PHOTOSYNTHETICA 36 (1-2): 51-60, 1999

Protective action of abscisic acid against the inhibition of photosynthesis of barley leaves by bisulphite

C.N. N'SOUKPOÈ-KOSSI*, A.G. IVANOV**, K. VEERANJANEYULU*,*** and R.M. LEBLANC*,***,+

*Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec, Canada, G9A 5H7**
*Department of Chemistry, University of Miami, P.O. Box 249118, Coral Gables, Florida 33124-0431, USA****

Abstract

The inhibition of photosynthetic activity by bisulphite was studied in intact leaves of abscisic acid (ABA)-treated and non-treated (control) barley plants. ABA inhibited the photosynthetic process as evidenced by lower values of chlorophyll fluorescence kinetic parameters F_v/F_m (photosystem 2 activity) and R_{fd} (vitality index, related to the whole photosynthetic activity) compared with ABA-non-treated plants. After bisulphite treatment, the extent of inhibition was smaller in ABA-treated plants than in the control ones indicating a protective effect of ABA. The protective action sites of ABA were the Q_A reduction and the Calvin cycle.

Additional key words: chlorophyll fluorescence; *Hordeum vulgare*; photoacoustic spectroscopy; photosystem 2.

Introduction

The study of biochemistry and physiology of abscisic acid (ABA), originally considered as a growth regulator, has undergone a renaissance in the 1980s. Like other plant hormones, ABA has a multiple role during the life cycle of a plant. Each of its functions is determined developmentally and environmentally. ABA is ubiquitous in higher plants, but it is also found in certain algae (Tietz and Kasprik

Received 12 March 1998, *accepted* 14 May 1998.
**Present address*: Department of Plant Sciences, University of Western Ontario, London, Ontario, N6A 5B8, Canada.
+Author for correspondence; fax 305-284-4571, e-mail: mmodrono@umiami.ir.miami.edu
Abbreviations: ABA - abscisic acid; Chl - chlorophyll; F_0 - initial fluorescence; F_m - maximum fluorescence; F_s - steady state fluorescence; F_v - variable fluorescence; PA - photoacoustic; PES - photochemical energy storage; q_{NP} - non-photochemical fluorescence quenching coefficient; q_P - photochemical fluorescence quenching coefficient; RuBPCO - ribulose-1,5-bisphosphate carboxylase/oxygenase.

1986) and phytopathogenic fungi (Dörffling *et al.* 1984). ABA elicits two responses in plants: (*1*) closure of stomata (Milborrow 1980, Hornberg and Weiler 1984) by acting on the outer surface of the plasmalemma (Hartung 1983), and (*2*) synthesis of a set of new proteins (Singh *et al.* 1987, Gomez *et al.* 1988, Mohaparta *et al.* 1988a,b, Zeevaart and Creelman 1988, Parthier 1989). ABA has been viewed as a stress hormone since it enhances adaptation to various stresses. For instance, freezing tolerance was increased by ABA both in plants (Chen *et al.* 1983, Lalk and Dörffling 1985, Hughes *et al.* 1992) and in cell cultures (Chen and Gusta 1983, Orr *et al.* 1986, Reaney and Gusta 1987, Churchill *et al.* 1992). The increased cold tolerance seems to be related to synthesis of new proteins (Chen *et al.* 1983, Robertson and Gusta 1986, Robertson *et al.* 1987). Besides, during cold hardening, plants show a transient increase in ABA content (Chen *et al.* 1983) indicating that ABA may be an endogenous regulator of adaptation to cold stress. It may be associated with resistance to drought (Kahn *et al.* 1993), to chilling (Xin and Li 1993), and to desiccation (Ooms *et al.* 1993), to accelerate the adaptation of cultured cells to salt stress (LaRosa *et al.* 1985), and to decrease the heat induced damage in isolated chloroplasts (Ivanov *et al.* 1992). As far as we know, there is no work done on the effect of ABA on damage caused by atmospheric pollutants to plants.

Sulphur dioxide is one of the major atmospheric pollutants that affect plant growth and development (Darrall 1989). Upon absorption, it results in the accumulation of its anions such as HSO_3^-, SO_3^{2-} and SO_4^{2-} in the cell. Although most of sulfur anions are directly or/and indirectly inhibit photosynthesis (Cerović *et al.* 1982, Marques and Anderson 1986, Alscher *et al.* 1987, Veeranjaneyulu *et al.* 1992), HSO_3^- is more toxic than the other two anions and damages the Calvin cycle more (N'soukpoé-Kossi *et al.* 1994). Hence, in the present study we have selected HSO_3^- to investigate the protective role of ABA, if any, against the sulfur anion damage to the photosynthetic apparatus.

Materials and methods

Plants and treatments: Barley (*Hordeum vulgare* L.) seedlings were grown in distilled water (control) or in 10^{-5} M ABA solution (ABA-treated plants) as described by Ivanov *et al.* (1992) in a growth room (temperature 22 ± 2 °C, 60 % R.H., 14-h photoperiod, irradiance 50 W m^{-2}, *i.e.*, 230 μmol m^{-2} s^{-1}). ABA treatments lasted 7 d during which the solutions were renewed daily. For bisulphite treatments, two segments (1 cm long) were cut in the middle region of the same leaf from control and ABA-treated plants; one of each pair of leaf segments was floated (the abaxial surface exposed to the solution) on 50 mM phosphate buffer, pH 5.9 (blank) and the other segment on 20 mM bisulphite solution in 50 mM phosphate buffer, pH 5.9, for 1 h under "white light" (100 W m^{-2}), temperature of 22 ± 2 °C, and 60 % R.H. Bisulphite solutions were prepared extemporaneously and bubbled with argon or nitrogen gas in order to avoid oxidation of the compound by O_2 (Beauregard and Popovic 1987). The same samples were used for modulated fluorescence and photoacoustic measurements. Before fluorescence measurements, samples were dark

adapted for 20 min. Fluorescence and photoacoustic values for bisulphite treated leaves (first subjected to ABA application or not) were expressed as percent of the blank (*i.e.*, without bisulphite).

Photoacoustic (PA) spectra were measured with a laboratory-built portable photoacoustic and fluorescence photometer (Bélanger *et al.* 1993). It consists of a PA cell (*MTEC*, model *100*, Ames, IA, USA) receiving modulated red radiation (650 ± 12.5 nm) provided by a light-emitting-diode (LED) *Hewlett Packard HLMP-4101* (Mississauga, ON, Canada) through a branch of a trifurcated fiber optic bundle. The second branch is used to apply saturating non-modulated background radiation, while the third branch collects the fluorescence emission. Since a sine wave is applied to the LED, the phase value read on the lock-in amplifier is very precise and used to calculate the in-phase (cosine) and out-of-phase (sine) signal amplitudes. The photosynthetic energy storage was quantified using the formula

$$PES = Q_{ma} - Q_m/Q_{ma}$$

where Q_{ma} and Q_m are, respectively, the PA signals obtained at high frequencies with and without saturating background radiation. PES represents the energy stored by PS2 and PS1. The frequency and density of the modulated beam were 75 Hz (for this species) and 4 mW m^{-2}, respectively. For details on the instrumental set-up and characteristics see Bélanger *et al.* (1993) and N'soukpoé-Kossi *et al.* (1994). The photosynthetic energy storage was measured at CO_2 compensation concentration since the PA experiments were made in a closed vessel.

Modulated fluorescence: The potential yield of the photochemical reaction of photosystem 2 (PS2) was measured as (Krause and Weis 1991)

$$\phi P_o = k_P/(k_F + k_D + k_T + k_P) = (\phi F_m - \phi F_0)/\phi F_m = F_v/F_m$$

where k_P, k_F, k_D, and k_T are, respectively, the rate constants of photochemical reaction, fluorescence, thermal deactivation, and excitation energy transfer to non-fluorescent pigments.

The F_v/F_m ratio is an important and easily measurable parameter of the physiological state of PS2 in green leaves. The ratio of the fluorescence decrease, F_d, to the steady-state fluorescence, F_s (*i.e.*, $R_{fd} = F_d/F_s$), known as the vitality index, measures the whole photosynthetic efficiency from the primary photochemical event to the activity of Calvin cycle enzymes (Lichtenthaler and Rinderle 1988).

The modulated fluorescence set-up consisted of a 12-W lamp providing 375-585 nm radiation by means of a filter (*SP 580, Ditric Optics*, Hudson, MA, USA) of flux density varying between 4 and 150 mW m^{-2}, modulated with a mechanical chopper, an actinic radiation (λ<640 nm, 0-30 W m^{-2}) from a 100-W halogen lamp passing through a short pass filter (*SP 640, Ditric Optics*, Hudson, MA, USA), a saturating irradiance (λ<640 nm, 0-400 W m^{-2}) obtained from a 150-W arc lamp (*ILC Technology*, Sunnyvale, CA, USA), photodetectors (no. *18706, 0187, Optikon*, St-Laurent, Qc, Canada), a PC lock-in amplifier (*Ithaco*, model *3981*, Ithaca, NY, USA), and an *IBM* PC computer. The irradiances were varied by controlling the opening size of the shutters. Both actinic and saturating radiations were transported by a multifurcated bundle. For modulated fluorescence measurements, leaf discs

were dark-adapted for 20 min. The quenching coefficients q_P and q_{NP} were calculated as described by Schreiber *et al.* (1986). F_0' was the fluorescence intensity measured under the modulated beam after closure of both actinic and saturating radiations at the end of the modulated fluorescence cycle. The fluorescence measurements were made in an open system, *i.e.*, at normal atmospheric CO_2 concentration.

Results and discussion

We first compared the photosynthetic activity of ABA-treated plants with the ABA-non-treated (control) plants (Table 1), in the absence of bisulphite. ABA-treated barley plants, compared with ABA-non-treated ones, exhibited lower (from the analysis of variance, ANOVA) fluorescence levels, F_0' ($p=7.7\times10^{-4}$), F_m ($p<10^{-6}$) and F_s ($p=2.3\times10^{-4}$), and lower photosynthetic activity as evident from F_v/F_m ($p=1.59\times10^{-4}$) and R_{fd} ($p=2.34\times10^{-4}$). The differences in q_P and q_{NP} values were not significant which indicated no inhibition by ABA. (The smaller values of F_0' and F_m obtained for ABA-treated plants compared to the non-treated plants can be explained

Table 1. Fluorescence and photoacoustic values for control and ABA-treated barley plants. * indicates that ABA-treated plants differ significantly from ABA-non-treated plants. Means values ± S.D. were calculated from seventeen independent experiments. The negative sign (-) for the % inhibition indicates a signal increase instead of a decrease due to ABA. For abbreviations see the text.

Parameter	ABA-non-treated	ABA-treated	% inhibition
F_0` [mV]	4.40 ± 0.56	3.80 ± 0.23*	14
F_m [mV]	20.70 ± 2.36	15.54 ± 3.64*	25
F_s [mV]	5.66 ± 0.87	4.64 ± 0.40*	18
F_v/F_m	0.78 ± 0.02	0.75 ± 0.03*	4
q_P	0.71 ± 0.03	0.73 ± 0.05	-3
q_{NP}	0.69 ± 0.05	0.77 ± 0.10	-12
R_{fd}	2.69 ± 0.45	2.34 ± 0.43*	13
PES	0.17 ± 0.02	0.16 ± 0.03	6

by the smaller dimensions of the leaves from the ABA-treated plants.) The differences in F_v/F_m and R_{fd} values showed a significant inhibition. Besides, the q_{NP} curve reached its maximum for ABA-non-treated plants in about 70 s (Fig. 1*A*) while this maximum was not attained before 200 s for ABA-treated plants (Fig. 1*B*). The decrease in the q_{NP} curve after having reached its maximum is due to the onset of the Calvin cycle (Schmidt *et al.* 1988), and thus ABA acts in this sense. In the presence of ABA, q_{NP} was even larger than q_P (Figs. 1*B,D*) showing that ABA does affect the Calvin cycle at the level of the thylakoid membrane energization.

These findings are not in agreement with the report of Downton *et al.* (1988) stating that stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. In our experiments ABA affected photosynthesis and more specifically

delayed the onset of the energization of the thylakoid membrane. Were the inhibition only due to stomatal closure, all photosynthetic parameters would be affected in a non-specific way and the energization of the membrane would not be only delayed but also depressed. The fact that q_{NP} for ABA-treated plants was higher than for non-treated plants and even larger than q_P in the presence and in the absence of bisulphite means that membrane energization is a preponderant process in ABA-treated plants. Our results agree with findings of some direct influence of *in vivo*-applied ABA on photosynthetic electron transport (Bauer *et al.* 1976, Maslenkova *et al.* 1989), on oxygen evolution (Maslenkova *et al.* 1989), on CO_2 uptake (Cornic and Miginiac 1983, Raschke and Hedrich 1985), and on carboxylation efficiency (Ward and Bunce 1987).

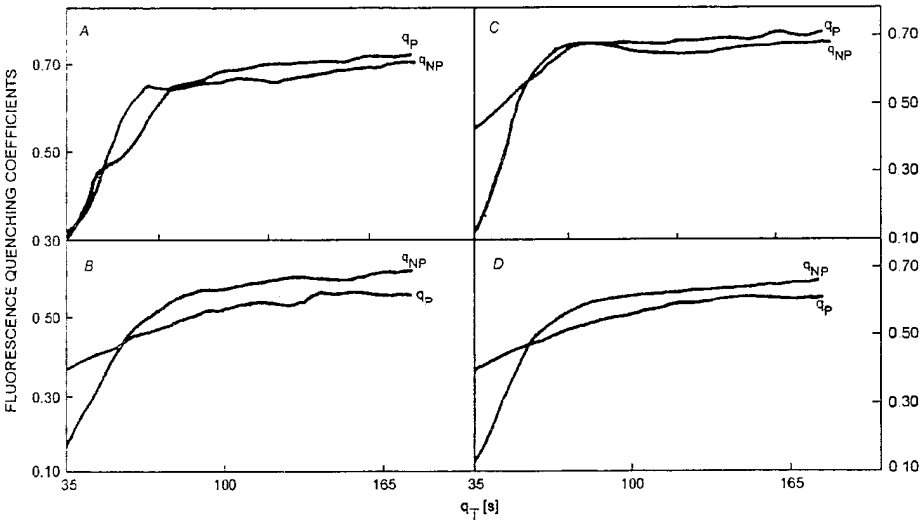

Fig. 1. Chlorophyll fluorescence quenching coefficients in ABA-non-treated (*A, C*) and ABA (10^{-5} M)-treated (*B, D*) barley leaves without (*A, B*) and after a 20 mM bisulphite treatment for 1 h in "white light" (100 W m^{-2}) (*C, D*). Parameters q_P and q_{NP} stand for the photochemical and non-photochemical quenching coefficients, respectively; q_T is the quenching time after the application of the actinic radiation. Modulated radiation: 375-585 nm, 50 mW m^{-2}, 400 Hz. Actinic radiation: $\lambda <$ 640 nm, 11 W m^{-2}. Saturating radiation: $\lambda <$ 640 nm, 40 W m^{-2}, for 2 s at every 10 s.

As concerns the effect of ABA treatment on the inhibitory action of bisulphite on the photosynthetic process in barley (Table 2), significantly higher values of F_m (p=0.019), F_v/F_m (p=0.06), and R_{fd} (p=0.038) were obtained for ABA-treated plants subjected to bisulphite exposure than for non-ABA-treated ones. This observation points to a protective action of ABA toward the harmful effect of bisulphite. One may argue that the so-called protective action of ABA is merely due to stomatal closure. Of course the stomatal effect cannot be avoided. But provided that the leaf samples used in this study have been cut, thus presenting injured areas, bisulphite could not be prevented from entering into the leaves, especially after a 1-h exposure.

For ABA-treated and non-treated barley plants (Table 2), the inhibitory action of bisulphite was strong on R_{fd} and insignificant as concerns PES (related to PS2 and

PS1), q_P, and q_{NP}. The fact that R_{fd} was much more affected than F_v/F_m implies that the activity of Calvin cycle enzymes was more affected than other photosynthetic processes in both ABA-treated and non-treated plants. These findings are in agreement with our results on sugar maple leaves (N'soukpoé-Kossi *et al.* 1994).

Table 2. Effect of 20 mM bisulphite treatment on the photosynthetic activity of ABA-treated (T) and non-treated (C) barley leaves. The results are expressed as % of blank (without bisulphite), and % inhibition in each case is calculated in relation to values obtained for the same sample without ABA-treatment or without bisulphite [% inhibition]. * indicates that ABA-treated plants differ significantly from ABA-non-treated plants. Mean values ± S.D. were calculated from seventeen independent experiments. For abbreviations see the text.

Parameter	ABA-non-treated (C) (+ bisulphite)	% inhibition	ABA-treated (T) (+ bisulphite)	% inhibition	T/C
F_0'	96 ± 11	4	94 ± 6	6	98
F_m	67 ± 12	33	78 ± 13*	22	116
F_v/F_m	86 ± 9	14	92 ± 7*	8	107
q_P	78 ± 12	22	81 ± 15	19	104
q_{NP}	92 ± 9	8	95 ± 7	5	103
R_{fd}	54 ± 17	46	67 ± 16*	33	124
PES	80 ± 10	20	68 ± 23	32	85

The q_{NP} was significantly less affected than q_P in both ABA-treated and non-treated plants (Table 2). The main component of q_{NP} is the energy-dependent quenching coefficient termed q_E which is related to the energization of the thylakoid membrane as a result of the light-induced build-up of proton gradient in favour of the thylakoid lumen. Although ΔpH is a strict requirement for q_E formation, Moss and Bendall (1984) show that q_E is subjected to a redox control. A role of zeaxanthin in the thylakoids has been postulated for q_E, the major component of which is characterized by a decrease in F_0 in leaves. The q_{NP} is predominant quenching factor under conditions that limit utilization of the ΔpH by photophosphorylation (Krause *et al.* 1981, 1982). The use of the accumulated protons by the coupling factor CF_1 (or ATP synthase) to produce ATP should lead to membrane relaxation and decrease or suppression of q_E (and consequently of q_{NP}). The fact that q_{NP} is less affected than q_P may be an indication that the use-up of protons as driving force for ATP synthesis is slowed down. Thus, ATP synthase may be considered as a possible site of action of bisulphite in line with previous observation that sulphite and glyoxal inhibit phosphorylation, reduce ATP synthesis, and completely abolish $^{14}CO_2$ fixation in spinach chloroplasts (Asada *et al.* 1965, Cerović *et al.* 1982).

RuBPCO may also be a site of action of sulfur anions (Marques and Anderson 1986, Schmidt *et al.* 1988): both situations can be reconcilable. Indeed, if the activity of RuBPCO is impaired by bisulphite, ATP requirement in Calvin cycle would decrease, and consequently ATP synthesis will be reduced as suggested by our results. The reverse reaction could also be possible: inhibition of ATP synthesis by bisulphite leading to reduced RuBPCO activity. But the latter possibility is not supported by the finding that RuBPCO activity and content of potato leaves have

decreased in presence of another strong oxidant, ozone (Dann and Pell 1989, Pell *et al.* 1990). Therefore it appears likely that RuBPCO would be a site of bisulphite action, and inhibition of the use-up of accumulated H^+ would be a consequence of RuBPCO inhibition. This helps to understand the mechanism of the effect of ABA in plants relative to bisulphite treatment.

We did not find a marked effect of bisulphite on F_s (reoxidation of plastoquinone, PQ) for ABA-treated (5.4 mV) and non-treated (6.3 mV) plants, while observing a significant difference in F_m (reduction of Q_A) with a lesser effect of bisulphite on F_m in the presence of ABA (Table 2). Hence the protective action of ABA against the inhibition of the photosynthetic activity by bisulphite was reflected by a relatively higher Q_A reduction rate compared with the results obtained in the absence of ABA. As a consequence, the R_{fd} was higher for ABA-treated plants than for ABA-non-treated ones due to the higher F_m. This increased reduction of Q_A was accompanied by a higher q_P value even though it is not significantly different from that of ABA-non-treated leaves (Table 2). Therefore the ABA-protective effect may be targeted at two sites at least, *i.e.*, the reduction site of Q_A and the acceptor of PS2, and the carbon reducing cycle as the recovering of activity (Table 2, T/C) was higher at the level of R_{fd} compared with F_v/F_m and q_P.

Thus there is an experimental evidence that (*1*) ABA affects the photosynthetic activity of barley leaves as monitored by photoacoustic and fluorescence methods, (*2*) ABA-treatment lowers the use of proton gradient as driving force in ATP synthesis, as reflected by a higher q_{NP} value (probably due to inhibition of RuBPCO activity) and by a delay in the onset of the Calvin cycle activity, and (*3*) ABA, to some extent, protects Q_A reduction and the Calvin cycle against bisulphite action.

Although the molecular mechanism of this ABA protective action has to be clarified, it may be related to new proteins synthesized in the presence of ABA. Indeed, ABA induces the synthesis of new proteins in plants that are very basic or rich in glycine (Gomez *et al.* 1988, Hong *et al.* 1988, Mundy and Chua 1988), and thus possibly positively charged (Kicheva and Ivanov 1992). Besides, an enhanced amount of positively charged light-harvesting chlorophyll *a/b* protein complex of PS2 (LHC*a/b*) in ABA-treated barley plants has been reported (Ivanov *et al.* 1992). The LHC*a/b*, and especially its positively charged portion, is required for the formation of grana appressions, and hence ABA may induce a better development of the PS2-enriched granal structure in barley chloroplast (Ivanov *et al.* 1992). If this is so, at least part of the protective action of ABA in the presence of bisulphite could be explained by PS2-enrichment.

In conclusion, ABA has proved to be once more a stress hormone. It also protects the photosynthetic apparatus from a severe inhibition by bisulphite. The action seems to be located at the Calvin cycle and at the site of Q_A reduction. We have also observed an inhibitory effect of ABA on the photosynthetic process of barley leaves in the absence of bisulphite, the mechanism of which has to be clarified.

References

Alscher, R., Franz, M., Jerke, C.W.: Sulfur dioxide and chloroplast metabolism. - In: Saunders, J.A., Kosak-Channing, L., Conn, E.E. (ed.): Phytochemical Effects of Environmental Compounds. Pp. 1-28. Plenum Publishing Corp., New York 1987.

Asada, K., Kitoh, S., Denra, R., Kasai, Z.: Effect of α-hydroxyl-sulfonates on photochemical reactions of spinach chloroplasts and participation of glyoxylate in photophosphorylation. - Plant Cell Physiol. **6**: 615-629, 1965.

Bauer, R., Huber, W., Sankhla, N.: Effects of abscisic acid on photosynthesis in *Lemna minor*. - Z. Pflanzenphysiol. 77: 237-246, 1976.

Beauregard, M., Popovic, R.: Artificial inhibitory effects of sulfite on photosystem II activity measured by oxygen evolution in chloroplasts. - Photosynth. Res. **14**: 89-94, 1987.

Bélanger, R., Paquette, A., N'soukpoè-Kossi, C.N., Leblanc, R.M.: New portable photoacoustic and fluorescence photometer for field measurement of photosynthesis. - Rev. Sci. Instrum. **64**: 1175-1181, 1993.

Cerović, Z.G., Kalezić, R., Plesničar, M.: The role of photophosphorylation in SO_2 and SO_3^{2-} inhibition of photosynthesis in isolated chloroplasts. - Planta **156**: 249-254, 1982.

Chen, H.H., Li, P.H., Brenner, M.L.: Involvement of abscisic acid in potato cold acclimation. - Plant Physiol. **71**: 362-365, 1983.

Chen, T.H.H., Gusta, L.V.: Abscisic acid-induced freezing resistance in cultured plant cells. - Plant Physiol. **73**: 71-75, 1983.

Churchill, G.C., Ewan, B., Reaney, M.J.T., Abrams, S.R., Gusta, L.V.: Structure-activity relationships of abscisic acid analogs based on the induction of freezing tolerance in bromegrass (*Bromus inermis* Leyss) cell cultures. - Plant Physiol. **100**: 2024-2029, 1992.

Cornic, G., Miginiac, E.: Non stomatal inhibition of net CO_2 uptake by (±) abscisic acid in *Pharbitis nil*. - Plant Physiol. **73**: 529-533, 1983.

Dann, M.S., Pell, E.J.: Decline in activity and quantity of ribulose bisphosphate carboxylase/oxygenase and net photosynthesis in ozone-treated potato foliage. - Plant Physiol. **91**: 427-432, 1989.

Darrall, N.M.: The effect of air pollutants on physiological processes in plants. - Plant Cell Environ. **12**: 1-30, 1989.

Dörffling, K., Petersen, W., Sprecher, E., Urbasch, I., Hanssen, H.-P.: Abscisic acid in phytopathogenic fungi of the genera *Botrytis, Ceratocystis, Fusarium*, and *Rhizoctonia*. - Z. Naturforsch. **39c**: 683-684, 1984.

Downton, W.J.S., Loveys, B.R., Grant, W.J.R.: Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. - New Phytol. **108**: 263-266, 1988.

Gomez, J., Sanchez-Martinez, D., Stiefel, V., Rigan, J., Puigdomenech, P., Pages, M.A.: A gene induced by the plant hormone abscisic acid in response to water stress encodes a glycine-rich protein. - Nature **334**: 262-264, 1988.

Hartung, W.: The site of action of abscisic acid at the guard cell plasmalemma of *Valerianella locusta*. - Plant Cell Environ. **6**: 427-428, 1983.

Hong, B., Uknes, S.J., Ho, T.D.: Cloning and characterization of a cDNA encoding mRNA rapidly-induced by ABA in barley aleurone layer. - Plant mol. Biol. **11**: 495-506, 1988.

Hornberg, C., Weiler, E.W.: High-affinity binding sites for abscisic acid on the plasmalemma of *Vicia faba* guard cells. - Nature **310**: 321-324, 1984.

Hughes, M.A., Dunn, M.A., Pearce, R.S., White, A.J., Zhang, L.: An abscisic-acid-response, low temperature barley gene has homology with a maize phospholipid transfer protein. - Plant Cell Environ. **15**: 861-865, 1992.

Ivanov, A.G., Kitcheva, M.I., Christov, A.M., Popova, L.P.: Effects of abscisic acid treatment on the thermostability of the photosynthetic apparatus in barley chloroplasts. - Plant Physiol. **98**: 1228-1232, 1992.

Kahn, T.L., Fender, S.E., Bray, E.A., O'Connell, M.A.: Characterization of expression of drougth- and abscisic acid-regulated tomato genes in the drought-resistant species *Lycopersicon pennellii*. - Plant Physiol. **103**: 597-605, 1993.

Kicheva, M.I., Ivanov, A.G.: A comparative analysis of the effects of *in-vivo* and *in-vitro* abscisic-acid treatment on the surface electrical properties of barley chloroplast membranes. - Planta **188**: 232-237, 1992.

Krause, G.H., Briantais, J.-M., Vernotte, C.: Two mechanisms of reversible quenching in chloroplasts. - In: Akoyunoglou, G. (ed.): Photosynthesis. Vol. I. Pp. 575-584. Balaban Int. Sci. Serv., Philadelphia 1981.

Krause, G.H., Vernotte, C., Briantais, J.-M.: Photoinduced quenching of chlorophyll fluorescence in intact chloroplasts and algae. Resolution in two components. - Biochim. biophys. Acta **679**: 116-124, 1982.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Lalk, I., Dörffling, K.: Hardening, abscisic acid, proline and freezing resistance in two winter wheat varieties. - Physiol. Plant. **63**: 287-292, 1985.

LaRosa, P.C., Handa, A.K., Hasegawa, P.M., Bressan, R.A.: Abscisic acid accelerates adaptation of cultured tobacco cells to salt. - Plant Physiol. **79**: 138-142, 1985.

Lichtenthaler, H.K., Rinderle, U.: The role of chlorophyll fluorescence in the detection of stress condition in plants. - CRC crit. Rev. anal. Chem. **19**: S29-S85, 1988.

Marques, I.A., Anderson, L.E.: Effects of arsenite, sulfite, and sulfate on photosynthetic carbon metabolism in isolated pea (*Pisum sativum* L., cv Little Marvel) chloroplasts. - Plant Physiol. **82**: 488-493, 1986.

Maslenkova, L.T., Zanev, Y., Popova, L.P.: Effect of abscisic acid on the photosynthetic oxygen evolution in barley chloroplasts. - Photosynth. Res. **21**: 45-50, 1989.

Milborrow, B.V.: Pathways to and from abscisic acid. - In: Addicott, T.F. (ed.): Abscisic Acid. Pp. 79-111. Praeger, New York 1980.

Mohaparta, S.S., Poole, R.J., Dhindsa, R.S.: Detection of two polypeptides induced by abscisic acid and cold acclimation. Possible role in freezing tolerance. - Plant Cell Physiol. **29**: 727-730, 1988a.

Mohaparta, S.S., Poole, R.J., Dhindsa, R.S.: Alterations in membrane protein profile during cold treatment of alfalfa. - Plant Physiol. **86**: 1005-1007, 1988b.

Moss, D.A., Bendall, D.S.: Cyclic electron transport in chloroplasts. The Q-cycle and the site of action of actimycin. - Biochim. biophys. Acta **767**: 389-395, 1984.

Mundy, J., Chua, N.H.: Abscisic acid and water stress induce the expression of a novel rice gene. - EMBO J. **7**: 2279-2286, 1988.

N'soukpoé-Kossi, C.N., Leblanc, R.M. : Application of photoacoustic spectroscopy in photosynthesis research. - J. mol. Struct. **217**: 69-84, 1990.

N'soukpoé-Kossi, C.N., Veeranjaneyulu, K., Leblanc, R.M.: Sites of action of sulphite and bisulphite in the photosynthetic apparatus of sugar maple leaves as studied by photoacoustic and modulated fluorescence methods. - Plant Cell Environ. **17**: 731-738, 1994.

Ooms, J.J.J., Lèon-Kloosterziel, K.M., Bartels, D., Koornneef, M., Karssen, C.M.: Acquisition of dessication tolerance and longevity in seeds of *Arabidopsis thaliana* . A comparative study using abscisic acid-insensitive *abi3* mutants. - Plant Physiol. **102**: 1185-1191, 1993.

Orr, W., Keller, W.A., Singh, J.: Induction of freezing tolerance in an embryogenic cell suspension culture of *Brassica napus* by abscisic acid at room temperature. - J. Plant Physiol. **126**: 23-32, 1986.

Parthier, B.: Hormone-induced alterations in plant gene expression. - Biochem. Physiol. Pflanz. **185**: 289-314, 1989.

Pell, E.J., Enyedi, A., Eckardt, N., Landry, L.: Ozone-induced alterations in quantity and activity of Rubisco: implications for foliar senescence. - In: Reddy, C.C., Hamilton, G.A., Madyastha, K.M. (ed.): Proc. Int. Symp. Biol. Oxidation Systems. Pp. 389-403. Academic Press, San Diego 1990.

Raschke, K., Hedrich, R.: Simultaneous and independent effects of abscisic acid on stomata and the photosynthetic apparatus in whole leaves. - Planta **163**: 105-118, 1985.

Reaney, M.J.T., Gusta, L.V.: Factors influencing the induction of freezing tolerance by abscisic acid in cell suspension cultures of *Bromus inermis* Leyss and *Medicago sativa* L. - Plant Physiol. **83**: 423-427, 1987.

Robertson, A.J., Gusta, L.V.: Abscisic acid and low temperature induced polypeptide changes in alfalfa (*Medicago sativa*) cell suspension cultures. - Can. J. Bot. **64**: 2758-2763, 1986.

Robertson, A.J., Gusta, L.V., Reaneej, M.J.J., Ishikawa, M.: Protein synthesis in bromegrass (*Bromus inermis* Leyss) cultured cells during the induction of from tolerance by abscisic acid or low temperature. - Plant Physiol. **84**: 1331-1336, 1987.

Schmidt, W., Schreiber, U., Urbach, W.: SO_2 injury in intact leaves, as detected by chlorophyll fluorescence. - Z. Naturforsch. **43c**: 269-274, 1988.

Schreiber, U., Schliwa, U., Bilger, W.: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. - Photosynth. Res. **10**: 51-62, 1986.

Singh, N.K., LaRosa, P.C., Handa, A.K., Hasegawa, P.M., Bresson, R.A.: Hormonal regulation of protein synthesis associated with salt tolerance in plant cells. - Proc. nat. Acad. Sci. USA **84**: 739-743, 1987.

Tietz, A., Kasprik, W.: Identification of abscisic acid in a green alga. - Biochem. Physiol. Pflanz. **181**: 269-274, 1986.

Veeranjaneyulu, K., Charlebois, D., N'soukpoé-Kossi, C.N., Leblanc, R.M.: Sulfite inhibition of photochemical activity of intact pea leaves. - Photosynth. Res. **34**: 271-278, 1992.

Ward D.A., Bunce, J.A.: Abscisic acid simultaneously decreases carboxylation efficiency and quantum yield in attached soybean leaves. - J. exp. Bot. **38**: 1182-1192, 1987.

Xin, Z., Li, P.H.: Relationship between proline and abscisic acid in the induction of chilling tolerance in maize suspension-cultured cells. - Plant Physiol. **103**: 607-613, 1993.

Zeevaart, J.A.D., Creelman, R.A.: Metabolism and physiology of abscisic acid. - Annu. Rev. Plant Physiol. Plant mol. Biol. **39**: 439-473, 1988.