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High Sensitivity to Auxin is a Common Feature of Hairy Root[1]

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ABSTRACT

The responses to auxin of *Lycopersicon esculentum* roots transformed by (T_L+T_R)-DNA of the Ri plasmid of agropine-type *Agrobacterium rhizogenes* strain 15834 and *Catharanthus trichophyllus* roots transformed by the (T_L+T_R)-DNA, and by T_L- or T_R-DNA alone of the same bacterial strain were compared to that of their normal counterparts. The transmembrane electrical potential difference of root protoplasts was measured as a function of the concentration of exogenous naphthalene acetic acid. The sensitivity to auxin expressed by this response was shown to be independent of the measurement conditions and of the basal polarization of isolated protoplasts. According to this electrical response, as well as to the modulation by auxin of proton excretion by root tips and root tip elongation, roots transformed by (T_L+T_R) DNA are 100 to 1000 times more sensitive to exogenous auxin than normal roots, as is the case with normal and transformed roots from *Lotus corniculatus* (WH Shen, A Petit, J Guern, J Tempé [1988] Proc Natl Acad Sci USA 85: 3417–3421). Furthermore, transformed roots of *C. trichophyllus* are not modified in their sensitivity to fusicoccin, illustrating the specificity of the modification of the auxin sensitivity. Roots transformed by the T_R-DNA alone showed the same sensitivity to auxin as normal roots, whereas the roots transformed by the T_L-DNA alone exhibited an auxin sensitivity as high as the roots transformed by (T_L+T_R)-DNA. It was concluded that the high sensitivity to auxin is controlled by the T_L-DNA in agropine type Ri plasmids.

Infection of dicotyledonous plants by *Agrobacterium rhizogenes* causes abundant adventitious root proliferation at the site of inoculation. This response results from the expression of specific DNA sequence(s) called T-DNA (transferred DNA), originally located in the pathogenic bacterium on the Ri (root inducing) plasmid, and which are transferred and stably integrated into the plant genome (see the review in ref. 29). The most studied *A. rhizogenes* strains, which belong to the group of agropine strains, have on their Ri plasmid two T-DNA regions, called T_L- and T_R-DNA, which can be independently transferred to the nuclear genome of infected plant cells. The T_R-DNA carries the genes responsible for opine synthesis (11), and sequences homologous to auxin synthesis genes (*iaaM* and *iaaH*) of *Agrobacterium tumefaciens* T-DNA (14, 15). Within the T_L-DNA, three genes, *rol A*, *B*, and *C*, have been shown to have a fundamental role in the induction of rhizogenesis in tobacco (27, 28, 30), although nothing is known as to the functions of the proteins encoded by these *rol* genes. Tobacco roots induced by the T_L-DNA alone exhibit the typical syndrome of hairy-root, whereas roots transformed by the T_R-DNA alone have the phenotype of nontransformed roots (30).

We previously showed that *Lotus corniculatus* roots transformed by different *A. rhizogenes* strains are 100 to 1000 times more sensitive to exogenous auxin than normal roots of the same species (26). As it was suggested that this phenomenon could be a major determinant of the abundant secondary root formation of the hairy root disease, we address in this paper the question whether this increase in sensitivity is specific to *L. corniculatus* hairy roots or can be considered as a general feature of different species transformed by *A. rhizogenes*. The possibility that T_L-DNA genes, responsible for the hairy root syndrome, could control the increase in sensitivity was explored by comparing transformed roots containing only T_L- or T_R-DNA, or the whole (T_R+T_L)-DNA of *A. rhizogenes* strain 15834. For these comparative studies, the use of the auxin-induced electrical response of root protoplasts as a marker of their sensitivity to the hormone was also critically evaluated and further substantiated.

MATERIALS AND METHODS

Materials

On *Lycopersicon esculentum*, a hairy-root line established from stem inoculation with *Agrobacterium rhizogenes* 15834 was provided by A. Petit in our laboratory. On *Catharanthus trichophyllus*, six root lines (T1–T6) were established from one site of inoculation with *A. rhizogenes* 15834 on stems (10). For each species, one normal root line was propagated as the source of normal material. The normal and transformed roots were maintained by subculturing every month on the medium described for *Lotus corniculatus* roots culture (26). Root tips, taken from about 20-d-old cultures, were used either directly in elongation and proton extrusion experiments, or for protoplast isolation.

Opine Analysis, DNA Preparation and T-DNA Hybridization

The presence of opines was investigated by high voltage paper electrophoresis (19, 20). DNA was prepared from 5 to 10 g of *in vitro* grown roots. DNA isolation and purification

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by isopycnic centrifugation, dot blot and Southern hybridization on Genescreen plus membrane (New England Nuclear) were as described (8, 26). The probes, plasmid pLJ1 (15) and pMP27 (21), which cover the whole T_L-DNA and T_R-DNA of agropine-type Ri plasmid, respectively, were labeled with ^{32}P to about 1 to 3 × 10^9 dpm/μg of DNA by a multiprime DNA labeling system (Amersham).

Effects of NAA[3] on Root Elongation and Proton Excretion

The effect of NAA on *C. trichophyllus* root elongation was measured on 1-cm long root tips as described (26). For measuring proton excretion, 0.5-cm long root tips were preincubated for 1 h in 0.5 mM $CaSO_4$ and 10 mM glucose under vigorous aeration. Random lots of 40 to 60 segments were then transferred to 1 mL of 1 mM $CaSO_4$, 10 mM K_2SO_4, 10 mM glucose (pH 6.0), with various concentrations of NAA. Proton excretion was measured by back titration of the incubation medium to the initial pH.

Effect of IAM on Root Growth

C. trichophyllus normal roots and root lines T4, T5, and T6 were cultivated on agar medium in the dark for a 30-d period. Random lots of ten segments were transferred to Petri dishes containing a medium supplemented with various concentrations of IAM (3 × 10^{-4} to 10^{-5} M), and incubated in the dark at 22°C.

Effects of NAA on the Transmembrane Em of Protoplasts

Protoplasts from *C. trichophyllus* and *L. esculentum* root tips were prepared as described (26), except that the concentrations of cellulase RS and pectolyase Y23 were doubled (to 3% and 0.1%, respectively) in the case of *L. esculentum*, and that the protoplasts were collected by centrifugation at 100*g*. Three classes of protoplasts can be identified in the suspensions obtained from roots of both species: (a) cortex protoplasts, coming from the elongation zone, with a large central vacuole and the largest diameter (30-40 μm); (b) protoplasts from the root cap, of smaller size (10-20 μm diameter), with many amyloplasts; and (c) small meristematic protoplasts (10 μm diameter), very dense and refringent. Only the large protoplasts issued from root cortex cells were used to measure the Em by the microelectrode technique. Protoplasts were immobilized in a microholder as described by Rona *et al.* (23) and impaled with a glass micropipette filled with 1 M KCl and connected to an Ag/AgCl electrode. Em was measured between the microelectrode inserted in the protoplast and a reference Ag/AgCl electrode immerged in the bathing medium through an agar (1%)-KCl (1 M) bridge. Measurements were carried out at room temperature on aliquots of the stock protoplast suspension immediately after dilution to 10^4 protoplasts·mL^{-1}. NAA effects on Em were measured according to the procedure used by Ephritikhine *et al.* (12) for tobacco mesophyll protoplasts. To study the influence of electrolytes on membrane potential and NAA effects, Cl^- in the micropipette was diluted or substituted with SO_4^{2-} or CH_3COO^-, without modifying the electrode half-cells (1 M KCl/Ag-AgCl).

[3] Abbreviations: NAA, 1-naphthaleneacetic acid; FC, fusicoccin; IAM, indole-3-acetamide; Em, electrical potential difference.

RESULTS AND DISCUSSION

Preparation and Characterization of Transformed Root Material

When cultured *in vitro* without phytohormones, the transformed line of *Lycopersicon esculentum* and five of the selected lines (T1–T5) of *Catharanthus trichophyllus* exhibited a typical hairy root phenotype, *i.e.* fast-growing and highly branched roots. In contrast, in the same conditions, roots from the line T6 of *C. trichophyllus* grew more slowly than normal roots, and spontaneously formed small calli.

The presence of T_L- and T_R-DNA of pRi15834 in the genome of these root lines was investigated by analysis of the

Table I. *Characteristics of the Root Lines of L. esculentum and C. trichophyllus*

Aqueous extracts from normal (N) and transformed (T) roots were analyzed for the presence of opines by high voltage paper electrophoresis (19, 20). The presence of T-DNAs from the *A. rhizogenes* strain 15834 was assessed by dot blot (*L. esculentum*) or Southern (*C. trichophyllus*) hybridization with T_L-DNA or T_R-DNA probes as described in "Materials and Methods."

Plant Species	Root Line	Phenotype	Opine	Hybridization		T-DNA Present
				T_R-DNA	T_L-DNA	
L. esculentum						
	N	Normal	−	NT[a]	−	−
	T	Hairy-root	+	NT	+	T_R+T_L
C. trichophyllus						
	N	Normal	−	−	−	−
	T4	Hairy-root	−	−	+	T_L
	T5	Hairy-root	+	+	+	T_R+T_L
	T6	Tumors	+	+	−	T_R

[a] Not tested.

opine content and T-DNA hybridization. Mannopine and agropine, used as chemical markers of the presence of T_R-DNA, were detected in the extracts from the transformed root line of *L. esculentum* and two transformed lines (T5, T6) of *C. trichophyllus*. With the DNA of *L. esculentum* transformed roots, a strong hybridization signal was obtained on dot blots with the T_L-DNA probe. The transformed *L. esculentum* root line thus contained both T_R- and T_L-DNA of pRi 15834 (Table I).

To assess the presence of T-DNA in *C. trichophyllus* roots, DNA from each root line was digested with the restriction endonuclease *Bam*HI and 'Southern' hybridized against the T_R- or T_L-DNA probes. Hybridization signals against the T_R-DNA probe were detected with DNAs from lines T5 and T6, but no signal was observed with DNAs from the lines T1 to T4. With the T_L-DNA probe, hybridization signals were detected in the root lines T1 to T5, whereas no hybridization was observed with the root line T6. From these results, three root lines were chosen for further investigation: T5 containing both T_L- and T_R-DNAs, T4 containing only the T_L-DNA, and T6 exhibiting only the T_R-DNA from pRi 15834 (Table I).

The phenotype of the T_R-DNA transformed root line T6 (slow growth and calli formation) was suggestive of the expression of the *iaaM* and *iaaH* genes located on the T_R-DNA. The expression of the *iaaH* gene in transformed roots was examined with indole-3-acetamide as an auxin precursor. At 0.1 mM, indole-3-acetamide partially inhibited the growth of the root line T5, transformed by (T_L+T_R)-DNA and stopped completely the growth of the line T6 transformed by T_R-DNA alone. In contrast, the growth of normal roots and of line T4, transformed by T_L-DNA alone, was not affected at this IAM concentration. These results suggest that the *iaaH* gene is expressed in the root lines T5 and T6.

Evidence for an Increased Sensitivity to Auxin of Ri-Transformed Root Tips and Root Protoplasts with Regard to Normal Roots

Effects of NAA on root tip elongation and proton excretion were tested on *C. trichophyllus*, with normal roots and transformed roots of line T4. For normal roots, NAA at low concentrations caused a slight but significant increase in root elongation (Fig. 1A) and proton excretion (Fig. 1B). The maximal stimulation (60% for both elongation and proton excretion) was obtained at about 10^{-9} M NAA for root elongation and 5×10^{-10} M NAA for proton excretion. At concentrations above 10^{-8} M, NAA inhibited both responses. Transformed roots exhibited only a concentration-dependent inhibition for both elongation and proton excretion at the concentrations tested from 10^{-11} to 10^{-6} M (Fig. 1, A and B). These results indicate that *C. trichophyllus* transformed roots of line T4 are 100 to 1000 times more sensitive to NAA than normal ones, as judging from the exogenous concentration of auxin necessary to get the incipient inhibition of either elongation (10^{-8} and 10^{-11} M for normal and transformed roots, respectively) or proton excretion (10^{-8}–10^{-7} M and 10^{-10} M for normal and transformed roots, respectively).

The effect of auxin on the transmembrane potential difference of root cortex protoplasts from *L. esculentum* and *C.*

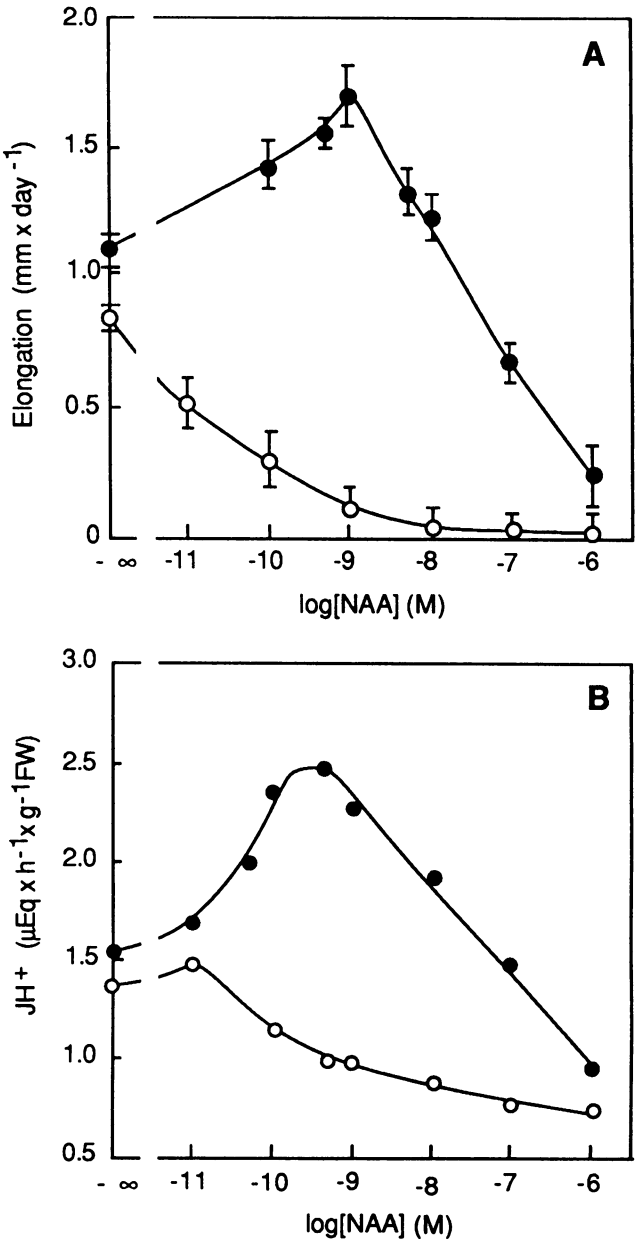

Figure 1. Effect of NAA on the elongation (A) and proton excretion (B) of apical root segments of *C. trichophyllus*. Elongation was measured as the difference between the mean length of 20 root segments (initial length: 1 cm) before and after 24 h incubation. The standard error is indicated by vertical bars. Essentially identical results were obtained in two other independent experiments. For proton excretion measurement, random lots of 40 to 60 segments (0.5 cm) were incubated as described in "Materials and Methods." The pH of the medium was monitored with a standard digital pH meter (Heito). The net flux of protons excreted during 1 h (JH^+) was determined by titrating the incubation medium to the initial pH with 0.001 N NaOH and was expressed as the number of μEqH^+ excreted per g of material fresh weight and per h. The mean value calculated from the results of three independent experiments is presented here. The standard error was inferior to 0.1 $\mu Eq \cdot h^{-1} \cdot g^{-1}$ fresh weight. Normal roots (●); transformed roots line T4 (○).

trichophyllus was investigated. These protoplasts exhibited a small electrical polarization, with Em values ranging from +3 to +10 mV when suspended in the measurement medium without auxin. No significant difference could be noted on the Em values between the different root lines. As to the effect of auxin on the membrane potential, both normal and transformed root protoplasts displayed a nonmonotonous response as a function of NAA concentration in the measurement medium: a hyperpolarization and a relative depolarization (Fig. 2). For *L. esculentum* protoplasts, the maximal hyperpolarization ($\Delta Em = -5.0$ mV) was observed for 10^{-8} M for normal and 5×10^{-11} M NAA for transformed root protoplasts (Fig. 2A). On *C. trichophyllus* protoplasts, the optimal NAA concentrations were 5×10^{-8} M for normal protoplasts and protoplasts of line T6, and 5×10^{-10} M NAA for transformed protoplasts of lines T4 and T5 (Fig. 2B). Thus, the protoplasts from transformed roots containing (T_L+T_R)-DNA, as well as those containing only the T_L-DNA, appeared about 100 times more sensitive to NAA than normal protoplasts. However, the transformed protoplasts of *C. trichophyllus* line T6, transformed by the T_R-DNA alone, exhibited the same sensitivity to NAA than protoplasts from normal roots.

In both *L. esculentum* and *C. trichophyllus*, hairy roots exhibited a sensitivity to NAA remarkably higher than that of normal roots, by a factor of 100 to 1000 according to the auxin-induced response considered. These results are quite comparable to those obtained in *L. corniculatus* where Ri-transformed roots (by agropine or mannopine *A. rhizogenes* strains) were shown to be about 500 times more sensitive to NAA than normal roots (26). The modifications induced by *A. rhizogenes* transformation appeared specific to the auxin response, as they did not affect the response of *C. trichophyllus* transformed roots to fusicoccin in terms of H^+-excretion (Fig. 3), as already observed for *L. corniculatus* roots, both in terms of H^+-extrusion (26) and protoplast hyperpolarization (3).

Critical Evaluation of the Auxin-Induced Electrical Response as a Marker of Protoplast Sensitivity to the Hormone

The NAA-induced electrical response on isolated protoplasts was used here as one of the criteria to characterize and compare the sensitivities to auxin of normal and *A. rhizogenes*-transformed roots. This comparison could appear questionable, due to the very small polarization of root protoplasts with regard to cells, as well as to the low amplitude of the auxin-induced hyperpolarization (see 25).

A depolarized state appears as a general feature of isolated protoplasts, as Em values near zero have already been described for protoplasts isolated from roots (26), leaves (1, 2, 12, 16, 22), and cultured cell suspensions (9, 24). Several possibilities, such as nonspecific permeabilization of the plasma membrane during cell wall digestion (9) or salt leakage from microelectrodes (4, 5), could account for such properties. To gain further insight into the origin of the depolarized state of isolated root protoplasts, we examined the possible role of salt leakage from microelectrode tips, using micropipettes filled with various electrolytes (KCl, K_2SO_4, and K-acetate) at different concentrations, on protoplasts isolated from the transformed *C. trichophyllus* root line T4. Although the mi-

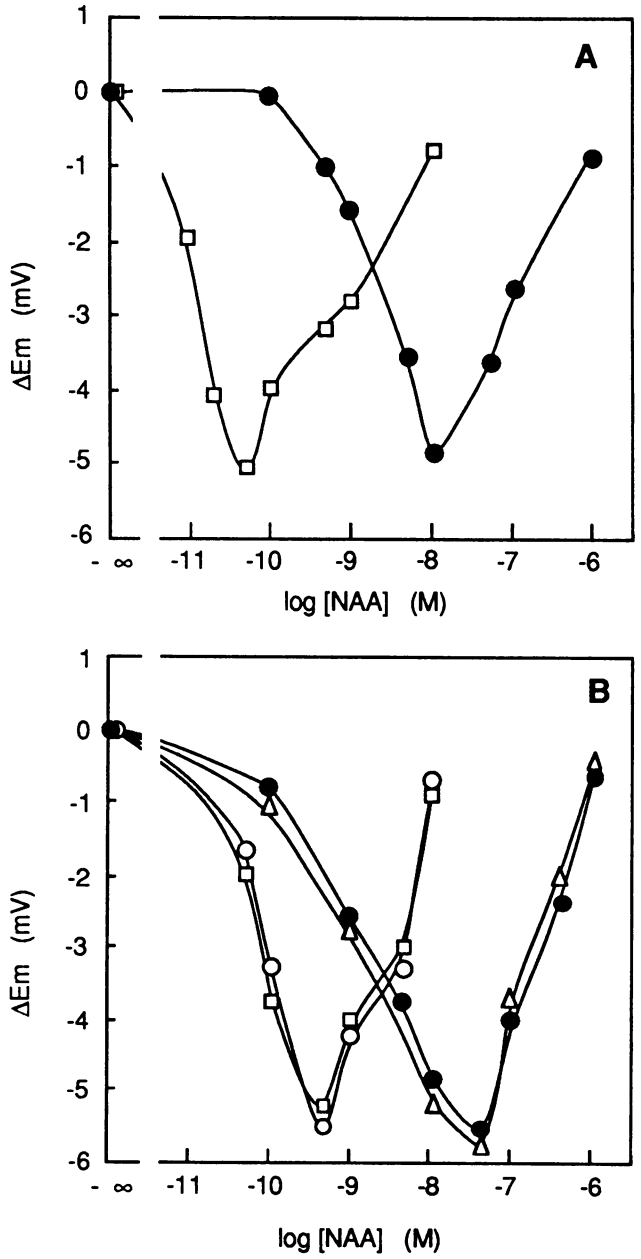

Figure 2. Effect of NAA on the transmembrane electrical potential difference of *L. esculentum* (A) and *C. trichophyllus* (B) root protoplasts. The transmembrane Em of the protoplasts isolated from normal and transformed roots were measured with microelectrodes filled with 1 M KCl. For each condition, a mean Em value was calculated from 15 measurements on individual protoplasts. For each experiment, a complete range of concentrations was tested on the same protoplast suspension. NAA-induced Em variations (ΔEm, mV) were calculated from the reference value measured in absence of auxin. The maximal standard error for each point was less than 0.3 mV. Results from one representative experiment are presented in the figure. Identical results were obtained in three independent experiments for each root line. A, *L. esculentum* protoplasts from normal (●) and Ri-15834 transformed (□) roots; B, *C. trichophyllus* protoplasts from normal (●) and transformed roots, line T4 (□), line T5 (○) and line T6 (△).

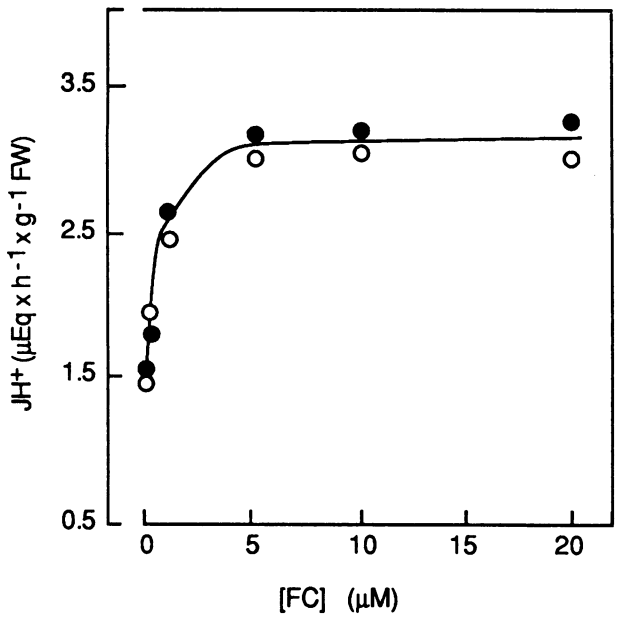

Figure 3. Effect of fusicoccin on proton excretion by apical root segments of *C. trichophyllus*. The net proton efflux (JH^+) was measured as in Figure 1. The mean value calculated from the results of three independent experiments is presented. The standard error was less than 0.1 $\mu Eq \cdot h^{-1} \cdot g^{-1}$ fresh weight. Normal roots (●); transformed roots line T4 (○).

cropipettes filled with diluted or assymetrical electrolytes have higher electrical resistances and tip junction potentials, stable Em values could be recorded with all types of microelectrodes (Table II). For each electrolyte, decreasing the concentration in the micropipettes induced more negative Em values for root protoplasts (Table II), indicating an influence of anion rather than cation leakage on the measured potential. The initial polarization was also strongly increased by the removal of chloride ions from the microelectrode and its replacement by acetate or sulfate (Table II). These results support the idea that the depolarization of root protoplasts would be due to an increased anion efflux through the plasmalemma, fed by the diffusion of chloride from the microelectrode tip when classically filled with 1 or 3 M KCl. This type of depolarization due to KCl leakage from microelectrode tips has been observed on cells of the mycelial fungus *Neurospora crassa* (5) and stomatal guard cells of *Vicia faba* and *Commelina communis* (4). A shift of −51 mV of Em values observed on root protoplasts (Table II), when KCl concentration in the microelectrodes was diluted from 1 M to 100 mM, is comparable to that described on plant stomatal guard cells (−52 mV) (4). The decay in polarization, as suggested (4, 5), can be accounted for if the Cl^- conductance comes to dominate the overall membrane conductance as the internal $[Cl^-]$ rises. Stretch-activated anion channels, recently described at the plasma membrane of plant cells (see 13 for a review) could mediate this anion conductance.

As Em values near the values described for plant cells could be obtained on root protoplasts with the micropipettes filled with the electrolytes at low concentration, the auxin effects on Em were examined in these conditions, and compared to those observed using classical 1M KCl-filled electrodes. The micropipettes filled with 50 mM K-acetate were selected according to the most negative Em values they gave (−45 to −60 mV). Figure 4 shows dose-response curves for NAA measured with both types of electrodes on protoplasts isolated from normal and transformed *C. trichophyllus* roots (line T4). The use of acetate instead of chloride led to more negative Em values in the absence of auxin, and increased the amplitude of the maximal NAA-induced hyperpolarization (ΔEm = −20 mV) together with the variability of the measured values (Fig. 4). As to its amplitude, this response can be compared to that measured on organs in the presence of 10 μM IAA: −17 mV on internode segments of *Pisum sativum* (17) and −20 to −25 mV on coleoptiles of *Zea mays* (18) or *Avena sativa* (25). Interestingly, regardless of the electrolyte, the shape and the position of the dose-response curves along the NAA concentration range were not modified, the only change concerning the amplitude of the response. The protoplasts from transformed roots thus appeared about 100 times more sensitive to auxin than normal protoplasts, independently of the measurement conditions. This confirms that the transmembrane electrical response provides a reliable estimation of protoplast sensitivity to auxin.

TL-DNA is Responsible for the Increased Sensitivity to Auxin of Hairy Roots

We previously proposed (26) that in *L. corniculatus* roots transformed with the agropine Ri plasmid 15834, high sensitivity to auxin was controlled by T_L-DNA. This hypothesis

Table II. *Influence of Electrolytes Filling the Microelectrodes on the Transmembrane Em of Root Protoplasts*

Potential difference measurements were carried out on protoplasts isolated from the transformed root line T4 of *C. trichophyllus* and suspended in 0.5 M mannitol, 0.5 mM Ca SO_4, 0.5 mM Mes-Bis Tris propane (pH 5.6), with micropipettes filled with KCl, K-acetate, or K_2SO_4 at different concentrations. The electrode half-cells (1 M KCl/Ag-AgCl) were not modified. The electrode tip potential was defined as the potential recorded with the electrode in the bathing solution with regard to circuit ground. The transmembrane potential Em is given as the difference between the potential registered with the electrode in the protoplast and the tip potential.

Electrolyte	Electrode Tip Junction Potential Mean ± SE[a]	Em Mean ± SE[b]
	mV	
KCl		
1 M	−4.4 ± 1.4 (11)	+20.0 ± 1.4 (31)
100 mM	−9.9 ± 2.1 (11)	−31.1 ± 1.9 (29)
50 mM	−16.6 ± 2.2 (14)	−42.0 ± 2.0 (29)
K-acetate		
1 M	−4.8 ± 1.8 (16)	−15.5 ± 1.7 (15)
200 mM	−16.0 ± 2.4 (15)	−35.4 ± 2.3 (27)
50 mM	−35.0 ± 3.7 (13)	−61.9 ± 4.0 (31)
K_2SO_4		
500 mM	−5.0 ± 2.6 (4)	−17.6 ± 3.3 (15)
50 mM	−12.8 ± 2.7 (5)	−47.8 ± 3.6 (16)

[a] Number of micropipettes used. [b] Number of protoplasts measured.

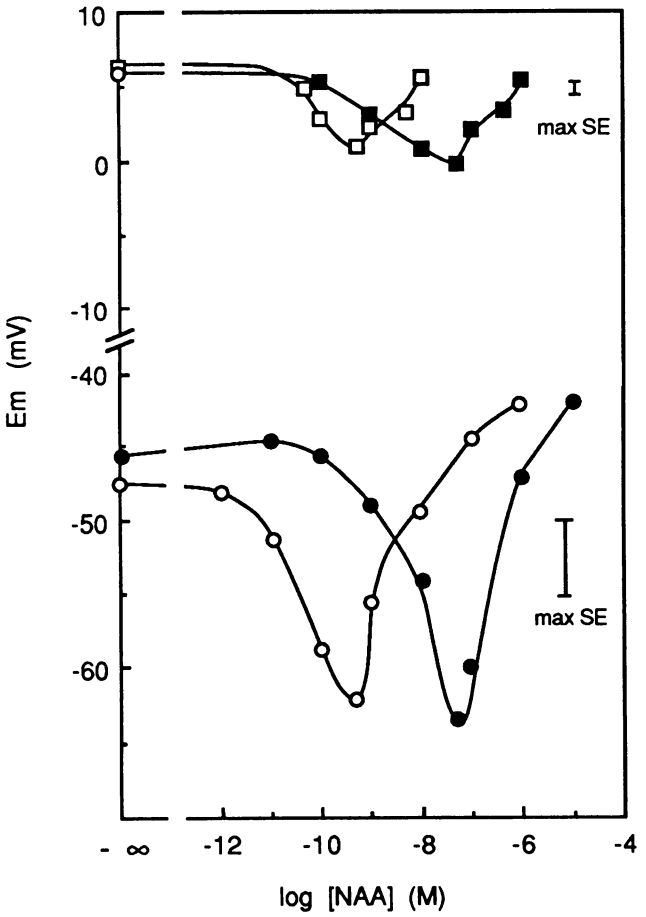

Figure 4. Effect of the nature of microelectrode-filling electrolytes on the dose-response curve to NAA of *C. trichophyllus* protoplasts isolated from normal or transformed (line T4) roots. The transmembrane potential differences (Em) were measured with 1 M KCl-filled (squares) or 50 mM K-acetate-filled micropipettes (circles), as a function of NAA concentration in the external medium. Each Em value was calculated as the mean from 15 measurements on individual protoplasts, and maximal standard errors (max SE) are given for each type of electrolyte. Results from one representative experiment are given in the figure. Identical results were obtained in three independent experiments for each root line. Normal roots (■, ●); transformed root line T4 (□, ○).

was based on the observations that roots transformed by agropine Ri plasmid 15834 and by mannopine Ri plasmid 8196 exhibited the same increase in sensitivity to auxin, and that the single T-DNA region of mannopine Ri plasmid presented homology to the T_L-DNA (6). It had already been demonstrated in tobacco that the presence of *iaaM* and *iaaH* genes of T_R-DNA is not necessary for hairy root formation (7) and that the hairy root phenotype is controlled by T_L-DNA (30) in agropine-type Ri plasmid. We tried here to dissect in *C. trichophyllus* the respective roles of T_R- and T_L-DNAs in inducing the increase in sensitivity to auxin observed on roots transformed by the whole (T_R+T_L)-DNA. The roots transformed by T_R-DNA alone (line T6) presented a sensitivity identical to that of normal roots (Fig. 2), whereas the roots transformed by T_L-DNA alone (line T4) presented the same increase in their sensitivity to auxin than the roots transformed by (T_L+T_R)-DNA (line T5) (Fig. 2). These results confirmed that the *iaaH* gene, which is present and functional in the line T6, has no role in the modifications of sensitivity induced by Ri-transformation of *C. trichophyllus*. They demonstrate that T_L-DNA within the agropine Ri plasmid is responsible for the observed increase in sensitivity to auxin of hairy roots.

CONCLUSION

According to the present study, high sensitivity to auxin is a common feature of hairy roots, regardless of the plant species and of the auxin-induced responses tested. Among these, the electrical membrane response of isolated protoplasts, demonstrated here to provide a reliable estimation of the sensitivity to auxin, actually represents the most accurate reading of differential sensitivities to the hormone (2, 3, 12, 26). In *C. trichophyllus* transformed roots, approximately the same increase in sensitivity to auxin was obtained for short-term (less than 2 min for Em modification), medium-term (1 h for proton excretion), and long-term (24 h for root elongation) responses. These results, in agreement with those observed on *L. corniculatus* roots (26), support our previous suggestion that the modification of the response to auxin by hairy root transformation might concern early events of the hormone action, possibly in the reception-transduction pathway.

An increased responsiveness to auxin has also been reported for leaf cells of *Nicotiana tabacum* transformed by an agropine type *A. rhizogenes* strain (1855), as measured by comparison of the effective exogenous auxin concentration inducing rhizogenesis on normal and transformed leaf fragments (27). Mesophyll protoplasts isolated from this transformed tobacco genotype were recently shown to display an increased sensitivity to exogenous auxin (2). We reported here for *C. trichophyllus* that the T_L-part of the T-DNA controls both the hairy root phenotype and the high sensitivity to auxin. In tobacco, only three T_L-DNA genes, *rol A*, *B*, and *C*, when transferred together, are able to induce the full hairy-root syndrome (27, 28, 30), and, as single genes, to direct developmental abnormalities (28). Investigating the effects of these genes on protoplast sensitivity to auxin and searching for possible associated modifications in the hormone reception/transduction pathway will provide some clues to understanding their role in rhizogenesis.

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