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Characterization of three hormone mutants of *Nicotiana plumbaginifolia*: evidence for a common ABA deficiency

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Summary. Various auxin-resistant *Nicotiana plumbaginifolia* mutants have already been isolated, including I217 which shows cross-resistance to paclobutrazol. Recently, a cytokinin-resistant mutant, CKR1, has been characterized and has been shown to be affected in abscisic acid (ABA) biosynthesis. We have isolated a new mutant, Esg152, which was selected on the basis of its early germination. In each of these mutants, resistance is due to a recessive nuclear mutation at a single locus. Complementation analysis indicated that mutants I217, CKR1 and Esg152 belong to the same complementation group. They have a similar phenotype, which includes a reduction in seed dormancy and an increased tendency to wilt. These mutants display an increased auxin tolerance and enhanced root formation when leaf or hypocotyl sections are cultivated on auxin. By immunoenzymatic methods, we show that the endogenous levels of ABA are significantly lower than in the wild-type. We have assigned the symbol *aba1* to the recessive alleles of the locus affected in the three mutants. The complexity of hormonal interactions is discussed briefly emerging from a consideration of this class of mutants.

Key words: *Nicotiana plumbaginifolia* – Abscisic acid – Auxin resistance – Plant hormone – Wilty mutant

Introduction

Plant mutants with altered responses to exogenously applied plant hormones can be used to study hormone-mediated processes to identify hormone receptors and components of signal transduction pathways (Reid 1990), and to study hormone metabolism. Various auxin-resistant *Nicotiana plumbaginifolia* mutants have already been isolated (Bitoun et al. 1990; Blonstein et al. 1991 b). One of these, mutant I217, was found to be cross-resistant to abscisic acid (ABA) and paclobutrazol (Bitoun et al. 1990). Recently, a cytokinin-resistant mutant, CKR1, was isolated (Blonstein et al. 1991 a) and found to be affected in ABA biosynthesis (Parry et al. 1991). A third mutant, Esg152, was isolated, as described in this paper, from ethyl methane sulfonate (EMS)-mutagenised seeds on the basis of its early and synchronous germination. Despite apparently different phenotypes, the three mutants were found to be alleles of the same genetic locus. This observation suggested that further characterization of the mutants I217, CKR1 and Esg152 was necessary.

Many wilty mutants of higher plants have been isolated. ABA deficiency is one of the causes of wiltiness (Quarrie 1987). Some of these mutants are primarily defective in carotenoid biosynthesis, as is the ABA-deficient mutant *aba* of *Arabidopsis thaliana* (Rock and Zeevaart 1991). Other mutants do not accumulate ABA and are unable to close their stomata in response to water deficit, as is the case with the *notabilis*, *flacca* and *sitiens* mutants of tomato (Tal and Nevo 1973) or the *droopy* mutant of potato (Quarrie 1982). These mutants are also deficient in ABA and have an abnormal stomatal behavior. Parry et al. (1991) showed that the mutant CKR1 of *N. plumbaginifolia* belongs to this type.

The present paper describes the further characterization of the three ABA-deficient mutants I217, CKR1 and Esg152 of *N. plumbaginifolia*. The genetic relationship of these mutations, all conferring a wilty phenotype, reduced dormancy and resistance to auxin, was established. The endogenous levels of ABA were determined, and the physiological effects of auxin application were studied. The modification of ABA biosynthesis is discussed in the light of the observed phenotypic alterations.

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Materials and methods

Chemicals

ABA, 1-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA) and gibberellic acid were purchased from Sigma. Paclobutrazol was a gift from Sopra. The hormones were dissolved in either ethanol or dimethyl sulphoxide (DMSO). The ethanol or DMSO added to the medium after autoclaving had no effect on either germination or seedling growth.

Plant material

The seeds and plants of *N. plumbaginifolia* cv. Viviani used throughout these studies were derived from the haplo-diploidized strain PbH1D (Bourgin et al. 1979). Plant growth studies were performed according to Bitoun et al. (1990). The mutants I217, CKR1 and Esg152 were isolated from EMS-mutagenised seeds. The analyses were carried out with mutants reselected after backcrossing to the wild-type. The transpiration rate of detached leaves was measured by exposing excised leaves to a stream of air and weighing at intervals. The water-stress protocol adopted was similar to that of Parry et al. (1991). Water-stress experiments were continued until the leaves had lost 10% of their initial fresh weight.

Mutagenic treatment and mutant selection

Mutagenic treatment and mutant selection were performed according to the established strategy for the isolation of auxin-resistant mutants (Bitoun et al. 1990), cytokinin-resistant mutants (Jullien et al. 1991), ethanol-resistant mutants (Rousselin et al. 1990), and nitrate reductase mutants (Pelsy et al. 1991), from EMS-mutagenised seeds of diploid plants of *N. plumbaginifolia* PbH1D. Mutant Esg152 was isolated by this same procedure in a subsequent experiment. The seeds were mutagenized by soaking in distilled water containing 0.5% EMS (Pelsy et al. 1991). Screening of M2 progeny of 5,000 EMS-mutagenised seeds resulted in the isolation of the mutant Esg152, which exhibits abnormal germination and growth.

Selection and genetic analysis were performed with surface-sterilized seeds plated on B medium (Bourgin et al. 1979) and incubated for 2–3 weeks in culture chambers with defined temperature and light conditions (Gabard et al. 1987).

Dose-response tests

To quantify the response of mutants and wild-type to several hormones, seeds were plated on B medium supplemented with various concentrations of hormone. The state of germination was scored after 2 weeks and the phenotype was recorded after 3 weeks, with the exception of experiments directed to germination kinetics.

In-vivo test of the sensitivity to ABA

ABA treatment was performed on plants in the greenhouse with a solution of 40 µM ABA and 0.01% tween 80. ABA was applied to the mutant plants by foliar spray (approximately 5 ml per plant per day).

Protoplast, cell and tissue cultures

The preparation of mesophyll protoplasts and their culture at low cell density were performed according to Muller et al. (1983). The effects of various paclobutrazol or ABA concentrations on the plating efficiency were recorded after a 6-week incubation period.

Assay of root neogenesis from excised plant segments

Explants for the hypocotyl assay were obtained by germinating seeds, pretreated with gibberellic acid (GA_3), in the dark. This procedure resulted in an elongation of the hypocotyls before excision. Explants were then placed on medium containing various concentrations of NAA. The test was performed either on leaf discs (1 cm) or petioles from in-vitro plants.

Extraction, purification and ELISA assay of plant growth substances

This study was performed with either leaves or seeds depending upon the experiment. The tissues were frozen in liquid N_2 and lyophilized prior to grinding into powder.

Extraction, purification and fractionation were performed according to the procedure described by Pelese et al. (1989). The solid phase-based ELISA procedure used for the detection of ABA, auxin and cytokinin, has already been described (Maldiney et al. 1986; Pelese et al. 1989). The competition step for ABA analysis was performed using a monoclonal anti-ABA antibody purchased from Phytoscience (Angers, France).

Results

Mutant isolation and phenotypes

Seeds were mutagenized with 0.5% EMS (Pelsy et al. 1991). EMS treatment resulted in 97% fertile plants and 2% albino chimeras in the M1 generation. Abnormal germination on medium without hormones was chosen as the criterion on which to screen the 5,000 M2 families. One mutant, Esg152, had a strikingly early and synchronous germination relative to the wild-type. Seven days after sowing in hormone-free medium the wild-type had not started to germinate whereas Esg152 had already reached the 2-cotyledon stage (Fig. 1 A). The phenotype of the other two mutants germinated in vitro was similar to that of Esg152 (Fig. 1 A).

To study the phenotypes of the mutants, concentrations of hormones were used that are known to produce characteristic disturbances in the germination or development of young wild-type seedlings. In the case of I217, Bitoun et al. (1990) used concentrations of auxin which prevented germination of the wild-type. Under these conditions (150 µM IBA) germinated seedlings of the mutants I217, CKR1 and Esg152 are blocked at the cotyledon stage although they germinate properly (Fig. 1 B). In the presence of 10 µM 6-benzylaminopurine (BAP) root development in the mutant CKR1 was substantial whereas root and shoot growth of wild-type seedlings was blocked (Fig. 1 C). The other two mutations behaved like CKR1 in the presence of 10 µM BAP. The kinetics of germination of the three mutants, compared to the wild-type, clearly show a tendency towards precocious germination. The criterion used to assess germination capacity was radicle breakthrough and by this criterion all three mutants germinated precociously (Fig. 2).

In the greenhouse the mutant plants were much shorter than the wild-type, had abundant branching at

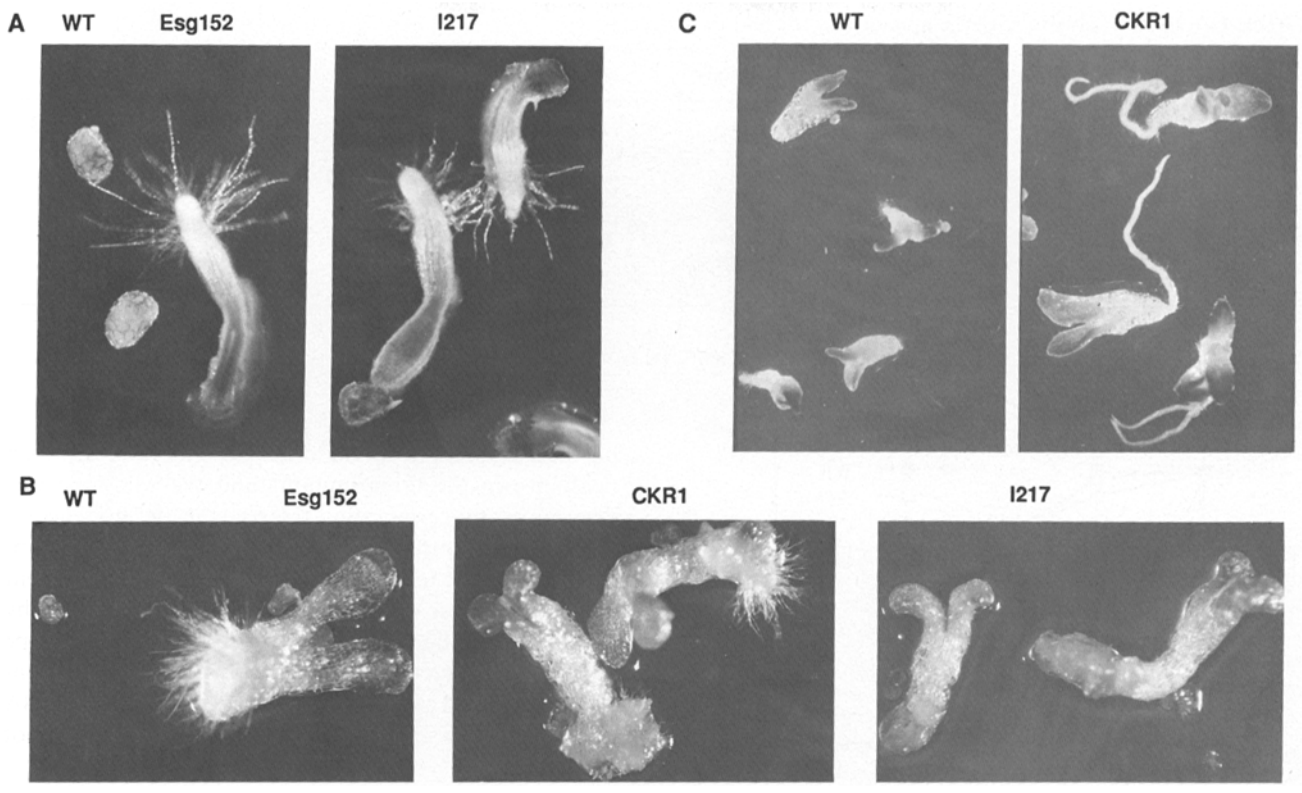

Fig. 1A–C. The phenotype of the wilty mutants compared to the wild-type. **A** The early germination phenotype of the mutants Esg152 and I217 compared to the wild-type (*WT*) after 7 days on hormone-free medium. **B** The phenotype of wild-type (*WT*) and mutants I217, CKR1 and Esg152 seedlings after 21 days on 150 μM IBA. **C** The phenotype of wild-type (*WT*) and mutant CKR1 seeds after 21 days on 10 μM BAP. Roots of the mutant CKR1 grow on 10 μM BAP

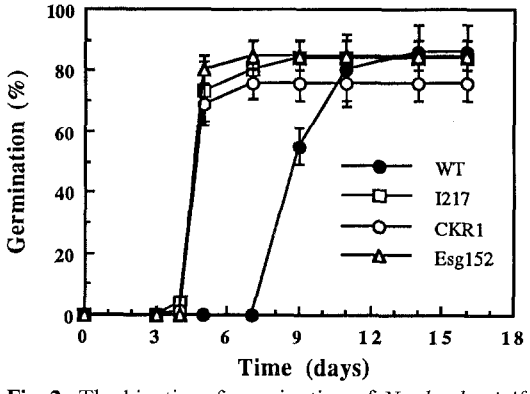

Fig. 2. The kinetics of germination of *N. plumbaginifolia* seeds Germination percentage of the wild-type (*WT*) and the mutants I217, CKR1 and Esg152 was scored in terms of radicle emergence

the crown (on the average four secondary stems; data not shown) and had less-rigid stems. Moreover, these plants were hypersensitive to water-stress and had a strong wilting tendency (Fig. 3). The wilty phenotype is typical of ABA-deficient mutants and is the consequence of the failure of stomatal closure during water-stress (Parry et al. 1991). The abundant branching and the reduced apical dominance are reminiscent of cytokinin overpro-

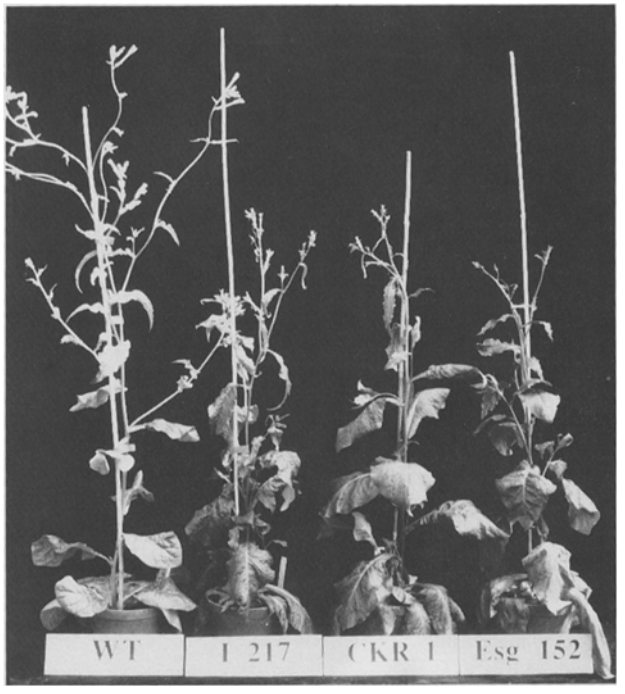

Fig. 3. Morphological characteristics of 3-month-old wild-type (*WT*) and mutant I217, CKR1 and Esg152 plants. These plants were germinated and grown in vitro, and then transferred to the greenhouse

Table 1. Results of crosses with the mutant Esg 152 and its progenies

Cross	Number of germinated seeds					
	Precocious	Normal	χ^2 1:3	Auxin^r	Auxin^s	χ^2 1:3
esg/esg	76	0	–	84	0	–
esg/esg × +/+	0	66	–	0	62	–
esg/+ × *esg/+*	23	75	0.12[b]	42	147	0.77[b]
esg/esg × *ckr1/ckr1*	64	0	–	73	0	–
esg/esg × *iba1/iba1*[a]	62	0	–	60	0	–

[a] The locus of mutant I217
[b] The observed ratio is not significantly different at the 5% level from the tested ratio

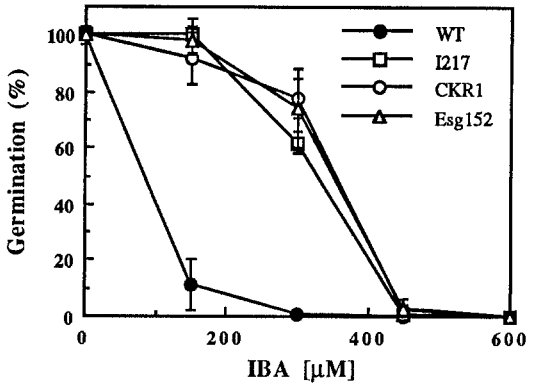

Fig. 4. The effect of exogenous IBA on the germination of *N. plumbaginifolia* seeds. The germination of the wild-type (*WT*) and the mutants I217, CKR1 and Esg152 was scored after 21 days of incubation and was based on radicle emergence

duction, and also of a deficiency in indole-3-acetic acid (IAA) content.

Genetic analysis of ABA-deficient mutants

In each of the three mutants, hormonal resistance, used as the criterion for isolation, was due to a recessive nuclear mutation at a single locus. The early and synchronous germination phenotype of mutant Esg152, as well as its auxin resistance (see below), were determined by a single recessive nuclear mutation (Table 1). Complementation analyses indicated that mutants I217, CKR1 and Esg152 are all alleles of the same genetic locus (Table 1). Because of the wilty phenotype and the ABA deficiency (see following pages) we have designated the locus affected by the mutation in all three mutants as *aba1*, in accordance with the terminology adopted for the ABA-deficient mutants of *A. thaliana* (Koornneef et al. 1982).

Resistance to hormones during seed germination

The *aba1* mutation confers a significant level of resistance to auxin and paclobutrazol at the seedling stage. We have compared the three different alleles of the *aba1* locus by testing the effect of several exogenous hormones on the germination of the mutants. The level of auxin resistance in the three mutants and the wild-type was determined by germinating the seeds in the presence of different concentrations of IBA. All three mutants appeared resistant to auxin (Fig. 4). Seedling development and root growth in the mutants were less inhibited by toxic cytokinin concentrations than that of the wild-type (Fig. 1 C). In agreement with the observations of Blonstein et al. (1991 a), the sensitivity of the mutant to ABA was found to be similar to that of the wild-type (data not shown).

Resistance to hormones at the cellular level

We had previously established that auxin resistance of both the mutant I217 and the wild-type is similar at the cellular level (Bitoun et al. 1990). In order to determine whether ABA and paclobutrazol resistance, as observed at germination (Bitoun et al. 1990), were similarly expressed at the cellular level, protoplast-derived cells were cultivated in the presence of various concentrations of each molecule. Protoplast-derived cells of I217 behaved in a manner similar to the wild-type. It was concluded that resistances were not expressed at the cellular level (Fig. 5).

Effect of auxin on the rhizogenesis of several explants

Auxin tolerance in the mutants was detected during seed germination. To study whether this tolerance was restricted to that stage of development where ABA plays a critical role we studied auxin tolerance in other tissues. We have tested the effect of exogenously applied auxin on the root forming capacity of a variety of explants, namely petioles (still attached to the leaf), leaf discs, and hypocotyls. We assumed that the observed effects were due solely to the exogenously applied auxin since the endogenous auxin concentrations were relatively similar in the mutants and wild-type (see below). The sensitivity of excised hypocotyl segments, petioles, and leaf discs to NAA during rhizogenesis and callus formation was determined in the wild-type and in the mutants I217, CKR1

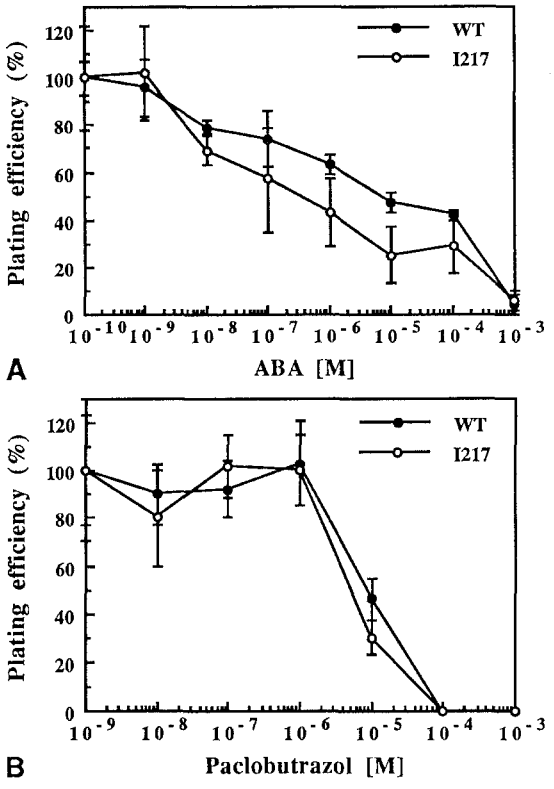

Fig. 5A, B. The effect of exogenous ABA (**A**) and paclobutrazo (**B**) on cell division of *N. plumbaginifolia* protoplasts. Averag values of plating efficiencies of wild-type and I217 cells ar compared

and Esg152 (Table 2). For instance, whereas excised wild type hypocotyls regenerated roots in the presence of con centrations no higher than 0.01 μM NAA, mutan hypocotyls still generated roots in the presence of 0.1 μM NAA (Table 2). Whereas only callus formation was obtained with wild-type petioles incubated in 1–10 μM NAA (Fig. 6), abundant root formation was observed in mutant I217 petioles under similar growth conditions (Fig. 6). We even obtained root formation on mutant leaf discs incubated in the presence of 10 μM NAA, a concentration far too high to promote root development in wild-type leaf explants (Table 2). The mutants were found to be up to ten-fold less-sensitive to auxin inhibition of root formation than the wild-type (Table 2). Together, these results suggest that the auxin tolerance of the mutants is not restricted to germination.

Biochemical studies

The phenotypes displayed by mutants I217, CKR1, and Esg152 suggested an ABA deficiency. Data relating to the level of this compound were available only for CKR1 (Parry et al. 1991). We therefore investigated the endogenous levels of abscisic acid, auxin and cytokinins in the

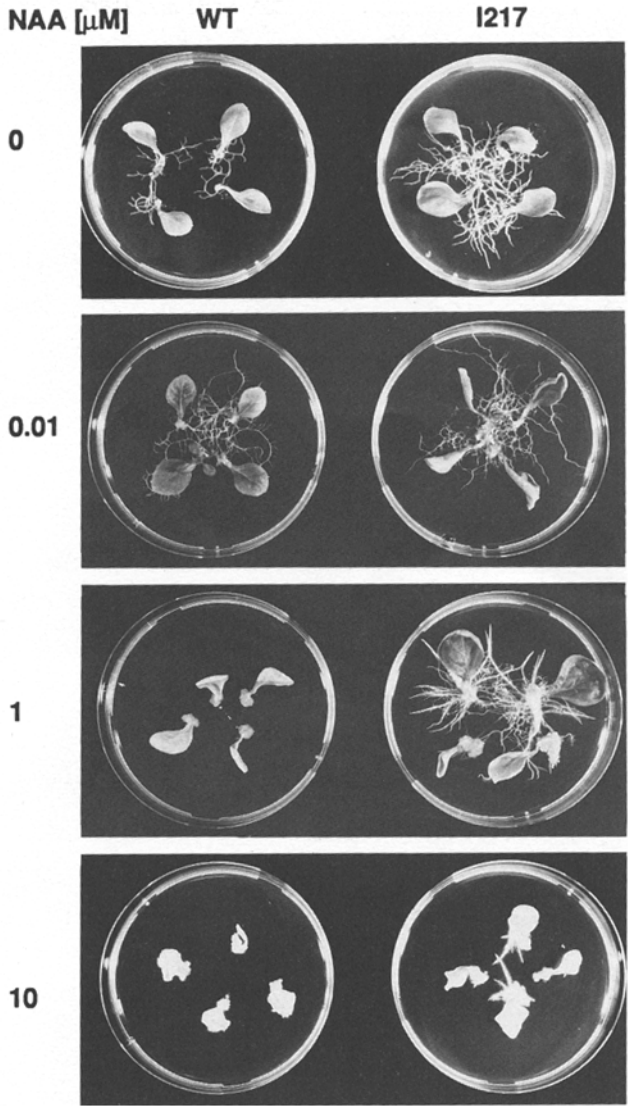

Fig. 6. Root formation on petioles of the wild-type (*WT*) and the mutant I217 in the presence of various concentations of NAA

Table 2. The effect of exogenously applied auxin on the root formation capacity of hypocotyl, petiole and leaf disc explants. The presence (+) or absence (−) of roots was observed after 21 days of incubation

Explants	NAA μM	0	0.01	0.1	1	10	100
Hypocotyls	WT	+	+	−	−	−	−
	I217	+	+	+	−	−	−
	CKR1	+	+	+	−	−	−
	Esg152	+	+	+	−	−	−
Petioles	WT	+	+	+	−	−	−
	I217	+	+	+	+	−	−
	CKR1	+	+	+	+	−	−
Leaf discs	WT	+	+	+	+	−	−
	I217	+	+	+	+	+	−
	CKR1	+	+	+	+	+	−
	Esg152	+	+	+	+	+	−

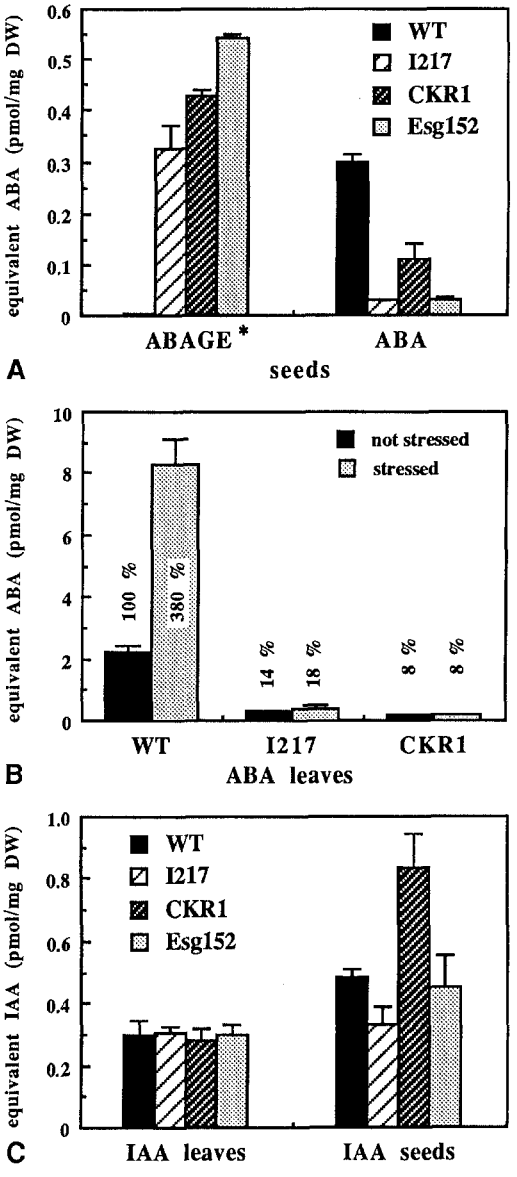

Fig. 7A–C. Hormonal concentrations in seeds and leaves of the wild-type (*WT*) and the three wilty mutants of *N. plumbaginifolia*. Results are an average value of five replicates. **A** Endogenous levels of ABA and ABAGE in seeds (*: ABAGE-like compounds) of the wild-type (*WT*) and the mutants I217, CKR1 and Esg152 of *N. plumbaginifolia*. **B** Levels of ABA in non-stressed and stressed leaves of the wild-type (*WT*) and the mutants I217 and CKR1. The values were expressed as a percentage of the non-stressed wild-type level. **C** Endogenous levels of IAA in leaves and seeds of the wild-type (*WT*) and the mutants I217, CKR1 and Esg152

three wilty mutants of *N. plumbaginifolia* and the wild-type. The hormonal concentrations were evaluated by an immunoenzymatic method, enzyme-linked immunosorbent assay (ELISA), following high performance liquid chromatography (HPLC). Measurements were made on leaves and seeds (Fig. 7). As expected the endogenous level of ABA was reduced in both seeds and leaves of the

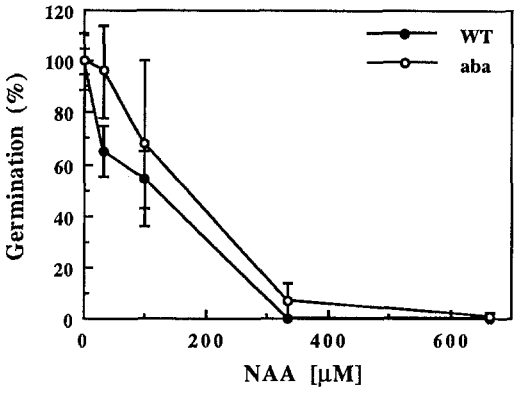

Fig. 8. The effect of exogenous NAA on germination of *A. thaliana* seeds. The germination of wild-type (*WT*) and mutant *aba* was scored after 6 days of incubation

mutants I217, CKR1 and Esg152 when compared to the wild-type. (Fig. 7A, B), suggesting that the reduced seed dormancy and the wilty leaf phenotype may depend on the low ABA level. The basal ABA level in seeds of the mutants was approx 10–30% that of the wild-type. In contrast, significantly high levels of immunoreactive substance(s) eluting at the β-D-glucopyranosyl abscisate (ABAGE) retention time was (were) found to accumulate in the mutants.

The level of ABA in non-stressed and stressed leaves of the wild-type and the mutants I217 and CKR1 of *N. plumbaginifolia* was also determined (Fig. 7B). We found an increased level of ABA in stressed leaves compared to non-stressed leaves. Following stress, the ABA level in wild-type leaves increased four-fold while the values from mutants I217 and CKR1 did not increase.

The endogenous levels of IAA in leaves and seeds of the wild-type and the mutants I217, CKR1 and Esg152 were compared (Fig. 7C). No significant difference was observed in the IAA content of the leaves whereas the mutant CKR1 seeds were richer in IAA content than those of the wild-type and the other two mutants. We were not able to detect cytokinins in seeds, presumably because of the very low endogenous levels of these substances.

Effect of ABA on restoration of the wilty phenotype

The *N. plumbaginifolia* mutants wilt rapidly under water stress due to abnormal stomatal behavior. In order to test the possible induction of stomatal closure in the wilty mutants, exogenous ABA (40 μM) was applied once a day to 2-month-old plants in the greenhouse. The three mutants treated with ABA during development were compared with control mutant plants. Changes in the growth habit of the mutants were evident after 3 weeks. The ABA-treated mutant plants looked very much like the wild-type in terms of stem rigidity, leaf turgidity and

Table 3. The effect of ABA treatment on the transpiration rate of detached leaves of *N. plumbaginifolia* wild-type (WT) and mutants I217, CKR1 and Esg152

Hours after leaf detachment	Weight of detached leaves from *N. plumbaginifolia* plants							
	WT		I217		CKR1		Esg152	
	Nt	T	Nt	T	Nt	T	Nt	T
1	92.1 (2.5)	94.9 (3.2)	84.2 (2.5)	94.2 (1.2)	84.6 (3.1)	93.3 (2.9)	86.2 (3.9)	94.0 (3.5)
2	89.1 (3.0)	93.0 (3.1)	80.4 (2.7)	91.8 (1.3)	79.8 (5.0)	90.6 (3.3)	79.8 (3.5)	91.3 (4.0)
3	87.5 (2.5)	90.8 (3.7)	76.5 (2.2)	88.7 (1.3)	73.5 (5.0)	87.5 (4.2)	76.7 (5.4)	89.0 (4.2)

The values are an average of weight determinations performed on 3–6 leaves and expressed as a percentage of the weight of the same leaves when freshly detached (in brackets the standard error)
Nt, Non-treated control plant; T, ABA-treated plant

transpiration rate (Table 3). ABA treatment induced an increase in both apical dominance and tolerance to water-stress. Therefore, these wilty mutants of *N. plumbaginifolia* appear to be ABA-sensitive, unlike the *abi* mutants of *A. thaliana* (Koornneef et al. 1984) or the *vp-1* mutant of maize (McCarty et al. 1989).

Effect of auxin on germination of an ABA-deficient mutant of A. thaliana

Since ABA-deficient genotypes exist in other species, such as *A. thaliana* (Koornneef et al. 1982), auxin sensitivity was compared to the corresponding wild-type. No obvious difference, in terms of auxin sensitivity, was observed in the germination of the wild-type and the *aba* mutant (Fig. 8). However, the precocious germination of *A. thaliana,* (within 3 days) compared to *N. plumbaginifolia* (6–8 days, Fig. 2), may have allowed these seeds to escape auxin sensitivity.

Discussion

In this report, we describe the isolation of a monogenic recessive mutant Esg152 of *N. plumbaginifolia* selected on the basis of its precocious germination. The mutant was found to have reduced seed dormancy and a wilty phenotype, similar to the mutants I217 and CKR1 previously described by Bitoun et al. (1990) and Blonstein et al. (1991 a) respectively. Table 1 suggests that in agreement with the data from Bitoun et al. (1990) a strict correlation exists between the precocious germination phenotype and resistance to auxin.

Wilty mutants of N. plumbaginifolia are ABA-deficient

The typical wilty phenotype could be the consequence either of an ABA insensitivity or an ABA deficiency. Application of ABA to mutant plants changes their morphology towards that of the wild-type. The treated mutant plants do not show wilting symptoms, in agreement with the observations of Imber and Tal (1970) on wilty tomato mutants and the previous results of Parry et al. (1991) on the CKR1 mutant. All three mutants reverted to the normal phenotype when treated with ABA, showing that they are sensitive to exogenous ABA. The concentration of endogenous ABA was four times lower in the mutants than in the wild-type (Fig. 7). This observation suggests that the excessive opening of the stomata is caused by an insufficient amount of ABA. The observation that wilty mutants with impaired stomatal functioning were ABA-deficient, and that treatment of these mutants with ABA led to phenotypic reversion (Table 3), confirms the well-established fact that ABA plays a crucial role in the regulation of plant water relations.

The resistance to auxin and cytokinin, the wilting tendency, and the reduced dormancy traits are pleiotropic effects expressed by mutants of a single genetic locus. ABA is known to interact with other plant growth substances. Tal and co-workers have shown that in tomato the reduced ABA level of the *flacca* mutant induces a high level of auxin and cytokinin activity and high ethylene production (Tal and Nevo 1973; Tal et al. 1979). The regulation of stomatal behaviour is another physiological process which seems to be influenced by several plant growth regulators: cytokinins stimulate stomatal opening whereas abscisic acid induces closure (Davies et al. 1986).

Interaction between ABA and other hormones

Several examples of cross-resistance among mutants selected on the basis of their resistance to a specific hormone have been reported recently (Bitoun et al. 1990; Wilson et al. 1990; Blonstein et al. 1991 a). In fact auxin- or cytokinin-resistant mutants have been found to be cross-resistant to, or to have an alteration in the production of, other hormones. These observations underline the complexity of phytohormone interactions and explain the observed pleiotropic effects of hormone mutants.

In this context, the question arises as to whether the mechanism of auxin or cytokinin resistance in the ABA-deficient mutants is specific, or merely a trivial consequence of a change in the rate of germination caused by ABA deficiency? In our experiments, the alteration in auxin sensitivity was detected not only at the germination level but also on excised hypocotyl segments. This observation indicates that alterations in ABA biosynthesis lead to a range of physiological modifications affecting both water relations and different stages of development, such as germination and seed maturation, which will modify the response of the corresponding tissues to auxin. From the data presented here, we can postulate that a link exists between ABA effects and auxin-stimulated root formation.

Another interesting observation was that mutants accumulated putative ABAGE. Work is in progress to confirm by mass spectometry analysis that this compound is authentic *cis*-ABAGE.

Based on available results we favour the hypothesis that the *Aba1* gene is directly involved in ABA biosynthesis. The site of the mutation in the ABA biosynthetic pathway may be of importance to the physiological consequences of the mutation. Rock and Zeevaart (1991) have shown that the *aba* mutant of *A. thaliana* is affected in carotenoid biosynthesis, a step different from the one affected in one of our mutants. Indeed, Parry et al. (1991) have shown that the lesion in the wilty mutant CKR1 of *N. plumbaginifolia* affects the last part of the ABA biosynthesis pathway, the conversion of ABA-aldehyde to ABA. Rock et al. (1991) showed that in a shunt pathway from abscisic aldehyde, abscisic alcohol can be an intermediate in abscisic acid biosynthesis. The accumulation of putative ABAGE in the mutants can be explained either by assuming that the ABA synthesized from abscisic alcohol is preferentially converted to ABAGE, as opposed to ABA derived directly from abscisic aldehyde. Alternatively, the mutant may not be affected in a structural component of the pathway but in a regulatory aspect of the control of ABA synthesis. This analysis requires further investigation.

The response of the *flacca* and *sitiens* mutants of tomato or the *droopy* mutant of potato to exogenously applied auxin must also be considered, because the lesion in these mutants affects the same step in ABA biosynthesis, the conversion of ABA-aldehyde to ABA (Taylor et al. 1988; Duckham et al. 1989; Parry et al. 1991).

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References

Bitoun R, Rousselin P, Caboche M (1990) A pleiotropic mutation results in cross-resistance to auxin, abscisic acid and paclobutrazol. Mol Gen Genet 220:234–239

Blonstein AD, Parry AD, Horgan R, King PJ (1991a) A cytokinin-resistant mutant of *Nicotiana plumbaginifolia* is wilty. Planta 183:244–250

Blonstein AD, Stirnberg P, King PJ (1991b) Mutants of *Nicotiana plumbaginifolia* with specific resistance to auxin. Mol Gen Genet 228:361–371

Bourgin JP, Chupeau Y, Missonier C (1979) Plant regeneration from mesophyll protoplasts of several *Nicotiana* species. Physiol Plant 45:288–292

Davies WJ, Metcalfe J, Lodge TA, Da Costa AR (1986) Plant growth substances and regulation of growth under drought. Aust J Plant Physiol 13:105–125

Duckham SC, Taylor IB, Linforth RST, Al-Naieb RJ, Marples BA, Bowman WR (1989) The metabolism of *cis* ABA-aldehyde by the wilty mutants of potato, pea and *Arabidopsis thaliana*. J Exp Botany 40:901–905

Gabard J, Marion-Poll A, Chérel I, Meyer C, Müller A, Caboche M (1987) Isolation and characterization of *Nicotiana plumbaginifolia* nitrate reductase-deficient mutants: genetic and biochemical analysis of the *NIA* complementation group. Mol Gen Genet 209:596–606

Imber D, Tal M (1970) Phenotypic reversion of flacca, a wilty mutant of tomato, by abscisic acid. Science 169:592–593

Jullien M, Lesueur D, Laloue M, Caboche M (1991) Isolation and preliminary characterization of cytokinin-resistant mutants of *Nicotiana plumbaginifolia*. In: Kaminek M, Mok DWS, Zazimalova E (eds) Physiology and Biochemistry of Cytokinins in plants. SPB Academic Publishing bv, The Hague, The Netherland, pp 157–162

Koornneef M, Jorna ML, Brinckhorst-van der Swan DLC, Karssen CM (1982) The isolation of abscisic acid (ABA)-deficient mutants by selection of induced revertants in non-germinating gibberellin-sensitive lines of *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 61:385–393

Koornneef M, Reuling G, Karssen CM (1984) The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. Physiol Plant 61:377–383

Maldiney R, Leroux B, Sabbagh I, Sotta B, Sossountzov L, Miginiac E (1986) A biotin-avidin-based enzyme immunoassay to quantify three phytohormones: auxin, abscisic acid and zeatin-riboside. J Immunol Methods 90:151–158

McCarty DR, Carson CB, Stimard PS, Robertson DS (1989) Molecular analysis of *viviparous-1*; an abscisic acid-insensitive mutant of maize. Plant Cell 1:523–532

Muller JF, Missonier C, Caboche M (1983) Low density growth of cells derived from *Nicotiana* and *Petunia* protoplasts: influence of the source of protoplasts and comparison of the growth-promoting activity of various auxins. Physiol Plant 57:35–41

Parry AD, Blonstein AD, Babiano MJ, King PJ, Horgan R (1991) Abscisic acid metabolism in a wilty mutant of *Nicotiana plumbaginifolia*. Planta 183:237–243

Pelese F, Megnegneau B, Sotta B, Sossountzov L, Caboche M, Miginiac E (1989) Hormonal characterization of a nonrooting naphtalene-acetic acid tolerant tobacco mutant by an immunoenzymic method. Plant Physiol 89:86–93

Pelsy F, Kronenberger J, Pollien J-M, Caboche M (1991) M2 seed screening for nitrate reductase deficiency in *Nicotiana plumbaginifolia*. Plant Sci 76:109–114

Quarrie SA (1982) *Droopy*: a wilty mutant of potato deficient in abscisic acid. Plant Cell Environ 5:23–26

Quarrie SA (1987) Use of genotypes differing in endogenous abscisic acid levels in studies of physiology and development. In: Hoad GV, Lenton JR, Jackson MB, Atkin RK (eds) Hormonal action in plant development. Butterworths, London, UK, pp 89–105

Reid JB (1990) Phytohormone mutants in plant research. J Plant Growth Regul 9:97–111

Rock CD, Zeevaart JAD (1991) The *aba* mutant *Arabidopsis thaliana* is impaired in epoxy-carotenoid biosynthesis. Proc Natl Acad Sci USA 88:7496–7499

Rock CD, Heath TG, Gage DA, Zeevaart JAD (1991) Abscisic alcohol is an intermediate in abscisic acid biosyntheis in a shunt pathway from abscisic aldehyde. Plant Physiol 97:670–676

Rousselin P, Lepingle A, Faure JD, Bitoun R, Caboche M (1990) Ethanol-resistant mutants of *Nicotiana plumbaginifolia* are deficient in the expression of pollen and seed alcohol dehydrogenase activity. Mol Gen Genet 222:409–415

Tal M, Nevo Y (1973) Abnormal stomatal behavior and root resistance, and hormonal imbalance in three wilty mutants of tomato. Biochem Genet 8:291–300

Tal M, Imber D, Erez A, Epstein E (1979) Abnormal stomatal behavior and hormonal imbalance in *flacca*, a wilty mutant of tomato. V. Effect of abscisic acid on indolacetic acid metabolism and ethylene evolution. Plant Physiol 63:1044–1048

Taylor IB, Linforth RST, Al-Naieb RJ, Bowman WR, Marples BA (1988) The wilty tomato mutants *flacca* and *sitiens* are impaired in the oxidation of ABA-aldehyde to ABA. Plant Cell Environ 11:739–745

Wilson AK, Pickett FB, Turner JC, Estelle M (1990) A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. Mol Gen Genet 222:377–383