

Cation Absorption by Slices of Stem Tissue of Bean and Cotton

The extent to which Na and K are translocated from the roots to the shoots varies with plant species¹. Bean plants translocate considerable amounts of K to their tops but little Na, whereas cotton plants translocate both Na and K.

The regulation of Na translocation has been considered to be a function of the roots², the stem tissue^{3,4}, or both⁵. JACOBY^{3,4} made a strong case that the stem tissue is the site of Na regulation. PEARSON⁵ has suggested that in Na-excluding plants such as bean, both stem and root tissue might be instrumental in restricting free movement of Na. BERNSTEIN et al.⁶ proposed that cells next to the conducting tissue might be directly involved in removing Na from the transpiration stream, thus excluding Na from the tops of the plants. There appears to be no direct evidence as to which of the stem tissues are involved in Na exclusion.

An investigation was undertaken of the absorption of K and Na by stem tissues of cotton and bean plants. The tissues used were whole stem, extra-cambial tissue (phloem, cortex, and epidermis), and xylary tissue (xylem and pith).

Various parameters of Na and K absorption by these 3 tissues were studied to obtain more information on possible mechanisms involved in selective regulation of ion translocation.

Brittle wax bean (*Phaseolus vulgaris* L.) and cotton SJI (*Gossypium hirsutum* L.) plants were grown in nutrient solution containing 0.4 mM KNO₃, 0.8 mM Ca(NO₃)₂, 0.4 mM NaH₂PO₄, 0.2 mM MgSO₄, and 2 ml micro-nutrient stock solution⁷.

After 3 weeks the plants were harvested and the upper and lower hypocotyl collected and placed in water until sampled. The hypocotyl tissue was cut into 3-cm lengths and a cut made along each entire length with a razor. The blade was inserted until there was some resistance, and the cut was made. The outer tissue was peeled from the inner cylinder, and microscopic examination showed that separation had occurred at the cambium. The outer tissue (designated extracambial tissue) thus contained epidermal, cortical and phloem tissue, and the inner cylinder (designated xylary tissue) contained xylem tissue and associated cells, along with pith tissue.

Slices 400 μ wide were cut across the stem tissue with a hand microtome as detailed in previous papers^{8,9}. Approximately 40 slices from each tissue made up each sample, and the slices were placed in a cheesecloth bag¹⁰.

The samples were suspended for 1 h at 30°C in an aerated solution of 0.5 mM CaSO₄. The samples were then exposed to solutions of various concentrations of Na labeled radioactively with sodium-22, or K labeled radioactively with potassium-42 or rubidium-86. There was no qualitative difference in results of experiments when rubidium-86 or potassium-42 was used to label K. The use of rubidium-86 to label K is valid and well documented for species of higher green plants^{9,11}. This labeling procedure was used in all subsequent experiments. At the end of the absorption period the samples were placed in desorption solutions containing 1 mM K or 1 mM Na, non-radioactive, and 0.5 mM Ca for 30 min. This was done to remove any freely exchangeable ions¹⁰. All solutions contained 0.5 mM CaSO₄.

The samples were analyzed for radioactivity, and the counts were compared with a standard. The results are expressed on the basis of μ moles/g fresh weight.

Figure 1 presents results on absorption. Sodium and K were present at a concentration of 0.1 mM. The rate of

K absorption by both cotton and bean was very low for all 3 stem tissues: whole stem, extracambial, and xylary tissue. The rate of Na absorption was also quite low for the 3 stem tissues of cotton but not for bean stem tissue. In bean stems, Na uptake was much greater than K uptake. When the stems were separated into extracambial and xylary portions, the xylary tissue was found to be mainly responsible for the greater rate of Na absorption of the whole bean stem.

Could the results be explained by differences between the 2 species in the initial cation status of the tissues? Tissue analysis for K and Na was carried out by flame spectrophotometry. Absorption of Na and K did not appear to be directly correlated with the initial cation content. The distribution of Na in the 2 bean stem tissues shows no correlation with the amount of Na absorbed by these tissues. If the cation content affected the rate of absorption of Na there should be no difference between extracambial and xylary tissue in absorption of Na since their initial Na content is the same. Also the K content was lower in the bean stem tissue than in cotton stem tissue but this had no significant effect on the rate of K absorption by stem tissue of these 2 plant species.

The effect of antimetabolites on Na absorption by bean stem tissue was tested. It was found that low temperatures (8°C), anaerobic conditions, and carbonyl cyanide *m*-chlorophenylhydrazone at a concentration of 1 μ molar

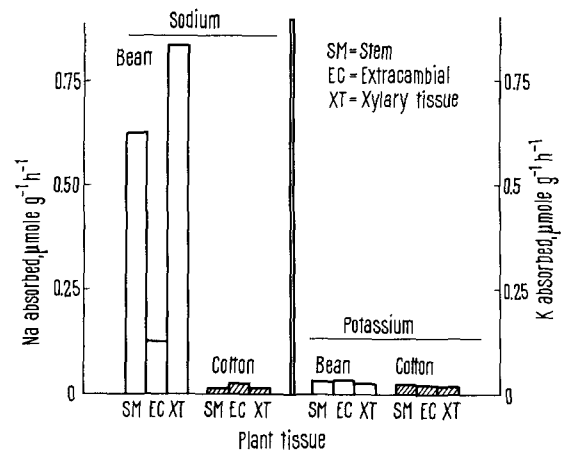


Fig. 1. The rate of K and Na absorption by stem tissues of cotton and bean. Potassium and Na are present at a concentration of 0.1 mM, Ca, 0.5 mM. The legend for the stem tissues is given on the Figure. The shaded bar graphs represent the data for cotton and the unshaded for bean stem tissues. Absorption time 1 h.

¹ R. COLLANDER, Pl. Physiol., Lancaster 16, 691 (1941).

² H. G. GAUCH and C. H. WADLEIGH, Soil Sci. 59, 139 (1945).

³ B. JACOBY, Pl. Physiol., Lancaster 39, 445 (1964).

⁴ B. JACOBY, Physiol. Plant. 18, 730 (1965).

⁵ G. A. PEARSON, Pl. Physiol., Lancaster 42, 1171 (1967).

⁶ L. BERNSTEIN, J. W. BROWN and H. E. HAYWARD, Proc. Am. Soc. hort. Sci. 68, 86 (1956).

⁷ C. M. JOHNSON, P. R. STOUT, T. C. BRODER and A. B. CARLTON, Pl. Soil 8, 337 (1957).

⁸ R. C. SMITH and E. EPSTEIN, Pl. Physiol., Lancaster 39, 338 (1964).

⁹ D. W. RAINS, Pl. Physiol., Lancaster 43, 394 (1968).

¹⁰ E. EPSTEIN, W. E. SCHMID and D. W. RAINS, Pl. Cell Physiol., Tokyo 4, 79 (1963).

¹¹ D. W. RAINS and E. EPSTEIN, Aust. J. biol. Sci. 20, 847 (1967).

resulted in approximately 80% inhibition of Na absorption. The uptake of Na by bean stem tissue appears to be mediated by metabolic processes.

The possibility of a sodium-specific transport mechanism was tested. Since the whole stem reflects the absorption characteristics of the xylary tissue, whole stem tissue was used in this experiment.

Figure 2 illustrates the results of an experiment on the effect of K on Na absorption by bean stem tissue. The concentration of Na varied from 0.01–0.2 mM in the presence or absence of 0.2 mM K. The presence of 0.2 mM KCl resulted in no depression of Na absorption by stem tissue. This is quite different from what is observed with other plant tissues¹². In root tissue, for instance, K interferes greatly with Na absorption^{13,14}.

The above results seem to support the contention of BERNSTEIN et al.⁶ that the regulation of Na translocation is mediated by cells closely associated with the xylem tissue. These authors suggested that these cells are capable of removing Na from the transpiration stream, thereby diminishing the transport of Na to the tops. The results shown in Figure 2 indicate that the mechanism for

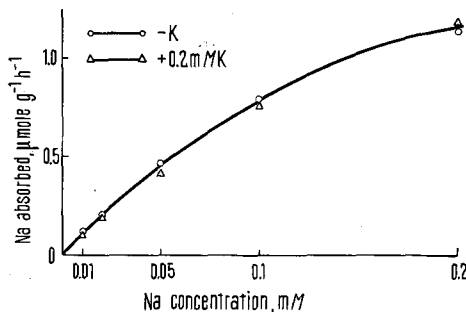


Fig. 2. The rate of Na absorption by stem tissue of bean as a function of increasing Na concentration in the presence and absence of 0.2 mM K. Sodium concentration varied from 0.01–0.2 mM. Ca, 0.5 mM. Absorption time 1 h.

Na transport is quite selective, at least in the range of concentrations used in these experiments. Also the data might reflect an absorption mechanism which appears to saturate at fairly low concentrations, as proposed by JACOBY^{3,4} but the quantitative aspects of this will have to await further investigation.

The data presented do not gainsay the importance of roots in the regulation of Na distribution, and as PEARSON⁵ suggested, both roots and stems are probably involved in the regulation of Na in Na-non-accumulator plants such as beans. The information presented here does suggest that a metabolically mediated specific mechanism for the regulation of Na translocation is located in the bean stems and is closely associated with xylary tissue. The lack of such a mechanism in cotton plants is to be expected since these plants readily translocate Na as well as K¹⁵.

Zusammenfassung. Stengelgewebe von *Phaseolus* und *Gossypium* wurden in einen extrakambialen Teil (Phloem, Rinde und Epidermis) und in einen Xylemteil (Xylem und Mark) getrennt. Die Aufnahme von K und Na in diese Gewebe ist bei *Gossypium* sehr gering, ebenfalls die K-Aufnahme bei *Phaseolus* wegen des Na-Aufnahme-Vermögens der Xylemgewebe wesentlich höher. Die Na-Aufnahme wird durch K nicht beeinflusst und verläuft metabolisch.

D. W. RAINS

Kearney Foundation of Soil Science, University of California, Davis (California 95616, USA),
30 August 1968.

¹² E. EPSTEIN, *Nature* 212, 132 (1966).

¹³ D. W. RAINS and E. EPSTEIN, *Science* 148, 1611 (1965).

¹⁴ D. W. RAINS and E. EPSTEIN, *Pl. Physiol.*, Lancaster 42, 314 (1967).

¹⁵ I thank Dr. E. EPSTEIN and Dr. A. LÄUCHLI for advice on preparation of this manuscript.

Nachweis hoher Aktivitäten von Kreatin-Kinase bei dem Chaetognathen *Sagitta setosa*

Kaum eine Tiergruppe bereitet bei der Einordnung im System der Tiere ähnliche Schwierigkeiten wie die Chaetognathen¹. In der vorliegenden Arbeit wird versucht, zur systematischen Gruppierung der Chaetognathen aus vergleichend biochemischer Sicht Stellung zu nehmen. Bekanntlich ist die Verteilung der Guanidinphosphate im Tierreich verschiedentlich systematisch ausgewertet worden². Kreatinphosphat ist das einzige Phosphagen der Wirbeltiere. Es findet sich, z. T. neben Argininphosphat in der «Chordatenlinie» bei Echinodermen und Prochordaten. Generell besitzen sonst Invertebraten das Argininphosphat, nur Anneliden zeigen eine bemerkenswerte Mannigfaltigkeit anderer Phosphagene³. Wir weisen hier das Vorhandensein hoher Aktivitäten der ATP-Kreatin-Phosphotransferase (Kreatin-Kinase) bei gleichzeitigem Fehlen der ATP-Arginin-Phosphotransferase (Arginin-Kinase) in Geweben der Chaetognathen nach.

Die Kreatin-Kinase-Aktivität wurde im optischen Test bestimmt, wobei Pyruvat-Kinase als Hilfsystem und Laktat-Dehydrogenase als Indikatorsystem dienten⁴.

Einzelheiten der Methode wurden mehrfach beschrieben⁵. Die Bestimmung von Arginin-Kinase-Aktivität erfolgte analog dem Kreatin-Kinase-Ansatz nach WIESMANN und RICHTERICH⁶.

Die Aktivitätsbestimmungen wurden direkt und fortlaufend im Spektrophotometer bei 340 nm und 20°C durchgeführt. Die Küvetten (d = 1 cm) enthielten in 2,4 ml Inkubationsvolumen Homogenat von 1,4 bzw. 0,7 mg Gewebe (s. Figur 1), Glycinpuffer 0,45 M, pH 9,0; ATP 1,25 mM; Kreatin bzw. Arginin 30 mM; Phospho-

¹ W. KÜHL, *Bronn's Kl. Ordn. Tierreichs* 4, Abt. 4, Buch 2, T 1, 1 (1938).

² D. C. WATTS, *Adv. comp. Physiol. Biochem.* 3, 1 (1968).

³ N. VAN THOI und J. ROCHE, in *Taxonomic Biochemistry* (Ed. C. A. LEONE; The Ronald Press Co., New York 1964).

⁴ M. L. TANZER und C. GILVARG, *J. biol. Chem.* 234, 3201 (1959).

⁵ J. P. COLOMBO, R. RICHTERICH und E. ROSSI, *Klin. Wschr.* 40, 37 (1962).

⁶ U. WIESMANN und R. RICHTERICH, *Helv. Physiol. Acta* 22, 1 (1964).