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Water Relations and Drought Adaptations in *Pisum sativum*

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Abstract.

The work reported here concerns the analysis of some aspects of the water relations of the adapted plants, The pressure chamber technique during and after water stress was used and light microscopical observations were made to study the cellular and anatomical adaptations . *Pisum sativum* plants were used for this work as a model system for drought adapted plants. We reported earlier that these plants are able to attain drought adaptation.

Important features identified in this study as indicators for drought adaptation in this study are the ability of the plants to: 1) maintain high water potential (-6 to -8 bars) and turgor pressure at water deficit conditions; 2) rapid close stomata under water shortage conditions; 3) accumulate solute during a long recovery period; 4) accumulate K^+ in the intercellular space, 5) retain more living stem parenchyma cells under water deficit conditions, and to develop smaller vascular bundles and xylem vessels.

Despite the development of these features during adaptation, small changes in internal water balance (both water and osmotic potential) were found to be related to larger changes in membrane properties especially those involved in K^+ uptake from the medium. This is in line with our earlier observation that membrane adaptation increased lipophily which could be an important factor for drought resistance.

Introduction.

Mild water deficit conditions for higher plants are often reported to show beneficial effects on plant productivity, but the physiological bases for these effects are still little known.

Drought hardening is based on the ability of plants to adapt to water deficit conditions, and generally is thought undesirable because drought hardening is frequently associated with a reduction in productivity. The ability of plants to adapt depends greatly on the growth and developmental stage (Lee-Stadelmann and Stadelmann, 1979a). Recent reports emphasize that drought received during the early vegetative developmental stage is beneficial to productivity. Most studies on water stress adaptation so far relate mainly to the productivity during water stress conditons.

Osmotic adjustment is frequently associated with other features of drought adaptation, such as the potential to develop deeper roots under stress conditions e.g. in some relatively drought resistant species or varieties e.s. Sorghum; Lee, 1975; Turner and Jones, 1980; Acevedo et al., 1979). These plants are able to grow continuously under stress conditions by maintaining turgor potential with decreasing osmotic potential (Hsiao, 1973). Some drought sensitive crop species with more shallow root development (Pisum sativum, Solanum tuberosum, Phaseolus) are unable to continue growth under water stress conditions. Solanum tuberosum, however, resumed leaf growth at the same rate or even faster after rewatering following the stress treatment (Lee-Stadelmann et al., 1979; Levi, 1983).

Protoplasmic tolerance on the other hand was significantly increased in Pisum sativum plants when they are stressed during the early vegetative stage.

In this study, we specifically investigated the water relations of drought adapted plants during the recovery stage, especially the role of osmoregulation. We present data on cellular and membrane qualities which can be used as a basis for evaluating drought adaptation features which may be involved in producing the beneficial effect of drought after recovery.

Material and Methods.

Plant Growth and Water Stress

Pisum sativum L. var. Alaska (pea) plants were grown in vermiculite in a controlled environment with a 15 h. photoperiod. Plants were water stressed when seedlings were 7 days old by withholding water for 10 or 17 days because of the high water withholding capacity of vermiculite this leads to a slow drying of the plants. Details of planting and the water stress regime were the same as described elsewhere (Lee-Stadelmann and Stadelmann, 1979a). After 10-17 days of water stress, plants were rewatered, and during this period water relation parameters were measured. The early stages of plant development, therefore, occurred under water stress conditions.

For the late stress experiments it was necessary for the growing medium to dry fast. To make this possible, soil was used instead of vermiculite. A green house soil mixture (soil:sand:peat = 1:2:1) was used. In this case, water was withheld from plants 2 days after planting. The same stress effect was reached in soil mixture at 3 to 5 days after stopping of watering as in vermiculite after 7 to 10 days.

Plant Productivity

Leaf growth is extremely sensitive to water stress and dependent on turgor pressure and photosynthetic acitivity. Therefore, leaf growth is generally used as an indicator for plant productivity. For *Pisum sativum*, because of the small leaf size, we found plant height (from first internode to shoot tip) to be a more accurate measure than the length or size of a small expanding leaflet.

Leaf Water Potential Measurement

Leaf water potential was measured by the pressure chamber method. Petioles with a pair of leaflets (length 1 cm) were cut with a razor blade and the pressure measured in a moisturized pressure chamber. Measurements were made in mid-photoperiod.

Stomatal Opening

For testing the stomatal opening, either porometer, silica impression, or direct microscopic observation by incident light microscopy, were used. For larger leaves (e.g., beans or potatoes) the porometer was used. Small leaves (peas), were evaluated using silica impression and by direct microscopic observation.

Leaf elongation

For measuring leaf elongation the youngest expanding leaves were used by determining their length with a ruler with millimeter divisions.

Measurement of Solute Potential by Pressure-Volume Curve Analysis

The fourth or fifth leaf with 1 paired leaflet was removed from the plants. The cut end of the petiole was immediately immersed in a beaker with water and placed in a bell jar which was lined with wet paper towels in order to maintain 100% humidity. The bell jar was kept overnight in a cooler (4 °C), usually for 12 hours before the measurement began.

The next day the fully saturated leaves were first weighed, the leaf was mounted in the usual way into the pressure chamber, and by stepwise increase of the air pressure the xylem sap of the leaf was collected for 10 minutes at each pressure step in a preweighed cotton ball stuffed in the top part of a plastic pipette tip. It was previously determined that the pressure equilibrium time was about 10 minutes. The pressure was increased by an increment of 2 bars usually up to 16 bars or as far as the tissue could withstand the pressure. The whole procedure for the sap collection took about 2 hours. After the sap collection and measurement, the leaf sample was oven dried for the dry weight measurement.

The amount of leaf tissue water was calculated as relative water content (RWC):

RWC = (saturation wt. - dry wt. - Amt. of cumulative water collected in cotton balls) / (saturation wt. - dry wt.)

The pressure-volume curve was constructed following the Richter method as a type I graph, (Richter 1978; Kim and Lee-Stadelmann, 1984; Stadelmann, 1984) by plotting the inverse of the RWC against the external equilibrium pressure for individual measurements (see Fig. 1.)

The measurements were made with leaves from the stress-rewatered plants (10 days of water stress followed by eighter 10 or 18 days of rewatering), and the control plants of corresponding age for comparison. Important water relation parameters such as Ψ_s(sat), leaf water potential at full saturation, and $\Psi_{s(R)}$, leaf water potential at the cell relaxation point, were derived from the regression analysis of each graph (Tables 1 and 2). Ten measurements were made for each of the two rewatering regimes (10 days and 18 days following 10 days of water stress) and ten measurements for the ccontrol of each corresponding age. Therefore, a total of 40 individual measurements were performed.

Test for K^+-Accumulation

Tissue strips of the stem basis of pea plants were placed in dishes with 100 mosM KCl for three hours, next the cells were plasmolyzed in mannitol solution. The solute potential of individual subepidermal cells was calculated before and after KCl treatment from measurements of the cell sap concentration using the plasmolytic method. The difference in solute potential before and after KCl treatment is considered to be an indicator of the accumulation of KCl within the vacuole.

Histochemical techniquees specific for K^+, using sodium cobaltnitrite and $(NH_4)_2S$ were used, according to Willmer and Mansfield (1970). This test shows the localization of K^+ within the tissue, and is especially useful to test K^+ presence in the intercellular space.

Stem (first internode) sections of pea were made freehand, sufficiently thick so that one intact cell layer was obtained (usually 100-150 micrometers). Leaf cross sections were made, using the Vibratome, usually 80 to 100 micrometers thick. After the K^+ treatment, the tissues were examined for K^+ localization under the light microscope.

Anatomical characteristics of leaves and stems of drought adapted pea were also observed under the light microscope.

Turgor Pressure Measurement by the Tissue Curling Method

A simple technique was used to indirectly measure turgor pressure: Internode stem segments (7 mm in length) were prepared from the first internode and infiltrated in spring water for 2 minutes (Lee-Stadelmann and Stadelmann, 1979a). From this stem section tissue strips (1 x 7 mm) were prepared using a curved tweezer with fine pointed tips. The tissue strips contained 3 cell layers (epiermis, subepidermis and parenchyma). Infiltration of the stem segments removes the air from the intercellular space and also facilitates the stripping off of the tissue. The tissue strips were floated on spring water for 3 hrs. under laboratory conditions. After 3 hrs., the curling index of the strips was recorded (∅ to 4; 1 = not curled, 4 = most curled ;see Fig. 8).

The curling of tissue strips is caused by unequal wall extension of the different cell types in the tissue. Parenchyma cell walls are thin and expandable while the outer walls of epidermal cells are thick, covered with a cuticle and therefore unable to expand. Thus, when cells take up water, only the parenchyma cell walls expand causing the convex curvature. The curling is a result of turgor pressure of the tissue.

The same tissue strips were plasmolyzed in mannitol, and solute potential and permeability for methylurea were measured for the subepidermal cells. The procedures for the determination of solute potential and permeability are described elsewhere in detail (Lee-Stadelmann and Stadelmann, 1976; Stadelmann, 1966).

Results.

Plant Growth Pattern During Early Water Stress and Recovery Period:

Because water stress began at the time of the emergence of the shoot from the soil line (when the shoot was about 1 cm long), most internodes were developed under water deficit conditions. The relative water content of vermiculite during the 2 week stress period decreased from 400% (on dry weight basis) to 100%, and 400% to 220%

for water-stressed plants and well-watered plants respectively (Lee-Stadelmann and Stadelmann 1979a). Growth of stressed plants during the 2 week stress period was only about 60% of the control plants. All leaves remained completely turgid during the stess period, and while the leaf size and length of the internode of stressed plants were reduced, the number of internodes was not. On rewatering, regrowth, mainly resumption of reduced leaf and internode growth took place. This was noticeable after one week. The stressed plants were completely recovered 18 days after rewatering, without any loss of lower leaves.

Water Relations of Drought Adapted Plants.

Leaf Water Potential Ψ_w of Drought Hardened Plants

Figure 2 shows the leaf water potential of pea plants during the complete vegetative growth period for (1) continuously watered plants (controls), (2) for plants which were submitted to a 17 day stress period (from day 7 to day 24) i.e. rewatered from day 25 on, and (3) plants under continuous water stress from 7 day on.

The leaf Ψ_w of well watered control plants remained nearly constant at -0.5 MPa throughout the entire growth period for all leaf levels. The Ψ_w of leaves from level 2 (old leaf) of plants under continuous water stress decreased from -0.5 MPa after 1 week of stress to -1.2 MPa after 4 weeks of stress, but no wilting occurred. For mature and young leaflets (leaf level 4 and 6), the measurements were possible onlv 21 and 28 days after planting, respectively. It is noteworthy that for all plant and leaf levels under continuous stress, the Ψ_w value increased substantially at around 29 days (flower initiation stage). This is shown as a sharp peak followed by a steep decrease. Obviously, there seems to be a reallocation of the internal water within the plants.

Upon rewatering the drought hardened plants, the Ψ_w at all leaf levels recovered completely to the control values within 3 days, but later underwent a transient lowering of the water potential. After the 3rd day of watering Ψ_w decreased gradually with increasing days of rewatering (see Fig. 1) The Ψ_w of the leaf of the drought hardened plants was clearly lower (by about -0.2 MPa to -0.3 MPa) than those of the control plants between 4 to 7 days after rewatering. Leaf Ψ_w completely recovered after 9 days to the well watered control values.

<u>Osmoregulation</u>

The solute potential of the stressed plants at the end of the water stress period was only about 0.1 MPa lower than that of well watered control plants. The stressed plants recovered their solute potential almost completely within 3 days of rewatering (see Fig. 3). The solute potential of the stressed plants, however, subsequently began to decrease from -0.8 MPa to -10.5 MPa during 18 days of re-watering. The solute potentials of the control (continuously watered) plants decreased only from -0.82 MPa to -0.9 MPa. This can be inter-preted as a delayed osmoregulation in the drought adapted plants.

<u>Leaf Water Potential Gradient along Leaf Levels</u>

The drought adapted plants (Fig. 4) showed a characteristically different leaf water potential gradient than the unadapted plants (Figure 5) for the leaf levels from the bottom (old) to the top of the plant (young): During the drought adaptation period when growth is still taking place (but reduced) the youngest leaves showed the lowest potential and the lower mature leaves the higher potential (Fig 4a).

Under more stress where growth completely stopped, the water potential gradient from the bottom to the top leaves was very small (Fig. 4b,c) The unadapted plants (well watered control) showed a different gradient pattern at age 28 days (Fig. 4b): a kind of reversed gradient developed at the level of the leaves which were actively growing. There was a tendency to show a low potential in the youngest leaves and the highest in the mature leaves (middle), whereas the lowest leaves (closest to soil line) showed high potential of about of the same magnitude as the youngest leaves (Fig. 4b).

When 28 days old unadapted plants were subjected to late drought stress (Fig. 5), the leaf water potential quickly declined in all leaves, and the lowest water potential appeared in the youngest leaves which were still qowing at the expense of water and nutrients from the mature, dehydrating leaves.

K^+ Accumulation in the Drought Adapted Plants

Stem cells of drought adapted plants accumulated more K^+ than the cells of unadapted plants when K^+ was externally applied (Table 3), resulting in greater differences in solute potential between the adapted and control cells.

Cells from mature unadapted plants which were subjected to such severe late water stress that they remained wilted, lost the ability to take up K^+ from external medium.

The cytochemical determination of K^+ showed that the xylem parenchyma cells have accumulated more K^+ in the adapted plants than in the unadapted plants (Figure 6). A higher K^+ accumulation was also observed in the extracellular spaces of the drought adapted plants.

Increased Turgor Potential and Membrane Activities

A linear relationship of relative turgor pressure (expressed as curling index) to osmotic potential as well as to permeability for methyl urea (Figure 8) was found: tissue strips with little or no curling after tissues were allowed to become fully turgid, showed lower osmotic potential and lower permeability for methylurea than the curled tissue with apparently higher turgor pressure. This relationship also indicates that there is a proportionality between permeability and solute potential. The cells with higher solute concentrations showed generally higher permeability for methyl urea than those with low solute concentration.

Anatomical and Cellular Changes During Drought Adaptation

The cross section of stems from drought adapted plants which grew under water deficient conditions (between -6 to -8 bars), revealed reduced vascular systems both in number of vascular bundles and number of vessels (Figure 7). This reduction of the conducting system may be important for reducing water loss. Mature stems of unadapted control plants usually have a central cavity. The stems of adapted plants had a smaller central cavity, an indication of an increasing number of living pith parenchyma cells that may be related to a higher degree of nutrient accumulation. The adapted leaves have a slightly thicker cuticule, both palisade and spongy layers are thinner than in the controls. The number of stomata per unit area was also higher in the adapted leaves.

Discussion

Drought hardening has been practiced by subjecting plants to several cycles of drought (e.g. potatoes, Levi, 1983). Our results demonstrates however, that one cycle of drought is sufficient to induce drought adaptation which is persistent during the complete vegetative life cycle after rewatering. One cycle hardening was also found sufficient for potato plants (Lee-Stadelmann, et al., 1979).

The delayed osmoregulation of stress conditioned plants, observed after an extended recovery period, is reported here for the first time. Since the change in osmotic potential was only a few bars, it is important to ensure that this decrease is not an artifact of technique. To prove this, the decrease in osmotic potential was confirmed by using the plasmolytic method as a second method, which also indicated a decrease of the solute potential fo a few bars below the control value (Lee-Stadelmann and Stadelmann 1983).

Decreased solute potential, or osmotic adjustment under water deficit, has been cited as an important basis for drought resistance (Turner and Jones, 1980; Acevedo, et al., 1979), and species or varietal differences in osmotic adjustment has been reported (Acevedo, et al., 1979; Levi, 1983). Osmotic adjustment is thought to be important to maintain cell turgor, which, in turn, may be important for photosynthesis and leaf growth (Hsiao, 1973).

Relations between osmotic potential and turgor pressure have to be interpreted with caution, especially when only small differences of the osmotic potential are measured. Turgor pressure is a result of the osmotic potential produced by solute accumulation inside the vacuole. Most osmotic potentials reported in the literature are calculated, however, from the press sap, which is the sum of the total solute concentration in the tissue, extracellular space, vessels and vacuoles.

Osmotic adjustment (solute accumulation) under stress conditions did not appear to play a role in turgor maintenance and growth. Levi (1983) have clearly demonstrated that osmotic potential and turgor pressure has no relation to drought resistance in 5 potato varieties tested under water stress conditions. Furthermore, the increased solute accumulation under stress conditions which is responsible for increased turgor pressure, seems to be associated with deeper root systems and therefore with a better supply for water and ions (cf. Van Loon, 1981; Levi, 1983). Krug and Wiese (1972) reported that low soil moisture (only when applied during the early growth stages) increased productivity partly by lengthening the period of high growth rate and partly by increasing rates of growth and net assimilation in potato leaves. They have clearly demonstrated the beneficial effects of early stage drought on productivity.

It is suggested that two types of drought acclimation should be distinguished in crop species:

1) drought acclimation by deep rooting and continuous root and shoot growth under water stress conditions by maintaining turgor and stomata opening, such as in Sorghum (e.g., Avecedo, et al., 1979); and

2) species that usually have more shallower root systems (e.g., Pisum sativum, Solanum tuberosum, Phaseolus sp.), and acclimate to the drought by condition early stomatal closure and by stopping growth, which leads under certain conditions to the accumulation of solutes.

We will elaborate on the second type of adaptation, because this type has been so far considered as a drought sensitive species, judging from their performances under water stress conditions. We have observed that during the vegetative growth period of S. tuberosum, leaves which were severely wilted completely recovered after rewatering, and their growth was even enhanced after several weeks of rewatering (compared to continuously watered plants). Levi (1983) also observed that potatoes subjected to stress of up to 30% relative humidity had a higher yield, after recovery from stress. Similar observations on yield were made with tomatoes (Rasco, J.R., 1976), where hardening furthermore resulted in lower transpiration rates and larger proline content. Whether the enhanced resistance to drought (including protoplasmic tolerance) and wilting is characteristic of the Solanaceae needs to be investigated. Nevertheless, from these observations it seems clear that the sensitivity of leaf growth and stomatal closure during the water stress period is rather beneficial for the performance of the plant after removal of water stress.

One typical response of the "drought sensitive" species (e.g. Pisum or Phaseolus) is yellowing or drying of lower leaves under water deficit, irreversibly reducing the assimilating organs. We have demonstrated that this yellowing can be prevented in Pisum sativum plants by hardening, i.e. by subjecting the plants during early seedling stages to very mild stress conditions. This acclimation produces only a small decrease in osmotic potential usually -2 to -4 bars. This is too small to account for the differences in their resistance to water stress (Cutler and Rains, 1978).

Our results indicate that K^+ is an important osmotic component in drought adaptation. Both the ability to accumulate K^+ or the prevention of cellular K^+ leakage under water stress conditions are integral components of drought adaptation. K^+ is the only inorganic cation known to increase under stress conditions in the tissue above the level of well watered tissue (Munns et al., 1979). Our results are the first to show more K^+ accumulated in hardened tissue.

We proposed that cellular and membrane alterations which promote K^+ accumulation are brought about during hardening (Lee-Stadelman and Stadelmann, 1979b). It remains to be tested whether this increase of extracellular K^+ has a direct impact on membrane functions by protecting membrane enzymes, or indirectly by interacting with other membrane-protecting substances. Proline and betaine are frequently observed solutes during stress and are often related to drought resistance (Jones, et al., 1981).

K^+-localization tests revealed that K^+ in control and stressed tissue was present, especially in chloroplast-containing parenchyma in the stem tissue. There are reports that in Pisum sativum plants, chloroplasts have higher KCl concentrations (250mM) than the cytoplasm (100mM). High concentrations of KCl (250 mM) was inhibitory to many cytoplasmic enzymes, except for enzymes in the chloroplast (Larrkum, 1968; Larkum and Bonner, 1972). It maybe important to maintain high K^+ levels in the chloroplast for the normal functioning of chloroplasts. The level depends, however, on the cytoplasmic pools of K^+. Under stress conditions when K^+ leaves the cell, a corresponding amount of water also leaves the cell. The extracellular K^+ may prevent further leaking of potassium. Extracellular accumulation of K^+ is possible when stomata are closed, while ion uptake through roots still takes place under stress conditions. Ion uptake into the roots is generally less sensitive to stress than the migration of the ions in the root to the vascular bundle (e.g., Greenway et al., 1969). Therefore, early stomatal closure seems to be an important factor for a higher degree of accumulations of K^+, and thus, for drought adaptation.

The hypothesis of the key role of K^+ in drought stress preventation, however, cannot explain the results of Cutler and Rains (1978) who found that hardened cotton leaves with -2 to -4 bars lower osmotic potential showed no significant differences in the main osmotic components of the cell sap (K^+, NO_3, sucrose) from unhardened plants. They concluded that the drought resistance was partly contributed to by cell structrual changes during hardening.

Cell wall elasticity may also play a role in drought resistance (Kim and Lee-Stadelmann, 1984; Stadelmann, 1984). It could be visualized that specific substances act as stress protectants, (prolins and betaine) while K^+ accumulated in the cell wall may be involved in altering the cell wall elasticity. But we were yet unable to numerically relate the elastic modulus to stress adaptation because of high tissue to tissue variability. However, qualitatively, the hardened tissues are generally more elastic (i.e., have a lower elastic modulus) than unhardened tissue.

It is worth noting that in tomato petiole tissues, collenchyma cells are more sensitive to drought stress than the thin walled pith or xylem parenchyma cells under the same extreme water stress conditions. When collenchyma cells are decreasing their volume under severe stress conditions they do not more recover upon later availabilities of water as do the parenchyma cells (unpublished data).

Conclusions.

Our data show that the osmoregulation (osmotic adjustment) attained during the 10 day water stress period is lost temporarily within 3 days after rewatering. However, the osmoregulatory ability is regained and a significant decrease in the osmotic potential results about 10 days after rewatering ("delayed osmoregulation"; Fig. 3).

The solute increase in the vacuole during the water stress may be a passive accumulation as a result of starch hydrolysis and growth cessation. This can be reversed relatively quickly upon removal of water stress, as observed after about 3 days Small decreases in osmotic potential per se do not seem to be an important factor for drought resistance in Pisum sativum.

The later observed decrease in the osmotic poteintial may be caused by a solute increase during the following days of the re-watering period and may result from an active accumulation of K^+-ion as caused by the "acclimation" of the membranes which took place during the water stress.

In peas, both greater cell wall elasticity, and osmotic adjustments contribute to the cellular adaptation to drought resistance. A decreased bulk elastic modulus (more elastic) is an important cell wall elasticity parameters for drought adaptation.

Early closure of stomata may lead to the accumulation of K^+ in the cell wall or intercellular spaces, which may result in the protection of the membrane under water stress conditions. Our results on recovery of growth after the end of the stress period indicates that growth reduction under water stress, is not a reliable criterion for sensitivity of crops to water stress, as frequently assumed.

The physiological basis for the renewed osmoregulation several days after recovery from water stress has to thought to be related to an enhanced membrane activity (membrane acclimation) during the adaptation period. One cycle of water stress, with a lowering of the osmotic potential for only a few bars (-1 to -2 bar) was sufficient to introduce this quality into the membrane.

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Table 1. Summary of leaf osmotic potentials for control and stress-hardened Pisum sativum.

Material: 32 to 37 days old plants

$\Psi_{s(sat)}$ and $\Psi_{s(R)}$ are the osmotic potentials at full water saturation and at the cell relaxation point respectively obtained from regression analysis of the pressure-volume curves illustrated in Figure 1. $\Psi_{s(pl)}$ is the osmotic potential obtained by plasmolysis.
SE = Standard Errors of Estimation. Values in bars.

Expt. No.	Control $\Psi_{s(sat)}$	$\Psi_{s(R)}$	Ψ_s	$\Psi_{s(pl)}$	Rewatered after stress (10 days of stress followed by 10 days of rewatering) $\Psi_{s(sat)}$	$\Psi_{s(R)}$	Ψ_s	$\Psi_{s(pl)}$
1	-9.45	-11.2	-1.75	-12.3	-11.11	-14.4	-3.29	--
2	-9.39	-10.0	-0.61	-12.3	-11.11	-14.3	-3.19	-13.6
3	-8.28	-8.3	-0.10	-12.9	-11.13	-12.5	-1.37	-13.6
4	-8.43	-8.0	0.43	-10.3	-8.83	-13.4	-4.57	-16.0
5	-8.10	-9.9	-1.8	-10.3	-14.27	-10.9	-3.37	--
6	-9.23	-10.3	-1.07	-11.0	-11.08	-13.5	2.42	--
7	-8.93	-10.3	-1.37	-9.6	-10.36	-12.4	2.04	-12.3
8	-9.22	-10.4	-1.18	-11.0	-9.92	-10.5	0.58	-11.0
9	-8.17	-9.3	-1.13	-9.6	-9.03	-10.4	1.37	-11.0
10	-10.75	-9.5	-1.25	-11.0	-9.19	-10.4	1.21	-11.0
Means	-9.00	-9.72	1.09	-11.03	10.54	12.09	2.16	-13.06
SE	0.25	0.31	0.15	0.36	0.45	0.49	0.39	0.74

1) $\Psi_s = \Psi_{s(R)} - \Psi_{s(sat)}$

Table 2. Summary of leaf mesophyll cells osmotic potentials for control and stress-hardened Pisum sativum.

Plant material: 26 to 28 days old.

$\Psi_{s(sat)}$ and $\Psi_{s(R)}$ are the osmotic potentials at full water saturation and at the cell relaxation point obtained from regression analysis of the pressure volume curves illustrated in Figure 1. $\Psi_{s(pl)}$ is the osmotic potential obtained by plasmolysis. SE = Standard Errors f Estimation. Pressure values in bars.

		Control				Rewatered after stress (10 days of stress followed by 10 days of rewatering)			
Expt. No.	age days	$\Psi_{s(sat)}$	$\Psi_{s(R)}$	Ψ_{s1}	$\Psi_{s(pl)}$	$\Psi_{s(sat)}$	$\Psi_{s(R)}$	Ψ_{s1}	$\Psi_{s(pl)}$
1	28	-9.00	-10.3	-1.3	-11.0	-10.76	-11.3	0.54	-14.6
2	28	-8.31	-9.3	-0.99	-11.0	-8.38	-10.3	1.92	-15.0
3	26	-8.81	-11.3	-2.49	-12.3	-9.82	-12.5	2.68	-14.6
4	26	-8.30	-9.3	-1.0	-12.3	-10.22	-12.5	2.28	-14.6
5	28	-7.69	-8.3	-0.61	-11.0	-8.01	-10.5	2.49	-13.9
6	28	-8.52	-9.4	-0.88	-11.6	-9.54	-10.3	0.76	-13.9
7	26	-8.96	-10.3	-1.34	-12.9	-9.26	-10.4	1.14	-13.9
8	26	-7.59	-8.3	-0.71	-12.9	-8.69	-9.3	0.61	-13.6
9	26	-8.20	-9.3	-1.10	-11.6	-8.27	-9.4	1.13	-13.7
10	28	-8.71	-9.3	-0.59	-11.6	-8.61	-10.1	1.49	-13.9
Means		-8.41	-9.51	-1.10	-11.82	-9.16	-10.66	1.50	-14.17
SE		0.15	0.29	0.17	0.23	0.20	0.35	0.25	0.15

$\Psi_s = \Psi_{s(R)} - \Psi_{s(sat)}$

Table 3. K^+-uptake in subepidermal stem base cells from medium (1) spring water; (2) 100 mosM KCl), expressed as solute potential after 3 hrs. 24 day old plants (2.5 week water stress). Each value is the result of 4 to 5 experiments, with 5 to 10 cells per each experiment.

Solute Potential (Bars)

	Without KCl (1)	With KCl (2)	(2)-(1) *)
control well-watered	-11.50 ± 1.51	-13.25 ± 1.65	1.75
adapted	-12.46 ± 1.85	-16.65 ± 2.82	4.19

*) Difference between control and KCl treated.

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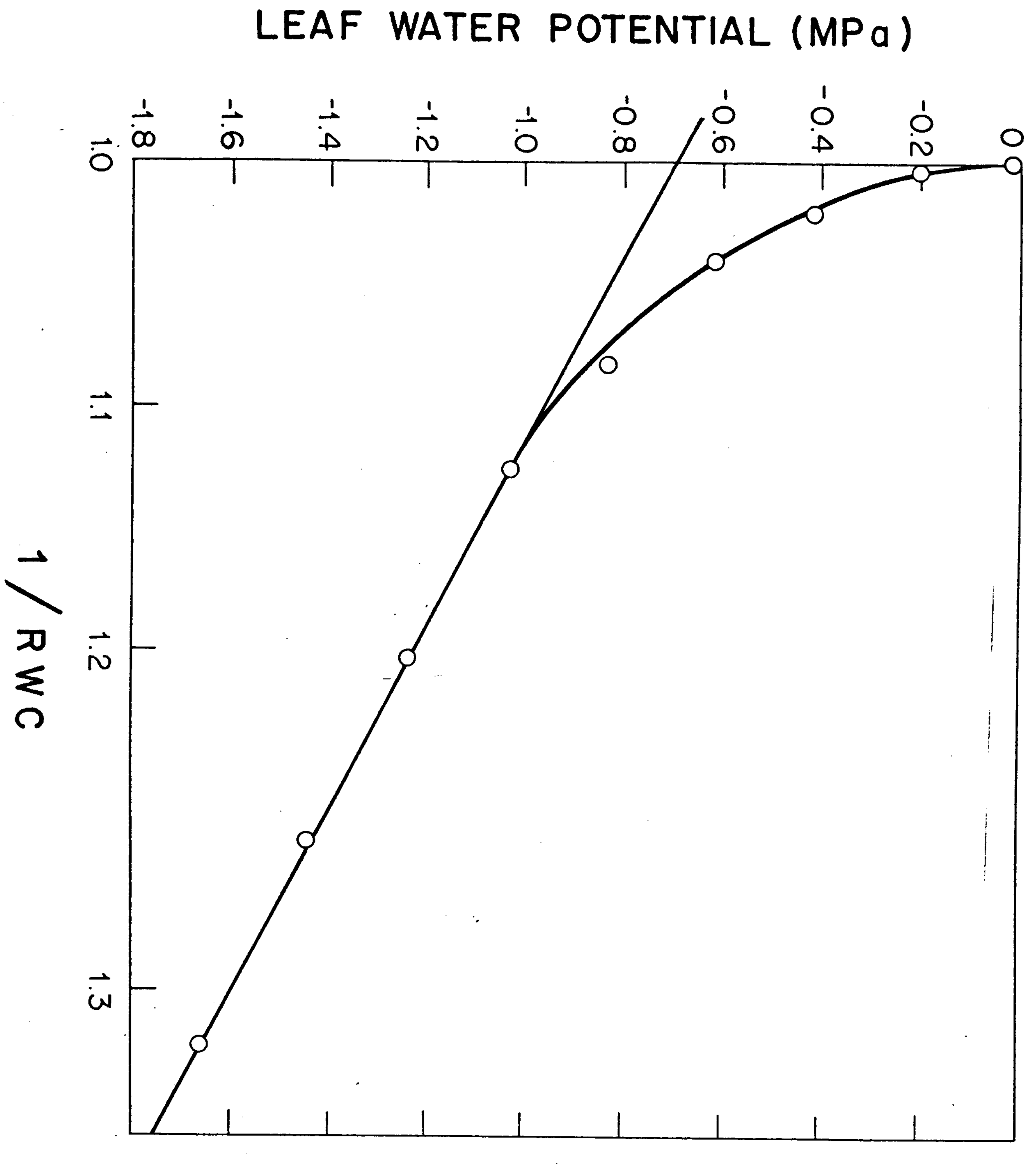

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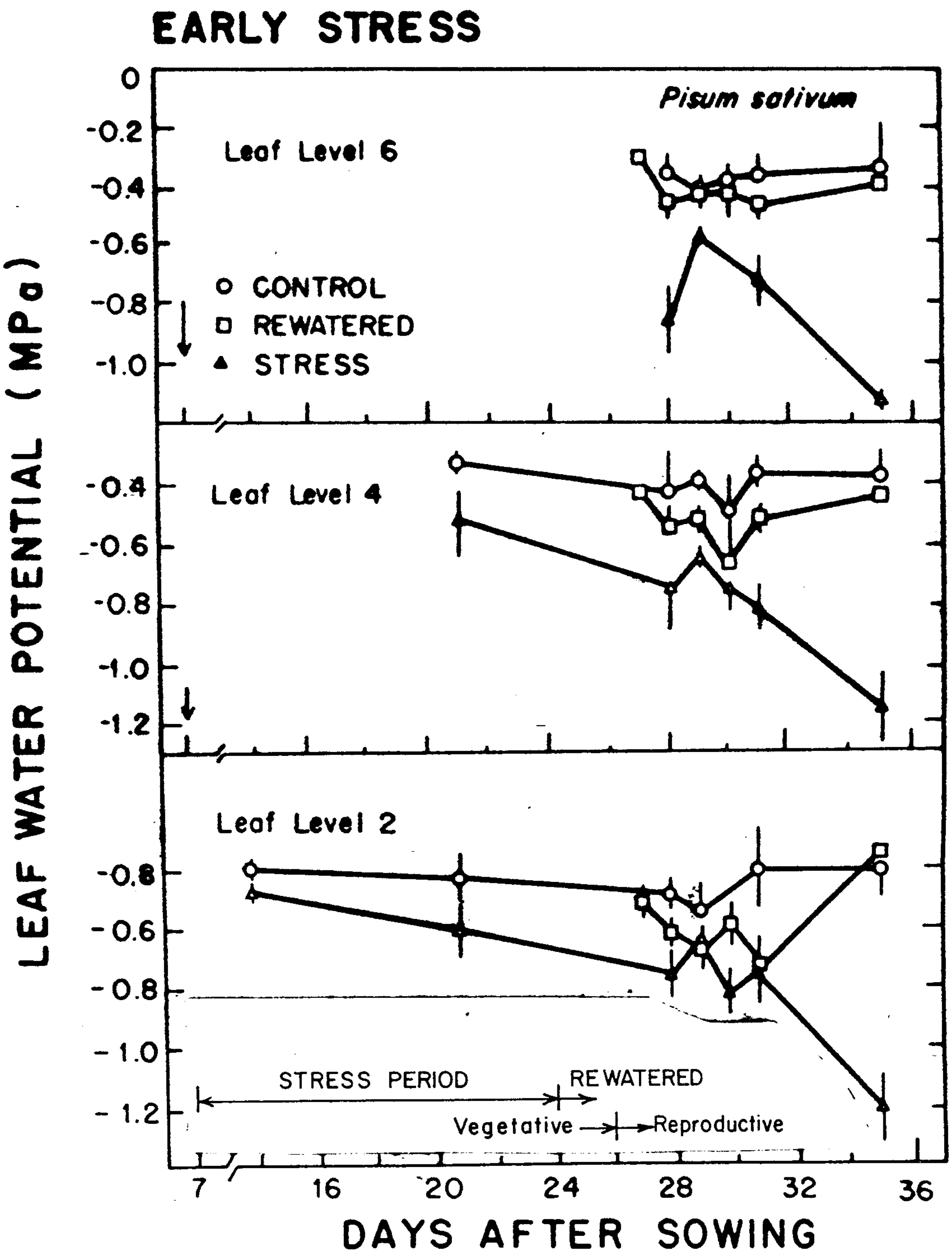

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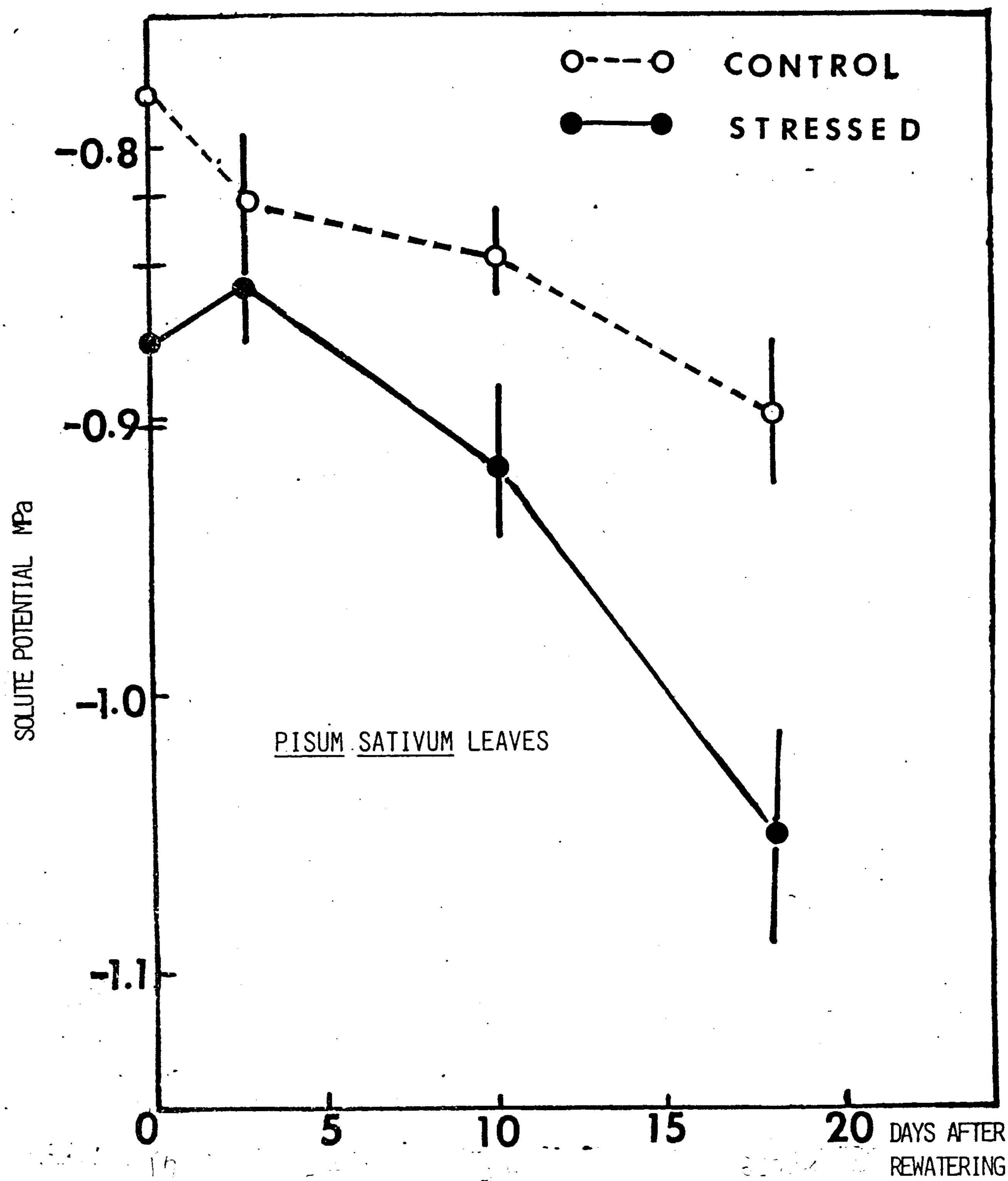
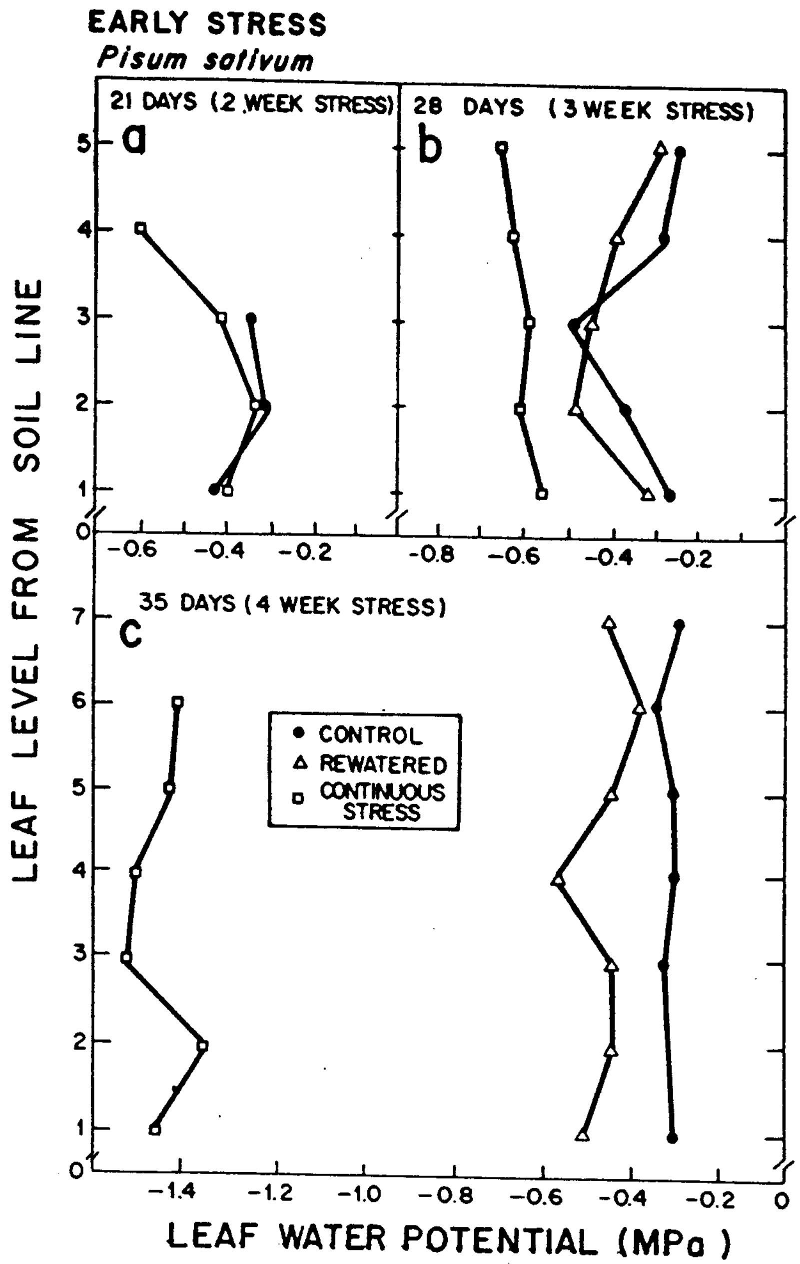


Fig. 3. Leaf solute potential of water stress adapted and unadapted (control) plants.



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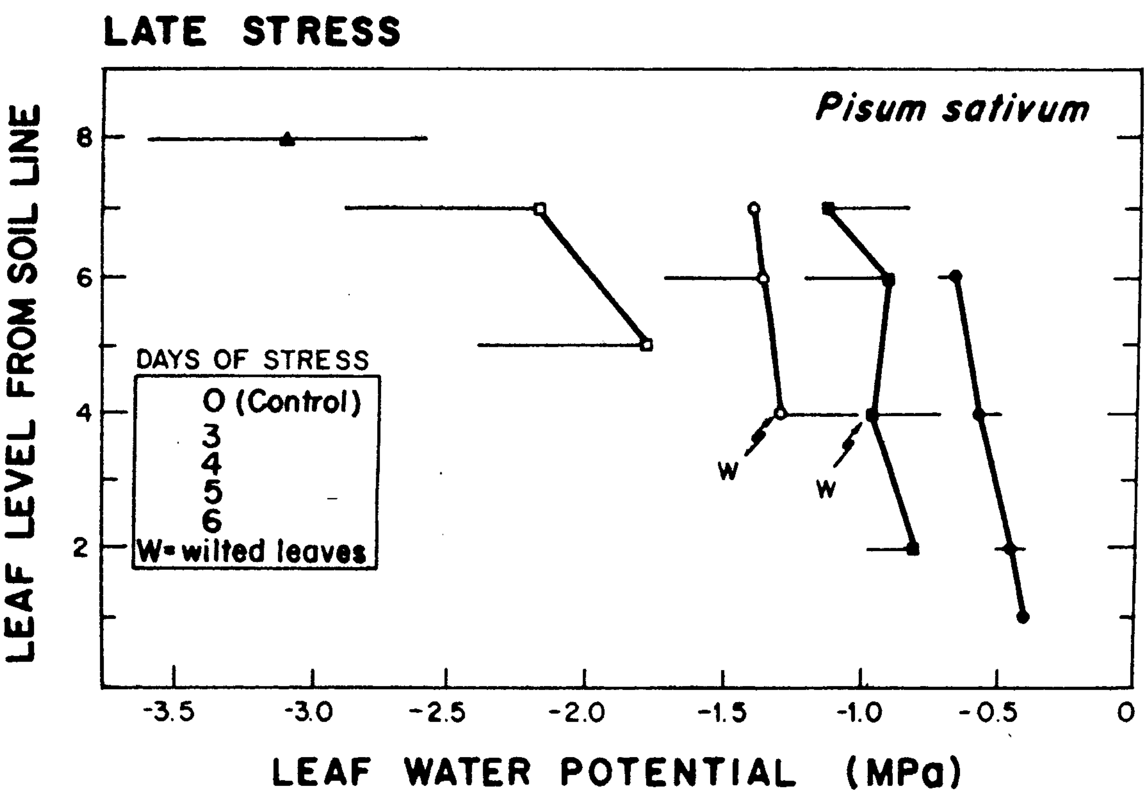

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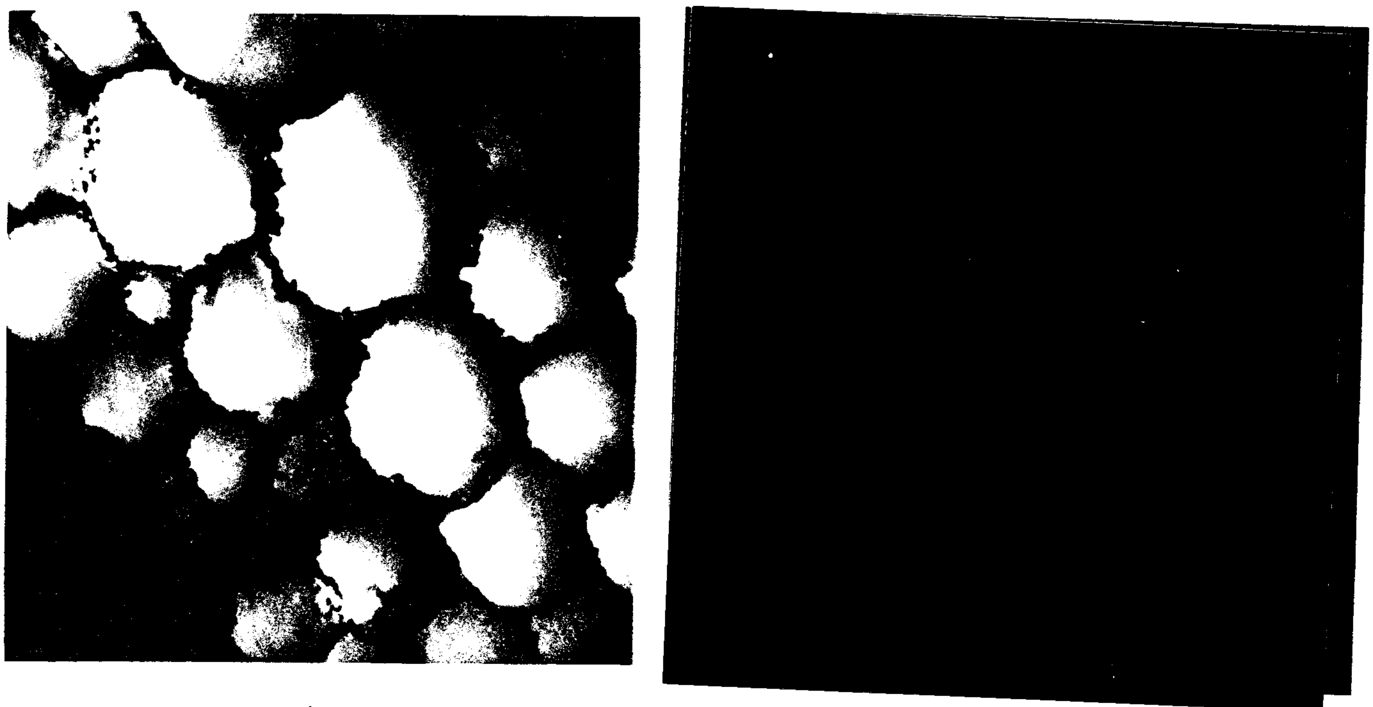

A B

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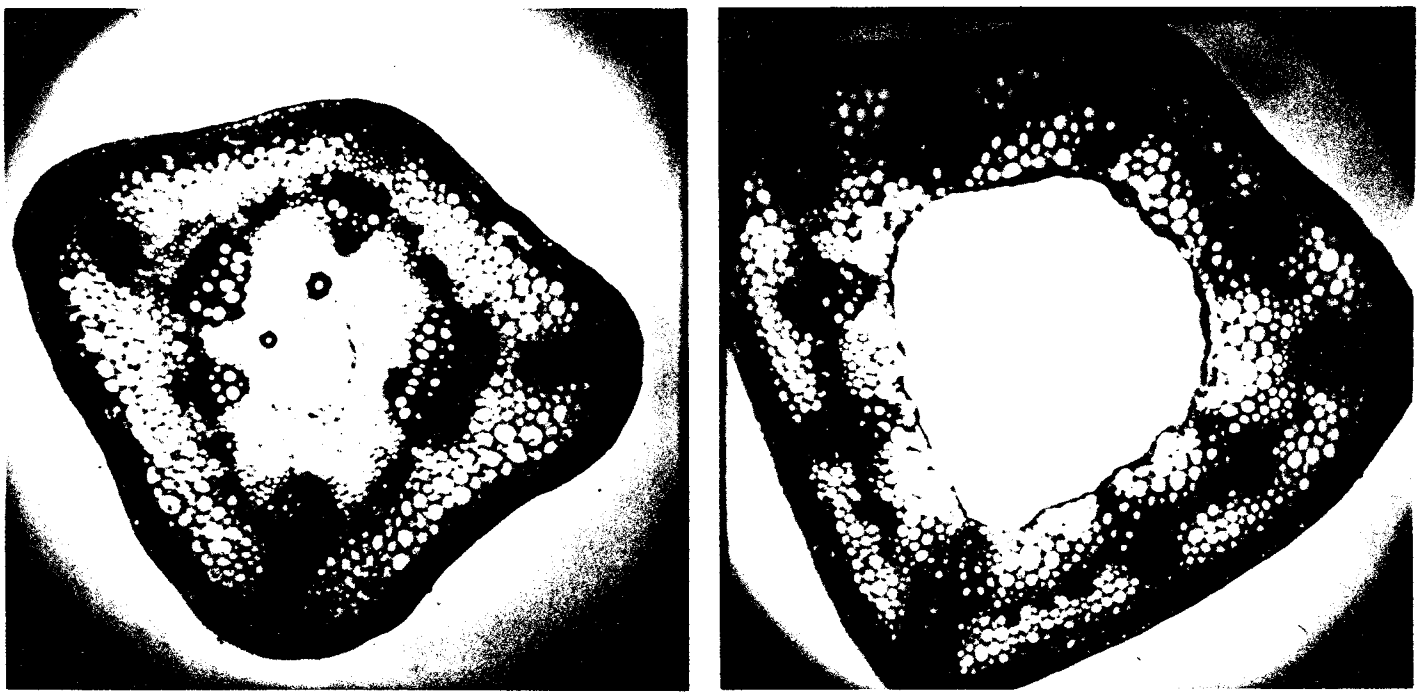

A B

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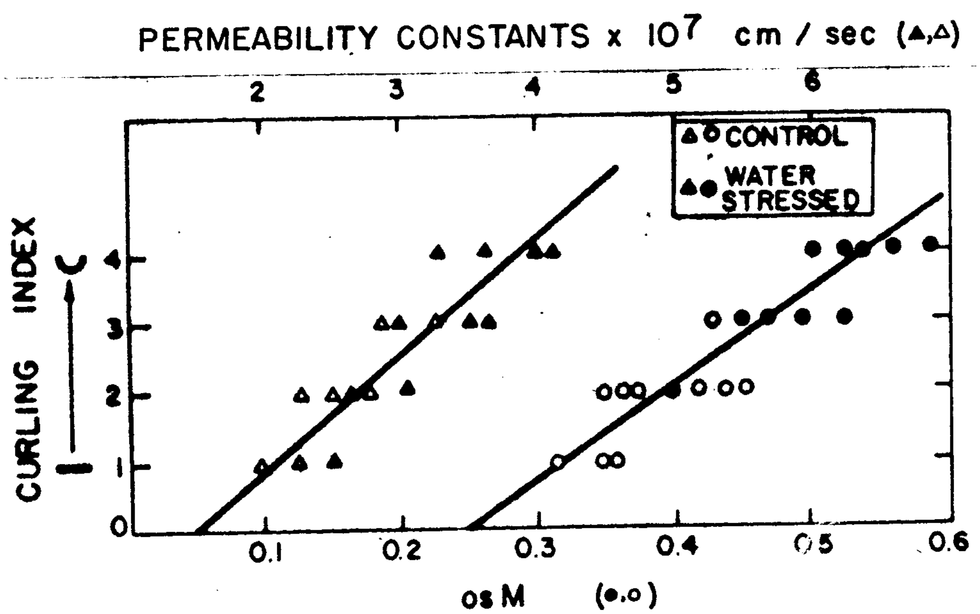

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