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WATER UPTAKE OF HIGHER PLANTS AS AFFECTED BY ROOT TEMPERATURE

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1. INTRODUCTION

The effect of root temperature on water uptake has been studied in many plant species (see e.g. DÖRING, 1934; KRAMER, 1956). In general, plants from a warm environment show a greater reduction in water uptake by low root temperature than plants grown under cooler conditions.

According to KRAMER (1942), a decrease in water uptake at lower temperatures is caused by a decrease in root growth and in water permeability of the cell membrane and an increase in viscosity of water and of protoplasm.

The effect of the viscosity of water has been observed in experiments, as shown in fig. 1, taken from various sources. The experimental data on water uptake as affected by root temperature are given for white pine (A), cabbage (B), cotton (C), and water melon (D; full-drawn curves). The broken curves of this figure are corrected for differences in viscosity of water between the various temperatures. Fig. 1E contains all these corrected curves, as obtained from KRAMER (1948) and others (*Helianthus annuus:* CLEMENTS and MARTIN, 1934; citrus: BIALOGLOWSKI, 1936). The decrease in water absorption of white pine (A) and cabbage (B) in the range of soil temperatures from 5° to 25°C seems to be entirely due to an increase in viscosity of water. The temperature range of this effect is smaller in citrus, sunflower, and loblolly pine, while it is nearly negligible in cotton and water melon (fig. 1E). After correction, the water uptake curves reveal the effect of temperature on the water permeability of the cytoplasma membranes of the root cells, since in these short-time experiments the effect of temperature on root growth can be left out of consideration (see, e.g. KRAMER, 1942, 1948, 1956). The effect on the water permeability becomes manifest at temperatures lower than 5°C (white pine, cabbage), 10°C (citrus), 20°C

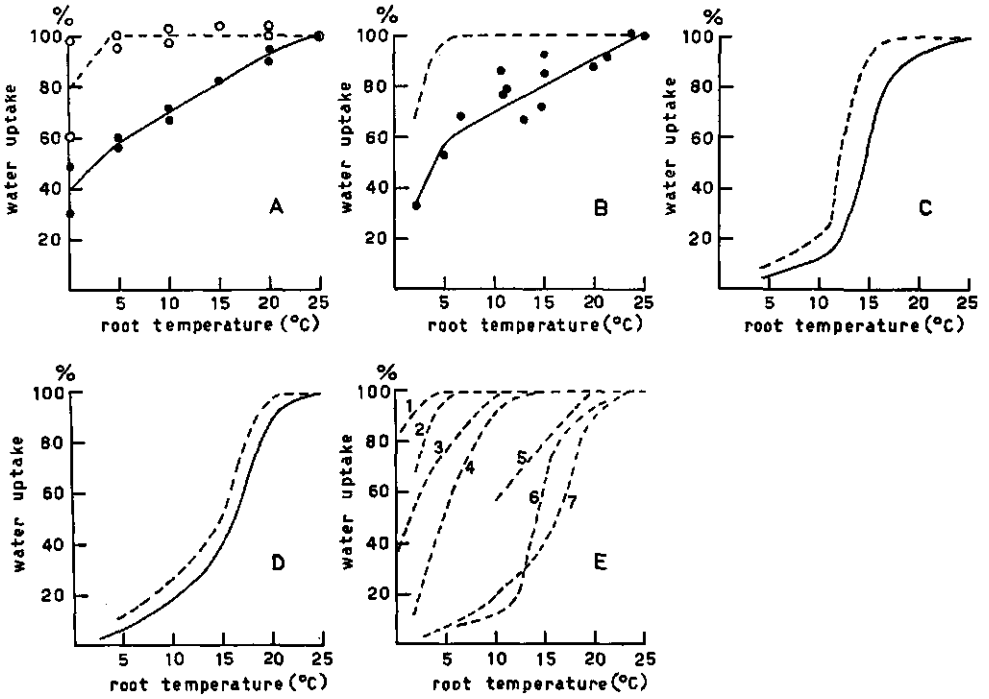

FIG. 1. The effect of root temperature on water uptake of white pine (A), cabbage (B), cotton (C), and water melon (D); full-drawn curves according to KRAMER (1942). The broken curves are corrected for differences in viscosity of water between the various temperatures. E: corrected curves for white pine (1), cabbage (2), citrus (3; BIALOGLOWSKI, 1936), *Helianthus annuus* (4; CLEMENTS and MARTIN, 1934), loblolly pine (5; KRAMER, 1942), cotton (6), and water melon (7). The value at 25 °C has been taken as 100%.

(loblolly pine), and about 22 °C (cotton, water melon). The temperature dependency (Q_{10}) is roughly the same for all species. ABD EL RAHMAN et al. (1959) give a similar analysis of the effect of root temperature on water uptake of tomato.

With regard to the effect of the viscosity of water, it still remains to be decided whether the picture of mass flow of water through the pores of the root cell membranes is valid. This assumption is required to explain the effect of viscosity of water in water absorption, but it seems difficult to bring it into accord with the well-known selectivity of root cell membranes for uptake of ions and small non-electrolytes. This problem will be discussed later.

Our experiments aimed at obtaining more information on the effect of temperature on water transport across the root system. Young bean plants were grown at different root temperatures and different oxygen supply to the roots under controlled experimental conditions. Some additional experiments with lettuce plants and artificial membranes were made.

2. Material and Methods

Bean plants (*Phaseolus vulgaris*, var. 'Wagenaar') were grown in coarse sand from germination. About 7 days after germination some of the seedlings were kept in coarse sand, while the others were transplanted to a water culture, or to an equipment in which the roots could grow on a wet nylon sheet in moist air. The temperature of the growth room was 24° ± 2°C, while light intensity at the leaf surface was about 3×10^4 erg. $sec.^{-1}$. cm^{-2} (400–700 mμ), using fluorescent daylight tubes. The illumination period was 14 hours a day. Different root temperatures were obtained from thermostatic baths. The temperature of the latter could be regulated with tap water and electric heating in the range of 14°C to 31°C, and was kept constant during the growth period with a deviation of less than 1°C. The nutrient solution of the culture was aerated with normal air or oxygen.

The experimental plants were used when the first pair of leaves were full-grown (about 14 days after germination). In one experiment, young lettuce plants (var. 'Meikoningin') with a leaf area of about 125 cm^2 were used.

Water uptake was measured in potometers, containing about 50 cm^3 of aerated water in which the roots were. An air-tight fit of stem and potometer was obtained with a rubber stopper and a mixture of beewax, vaseline, and cotton wool. Under constant conditions water uptake remained the same for at least 4 hours. The potometers were placed in a thermostatic bath, the temperature of which could be regulated with cooling (tap water or ice water) and electric heating in the range of 0°C to 35°C with an accuracy of about 0.02°C. The time course of temperature changes in the potometer was followed with small thermocouples, placed between the roots. The effect of temperature on the water volume of the potometer has been taken into account. During the experiment, the environment of the leaves was kept constant with regard to temperature, humidity of the air, and light intensity (see Kuiper, 1961). In some experiments, the stem was cut, and an artificial suction obtained from a water jet pump, was applied to the root stump; polyethylene tubing connected the stump and the pump. An air-tight fit of stem and tubing was again obtained with the beewax mixture. A more extensive description of this technique will be published later. Water transport through a copper ferrocyanide membrane, a collodion membrane, and dialysis tubing 'Visking' under suction was measured in a similar way.

In addition to the calculation of the well-known and useful Q_{10}-values of water uptake from our experiments, in some cases the activation energy is calculated according to the Arrhenius equation:

$$k_1 = k_0 . e^{\frac{\mu}{R} \cdot \frac{T_1 - T_0}{T_1 \cdot T_0}} \qquad \text{and} \quad \mu = 0.4577 . T_0 . T_1 . {}^{10}\log Q_{10}$$

in which

μ = activation energy (cal/Mol),
k_1, k_0 = rate of transport of water (mg/hr),
T_0, T_1 = absolute temperature (° Kelvin),
R = gas constant (1.99 cal/degree for 1 Mol).

As an example, the μ-values for water are given below, based on the temperature coefficient of its viscosity. According to Rodebush and Buswell (1959) the molecules in liquid water are attached by hydrogen bonds, which bonds must be broken before movement can occur. The low energy required to break this bond indicates that each hydrogen atom is bound to two or more oxygen atoms of neighbouring water molecules; it is necessary to break only one of these bonds in order to induce water movement. According to the following table, the number of bonds between a certain hydrogen atom and the oxygen atoms of surrounding water molecules increases with temperature.

Temperature range	Q_{10} of viscosity	Activation energy (μ, in cal/Mol)
0°–10°	1.37	4820
10°–20°	1.30	4320
20°–30°	1.26	4080
30°–40°	1.22	3750

For calculation of the activation energy, the log. rate of water transport should be plotted against the reciprocal of the absolute temperature. When a straight line results, its slope is a measure for the value of the activation energy. Sometimes, a broken line or a slightly curved line is observed, indicating that two processes with different activation energy levels (or different Q_{10}-values) occur (JOHNSON et al., 1954).

3. EXPERIMENTAL RESULTS

In the first experiment, the initial effect of temperature on water uptake of intact bean plants with a leaf area of about 95 cm^2 and grown on wet nylon sheet was measured. First, water uptake was measured at 25 °C root temperature (leaf temperature was about 28 °C) in strong light; a limitation of water uptake by transpiration is not likely to occur. Change in root temperature induced an immediate change in water uptake giving rise to a new, constant, level after about 15 to 30 minutes. Then, mostly another change in water uptake occurred, continuing until a new, constant, level was reached after 30 to 60 minutes. This picture is in agreement with ROUSCHALL's observations (1935), except that, in his experiments, the first period of constant water uptake was somewhat longer.

In fig. 2, the initial water uptake, measured within 5 minutes after change in temperature, is plotted against the fluidity of water. For comparison, the temperature scale is added in this figure. The data scatter around a straight line through the origin.

As a rule, the suction tension in the xylem vessels in intact plants is unknown. In order to localize this temperature effect on water transport through the plant,

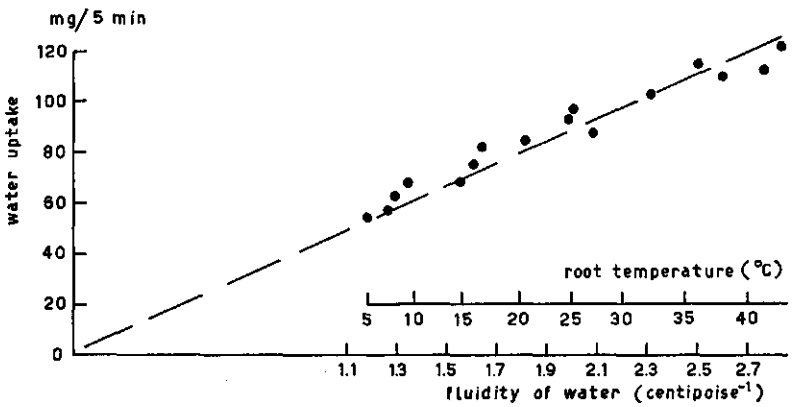

FIG. 2. The effect of fluidity of water on the initial water uptake of intact bean plants, measured within 5 minutes after change of temperature. Light intensity 85000 erg.sec.$^{-1}$.cm^{-2}; vapour pressure of the air 10 mm Hg; leaf temperature about 29 °C.

the above mentioned experiment was repeated with roots, the cut end of the stem being connected to a constant suction tension of 50 cm Hg. The latter value was found adequate for our experiments; a more extensive consideration of the effect of an artificial suction on water uptake of an excised root system will be discussed elsewhere. Results analogous to those of fig. 2 were obtained, indicating that the temperature effect was located in the roots. These results indicate, that an effect of the viscosity of water may be observed in living root cells, as in artificial membranes (BIGELOW, 1907) and in dead root cells (KRAMER, 1940). Thus, after changing the root temperature in our experiments, first the effect of viscosity is visible, while after 15 to 30 minutes another effect, the water permeability of the cytoplasmic membranes, becomes manifest.

Next to the initial effect the stationary effect of root temperature on water uptake in intact bean plants, grown in strong light with the roots on wet nylon sheet, was studied; root temperatures were 17° and 24°C. Change in temperature induced a change in water uptake until a constant rate was observed after 30 to 90 minutes. Fig. 3 represents some data, the water absorption being plotted on a logarithmic scale. Plants grown at 24°C root temperature showed a logarithmic relation between water uptake and temperature within the entire range studied. The Q_{10}-value is about 4.0; the activation energy, μ, is about 24400 cal per Mol.

Plants grown under the same conditions were used for the determination of water uptake of roots under an artificial suction tension of 50 cm Hg. The effect of temperature on water uptake was similar to that on water uptake of intact plants. The Q_{10}-value varied from 4.0 and 5.0 between 5° and 32°C, for individual root systems.

Bean plants, grown at 17°C show another picture; see fig. 3. Below 15°C a logarithmic relation between water uptake and temperature exists, with a Q_{10}-

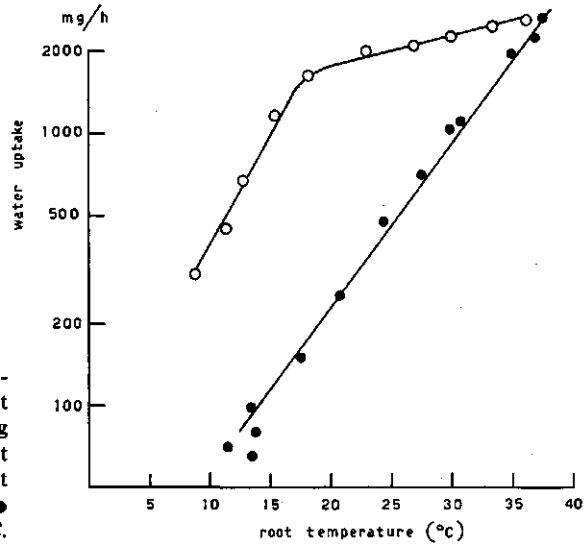

FIG. 3. The effect of root temperature on water uptake of intact bean plants, grown in strong light and with the roots on wet nylon sheet; o——o grown at root temperature 17°C; ●——● grown at root temperature 24°C.

value of about 5.0, corresponding to an activation energy of 25600 cal per Mol. Above 15 °C, the effect of temperature on water uptake is much smaller. A Q_{10}-value of about 1.32 is observed, while the activation energy is around 4300 cal per Mol. The same relation was observed in excised root systems of plants, grown under similar conditions under a suction tension of 50 cm Hg. In accordance with the experimental results on the effect of temperature on the initial water uptake, it is likely, that the temperature effect above 15 °C is due to a change in the viscosity of water. Consequently, it seems likely that the water permeability of the root cell plasma membranes reaches saturation at 15 °C, while this is above 32 °C in plants, grown at 24 °C root temperature.

At low temperatures, plants grown at 17 °C root temperature show a higher water uptake than plants grown at 24 °C root temperature. This kind of adaptation to lower temperatures is already visible about 36 hours after transfer from 24° to 17 °C root temperature. During this period, root elongation appeared somewhat retarded as compared with control roots at 24 °C. The water uptake versus temperature curve shifts to lower temperature ranges without much effect on the Q_{10}-value.

The results obtained with plants grown in coarse sand or in water culture, continuously aerated with oxygen, were closely similar to those described above.

Fig. 4 represents some data on the effect of temperature on water uptake of intact bean plants grown in water culture and aerated with normal air either continuously or one half hour every 12 hours (root temperature 24 °C). Slightly curved lines indicate differences in sensitivity to temperature. Above 20 °C again the effect of viscosity of water is observed; the Q_{10}-value is about 1.25. Below 20 °C, the Q_{10}-value is again about the same as in the previous experiments, viz. 3.8 to 4.0. In the same figure, a curve is given for bean plants which had been grown in water culture with continuous aeration and 17 °C root temperature. The figure shows that in this case above 12 °C the effect is determined by the

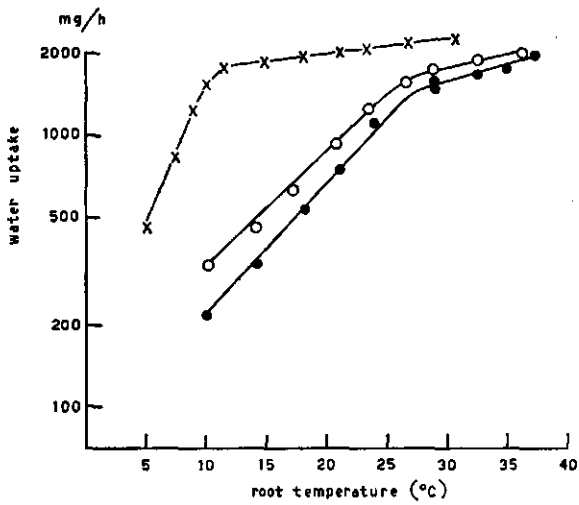

FIG. 4. The effect of root temperature on water uptake of intact bean plants, grown in water culture at 24 °C root temperature with continuous aeration (o—o), or with aeration one half hour each 12 hours (•—•); x—x bean plants, grown in water culture with continuous aeration at 17 °C root temperature.

viscosity of water, while below 12°C a stronger temperature effect on water uptake is observed. All high Q_{10}-values (3.8–4.0) given in fig. 4 for intact plants were also obtained in experiments on water uptake of excised roots under a suction tension of 50 cm Hg.

Additional observations have shown that also after transfer of plants from wet nylon to water culture adaptation to lower temperatures occurred within 36 hours.

It may be concluded that the temperature sensitivity as expressed by the value of Q_{10} and activation energy is not affected by the aeration conditions and the root temperature during growth. However, the temperature, at which the water permeability of the root cell membranes reaches its saturation level, strongly depends on the culture conditions. This level is reached at 12° (1), 15° (2), 20° (3), or above 32°C (4) respectively for the following growth conditions: 17°C root temperature, water culture (1), 17°C root temperature, wet nylon sheet (2), 24°C root temperature, water culture (3), and 24°C root temperature, wet nylon sheet (4). It is tempting to suggest that the mentioned 'critical' temperature is determined by the relative capacity of aerobic respiration or some related metabolic activity of the root cells.

Incidentally, some experiments on the effect of root temperature on water uptake of lettuce plants were made. Fig. 5 gives the results of such an experiment, showing a similar type of curve as in the case of the bean plants. The water permeability of the root cells reaches its saturation level at 10°C; the Q_{10}-value is very high, viz. 8.0. Above 10°C, again the effect of viscosity of water can be observed. In agreement with results on other crops from cool environments, water uptake is only strongly affected at root temperatures lower than 10° C (KRAMER, 1942).

In some experiments, the effect of temperature on water transport across artificial membranes was studied. The Q_{10}-value varied between 1.2 and 1.3,

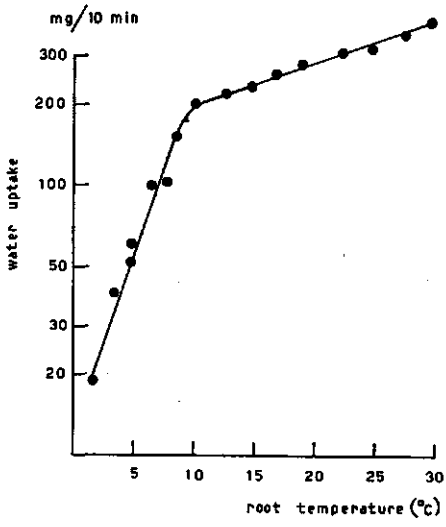

FIG. 5. The effect of root temperature on water uptake of a small lettuce plant. Light intensity 70000 erg.sec.$^{-1}$.cm^{-2}; leaf area 133 cm^2; difference in vapour pressure between leaf and air 19 mm Hg.

indicating the effect of viscosity of water on water transfer. This was the case in relatively coarse membranes, e.g. in a collodion membrane, as well as in membranes with fine pores, e.g. a copper ferrocyanide membrane. The latter proved to be impermeable to sucrose. This is in agreement with BIGELOW's results (1907). Thus, the effect of viscosity of water cannot only be observed in experiments on mass flow through the large pores of coarse membranes, but also in experiments on water flow through membranes with fine pores, the size of which is of the same order as in living membranes.

4. DISCUSSION

We have seen in the experimental part of this paper that, in general, the temperature curves for water uptake consist of a low-temperature part with (even excessively) high Q_{10}-values, and a high-temperature part with low Q_{10}-values. The change is rather abrupt, at a 'critical temperature' which differs according to experimental growth conditions. This situation is very common in biological processes (cf. e.g. WASSINK, 1934; BOTTELIER, 1935). The general explanation is that in the high Q_{10}-part of such curves the rate of the physiological process studied (in our case water uptake) is determined by the capacity of a metabolic process (the possible nature of which will be discussed further below) while in the low Q_{10}-part this capacity is sufficiently large to take care of the whole amount of reactant available. Further increase of temperature then affects the overall rate only in so far as it increases the supply of reactant (i.c. water).

The following serves to outline a possible mechanism to account for the high temperature coefficient part of the curves.

High Q_{10}-values for water uptake, as observed below a 'critical temperature' in our experiments correspond to high values for the activation energy and indicate a high potential energy barrier for the strongly polar substance, water. Probably, this barrier is made up by the lipid layers of the cell membrane, consisting of long lipid molecules in regular orientation (KUYPER, 1962). Owing to thermal agitation of these lipid molecules, the VAN DER WAALS forces between the lipid molecules will be broken and small gaps appear. These 'statistical pores' may allow passage of a single string of water molecules. A pore will close again when the kinetic energy of the lipid molecules surrounding it exceeds the kinetic energy of the hydrogen bonds between two successive water molecules which pass the pore.

Certainly, these pores are small, as e.g. is obvious from the selectivity for ions. Moreover, pores of living cell membranes have never been observed in electron microscopy which resolves down to 10–15 Å. FRENZEL (1929) concluded from plasmolysis experiments that the size of the pores of plant cell membranes is less than 10 Å. COLLANDER and BÄRLUND (1933) mention 4 Å as the effective pore diameter of the tonoplast of *Chara*-cells which are very similar to those of higher plants with respect to permeability for ions and non-electrolytes.

Above the 'critical temperature', a low temperature coefficient was observed, indicating limitation of water transport by diffusion or mass flow, at least by

some physical characteristic of 'substrate' supply. In relation to the above picture this means that, in the range of temperatures concerned, the number and size of the supposed pores statistically is sufficient to take care of transport of all water available to the membrane surface. The viscosity of water depends on the mutual forces between water molecules. Contrary to the sliding movement of molecules in the flow of organic liquids, as e.g. benzene, the movement of water molecules is rolling, since at least one hydrogen bond should be broken before any water flow can occur (see RODEBUSH and BUSHWELL, 1958). The energy required to break these bonds, is, of course, independent of the size of membrane pores. For this reason, the effect of viscosity of water is the same for transport of water through membranes of different porosity, as can be concluded from BIGELOW's experiments (1907). This appears to describe the situation in our experiments above the critical temperature; the membrane as such does not constitute the limiting factor for the rate of transport.

According to COLLANDER (1959), the opinion that the assumed pores of the plasma membranes are water-filled channels, in general, can hardly be correct. According to what has been said above, it may well hold, however, above the 'critical temperature'. Water flow through permanent water-filled pores is characterized by a low temperature coefficient, viz. that for the viscosity of water. We may thus well assume that above the critical temperature the pores are permanently filled with water, passing the membrane.

The behaviour of root cell membranes above the critical temperature is similar to that in *Beggiatoa* cells, studied by RUHLAND et al. (1925), while other examples (diatoms, *Oscillatoria*) are mentioned by COLLANDER (1959).

Our discussion may be summarized in stating that the root cell membranes act as being distinctly porous above the critical temperature, while below this temperature these membranes behave as being more homogeneous and characterized by statistical pores, appearing in the oriented lipid layer. It is evident from the broken curves of figs. 3, 4 and 5, that the transition zone between the two states of the membrane is short.

In our experiments, the critical temperature, viz. that at which the water permeability of the root cell membranes reaches its saturation level, was found to be much affected by culture conditions during growth, viz. root temperature and aeration. Below the critical temperature the variation in Q_{10}-values for different growth conditions was insignificant. This means that the structure of the lipid layers with regard to water permeability is essentially independent of growth conditions. The shift of the critical temperature to lower values with decreasing root temperature and/or decreasing aeration conditions during growth may be interpreted as due to increase in number of pores available for water transfer. Since root growth decreases after transfer from high root temperature to lower, a longer lifetime of the root cells probably provides the explanation. Due to delayed suberization, the endodermis cells may function for a longer time as a pathway for water from the root environment to the xylem vessels.

In the first experiment, the initial effect of a temperature change on water uptake was measured. It was shown that first an effect of the viscosity of water

was manifest, after 15 to 30 minutes followed by the effect on the water permeability of the cell membranes. This appears as a kind of 'memory' of the membrane for temperature. Similar hysteresis effects with regard to hydrostatic and osmotic suction tension were observed by two coworkers of the present author; they will be presented and discussed in due time.

Finally, we may remark that in water uptake and water transport two main items should be distinguished for a correct evaluation of the results presented and their discussion, as given.

The magnitude of water uptake is determined on the one hand by a driving force, viz. transpiration (which may be replaced by applying suction to the decapitated stem), and on the other hand by the resistance in the various tissues of the plant. The above discussion pertains solely to properties of the resistance. It is, therefore, easy to see that the measured water uptake will, in general, vary according to the suction value (transpiration conditions or applied suction), and that such variation (observed in preliminary experiments) does not violate the application of the principle of 'limiting factors' (or rather limiting processes) as attempted in the preceding discussion. For, it is to be expected that at any value of the resistance as determined by metabolic and/or physical properties, increase or decrease of the suction force will increase or decrease the amount of water uptake as measured in experiments such as ours. Which type of relation exactly exists between both remains to be established.

In all such considerations it should be kept in mind that water transport in a higher plant largely passes 'outside' the metabolic functions of the plant which take up only a very small part of it (less than 1%, as expressed, e.g., by established values of 'water requirement', see, e.g. ABD EL RAHMAN et al. 1959). In view of water uptake and transport, therefore, the plant can be roughly considered as a tubing system with certain 'filters', connected to a driving force. Metabolic functions enter into it only so far as they affect the properties of the 'filters' and 'tubes', and herewith, the values of their resistance to transport; not with respect to the 'use' of water in metabolism which is quantitatively unimportant in relation to the amount transported.[1])

The excessively high Q_{10}-value found in the 'metabolically determined' part of the temperature curve may well be looked upon from this viewpoint.

[1]) For this general consideration it does not appear relevant whether metabolic functions additionally produce forces promoting water flow or are restricted to affecting the sensu stricto transmission properties of the 'filters'. The more so, since the 'mechanical' suction forces, such as produced by transpiration, seem quantitatively predominant for determining the water flow through a plant.

It remains to be seen in how far such a possible metabolic part in the development of suction forces may influence Q_{10}-values as obtained in the metabolically determined part of the temperature curve. Since data pertinent to this question are not yet available, it did not appear appropriate to introduce this complication into the consideration presented, in order not to withdraw attention from the general outline of the situation.

Summary

The effect of root temperature on water uptake of intact bean plants under high transpiration conditions has been studied. Similar experiments were conducted on water uptake of root systems while a constant suction tension of 50 cm Hg was applied to the cut end of the stem. In general, two temperature ranges could be distinguished, one with a high, and one with a low Q_{10}-value. Above a certain 'critical temperature' only the effect of viscosity of water could be observed as limiting water uptake, indicating the existence of permanent water-filled pores in the root cell membranes. Below this critical temperature the root cell membranes are assumed to be more homogeneous and characterized by a high potential energy barrier for water. Water transport then appears to be limited by the capacity of a metabolic process involved in the establishment of the membrane structure. The critical temperature strongly depends on culture conditions during growth; it was found to shift to lower values, e.g., with decreasing root temperature and/or decreasing aeration conditions during growth.

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