ing adequate rates of pollen hydration. The SC phenotype of *mod* stigmas would result from a lack of channel molecules that can serve as targets of SRK activity. Although a mechanism of self-incompatibility based on the regulation of water availability is consistent with pollination biology, some MIPs are permeable to small molecules other than water (*15*). Therefore, the MOD channel might promote either the efflux and localized accumulation at the pollen–papillar cell interface of substances inhibitory to pollen germination and tube ingress, or the influx, and therefore localized depletion from the cell wall, of substances required for pollen germination and tube growth. The rapid modulation of membrane permeability in response to self-pollination could be brought about by reversible phosphorylation of MOD channel proteins, resulting in an increase in their transport activity (*20*) or in their rapid recruitment to the plasma membrane (*21*).

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Aluminum Tolerance in Transgenic Plants by Alteration of Citrate Synthesis

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Aluminum when in soluble form, as found in acidic soils that comprise about 40 percent of the world's arable land, is toxic to many crops. Organic acid excretion has been correlated with aluminum tolerance in higher plants. Overproduction of citrate was shown to result in aluminum tolerance in transgenic tobacco (*Nicotiana tabacum*) and papaya (*Carica papaya*) plants.

When solubilized in acid soils, Al (primarily in the form of Al^{3+}) is toxic to many crops and is the major limiting factor for plant productivity on these soils. Soil acidification accelerated by certain farming practices and by acid rain affects about 40% of the arable land worldwide (*1*, *2*). Although crop production on acid soils can be sustained by application of lime, runoff pollution is an undesirable side effect. Thus, the production of Al-tolerant plant varieties either by conventional breeding or genetic engineering appears to be the best solution.

Symptoms of Al toxicity are similar to those of nutrient deficiency (*3*, *4*), probably owing to the inhibition of root development caused by the action of Al at the root tip (*2*, *5*). In simple nutrient solutions, micromolar concentrations of Al can begin to inhibit root growth within 60 min (*6*).

Plants show a range of natural resistance to Al toxicity (*4*, *7*). Tolerance may occur by Al exclusion from the root tip (*8*) and in several cases has been closely correlated with an increased capacity to release organic acids, such as citric acid (*9*, *10*), which may chelate Al^{3+} outside the plasma membrane, thereby preventing its uptake (*10*). In vitro, organic acids do indeed decrease the toxic effect of Al, citrate being more effective than succinate or malate (*11*).

To examine whether increased citrate production and release in transgenic plants could confer Al tolerance, we produced transgenic tobacco and papaya plants that overexpress a citrate synthase (CS) gene from *Pseudomonas aeruginosa* in their cytoplasm. We targeted the bacterial CS to the cytoplasm rather than the mitochondria to avoid redistribution of carbon from citrate synthesis to other components of the Krebs cycle.

We constructed a chimeric gene with the coding sequence of the *P. aeruginosa* CS gene (CSb) (*12*) fused to the 35*S* promoter from the cauliflower mosaic virus and *nos* 3′-end sequences (35S-CSb). We introduced the 35S-CSb gene into the genome of tobacco (*Nicotiana tabacum* L var. xanthi) plants using a Ti plasmid–derived transformation system (*13*). The presence of the transgene was confirmed in 30 independent transgenic lines by Southern (DNA) blot hybridization analysis (*14*). The T2 progeny of homozygous plants harboring a single copy of the transgene were selected for further analysis. No obvious phenotypical differences were observed between the plants harboring the 35S-CSb construct and the controls when grown under greenhouse conditions. Four lines expressing between two- to threefold higher levels of CS (*15*) than the control were further analyzed (Fig. 1).

To determine whether the cytoplasmic

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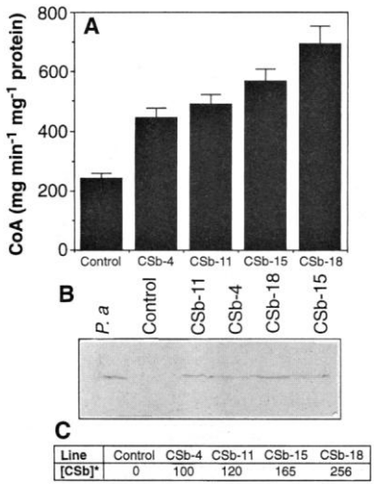

Fig. 1. Level of citrate synthase in CSb and control tobacco lines. (**A**) Level of citrate synthase activity in transgenic tobacco plants (*14*). (**B**) Protein immunoblot analysis of root total protein extracts with a *P. aeureginosa* CS-specific antiserum (*12*). (**C**) Relative concentration of bacterial CS ([CSb]*) in transgenic tobacco lines as determined by densitometric analysis of protein immunoblot assay shown in (B). CSb-4, CSb-11, CSb-15, and CSb-18 are transgenic tobacco plants harboring the 35S-CSb construct. The control is a tobacco line transformed with the same vector but without CSb coding sequence. Data are the mean ± SD of three independent experiments.

Table 1. Level of citrate and citrate efflux in roots of transgenic 35S and control plants. Data are the mean ± SD of three independent experiments.

Line	Citrate levels (mmol per gram fresh weight)	Citrate efflux (nmol per seedling per hour)
Control	0.43 ± 0.05	57 ± 7.2
CSb-4	1.41 ± 0.07	105 ± 12.2
CSb-11	1.62 ± 0.08	111 ± 12.6
CSb-15	2.31 ± 0.10	163 ± 14.3
CSb-18	4.47 ± 0.35	231 ± 15.3

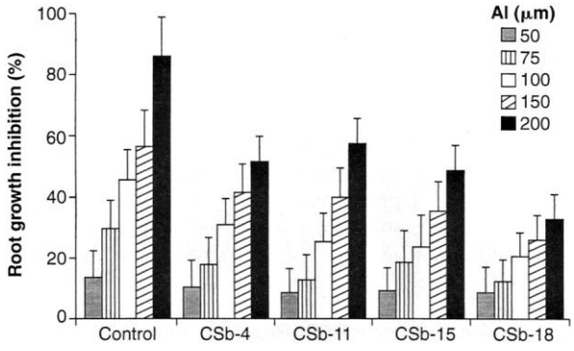

Fig. 2. Root growth inhibition of 35S-CSb and control tobacco lines by different concentrations of Al. Data are the mean ± SD of three different experiments in which 100 seedlings were included per experiment.

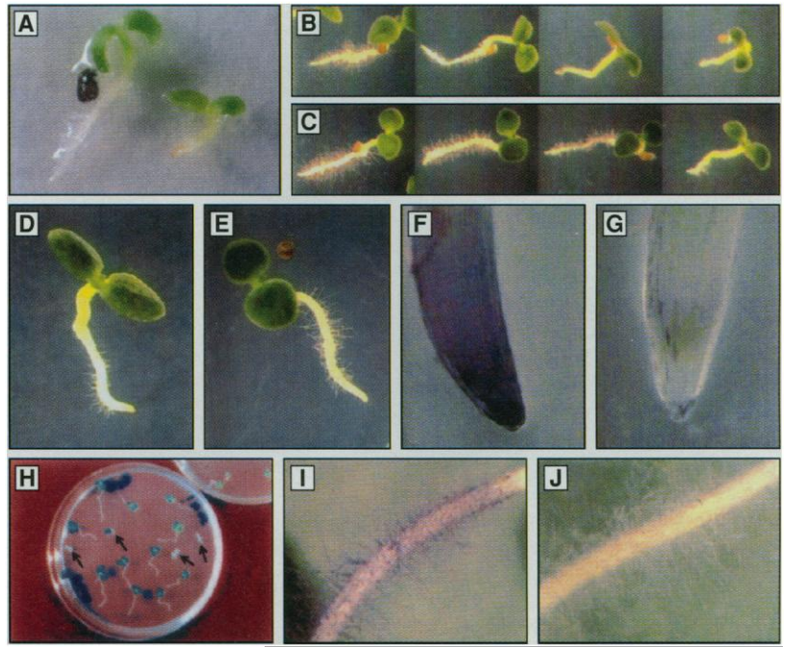

Fig. 3. Growth of tobacco CSb and control lines germinated in Al-containing media and detection of Al uptake. (**A**) Control plants germinated in media with (right) or without 300 μM Al, 2 weeks after germination. (**B** and **C**) Control (B) and CSb-18 (C) plants germinated in media containing 0, 75, 300, and 1000 μM Al 10 days after germination. (**D** and **E**) CSb-4 and CSb-18 1-week-old seedlings germinated in 200 μM Al. (**H**) Segregation of Al-tolerance phenotype in the CSb-18 T1 progeny after 4 weeks of growth in medium containing 300 μM Al (pH 4.3); arrows indicate susceptible seedlings. (**F**, **G**, **I**, and **J**) Hematoxylin staining of root hairs and root tips of 7-day-old seedlings treated for 1 hour with 100 μM Al; (F) and (I) are control and (G) and (J) CSb-18 seedlings.

expression of CS results in increased citrate production, we examined total and root extracts of the 35S-CSb lines by high-pressure liquid chromatography (HPLC) (*16*). Lines expressing the 35S-CSb construct had up to 10-fold higher levels of citrate in their root tissue (Table 1).

Because the protective effect of citrate is probably produced when citrate is released extracellularly, we examined citrate efflux in CSb plants. Fifty plants of each line were germinated on solid media and then transferred to sterile water for 12 hours, and the amount of citrate released into the water was determined by HPLC (*16*). Plants from the selected 35S-CSb lines released up to fourfold more citrate than control plants (Table 1). The chemical identity of citrate in CSb exudates was confirmed by mass spectrometry (*14*). The CSb-18 line, which accumulates 10-fold more citrate than the control lines, releases only fourfold more, suggesting that the citrate transport system might be saturated in this line.

To determine whether the 35S-CSb lines with increased levels of synthesis and excretion of citrate were more tolerant to Al, we measured the effect of Al on their root growth. One hundred seeds from the T2 progeny of homozygous 35S-CSb and control plants were germinated on plates covered by filter paper dampened with Blaydes nutrient solution at pH 4.3 (*17*). Seven days after germination, the plates containing the seedlings were rotated 90°, and the nutrient solution was replaced by Al-containing nutrient solution adjusted to pH 4.3 (*17*). After 7 days of growth in the presence of Al, during which the pH varied by no more than 0.2 pH units, root growth was evaluated. Inhibition of root growth by Al was lower in the 35S-CSb lines than in the control (Fig. 2). Statistical analysis with the single degree of freedom contrast from a combined analysis of variance of two independent experiments indicated significant differences between the control and CSb lines at Al concentrations greater than 50 μM ($P < 0.01$).

To examine whether citrate overproduction also protects root development in seeds

germinated on Al-containing media (pH 4.3), we germinated seeds from the CSb and control lines on media containing 0.1 to 1 mM Al. At concentrations higher than 300 μM, control seeds germinated but did not develop a root system (Fig. 3A), and at low Al concentrations (50 to 75 μM) root growth was only slightly affected, but root-hair development was severely impaired (Fig. 3B). CSb lines germinated on Al-containing medium were more resistant to these effects (Fig. 3C). Root growth and root hair development of CSb lines exposed to Al correlated with their level of CS activity and citrate content (Fig. 3, D and E). Tolerant and susceptible phenotypes segregated with a 3:1 ratio for the lines containing a single transferred DNA (T-DNA) insertion (Fig. 3H). Al tolerance cosegregated with the kanamycin resistance gene also present in the T-DNA of the binary vector used to produce the CSb lines (*14*).

To analyze Al uptake, we exposed roots from 14-day-old homozygous CSb plantlets for 1 hour to a 100 μM solution of Al (pH 4.3), then washed the roots extensively with water and stained them with hematoxylin (*18*). CSb lines showed less staining than did controls (Fig. 3, F, G, I, and J), indicating that lower amounts of Al penetrated the root tip and root hairs of CSb lines (hematoxylin turns violet only in the presence of Al).

The 35S-CSb construct was also introduced into the genome of papaya plants by particle bombardment (*19*). CSb transgenic papaya lines contained two- to threefold higher levels of citrate synthase compared with controls transformed with the vector alone (*14*). Twenty regenerated plants from each papaya CSb line were transferred to rooting media (pH 4.3) containing different concentrations of Al. Root development failed in control plants exposed to 50 μM or higher concentrations of Al, whereas CSb lines were able to form roots and grow normally on concentrations of Al of up to 300 μM (Fig. 4).

Fig. 4. Control and citrate-overproducing papaya plants after 30 days of culture in the presence of 300 μM Al. (Left) CSb transformant; (right) transformant containing the vector without the CS coding sequence.

Our data provide a direct demonstration that organic acid excretion is indeed a mechanism of Al tolerance in higher plants and that this trait can be engineered transgenically. This finding opens the possibility of applying this technology to important crop plants, such as maize, rice, and sorghum, which are often grown in acidic soils in which Al toxicity is a major problem.

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16. For measurement of citrate content, 1 g of roots was ground in liquid nitrogen and extracted with 10 ml of boiling 80% ethanol. The homogenate was centrifuged in a tabletop centrifuge at 1000 rpm for 10 min and the supernatant filtered through a 0.45-μm Millipore filter. Organic acid content was analyzed by HPLC as described [H. D. Picha, *J. Agric. Food Chem.* **33**, 743 (1985)].
17. One hundred seeds were germinated on plates placed in a vertical position inside a container with nutrient solution. When the plantlets were exposed to Al-containing Blaydes solution [W. A. Parrot and H. J. Bouton, *Crop Sci.* **30**, 387 (1990)] (adjusted to pH 4.3 by addition of 0.1 N KOH), the plates containing the seedlings were rotated 90°, where a change in the direction of root growth occurred due to gravitropism, allowing the measurement of root growth after exposure to Al. This technique was described by L. Taiz and A. Murphy [*Plant Physiol.* **108**, 29 (1995)]. The pH of the nutrient solution was monitored throughout the experiment and found to vary by no more than 0.2 pH units.
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Isolation of a Bacterium That Reductively Dechlorinates Tetrachloroethene to Ethene

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Tetrachloroethene is a prominent groundwater pollutant that can be reductively dechlorinated by mixed anaerobic microbial populations to the nontoxic product ethene. Strain 195, a coccoid bacterium that dechlorinates tetrachloroethene to ethene, was isolated and characterized. Growth of strain 195 with H_2 and tetrachloroethene as the electron donor and acceptor pair required extracts from mixed microbial cultures. Growth of strain 195 was resistant to ampicillin and vancomycin; its cell wall did not react with a peptidoglycan-specific lectin and its ultrastructure resembled S-layers of Archaea. Analysis of the 16S ribosomal DNA sequence of strain 195 indicated that it is a eubacterium without close affiliation to any known groups.

The solvent tetrachloroethene [perchloroethylene (PCE)] is a common groundwater pollutant (*1*, *2*) that is highly toxic and is suspected to be a human carcinogen. It is nonbiodegradable by aerobes but can be reductively dechlorinated by natural microbial communities and mixed microbial enrichment cultures under anaerobic conditions according to the reaction sequence shown in Fig. 1 (*3*). The formation of nontoxic products such as ethene (ETH) (*4*) and ethane (*5*) indicates the potential for complete anaerobic detoxification of chloroethenes in situ.

Slow reductive dechlorination of chlo-

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