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Light Sensitivity of ^{22}Na, ^{86}Rb, and ^{42}K Absorption by Different Tissues of Bean Leaves[1]

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BENJAMIN JACOBY
Department of Agricultural Botany, The Hebrew University, Rehovot 76 100, Israel

ABSTRACT

Autoradiographs of ^{22}Na-loaded bean (*Phaseolus vulgaris* L. cv. 'Brittle Wax') leaf slices showed that most of the tracer was concentrated in vascular tissue. Rubidium-86 was uniformly distributed in slices that had been incubated in darkness; after incubation in the light many small dark spots appeared on the autoradiographs, apparently corresponding with the stomata. Autoradiographs of ^{42}K-loaded slices showed a rather uniform distribution of the tracer, whether the slices had been incubated in light or in darkness.

Metabolic energy for ion fluxes in green plant cells can apparently be supplied by photosynthesis. Light usually enhances ion fluxes in algae (*cf.*, 18, 24), but in leaves of aquatic plants or leaf-slices of terrestrial higher plants, a variety of possible relations between light and metabolic ion absorption has been observed. In some cases light enhances ion absorption only under anaerobic conditions (15, 23, 25); in other cases light enhancement was observed under aerobic conditions as well (1, 14, 16, 17, 20, 22). In barley-leaf slices, ion absorption was found to be stimulated only after aging the slices overnight (21).

In unaged bean-leaf slices, the absorption of ^{86}Rb and ^{42}K was enhanced by light but, on the other hand, ^{22}Na absorption was light insensitive under aerobic conditions (9); the ATP content of these slices was also unaffected by illumination under aerobic conditions (25). Under anaerobic conditions, illumination restored the ATP content as well as the ^{22}Na absorption rate to those occurring under aerobic conditions. In view of these results, the light enhancement of ^{86}Rb and ^{42}K absorption under aerobic conditions by similar bean-leaf slices (9) cannot be explained simply by an effect of light on the ATP content of the cytoplasm of leaf cells.

During the present work, we set out to investigate whether or not the light-enhanced ^{86}Rb and ^{42}K absorption in bean-leaf slices and the light-insensitive absorption of ^{22}Na occur in the same leaf tissues.

MATERIALS AND METHODS

Bean plants (*Phaseolus vulgaris* L. cv. 'Brittle Wax') were grown at 25 C and with a 16-hr photoperiod as described in detail previously (11). Absorption experiments were carried out with leaf slices (10) aerated for 1 hr in 0.2 mM $CaSO_4$ before use. All incubation media also contained 0.2 mM $CaSO_4$.

Leaf slices were incubated 1 hr in 0.1 mM chlorides of ^{22}Na, ^{42}K, or ^{86}Rb at 30 C in light or darkness. The free space was exchanged with cold (0–1 C) 10 mM $CaSO_4$. Detailed procedures have been described previously (25). For the autoradiographic location of absorbed ions, tracer-loaded leaf slices were rapidly frozen in liquid nitrogen and freeze-dried for 24 hr at −25 C. Dry slices were placed on AR-10 Kodak stripping films on microscope slides.

RESULTS

Leaf slices were incubated in 0.1 mM $^{22}NaCl$ in darkness and were freeze-dried after exchange of the free space. Autoradiographs of such slices (Fig. 1) showed that most of the absorbed ^{22}Na was concentrated in the leaf veins. The same results were obtained when leaf slices were incubated in ^{22}Na in the light.

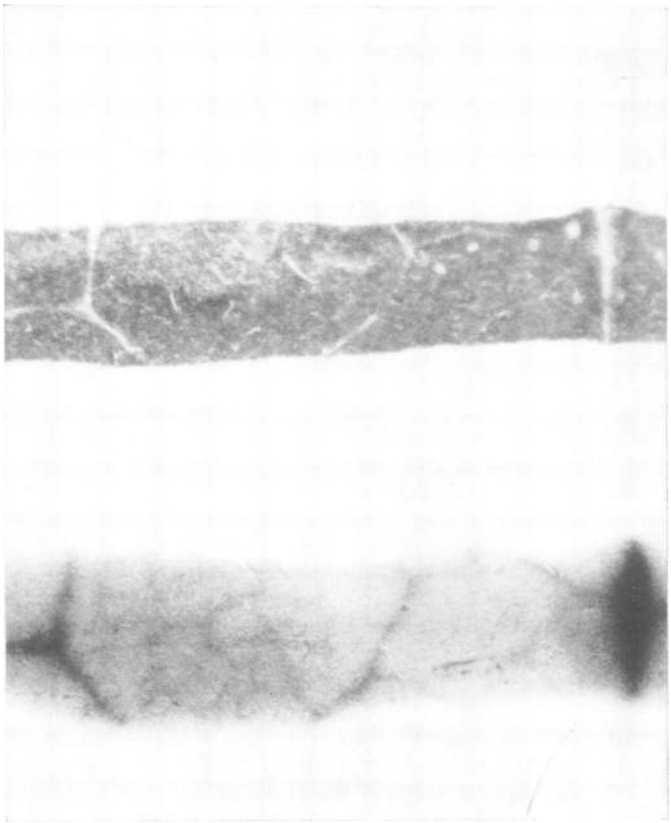

FIG. 1. Photograph (above) and autoradiograph (below) of a ^{22}Na-loaded bean leaf slice incubated in light, 1 hr in 0.1 mM $^{22}NaCl$ at 30 C. Autoradiography, 24 hr; ^{22}Na concentration, 2.5 $\mu Ci\ cm^{-2}$ of leaf slice. × 16.

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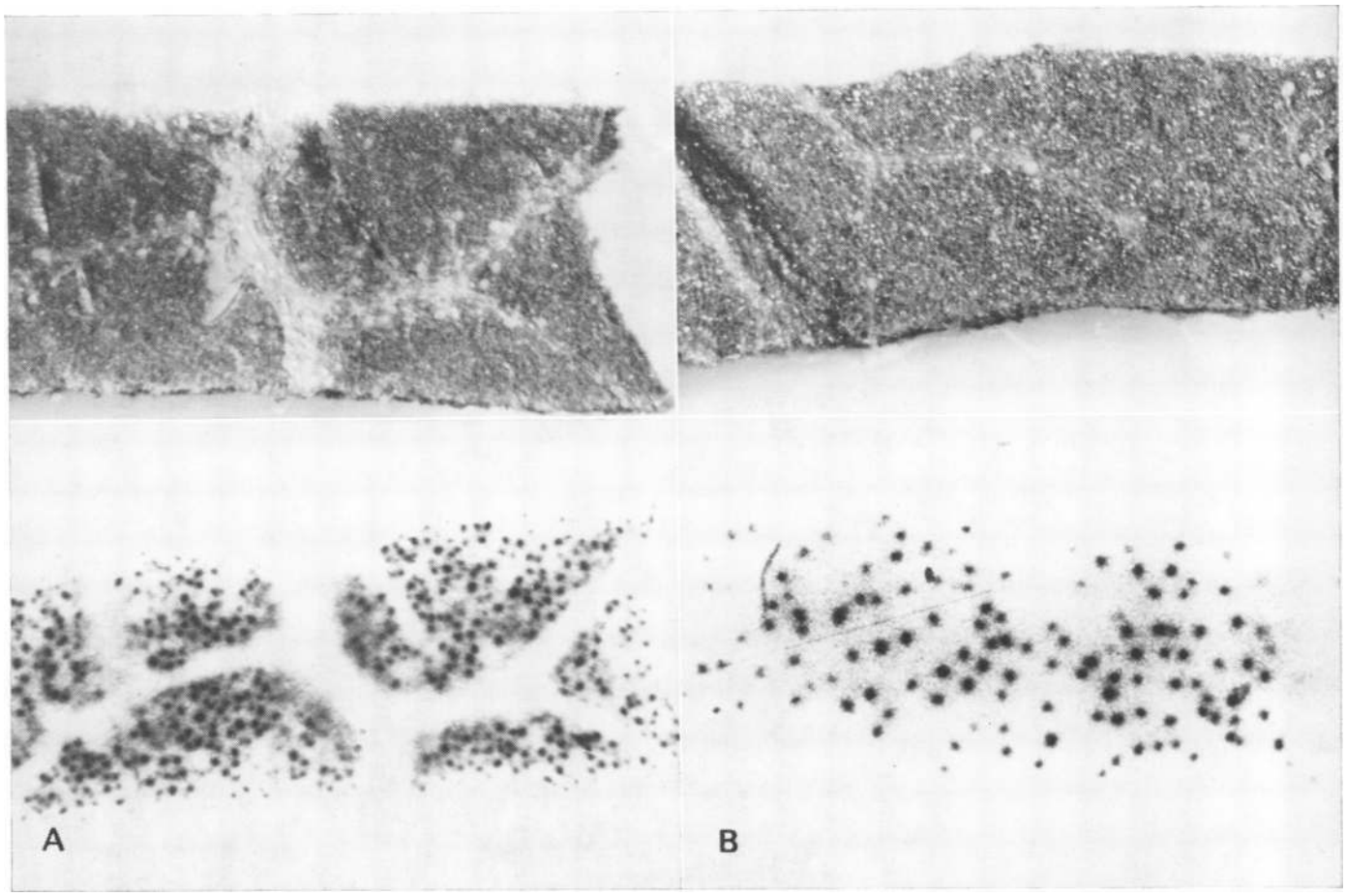

FIG. 2. Photograph (above) and autoradiograph (below) of ^{86}Rb-loaded leaf slice incubated in light, 1 hr in 0.1 mM ^{86}RbCl at 30 C. Abaxial (A) or adaxial (B) surface of leaf slice in contact with the film during exposure. Autoradiography, 24 hr; ^{86}Rb concentration, 1 μCi × cm^2 of leaf slice. × 40.

A very different autoradiographic pattern was obtained when the leaf slices were incubated in ^{86}RbCl in the light (Fig. 2). On these autoradiographs many dark spots appear between the areas corresponding to the leaf veins and some diffusive darkening is evident between these spots. The concentration of the dark spots on the autoradiographs was about 40,000/cm^2 of leaf area exposed to the X-ray film when the abaxial surface of the leaf slice was in contact with the film during exposure (Fig. 2A); the concentration was about 12,000/cm^2 when the film had been in contact with the adaxial surface (Fig. 2B). The concentrations of the silver-grain spots on the autoradiographs were the same as the respective concentrations of stomata on similar leaf slices. Autoradiographs of ^{86}Rb-loaded slices which had been incubated in darkness (Fig. 3) do not show the dark spots found on autoradiographs of light-incubated slices; instead a uniform distribution of the tracer is evident. The autoradiographic pattern was also the same whether the abaxial or the adaxial epidermis had been in contact with the film. Rubidium-86 emits hard β as well as γ radiation which should not be self-absorbed by the leaf tissue; good resolution of the location of the tracer would, however, be expected only for the layer of cells in direct contact with the X-ray film. This was ascertained by exposing ^{86}Rb-loaded slices directly to the film, or with an unloaded piece of dried leaf between the ^{86}Rb-loaded slice and the film (Fig. 4). In the latter case, a diffused image of the slice was obtained and the spots of ^{86}Rb concentrations were not resolved. The autoradiographs of ^{86}Rb-loaded slices probably indicate that light-enhanced ^{86}Rb absorption occurs primarily into the stomatal guard cells. An approximate calculation (Appendix) shows that this corresponds with a light-induced absorption rate of about 30 μeq of Rb/ml of guard cell volume during 1 hr from 0.1 mM ^{86}RbCl.

Results with slices which had been incubated in 0.1 mM ^{42}KCl differed very much from those obtained with ^{86}Rb. These autoradiographs (Fig. 5) reveal a rather uniform distribution of ^{42}K whether the slices had been incubated in light or in darkness. The effects of light on ^{42}K and ^{86}Rb absorption by bean leaf slices are compared in Table I (*cf.* ref. 9). Light enhances the absorption of both ions, but not to the same extent. In darkness ^{42}K was absorbed at a rate about 12 times higher than ^{86}Rb. The amount of light-enhanced ^{42}K absorption was less than three times larger than the light-enhanced ^{86}Rb absorption. Substantial amounts of ^{42}K are absorbed in the dark, but the light-dependent amount is only about 40% of the total absorbed in light, whereas it is almost 80% in the case of ^{86}Rb. Light-enhanced ^{42}K absorption by guard cells may not be discernible on the autoradiographs because of the relatively high light-dependent ^{42}K background attributable to absorption by other leaf cells.

DISCUSSION

Considerable heterogeneity was displayed by bean-leaf tissues with respect to the selective absorption of monovalent cations from dilute solutions.

Most cells in bean-leaf slices seemed to be capable of ^{42}K ab-

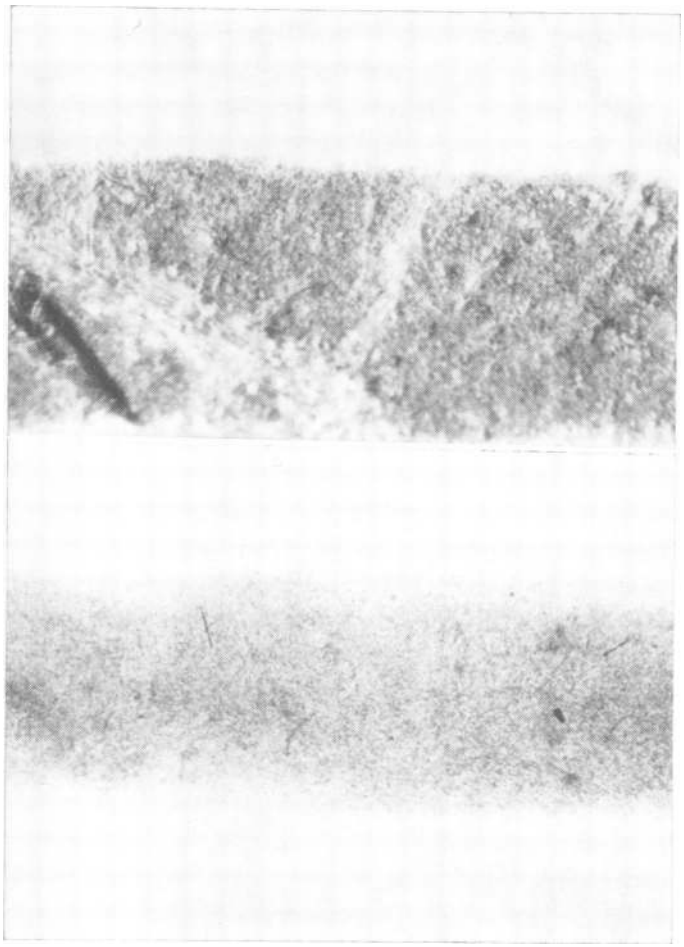

FIG. 3. Photograph (above) and autoradiograph (below) of ^{86}Rb-loaded bean leaf slice incubated in darkness, 1 hr in 0.1 mM ^{86}RbCl at 30 C. Abaxial surface of leaf in contact with the film during exposure. Autoradiography, 24 hr; ^{86}Rb concentration, 1 μCi cm^{-2} of leaf slice. × 40.

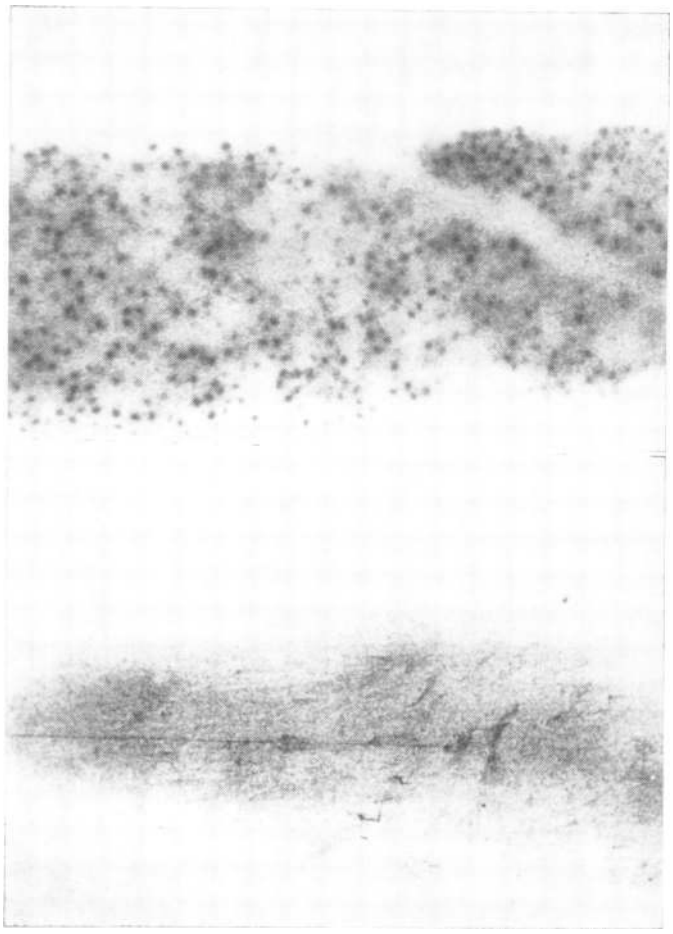

FIG. 4. Autoradiographs of leaf slices incubated in 0.1 mM ^{86}RbCl in the light. Above: abaxial epidermis in contact with the film. Below: a piece of dry leaf between abaxial epidermis and film. Autoradiography, 48 hr; ^{86}Rb concentration, 1 μCi cm^{-2} of leaf slice. × 40.

sorption, whereas active ^{22}Na absorption was restricted to some leaf-vein tissue. When Na was applied as a droplet to bean leaves on intact plants (12), it was also directly transferred to the veins. Similar vascular-bundle tissues may be responsible for the retention of Na in bean roots and stems (3, 5, 7, 8, 13).

Bean leaves absorbed very small amounts of ^{86}Rb in the dark as compared to ^{42}K; the larger light-enhanced ^{86}Rb absorption seemed to be restricted to specific cells, *viz.*, the guard cells which are well known to accumulate K or Rb in the light (4). Bean leaf cells discriminate between K and Rb also at higher salt concentrations (9) when net K absorption can be shown. The capability for differential uptake of K and Rb was previously shown for *Phaseolus vulgaris* by Cline and Hungate (2), as well as for other plants (*cf.* 19). Bean mesophyl cells resemble some algae (27) in the extent of their selectivity between Rb and K.

The restriction of ^{22}Na and the light-enhanced ^{86}Rb (and perhaps also ^{42}K) absorption to different tissues may help to explain the different light sensitivity of these processes. Light does not affect the ATP content measured on bulk bean-leaf slices (25). It is possible that the ATP content of guard cells is affected. Alternatively, a more specific effect of light on the stomatal apparatus could be involved. The selectivity of the stomatal apparatus for K or Rb against Na was previously shown for *Vicia faba* (6) and for tobacco (26).

Table 1. *Effect of Light on ^{86}Rb and ^{42}K Absorption by Bean Leaf Slices*

Incubation was 1 hr in 0.1 mM chlorides, and 0.2 mM $CaSO_4$ at 30 C.

Salt	Absorption		
	Light	Dark	Light-Dark
	neq g fresh wt^{-1} hr^{-1}		
^{86}RbCl	330 ± 20	90 ± 7	240
^{42}KCl	1770 ± 130	1070 ± 40	700

The diverse relationships of Na, K and Rb absorption by bean leaf slices to leaf age (9) were previously assumed to depend on selective compartmentation of these ions in the same cells. This diversity should now apparently also be ascribed to the different characteristics of the cells in bean leaves by which the specific ions are absorbed.

APPENDIX

Approximate calculation of light-enhanced ^{86}Rb absorption by bean leaf guard cells from 0.1 mM ^{86}RbCl.

1. Light-enhanced Rb absorption: 0.24 μeq × g^{-1} × hr^{-1}

FIG. 5. Photograph (above) and autoradiograph (below) of ^{42}K-loaded bean leaf slice incubated in light, 1 hr in 0.1 mM ^{42}KCl at 30 C. Abaxial surface of leaf slice in contact with film during exposure. Autoradiography, 24 hr; ^{42}K concentration at beginning of exposure, 1.1 μCi cm^{-2} of leaf slice. × 40.

2. Number of guard cells: $1.12 \times 10^5 \times$ cm^{-2} of leaf area (44,000 + 12,000 stomata on abaxial and adaxial epidermis, respectively)

3. Approximate volume of guard cells: 11.5 μm × 9 μm × 9 μm = 930 μm^3 = 9.3×10^{-10} ml

4. Area/weight ratio of leaves: 71.5 cm^2 × g^{-1}

5. Guard cell volume per g of fresh leaf slices: $1.12 \times 10^5 \times 71.5 \times 9.3 \times 10^{-10} = 7.5 \times 10^{-3}$ ml × g^{-1}

6. Light-enhanced change of ^{86}Rb concentration in guard cells, assuming that all light-enhanced ^{86}absorption is localized in guard cells, and that none of the ^{86}Rb absorbed in the dark is transferred to the guard cells:

$$\frac{0.24}{7.5 \times 10^{-3}} \simeq 30\ \mu\text{eq} \times \text{ml}^{-1} \times \text{hr}^{-1}$$

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