contains only a subset of the antigens expressed by the pathogen population (*17*) such as those currently planned for *N. meningitidis* (*18*) and *Streptococcus pneumoniae* (*19*). Attributing observed changes in strain structure following mass immunization to the intervention may be problematic, given the complex nonlinear dynamics suggested by our analyses. The intricate behavior of these multistrain systems is a consequence of the selective pressures imposed on the pathogen population by the profile of herd immunity in the host population. This profile is, in turn, conditioned by the prevailing antigenic structure of the pathogen population. It is the subtle interplay between these two factors that leads to the unstable evolutionary dynamics we describe.

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4. The proportion immune to a given strain *i*, z_i, is simply given by

$$\frac{dz_i}{dt} = (1 - z_i)\lambda_i - \mu z_i$$

Here, λ_i represents the force of infection of strain *i*. We assume, for simplicity, that immunity is lifelong. We then define an additional compartment, w_i, which represents those immune to any strain *j* that shares alleles (at the relevant polymorphic loci) with strain *i* (including *i* itself). The dynamics of w_i are then given by (where $j \sim i$ means *j* shares alleles with *i*)

$$\frac{dw_i}{dt} = (1 - w_i) \sum_{j \sim i} \lambda_i - \mu w_i$$

Individuals who have never been exposed to any strain sharing alleles with strain *i* (that is, $1 - w_i$) are completely susceptible to strain *i*. However, those that have been exposed to a strain sharing alleles with *i*, but not exposed to strain *i* itself (that is, $w_i - z_i$), will become infectious with a probability $1 - \gamma$ when they are infected by strain *i*. With σ being the rate of loss of infectiousness of the host, the dynamics of the proportion of the population infectious for strain *i* may therefore be represented as

$$\frac{dy_i}{dt} [(1 - w_i) + (1 - \gamma)(w_i - z_i)]\lambda_i - \sigma y_i$$

The impact of genetic exchange on the population structure of infectious disease agents may be examined within this framework by modifying the force of infection term, λ, to include the assumption that the progeny of parasites within hosts infectious for two or more strains will consist of defined fractions, Ω_{ijk}, of the various combinations of the different strains, *j* and *k*, that may generate strain *i* through recombination. Because the proportions of infectious hosts are very small, the force of infection of strain *i* may be approximately represented as $\lambda_i = \beta_i (y_i + \Sigma \Omega_{ijk} y_j y_k)$, where β_i is a combination of parameters affecting the transmission of strain *i*. The behavior of the model is largely unaffected by the inclusion or precise functional form of the recombination term.
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Identification of Non–Heme Diiron Proteins That Catalyze Triple Bond and Epoxy Group Formation

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Acetylenic bonds are present in more than 600 naturally occurring compounds. Plant enzymes that catalyze the formation of the Δ12 acetylenic bond in 9-octadecen-12-ynoic acid and the Δ12 epoxy group in 12,13-epoxy-9-octadecenoic acid were characterized, and two genes, similar in sequence, were cloned. When these complementary DNAs were expressed in *Arabidopsis thaliana*, the content of acetylenic or epoxidated fatty acids in the seeds increased from 0 to 25 or 15 percent, respectively. Both enzymes have characteristics similar to the membrane proteins containing non–heme iron that have histidine-rich motifs.

Over 600 naturally occurring acetylenic compounds are known, many of which occur in higher plants and mosses (*1*, *2*). Knowledge of the enzymatic reactions leading to the formation of a carbon-carbon acetylenic (triple) bond is limited; it is known, however, that in the moss *Ceratodon purpurea*, an acetylenic bond at the Δ6 position in a C_{18} fatty acid is formed from a carbon-carbon double bond (*3*), whereas in *Crepis rubra*, oleate is a substrate in the synthesis of 9-octadecen-12-ynoic acid (crepenynic acid) (*4*).

We have studied the synthesis of acetylenic and epoxy fatty acids in plants of the genus *Crepis* (*2*, *5*). *Crepis alpina* seed oil is made up of about 70% crepenynic acid, and *Crepis palaestina* seed oil is made up of about 60% vernolic acid (12,13-epoxy-9-octadecenoic acid). Here we characterized the enzymes involved in the biosynthesis of crepenynic and vernolic acid in *C. alpina* and *C. palaestina* (*6*). Microsomes from developing seeds of *C. alpina*, prepared in the presence of reduced nicotinamide adenine dinucleotide (NADH), converted [^{14}C]linoleate into [^{14}C]crepenynate (Table 1). Similarly, microsomes from developing seeds of *C. palaestina* converted [^{14}C]linoleate into [^{14}C]vernoleate. The results indicated that both the Δ12 acetylenic bond–forming and Δ12 epoxidation reactions used acyl chains with a carbon double bond at the Δ12 position as substrate.

These reactions, and the Δ12 desaturase, required NADH or NADPH and were inhibited by cyanide (Table 1). Unlike the *Euphorbia lagascae* epoxygenase, which is likely to be a cytochrome P-450–type enzyme (*7*), the *C. palaestina* epoxygenase was unaffected by carbon monoxide or antibodies to cytochrome P-450 reductase (*8*). Both the Δ12 epoxygenation and Δ12 acetylena-

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tion enzymes of *Crepis* had biochemical characteristics typical of fatty acid desaturases and might therefore, we thought, be structurally similar to these enzymes (*9*, *10*).

To clone the gene encoding the acetylenase, we used cDNA from *C. alpina* developing seed and primers derived from endoplasmic reticular (ER) desaturases (*11*) for polymerase chain reaction (PCR). DNA sequencing of the products revealed a likely ER Δ15 desaturase sequence, a likely Δ12 desaturase sequence (D12N), and a variant ER Δ12 sequence (D12V). Northern (RNA) blot analysis showed that expression of the D12V sequence was seed specific in *C. alpina*, as was the presence of crepenynic acid.

The D12V fragment was used to isolate a full-length cDNA (Crep1) from a *C. alpina* developing-seed library from which the plasmid pCrep1 was produced (*12*). Sequencing of Crep1 revealed a putative protein of 375 amino acids (Fig. 1). This protein had 59% identity to the castor bean Δ12-hydroxylase and 56% identity to the *Arabidopsis* ER Δ12 desaturase when compared pairwise.

The Crep1 gene was expressed in *Saccharomyces cerevisiae* (YN94-1 strain) with the plasmid pVT-Crep1 (*13*). Yeast were cultivated in media with linoleic acid and their fatty acid composition determined (*14*). A peak with the same retention time as the methyl ester of crepenynic acid (up to 0.3% of the total peak area) was identified in the pVT-Crep1–transformed yeast but not in yeast transformed with vector alone. In addition, only the pVT-Crep1–transformed yeast had fatty acid diethylamides (FADEAs), prepared from the total fatty acids and analyzed by gas-liquid chromatography–mass spectrometry (GLC-MS) (*15*), that contained a compound with the same retention time and mass of 9-octadecen-12-ynoic acid diethylamide (Fig. 2, A and B). The mass spectrum of this

Table 1. Properties of different fatty acid modification enzymes [some of these results were published earlier (*6*, *7*)]. The amount of enzyme activity is given as the amount of radioactive substrate converted into product relative to a control, which was in each case the incubation conditions giving the highest activity. The amount of substrate conversion in these controls was *C. alpina* acetylenase, 12%; *C. palaestina* epoxygenase, 26%; *C. alpina* desaturase, 30%; and *E. lagascae* epoxygenase, 13%.

Treatment	Enzymatic activity (% of control)			
	Δ12 oleate desaturase *C. alpina*	Δ12 linoleate acetylenase *C. alpina*	Δ12 linoleate epoxygenase *C. palaestina*	Δ12 linoleate epoxygenase *E. lagascae*
CO	85	84	88	3
P-450 reductase antibodies	96	91	94	33
KCN	16	0	35	92
Minus NADH plus NADPH	95	73	94	100
Minus NADPH plus NADH	100	100	100	11

```
     1                                                                  80
acet --g---.... ..grtsq-.. ..plme-vsv dp.p-tvsd- -q-------k --vi--sy-i vh-aiiayi- -fl-dkyipi
epox --a---.... ..grtse-.. ..svme-vsv dpvt-slse- -q-------q --vi--sy-v vq-liiayi- -fl-ntyipt
cpde --a---mqd. ...psnd-.. .t.tte-vpy qkpp-tvge- -k-------n --vi--fs-v vy-ltiasi- -yi-tnyiht
atde --a---mpv. ...ptss-ks etdttk-vpc ekpp-svgd- -k-------k --ip--fs-l is-iiiasc- -yv-tnyfsl
hydr --g---mstv itsnnse-kg gsshlk-aph tkpp-tlgd- -r-------e --fv--fs-v ay-vclsfl- -si-tnffpy
cons MG-GGR---- -------K-- ------R--- ----F----L K-AIPPHCF- RS--RS--Y- --D------F Y--A------

     81                                                                160
acet lpap-a-l-- pl--fc-asi ---l-vig-- -------dyq wv--t--f-l --f-mt---- --y---n--a -tn-ldn---
epox lpts-a-l-- pv--fc-asv ---l-ilg-- -------nyt wf--t--f-l --f-lt---- --f---n--s -ts-idn---
cpde lpqp-t-f-- pi--al-gcv ---v-via-- -------dhq wl--t--l-l --f-lv---- --y---r--s -tg-ieh---
atde lpqp-s-l-- pl--ac-gcv ---i-via-- -------dyq wl--t--l-f --f-lv---- --y---r--s -tg-ler---
hydr issp-s-v-- lv--lf-gci ---l-vig-- -------eyq la--i--l-v --a-lv---- --y---r--s -ig-ler---
cons ----L-Y-AW --YW--Q--- LTG-W---HE CGHHAFS--- --DD-VG-I- HS-L--PYFS WK-SHR-HH- N--S---DEV

     161                                                               240
acet yi--s-akva lyy-vl-h-p --llimfitf t--f----ft -i--kk-er- -n-fd-ms-- fke---f-vl ls-l-ll-vl
epox yi--s-skla riy-ll-n-p --llvliimf t--f----lt -i--kk-dr- -n-fd-ms-- fke---f-vf ls-l-ll-vf
cpde fv--l-sdlr sta-yl-n-p --iltllvtl t--w----mf -v--ry-dr- -c-fd-ns-- ysn---a-if is-a-if-vl
atde fv--q-saik wyg-yl-n-l --immltvqf v--w----af -v--rp-dg- -c-ff-na-- ynd---l-iy ls-a-il-vc
hydr fv--s-skis wys-ys-n-p --vltlaatl l--w----af -v--rp-dr- -c-yd-yg-- fse---l-iy ia-l-if-tt
cons --PK-K---- ---K--N-P- GR-------- -LG-PLYL-- N-SG--Y--F A-H--P--PI ---RER-Q-- --D-G--A--

     241                                                               320
acet ygvklavaak -aawvtci-- i-v-g-fi-f di--y-h--- lsl-h-d-s- -n-l---lst i---f-f--s -l-dvth---
epox ygikvavank -aawvacm-- v-v-g-ft-f dv--f-h--- qss-h-d-t- -n-i---lsa i---f-f--s -f-dvth---
cpde yglfrlatik -lgwvlam-- g-l-v-ng-l vl--f-q--- asl-h-d-t- -d-l---lat i---y-i--k -f-nitd---
atde fglyryaaaq -masmicl-- v-l-i-na-l vl--y-q--- psl-h-d-s- -d-l---lat v---y-i--k -f-nitd---
hydr fvlyqatmak -lawvmri-- v-l-i-nc-l vm--y-q--- pai-r-g-s- -d-l---mvt v---y-v--k -f-niad---
cons ---------- G-------YG -P-L-V--F- --IT-L-HTH ---P-Y-S-E W-W-RGA--- -DRD-G-LN- V-H----THV

     321                                                          388
acet m----syi-- -h-k--rd-- ntvl-df-ki -r-pilk-mw --ak--ifie pekgresk-- y--.n-f-
epox m----syi-- -h-k--rd-- kpil-df-mi -r-pilk-mw --gr--myie pds..klk-- y--.h-l-
cpde t----stm-- -h-m--tk-- kpil-dy-qf -g-svfk-my --tk--iyvd kdae.vkd-- y--rn-ie
atde a----stm-- -n-m--tk-- kpil-dy-qf -g-pwyv-my --ak--iyve pdregdkk-- y--nn-l-
hydr a----atv-- -h-m--tk-- kpim-ey-ry -g-pfyk-lw --ak--lfve pdegaptq-- f--rn-y-
cons -HHLF---PH Y-A-EA--AI ----G--Y-- D-T----A-- RE--EC---- --------GV -WY--K--
```

Fig. 1. Alignment of derived amino acid sequences. The amino acid sequences of the *C. palaestina* epoxygenase (epox), *C. alpina* acetylenase (acet), *C. palaestina* putative Δ12 desaturase (cpde), *A. thaliana* Δ12 desaturase (atde), and castor bean oleate hydroxylase (hydr) (*30*) were aligned and from this a consensus sequence (cons) generated with the programs Pileup and Pretty (*31*). The consensus sequence is in uppercase and variant residues are in lowercase. Dots represent gaps introduced to optimize alignment, and hyphens indicate residues that are identical to the analogous amino acid in the consensus sequence. The three histidine-rich motifs [HX(3 or 4)H, HX(2 or 3)HH, HX(2 or 3)HH] (*22*) are underlined. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

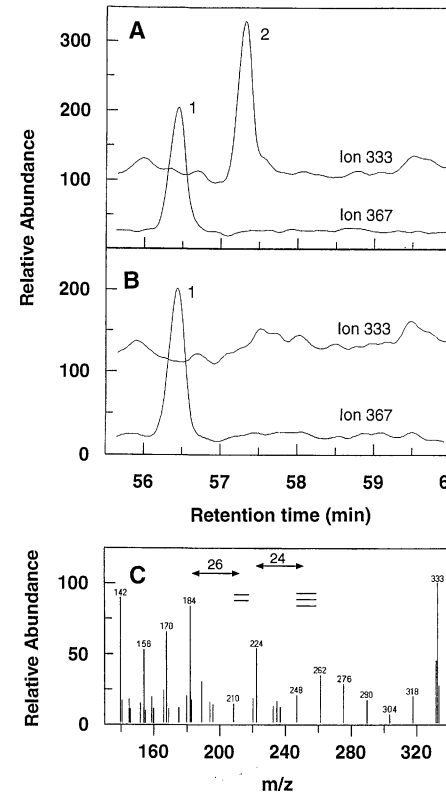

Fig. 2. GLC-MS analysis of fatty acid derivatives from transgenic yeast. (**A**) Single-ion chromatograms [367 atomic mass units (amu) and 333 amu] of FADEA derivatives from yeast transformed with pVT-Crep1 and cultivated with linoleic acid. The peaks indicated correspond to mass ions of eicosanoic acid (367 amu, peak 1) and crepenynic acid (333 amu, peak 2). (**B**) Single-ion chromatograms of FADEA derivatives from yeast with vector alone and cultivated with linoleic acid. Peak 1 is the mass ion of eicosanoic acid (367 amu). (**C**) Mass spectrum of the compound giving rise to peak 2 in chromatogram (A). m/z, mass-to-charge ratio.

FADEA was identical to the FADEA derivative of crepenynic acid (Fig. 2C). Thus, the Crep1 gene encodes an enzyme that catalyzes the formation of an acetylenic bond from the Δ12 double bond of linoleate to give crepenynic acid. We designated this type of enzyme acetylenase, because it is capable of converting a carbon-carbon double bond into an acetylenic bond and is distinct from the related desaturases that convert carbon single bonds into double bonds.

Yeast containing pVT-Crep1 and grown without linoleic acid could produce linoleic acid (up to 0.4% w/w of total fatty acids) in contrast to the control yeast. Although the C. *alpina* acetylenase has Δ12 desaturase activity, crepenynic acid was not detected in these yeast, implying that the linoleate produced was not used to synthesize crepenynic acid. The D12N sequence, also expressed in developing C. *alpina* seeds, probably represents the ER Δ12 desaturase that produces the linoleate used by the acetylenase.

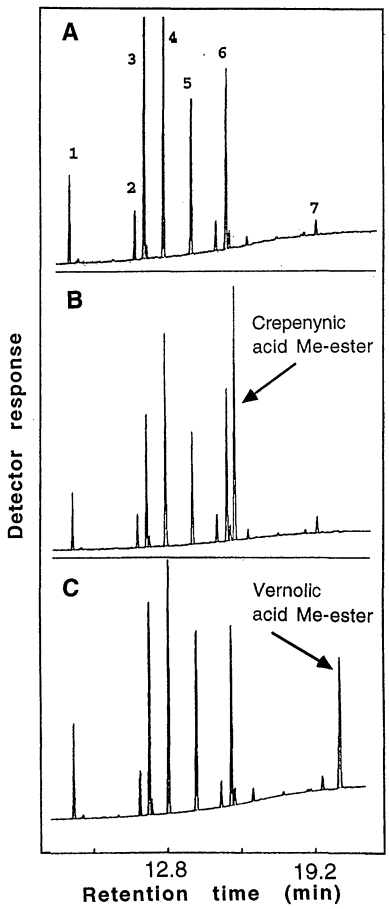

Fig. 3. GLC analysis of *A. thaliana* seed fatty acids. Methyl esters of seeds from a plant transformed with (**A**) empty vector, (**B**) the *C. alpina* acetylenase, and (**C**) the *C. palaestina* epoxygenase were analyzed and their fatty acid profiles determined. In (A) the indicated peaks correspond to methyl ester (Me) derivatives of hexadecanoic acid, 1; octadecanoic acid, 2; 9-octadecenoic acid, 3; 9,12-octadecadienoic acid, 4; 9,12,15-octadecatrienoic acid, 5; 11-eicosaenoic acid, 6; and 13-docosaenoic acid, 7. In (B) and (C) the peaks corresponding to the methyl ester derivatives of crepenynic and vernolic acid are indicated.

We expressed the acetylenase gene in *Arabidopsis* with the seed-specific napin promoter (*16*). Total fatty acids from seeds of individual T_0 transgenic plants contained up to 25% (w/w) crepenynic acid in contrast to control plants (Fig. 3). No other acetylenic fatty acids were detected (*17*).

To clone the Δ12 epoxygenase gene, we used the D12V fragment to screen a cDNA library from C. *palaestina* developing seeds (*18*). A clone (Cpal2) was isolated that encoded a putative protein of 374 amino acids. This amino acid sequence was similar to the C. *alpina* acetylenase (81% identity), the *Arabidopsis* ER Δ12-desaturase (58% identity), and the castor bean Δ12-hydroxylase (53% identity) when compared pairwise. A putative Δ12 desaturase clone was also isolated from this C. *palaestina* library (Fig. 1).

We transformed the Cpal2 cDNA into *Arabidopsis* (*19*). Total fatty acids from seeds of T_0 transgenic plants contained up to 15% (w/w) of vernolic acid (Fig. 3). Seeds from some plants also had up to 1% (w/w) of an epoxy fatty acid identified tentatively as 12-epoxy-9,15-octadecadienoic acid (*20*). This fatty acid probably arose through Δ15 desaturation of vernolic acid by the endogenous *Arabidopsis* enzyme because developing linseeds can desaturate added vernolic acid at the Δ15 position (*21*).

Despite the homology between the Crep1 and Cpal2 sequences, acetylenic and epoxy fatty acids were not detected in seeds from *Arabidopsis* plants carrying the epoxygenase or acetylenase gene, respectively. It appears that only moderate changes in amino acid sequence may determine whether these enzymes are acetylenases or epoxygenases. Their shared sequence differences to the Δ12 desaturases might reflect the changes needed for the recognition of a linoleate instead of an oleate substrate. Sequence comparison of the oleate hydroxylase and the expression of a mutant enzyme suggested that as few as six amino acids might determine if this enzyme is a desaturase or hydroxylase (*22*). That both crepenynic and vernolic acid can occur in the seed of some *Crepis* species (*2*) may indicate that these species contain both an acetylenase and an epoxygenase or a dual-functional enzyme.

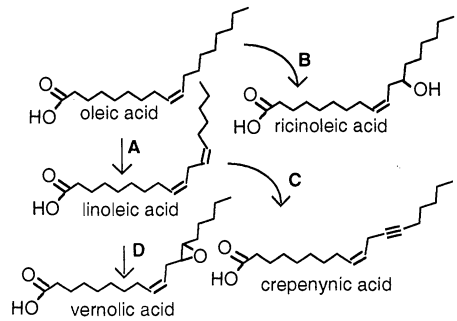

Fig. 4. Reactions catalyzed by plant Δ12 desaturase and Δ12 desaturase-like enzymes. Reactions A, B, C, and D can be catalyzed by a Δ12 desaturase, Δ12 hydroxylase, Δ12 acetylenase, and Δ12 epoxygenase, respectively.

The sequence and biochemical characteristics of the acetylenase and epoxygenase suggest that they are likely to be non–heme diiron proteins (Fig. 1) (*23, 24*). This group of proteins includes desaturases, hydroxylases, and epoxygenases found in animals, fungi, plants, and bacteria (*9, 25–27*). The diverse reactions that these enzymes catalyze probably use a common reactive center (*10*). Histidine-rich motifs are thought to form part of the diiron center where oxygen activation and substrate oxidation occur (*26, 28*). At least four reactions (Fig. 4) can be catalyzed by Δ12 desaturase–like plant enzymes.

Many of the unusual plant fatty acids are potentially valuable for the production of paints, varnishes, plastisizers, resins, lubricants, and polymers. Crepenynic acid, for example, can be converted by alkali isomerization to 8,10,12-octadecatrienoic acid and C_{18} cyclohexadiene monocarboxylic acid (*29*), which are valuable in high-quality coatings and cold weather ester-type lubricants, respectively. Similarly, epoxidized fatty acids are widely used as plastisizers. The production of large amounts of epoxy and acetylenic fatty acid in transgenic seeds may contribute to converting traditional agricultural crops into efficient producers of more valuable chemical commodities. Commercial production of such crops might be a reality in the near future.

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12. Cotyledons from seeds harvested 17 to 20 days after flowering, were used to make a cDNA library with a Uni-ZAP XR cDNA cloning kit from Stratagene. After screening with a random primed probe, pBluescript phagemid was excised. This was used to create a double-stranded DNA plasmid.
13. Plasmid pVT-Crep1 was constructed by placing the insert from pCrep1 into the vector pVT100U, which contains the constitutive alcohol dehydrogenase promoter [T. Vernet, D. Dignard, D. Y. Thomas, *Gene* **52**, 225 (1987); R. Elble, *Biotechniques* **13**, 18 (1992)].
14. Yeast were grown in liquid medium without uracil at 28°C for 5 hours before linoleic acid and Tween 40 were added to a final concentration of 0.03% (w/v) and 1% (w/v), respectively. After a further 78 hours of cultivation, cells were washed and the lipids extracted. Methyl esters were prepared with 4% w/w methanolic HCl and analyzed by GLC with a glass column (2.5 m long with a 2-mm inner diameter) packed with 3% SP-2300 on Supelcoport 100/120 mesh (Supelco, Bellefonte, PA).
15. FADEA was prepared [R. Nilsson and C. Liljenberg, *Phytochem. Anal.* **2**, 253 (1991)] and injected directly for GLC-MS [Hewlett-Packard 5890 II GLC with a DB225 (J & W Scientific, Folsom, CA) in series with a Rtx 2330 (Restek, Bellefonte, PA) fused-silica capillary column coupled to a Hewlett-Packard 5989A mass spectrometer working in electron impact mode at 70 eV].
16. A binary vector for the acetylenase consisted of the Crep1 cDNA placed downstream of the −309 fragment from the napin promoter [K. Stålberg, M. Ellerström, L. Josefsson, L. Rask, *Plant Mol. Biol.* **23**, 671 (1993)] in the vector pGPTV-KAN [D. Becker, E. Kemper, J. Schell, R. Masterson, *ibid.* **20**, 1195 (1992)]. *Arabidopsis thaliana* Columbia (C-24) was transformed with *Agrobacterium tumefaciens* [D. Valvekens, M. Van Montagu, Van Lusbettens, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5536 (1988)].
17. Methyl esters were prepared by heating 10 to 30 whole seeds at 85°C for 90 min in 1 ml of 0.1 M sodium methoxide. Methyl esters were extracted with hexane and analyzed by GLC through a 50 m by 0.32 mm CP-Wax58-CB fused-silica column (Chrompack).
18. A *C. palaestina* library was prepared and screened as described (*12*).
19. The Cpal2 transformation was done as described (*16*) except that the binary vector used was pBI121 (Clonetech).
20. Diols were prepared from epoxy fatty acids purified from Cpal2-transformed *Arabidopsis*. They were converted further to trimethylsilyl ethers and analyzed by GLC-MS (*15*) with a DB23 fused-silica capillary column. The total ion chromatogram showed two peaks. The mass spectrum of the first eluting peak had prominent ions of mass 73, 172, 275, and 299. This indicated that the epoxy group was positioned at C-12 of a C_{18} fatty acid and that a double bond occurred between the epoxy group and the COOH-terminus. This mass spectra was identical to the spectra of a trimethylsilyl ether derivative of diols prepared from vernolic acid. The second peak had prominent ions of mass 73, 171, 273, and 299. This indicated the presence of two double bonds and an epoxy group positioned at C-12 in a C_{18} fatty acid.
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30. European Molecular Biology Laboratory (EMBL) accession numbers are as follows: *C. palaestina* epoxygenase, Y16283; *C. alpina* acetylenase, Y16285; *C. palaestina* putative Δ12 desaturase, Y16284; *A. thaliana* Δ12 desaturase, L26296; and the castor bean oleate hydroxylase, U22378.
31. Pileup and Pretty, Wisconsin Package Version 9.0-UNIX, Genetics Computer Group, Madison, WI.
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A Role for the AKT1 Potassium Channel in Plant Nutrition

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In plants, potassium serves an essential role as an osmoticum and charge carrier. Its uptake by roots occurs by poorly defined mechanisms. To determine the role of potassium channels in planta, we performed a reverse genetic screen and identified an *Arabidopsis thaliana* mutant in which the *AKT1* channel gene was disrupted. Roots of this mutant lacked inward-rectifying potassium channels and displayed reduced potassium (rubidium-86) uptake. Compared with wild type, mutant plants grew poorly on media with a potassium concentration of 100 micromolar or less. These results and membrane potential measurements suggest that the AKT1 channel mediates potassium uptake from solutions that contain as little as 10 micromolar potassium.

Potassium absorption by roots is essential for plant growth. Current models, which are elaborations of classic studies (*1*), state that K^+ absorption is mediated by cotransporters at micromolar K^+ concentrations and channels at higher concentrations (*2–6*). This notion is supported by the finding that plant genes encoding channels or cotransporters could complement yeast K^+-uptake mutants (*6–8*), but such experiments do not address which mechanisms are operating in the plant. Here we report an in planta genetic dissection of the role of the AKT1 channel in the uptake of K^+ by a root.

A transferred DNA (T-DNA) mutagenized population of *Arabidopsis* was screened for plants containing an insertional mutation in the root-specific K^+-channel gene *AKT1* by using the polymerase chain reaction (PCR)–based, reverse genetic method of Krysan *et al.* (*9*, *10*). From a population of 14,200 different T-DNA lines, containing about 20,000 independent insertional events, we identified and isolated a single mutant plant (*akt1-1*) with a T-DNA insertion in *AKT1*. Southern blot analysis of the *akt1-1* locus (*11*) revealed a T-DNA insertion within the last exon of the coding region (Fig. 1). Sequence analysis reveals the T-DNA insertion site to be 4071 bases downstream of the start codon, and Northern blot analysis confirms that the mutation truncates the transcript by about 400 bases (*12*).

We examined the K^+ conductance of the plasma membrane in root cells, where high expression of *AKT1* was previously found (*13*). Microelectrodes inserted into cells approximately 150 μm from the apex of roots, which were bathed in 10 μM K^+ (*14*), revealed very negative resting membrane potentials (V_m) in both wild-type and *akt1-1* seedlings (Fig. 2B). A 10-fold increase in the extracellular K^+ concentra-

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