isolates grow in an apparent log phase at the about same rate for 28 h, but subsequently the avirulent leveled off at a higher concentration per leaf.

The avirulent Group II cells give the keto sugar test[11] characteristic of many isolates of *Agrobacterium* and have a typical *A. tumefaciens* colony morphology at room temperature. Preliminary observations by electron microscopy indicated no gross differences between room temperature grown Group II avirulent or virulent cells.

Although the growth rates were about the same at 25 °C, the avirulent cultures grew 4–8 times as fast as virulent cultures at 37 °C in nutrient broth shake culture. When grown on nutrient agar plates at 37 °C for 24 h, the larger avirulent colonies are easily distinguished from the pinpoint virulent colonies. This growth characteristic has proven to be a much more sensitive and precise method for the isolation of avirulents. If colonies of strain C-58 are grown at 37 °C for 24 h, the cells washed from the plates, and replated at 37 °C, 2–4% of the cells produce the large colony type avirulents. Preliminary data indicate that many of the colonies grown at 37 °C contain a few avirulent cells. Thus the conversion appears to be a high frequency event. All attempts to restore virulence by incubation of the avirulent cultures with lysates from the virulent cells, isolated DNA from virulent cells, Lv-1 phage DNA[12], or the intact Lv-1 phage have so far failed.

Although our results could be explained by the occurrence of a high frequency mutation, perhaps a more likely possibility is the loss of a virulence factor. In other strains this factor may become integrated more tightly with the bacterial DNA. It may be significant that strain C-58 of *A. tumefaciens* is also one of the most virulent strains we have tested. In any event these strains should be extremely useful in the investigation of virulence and tumor induction[13].

Zusammenfassung. Durch Inkubation bei hohen Temperaturen (37 °C) verliert das *Agrobacterium tumefaciens* C-58 allmählich die Fähigkeit zur Gallen- oder Tumorbildung in Pflanzen.

R. H. HAMILTON and M. Z. FALL

Department of Biology,
The Pennsylvania State University,
University Park (Pennsylvania 16802, USA),
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Localization of Ions in the Mesophyll Cells of the Succulent Halophyte *Suaeda monoica* Forssk. by X-Ray Microanalysis

Distribution of sodium, chloride, potassium and phosphorus between the cytoplasm and vacuoles of leaf cells of *Suaeda monoica* plants was investigated by X-ray microanalysis. Distribution of sodium and chloride in leaf cells of NaCl-treated plants did not coincide. Sodium tended to concentrate in the cytoplasm while chloride was evenly distributed in both cytoplasm and vacuole. Distribution of potassium in the cells of those plants was similar to that of sodium. Potassium content of vacuoles of leaf cells of plants grown under non-saline conditions was higher than that of the cytoplasm.

It is generally accepted that ions absorbed in excess by plant cells are accumulated inside their vacuoles. Halophytes are no exception in this respect and a considerable accumulation of ions in their vacuoles was frequently reported[1–4]. Moreover, transport of sodium and chloride from the cytoplasm into the vacuoles of such plants was believed to be a major cause for plant halosucculence and a mechanism for diminishing the effects of a high intracytoplasmatic salt content[4–6]. It was also frequently spoken of 'salt' effects denoting a similar transport of sodium and chloride. Since no experimental evidence for such assumptions was available, the problem whether ions remain inside the cytoplasm of the succulent leaf cells, or whether they are neutralized in the vacuoles, was investigated.

Seeds of *Suaeda monoica* Forssk. were germinated in Petri dishes and seedlings grown in water culture on a half-strength Hoagland's solution. Sodium chloride, 50 m*M*, was added to half of the plants. Plants were grown for 4 weeks in a growth-chamber under constant temperature (27 °C) and continuous illumination (12,000 lux). Cotyledons and leaves were then taken for analysis.

Material was mounted on top of a microtome blockholder, embedded in fresh rat brain or in liver slices[7], and frozen within 30 sec in a dry ice acetone mixture (− 70 °C). Blocks were cut in a cryostat and the frozen cross-sections placed on cold (− 20 °C) aluminium plates which had previously been coated with a thin layer of silicone grease. A second plate was loaded on top of the mounted sections. The material was left for a few hours in a deep freezer, transferred to a lyophilizer and dried under vacuum (10^{-2} Torr) overnight. A gradual dehydration of the frozen sections – while placed between 2 metal plates – was found to be essential for keeping the sections flat and intact. Strict care was taken to keep the material deeply frozen throughout the procedure. Vacuum was broken with dry air. Following dehydration, the sections were coated with carbon and the location of Na, K, P and Cl was determined by X-ray microanalysis (Jeolco JXA-3A X-ray microanalyzer). An accelerating voltage of 10 KV was used. Sample current was approximately 6×10^{-8} A. Beam diameter was less than 1 μm. Background level was determined by lowering the spectrometer 1° off the specific Bragg's angle. Results for undamaged cells were reproducable.

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Data presented (Figure 1) reveal that in the mesophyll cells of NaCl-treated plants, sodium was located mainly in the cytoplasm. Only small portions of the sodium and potassium contents of the cells were found inside the vacuoles. Content of phosphorus inside the vacuoles was negligible and thus below the detection limits of the instrument. It is interesting to note that the total content of chloride in cells of NaCl-treated plants seemed to be low. Distribution of chloride and sodium did not coincide; while relatively more sodium was concentrated in the cytoplasm, chloride was evenly distributed throughout the cell section.

A different distribution of ions was found in plants grown without NaCl in the growth medium (Figure 2). Apparently, the levels observed for sodium and chloride were background levels only. However, in such sodium-deficient cells, potassium seemed to substitute for sodium. Potassium was amply found inside the vacuoles with its content in the vacuole exceeding that of the cytoplasm. Phosphorus in such cells was located in the cytoplasm as well as in the vacuoles.

At present, it is still difficult to make an exact quantitative evaluation of the data. The method used is not yet refined enough to enable the distinction of the fine

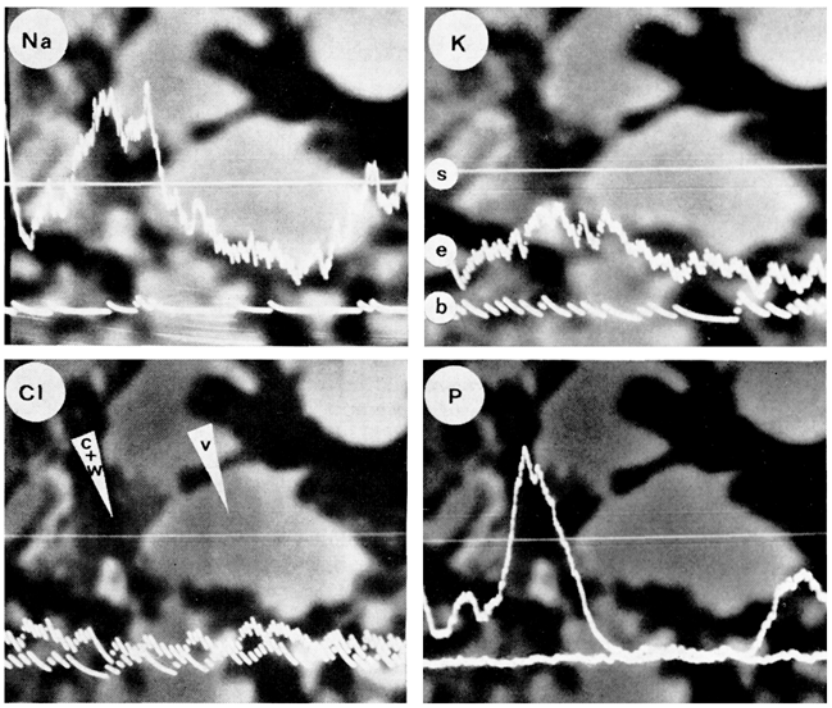

Fig. 1. Distribution of sodium, chloride, potassium and phosphorus in the mesophyll cells of *Suaeda monoica* plants grown on a half-strength Hoagland's solution + 50 m*M* NaCl. Absorbed electron image. ×850. Range of determinations (cpm): Na– 3×10^3; K– 10^3; Cl– 10^3; P– 10^4. s, scanning line; e, element distribution; b, background (2 Θ–1°); c + w, cytoplasm and cell wall; v, vacuole.

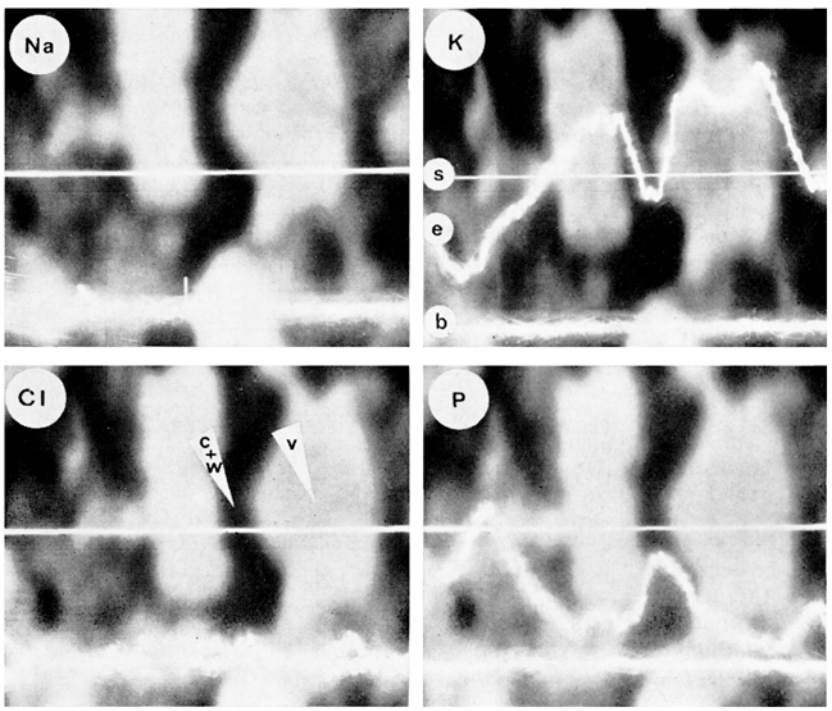

Fig. 2. Distribution of sodium, chloride, potassium and phosphorus in the mesophyll cells of *Suaeda monoica* plants grown on a half strength Hoaglands, NaCl-deficient solution. Range of determinations (cpm): Na– 10^3; K– 10^4; Cl– 10^3; P– 10^4.

cellular constituents. Nevertheless, the resolution obtained still permitted a gross distinction between 2 major compartments, i.e. cell vacuoles on one hand, and cell wall with the adhering cytoplasm on the other hand[8-10]. Conclusions based on these analyses imply that the cytoplasm of the mesophyll cells of *Suaeda monoica* absorb and retain high quantities of sodium rather than reject it. Neither sodium nor chloride were excessively accumulated and inactivated[4] in the vacuoles of cells of *Suaeda monoica* plants grown under saline conditions.

Furthermore, as satisfactory growth of *Suaeda monoica* plants depends on the presence of high concentrations of sodium chloride in the growth medium[11], it also seems reasonable to assume that sodium participates in physiological processes and thus will be concentrated in sites which actually affect growth, i.e. inside the cytoplasm[12].

Zusammenfassung. Die Verteilung von Natrium, Chlorid, Kalium und Phosphor im Zytoplasma und in den Vakuolen der Blattzellen von *Suaeda-monoica*-Pflanzen wurde mittels Röntgen-Mikrosonde untersucht. Natrium neigte zur Anreicherung im Zytoplasma, während Chlorid gleichmässig im Zytoplasma und in Vakuolen verteilt war.

Y. WAISEL and A. ESHEL

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Inhibition of Auxin Transport by a Morphactin

Among the various morphactins, IT 3456 has been shown to be the most active in stimulation of elongation of tissue segments and in interference with auxin transport[1,2]. In the present investigation, seedlings raised in the presence of, or tissue segments pretreated with methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate (IT 3456) have been used to study its effect on indoleacetic acid-2-^{14}C (IAA) transport.

Seeds of *Zea mays* L. (cv. Københavns Torve) were thoroughly washed and soaked for 4 h in tap water and planted on tap water saturated paper pads in plastic boxes. After 48 h the germinating seeds from one of the boxes were transferred onto paper pads saturated with $10^{-6}M$ morphactin solution. The seedlings were raised in complete darkness for 96 h, except between 48 and 54 h when they were exposed to red light. For transport experiments 8 mm sections, taken 1–2 mm below the tip of 96 h etiolated coleoptiles, were used.

Two sets of experiments were conducted, each in a basipetal and acropetal direction. 1. An assembly consisted of sections from tap water grown seedlings supplied with donor blocks ($5.5 \times 4 \times 1$ mm) containing either 1% sucrose + 0.4 mg/l IAA or 1% sucrose + 0.4 mg/l IAA + $10^{-6}M$ morphactin both in 1.5% agar on the apical or basal cut ends and the other end (receiver) placed on (basipetal) or covered with (acropetal) plain 1.5% agar blocks. After 30 min the original donor blocks were replaced with new radioactive donor blocks (1% sucrose + 0.4 mg/l IAA, specific activity 48.5 mCi/mM) and fresh receiver blocks replaced the old ones. 2. In this assembly, sections from seedlings raised in the presence of morphactin solution in tap water were used and provided directly with radioactive donor blocks (1% sucrose + 0.4 mg/l IAA) and the other cut end was covered or placed on receiver blocks.

Four assembly components were pooled after 90 min of transport for each measurement and the experiments were replicated 3 times. The temperature throughout was maintained at 24 ± 1 °C and only green (Cinemoid nos. 32, 21 and 5) safe light was used during manipulations. The radioactivity in donors, tissues and receivers was analyzed in a Packard liquid scintillation counter[3,4].

The seedlings raised in presence of $10^{-6}M$ morphactin showed lack of geotropic response, as was observed earlier[5,6]. From the Table it can be seen that morphactin treatments did not materially affect the total amount absorbed from the donor blocks but it did significantly reduce the percentage translocated through the tissue into the receiver block. Thus morphactin-treated tissues retained more auxin than the controls. The remarkable similarity between the two methods of morphactin treatment is worth noticing. Thus the action of morphactin is so rapid that essentially no difference can be demonstrated between sections pre-treated for 30 min and seedlings raised for 48 h in morphactin solution. The threshold level is being determined.

Effect of morphactin (IT 3456) on the transport of indoleacetic acid-2-^{14}C

Percentage of applied	Basipetal			Acropetal		
	C	P	M	C	P	M
In tissue	29.99	39.41	36.47	11.48	11.81	11.50
In receiver	11.22	0.42	0.54	0.00	0.00	0.00
Total	41.21	39.83	37.01	11.48	11.81	11.50

C, control sections treated with 1% sucrose + 0.4 mg/l IAA; P, sections pre-treated with 1% sucrose + 0.4 mg/l IAA + $10^{-6}M$ morphactin; M, sections from seedlings raised in presence of $10^{-6}M$ morphactin.

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