Chloride Uptake and Transport in Roots of Different Salt Status 1, 2

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In a previous report (8) it was shown that the rate of radiochloride transport to the exudation stream of single exuding roots was less in high salt roots than in low salt roots. Although similar data have been presented by other investigators, (5, 15, 16, 18), some studies show that the transport of various ions to plant shoots or xylem exudates in high salt plants is greater than in low salt plants (6, 7, 16, 17, 18).

The concentration of the external solution appears to play an important role in determining whether ion transport to shoots will be greater or smaller in high salt plants relative to low salt plants. Russell, Martin, and Bishop (18) and Russell and Shorocks (16) have shown that high salt barley plants transport 32P2- (salt not specified) to shoots at a greater rate than do low salt plants when the external concentration is low, but that the reverse is true when the external concentration is high. Furthermore, Russell and Shorocks showed that a relationship exists between nutrient transport to shoots and transpiration rates only when high salt plants or high external concentrations are used. Similar data were presented earlier by Broyer and Haogland (3). Hylmø and colleagues (9, 10, 11) have interpreted this type of relationship to mean that ions are passively swept into and across the root to the xylem with the transpiration stream. Broyer and Haogland and Russell and Shorocks, however, feel that the more rapid removal of ions from the vascular tissue caused by enhanced transpiration, serves to increase the overall rate of active ion transfer.

Since the possibility exists that the salt status of the xylem stream may affect the rate of ion entry into the xylem, this particular point has been investigated and the results are presented here. In addition, the effect of the chloride content of the root tissue on the rate of Cl- transport to the exudation stream of individual roots was investigated.

Materials and Methods

Individual exuding onion (Allium cepa, var. Early Grano) roots were used for all experiments. Culturing of the onion bulbs, experimental procedure, and sampling technique were as described in the previous report (8). Exudation rates were determined by weighing the sap which was collected in glass tubes and Cl36 in the exudate was determined directly by liquid scintillation spectroscopy. Radiochloride was extracted from the blotted and dried (60°) root tissue with 0.1N HNO3 for 48 hours and an aliquot of the extract was counted for Cl36 activity by liquid scintillation. Total chloride was determined with a Coastove chloridometer, Model 4-2000.

Results and Discussion

Different xylem concentrations of chloride can be obtained by varying the length of root exposed to the chloride solution (8). The remaining portion of the root, when exposed to a dilute CaSO4 solution, absorbs water which dilutes the chloride in the xylem stream. Figure 1 shows the transport of Cl36 to the exudation stream (period II) when different lengths of roots had been pretreated (period I) with nonlabeled chloride for 12 hours. The 4 pretreatments were as follows: 1) Entire roots in 0.3 mM CaSO4; 2) Root zone A in 0.3 mM CaSO4 plus 2.0 mM KCl—root zones; B and C in 0.3 mM CaSO4; 3) Root zones A and B in 0.3 mM CaSO4 plus 2.0 mM KCl—root zone C in 0.3 mM CaSO4; 4) Root zones A, B, and C in 0.3 mM CaSO4 plus 2.0 mM KCl.

![Figure 1](https://www.plantphysiol.org/)

**Fig. 1.** The effect of different xylem concentrations of nonlabeled chloride (period I) on the transport of Cl36 to the exudate (period II). Different xylem concentrations were obtained by exposing the A, B, and C root zones, the A and B zones, the A zone, or none of the root to nonlabeled chloride. During period II Cl36 was exposed to root zone B. During both periods the root zones not exposed to a chloride solution were bathed in 0.3 mM CaSO4.
The exudate concentrations at the end of period I were approximately 0, 2, 3 and 5 meq/liter for pretreatments 1, 2, 3, and 4, respectively. The transport of Cl\textsuperscript{36} to the exudation stream during period II (only zone B exposed to Cl\textsuperscript{36}, zones A and C to CaSO\textsubscript{4}) was essentially the same for the variously pretreated roots. Thus, it appears that the concentration of chloride in the xylem had no effect on the subsequent entry of Cl\textsuperscript{36} into the xylem. However, the indifference to xylem chloride concentration did not always obtain. If the pretreatment time was longer than 12 hours the subsequent rate of Cl\textsuperscript{36} transport to the exudate was least in roots having the highest xylem concentration of nonlabeled chloride. If, however, the pretreatment time was shorter than 12 hours, the reverse was true, i.e. Cl\textsuperscript{36} transport to the xylem was greatest in roots having the highest xylem concentration of nonlabeled chloride. In addition, it was found that roots from onion bulbs obtained from different sources did not respond to pretreatments the same as the roots we used routinely. In one batch of roots, the highest nonlabeled chloride concentration in the xylem resulted in the greatest rate of Cl\textsuperscript{36} entry into the xylem. In still a third batch of roots the reverse occurred, i.e. roots with the highest nonlabeled chloride concentration in the xylem had the lowest rate of Cl\textsuperscript{36} transport to the xylem. Analysis of the roots from these various bulbs indicated that the initial chloride levels (prior to pretreatment) were different. These very con-

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**Fig. 2.** The effect of pretreatment time with nonlabeled chloride on the rate of Cl\textsuperscript{36} transport to the exudate.

**Fig. 3.** The effect of pretreatment time with nonlabeled chloride on the rate of nonlabeled chloride release and transport to the exudate.
fusing results led us to study the rate of Cl\textsuperscript{36} transport to the xylem as affected by the general chloride status of the root tissue.

The effect of different pretreatment times with nonlabeled chloride on the subsequent rates of labeled and nonlabeled chloride transport to the exudate are shown in figures 2 and 3. For this experiment the roots were cut from the bulbs and placed in the experimental boxes, which contained 0.3 mm CaSO\textsubscript{4} 25 hours prior to the experiment. The CaSO\textsubscript{4} solution was then replaced with the nonlabeled chloride solution at 24, 20, 13, 8, 4, and 2 hours prior to the experiment. One set of roots remained in the CaSO\textsubscript{4} solution for the entire 25 hours. All roots were then exposed to the Cl\textsuperscript{36} solution for an additional 9 hours, during which the exudate was collected and analyzed for total chloride and Cl\textsuperscript{36}. At the termination of the experiment the root tissue was analyzed for total chloride and Cl\textsuperscript{36}. Nonlabeled chloride in the exudate and tissue was calculated by subtracting Cl\textsuperscript{36} from total chloride.

Figures 2 and 3 show that exudation rates of both labeled and nonlabeled chloride was essentially linear over the 9 hour experimental period. The relative amounts of labeled ion (from solution) and nonlabeled ion (previously accumulated by the tissue) appearing in the exudate were a function of pretreatment time. Nonlabeled chloride transport (fig. 3) increased with increasing pretreatment time while Cl\textsuperscript{36} transport (fig 2) was increased by the short pretreatments (2-8 hours) and decreased by the longer pretreatments (20-24 hours). This effect of pretreatment time on Cl\textsuperscript{36} transport undoubtedly accounts for the variable results noted earlier as well as the contrasting reports in the literature (see Introduction).

The data from this experiment are plotted as a function of pretreatment time in figure 4. All quantities shown are total amounts for the 9 hour experimental period. Total Cl\textsuperscript{36} absorbed is the sum of that accumulated by the root tissue plus the amount transported to the exudate. Total chloride delivered to the exudate reached a maximum in roots pretreated 4 to 8 hours. However, the relative amounts of labeled and nonlabeled chloride entering the xylem varied considerably depending on the pretreatment time. Tissue accumulation of Cl\textsuperscript{36} progressively declined with increasing pretreatment time as did total Cl\textsuperscript{36} absorption. It should be noted, however, that total Cl\textsuperscript{36} absorption was reduced only slightly by the short (2 and 4 hours) pretreatments.

The data for the 0 and 13 hour pretreated roots are of particular interest since Cl\textsuperscript{36} transport to the exudate was the same for both of these pretreatments. In the case of the 0 pretreatment the exuded chloride represented only about 1/3 of the total Cl\textsuperscript{36} absorbed. In other words, twice as much Cl\textsuperscript{36} was accumulated as was transported to the xylem. In the 13 hour pretreated roots the exuded chloride was about half of the total absorbed; the other half being accumulated by the tissue. In this case, though, total chloride exuded, which was twice the amount exuded in 0 pretreated roots, equaled the total Cl\textsuperscript{36} absorbed from the external solution. Therefore, no net gain in tissue chloride occurred and for each labeled ion accumulated by the tissue a nonlabeled ion was released and transported to the xylem. The equivalent amounts of nonlabeled and labeled ions transported to the xylem must mean that vacuolar ions moved back into the symplast and mixed with ions absorbed from the external solution.

Pretreatment to build up very high nonlabeled chloride concentrations in the tissue (20-24 hours pretreatment) serves to further reduce total Cl\textsuperscript{36} absorption. In these roots, as in the 13 hour pretreated roots, approximately equivalent amounts of Cl\textsuperscript{36} were accumulated by the tissue and transported to the exudate. The longer pretreatment times did not alter the total chloride exuded, therefore, the decreased transport of Cl\textsuperscript{36} to the exudate was compensated for by the greater rate of nonlabeled chloride release and transport.

The total chloride in the root tissue at the end of the experiment was similar for all pretreatments. The 0 and 20 hour pretreated roots did contain...
slightly more chloride than the intermediate length pretreatments, however, the significance of this was questionable.

The release of vacuolar ions and their transport to the xylem was investigated further by determining their rate of release in the presence and absence of external chloride. Roots (only zones A and B were used) were pretreated for 12 hours in the $^{35}\text{Cl}$ solution and then placed in either 2.0 mKCl plus 0.3 mCaSO$_4$ or just 0.3mKCl. In addition, one set of roots had root zone A (0–3 cm) exposed to the KCl solution and root zone B (3–6 cm) exposed to the CaSO$_4$ solution. Another set of roots had the location of these solutions reversed. The concurrent transport of $^{36}\text{Cl}$ and nonlabeled chloride to the exudate for the various treatments is shown in figures 5 and 6. The transport of previously accumulated $^{36}\text{Cl}$

![Fig 5](image)

**Fig. 5.** The effect of the presence or absence of nonlabeled chloride in the external solution on the transport of previously accumulated $^{36}\text{Cl}$ to the exudate. Roots were pretreated for 12 hours with 2.0 mKCl plus 0.3 mCaSO$_4$.

**Fig. 6.** The transport of nonlabeled chloride to the exudate for the experiment described in figure 5.

to the exudation stream was least when entire roots were bathed in KCl and greatest when entire roots were bathed in CaSO$_4$. With only a part of the root exposed to KCl, the $^{36}\text{Cl}$ movement from root to exudation stream was at an intermediate rate. The transport of nonlabeled Cl to the exudation stream was obviously greater when KCl was present during the second period of the experiment (fig 6). Nevertheless, the nonlabeled Cl transported to the exudation stream, when the entire root was exposed to KCl, was only slightly greater than the $^{36}\text{Cl}$ movement from root to exudation stream when the entire root was in CaSO$_4$. For the latter three cases, the total chloride transported to the exudation stream was essentially the same (fig 7).

Slightly more $^{36}\text{Cl}$ moved to the external solution from roots exposed to KCl during the experimental period than from roots exposed to CaSO$_4$ only. However, this difference in exchange was insufficient to account for the different amounts of labeled and nonlabeled chloride in the exudation stream.

This experiment, like the previous one, illustrates that vacuolar ions can supply a major portion of the ions transported to the xylem. It also illustrates that the actual amount of vacuolar ions released and transported to the xylem is dependent on the amount of external ions absorbed and transported.

**Discussion**

The experiments reported here help to explain the contrasting reports which have been made concerning the effect of plant salt status on ion transport to xylem exudates or plant shoots (5, 6, 7, 15, 16, 17, 18). Low chloride roots transported $^{35}\text{Cl}$ to the exudate at the same rate as similar roots pretreated for 13 hours with nonlabeled chloride (fig 2, 4). However, pretreatments shorter than 13 hours resulted in enhanced rates of $^{36}\text{Cl}$ transport to the exudate, while pretreatments longer than 13 hours resulted in decreased rates of $^{36}\text{Cl}$ transport.

By exposing different lengths of roots to nonlabeled chloride it was possible to show that the concentration of nonlabeled chloride in the xylem had no effect on the subsequent rate of $^{36}\text{Cl}$ entry into the xylem (fig 1). The results of this experiment depended on the fact that root zone B, from which $^{36}\text{Cl}$ absorption occurred, was exposed to nonlabeled chloride for either 0 or 12 hours, i.e. times which should have resulted in approximately equal rates of $^{36}\text{Cl}$ transport (figures 2, 4) providing the xylem chloride concentration had no regulatory effect. These results illustrate that the chloride status of
cortical tissue and not the xylem are responsible for salt status responses such as those presented in figure 4. The initial experiments concerning the effect of xylem concentrations of chloride, using different pretreatment times and roots from onion bulbs of different sources, obviously gave variable results because of the different amounts of chloride in the cortical tissue.

The data of figure 4 are quite revealing with respect to the path and mechanism of ion transfer across the root. It is apparent that ions can move to the xylem directly through the symplast (Cl\(^{36}\) transport following short pretreatments) or after having been accumulated into vacuoles (nonlabeled chloride transport following long pretreatments). The amount of chloride released from vacuoles appears to regulate the amount of absorption, accumulation, and transport of external chloride. Likewise, figures 5 and 6 show that the absorption of external chloride alters the amount of vacuolar released chloride which is transported to the xylem. As suggested in the previous paper (8) it appears that a system of transport sites exist in the cytoplasm which can be occupied by either internal (vacuolar released) or externally absorbed chloride. It is suggested that with increasing pretreatment time, progressively more of the sites would be occupied by vacuolar released chloride and correspondingly fewer sites would be free for occupancy by external chloride.

Total chloride transport to the exudate did not reach a maximum until roots had been pretreated for 4 to 8 hours (fig 4). For shorter pretreatments, the rate of external ion (Cl\(^{36}\)) entry into the cytoplasm and the amount of vacuolar released ions were apparently insufficient to saturate the transport sites. This is also suggested by the preferential accumulation of Cl\(^{36}\) following the 0, 2, and 4 hour pretreatments (fig 4). However, with increasing pretreatment time up to 8 hours, the transport sites occupied by vacuolar released chloride did progressively reduce tissue (vacuolar) accumulation of Cl\(^{36}\). The lack of transport site saturation, coupled with the reduced vacuolar accumulation of Cl\(^{36}\) following short pretreatments, enabled more of the absorbed Cl\(^{36}\) to be transported to the xylem (fig 4). This situation is apparently analogous to other reports which show a greater rate of ion transport in high salt plants than in low salt plants (6, 7, 16, 17, 18). Such results were generally obtained only when using low external concentrations, thus transport site saturation would not have prevailed in the low salt plants.

When sufficient vacuolar and external chloride ions are available for saturating the transport sites, then any additional vacuolar chloride, resulting from a pretreatment (in the present case longer than 8 hours), simply serves to reduce the number of sites available for absorption, accumulation, and transport of external Cl\(^{36}\). This situation is similar to reports which show that high salt plants transport ions at reduced rate relative to low salt plants (5, 15, 16, 18). These particular results were most frequently observed when high external concentrations were used, thus sufficient external ions were apparently available for saturating the transport sites in the low salt plants and any additional internal ions (in high salt plants) would only lower the number of sites available for external ions.

Roots pretreated for 13 hours appeared to have half the transport sites occupied by vacuolar released chloride and half by externally absorbed chloride. Such a situation would account for Cl\(^{36}\) accumulation being reduced by one-half relative to that in 0 pretreated roots and for the equivalent amounts of labeled and nonlabeled chloride transported to the exudate (fig 4).

As pointed out earlier, the concentration of nonlabeled chloride in the xylem had no effect on the entry of Cl\(^{36}\) into the xylem. In addition, it was shown in the previous report (8) that very little chloride moved from the xylem to the cortical tissue in the exuding roots. However, it is possible that in intact plants, which represent more of a closed system than the exuding roots used here, that ion concentrations in the xylem could become sufficiently high to result in a significant movement of ions from xylem to cortical tissue. Since these ions could also occupy transport sites, the absorption of external ions would be retarded. This situation would undoubtedly be most pronounced in high salt plants and under conditions of low transpiration rates. A slight increase in transpiration would then serve to reduce the efflux of ions from xylem to cortical tissue and consequently free transport sites for absorption of external ions. This would explain why a relationship frequently exists between salt transport and transpiration rates in high salt plants but not in low salt plants (3, 16, see also 9, 10, 11).

The data presented here support Lundegardh's (12, 13, 14) contention that ions can be accumulated into vacuoles prior to their movement to the xylem. They also support the concept of a direct movement of ions through the symplast as emphasized by Arisz (1) and Broyer (2). Furthermore, scheme III of Fried et al. (4) will, in general, account for the movement of chloride observed here. The present work merely provides clarification of the quantitative aspects of these phenomena and emphasizes the regulating effect of endogenous ions on the absorption of external ions.

**Summary**

The absorption, accumulation and transport to the exudation stream of Cl\(^{36}\) in exuding onion (Allium cepa) roots as a function of the chloride content of the tissue and the xylem has been investigated. The concentration of nonlabeled chloride in the exudate had no discernable effect on the Cl\(^{36}\) transport to the exudation stream.

The chloride content of the root tissue had a major effect on the absorption, accumulation and transport of Cl\(^{36}\). Small amounts of chloride in the tissue accelerated the rate of Cl\(^{36}\) transport to the exudation stream. Large amounts of chloride in the tissue...
decreased the rate of Cl\textsuperscript{36} transport to the exudation stream.

The rate of movement of the previously accumulated chloride to the exudation stream progressively increased with increasing amounts of chloride in the tissue, while the accumulation of Cl\textsuperscript{36} by the root tissue progressively decreased.

These results have been interpreted to mean that a system of transport sites, which absorb external chloride and transport it to either vacuoles or xylem, exists throughout the cytoplasm. These transport sites appear to be occupied by either internal (from vacuoles) or externally absorbed ions. It is suggested that the number of sites occupied by internal ions serves to regulate the uptake, accumulation and transport to the xylem of external ions.

Acknowledgment

The authors would like to thank Professors C. R. Stocking and E. Epstein for their advice throughout the course of this investigation and Professors J. B. Hanson and R. H. Hageman for their advice and criticism in preparing the manuscript.

Literature Cited