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Characterization of the ABA-deficient *Pisum sativum* 'wilty' mutant

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SUMMARY

In this paper, an ABA-deficient *Pisum sativum* mutant is physiologically characterized. A wilty pea line (*wil*) was backcrossed six times with a non-wilty line, and then selfed to obtain near-isogenic lines. These lines were used for further studies. The plants were grown hydroponically and at high relative humidity. The ABA-deficient mutant grew more slowly in mass and height and invested less dry matter in its roots. Mutant leaves wilted rapidly under drought conditions, and had a lower water content than wild-type leaves. ABA-deficient plants had fewer and smaller seeds than wild-type plants, but the weight ratio of reproductive to vegetative parts was similar in both lines. Seeds of mutant plants contained about five times less ABA than wild-type seeds. It is suggested that the lower growth rate of both vegetative and reproductive parts is not directly caused by the lower ABA content, but by disturbed water relations.

Key-words: ABA-deficient (*wil*) mutant, abscisic acid, growth, seed development, water relations.

INTRODUCTION

During the last two decades a series of mutants that are either ABA-deficient or altered in their sensitivity to ABA have become available (Koornneef 1986). Although only a few of them are well-characterized (Zeevaart & Creelman 1988), it is clear that these mutants play a significant role in physiological research. Comparisons of mutants with their wild types have successfully answered questions on the role of ABA in water relations (e.g. in tomato, Tal & Nevo 1973) and the biosynthetic pathway of ABA (e.g. in *Arabidopsis*, Rock *et al.* 1992). Also, several aspects of the role of ABA in seed development have been elucidated with the aid of hormone mutants. For example, the development of seed desiccation tolerance was found to be absent in an ABA

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Abbreviations: ABA, abscisic acid; ELISA, enzyme-linked immunosorbent assay; TRIS, tris(hydroxymethyl) aminomethane; TES, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; BSA, bovine serum albumin.

biosynthesis and responsiveness double mutant of *Arabidopsis* (Koornneef *et al.* 1989). The role of mutants in research on ABA and seed dormancy and germination was recently reviewed by Hilhorst & Karssen (1992).

It is obvious, however, that not all the differences between ABA mutants and their wild types can be directly attributed to the absence of, or insensitivity to, ABA. Several pleiotopic effects are described (Koornneef 1986), and even in monogenic mutants, cross-resistance to other hormones has been reported (Blonstein *et al.* 1991). Marz (1976) described a recessive gene mutation ('wilty', *wil*) in pea, that caused early and dramatic wilting during drought stress. Leaves of this mutant appear to contain considerably less ABA compared to other pea lines (Wang *et al.* 1984; Jackson & Hall 1987). Unfortunately, the original breeding line is not available (Donkin *et al.* 1983). In the present study, the mutant is compared with a non-wilty line that has been backcrossed with the wilty parent line to obtain near-isogenic lines. Since we are primarily interested in the role of ABA on assimilate partitioning, special attention is paid to the dry matter distribution in the mutant, with respect to both vegetative and reproductive organs. To determine whether this mutation is organ-specific (for example, some GA-deficient mutants have wild-type GA levels in their seeds, Potts & Reid 1983), ABA concentrations in seeds have been determined during their development.

MATERIALS AND METHODS

Plant material

Seeds of ABA-deficient pea plants (*Pisum sativum, wil* mutant, line B78-918) were kindly provided by Professor G. A. Marx (NYSAES, Geneva, NY, USA). The original non-wilty wild-type line (B1777-155) that was used for the backcrosses was not an isogenic line but closely approximated the phenotype of the wilty lines (G. A. Marx, personal communication). The heterozygote F_1 that resulted from a cross between the wilty line and the wild-type line was backcrossed with the wilty parent line. To discern in the offspring between heterozygous plants and homozygous recessive ones, some leaflets were detached from each plant, and the decrease in fresh weight was monitored. Leaves of ABA-deficient plants transpire at a higher rate (Wang *et al.* 1984; cf. Fig. 1). The heterozygotes were backcrossed again with the wilty parent line, and this procedure was repeated four times. Heterozygotes that resulted from the sixth backcross were selfed, and the progeny were selfed again to obtain reasonably homozygous lines for the characterization experiments. According to Marx (1976), the gene symbol *wil* is used to designate this mutant.

About 250 seeds of each line were disinfected with a fungicide (thiram) and germinated in a glasshouse at ambient conditions, in a large Petri dish with glass beads and half-strength Hoagland medium (Hoaglund & Arnon 1950), covered with wetted filter paper. Seven to ten days after germination each seedling was transferred to a 2-litre jar with well aerated half strength Hoagland medium, supplemented with a fungicide (propamocarb, 5 mg l^{-1}). During the growth period, the medium in the jars was replaced three times, with due care to prevent damage to the roots. Iron deficiency was prevented by adding a few droplets of a 0·1 M Fe, Na-EDTA solution to the nutrient solution each week. Plants were raised in a glasshouse with minimum day temperatures of 19°C and night temperatures of 16°C, under natural light, supplemented with artificial illumination from high-pressure sodium lamps (Philips, SON/T), to give a 16-h day

length. Plants were protected from direct sunlight. The floors of the greenhouse were regularly sprinkled with water and care was taken to keep the relative humidity in the glasshouse above 60%.

Growth characteristics

At eight different stages after sowing, samples of randomly selected plants of both lines were harvested. The number of plants in wild-type samples varied from 8 to 15, in mutant samples from 11 to 15. For the sake of security, the genotype of each plant was checked by determining the rate of decrease in fresh weight of detached leaflets (Wang *et al.* 1984; cf. Fig. 1) prior to the harvest.

Measurements were made of fresh and dry (after 24 h at 105°C) weights of roots, stems, leafs, pods, seeds and lateral shoots, as well as the number of internodes, leaves, pods and seeds. Leaf areas were determined with a LI-COR model 3100 Area Meter. Flowers were tagged at the day of flowering, and seeds were only harvested separately from the fruit when the fruit length exceeded 5 cm; at earlier times the seeds were too small to weigh, i.e. fresh weight 1 mg or less.

ABA determinations

Flowers were tagged on plants of both lines, and 80 fruits were harvested at various ages, ranging from 11 to 35 days after flowering. Directly after harvest, the seeds were separated in half. One-half of the harvested material was weighed and immediately frozen in liquid nitrogen and stored at −70°C for later determination of ABA levels. The other half was used for both fresh and dry weight determinations. The dry matter content was used to select a series of 34 representative fruits. The harvested material of individual fruits was homogenized in 10 ml 70% (v/v) methanol and 0·2 g purified seasand, for about 10 min in a Pulverisette (Fritsch, Germany) (since older seeds could not be homogenized in a mortar), at 4°C. The homogenate was centrifuged (400 ***g***, 5 min), and the volume of the supernatant was reduced to 0·5–1 ml under a flow of nitrogen.

A series of six dilutions of the residue was subsequently analysed in triplicate using an indirect enzyme-linked immuno sorbent assay (ELISA) as described by Ross *et al.* (1987). In short: microtitre plates were coated with 50 mg l^{-1} KLH-ABA conjugate (ABA C_1-linked to keyhole limpets haemocyanin) dissolved in a coating buffer (34·8 mM $NaHCO_3$, 16·0 mM Na_2CO_3, 6·5 mM NaN_3 and 0·5 mM $MgCl_2 \cdot H_2O$, pH 9·6). The plates were incubated for at least 24 h. After rinsing with water and PBST (phosphate buffered saline with Tween: 8·1 mM $Na_2HPO_4 \cdot 2H_2O$, 1·5 mM KH_2PO_4, 137 mM NaCl, 2·7 mM KCl, 6·5 mM NaN_3, 0·05% (v/v) Tween-20, pH 7·4), 100 µl samples of ABA (standard curve) or pea seed homogenate dilutions were added to the wells, followed by 100 µl of a mouse monoclonal antibody raised against ABA (McAb-3G4, Boonekamp *et al.* 1990). The plates were sealed, covered with a lid, wrapped in aluminium foil and incubated overnight at 4°C. After washing the plates with PBST, 200 µl aliquots of a goat anti-mouse IgG (whole molecule) conjugated to alkaline phosphatase (Sigma), diluted in TTBST (TRIS and TES buffered saline with Tween and BSA: 20 mM TRIS, 20 mM TES, 137 mM NaCl, 2·7 mM KCl, 6·5 mM NaN_3, 0·5% (w/v) BSA, 0·05% (v/v) Tween-20, pH 7·4) were added. After sealing and wrapping the plates were incubated for 3 h at 35°C. Then, after washing, 200 µl *p*-nitrophenyl phosphate (1 g l^{-1}) in coating buffer were added. After an hour's incubation at 35°C the plates were read at 405 nm with a MIOS (Merck) reader.

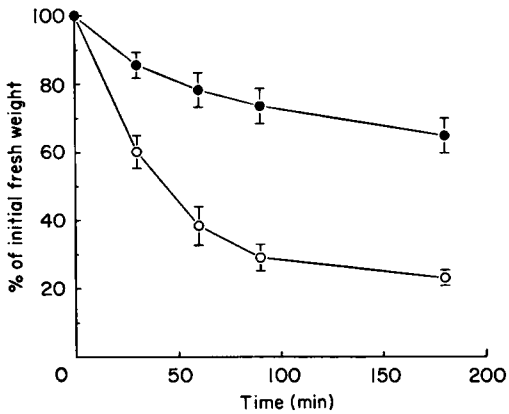

Fig. 1. Time-course of the loss of fresh weight (mean ± SD, $n=12$) from detached leaflets of wild-type (●) and ABA-deficient (○) *Pisum sativum* plants over 3 h.

The antibodies used in this ELISA were very sensitive (range: 1–100 pmol) and highly specific to *cis*-(+)-ABA (cross-reactivity of *cis*-(±)-ABA: 50%; of *trans*-(−)-ABA: 0·9%) (Boonekamp *et al.* 1990). Cross-reactivity to the glucose-ester of ABA was rather high, but levels of these conjugates are low and constant during seed development in pea (Ross & McWha 1990). Since the antibodies were raised against protein conjugates using the C-1 moiety of ABA, cross-reactivity of phaseic acid and interference with organic acids (as reported by Belefant & Fong (1989) for C-4′-conjugated ABA) is expected to be low (M.H.M. Cornelussen, personal communication). Validation of the ABA levels by gas-chromatography (Vermeer *et al.* 1987) resulted in lower values than the levels measured by ELISA (due to lower recoveries), but the difference between the pea lines was of the same order of magnitude.

RESULTS AND DISCUSSION

Water relations

Originally, the *wil* mutant was described as 'wilty', since its wilting behaviour is one of the most obvious characteristics. In general, the wilting response of intact plants was only observed at conditions of relatively severe water stress. Wang *et al.* (1984) described special treatments to induce wilting symptoms. We experienced that these conditions were indeed necessary when the plants were cultured in soil. The experiments described here were performed on plants cultured on hydroponics, and these plants had a remarkably different wilting behaviour. Figure 1 shows the loss of water from detached leaflets over time, for both lines. Within 45 min, the mutant leaves had lost half of their initial fresh weight, whereas wild-type leaves lost less than 20% during the same period. For comparison, the mutant plants grown in soil used by Wang *et al.* (1984) needed 3 h to lose 30% of their initial fresh weight; the conditions during the measurement were identical to our conditions. It seems likely that the plants cultured in soil became to some extent acclimatized to water shortage and needed more time and more extreme conditions to show wilting symptoms, whereas the optimal availability of water in hydroponic culture prevented the plants from stress accommodation.

The role of ABA in maintaining the water balance in plant leaves has been much studied (see: Davies & Mansfield 1983; MacRobbie 1991). It seems self-evident that the

wilting symptoms in ABA-deficient mutants are caused by an inability, relative to wild-type plants, to close their stomata. Although stomata of the wilty pea mutant and non-wilty lines respond in similar ways to ABA, KCl and CO_2 (Donkin *et al.* 1983), the mutant leaves fail to accumulate ABA and consequently maintain a higher stomatal conductance during water deficiency (Wang *et al.* 1984; Jackson & Hall 1987), which leads to rapid wilting. In unstressed conditions, mutant leaves possess a more negative water potential and larger stomatal apertures than those of the wild type (Donkin *et al.* 1983). The resulting lower leaf dry matter content of wild-type plants (Donkin *et al.* 1983) has been partially confirmed by the present study (Table 1). The percentage dry matter content in our plants was, however, considerably lower than that reported by Donkin *et al.* (1983) (14·3% compared to 21·3% for mutant plants). This difference may be related to the hydroponics system we used.

Vegetative parts

Because mutant plants wilted so rapidly, the fresh weight determinations on harvested mutant plants were done quickly (within 10 min). Directly after harvesting, the roots were separated from the shoot, fruits and flowers were detached and seeds were isolated from the fruits. All parts were weighed separately.

Figure 2 illustrates the most important differences between the lines. Clearly, wild-type plants grew faster than ABA-deficient plants, both on a fresh and on a dry weight basis. Wild-type plants always had smaller shoot/root ratios than mutant plants (Table 1). Similar effects of ABA on shoot/root ratios were found in several other systems (Creelman *et al.* 1990; Saab *et al.* 1990) and in ABA-deficient mutants of potato (*droopy*, Waggoner & Simmonds 1966) and tomato (*flacca*, Bradford 1983). These findings favour the hypothesis that ABA promotes the growth of roots as a measure to increase the volume of soil explored under dry conditions, and to prevent an undesirable water status of the leaves (Davies & Mansfield 1983). The relatively high values for the shoot/root ratio in both genotypes in our system are most probably caused by the hydroponic culture and are indicative of a low degree of water deficiency (Setter 1990).

In the present study, ABA-deficient plants showed more outgrowth of the axillary buds, especially at the base of the plant. The number of lateral shoots per plant was not significantly different, but the absolute lateral shoot weight per plant and the ratio of lateral shoot weight to total shoot weight were considerably higher in mutant plants (Table 1). This reduced degree of apical dominance has not been reported for other ABA-deficient mutants. On the contrary, ABA-deficient mutants of *Arabidopsis thaliana* had smaller numbers of side shoots than wild-type plants (Koornneef *et al.* 1982). Information on the effect of ABA on apical dominance is still somewhat inconsistent since both stimulation and inhibition of lateral bud growth by ABA application have been reported (e.g. Hartung & Fünfer 1981; Everat-Bourbouloux & Charnay 1982; Cline 1991). It cannot be excluded, however, that loss of apical dominance in mutant pea plants was partly due to incidental wilting of the apex, or to accelerated senescence of the basal leaves observed in mutant plants. The reduced apical dominance in mutant plants also finds expression in a larger number of leaves and a smaller leaf area per leaf (Table 1), since the lateral shoots had many small leaves. ABA-deficient tomato mutants also have a larger number of leaves than wild-type plants (Quarrie 1987).

Other parameters, viz., the leaf area ratio (leaf area/plant weight), the specific leaf area (leaf area/leaf weight) and the leaf weight ratio (leaf weight/plant weight) showed only minor differences between the genotypes (Table 1).

Table 1. Comparison of both vegetative and reproductive parts of the wild-type and ABA-deficient *Pisum satvum* plants (mean ± SD (*n*)). Unless otherwise indicated, calculations are based on dry weights of organs. The letters Y or N in the last column indicate whether or not significant differences exist at the $P=0{\cdot}05$ level, respectively (Student's *t*-test)

Parameter	Age (days)	Wild type	Mutant	S
Vegetative parts				
Dry matter content of leaves (%)		11·2 ± 3·7 (55)	14·3 ± 3·4 (82)	Y
Shoot/root ratio	50	6·3 ± 0·8 (10)	7·9 ± 1·0 (14)	Y
	57	6·1 ± 1·1 (9)	9·9 ± 2·0 (12)	Y
	69	8·8 ± 1·8 (9)	14·2 ± 2·4 (13)	Y
Number of lateral shoots		2·1 ± 2·0 (57)	1·9 ± 1·8 (91)	N
Lateral shoot fresh weight (g)		0·6 ± 0·9 (122)	2·4 ± 4·3 (171)	Y
Fresh weight ratio of lateral shoots to whole shoot		0·02 ± 0·03 (57)	0·15 ± 0·26 (91)	
Number of leaves per plant	50	88 ± 6 (10)	71 ± 12 (14)	Y
	57	92 ± 10 (9)	96 ± 27 (12)	N
	69	134 ± 34 (9)	150 ± 57 (13)	N
	86	121 ± 14 (9)	180 ± 72 (13)	Y
Leaf area per leaf (cm^2)		9·36 ± 1·85 (46)	5·01 ± 1·25 (16)	Y
Leaf area ratio ($cm^2\ g^{-1}$)	50	226 ± 19 (10)	245 ± 19 (14)	Y
	57	223 ± 37 (9)	205 ± 17 (12)	N
	69	165 ± 28 (9)	160 ± 23 (13)	N
Specific leaf area ($cm^2\ g^{-1}$)	50	519 ± 33 (10)	502 ± 32 (14)	N
	57	489 ± 59 (9)	450 ± 31 (12)	N
	69	468 ± 29 (9)	413 ± 38 (13)	Y
Leaf weight ratio	50	0·44 ± 0·02 (10)	0·49 ± 0·02 (14)	Y
	57	0·45 ± 0·03 (9)	0·45 ± 0·02 (12)	N
	69	0·35 ± 0·05 (9)	0·39 ± 0·04 (13)	N
Reproductive parts				
Flowering date (days after sowing)		57·1 ± 4·4 (45)	57·2 ± 3·6 (59)	N
First flowering node (counted from basis upwards)		20·9 ± 1·4 (54)	21·5 ± 1·8 (72)	N
Weight ratio of reproductive to vegetative parts	69	0·16 ± 0·08 (9)	0·16 ± 0·08 (13)	N
	78	0·41 ± 0·07 (9)	0·32 ± 0·12 (13)	Y
	86	0·54 ± 0·08 (9)	0·39 ± 0·13 (13)	Y
	93	0·62 ± 0·04 (8)	0·59 ± 0·06 (11)	N
Number of fruits per plant		5·4 ± 3·3 (44)	5·0 ± 3·0 (62)	N
Number of seeds per plant		36·9 ± 12·7 (35)	18·4 ± 10·9 (50)	Y
Seed weight (mg)	69	6·3 ± 8·5 (273)	5·9 ± 10·2 (245)	N
	78	42·6 ± 41·8 (397)	24·2 ± 36·0 (372)	Y
	86	99·5 ± 75·2 (366)	61·7 ± 74·8 (340)	Y
	93	135·8 ± 79·4 (393)	94·5 ± 84·3 (464)	Y

Reproductive parts

Both wild-type and mutant plants started to flower at the same age, and in both lines the first flowers appeared in the same node (i.e. 21–22 nodes above the cotyledons).

ABA-deficient plants invested less dry matter in their pods and seeds. The final seed dry weight per plant was 65% higher in wild-type plants (Fig. 3), whereas the ratio of reproductive to vegetative parts was also higher in this genotype (Table 1). However, at the end of development this difference was less pronounced: it seemed that mutant

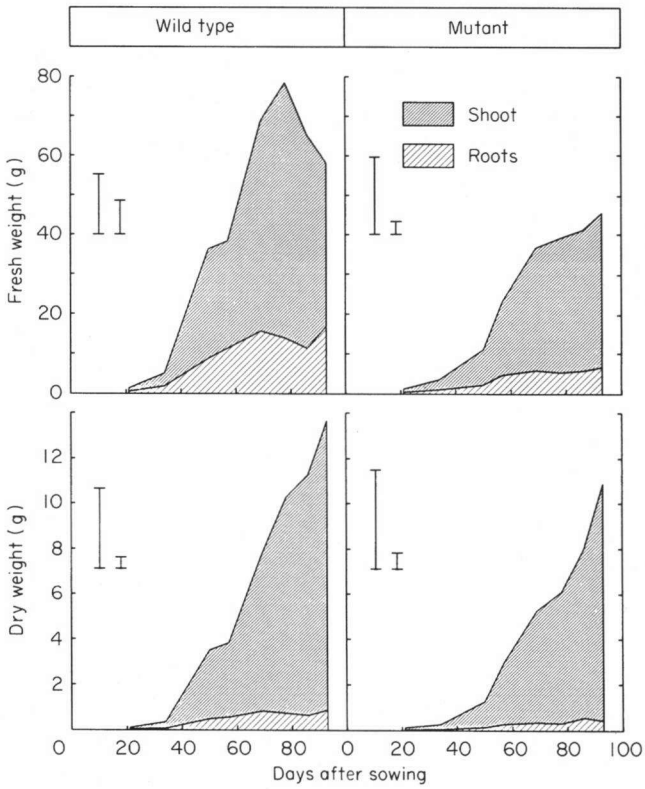

Fig. 2. Growth patterns of shoots and roots of wild-type (left panels) and ABA-deficient (right panels) *Pisum sativum* plants, both on a fresh weight (upper panels) and a dry weight (lower panels) basis. The error bars in each panel indicate the largest standard deviation of the measurements on shoots (left bar) and roots (right bar), respectively.

plants were slower but finally reached similar ratios as wild-type plants. Since seed number per plant was smaller in mutant plants, the average weight per seed was only slightly (but significantly) lighter for mutant seeds. The number of fruits was not significantly different between the lines (Table 1). In general, ABA-deficient plants carried fewer seeds per fruit. Figure 4 displays the frequency distribution of the number of seeds per fruit for both lines.

Previous studies on tomato and *Arabidopsis* ABA-deficient mutants revealed no effects of the reduced ABA levels on seed fresh and dry weight (Karssen *et al.* 1983; Groot *et al.* 1991). Seeds of *Arabidopsis* mutants that were both ABA-deficient and ABA-insensitive were impaired in several developmental processes such as ripening and desiccation tolerance (Koornneef *et al.* 1989), but still attained fresh and dry weights similar to those of wild-type seeds (De Bruijn *et al.* 1993). Apparently, in these species, ABA does not determine the sink strength of the seeds.

Although the pea mutant in the present study has a somewhat lighter seed weight and a reduced mass of reproductive organs per plant, it remains to be seen whether this is a direct effect of the lower ABA level in the seeds. Since by the end of development, the weight ratio of reproductive to vegetative plant parts is similar in both lines (Table 1), the lower seed weight seems to be the indirect consequence of

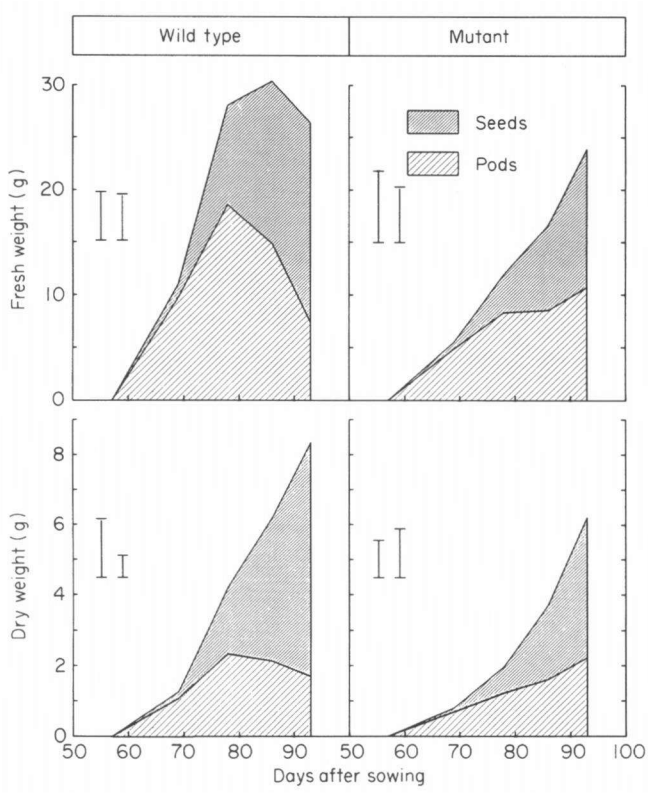

Fig. 3. Growth patterns of pods and seeds on wild-type (left panels) and ABA-deficient (right panels) *Pisum sativum* plants, both on a fresh weight (upper panels) and a dry weight (lower panels) basis. The experiment was finished just before full maturation of the mutant seeds. The error bars in each panel indicate the largest standard deviation of the measurements on seeds (left bar) and pods (right bar), respectively.

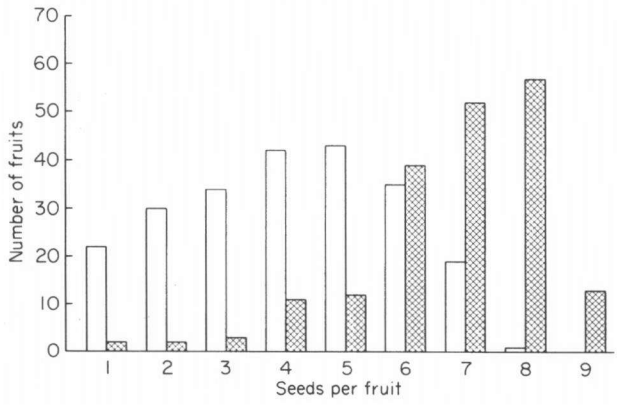

Fig. 4. Frequency distribution of the number of seeds per fruit in wild-type (hatched bars) and ABA-deficient (open bars) *Pisum sativum* plants.

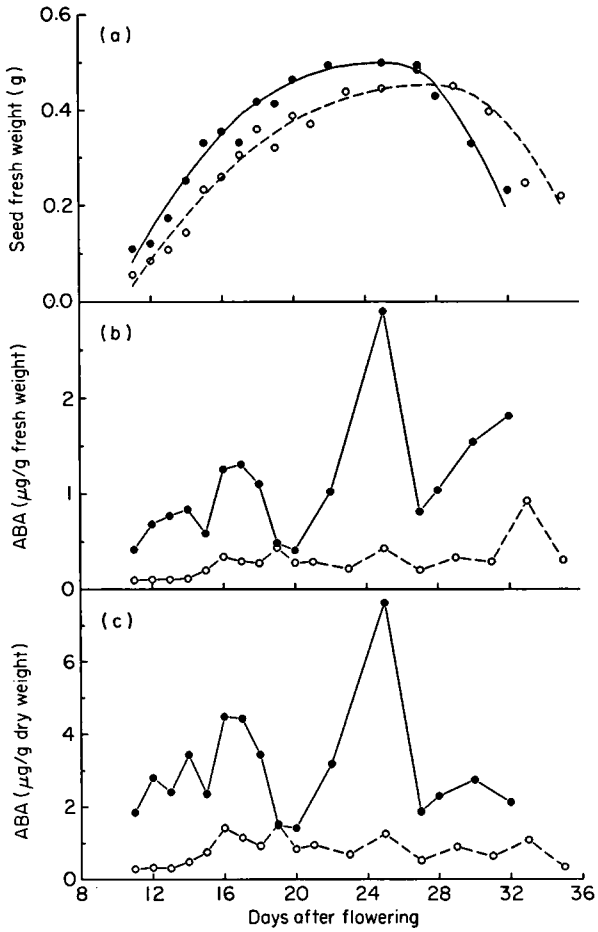

Fig. 5. Fresh weight (a), ABA content expressed on a fresh weight basis (b) and on a dry weight basis (c) of developing wild-type (●) and ABA-deficient (○) seeds.

the disturbed water relations, and not to be a primary ABA effect. This conclusion is supported by the recent finding that pea wild-type seeds developing in an ABA-deficient pod have the same growth rate as ABA-deficient seeds in the same pod (De Bruijn & Vreugdenhil 1992).

ABA levels in developing seeds

From plants of both genotypes, 34 fruits were selected. The fresh weight of their seeds and their concentration of ABA measured by ELISA are presented in Fig. 5. Wild-type seeds showed two distinct peaks, one during the initial rapid-growth phase and the other just preceding the maturation phase. The ABA concentration of the mutant seeds was much more constant and typically a fifth of wild-type values. The increase in ABA at the end of development in both genotypes when expressed on a fresh weight basis (Fig. 5b) coincided with a decrease of fresh weight during maturation, due to water loss of the seeds. The oldest seeds have dry matter contents of about 85%. This increasein ABA was much less apparent when the data are expressed on a dry weight basis (Fig. 5c).

Previous studies of endogenous ABA levels in developing seeds have shown two peaks (Hsu 1979; Browning 1980; Wang *et al.* 1987; Groot *et al.* 1991). There is evidence in *Arabidopsis* and tomato that the first one originates from maternal tissues, and the second from the embryo itself (Karssen *et al.* 1983; Groot *et al.* 1991). In those studies, the first ABA peak was shown not to be present in heterozygous seeds from an ABA-deficient mother plant, while the second ABA peak was retained (Karssen *et al.* 1983; Groot *et al.* 1991). This mechanism will probably also hold for pea seeds, since Wang *et al.* (1987) demonstrated that at the time of the first ABA peak, the hormone was also present in large amounts in the pod and the testa, whereas at the end of development both these tissues contained little ABA. Similar results were obtained from *Phaseolus* (Hsu 1979).

The ABA levels presented in this study deviate to some extent from literature data. The main differences with previous reports are the relatively high ABA concentrations in wild-type seeds at the end of development. Data from ripe pea seeds are absent from the literature, since most authors did not collect seeds with dry matter contents exceeding 50% (Eeuwens & Schwabe 1975; Browning 1980; Wang *et al.* 1987; Ross & McWha 1990). Ripe *Phaseolus* and *Vicia* seeds, however, contain almost no ABA (Hsu 1979; Gräbner *et al.* 1980). Nevertheless, several species have been described that maintain considerable amounts of ABA in ripe seeds (e.g. Piaggesi *et al.* 1991), although in these species the presence of ABA is generally coupled to dormancy (Black 1983). Whether the observed differences in ABA level between mature seeds of ABA-deficient and wild-type plants in the present study have led to deeper dormancy for wild-type seeds could not be detected, since pea seeds of both lines usually germinated immediately. In some cases, however, mutant seeds germinated viviparously in the fruits, which was never seen in wild-type seeds.

The five-fold difference between the ABA content of ABA-deficient and wild-type pea seeds corresponds with previous reports of 9–15 or 2–3 times less ABA (Wang *et al.* 1984, and Jackson & Hall 1987, respectively) in leaves of this mutant as compared to those of wild-type. A remarkable difference between the pea mutant and other ABA-deficient plants is that pea mutant leaves will accumulate at least some ABA after prolonged exposure to water deficiency or flooding (Wang *et al.* 1984; Jackson & Hall 1987), whereas other mutants do not (Quarrie 1982; Neill & Horgan 1985; Rock *et al.* 1992). A slight increase in ABA level upon stress was reported in a wilty tobacco mutant (Parry *et al.* 1991).

In general, ABA levels in the seeds of ABA-deficient mutants are very variable, and range from half of that of the wild type level in embryos of a viviparous maize mutant (Brenner *et al.* 1977; Smith *et al.* 1989) to less than 5% in the *Arabidopsis aba-1* mutant and 3% in the tomato *sit*^{w} mutant (Karssen *et al.* 1983; Groot *et al.* 1991). The extent to which various ABA mutants are 'leaky' will partly depend on how the mutations interfere with the biosynthetic pathway of ABA. This has been demonstrated in a series of tomato mutants (Taylor & Tarr 1984) but, so far, the step in ABA biosynthesis where the pea mutation acts has not been identified. Duckham *et al.* (1989) have shown that the biochemical basis of the *wilty* pea mutant is not the same as in the *flacca* and *sitiens* tomato mutants and the *droopy* potato mutant, which are impaired in the last step of ABA biosynthesis, the conversion of ABA-aldehyde to ABA. Moreover, leaves of the pea mutant have wild-type xanthophyll levels, in contrast of *Arabidopsis aba-1* mutants which accumulated zeaxanthin levels indicating a block early in the ABA biosynthetic pathway (Duckham *et al.* 1991).

Conclusion

The most striking characteristics of the ABA-deficient pea mutant in comparison with its wild type can be summarized as follows: the mutant wilts much faster, grows more slowly, has less apical dominance, has larger shoot/root ratios, invests less dry matter in its roots and seeds, and has five times less ABA in its seeds.

Does ABA influence assimilate partitioning? Several growth parameters differed significantly between the genotypes, but the question remains still unanswered whether these differences were directly caused by the lack of ABA in the mutant. In a similar study, the 25% slower relative growth rate of the ABA-deficient tomato *sitiens* mutant was primarily attributed to altered water relations, and not to an effect of ABA on sink strength of different plant organs (Nagel *et al.* 1991). Although the experimental conditions in our study were made as favourable as possible, the higher dry matter content in mutant leaves indicate a disturbed water balance in ABA-deficient plants, which may have caused the retarded development of both vegetative and reproductive parts. Because of the water deficiency, dry matter production and its partitioning into the pods and seeds may be slower. However, at the end of plant development the weight ratio of reproductive to vegetative parts in wild-type plants is equalled by mutant plants (Table 1). This finding does not support the idea that ABA influences the sink strength of reproductive organs.

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REFERENCES

Belefant, H. & Fong, F. (1989): Abscisic acid ELISA: organic acid interference. *Pl. Physiol.* **91:** 1467–1470.

Black, M. (1983): Abscisic acid in seed germination and dormancy. In: Addicott, F.T. (ed.): *Abscisic Acid.* 331–363. Praeger Publishers, New York.

Blonstein, A.D., Parry, A.D., Horgan, R. & King, P.J. (1991): A cytokinin-resistant mutant of *Nicotiana plumbaginifolia* is wilty. *Planta* **183:** 244–250.

Boonekamp, P.M., Pomp, H., Davelaar, E. & Vonk, C.R. (1990): Selection of monoclonal antibodies to the plant growth regulators indole-3-acetic acid and abscisic acid after immunization with conjugates coupled through the carboxylic group. In: Schots, A. (ed.): *Monoclonal Antibodies in Agriculture. LMA Symposium 30 May 1990.* 37–46. Pudoc, Wageningen.

Bradford, K.J. (1983): Water relations and growth of the *flacca* tomato mutant in relation to abscisic acid. *Pl. Physiol.* **72:** 251–255.

Brenner, M.L., Burr, B. & Burr, F. (1977): Correlation of genetic vivipary in corn with abscisic acid concentration. *Pl. Physiol. Suppl.* **59:** 76.

Browning, G. (1980): Endogenous *cis,trans*-abscisic acid and pea seed development: evidence for a role in seed growth from changes induced by temperature. *J. Exp. Bot.* **31:** 185–197.

Cline, M.G. (1991): Apical dominance. *Bot. Rev.* **57:** 318–358.

Creelman, R.A., Mason, H.S., Bensen, R.J., Boyer, J.S. & Mullet, J.E. (1990): Water deficit and abscisic acid cause differential inhibition of shoot *versus* root growth, in soybean seedlings: Analysis of growth, sugar accumulation, and gene expression. *Pl. Physiol.* **92:** 205–214.

Davies, W.J. & Mansfield, T.A. (1983): The role of abscisic acid in drought avoidance. In: Addicott, F.T. (ed.): *Abscisic Acid.* 237–268. Praeger Publishers, New York.

De Bruijn, S.M. & Vreugdenhil, D. (1992): Abscisic acid and assimilate partitioning to developing seeds. I. Does abscisic acid influence the growth rate of pea seeds? *J. Pl. Physiol.* **140:** 201–206.

De Bruijn, S.M., Ooms, J.J.J., Basra, A.S., Van Lammeren, A.A.M. & Vreugdenhil, D. (1993): Influence of abscisic acid on storage of lipids and carbohydrates in developing *Arabidopsis* seeds. In: Côme, D. & Corbineau, F. (eds.): *Fourth International Workshop on Seeds—Basic and Applied Aspects of Seed Biology, Angers, France, 20–24 July 1992.* Vol. 1, 103–108. Angers, France.

Donkin, M.E., Wang, T.L. & Martin, E.S. (1983): An investigation into the stomatal behaviour of a wilty mutant of *Pisum sativum. J. exp. Bot.* **34:** 825–834.

Duckham, S.C., Taylor, I.B., Linforth, R.S.T., Al-Naieb, R.J., Marples, B.A. & Bowman, W.R. (1989): The metabolism of *cis* ABA-aldehyde by the wilty mutants of potato, pea and *Arabidopsis thaliana. J. exp. Bot.* **40:** 901–905.

Duckham, S.C., Linforth, R.S.T. & Taylor, I.B. (1991): Abscisic-acid-deficient mutants at the *aba* gene locus of *Arabidopsis thaliana* are impaired in the epoxidation of zeaxanthoxin. *Pl. Cell Environ.* **14:** 601–606.

Eeuwens, C.J. & Schwabe, W.W. (1975): Seed and pod wall development in *Pisum sativum*, L. in relation to extracted and applied hormones. *J. exp. Bot.* **26:** 1–14.

Everat-Bourbouloux, A. & Charnay, D. (1982): Endogenous abscisic acid levels in stems and axillary buds of intact or decapitated broad-bean plants (*Vicia faba* L.). *Physiol. Pl.* **54:** 440–445.

Gräbner, R., Dathe, W. & Sembdner, G. (1980): Endogene Pflanzenhormone der Ackerbohne, *Vicia faba* L. I. Abscisinsäure und andere Wachstumsinhibitoren in sich entwickelnde Samen. *Biochem. Physiol. Pflanzen* **175:** 447–459.

Groot, S.P.C., van Yperen, I.I. & Karssen, C.M. (1991): Strongly reduced levels of endogenous abscisic acid in developing seeds of tomato mutant *sitiens* do not influence *in vivo* accumulation of dry matter and storage proteins. *Physiol. Pl.* **81:** 73–78.

Hartung, W. & Fünfer, C. (1981): Abscisic acid and apical dominance in *Phaseolus coccineus* L. The role of tissue age. *Ann. Bot.* **47:** 371–375.

Hilhorst, H.W.M. & Karssen, C.M. (1992): Seed dormancy and germination: the role of abscisic acid and gibberellins and the importance of hormone mutants. *Plant Growth Regul.* **11:** 225–238.

Hoagland, D.R. & Arnon, D.I. (1950): The water culture method for growing plants without soil. *Coll. Agric. UC Berkeley USA Cal. Agric. Exp. Stn. Circ.* **347:** 1–32.

Hsu, F.C. (1979): Abscisic acid accumulation in developing seeds of *Phaseolus vulgaris* L. *Pl. Physiol.* **63:** 552–556.

Jackson, M.B. & Hall, K.C. (1987): Early stomatal closure in waterlogged pea plants is mediated by abscisic acid in the absence of foliar water deficits. *Pl. Cell. Environ.* **10:** 121–130.

Karssen, C.M., Brinkhorst-van der Swan, D.L.C., Breekland, A.E. & Koornneef, M. (1983): Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. *Planta* **157:** 158–165.

Koornneef, M., Jorna, M.L., Brinkhorst-van der Swan, D.L.C. & Karssen, C.M. (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. (1986): Genetic aspects of abscisic acid. In: Blonstein, A.D. & King, P.J. (eds): *Plant Gene Research—A Genetic Approach to Plant Biochemistry.* 35–54. Springer-Verlag, Vienna.

Koornneef, M., Hanhart, C.J., Hilhorst, H.W.M. & Karssen, C.M. (1989): *In vivo* inhibition of seed development and reserve protein accumulation in recombinants of abscisic acid biosynthesis and responsiveness mutants in *Arabidopsis thaliana. Pl. Physiol.* **90:** 463–469.

MacRobbie, E.A.C. (1991): Effect of ABA on ion transport and stomatal regulation. In: Davies, W.J. & Jones, H.G. (eds): *Abscisic Acid: Physiology and Biochemistry.* 153–168. BIOS Scientific Publishers, Oxford.

Marx, G.A. (1976): 'Wilty': a new gene of *Pisum. Pisum Newslett.* **8:** 40–41.

Nagel, O.W., Konings, H. & Lambers, H. (1991): Growth rate, plant development and water relations of the *sitiens* tomato mutant in relation to abscisic acid. *Abstracts 14th International Conference on Plant Growth Substances* 119.

Neill, S.J. & Horgan, R. (1985): Abscisic acid production and water relations in wilty tomato mutants subjected to water deficiency. *J. exp. Bot.* **36:** 1222–1231.

Parry, A.D., Blonstein, A.D., Babiano, M.J., King, P.J. & Horgan, R. (1991): Abscisic-acid metabolism in a wilty mutant of Nicotiana plumbaginifolia. *Planta* **183:** 237–243.

Piaggesi, A., Perata, P., Vitagliano, C. & Alpi, A. (1991): Level of abscisic acid in integuments, nucellus, endosperm, and embryo of peach seeds (*Prunus persica* L. *cv* Springcrest) during development. *Pl. Physiol.* **97:** 793–797.

Potts, W.C. & Reid, J.B. (1983): Internode length in

Pisum. III. The effect and interaction of the *Na/na* and *Le/le* gene difference on endogenous gibberellin-like substances. *Physiol. Pl.* **57:** 448–454.

Quarrie, S.A. (1982): Droopy: a wilty mutant of potato deficient in abscisic acid. *Pl. Cell Environ.* **5:** 23–26.

Quarrie, S.A. (1987): Use of genotypes differing in endogenous abscisic acid levels in studies of physiology and development. In: Hoad, G.V., Lenton, J.R., Jackson, M.B. & Atkin, R.K. (eds): *Hormone Action in Plant Development.* 89–105. Butterworths, London.

Rock, C.D., Heath, T.G. & Zeevaart, J.A.D. (1991): 2-*trans*-abscisic acid biosynthesis and metabolism of ABA-aldehyde and xanthoxin in wild type and the *aba* mutant of *Arabidopsis thaliana*. *J. exp. Bot.* **43:** 249–256.

Ross, G.S., Elder, P.A., McWha, J.A., Pearce, D. & Pharis, R.P. (1987): The development of an indirect enzyme linked immunoassay for abscisic acid. *Pl. Physiol.* **85:** 46–50.

Ross, G.S. & McWha, J.A. (1990): The distribution of abscisic acid in *Pisum sativum* plants during seed development. *J. Pl. Physiol.* **136:** 137–142.

Saab, I.N., Sharp, R.E., Pritchard, J. & Voetberg, G.S. (1990): Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. *Pl. Physiol.* **93:** 1329–1336.

Setter, T.L. (1990): Transport/harvest index: photosynthate partitioning in stressed plants. In: Alscher, R.G. & Cumming, J.R. (eds): *Stress Responses in Plants: Adaptation and Acclimation Mechanisms.* 17–36. Wiley-Liss, Inc., New York.

Smith, J.D., Fong, F., Magill, C.W., Cobb, B.G. & Bai, D.G. (1989): Hormones, genetic mutants and seed development. In: Taylorson, R.B. (ed.): *Recent Advances in the Development of Germination of Seeds.* 57–69. Plenum Press, New York.

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

Taylor, I.B. & Tarr, A.R. (1984): Phenotypic interactions between abscisic acid deficient tomato mutants. *Theor. Appl. Genet.* **68:** 115–119.

Vermeer, E., Knegt, E. & Bruinsma, J. (1987): Determination of abscisic acid in small amounts of plant material. *J. Chromatogr.* **404:** 346–351.

Waggoner, P.E. & Simmonds, N.W. (1966): Stomata and transpiration of droopy potatoes. *Pl. Physiol.* **41:** 1268–1271.

Wang, T.L., Donkin, M.E. & Martin, E.S. (1984): The physiology of a wilty pea: abscisic acid production under water stress. *J. exp. Bot.* **35:** 1222–1232.

Wang, T.L., Cook, S.K., Francis, R.J., Ambrose, M.J. & Hedley, C.L. (1987): An analysis of seed development in *Pisum sativum*. VI. Abscisic acid accumulation. *J. exp. Bot.* **38:** 1921–1932.

Zeevaart, J.A.D. & Creelman, R.A. (1988): Metabolism and physiology of abscisic acid. *Annu. Rev. Pl. Physiol.* **39:** 439–473.