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ABA-DEFICIENT MUTANTS IN SUNFLOWER (*Helianthus annuus* L.)

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SUMMARY

The use of abscisic acid (ABA)-deficient mutants has contributed to elucidate the connection between the capacity to synthesize ABA and the drought response of plants. This review summarizes the present state of knowledge of two ABA-deficient mutants, recently isolated in sunflower (*Helianthus annuus* L.): non dormant-1 (*nd-1*) and wilty-1 (*w-1*).

The former is an albino mutant defective in carotenoid biosynthesis, induced by *in vitro* tissue culture. This mutation causes photobleaching of chlorophyll pigments, absence of seed dormancy, and inability to accumulate ABA in cotyledons and leaves. Among albino mutants of dicotyledon species, *nd-1* is the first mutant characterized by ABA deficiency.

The latter (*W-1*) is a genotype in which a spontaneous mutation confers a wilty phenotype. The wilting condition of the *w-1* mutant is due to abnormal stomatal behaviour, associated with low levels of endogenous ABA. Exogenous ABA treatments can induce a phenotypic reversion of the mutant. Partial phenotypic reversion was also observed in mutant scions grafted onto wild type rootstocks.

Detached leaves of *w-1* strongly dehydrated with a slight ABA increase. When water stress was imposed to potted mutant plants significant changes in ABA content and in stomatal conductance were found only at very low water potentials. Moreover, *w-1* started to accumulate ABA in the xylem sap and to close stomata when soil water content and leaf water potential were dramatically reduced.

The results suggest that the low endogenous ABA levels and the inability to synthesize the hormone rapidly either in the leaves or in the roots are responsible for the high susceptibility of *w-1* to water stress.

Key words: Sunflower (*Helianthus annuus* L.), ABA-deficient mutants, embryo dormancy, stomatal conductance, water relations.

INTRODUCTION

The high interest in studies on abscisic acid (ABA) is justified by its role in plant growth and development. ABA-deficient or ABA-insensitive mutants can provide an excellent plant material to increase the knowledge of the pathway of ABA synthesis, and to investigate the physiology of ABA in stress adaptation and seed development, two processes in which the hormone has a key role. Besides, the mutants offer a suitable

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Photo 1.Germination of wild type and nd-1 mutant

Photo 2. Germination of progeny heterozygots for the nd-1 mutation

Photo 3. Germination of progeny heterozygots for the nd-1 mutation

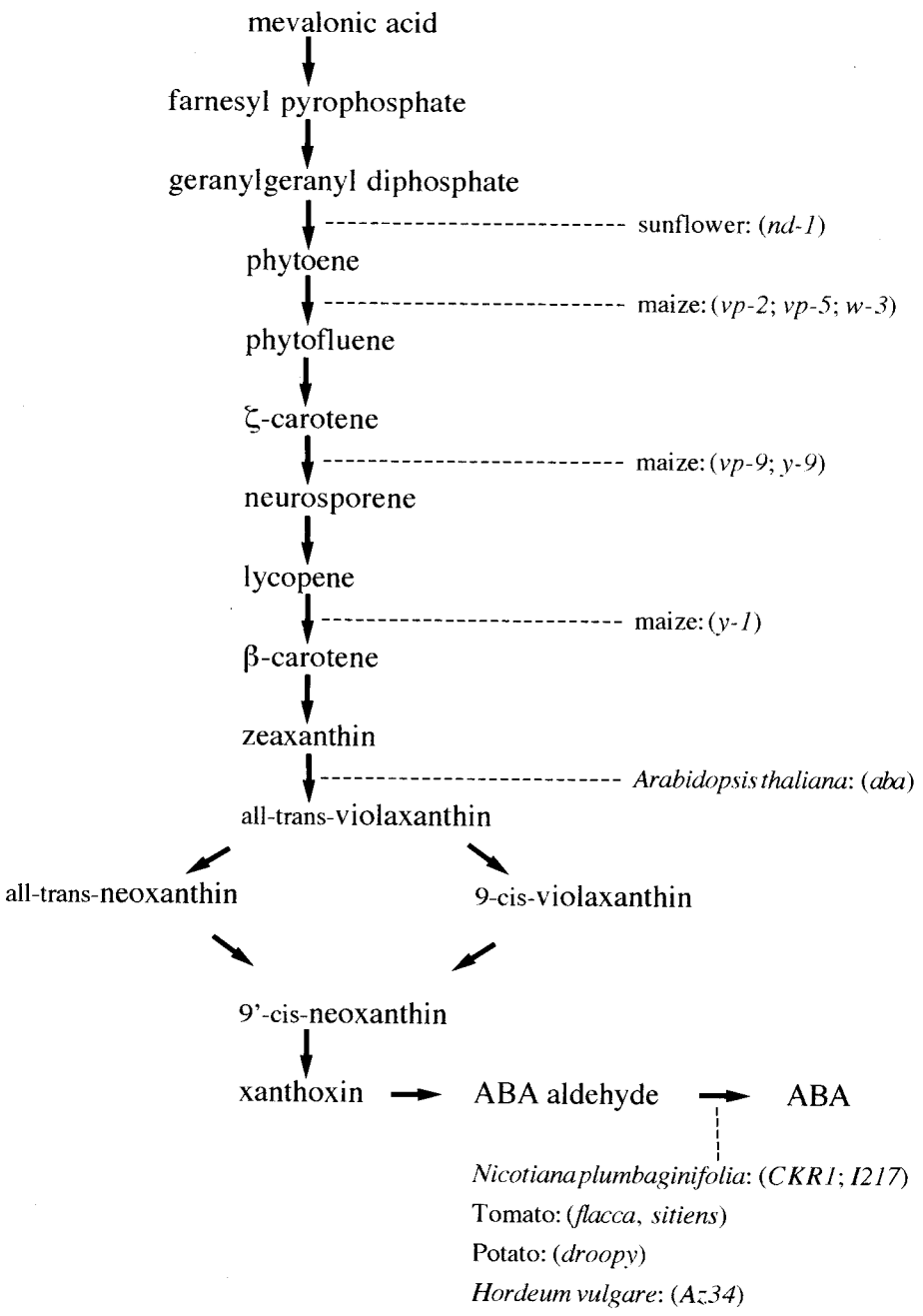

Figure 1. Carotenoid-abscisic acid (ABA) biosynthesis pathway. Not all intermediates are shown. The metabolic blocks in various ABA-deficient mutants are indicated. Adapted from Taylor (1991) and Bartley et al. (1994).

system to understand the mechanism by which ABA influences gene expression (Chandler and Robertson, 1994).

In relation to water deficiency, a plant undergoes a number of physiological and metabolic changes together with an increase in the biosynthesis of ABA. Since the raise of endogenous ABA is an early event after the onset of drought stress, it has been postulated that the hormone acts as a signal for the initiation of regulatory processes involved in adaptation during growth at low water potential (Cowan, 1989; Hartung and Davies, 1991).

Genotypes with low endogenous ABA content and insufficient capacity to synthesize the hormone in response to water stress show excessive transpiration induced by increased stomatal conductance (Quarrie, 1987; King, 1991). Phenotypic reversion of these mutants can be induced by exogenous ABA treatments (Imber and Tal, 1970; Zeevaart and Creelman, 1988).

Moreover, ABA plays an important role in the control of many events during embryogenesis and seed formation, including embryo morphogenesis (Quatrano, 1986), storage proteins synthesis (Black, 1991), desiccation tolerance (Kermode et al., 1986; Meurs et al., 1992), and the onset and maintenance of dormancy (Koornneef, 1986). In mature seed, there are evidences that reserves mobilization activity is regulated by native ABA, while germinability and dormancy depend, in some cases, on the degree of sensitivity to the hormone (Kermode, 1990; Black, 1991). Some of these processes have been clarified by the use of ABA mutants of maize (Robertson,1955; Robichaud et al., 1980; Robichaud and Sussex, 1986; Hattori et al., 1992; Paiva and Kriz, 1994), *Arabidopsis thaliana* (Koornneef et al., 1984 and 1989; Finkelstein and Sommerville, 1990; Meurs et al., 1992; Parcy et al., 1994) and tomato (Groot and Karssen, 1992).

In the present review, ABA-insensitive mutants will not be considered; we will examine the main characteristics of two sunflower (*Helianthus annuus* L.) ABA-deficient mutants recently isolated and characterized. The first, *non dormant-1 (nd-1)*, is defective in carotenoid biosynthesis and shows chlorophyll loss and absence of seed dormancy (Fambrini et al., 1993); the second, *wilty-1 (w-1)*, shows inefficient stomatal control of leaf transpiration in well-watered conditions (Pugliesi et al., 1994) due to the low endogenous levels of ABA and to the inability to synthesize the hormone rapidly in response to water deficit (Fambrini et al., 1994).

MUTATIONS OF THE CAROTENOID BIOSYNTHESIS

In some mutants of monocot-species, low ABA concentrations are accompanied by a large reduction in chlorophyll content and precocious germination. Some of the *viviparous* mutants of maize fall in this category. In these mutants, (e.g., *vp2*, *vp5*, *vp7*, *vp9* and *w3*) the embryo fails to become dormant showing precocious germination on the mother plant. The genetic lesions are identified with specific steps in the carotenoid pathway (Moore and Smith, 1985) and the mutants accumulate low amounts of ABA in embryo and endosperm (Neill et al., 1986). These evidences support the view that carotenoids are precursors of ABA in plants (Zeevaart et al., 1989; Sindhu et al., 1990).

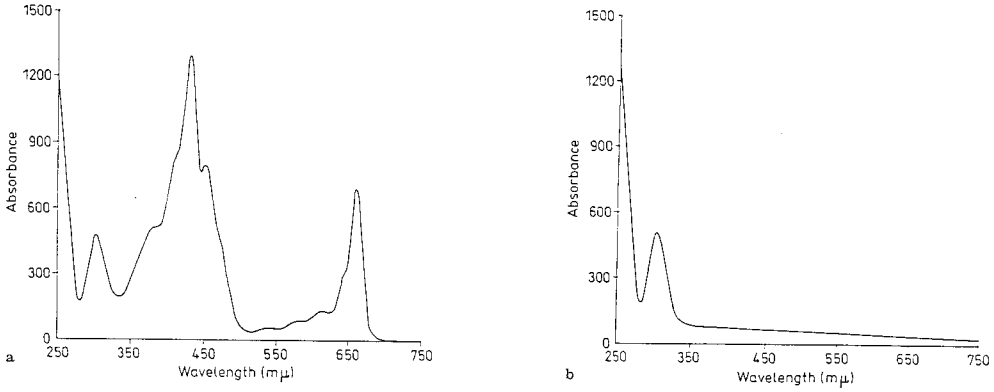

Figure 2. Absorption spectra of green-sib (a) and nd-1 mutant (b) seedlings. Seedlings were grown for 7 days after germination in a growth chamber at 25°C, 16 h photoperiod (180μmol $m^{-2}s^{-1}$). (Data from: Fambrini et al., 1993).

THE *nd-1* MUTANT

In the albino *nd-1* mutant of sunflower induced by *in vitro* tissue culture (Pugliesi et al., 1991), the genetic lesion seems to impair one of the early steps in the carotenoid biosynthesis, resulting in photobleaching, ABA deficiency and the lack of dormancy (Fambrini et al., 1993). In sunflower, a non-dormant, carotenoid-free, white mutant that has not been characterized with respect to ABA was described by Wallace and Habermann (1959).

Table.1. Effect of fluence rates on the chlorophyll content of the *nd-1* mutant and an isogenic line (wt-sibs). Seedlings were grown for 7 days after germination in a growth chamber at 25°C.

Fluence rate (μmol $m^{-2}s^{-1}$	*nd-1* mutant				wt-sibs			
	Proto-	Chl a	Chl b	Total	Proto-	Chl a	Chl b	Total
Total darkness	12.9±1.1 (54.89)	4.4±0.6 (18.72)	6.2±0.7 (26.38)	23.5	13.7±1.2 (64.62)	3.7±0.3 (17.53)	3.8±0.5 (17.92)	21.2
12	15±4.1 (11.11)	100±9.01 (74.07)	20±4.03 (14.81)	135	21±3.2 (10.34)	154±15.1 (75.86)	28±10.9 (13.76)	203
18	0	0	0	0	116±20.03 (8.52)	891±45. (65.51)	353±15.3 (25.95)	1,360

Proto-: Proto-chlorophyll; Chl a: Chlorophyll a; Chl b: Chlorpyll b.
Chlorophyll content: mg/kg fresh weight. Data are means of ten reπλicates±SE.
In brackets:percentage of pigment.
(Data from: Fambrini et al., 1993).

In the green line, the absorption spectra of pigment extract indicate the presence of chlorophyll and carotenoids, while in the *nd-1* mutant no evidence of the presence of any coloured pigments was found (Fig. 2). Pigment extracts from *nd-1* seedlings were fractionated by thin layer chromatography (TLC) and no compound comigrating with phytoene was observed in this system (Fambrini et al., 1993). These data indicate that

carotenoid biosynthesis is presumably arrested in the mutant before the formation of phytoene (Figure 1)(Bartley et al., 1994).

Carotenoids are known to act as accessory light-harvesting pigments, effectively extending the range of light absorbed by the photosynthetic apparatus. Besides, the photoprotective role of these pigments is essential for the organism's survival, providing protection against light-mediated stresses (Koyama, 1991; Young, 1991). In the *nd-1* mutant after a prolonged exposure to weak light (12μmol $m^{-2}s^{-1}$), chlorophyll content is 2/3 of that in green sibs and there is little or no difference between them regarding the clorophyll a/chlorophyll b ratio (Table 1), while chlorophyll is destroyed under high light intensity (180 μmol $m^{-2}s^{-1}$) (Fambrini et al., 1993).

As previously observed in corn (Moore and Smith, 1985) and *Arabidopsis* (Duckham et al., 1991), the relationship between the blocks in the carotenoid pathway and the effects on ABA has provided strong arguments for the indirect C_{40} (apo-carotenoid) route (Li and Walton, 1990; Duckham et al., 1991; Rock and Zeevaart, 1991; Parry and Horgan, 1992). Moreover, inhibitors of carotenoid biosynthesis, such as fluridone and norflurazon, which block the conversion of phytoene to phytofluene, also inhibit accumulation of ABA (Fong et al., 1983; More and Smith 1985; Oishi and Bewley 1992).

As expected, Fambrini et al., (1993) have shown that the endogenous ABA content in *nd-1* cotyledons and leaves is lower than that in the isogenic line and the capacity to accumulate the hormone in response to water stress is lacking (Table 2).

Table 2. Levels of ABA (ng g^{-1} fresh weight) in non-stressed (NS) and stressed (S) cotyledons and leaves of the *nd-1* mutant and its green sibs. Stress treatments (by detachment) were prolonged until the fresh weight of the tissues were about 50% of the initial.

Genotype	Tissue	Treatment	
		NS	S
nd-1 mutant	Cotyledons	5.1±0.5	4.3±0.5
	Leaves	2.2±0.6	0.5±0,2
Green sibs	Cotyledons	10.3±1.6	29.3±1.4
	Leaves	13.5±0.9	30.8±1.5

Data are means of ten replicates±SE.
(Data from: Fambrini et al., 1993).

Table 3. Summary of genetics results from the R_3 generation of the albino phenotype.

Growing conditions	Number of independent R_3 progenies	Number of selfed progeny plants		
		Green	Albino	x^2(3:1)
Field	7	935	148 (13.7)	84.2***
Greenhouse	21	2140	660 (23.6)	5.8

In brackets the percentage of albino seedlings.
***P>0.001.
(Data from: Fambrini et al., 1993).

Although the strong reduction of ABA level in the *nd-1* mutant did not show significant influence on seed development, the mutation caused vivipary and lack of dormancy in ripe and mature seed (Fambrini et al., 1993). In self-pollinated, field-grown heterozygous plants, before harvest, in the presence of high relative humidity, *nd-1* seeds showed premature germination on the head. These seedlings survived for 2-3 days, until

death was caused by the drying of the head. The percentage of albino seeds in heterozygous heads (13.67%) was not consistent with single recessive ratio as shown by the chi-square (x^2) test (Table 3). On the other hand, the segregation data for the seeds from self-pollinated plants grown in greenhouse, under controlled environmental conditions, gave an almost perfect single recessive ratio (Table 3). Moreover, when green seedlings derived from plants grown in the field were self-pollinated, the number of heads segregating for the albino character showed a ratio between heterozygous and homozygous plants of 1.97; this data confirmed that the albino phenotype is controlled by a single recessive gene (Table 3) (Fambrini et al., 1993).

Studies with ABA-deficient mutants of *Arabidopsis thaliana* (Karssen et al., 1983, Karssen and Laçka, 1986) and tomato (Groot and Karssen, 1992) provided clear evidence that the induction of dormancy, acquired during the development of seeds on the mother plant, depends on an increase of endogenous ABA. Furthermore, Le Page-Degivry et al., (1990) showed that in sunflower the endogenous ABA level increased sharply during embryo development, and an application of fluridone, an inhibitor of carotenoid biosynthesis, prevented both ABA synthesis and development of embryo dormancy. From this consideration Fambrini et al., (1993) drew the conclusion that in the *nd-1* mutant the lack of dormancy is correlated to the lack of ABA synthesis in developing embryos and it gave rise to the hypothesis that ABA is a breakdown product of carotenoid.

WILTY MUTANTS

Mutants with low endogenous content of ABA and reduced capacity to accumulate the hormone in response to water stress have been isolated from several species (Koornneef, 1986; Quarry, 1987; Taylor, 1991).

ABA-deficient mutants show wilty phenotype due to their abnormal stomatal behaviour. The best-characterized ABA-deficient mutants are the *notabilis (not), flacca (flc)* and *sitiens (sit)* mutants of tomato (Tal, 1966; Tal and Nevo, 1973; Neill and Horgan, 1985). Wilty ABA-deficient mutants of potato (*droopy:* Quarrie, 1982), pea (*wilty* Wang et al., 1984), *Arabidopsis thaliana (aba*: Koornneef et al., 1982), *Nicotiana plumbaginifolia* (*CKR1*: Bitoun et al., 1990; *I217*: Blonstein et al., 1991; *Esg 152*: Rousselin et al., 1992), *Hordeum vulgare* (*Az34*: Walker-Simmons et al., 1989) have also been reported.

Unlike *viviparous* albino mutants, wilty genotypes are viable, because they contain normal levels of carotenoids and are impaired in the last steps of ABA biosynthesis (Taylor, 1991: Reid, 1993). The tomato mutants *flacca* and *sitiens* are blocked at the oxidation step of ABA-aldehyde to ABA (Parry et al., 1988; Taylor et al., 1988; Sindhu and Walton, 1988). Similarly, the *droopy* mutant of potato and two ABA-deficient mutants of *Nicotiana plumbaginifolia* were also found to be unable to carry out this step and may code for the same gene product as *sitiens* (Parry et al., 1990; Duckham et al., 1989). On the contrary, *notabilis* possesses an enzyme with reduced substrate specificity which cleaves more all-*trans*- than 9'-*cis*-neoxanthin (Parry et al., 1992). The molybdenum cofactor mutant (*Az34*) was shown to cause a change in the activity of three enzymes of ABA biosynthesis (Walker-Simmons et al., 1989). In *Arabidopsis thaliana*, the wilty phenotype displayed by ABA deficient mutants (*aba-1*, -3 and-4) (Koornneef et al., 1982) is the result of a small ABA precursor pool of compounds that contain

oxygens on the ring. Quantitation of the carotenoids from mutant and wild type leaves of *Arabidopsis* showed that the mutations cause a deficiency of the epoxy-carotenoids violaxanthin and neoxanthin and an accumulation of their biosynthetic precursor, zeaxanthin (Rock and Zeevaart, 1991; Duckham et al., 1991).

THE *w-1* MUTANT

1) BEHAVIOUR OF THE *w-1* MUTANT IN WELL-WATERED CONDITIONS

The *w-1* sunflower mutant tends to wilt rapidly in the field, even in well-watered conditions as compared with other inbred lines (Pugliesi et al., 1994). The wilting was the result of excessive transpiration induced by increased stomatal conductance, in both light and dark cycles (Figure 3). The stomatal behaviour of *w-1*, in the light/dark cycles in well-watered conditions, resembles the situation found by Tal and Imber(1972) in the wilty mutant of tomato in which the stomata tend to open wider and to resist closure in darkness.

The genetic analysis has shown that the wilty phenotype is due to a recessive nuclear mutation at a single locus (Puglesi et al., 1994).

Levels of endogenous ABA, water potential (Ψ_w), osmotic potential (Ψ_π), turgor potential (Ψ_p and relative water content (RWC) (Table 4) were substantially lower in *w-1* than in control line (W-1). These observations taken together have suggested that the maintenance of open stomata during the dark cycles is caused by an insufficient amount of ABA (Pugliesi et al., 1994).

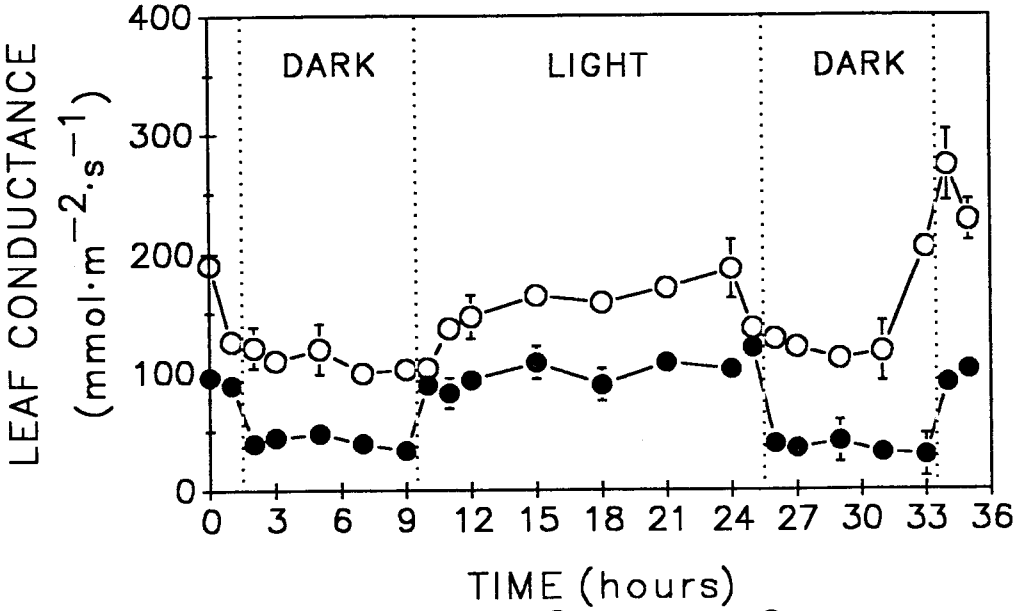

Figure 3. Changes in leaf conductance in mutant w-1 plants (○) and normal plants (●), during light/dark cycles. Conductance was determined on 3-week old plants grown in a growth chamber at 25°C, 1.0-1.1 kPa Vapour Pressure Deficit, 16 h photoperiod (180 μmol $m^{-2}s^{-1}$). Data are means of ten replicates±SE. (Data from: Pugliesi et al., 1994).

Table 4. ABA concentration, relative water content (RWC), water potential (Ψw), osmotic potential (Ψπ) and turgor potential (Ψp) of mutant (*w-1*) and normal (W-1)plants, in light or dark, in well - watered conditions. Plants were grown in a growth chamber at 25°C, 1.0-1.1 kPa Vapour Pressure Deficit (VPD), 16 h photoperiod (180 μmol $m^{-2}s^{-1}$).

	w-1		W-1	
	Light	Dark	Light	Dark
ABA (ng g^{-1} DW)	63.5±2.4 a	64.1±2.0 a	179.3±12.2 b	183.3±10.3 b
RWC (%)	81.8±0.5 a	82.1±0.5 a	88.4±0.5 b	89.3±0.5 b
Ψw (MPA)	-0.83±0.02 a	-0.81±0.03 a	-0.58±0.02 b	-0.57±0.02 b
Ψπ(MPa)	-0.95±0.02 a	-0.95±0.03 a	-0.79±-0.03 b	-0.80±-0.03 b
Ψp (MPa)	0.12±0.006 a	0.14±0.005 a	0.21±0.007 b	0.23±0.009 b

Values are means±SE of ten measurements; Values within the same line followed by the same letter are not different at the 0.001 level of significance.
(Data from: Pugliesi et al., 1994).

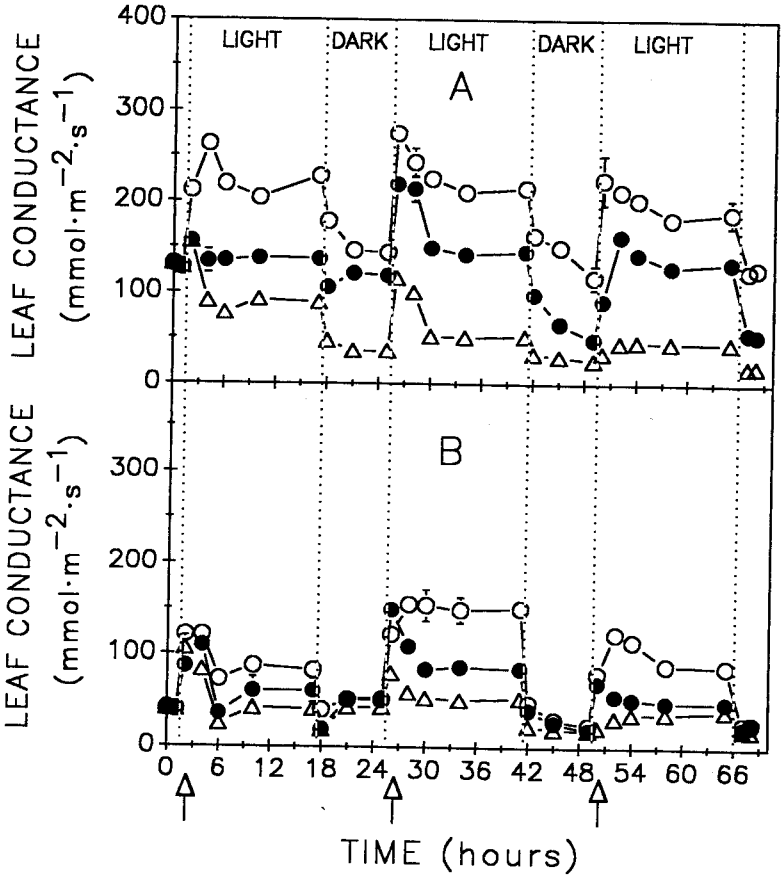

Figure 4. Effect of ABA treatments (●: 10^{-5} M; △: 10^{-4} M; ○: control) on leaf conductance of mutant w-1 plants (A) and normal plants (B), during light/dark cycles. Arrows indicate time of ABA applications. Conductance was determined on 3-week old plants grown in a growth chamber at 25°C, 1.0-1.1 kPa Vapour Pressure Deficit, 16 h photoperiod (18 μmol $m^{-2}s^{-1}$). Data are means of then replicates ±SE. (Data from: Pugliesi et al., 1994).

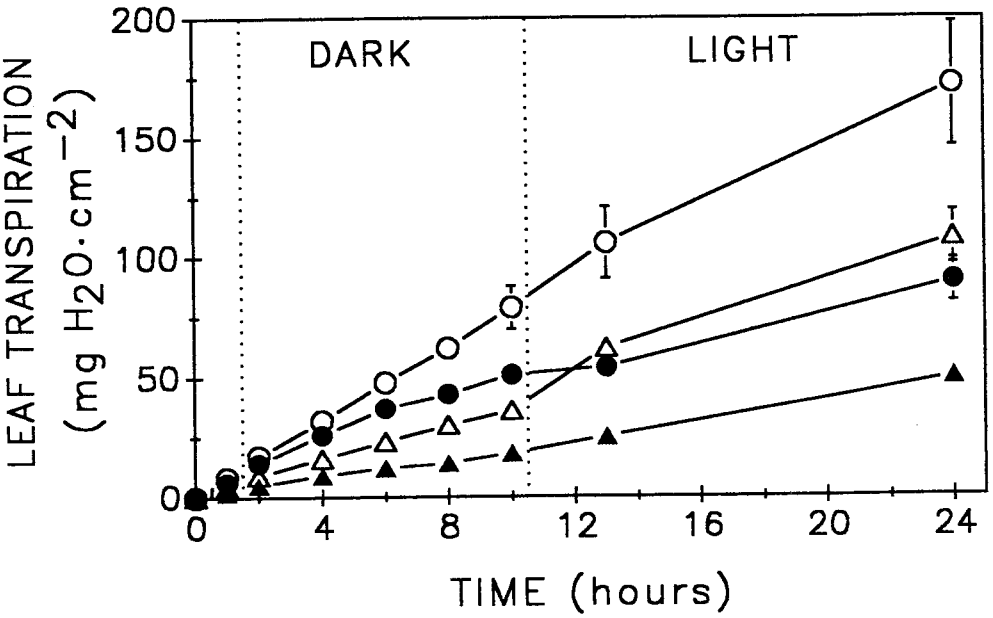

Figure 5. Effect of ABA (10^{-4}M) on transpiration rate of detached leaves of mutant (w-1) plants and normal (W-1) plants. (●: w-1 +ABA); (▲: W-1 + ABA); (○ : w-1 + water): (△ : W-1 + water). Transpiration rate was determined in the growth chamber at 25°C, 1.0-1.1 kPa Vapour Pressure Deficit, 16 h photoperiod (180 µmol $m^{-2}s^{-1}$). Data are means of ten replicates ±SE. (Data from: Pugliesi et al., 1994).

Wilty mutant that contain reduced levels of ABA can be phenotypically reverted by foliar treatments with ABA solutions (Imber and Tal, 1970; Tal and Nevo, 1973). The same response was observed in sunflower plant when ABA was applied by foliar spray (Pugliesi et al., 1994). In fact, stomatal conductance in leaves of the *w-1* genotype decreased proportionally with the hormone concentration either in the dark or light cycles (Figure 4A).A similar qualitative response was also obtained in the *w-1*genotype after foliar spray (Figure 4B). Besides, ABA application caused a reduction in the rate of transpiration by inhibiting stomatal opening (Figure 5). The transpiration rate of *w-1* leaves treated with ABA was still as great as that of control W-1 leaves; nonetheless, the results clearly demonstrate that the stomata of *w-1* leaves can respond to exogenous ABA by closing (Pugliesi et al., 1994).

Stomata of sunflower respond directly to the increase in leaf ABA, or to a signal from the roots (presumably ABA) under conditions of soil drought (Neales et al., 1989; Neales and McLeod, 1991; Zhang and Davies, 1989 and 1990), while the response of stomata to light-to-dark transition is likely due to a redistribution of existing ABA in the leaf (Zeevaart and Creelman, 1988) without any *de novo* synthesis. In both genotypes (*w-1* and W-1), no consistent light/dark trends were apparent in leaf ABA levels which, taken overall, remained constant (Table 4). These results confirm that the light-to-dark transition of stomatal movements and the stomatal closure under water stress share different mechanisms (Pugliesi et al., 1994).

2) RESPONSE OF THE *w-1* MUTANT TO WATER STRESS

ABA plays a key role in plant response to water deficit so, the behaviour of the *w-1* mutant to drought condition has been compared with the control line W-1 (Fambrini et al., 1994).

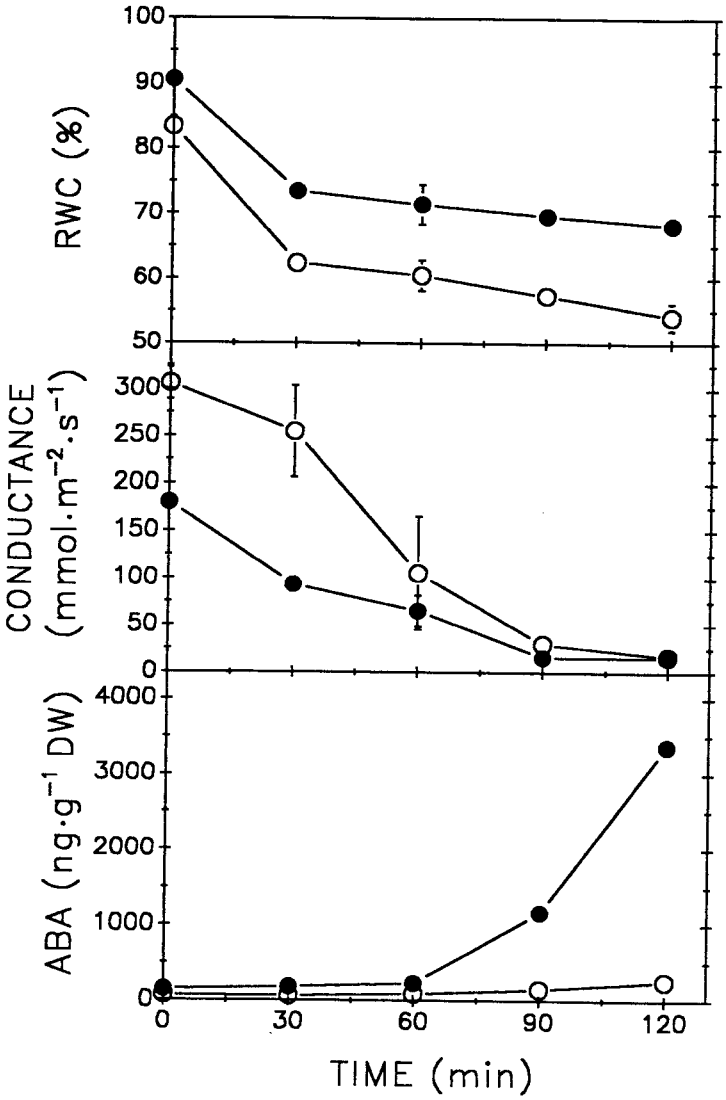

Figure 6. Changes in relative water content (RWC), leaf conductance and ABA content in detached leaves of the w-1 mutant (○) and wild type (●) placed on a bench at 21 ±°C, under light (180 μmol $m^{-2}s^{-1}$), with the abaxial surfaces uppermost and allowed to dehydrate. Data are means of ten replicates ±SE. (Data from: Fambrini et. al., 1994).

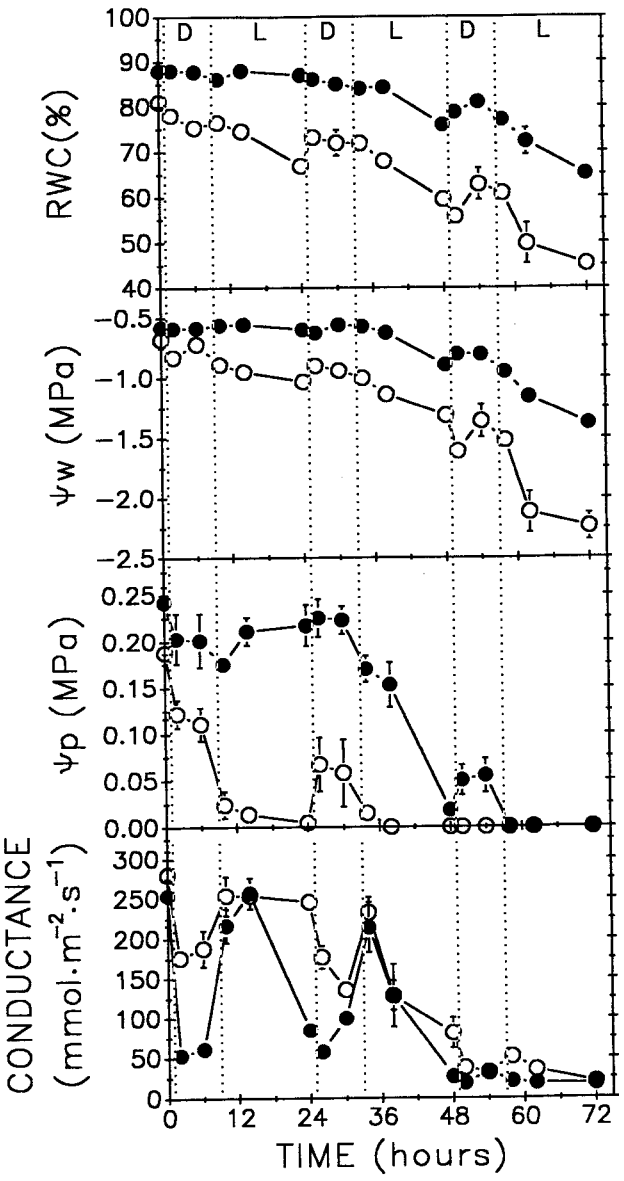

Figure 7. Changes in relative water content (RWC), water potential (Ψ_w), turgor potential (Ψ_p) and leaf conductance in potted plants of the w-1 mutant (○) and wild type (●) subjected to withholding water for 72 h. Stress treatment was carried out in a growth chamber at 21°C, 0.7 kPa Vapour Pressure Deficit, 16 h photoperiod (180μmol $m^{-2}s^{-1}$). Data are means of ten replicates ±SE. (Data from: Fambrini et al., 1994).

Experiments conducted with detached leaves (Fambrini et al., 1994) have indicated the inability of *w-1* to promptly respond to water loss by closing stomata (Figure 6). Leaves of the wild type also lost water during this period, but rapidly diminished stomatal aperture thus reducing the severity of the stress (Figure 6). The early stomatal closure in the wild type (W-1) was not associated with an increase in ABA levels, which started to rise only when leaf conductance was already very low. This agrees with previous reports

(Radin and Ackerson, 1982; Pardossi et al., 1992) and strengthens the idea that endogenous ABA has to be redistributed in response to water stress to induce the closure of stomata, while de novo ABA biosynthesis act to maintain stomata closed (Zeevaart and Creelman, 1988; Morgan, 1990; Harris and Outlaw, 1991). In detached leaves of the *wilty* mutant of pea, Wang et al., (1984) observed that ABA accumulation was completely inhibited. Leaves of *w-1* showed only a slight increase in ABA content after 2 h from detachment, indicating that this genotype is not able to respond to a very rapid water stress by accumulating ABA (Figure 6). Nevertheless this result suggests that *w-1* is a "leaky" mutant capable of limited ABA biosynthesis (Pugliesi et al., 1994; Fambrini et al., 1994). Stress-induced ABA accumulation was not found in detached leaves of *sitiens* and *flacca* tomato mutants, while *notabilis* showed a significant rise in ABA content 3h

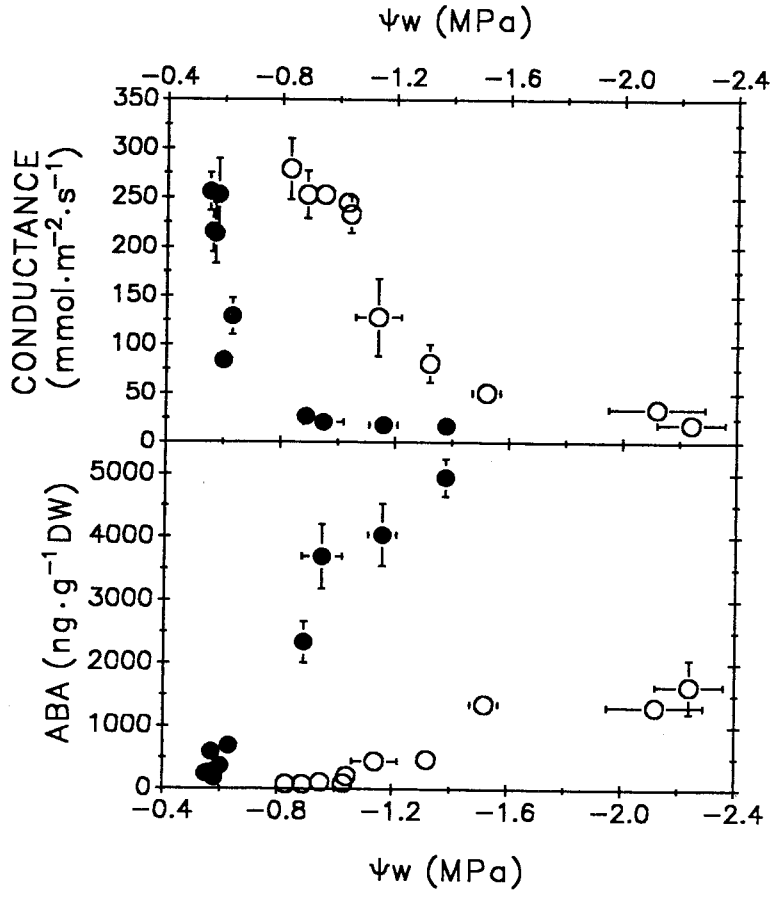

Figure 8. Relationships among leaf conductance, leaf ABA content and water potential (Ψw) in potted plants of the w-1 mutant (○) and wild type (●) subjected to withholding water. Stress treatment was carried out in a growth chamber at 21±C, 0.7 kPa Vapour Pressure Deficit, 16 h photoperiod (180 μmol^{-2}s^{-1}). Data are means of ten replicates±SE. (Data from: Fambrini et al., 1994).

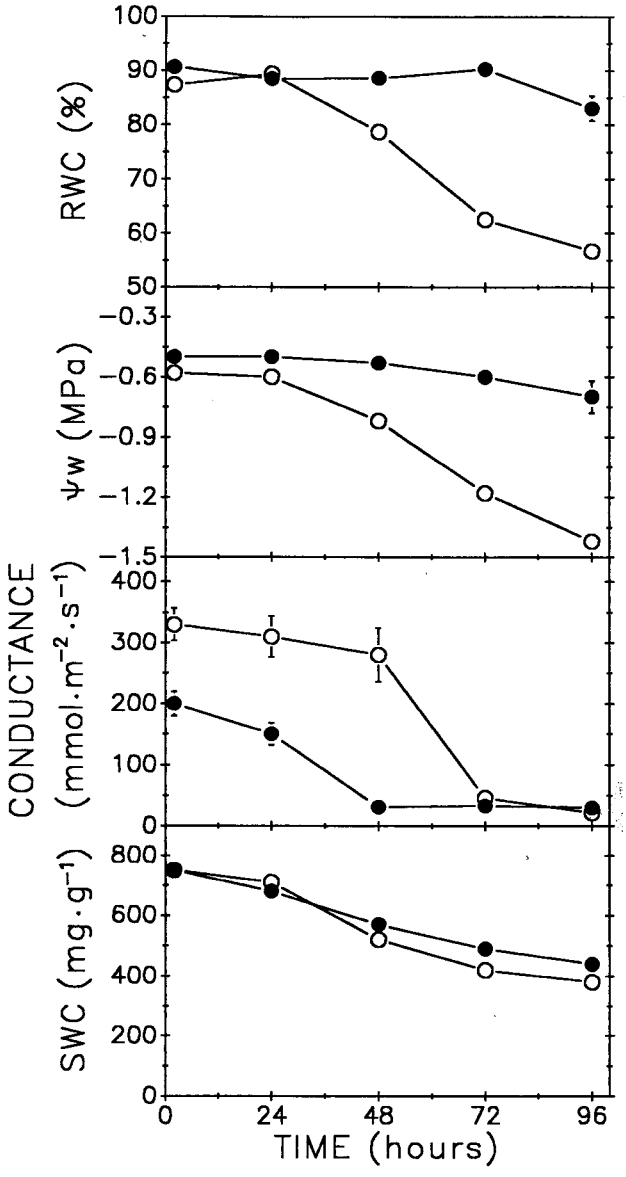

Figure 9. Changes in relative water content (RWC), water potential (Ψw), leaf conductance and soil water content (SWC) in potted plants of the w-1 mutant (○) and wild type (●) during 96 h of withholding water. Stress treatment was carried out in a growth chamber at 21°C, 0.2 kPa Vapour Pressure Deficit, 16 h photoperiod (180 μmol $m^{-2}s^{-1}$). Data are means of ten replicates ±SE. (Data from: Fambrini et al., 1994).

from detachment (Neill and Horgan, 1985). As far as the response to a very rapid stress is concerned, the *w-1* mutant seems to be similar to *notabilis* tomato mutant, but the different conditions under which water stress was imposed by Neill and Horgan (after 10% fresh weight loss leaves were sealed in plastic bags) have to be taken into account.

When drought stress was imposed to potted plants by withholding water (Fambrini et al., 1994), wild type plants maintained high values of RWC and Ψ_w until 36 h from

stopping irrigation, and during this period light-dark transitions caused a strong reduction in stomatal conductance; on the contary *w-1* leaves rapidly dehydrated as indicated by the drop in RWC and Ψ_w (Figure 7). The reduction in conductance during the dark period could partly explain the better control of leaf transpiration of W-1, which allowed this genotype to retain hydration for a longer period. Neither *w-1* nor the wild type showed active osmotic adjustment during the experiment; this suggests that the different sensitivity to water stress between the two genotypes mainly relies upon the inability of the mutant to reduce water losses by rapidly closing stomata.

Potted plants of *wilty* pea (Wang et al., 1984) and *notabilis* are able to accumulate ABA when slowly dehydrated, while in *flacca* and *sitiens* tomato water deprivation does not trigger ABA accumulation (Neill and Horgan, 1985). A similar situation was also observed in the ability of the two genotypes of *H. annuus* to synthesize ABA: in *w-1*, a noticeable increase in the concentration of the hormone was reached only when Ψ_w fell below -1.5 MPa (Figure 8), whereas in W-1 a strong rise in leaf ABA levels was induced by a relatively small reduction in Ψ_w (from -0.6 to -0.9 MPa). Moreover, the capacity of *w-1* to accumulate large amounts of ABA only when subjected to a slow dehydration confirms the "leaky" nature of this mutant (Fambrini et al., 1994).

In some plant species (for instance, maize and sunflower), strong evidences indicate that ABA (and/or other signals, produced by roots in drying soil) moves to the shoots through the transpiration stream and accumulates in guard cells, initiating the closure of stomata before any change in the leaf water potential occurs (Zhang and Davies 1989; Davies and Zhang, 1991; Munns and Sharp, 1993; Davies et al., 1994).

In experiments based on slow soil drying (Fambrini et al., 1994), wild type (W-1) stomata showed a fast response to changes in soil water content, even in the absence of

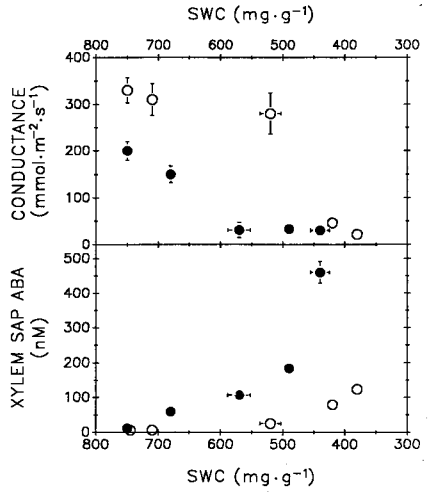

Figure 10. Relationships among leaf conductance, xylem sap ABA concentration and soil water content (SWC) in potted plants of the w-1 mutant (○) and wild type (●) subject to withholding water. Stress treatment was carried out in a growth chamber at 21°C, 0.2 kPa Vapour Pressure Deficit, 16 h photoperiod (180 μmol $m^{-2}s^{-1}$). Data are means of ten replicates ±SE. (Data from: Fambrini et al., 1994).

any appreciable symptom of leaf water deficit (Figure 9). On the contrary, stomata of *w-1* showed a slighter response to modifications of soil water content and a significant reduction in leaf conductance was detectable only in combination with an evident leaf dehydration. Regarding this different response, Fambrini et al., (1994) suggested the involvement of a root signal which is either lacking or poorly efficient in the mutant. Indeed the concentration of ABA in the xylem sap of the wild type plants increased promptly when soil water content started to decrease and the rise in the hormone concentration corresponded to a the reduction in leaf conductance (Figure 10). Also in *w-1* xylem sap ABA concentration showed a build up coincident with stomatal closure, but this phenomenon occurred after several hours of stress, when SWC and Ψ_w were already dramatically diminished.

3) PHENOTYPIC REVERSION OF THE *w-1* MUTANT BY GRAFTING

Grafts from genotypes with different endogenous ABA levels have helped to establish the role of roots in water stress perception and adaptation, especially the relative importance of the root and the shoot in controlling stomatal conductance, water potential and ABA level (Simmonds, 1965; Tal et al., 1970; Wang et al., 1984; Jones et al., 1987; Cornish and Zeevaart, 1988; Jackson, 1991).

Partial phenotypic reversion was observed in scions of *w-1* mutant grafted onto a wild type rootstock (Fambrini et al., 1995). In fact, in well-watered conditions, RWC, Ψ_w, and ABA levels were higher in *w-1*/W-1 grafts than the control (*w-1*/*w-1*) (Table 5) and stomata of *w-1*/W-1 grafts responded to light-to-dark transition by partial closure (Figure 11). Stomatal closure in dark seems to be mediated by a redistribution of the existing ABA in the leaf not depending upon *de novo* synthesis of the hormone (Hartung et al., 1988; Zeevaart and Creelman, 1988). Thus, the increase in basal leaf ABA content shown by *w-1*/W-1 (Table 5) may be responsible for the partial stomatal closure in the dark and, consequently, for the reduction in leaf transpiration that results in higher RWC and Ψ_w values in well-watered plants. By contrast, the mutant rootstock did not affect significantly the conductance of the normal scions. This seems attributable to the fact that leaf ABA level of this graft did not differ significantly from those of W-1/W-1 (Fambrini et al., 1995). Similar results were obtained in reciprocal grafts of tomato mutants (Tal et al., 1970; Jones et al., 1987).

Table 5. Leaf relative water content (RWC), leaf water potential (Ψw) and leaf ABA content in grafted mutant (*w-1*) and wild type (W-1) plants under well - watered conditions. Grafts were grown in a growth chamber at 21°C, 0.7 kPa Vapour Pressure Deficit, 16 h photoperiod (180 μmol $m^{-2}s^{-1}$).

Graft (scion/stock)	RWC (%)	Ψw(MPa)	ABA (ng g^{-1} DW)
w-1/*w-1*	78.2±1.7	-0.74±0.02	56±4.9
w-1/W-1	86.8±1.4	-0.59±0.01	130±10.4
W-1/W-1	89.5±1.2	-0.52±0.02	200±16.3
W-1/*w-1*	90.7±1.3	-0.52±0.03	186±18.0
Data are means of ten replicates±SE. (Data from Fambrini et al., 1995).			

In 1970, Tal et al. have shown that detached leaves of *flacca* scions grafted onto wild type stocks exhibited lower water loss than the mutant grafted onto itself. A similar

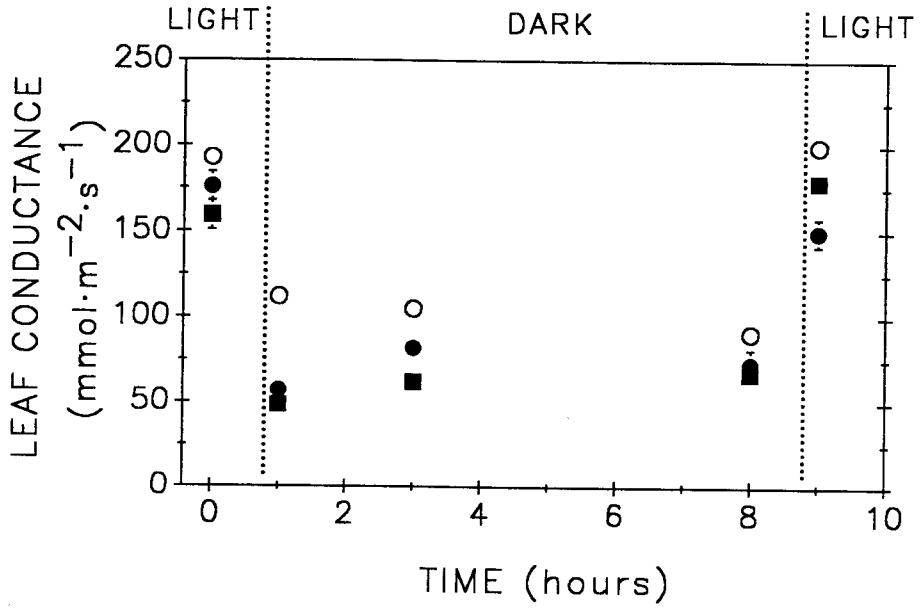

Figure 11. Changes in leaf conductance in grafted mutant (w-1) and wild type (W-1) plants during light-dark cycles. Grafts were (scion/stock): ○: w-1/w-1; ●: w-1/W-1; ■: W-1/W-1. Data are means of ten replicates ±SE. (Data from: Fambrini et al., 1995).

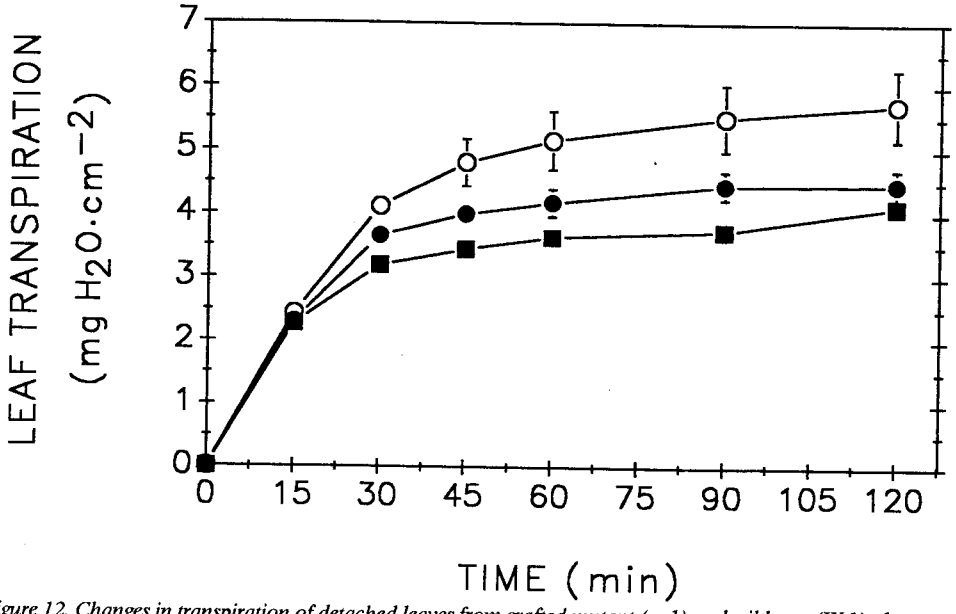

Figure 12. Changes in transpiration of detached leaves from grafted mutant (w-1) and wild type (W-1) plants. Leaves were placed on a bench at 21°C, under light (180 μmol m^{-2} s^{-1}), with the abaxial surfaces uppermost and allowed to dehydrate. Grafts were (scion/stock): ○: w-1/w-1; ●: w-1/W-1; ■: W-1/W-1. Data are means of ten replicates ±SE. (Data from: Fambrini et al., 1995).

situation was observed in detached leaves from *w-1* scions grafted onto the control stock (*w-1*/W-1) (Figure 12). On the contrary, scions grafted onto their own rootstocks maintained the behaviour of the non-grafted plants. However, water loss from detached leaves of the *w-1* mutant developed on normal roots was still higher than the one observed in W-1/W-1 leaves (Figure 12).

When drought stress was imposed on potted grafted plants, by withholding water (Fambrini et al., 1995), *w-1/w-1* plants were not able to control water loss by closing stomata and rapidly dehydrated; on the contrary, the mutant scions grafted onto the wild

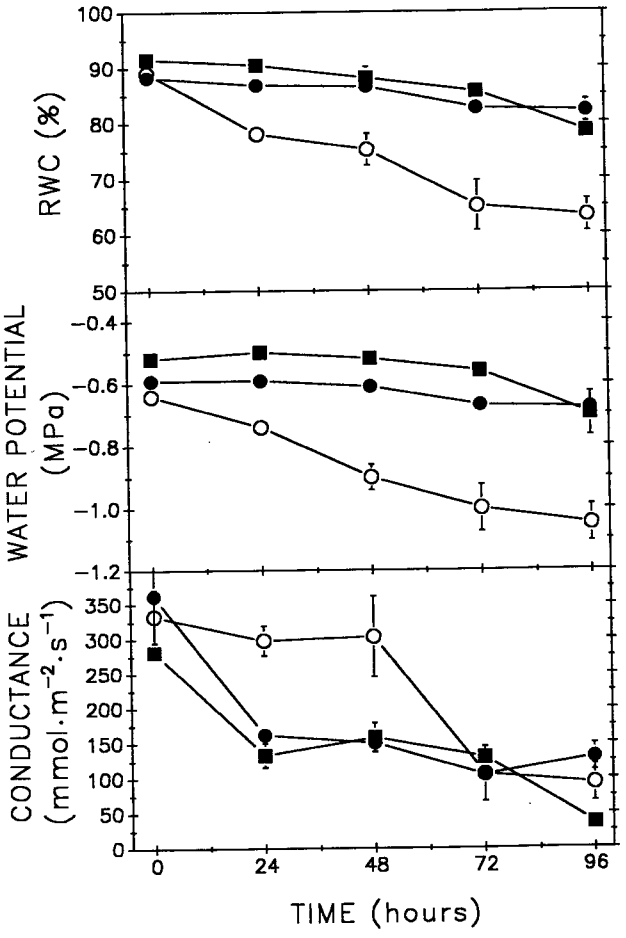

Figure 13. Changes in relative water content (RWC), water potential (Ψw) and leaf conductance in grafted mutant (w-1) and wild type (W-1) plants subjected to withholding water for 96 h. Stress treatment was carried out in a growth chamber at 21°C, 0.7 kPa Vapour Pressure Deficit, 16 h photoperiod (180 µmol $m^{-2}s^{-1}$). Grafts were (scion/stock): ○: w-1/w-1; ●: w-1/W-1; ■: W-1/W-1. Data are means of ten replicates ±SE. (Data from: Fambrini et al., 1995).

type rootstock responded similarly to the W-1/W-1 plants showing a rapid reduction in stomatal conductance and maintaining high values of RWC and Ψ_w throughout the experimental period (Figure 13).

In W-1/W-1 grafts, a small decrease in Ψ_w, from-0.50 to -0.56 MPa, caused a marked stomatal closure and a rise in ABA levels (Figure 14). In *w-1/w-1* grafts, a strong reduction in stomatal conductance and a significant increase in leaf ABA content were observed only when Ψ_w decreased below -0.9 MPa. Bu contrast, stressed grafts with mutant scions grafted onto control stocks (*w-1/W-1*) showed appreciable changes in ABA levels and stomatal conductance in correspondence to a decrease in Ψ_w from -0.6 to -0.7 MPa (Figure 14) though leaf ABA levels in this graft type did not reach the values found in normal leaves (W-1/W-1). The W-1 rootstock seems to "sensitize" leaves of *w-1* to water stress, inducing a rapid stomatal closure. The early stomatal closure observed in W-1/W-1 and *w-1*/W-1 before any significant change in leaf water potential and ABA content could suggest the involvement of a root signal. On the other hand, the observation that W-1/*w-1* grafts behaved as W-1/W-1 (data not shown) apparently does not support this view, but rather points out the importance of the basal level of leaf ABA.

To explain the mechanism through which wild type roots allow a higher ABA level in leaves of well-watered *w-1* scions and a rapid rise of the hormone in *w-1*/W-1 subjected to water deficit, some hypotheses can be formulated.

Sunflower roots are able to synthesize ABA in response to water deficit (Robertson et al., 1985; Neales and McLeod, 1991). We suggested that the partial phenotypic

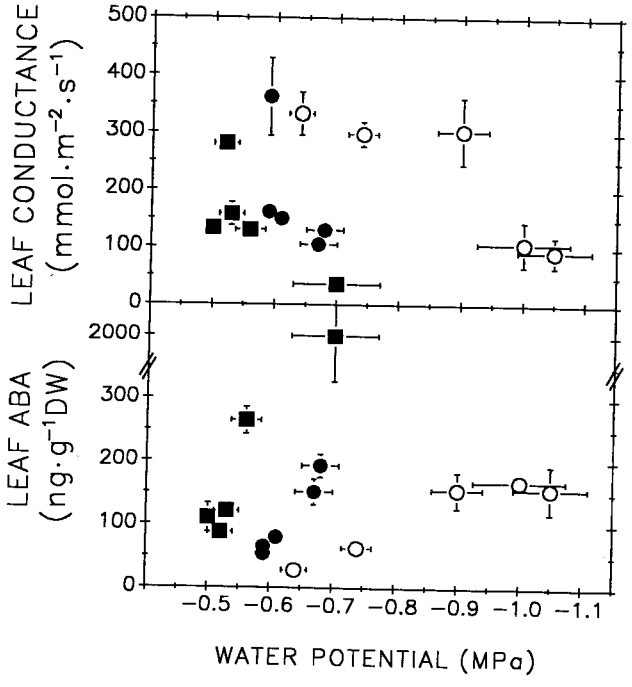

Figure 14. Relationships among leaf conductance, leaf ABA content and leaf water potential (Ψw) in grafted mutant (w-1) and wild type (W-1) plants subjected to with-holding water. Stress treatment was carried out in a growth chamber at 21°C, 0.7 kPa Vapour Pressure Deficit, 16 h photoperiod (180 $\mu mol\ m^{-2}s^{-1}$). Grafts were (scion/stock): ○: w-1/w-1; ●: w-1/W-1; ■: W-1/W-1. Data are means of ten replicates ±SE. (Data from: Fambrini et al., 1995).

reversion observed in *w-1/W-1* plants could be due to the higher amount of ABA synthesized by the normal rootstock, which migrates through the transpiration stream reaching the leaf tissues (Fambrini et al., 1995). This could be in agreement with the results obtained with barley and cotton plants grown in high salinity, where the roots were the source of at least a part of the ABA found in the leaves (Kefu et al., 1991).

On the other hand, *w-1* is a "leaky" mutant able of limited ABA biosynthesis, probably as a consequence of inefficient enzymatic activity in a step of the hormonal biosynthetic pathway. Another possibility is, therefore, that an ABA precursor, following the enzymatic block, is provided by normal roots through the transpiration stream and successively, converted in ABA to *w-1* leaves.

CONCLUSION

The study of the role of ABA in several physiological processes and in plant development, can be facilitated by the use of genotypes differing in endogenous ABA levels. The ABA-deficient mutants *nd-1* and *w-1*, which were recently isolated and characterized, represent a useful plant material to investigate, in sunflower, the importance of this hormone in seed physiology and in the response to water deficit.

The inability to produce ABA of *nd-1* albino mutant provides evidence of an indirect pathway of hormone biosynthesis in this species; furthermore, the *viviparous* phenotype of this mutant supported the involvement of ABA in the dormancy of sunflower embryo.

The *w-1* is a "leaky" mutant suitable to cast light on the relationships between endogenous ABA levels and the plant response to unfavourable environmental conditions. This high sensitivity of *w-1* to water stress, like in the other wilty mutants, the high sensitivity of *w-1* to water stress is largely dependent upon the inefficient stomatal control of leaf transpiration. This abnoraml stomatal behaviour is due to the low endogenous levels of ABA and to the inability to rapidly synthesize the hormone, in root and leaves, in response to water deficit.

We concluded that the ABA-deficient mutants of sunflower discussed in this reivew can offer an excellent system for assessing the effects of endogenous ABA on the mechanism of transduction between environmental signals and gene expresson.

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MUTANTES DEFICIENTES EN ABA EN GIRASOL (*Helianthus annuus* L.)

RESUMEN

El uso de mutantes deficientes de ácido abcísico (ABA) ha contribuido a elucidar la conexión entre la síntesis de ABA y las respuestas a la sequía de las plantas. Esta revisión resume el presente estado de conocimiento de dos mutantes deficientes de ABA, recientemente aislados en girasol (*Helianthus annuus* L.), no dominante-1 (*nd-1*) y wilty-1 (*w-1*).

El primero es un mutante analbino defectivo en biosíntesis en carotenoide, inducidos por cultivo de tejido *in vitro*. Esta mutación causa el blanqueo de pigmentos de clorofila, dormancia de semillas e inestabilidad para acumular ABA en cotiledones y hojas. Entre los mutantes albinos de las especies dicotiledóneas, *nd-1* es el primer mutante caracterizado por la deficiencia de ABA.

El segundo (*w-1*) es un genotipo en el cual la mutación espontánea confiere un fenotipo con marchitamiento. La condición de marchitamiento del mutante *w-1* es debido al comportamiento estomático anormal asociada con niveles bajos de ABA endógeno. Los tratamientos exógenos con ABA pueden inducir una reversión fenotípica del mutante. La reversión fenotípica parcial fue también observada en injertos de mutantes sobre patrones del tipo silvestre.

Las hojas cortadas de *w-1* se deshidrataron fuertemente con un incremento de ABA. Cuando el estrés hídrico fue impuesto en plantas mutantes en macetas se encontraron cambios significativos en el contenido en ABA y en la conductancia estomática se encontraron a niveles muy bajos de potencial de agua. Además *w-1* empezó a acumular ABA en la savia del xilema y a cerrar los estomas cuando el contenido de agua del suelo y el potencial de agua de la hoja fueron reducidos dramáticamente.

Los resultados sugieren que los bajos niveles de ABA endógeno y la incapacidad para sintetizar la hormona rápidamente en las hojas o en las raíces son responsables de la alta susceptibilidad del estrés hídrico de *w-1*.

MUTANTS ABA-DÉFICIENTS CHEZ LE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

L'utilisation de mutants déficients pour l'acide abscissique (ABA) a contribué à élucider la relation entre la synthèse de l'ABA et la réponse à la sécheresse des plantes. Cette mise au point résume l'état actuel des connaissances sur deux mutants ABA déficients, récemment isolés chez le tournesol (*Helianthus annuus* L.); non dormant-1 *(nd-1)* et wilty-1 *(w-1)*.

Le premier est un mutant albinos déficient pour la biosynthèse des caroténoides, issu de culture de tissus *in vitro*. Cette mutation provoque la décoloration des pigments chlorophylliens à la lumière, l'absence de dormance de la graine et l'incapacité d'accumulation de l'ABA dans les cotylédons et les feuilles. Parmi les mutants albinos des espèces dicotylédones, *nd-1* est le premier mutant caractérisé pour la déficience en ABA.

Le deuxième (*w-1*), est un génotype dans lequel une mutation spontanée confère un phénotype "flétri". L'état de flétrissement du mutant *w-1* est due à un comportement stomatique anormal associé à de faibles niveaux d'ABA endogène. Les traitement exogènes à l'ABA peuvent induire une réversion phénotypique du mutant. Une réversion phénotypique partielle a été aussi observée sur des greffons mutants greffés sur des porte-graffes de type sauvage.

Des feuilles détachées du mutant *w-1* fortement déshydratées présentent une faible augmentation de l'ABA Lorsque un stress hydrique est appliqué à des plantes mutantes en pots, on trouve des modifications significatives de la teneur en ABA et de la conductance stomatique, mais seulement dans le cas de très faibles potentiels hydriques. De plus, *W-1* commence à accumuler de l'ABA dans la sève du xylème et à fermer les stomates lorsque la teneur en eau du sol et le potentiel hydrique de la feuille sont fortement réduits.

Les résultats suggèrent que les faibles teneurs en ABA endogène et l'incapacité à synthétiser l'hormone rapidement, soit dans les feuilles ou dans les racines, sont responsables de la forte sensibilité su stress hydrique de *w-1*.