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Potassium Deficiency-induced Changes in Stomatal Behavior, Leaf Water Potentials, and Root System Permeability in *Beta vulgaris* L.[1]

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ABSTRACT

Studies of the water relations of potassium deficient sugarbeet plants (*Beta vulgaris* L.) revealed two factors for stomatal closure. One component of stomatal closure was reversible by floating leaf discs on distilled water to relieve the water deficit in the leaves; the other component was reversible in the light by floating the leaf discs on KCl solution for 1 hour or more. Potassium-activated stomatal opening in the light was observed when the guard cells were surrounded by their normal environment of epidermal and mesophyll cells, just as observed by previous workers for epidermal strips. Leaf water potentials, like stomatal apertures, appear to be strongly related to leaf potassium concentration. Potassium-deficient plants have a greatly decreased root permeability to water, and the implications of this effect on stomatal aperture and leaf water potential are discussed. In contrast, petiole permeability to water is unaffected by potassium treatment.

A number of authors (5–7, 9, 13, 15) have reported recently on the role of potassium in stomatal opening. Most studies have been made using excised epidermal strips (5–7, 9) and this technique has permitted rapid advances in the study of stomatal behavior (18).

From a purely mechanical point of view, guard cell activity is considered to be due to an increase in turgor relative to the adjacent epidermal cells (17) with which guard cells may exchange water deficits in very short time intervals as Raschke (14) has recently reported.

Such relationships between the guard cells and the epidermal cells are generally destroyed when epidermal strips are used as the test material. The mesophyll is removed and almost all epidermal cells are ruptured during separation (5).

In the present studies, the response of guard cells was observed in the intact leaf and this technique also confers the advantage of allowing the use of a porometer to estimate stomatal apertures. Measurements of the leaf water potential profile and the root system permeability are also included in this report as they contribute to the understanding of the stomatal responses.

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MATERIALS AND METHODS

Sugarbeet plants (*Beta vulgaris* L. MS NB1 × NB4) were grown in plant growth chambers in a complete nutrient culture solution (8) until they were well established (about 5 weeks after germination). The photoperiod was 16 hr, and the light intensity was 43,000 lux, supplied by fluorescent lamps supplemented with incandescent tungsten lamps. Some plants were then harvested, and half the remainder were transferred to solutions similar to the complete nutrient solution but lacking in potassium. Measurements and further harvests of plants were made at intervals up to 15 days after potassium cutoff. Night temperature was 20 C throughout, whereas day temperature, initially 25 C, was increased stepwise to 29 C to maintain the evaporative demand on the growing plants.

Leaf viscous resistance was measured with an Alvim-type porometer (1), similar to the one described by Bierhuizen *et al.* (3). Leaf discs (diameter 1.5 cm) were floated on water or KCl solution in Petri dishes at 25 C under lights and on removal, gently dried in a standard manner with absorbent paper tissue before being placed in the porometer. The light source and intensity were the same as in the plant growth chambers. Leaves are numbered from the oldest leaf.

Root system permeability was measured by placing the decapitated root systems in a pressure chamber containing the nutrient solution. The solution was aerated by a bleed tube, and a pressure of 2 bars was applied to the chamber. Fluxes were measured at the cut stump which was exposed to the atmosphere. Petiole permeability was measured likewise in a similar chamber with a 7.5 cm length of petiole. The leaf water potential was determined by using the Shardakov technique as described recently by Knipling (11).

Further experimental details have been published elsewhere (8).

RESULTS

In Figure 1, three sets of leaf viscous resistance measurements are presented for different leaves. Resistance is represented by the length of the bars, and, as there is an inverse relationship between resistance and stomatal aperture, a high resistance value is interpreted as a small stomatal aperture and vice versa. Each set of data shows that for intact plants the resistance to the movement of air through the leaf under pressure was much higher where the potassium status was low. Stomatal apertures of low K leaves were substantially increased when full turgor was restored. This is demonstrated as a decrease in leaf viscous resistance when leaf discs were floated on distilled water for 4 hr (2). Whereas this treatment

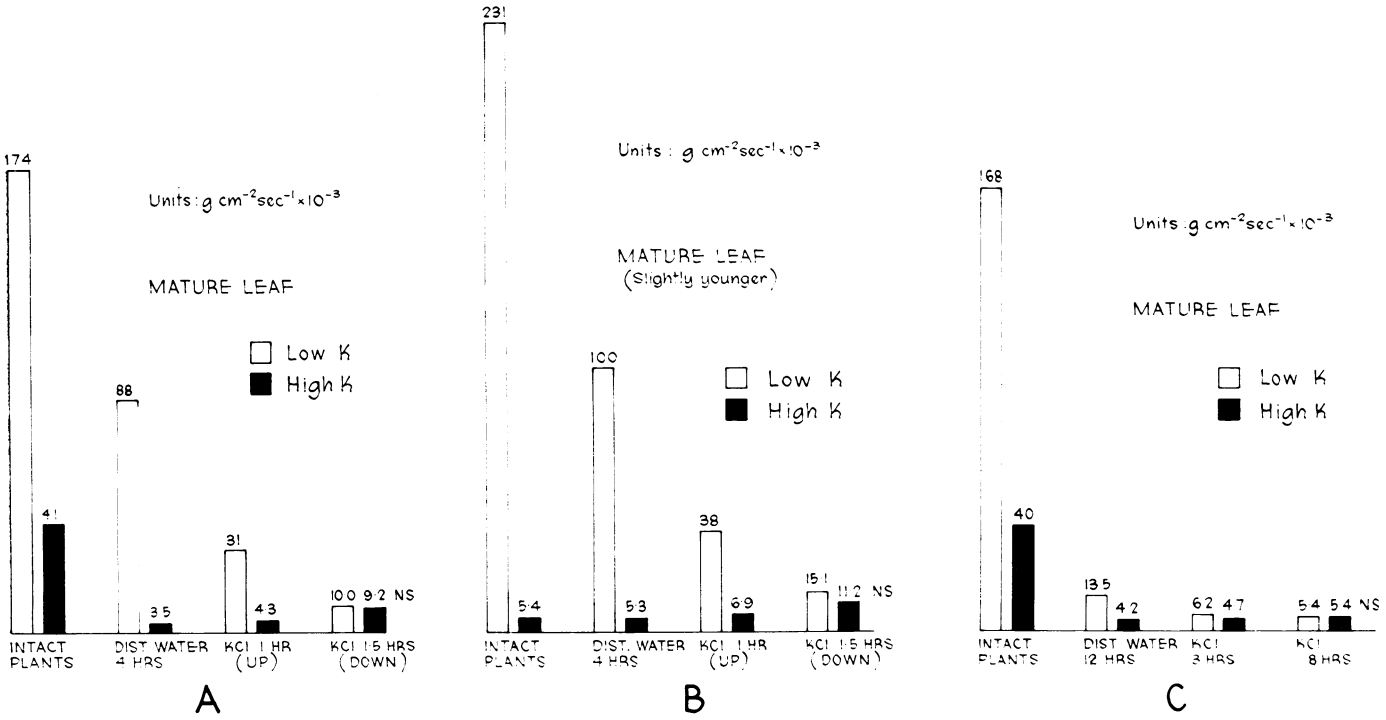

FIG. 1. Viscous resistance of intact leaves or of leaf discs floated on distilled water or 5 mM KCl solution for various times. A: Mature or intermediate aged leaf, 11 days after K cutoff, six replicates; B: mature leaf slightly younger than A, 11 days after K cutoff, six replicates; C: mature leaf, 9 days after K cutoff, three replicates; NS: nonsignificant difference between two means. The words UP and DOWN refer to the adaxial surface of the floating leaf disc.

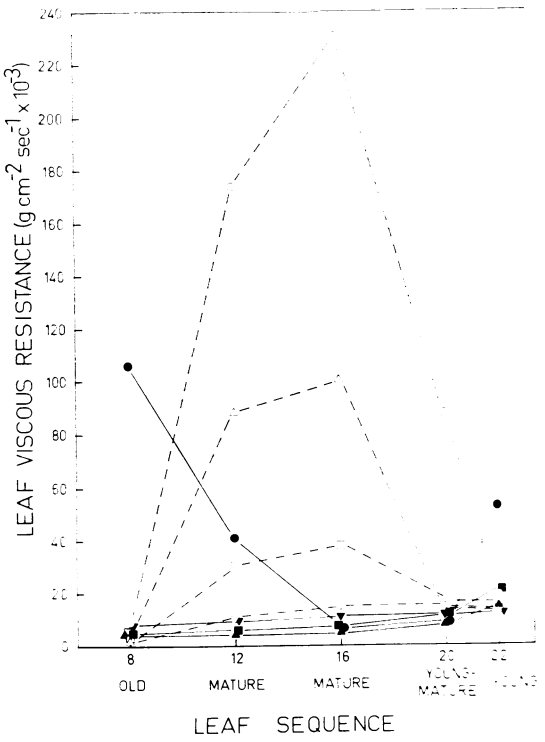

FIG. 2. Leaf viscous resistance profiles for low K and high K plants. Open symbols: low K; closed symbols: high K; ○, ●: intact plants; △, ▲: leaf discs floated on distilled water 4 hr; □, ■: leaf discs floated 1 hr on 5 mM KCl solution in the light with abaxial surface down; ▽, ▼: leaf discs floated a further 1.5 hr on 5 mM KCl solution in the light with adaxial surface down. The first five or six leaves had died.

always produced maximal stomatal opening in high K leaf discs, there was only partial recovery in K-deficient discs. The partial recovery is here termed water-reversible stomatal movement.

Potassium-activated stomatal movement was demonstrated when turgid, low K leaf discs were transferred to 5 mM KCl solution (containing 0.1 mM $CaCl_2$) in the light. Further marked decreases in resistance were observed for the low K discs, so that after a total of 2.5 hr in contact with the KCl solution, the resistance was not significantly different from that of the high K discs, which remained essentially unaffected by the KCl treatment. The slight increase in resistance of the high K discs after 2.5 hr on the KCl solution was considered to be due to repeated manipulations in the porometer. Increase in turgor of epidermal cells as a result of absorption of potassium might also explain this effect. The lower epidermis was left in contact with the solution for 1 hr, and then the disc was turned upper (or adaxial) surface down for another 1.5 hr (Fig. 1, A and B). Note that Figure 1B, giving data for a slightly younger leaf, shows that minimal resistance was obtained by the high K leaf while intact on the plant.

Figure 1C shows data for discs which were not turned over but left abaxial surface down throughout, and, in this situation, potassium must be transported across the leaf to the guard cells of the adaxial surface. In this experiment, replicate discs not previously used were available for the final measurement (8 hr) so that any adverse effect of repeated manipulations in the porometer, as previously mentioned, was eliminated. This graph also shows that as much as 12 hr on distilled water did not promote maximum stomatal opening in low K discs. In this set, as in Figure 1, A and B, the final measurements were not significantly different.

Viscous resistance profiles are shown in Figure 2 for five different ages of leaves of low K and high K plants. The vastly different profiles for intact high K and low K leaves is

the feature of this graph. Also clearly shown is the way in which the low K profile approaches that of the high K leaves with successive floating treatments.

It is most curious that in the intact high K plants, it is the mature leaves which have the minimal leaf resistance, whereas in the K deficient plants, the lowest resistance is found in the quite old and quite young leaves. Leaf resistance in low K plants appears to be inversely related to the leaf potassium levels. In low K plants, potassium concentration is lowest for the mature leaves (about 0.5% K) and increases to more than 3% K in both young and old leaves (Fig. 3). It should be noted that translocation of potassium from old leaves to the growing point is somewhat impeded, losses from the intermediate aged (mature) leaves being much greater. Leaf symptoms of K deficiency were apparent 11 days after K cutoff.

Table I shows the changes in potassium level in the leaf discs corresponding to the data of Figure 1C as well as the levels of the other cations. Note the low sodium levels. In 3 hr, low K leaf discs absorbed enough potassium to double the potassium concentration which, however, was not increased by a further 5 hr floating.

Leaf water potentials obtained by the Shardakov technique are shown in Figure 4. For two determinations, the leaf water potential was outside the range of solutions used, and these values are shown with arrows attached. The curve for the control plants shows an expected trend of increasing water potential with decreasing age while in the low K plants, values were high for old and young leaves, comparing favorably with the controls, and low for the intermediate aged leaves (which constitute most of the plants' leaf area). The pressure bomb technique (16), like the Shardakov method, also gave lower values, relative to controls, for the water potential of mature leaves of low K plants. The water potentials of leaves of K-deficient plants show a striking inverse relationship to leaf viscous resistance (Fig. 2) and appear to be directly related to the potassium content.

The resistance to the passage of water through the root system was measured in a pressure vessel. These data are

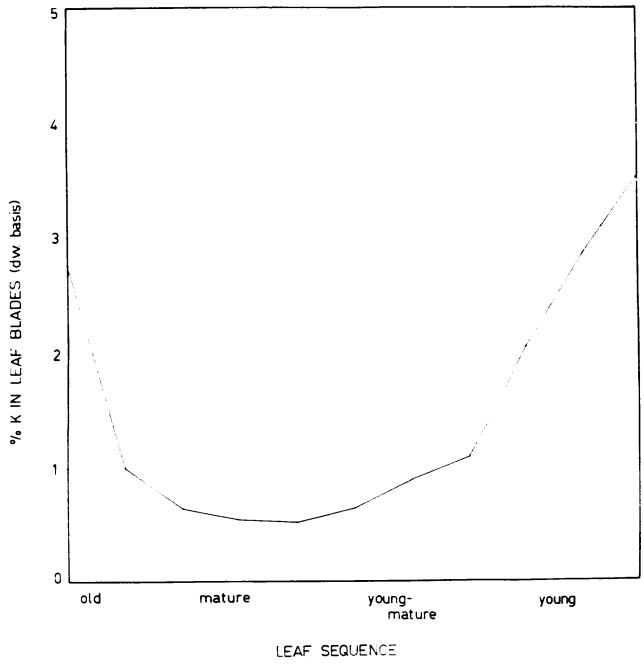

FIG. 3. Potassium concentration in various leaf blades of K deficient (low K) plants. Means of three replicates.

Table I. *Composition of Low K and High K Discs of Leaf 14, Corresponding to the Data in Figure 1C*

Treatment: Hours of Floating on 5 mM KCl Solution	Mineral Composition[1]							
	K		Na		Ca		Mg	
	Low K	High K	Low K	High K	Low K	High K	Low K	High K
	% of dry wt							
0	0.5	8.0	0.12	0.17	1.9	1.1	1.7	1.0
3	1.0	7.3	0.13	0.28	1.8	1.1	1.4	1.0
8	1.0	7.1	0.12	0.21	1.7	1.0	1.3	0.8

[1] Means of three replicates.

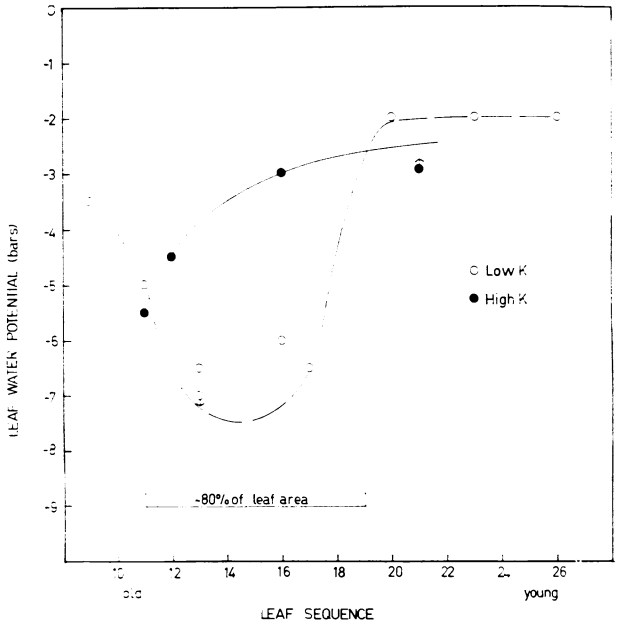

FIG. 4. Leaf water potentials determined by the Shardakov technique for various blades of K deficient (low K) and K sufficient (high K) plants. Arrows on points indicate true values were greater than (↑) or less than (↓) the value shown.

given in Table II, where they are expressed as root system permeabilities since area of absorbing surface was not determined. In fact, the information required is the amount of water per unit time made available to the shoot by the entire root system. The permeability of the control root systems was in every case significantly higher than that of the K deficient root systems. The permeability of high K roots was still about twice as high as that of low K roots when the data were expressed on the basis of unit fresh weight of fibrous roots. Fresh weight of fibrous roots was the only estimate of root absorbing surface available.

Corresponding to, and for comparison with the data of Table II, the mean daytime transpiration rates are shown in Table III. For the minus K treated plants, the mean daytime transpiration rates are of the same magnitude as the fluxes obtained experimentally after removal of the tops. Since a pressure difference of 2 bars was required to produce these fluxes experimentally, the mean daytime pressure potential at the root shoot interface in the intact plants must have been about −2 bars (the actual values at times of peak transpiration may be expected to be considerably more negative). By the same argument, potentials in the intact K sufficient

Table II. *Effect of Removing Potassium from the Culture Solution on the Permeability of the Root System to Water*

Culture Solution	Volume Flux[1]			
	Days after K cutoff			
	8[2]	11[3]	14[2]	15[3]
Minus K	1.1	1.0	2.2	1.1
Plus K	2.5	2.2	5.6	5.9

[1] Units: ml min^{-1} per root system for $\Delta\Psi_p$ = 2 bars; five or six replicates; all treatment differences are significant at the 5% level.
[2] First experiment.
[3] Second experiment.

Table III. *Effect of Removing Potassium from the Culture Solution on the Mean Daytime Transpiration Rate*

Culture Solution	Mean Daytime Transpiration Rate[1]			
	Days after K cutoff			
	8[2]	11[3]	14[2]	15[3]
Minus K	1.3	1.0	1.3	1.1
Plus K	1.9	1.5	2.0	1.9

[1] Units: ml min^{-1} per plant; five or six replicates; all treatment differences are significant at the 5% level.
[2] First experiment.
[3] Second experiment.

plants must be higher (mean values perhaps greater than −1 bar). Leaf areas of the plants corresponding to the data of Table III are published elsewhere (8).

The permeability to water of the petioles of these plants was also determined. Unlike the root systems, the permeability to water of the petioles was relatively unaffected by their potassium status, nor was there any definite trend with time. Measured fluxes (ml min^{-1} cm^{-1} bar^{-1}) were 6.9 for low K petioles and 6.5 for high K petioles. These figures are means of 12 replicates obtained over 2 days and are not significantly different.

DISCUSSION

The data presented show a dual-phased closure of stomata in K-deficient plants: the first is a water-reversible effect, and the second is a potassium-activated effect in the presence of light. That potassium is the specific physiological ion has already been demonstrated (9). The water-reversible closure would appear to be due to a leaf water deficit related partly to the low root permeability to water and so to an indirect effect of K deficiency.

The significance of the epidermal cells adjacent to the guard cells was mentioned in the introduction. Epidermal cells are known to exert pressure on the guard cells (10) though the magnitude of the effect varies among species. Milthrope and Spencer's (12) work suggests that stomatal opening can be induced by low turgor in the epidermal cells. In the present work, the epidermis was left intact on the leaf, and the effect of potassium was to induce full stomatal opening in the K-deficient leaves. The rate and direction of the response was similar to that which has been reported previously for isolated epidermal strips. It must be considered that the experimental conditions were favorable to the attainment of high turgor in the epidermal cells also, and if this is the case, the opening response was achieved against increasing epidermal cell pressure.

It may be inferred from the data of Figure 1, A and B, that contact between either the upper or lower epidermis and the KCl solution is required for as little as 1 hr for maximal stomatal aperture to be obtained. Other data (unpublished) for the sugarbeet plant grown in our growth chambers suggest that this is approaching the normal rate of opening in healthy plants under these conditions. Stomatal opening appears to occur in less than the 3 hr allowed by Fischer and others for maximal opening in epidermal strips (5, 6, 9).

That the low K leaf discs absorb significant amounts of potassium from the KCl solutions is shown by the data in Table I. Mean K concentration is doubled in 3 hr, and none of this accretion is due to exchange for sodium, though some may be due to exchange for calcium and magnesium which are high in K-deficient tissues.

The marked correlation of leaf viscous resistance and of leaf water potential with the K concentration in K-deficient plants is of particular interest. In K-deficient plants both leaf water potential and leaf K concentration are high in old leaves, both decrease to low values in mature leaves, and both increase again to high values in the young leaves (Figs. 3 and 4). In contrast, leaf resistance is high in mature leaves and decreases to low values in both old and young leaves (Fig. 2). The positive correlation of leaf water potential and the negative correlation of leaf viscous resistance with the K concentration suggests some direct relation with the leaf K content itself rather than an indirect effect elsewhere, for example, in the roots.

The direct effect of K content on leaf resistance has already been described but the nature of the relation between leaf water potential and leaf K concentration is not clear. It is suggested that decreased root permeability to water in low K plants contributes both to the high leaf resistances and low leaf water potentials in mature leaves, but this is not the only effect since the old and young leaves are not adversely affected. It appears that in the low K plants, water is perferentially retained by the old and young leaves still relatively high in potassium. These tissues may be as yet undamaged and capable of expending energy for the absorption of ions and retention of water at high water potentials. In contrast, the mature leaves are low in potassium and therefore may be incapable of retention of water at high water potentials.

The marked effect of K deficiency in decreasing root permeability to water was in contrast to the lack of an effect on the petiole permeability. Conductivity by the petiole was clearly localized in the vascular bundles and presumably the xylem dominated in this respect. If the xylem of the roots were assumed to be similarly unaffected by the minus K treatment, then the site of increased resistance to water flow in the low K root systems must be external to the xylem. This would be in agreement with the conclusions of Brouwer (4). It is of interest here that neither low K nor high K sugarbeet root systems show any evidence, when decapitated, of root pressure (metabolically mediated water transport) even after several hours. Nevertheless, potassium clearly plays an important role in root permeability to water.

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