

Miguel A. Naranjo · Carlos Romero  
José M. Bellés · Consuelo Montesinos  
Oscar Vicente · Ramón Serrano

## Lithium treatment induces a hypersensitive-like response in tobacco

Received: 16 September 2002 / Accepted: 12 February 2003 / Published online: 21 March 2003  
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**Abstract** Treatment of tobacco (*Nicotiana tabacum* L.) plants with lithium induces the formation of necrotic lesions and leaf curling as in the case of incompatible pathogen interactions. Further similarities at the molecular level include accumulation of ethylene and of salicylic and gentisic acids, and induced expression of pathogenesis-related PR-P, PR5 and PR1 genes. With the exception of PR1 induction, lithium produced the same effects in transgenic tobacco plants that do not accumulate salicylate because of overexpression of the bacterial hydroxylase gene *nahG*. On the other hand, inhibition of ethylene biosynthesis with aminoethoxyvinylglycine prevented lithium-induced cell death and PR5 expression. These results suggest that lithium triggers a hypersensitive-like response where ethylene signalling is essential.

**Keywords** Ethylene · Lithium · Necrotic lesions · *Nicotiana* · Pathogenesis-related proteins · Salicylic acid

**Abbreviations** AVG: aminoethoxyvinylglycine · HR: hypersensitive response · PR: pathogenesis related · SA: salicylic acid · SAR: systemic acquired resistance

M.A. Naranjo · C. Romero · J.M. Bellés · C. Montesinos  
O. Vicente · R. Serrano (✉)  
Instituto de Biología Molecular y Celular de Plantas,  
Universidad Politécnica de Valencia-C.S.I.C.,  
Camino de Vera s/n, 46022 Valencia, Spain  
E-mail: serrano@ibmcp.upv.es  
Fax: +34-96-3877859

*Present address:* M.A. Naranjo  
Departamento de Biotecnología,  
Universidad Tecnológica Metropolitana,  
Avda. José Pedro Alessandri 1242,  
Macul-Santiago, Chile

*Present address:* C. Romero  
Instituto Valenciano de Investigaciones Agrarias,  
Carretera de Moncada-Naquera Km 5,  
Apartado Oficial, 46113 Moncada, Spain

### Introduction

Lithium is a non-physiological cation which has many interesting *in vivo* effects, including its therapeutic action on manic-depressive psychosis (Nahorski et al. 1991), or the induction of developmental alterations in many organisms (Klein and Melton 1996). In plants,  $\text{Li}^+$  inhibits the rhythmic movements of pulvini and petals (Birch 1991), disrupts normal pollen development inducing symmetrical mitoses in the microspores (Zonia and Tupy 1995a), and blocks pollen germination (Zonia and Tupy 1995b). Although inhibition of glycogen synthase kinase-3 $\beta$  has been proposed as a possible mechanism for the effect of lithium on development (Klein and Melton 1996), the most widely accepted model for lithium action is the “inositol depletion hypothesis” (Berridge 1993), which is based on the inhibition of inositol monophosphatases by  $\text{Li}^+$ , leading to depletion of cellular inositol and a rundown of the inositol cycle and calcium signalling. Plant inositol monophosphatases are  $\text{Li}^+$ -sensitive (Gillaspy et al. 1995) and the effects of  $\text{Li}^+$  on plants have also been interpreted in terms of inhibition of the inositol cycle and calcium signalling (Zonia and Tupy 1995a, 1995b; Liang et al. 1996).

$\text{Li}^+$  has also been utilised as an analogue of  $\text{Na}^+$  for salt toxicity studies in yeast (Serrano et al. 1999).  $\text{Li}^+$  seems to share with  $\text{Na}^+$  some cellular targets such as the Hal2 nucleotidase (Murguía et al. 1996) and inositol monophosphatase (López et al. 1999) but, as it is much more toxic, it is used at lower concentrations which pose no osmotic problem.  $\text{Na}^+$  is inhibitory at higher concentrations and then there is both osmotic stress and cation toxicity, a fact that complicates the interpretation of salt-stress experiments.

In the course of studies to evaluate the salt tolerance of tobacco plants we have also used  $\text{Li}^+$  as a more toxic analogue of  $\text{Na}^+$  and made the fortuitous observation that  $\text{Li}^+$  treatment produces morphological alterations (leaf curling and necrotic spots) similar to those produced

during a plant's defence reactions in incompatible (avirulent) pathogen interactions, the so-called hypersensitive response or HR (Greenberg et al. 1994; Dangi and Jones 2001). As  $\text{Li}^+$  is widely employed in physiological studies to probe the role of the inositol cycle in different phenomena (Birch 1991; Berridge 1993), we have characterised these novel effects of  $\text{Li}^+$  in molecular terms. Our results suggest that lithium triggers an HR-like reaction with production of the same defence molecules that are induced by pathogens.

## Materials and methods

### Plant growth and lithium treatments

Seeds from wild-type tobacco (*Nicotiana tabacum* L.) plants, cv. Petit Havana SR1, Xanthi or Samsun, or from transgenic Samsun plants overexpressing a bacterial hydroxylase (*nahG* gene; Gaffney et al. 1993), kindly provided by Dr. Jonathan D.G. Jones (John Innes Centre, Norwich, UK), were used in the experiments. Seeds were sown in pots with a 1:1 mix of peat and vermiculite and bottom-watered periodically with nutrient solution, containing macronutrients [5 mM  $\text{KNO}_3$ , 2.5 mM  $\text{K}_3\text{PO}_4$ , 2 mM  $\text{MgSO}_4$ , 2 mM  $\text{Ca}(\text{NO}_3)_2$ , 0.036% (w/v) EDTA- $\text{FeSO}_4$ ] and micronutrients (70  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 14  $\mu\text{M}$   $\text{MnCl}_2$ , 0.5  $\mu\text{M}$   $\text{CuSO}_4$ , 1  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.2  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$ , 10  $\mu\text{M}$   $\text{NaCl}$ , 0.01  $\mu\text{M}$   $\text{CoCl}_2$ ). Approximately 4 weeks later, plantlets, grown at least to the four-leaf stage, were individually transferred to pots with vermiculite, which were maintained wet throughout the course of the experiments; after another 4 weeks, treatments were initiated by supplementing the nutrient solution with  $\text{LiCl}$  at different final concentrations. At the times indicated in the corresponding figures, leaf material was collected, weighed and used for measurements of cations, phenolic compounds or ethylene, or for RNA preparation, as described below. The figures show representative experiments, which were repeated at least twice with similar results.

### Aminoethoxyvinylglycine (AVG) treatment

*NahG* transgenic tobacco plants were watered with 30 mM  $\text{LiCl}$  in nutrient solution, or with nutrient solution alone. After 24 h, a solution containing 0.1 mM AVG was applied with a brush onto the surface of selected leaves from salt-treated and non-treated plants. Those leaves, and control leaves (not painted with AVG) from the same plants, were detached at different times to determine ethylene production, and for RNA preparation.

### Cation determinations

Leaf material (0.1 g FW), frozen in liquid nitrogen, was ground in a mortar until a fine powder was obtained, extracted with 0.1 N perchloric acid, and boiled for 10 min at 95 °C. Cell debris were removed by centrifugation for 10 min at 14,000 g, the supernatant was collected, cooled on ice and diluted 40-fold with sterile water; finally, a 100- $\mu\text{l}$  sample of this dilution was subjected to HPLC analysis in a chromatograph (Waters) with an IC-PAK CM/D column and a Waters 432 conductivity detector. Elution was made in an isocratic flux, using as a mobile phase 3 mM  $\text{HNO}_3$  containing 0.1 mM EDTA. Sample analysis and preparation of lithium ( $\text{LiOH}$ ) and potassium ( $\text{KCl}$ ) standards were performed as described by the manufacturer.

### Analysis of phenolic compounds

Free salicylic acid (SA) was extracted as previously described (Raskin et al. 1989), with some modifications. A 0.5-g sample of

leaf material was ground with a pestle in a mortar, in the presence of liquid nitrogen, and extracted with 1.5 ml of 90% methanol. The extract was then sonicated for 15 min and cell debris was removed by centrifugation at 14,000 g for 15 min. The supernatant was dried in 5-ml glass tubes at 40 °C, under nitrogen. The dried residue was dissolved in 1 ml of 5% (w/v) perchloric acid, and the sample was centrifuged again for 15 min at 14,000 g. The supernatant was extracted with 2.5 ml of cyclopentane/ethyl acetate (1:1, v/v), and the organic upper phase was collected and dried at 40 °C under nitrogen. The residue was finally resuspended in 200  $\mu\text{l}$  of methanol and filtered through a 0.45- $\mu\text{m}$  nylon filter (Waters).

Extraction of total salicylic or gentisic acids was carried out following the protocol described by Enyedi et al. (1992). After methanol extraction of the sample, as indicated above, the residue was dissolved in 4 M HCl (instead of in perchloric acid) and hydrolysed at 80 °C for 1 h. The hydrolysis mixture was then centrifuged at 14,000 g for 15 min, the supernatant was extracted with cyclopentane/ethyl acetate as indicated before for free SA, and the final residue was also resuspended in 200  $\mu\text{l}$  of methanol.

Salicylic and gentisic acids were analysed by HPLC following previous protocols, described in Bellés et al. (1999). Briefly, a 40- $\mu\text{l}$  aliquot from the final methanolic sample was injected into a Symmetry 5  $\mu\text{m}$  C18 column (4.6 $\times$ 150 mm; Waters), equilibrated in 1% (v/v) acetic acid. A linear gradient of methanol (0 to 100%) was applied over 20 min. The temperature of the oven was 30 °C. Salicylic and gentisic acids were detected with a Waters 470 fluorescence detector and quantified with Waters Millennium<sup>32</sup> software, using authentic standards.

### Analysis of ethylene

Approximately 1 g of leaf material was incubated at room temperature in a closed vial for 2 h. A 100- $\mu\text{l}$  sample of the gas in the vial was collected with a syringe and injected into a gas chromatograph (Pelkin Elmer, model 3920). The amount of ethylene in the sample was calculated from an ethylene standard curve.

### Northern blot analysis

Total RNA (20  $\mu\text{g}$ ), prepared from tobacco leaves essentially as described in Kiedrowsky et al. (1992), was separated by electrophoresis through formaldehyde-containing agarose gels and transferred to nylon membranes (Hybond N; Amersham) using standard protocols (Sambrook et al. 1989). Blots were hybridised in high-stringency SDS buffer (Church and Gilbert 1984) with dsDNA,  $\alpha$ -[<sup>32</sup>P]dCTP-labelled probes specific for tobacco PR1, PR5 and PR-P genes. These probes were synthesised by random-priming from templates prepared by PCR amplification of tobacco DNA with gene-specific primers.

### Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA (0.5  $\mu\text{g}$ ), isolated from tobacco leaves with TRIZOL reagent (Gibco-BRL) as recommended by the manufacturer, was digested with RNase-free DNase I, incubated at 65 °C for 10 min to inactivate the enzyme, and reverse-transcribed for 2 h at 37 °C with M-MuLV RTase, using a 3' oligonucleotide primer specific for the tobacco PR5 gene; control reactions without RTase were done in parallel. One-fifth of the reaction mixtures was then subjected to 27 cycles of standard PCR amplification with 5' and 3' PR5-specific primers. Reaction conditions, regarding dilution of RNA samples and number of PCR cycles, were previously optimised to ensure that the amplification reactions were stopped in the exponential phase of the PCR, so that the assay could be considered as semi-quantitative.

## Results

### Lithium treatment induces necrotic spots in tobacco

Treatment of tobacco plants with 50 mM LiCl progressively induced leaf curling and the appearance of necrotic spots in the leaves (Fig. 1a–f). These alterations were more obvious in older leaves, as expected from preferential Li<sup>+</sup> accumulation in those leaves. A similar effect was observed using lower LiCl concentrations (30 or 15 mM), although the formation of necrotic lesions was proportionally delayed (Fig. 1g, h). Li<sup>+</sup> also caused an overall, concentration-dependent inhibition of plant growth (Fig. 1g, h). These effects of lithium on tobacco were not cultivar-dependent; necrotic spots were observed in cv. Xanthi (Fig. 1), as well as in Samsun (see below) and Petit Havana SR1 (not shown) plants.

The appearance of morphological symptoms correlated with the accumulation of Li<sup>+</sup> by the leaves (Fig. 2a). Apparently, necrotic spots appear first when the Li<sup>+</sup> concentration in the leaves reaches 5–10 μmol/g FW, which corresponded to about 6 days for the 50 mM

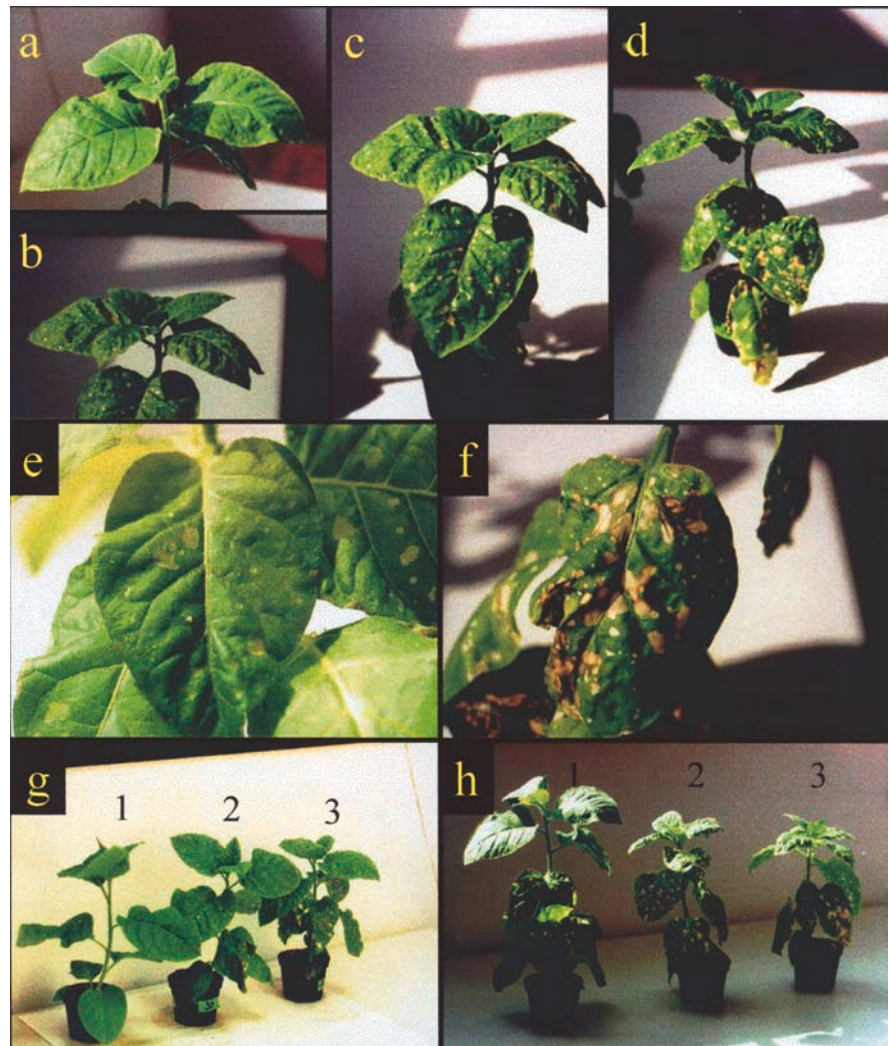
LiCl treatments, but more than 2 weeks when the plants were watered with 15 mM LiCl (Figs. 1, 2a). From then on, the number and size of the necrotic spots, and therefore the total necrotised surface, increased more or less in parallel with Li<sup>+</sup> accumulation.

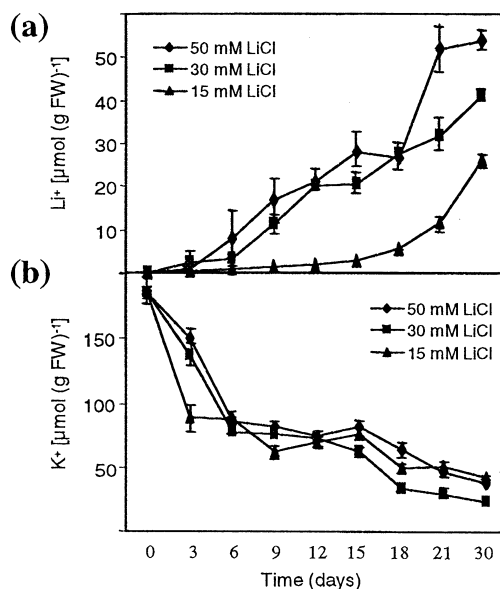
It is interesting to note that LiCl treatments also caused a rapid depletion of K<sup>+</sup> from the leaves (Fig. 2b). This decrease in potassium levels, however, was similar in plants treated with 15, 30 or 50 mM LiCl, and therefore this possible nutritional deficiency (Marschner 1995) does not correlate with the intensity of Li<sup>+</sup>-induced symptoms, which was greater at higher LiCl concentrations.

### Lithium activates pathogenesis-related (PR) genes

Necrotic spots are typical of incompatible (avirulent) plant–pathogen interactions (Greenberg et al. 1994; Dangl and Jones 2001), such as for example tobacco infection by tobacco mosaic virus (TMV) at 22 °C (Ward et al. 1991; Guo et al. 2000). The spots induced by TMV are smaller and more regular in shape and

**Fig. 1a–h** Induction by LiCl of leaf curling, necrotic spots, and overall inhibition of growth in tobacco (*Nicotiana tabacum*). **a–d** Time-course of the appearance of symptoms; plants were treated with 50 mM LiCl for 6 (**a**), 9 (**b**), 15 (**c**) or 30 (**d**) days. **e, f** Close-ups of leaves from plants treated for 9 (**e**) or 30 (**f**) days with 50 mM LiCl. **g, h** Concentration-dependence of the intensity of morphological symptoms and of growth inhibition; plants were treated with 15 (**1**), 30 (**2**) or 50 mM (**3**) LiCl, for 15 (**g**) or 30 (**h**) days. Untreated controls did not develop any of the symptoms





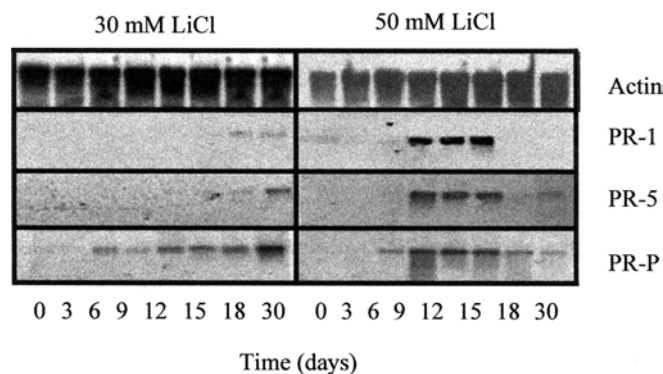
**Fig. 2a, b** Variation in cation levels during LiCl treatments. The graphs show Li<sup>+</sup>-accumulation (a) and K<sup>+</sup>-depletion (b) kinetics in leaves of tobacco plants treated with 15, 30 or 50 mM LiCl, as indicated. Values are the means of three independent samples (taken from different plants) per treatment. Standard deviations are indicated by vertical bars

size than those induced by Li<sup>+</sup> (compare Fig. 1 of Ward et al. 1991, with Fig. 1 of the present work). Nevertheless, the gross similarities between Li<sup>+</sup>-treated and pathogen-attacked plants prompted us to investigate the possible induction by lithium of several molecular markers typical of responses of plants to pathogens.

One important aspect of plant responses to pathogens is the induced expression of a series of PR genes encoding defence proteins (Ward et al. 1991; Maleck and Dietrich 1999; Guo et al. 2000). Expression of PR-proteins correlates with systemic acquired resistance (SAR) to pathogens and seems to be triggered by pathogen-induced localised necrosis or HR (Sticher et al. 1997; Maleck and Dietrich 1999). As shown in Fig. 3, Li<sup>+</sup> treatment activates three typical PR genes: PR1 (Payne et al. 1988a), PR5 (Payne et al. 1988b) and PR-P (Payne et al. 1990), encoding defence proteins with antifungal activities (Sticher et al. 1997). Induction followed the time course of necrosis and Li<sup>+</sup> accumulation (Figs. 1, 2), and maximum levels of the transcripts were detected at 9–15 days in plants treated with 50 mM LiCl, or after 30 days of treatment with 30 mM LiCl (Fig. 3).

#### Lithium stimulates production of ethylene and of salicylic and gentisic acids

Leaf curling, observed in tobacco plants treated with LiCl (Fig. 1) may reflect ethylene production (Boller 1990; Conejero et al. 1990) and this is one of the pathways triggered by pathogens. Actually, some plant defence genes are regulated by ethylene (Ecker and Davis



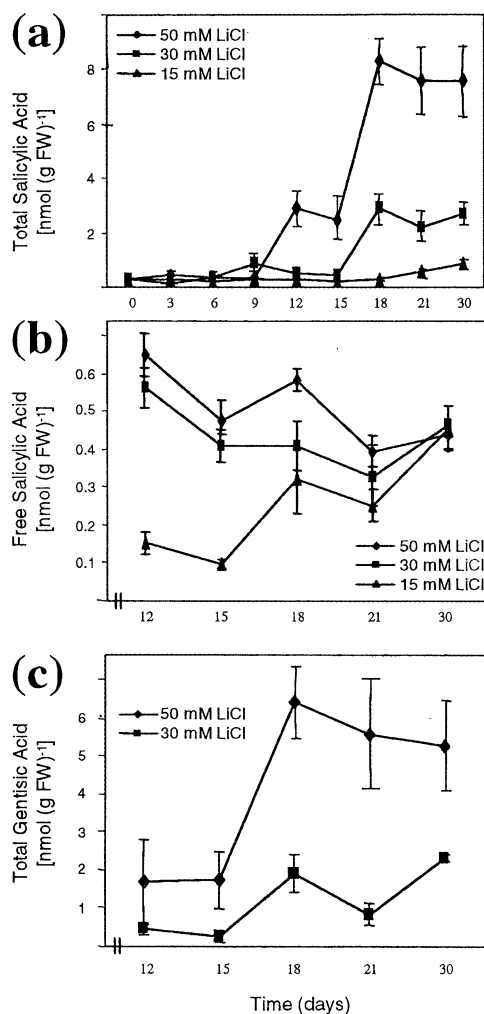
**Fig. 3** Activation of tobacco PR genes by lithium. Northern blot analysis of total RNA (20 μg per lane), isolated from leaves of tobacco plants watered for the indicated times with nutrient solution containing 30 or 50 mM LiCl. Blots were hybridized with <sup>32</sup>P-labeled double-stranded-DNA probes specific for tobacco PR-1, PR-5, PR-P, or (as control) actin genes. Untreated controls did not activate any of the genes

1987; Sticher et al. 1997). Li<sup>+</sup> has already been reported to increase ethylene production through induction of 1-aminocyclopropane-1-carboxylate synthase genes (Boller 1984; Liang et al. 1996). We have corroborated that Li<sup>+</sup> also increased ethylene in our experiments, and that its production in leaves was sensitive to the 1-aminocyclopropane-1-carboxylate synthase inhibitor AVG (see below, Fig. 7).

Another pathway triggered by pathogens involves SA, a phenolic compound which is essential for the induction of many PRs and for the overall SAR response (Gaffney et al. 1993; Delaney et al. 1994; Sticher et al. 1997; Maleck and Dietrich 1999). More recently, gentisic acid has been identified as an additional phenolic compound that may also be important for PR induction (Bellés et al. 1999). As indicated in Fig. 4, LiCl treatment induced the accumulation of both salicylic and gentisic acids, also in a concentration-dependent manner. However, total (including their conjugated forms) salicylic and gentisic acid accumulation (Fig. 4a, c) appeared to be somehow delayed with respect to the formation of necrotic lesions (Fig. 1) and to Li<sup>+</sup> accumulation (Fig. 2a), so that only after 12 days of treatment with 50 mM LiCl (or longer periods for lower concentrations) was a significant increase in the concentration of these compounds observed. In the case of salicylate, the free form was also measured (Fig. 4b); its levels in leaves were maintained below 0.6 nmol/g FW over the course of the experiment, and did not increase in parallel to those of total SA in the corresponding samples, which reached up to 8 nmol/g FW (Fig. 4a), indicating the rapid and efficient conversion of free SA into conjugated forms.

#### Salicylate is not required for the observed lithium effects

Our results indicate that Li<sup>+</sup> treatment not only induces leaf curling and necrotic spots as occurs in typical



**Fig. 4a–c** Induction by LiCl of phenolic compounds in tobacco leaves. Kinetics of accumulation of total SA (a), free SA (b) and total gentisic acid (c) in plants treated with the indicated salt concentrations. Values are the means of three independent samples (taken from different plants) per treatment. Standard deviations are indicated by vertical bars. Untreated controls did not induce any of the phenolic compounds

HR/SAR responses to pathogens, but also increases the production of several molecular markers of these responses such as ethylene, gentisic acid, salicylate and PR mRNAs. The central role of salicylate in SAR responses has been probed with transgenic plants expressing a bacterial hydroxylase (*nahG* gene) that converts salicylate into catechol (Gaffney et al. 1993; Delaney et al. 1994). These plants do not accumulate salicylate, do not develop SAR and are more susceptible to viral, fungal and bacterial pathogens.

We have tested the effect of Li<sup>+</sup> in *nahG* tobacco plants (cv. Samsun). After 2 weeks of treatment with 50 mM LiCl, as expected, the total levels of salicylic or gentisic acids in the leaves of these transgenics did not increase, as compared to non-treated *nahG* plants, while a 3-fold increase in both compounds was detected in the corresponding wild-type controls (Table 1). However, as



**Fig. 5** Ethylene-dependent formation of necrotic spots upon lithium treatment of transgenic tobacco plants that do not accumulate SA. The figure shows a *nahG* tobacco plant treated with 30 mM LiCl for 6 days. At 24 h after the initiation of the salt treatment, the ethylene biosynthesis inhibitor AVG was applied with a brush to the leaf marked with an arrow, which did not develop necrotic spots

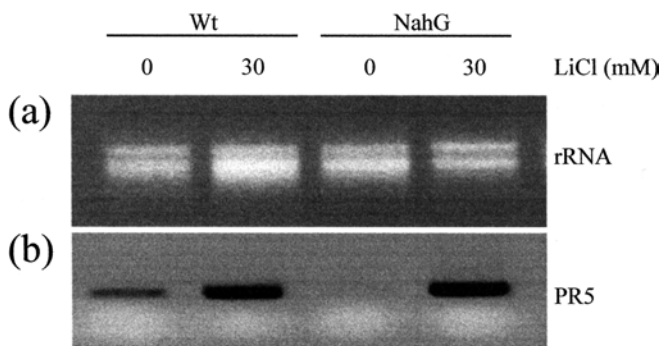
**Table 1** Effect of LiCl treatment on the accumulation of Li<sup>+</sup>, and of total salicylic and gentisic acids in leaves of wild-type (*wt*) and *nahG* tobacco (*Nicotiana glauca*) plants. Tobacco plants (cv. Samsun) were watered with nutrient solution, with or without 50 mM LiCl, for 14 days. Values are the means of two independent samples per treatment, taken from two different plants and differing in less than 10%. n.d. Not detectable

	LiCl	Li <sup>+</sup> (μmol/g FW)	Salicylic acid (nmol/g FW)	Gentisic acid (nmol/g FW)
wt	–	n.d.	0.62	0.41
	+	55.10	1.86	1.23
<i>nahG</i>	–	n.d.	0.39	0.26
	+	31.66	0.32	0.21

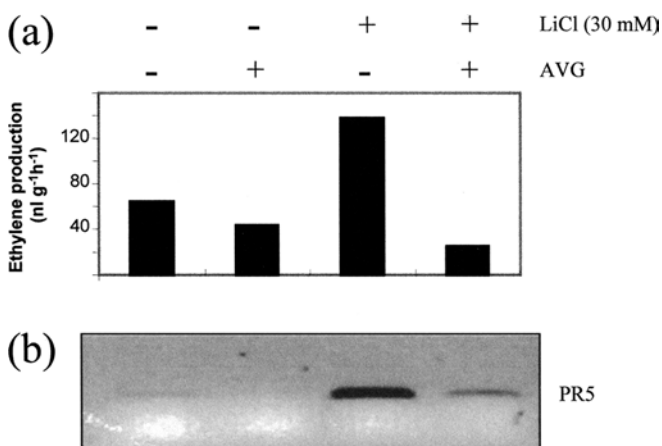
shown in Fig. 5, lithium still induced the formation of necrotic spots as in wild-type tobacco. The induction of PR1, which is known to be salicylate-dependent (Guo et al. 2000), was not observed (data not shown) but induction of PR5 by Li<sup>+</sup> was normal in both wild-type and *nahG* plants (Fig. 6). Therefore salicylate is only required for PR1 induction but not for the morphological alterations observed in the presence of lithium nor for the induction of PR5.

#### Ethylene is essential for lithium action

We have probed the role of ethylene in Li<sup>+</sup> action by using the ethylene biosynthesis inhibitor AVG. Painting a leaf of a LiCl-treated plant with a solution containing



**Fig. 6a, b** Induction by lithium of PR5 expression in wild-type (*Wt*) and transgenic *nahG* tobacco plants. Total RNA was prepared from leaves of plants treated for 6 days with 30 mM LiCl, or from non-treated controls, and the levels of the PR5 transcript in each sample were analysed by RT-PCR (b). Panel a shows an ethidium bromide-stained agarose gel of the corresponding RNA samples



**Fig. 7a, b** Ethylene is required for lithium induction of the PR5 gene in transgenic *nahG* tobacco plants. a Inhibition of basal and lithium-activated ethylene production in the presence of AVG. b Inhibition of the induction of PR5 expression by lithium in the presence of AVG. Levels of PR5 transcripts were analysed by RT-PCR. Salt treatment was performed as in Fig. 6

AVG greatly prevented the appearance of necrotic spots (Fig. 5), inhibited ethylene production (Fig. 7a) and partially blocked the induction of PR5 (Fig. 7b) in that leaf. AVG also reduced ethylene production in non-treated plants (Fig. 7a), as well as reducing the (already very low) non-induced levels of the PR5 transcript (Fig. 7b). These results strongly suggest that Li<sup>+</sup>-induced ethylene production is an essential component of Li<sup>+</sup> action. *NahG* transgenic tobacco was again used for these experiments, to exclude the possibility of both ethylene and salicylate inducing similar symptoms by independent mechanisms.

## Discussion

A conspicuous morphological alteration during plant responses to pathogens is a localised necrosis resulting

from recognition of the pathogen by the host in an incompatible interaction (programmed cell death or HR reaction; Greenberg et al. 1994; Dangl and Jones 2001). Cell death is somehow connected to accumulation of phenolic compounds such as SA, which has been demonstrated to mediate the local and systemic (SAR) induction of PR genes encoding defence proteins, and the induced resistance to pathogens (Sticher et al. 1997; Maleck and Dietrich 1999). More recently, another phenolic compound, gentisic acid, has been shown to accumulate during pathogen attack and to activate some PR genes in tomato (Bellés et al. 1999). Ethylene is also produced upon infection by pathogens and it can induce some of the PR genes (Ecker and Davis 1987; Boller 1990; Conejero et al. 1990), although it is usually considered to play a role secondary to that of salicylate (Lawton et al. 1994; Sticher et al. 1997).

A hypothesis to explain the effects of lithium described in the present work is that this toxic cation triggers an HR-like reaction. The ensuing production of ethylene and phenolic compounds would explain the induction of PR genes. These effects of lithium resemble the phenotype of lesion mimic mutants (Dietrich et al. 1994) and of transgenic plants with alterations in either the ubiquitin system (Becker et al. 1993), proton transport (Mittler et al. 1995) or sugar metabolism (Herbers et al. 1996; Tadege et al. 1998).

The mechanism of the HR reaction is not completely understood. After recognition of pathogen molecules (elicitors or avirulence determinants) by plasma membrane receptors there is a non-completely defined signal transduction involving G proteins, calcium and pH changes, and protein kinases and phosphatases. This leads to activation of a plasma membrane NADPH oxidase, which generates toxic superoxide radicals and hydrogen peroxide, which are assumed to trigger the HR response (Alvarez et al. 1998; Blumwald et al. 1998).

It is difficult to predict how lithium could interact with pathogen signalling. Lithium accumulates in leaves of treated plants, and inhibition of known targets of this cation, such as inositol monophosphatases (Gillaspy et al. 1995), Hal2-like nucleotidases (Murguía et al. 1996; Gil-Mascarell et al. 1999), and/or rRNA-processing enzymes (Dichtl et al. 1997) could be involved. Inhibition of inositol monophosphatases would affect the inositol cycle and calcium signalling (Nahorski et al. 1991; Liang et al. 1996), while inhibition of Hal2-like nucleotidases would affect sulphotransferase reactions and rRNA processing (Dichtl et al. 1997; Gil-Mascarell et al. 1999). Recently, we have provided evidence that pre-mRNA splicing is also a target of lithium (and sodium) toxicity in eukaryotic cells (Forment et al. 2002).

Plants activate genetic programs for cellular suicide under a variety of circumstances, such as organ senescence, tracheary element differentiation, seed development and pathogen invasion (the HR response; Beers and McDowell 2001). It has been argued that alteration of cellular homeostasis by either mutation or expression of different transgenes may be misinterpreted by host

cells as pathogen infection (Dietrich et al. 1994; Mittler et al. 1995; Tadege et al. 1998). We could assume that lithium also causes biochemical alterations that trigger the HR response. The elucidation of these alterations may throw light not only into the mechanism of lithium action in the cell, but also into important signalling pathways controlling programmed cell death and HR–SAR responses in plants. One aspect of lithium action is that the induction of necrotic spots seems to be mediated by ethylene, although this compound is usually considered to play a secondary role in the SAR response (Lawton et al. 1994; Sticher et al. 1997). Other conditions that have been reported to induce necrotic spots are toxic concentrations of manganese and of boron, and deficiency of magnesium, calcium, manganese, boron or copper (Marschner 1995). It would be interesting to investigate if these symptoms also correspond to an HR-like response with production of salicylate, ethylene and PRs as in the case of lithium. Treatment with NaCl (100–150 mM), although inhibitory for plant growth, did not produce the morphological alterations of leaves induced by lithium, although it has been reported to induce programmed cell death in root meristems (Huh et al. 2002).

**Acknowledgements** We thank Dr. Jonathan D.G. Jones (John Innes Centre, Norwich, UK) for providing seeds of the transgenic *nahG* tobacco plants. This work was supported by grants from the Conselleria de Agricultura, Pesca y Alimentación (Generalitat Valenciana, Valencia, Spain) to R.S., and from the Spanish Ministerio de Ciencia y Tecnología (Madrid, Spain, project BMC2000-1130) to O.V. C.R. was a fellow of the Spanish Ministerio de Educación y Cultura (Madrid, Spain). We thank V. Conejero, Jose L. Vaya and F.A. Culiñez-Macia for their help at different stages of the work.

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