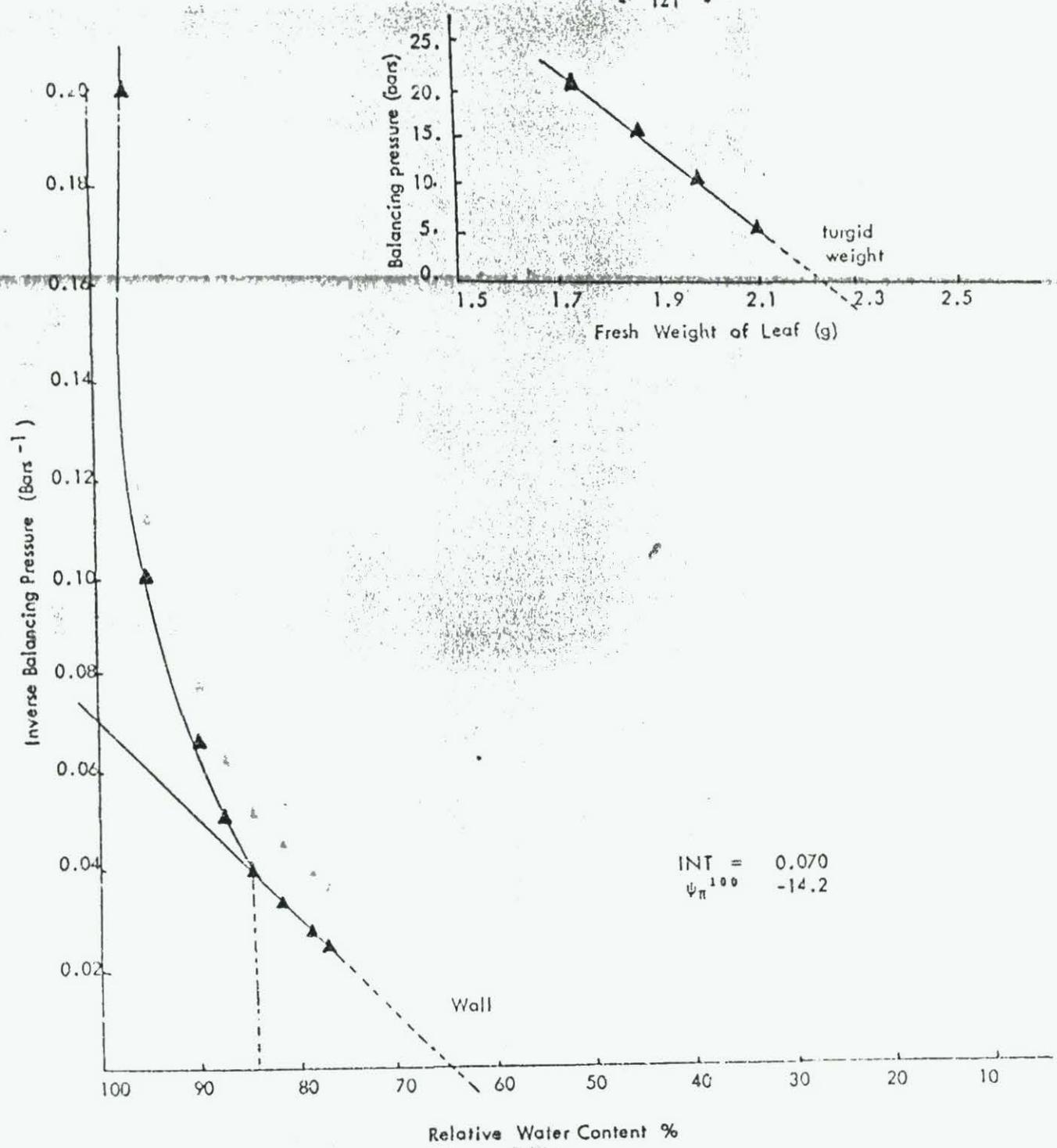


FIG. 4A.

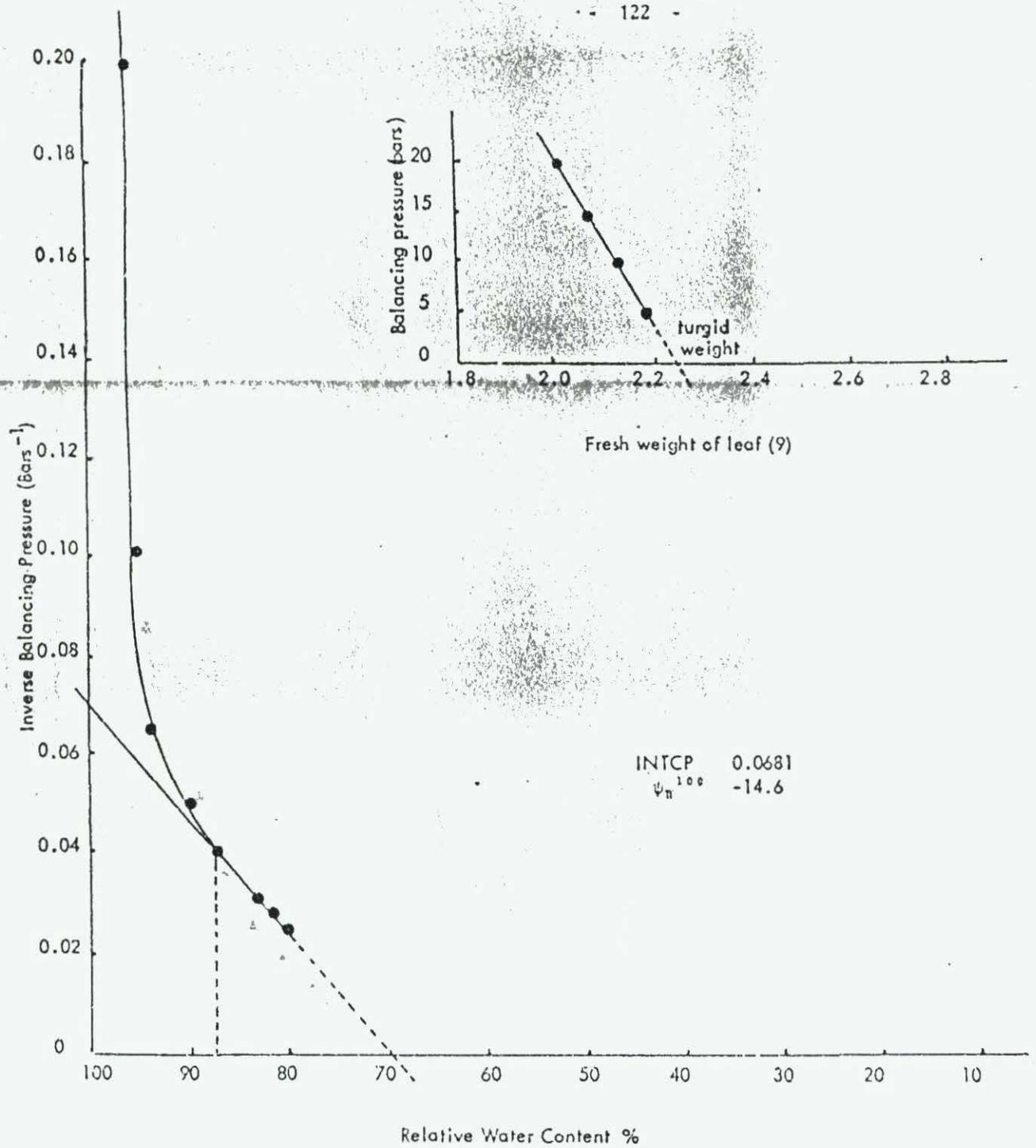


FIG. 4B. Relación de presión de balance y contenido relativo de agua en el tejido de hojas de M Mex 59, stressados por sequía.

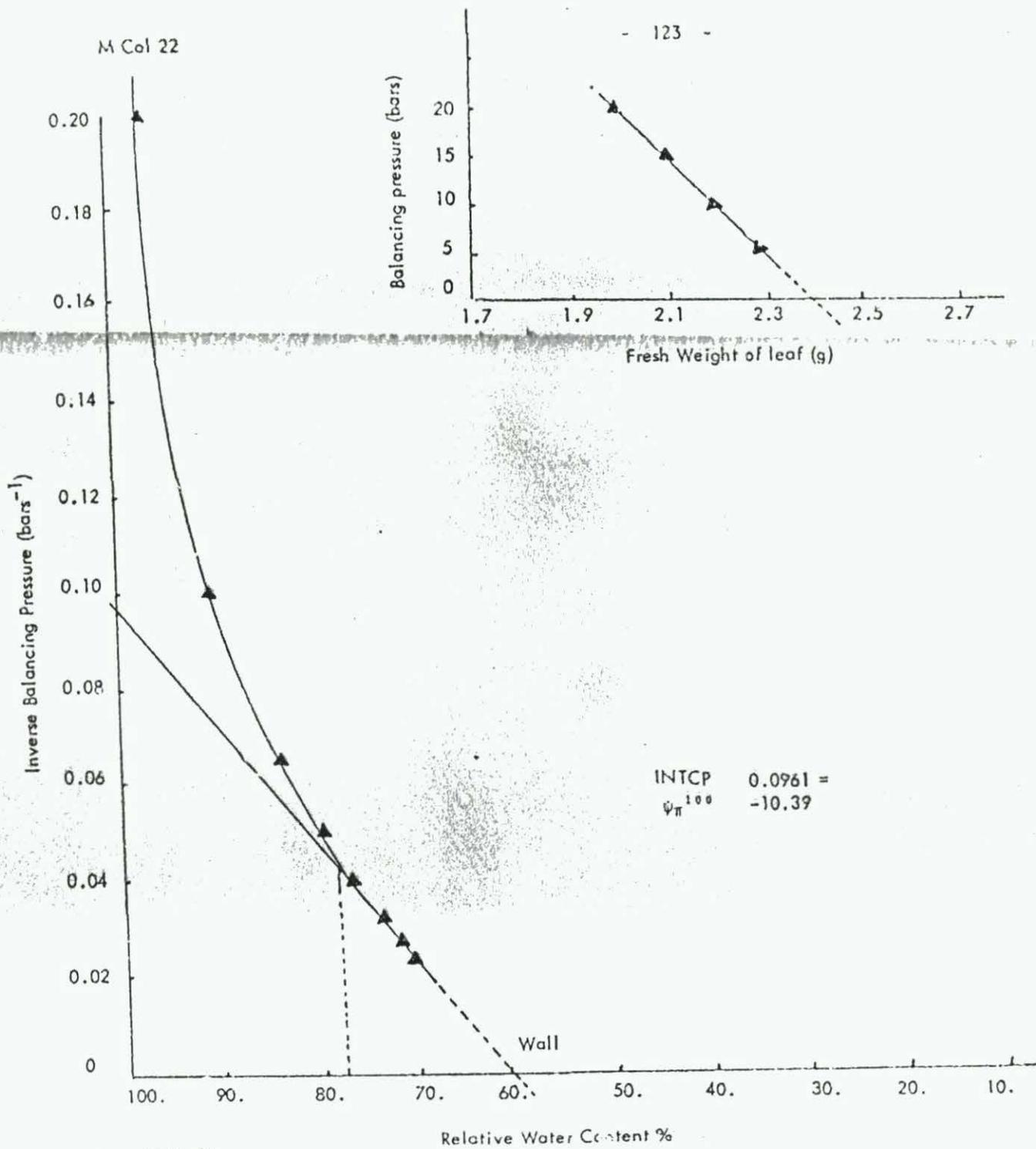


FIG. 5A  
 Relación de presión de balance y contenido relativo de agua en tejidos de hojas de M Col 22, dos semanas después de terminarse el ciclo de stress.

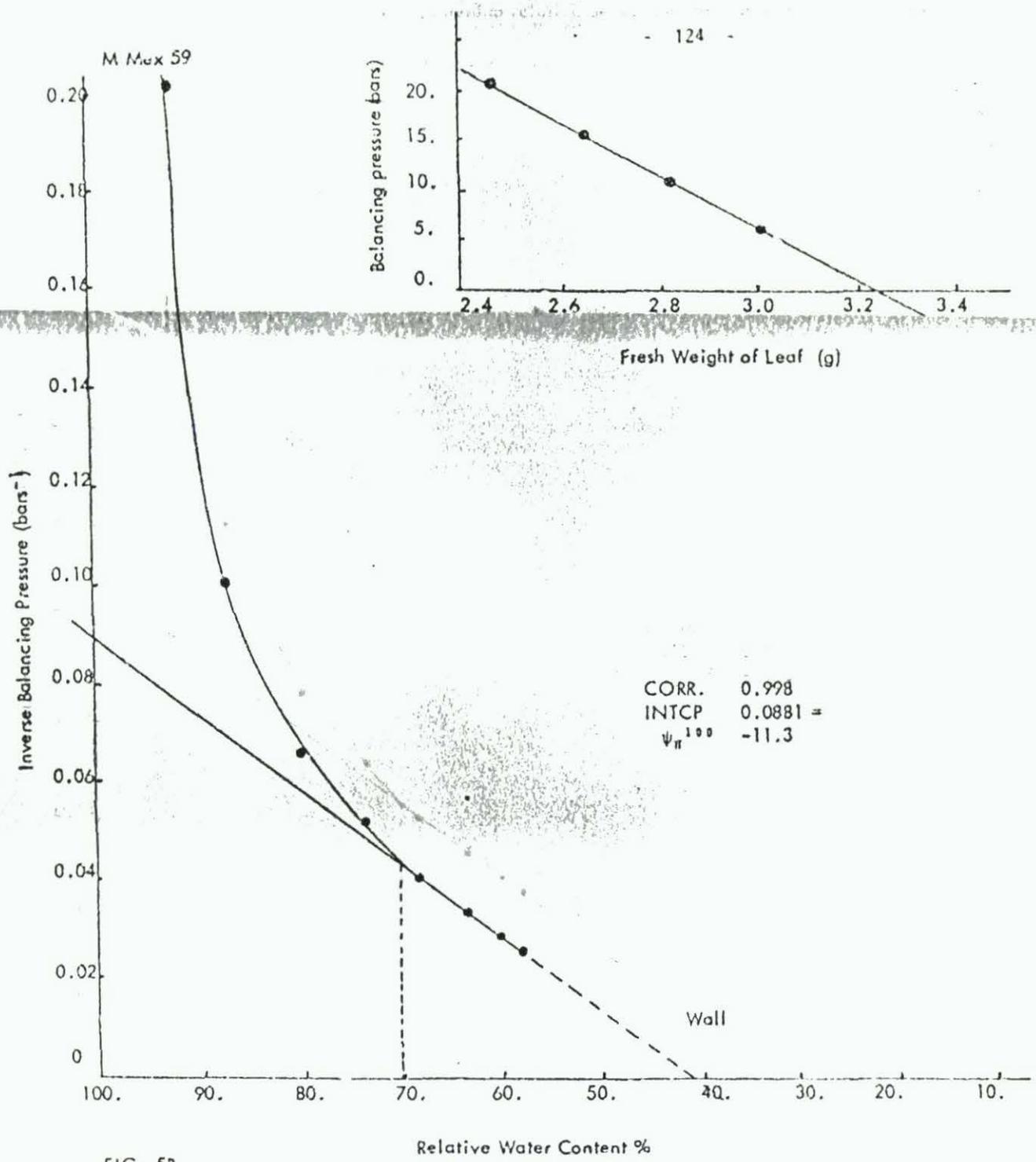


FIG. 5B  
 Relación de presión de balance y contenido relativo de agua en tejidos de hojas de M Mex 59,  
 dos semanas después de terminarse el ciclo de stress.