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Day-Night Periodicity of Exudation in Detopped Tobacco[1]

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Summary. Exudate was collected periodically from the root systems of detopped tobacco plants. Volume, cations, and ^{42}K or ^{86}Rb transfer were measured. According to measurements of K by ^{42}K and by the flame photometer, when concentrations of KCl and KNO_3 were lower than 10^{-2} M, the K in the exudate came mostly from a pool in the plant rather than from the external solution. With higher external KNO_3 solutions, within a few hours nearly all of the K in the exudate came directly from the external solutions. Studies with ^{86}Rb lead to the same conclusion. In contrast the maximum proportion of K in the exudate that came from KCl in the external solution was reached usually in many hours after detopping and amounted to from 50 to 75 %. The higher the external concentration the faster it was reached. These data for KCl are indicative of the ^{42}K passing through a K pool in the root cells. K and Rb from high concentrations of KNO_3 and $RbNO_3$, however, may not pass through such a pool. The addition of 10^{-2} M KNO_3 into the external solution during exudation essentially eliminated the effect of periodicity at least for a period of time and under the conditions of the experiments. Hydrochloric acid, mercuric chloride, anaerobiosis, and 2,4-dinitrophenol had the same effect and each resulted in a massive final exudation that usually persisted for 1 to 3 days before stopping. These results all lead to a hypothesis that periodicity is regulated at the tonoplast.

Detopped root systems of many plant species exhibit diurnal fluctuations in exudation volume (2, 3, 4). Much of the old literature of the subject has been reviewed by Van Andel (4) and recent work by Macdowall (2) and Vaadia (3). Vaadia showed that the rate of exudation was regulated osmotically by the rate of salt entry into the xylem from surrounding tissues and that diurnal variations in exudation were related to diurnal variations in salt entry into the xylem (3). The present paper gives some information on the pathway of salt movement to the xylem.

Materials and Methods

Seeds of *Nicotiana tabacum,* var. Virginia Gold were sown periodically on the surface of fine vermiculite in 15 cm pots placed in a warm humid chamber in a glasshouse. They were watered with tap water and germination occurred in about 5 days. The pots were then transferred to a glasshouse bench and watered every other day with one-half strength nutrient solution (full strength solution was in me/liter: 10 Ca, 5 K, 8 Mg, 12 S, 2 P, 2 NH^+_4-N, 11 NO^-_3-N, 0.01 Mn, 0.004B, 0.0025 Zn, 0.0003 Mo, and 0.1 Fe as EDDHA), and with water on alternate days. About 5 weeks from planting the plants were transferred from vermiculite to one-fourth strength nutrient solution in 2-gallon crocks with 4 plants per crock. After 2 weeks the plants were transferred to 3-gallon crocks in one-half strength nutrient solution with 1 plant per crock. After an additional 2 weeks the solutions were changed to full strength, and the plants were used for exudation studies 2 or 3 weeks thereafter. At this time they were in flower and were about 3 meters tall. Dry weight of tops of individual plants was about 100 g and that of roots was about 15 g. Solutions were changed weekly, and crocks were brought to volume daily with distilled water. All air entering the glasshouse was passed through activated charcoal filters to remove air pollutants.

The tops of the plants were removed by cutting about 10 cm above the root-stem junction. Roots were rinsed in deionized water and then placed individually into crocks containing 7 liters of aerated test solution in a constant temperature room maintained at 18°. Test solutions were renewed every 2 or 3 days so that they would not become depleted. A rubber tube was fastened to the stump and this was fastened to a peg in the crock cover to hold the root stump upright. Exudate was collected periodically from the rubber

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tubes with a rubber-bulb pipet assembly, and volume and composition were measured. Generally single salt external solutions were used in the study and in no case was lack of calcium a factor in the results. Previous studies (5) had indicated that under the conditions used, calcium was not essential to the integrity of the system studied and this was verified by detailed checking.

Planting of seed was staggered to give a weekly supply of from 6 to 40 plants and the experiments for this particular report covered about 3 years.

Where indicated, about 40 μc ^{42}K or 125 μc ^{86}Rb were added with K or Rb salts to the solution in which detopped plants were placed. ^{42}K and ^{86}Rb were assayed with a Nuclear Chicago Scintillation well counting system and corrections for decay were made, and the results were calculated from specific activity relationships as μmoles per ml exudate. The values obtained were compared with total K in the exudate and roots which was determined with a Perkin-Elmer Model 52C flame photometer.

Results and Discussion

Collections were made from plants for as many as 19 days (fig 1), and collections could have been made longer. There was no evidence of callus formation or microbial breakdown in 19 days at the temperature used. The rate of exudation was essentially undiminished for several days, diurnal periodicity remained unchanged, and only about 10 % of the cation content of the root was lost to the exudate, even for very high and very low endogenous levels of potassium in roots (table I).

The results with roots in distilled water emphasize that salt accumulation itself is not a necessary component of the exudation process, but that salt release (transport, loss, or diffusion) to the xylem vessels is the driving force that creates the difference in concentration between the xylem vessels of the external solution which results in exudation.

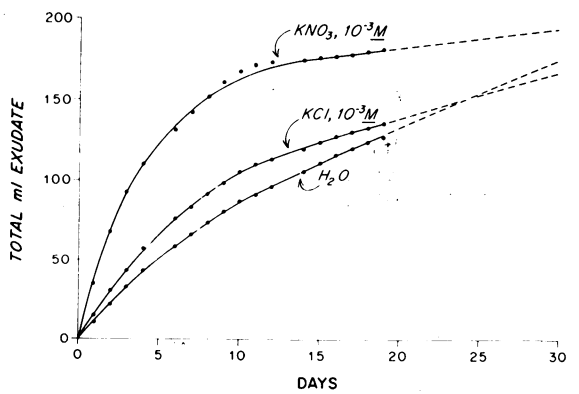

FIG. 1. Exudation from detopped plants from 3 different external solutions over a period of 19 days. The curves were extrapolated to 30 days to illustrate that they eventually may come to a common point.

Table I. *Balance Sheet for K in Detopped Roots in the Presence of Different Substrates and that Appearing in the Exudate in 96 Hours*

External solution M	K in exudate mmoles of K	Total K in roots	Ratio of K in exudate to K in root %
10^{-2}KCl	0.15	16.0	3
10^{-3}KCl	1.00	12.6	8
10^{-4}KCl	0.61	10.5	6
$10^{-2}KNO_3$	4.00	17.8	22
$10^{-3}KNO_3$	3.91	12.1	31
$10^{-4}KNO_3$	0.77	7.3	11
H_2O (control)	0.60	7.3	8

Typical day-night effects are illustrated in figure 2 for 10^{-2} M KCl and 10^{-4} M KNO_3. The peak periods were near noon and the low periods were near midnight. A 24-hour cycle was observed with respect to exudate volume, but conductivity remained reasonably constant for some salt solutions but not for 10^{-2} M KNO_3 (fig 3). Day-night periodicity was also obtained for root systems in distilled water and for those in low but not in high concentrations of KNO_3 (figs 2, 4).

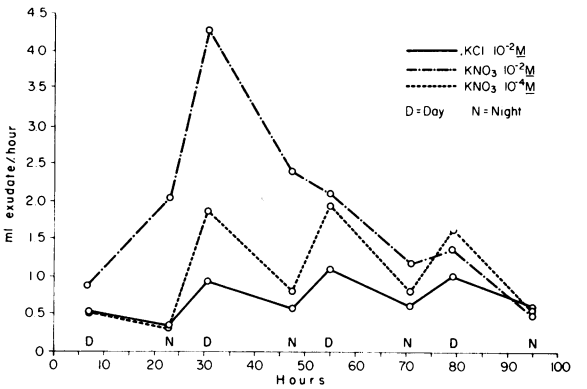

FIG. 2. Exudation from detopped plants in KCl and KNO_3 solutions.

Moderately high levels of KNO_3 and KCl in the external solution gave vastly different time curves. A high level of KNO_3 resulted in loss of periodicity under the conditions employed (fig 2). The conductivity of the exudate with KNO_3 was much higher than that with KCl (fig 3) corresponding with the higher rate of exudation with the nitrate salt. Several logical hypotheses that might explain the differential behavior between the 2 salts were explored and eventually 1 seemed to apply. The rates of appearance of ^{42}K compared with the total K in the exudate for absorption from solutions of potassium salts of chloride and

Table II. *Relationships of Anion Source to the Proportion of K from the External Solution (^{42}K) to the Total K in the Exudate*

Hrs	10^{-4} M KNO_3	5×10^{-4} M KNO_3	10^{-3} M KNO_3	10^{-4} M KCl	5×10^{-4} M KCl	10^{-3} M KCl	10^{-3} M $KHCO_3$
	K by $^{42}K/K$ by flame photometer, %						
5	0.9	6.6	9.2	0.1	0.8	0.9	0.5
21	3.8	57.7	97.0	1.3	13.8	23.5	16.5
29	10.3	66.0	102.7	3.3	29.0	67.6	43.3
44	11.0	60.8	94.2	7.3	43.1	75.9	59.0
52	10.8	46.4	101.2	4.9	42.9	65.6	61.8
68	12.7	48.6	94.9	4.0	42.7	57.8	60.7
77	8.9	60.0	116.0	4.3	31.6	56.8	34.6
92	5.5	74.0	105.8	4.0	36.2	40.2	34.5
Total vol., ml.*	66.0	96.0	130.0	48.0	40.0	52.0	57.0
Mean specific conductivity mmho/cm*	1.44	1.82	2.18	1.33	1.07	1.38	1.24
Mean K in exudate, M*	0.0028	0.0049	0.0083	0.0036	0.0029	0.0050	0.0035

* A water treatment simultaneously gave a volume of 51 ml, a mean conductivity of 1.22 mmho/cm and a mean K content of .0025M.

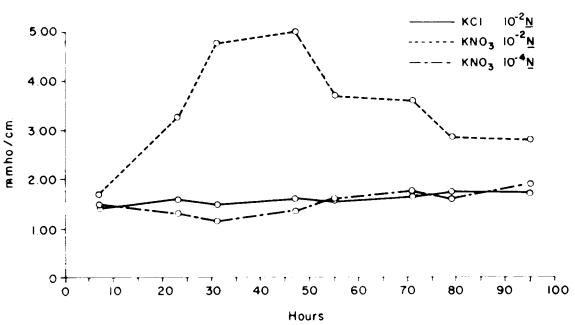

FIG. 3. Conductivities of exudates described in figure 2.

nitrate respectively were determined (table II). A value of 100 % in the table indicates that all of the K_m in the exudate came directly from the external solution. Values over 100 % represent experimental error. The results with $^{42}KNO_3$ indicate that the K in the exudate from 10^{-2} M KNO_3 tended to move directly there from the external solution without first being mixed in a pool with K already in the root. From the chloride source, in contrast, K was mixed with a pool and then transferred to the exudate at a much slower rate than from the nitrate source. Analyses of roots indicated that with chloride only a small component of the K that was taken up moved to the exudate (table I). The majority remained in the root. A larger portion with nitrate moved to the exudate. That portion which was moved to the exudate from nitrate did not seem to pass through a pool and this evidently resulted in lack of periodicity.

This was further checked with $^{86}RbNO_3$ and $^{86}RbCl$. Roots would not contain a rubidium pool and for this reason ^{86}Rb would allow a more direct evaluation of compartmentation than would ^{42}K. The results in table III indicate that the ^{86}Rb did not tend to equilibrate with a pool of K when nitrate was used (the proportion of Rb to Rb + K in the exudate approached 100 %) and that ^{86}Rb moved much more rapidly to the exudate with nitrate than with chloride. The ratio of ^{86}Rb from nitrate in the exudate (0.04) was very much lower than the same ratio for uptake (0.55). For this reason the conclusion is the same as for ^{42}K and that is, with nitrate, salts tend to bypass the vacuole enroute to the xylem vessels.

These data have resulted in the development of a working hypothesis that periodicity is regulated at the tonoplast. In this concept salts are ordinarily stored in the vacuole and differential salt release to the xylem during the day versus during the night results in periodicity of exudation. Exudation that results from transfer of salts to the xylem without passing through the tonoplast such as with high levels of KNO_3 or $RbNO_3$ may not result in periodicity.

It appears that metabolic inhibitors cause loss of salts from cells and such loss results in exudation which is not subject to periodicity. Other workers have reported that the effects of anaerobiosis (2, 4) and of 2,4-DNP in destroying periodicity are reversible (3).

H^+ when used in the external solution at 10^{-3} M also destroyed periodicity (fig 4) even though it resulted in temporary exudation, and some of our

Table III. *Effect of 10^{-2} N $^{86}RbCl$ and $^{86}RbNO_3$ on Exudation Characteristics*

Hrs from detopping	Volume of exudate from Cl	Volume of exudate from NO_3	^{86}Rb in exudate from Cl	^{86}Rb in exudate from NO_3	^{86}Rb from Cl / ^{86}Rb from NO_3	Rb/K in exudate from Cl	Rb/K in exudate from NO_3	Rb/K + Rb in exudate from Cl	Rb/K + Rb in exudate from NO_2
	ml		micro-equivalents		ratios	ratios		ratios	
0–16	0.0	4.7	...	10	0.000	...	0.4	0.00	0.27
16–40	0.8	30.5	1	641	0.001	0.16	9.0	0.16	0.85
40–64	2.1	12.4	2	246	0.008	0.20	12.3	0.20	0.92
64–88	3.9	9.6	6	136	0.04	0.50	17.0	0.33	0.92
88–112	4.4	8.2	12	87	0.15	1.50	43.5	0.60	0.97
112–136	4.2	7.2	12	60	0.20	3.00	30.0	0.75	0.97
136–160	4.0	7.0	10	55	0.22	2.50	18.3	0.75	0.94
160–168	2.0	2.6	4	20	0.25	2.00	10.0	0.67	0.93
Total	21.4	82.2	47	1255	0.04	1.0	9.9	0.51	0.89
Whole roots			13.1*	23.8*	0.55	1.0	1.9	0.49	0.65

* Calculated from loss from external solutions and given in milliequivalents per root.

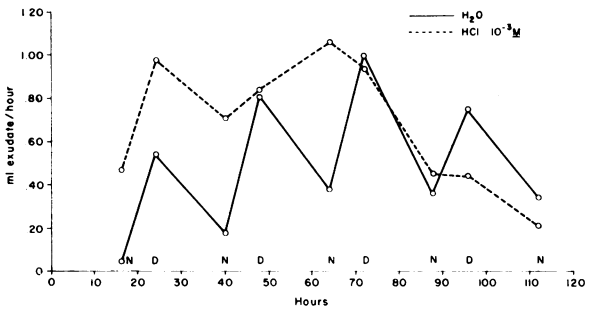

FIG. 4. Loss of diurnal periodicity in exudation as a result of 10^{-3} M HCl. Mean conductivities in mmho/cm for the entire period of exudation was 1.20 for H_2O and 1.67 for HCl.

results with sodium translocation in bush beans indicate that the effect of H^+ may be at the tonoplast (6). Sodium is ordinarily accumulated in roots of bush beans but not readily translocated to shoots. In the presence of 10^{-2} M HCl in the solution in which roots were placed, essentially all of the previously accumulated sodium was translocated to the shoots. Since sodium that is present in cells is largely located in the vacuole, and since nearly all the root sodium was lost to the shoots, the suggestion is that the H^+ effect is located at the tonoplast. If it can be assumed that the same holds for exuding tobacco, then the effect of H^+ in destroying periodicity may be due to its causing leakage from the vacuolar membrane (tonoplast).

Studies have been made of salt accumulation and translocation in the daytime and in the nighttime for intact tobacco and bush bean plants (7). In general more of each isotope was accumulated at night than in the daytime but with the intact plants more was transferred to the shoot during the day than at night. The results emphasize a general discontinuity between salt uptake and transfer to xylem and again indicate that the tonoplast is involved in periodicity of exudation. Salt uptake can increase the magnitude of exudation but in general salts are stored in a compartment, probably the vacuole, and salt release from the vacuole largely controls the rate and periodicity of exudation.

Considerable study has been made by other workers of the question of whether or not salts leak or are transported to the xylem vessels. Laties and Budd have produced evidence strongly favoring a hypothesis of leakage at the endodermis (1). Chemicals which caused salts to leak to the xylem did cause a proportionate increase in exudation as would be expected according to the osmometer theory. The increase was not subject to periodicity. $HgCl_2$ is used to cause cell leakiness and it seemed to have this effect for tobacco roots (table IV). H^+ and 2,4-DNP each had a similar effect although the mode of action of all of these may not be exactly the same (figs 4, 5). This

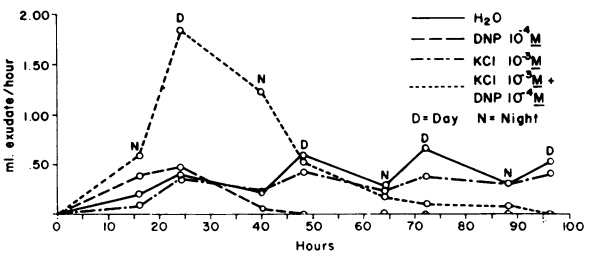

FIG. 5. Loss of diurnal periodicity in exudation as a result of 2,4-DNP. The mean conductivities of the exudate in mmho/cm, for the first 48 hours were 1.17 for H_2O, 1.40 for DNP, 1.13 for KCl, and 1.45 for KCl and DNP.

Table IV. *Effect of $HgCl_2$ on Tobacco Root Exudation Applied with 10^{-3} M KNO_3*

$HgCl_2$	Volume of exudate		Conductivity of exudate	
	0–6 hrs	6–168 hrs	0–6 hrs	6–168 hrs
M	ml	ml	mmho/cm	mmho/cm
0	9	233	1.70	2.33
10^{-5}	4	156	2.00	2.20
10^{-3}	39	6	1.90	2.20
Water only (no KNO_3)	1	69	1.35	1.18

same effect for anaerobiosis had been reported by Macdowall (2). All the treatments mentioned here destroyed periodicity and eventually stopped exudation.

The results above with H^+ and metabolic inhibitors indicate that there is a metabolic barrier at the tonoplast to salt loss to the xylem vessels. If this is so, then the endodermis probably allows free passage into the vessels as suggested by Laties and Budd (1).

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