J Plant Growth Regul (1992) 11:195–201

Journal of
Plant
Growth
Regulation

The Lipid Composition, Fluidity, and Mg^{2+}-ATPase Activity of Rice (*Oryza sativa* L. cv. Bahia) Shoot Plasma Membranes: Effects of ABA and GA_3

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Received May 22, 1992; accepted July 28, 1992

Abstract. Six-day-old rice plants (*Oryza sativa* L., cv Bahia) were grown for 5 days more in nutrient solution culture containing 10^{-5} M abscisic acid (ABA) or gibberellic acid (GA_3) (treated plants). Plasma membrane (PM) vesicles were isolated from the shoots of treated or control plants, and ATPase hydrolytic and proton-pumping activity, fluidity, and free sterol and phospholipid composition were determined. Both treatments resulted in modified plant growth and increases in PM fluidity. The ATPase hydrolytic activity was decreased by 25% of control values with ABA treatment and by 35% with GA_3. Both treatments reduced proton-pumping by 23%. GA_3 treatment reduced the relative amount (%) of stigmasterol in the PM from 38–34%, and increased Δ^5-avenasterol from 4–7%. ABA and GA_3 did affect the percentage composition of the fatty acyl chains of phosphatidylinositol (PI). GA_3 treatment also resulted in a reduction of the total amount of PI from 73–37 mg mg^{-1} protein, as well as a decrease in the overall PM C_{16}/C_{18} ratio and an increase in the degree of unsaturation. Our results show that there is no common link between a specific change in lipid composition and fluidity in these membranes. On the other hand, the reduction in Mg^{2+}-ATPase activity found with both hormones suggests that there is no relationship between membrane-bound enzyme activity and membrane fluidity.

It has been suggested that the activity of plasma membrane (PM)-bound enzymes, including ATPase, is regulated by membrane lipid composition (Carruthers and Melchior 1986). Two theories are currently postulated to explain the mechanism by which this regulation occurs. One suggests that changes in membrane lipid composition alter the fluidity of the membrane and thus the enzyme activity (Douglas and Walker 1984), whereas the other theory postulates a direct binding of lipids to the enzyme (Simmonds et al. 1982).

It has been found that changes in PM lipid composition caused by stress or xenobiotic treatment, in turn affect ATPase activity (Cooke and Burden 1990). These treatments also modify plant hormone levels. Thus, abscisic acid (ABA) has been shown to accumulate in plants subjected to different stresses, such as water stress (Creelman 1989), nitrogen stress (Chapin 1990), or heavy metal stress (Barceló et al. 1986). Under similar stress conditions, alterations to the plant lipid composition occurs (Davis 1972, Rooney et al. 1990).

On the other hand, xenobiotics, such as the triazole, paclobutrazol (a plant growth regulator), the pyrimidine methanol, nuarimol (a fungicide), or the norbornenodiazetine, tetcyclacis (an experimental plant growth regulator), not only inhibit the biosynthesis of the gibberellins, but also alter PM lipid composition (Cooke et al. 1989, Haughan et al. 1989). Furthermore, the ATPase activity of nuarimol- and tetcyclacis-treated plants is increased, compared with untreated plants (Cooke et al. 1989).

Therefore, there are grounds for supposing that plants may directly, or indirectly, modify the composition of membranes and may, thereby modulate the activities of membrane-bound enzymes. In this work we have studied the effect of exogenously applied gibberellic acid (GA_3) and ABA on rice plants to see whether a relationship between PM lipid composition, fluidity, and ATPase activity exists.

Materials and Methods

Plant Culture

Rice seeds (*Oryza sativa* L. cv. Bahia) were sterilized in 2% sodium hypochlorite and placed on stainless-steel grids suspended above a 5-L beaker containing an aerated culture solution comprising 0.1 mM KCl, 0.3 mM KNO_3, 0.1 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.1 mM $NaH_2PO_4 \cdot 2H_2O$, 0.05 mM $MgSO_4 \cdot 7H_2O$, 0.24 mM NH_4NO_3, 3 mg $\cdot$ dm^{-3} Fe-EDDHA (Sequestrene), 1 μM $MnSO_4 \cdot 4H_2O$, 3 μM H_3BO_3, 0.1 μM $CuSO_4 \cdot 5H_2O$, 0.1 μM $ZnSO_4$, and 0.03 μM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. The plants were grown in the dark in a controlled environment at 30°C. Six days after sowing light was provided to stimulate a minimum 16 h photoperiod, and either ABA (10^{-5} M) or GA_3 (10^{-5} M) was added to the culture solution. Shoots of both treated and untreated plants were harvested 5 days after treatments started and the PM isolated immediately.

PM Preparation

PMs were isolated and purified using the two-phase aqueous polymer technique of Larsson et al. (1987). Approximately 7 g of shoot material was chopped finely and vacuum infiltrated with 50 cm^3, 50 mM N-2-hydroxyethylpiperazine-N-2-ethane sulphonic acid (HEPES) (pH 7.6 with KOH) plus 500 mM sucrose, 5 mM ascorbic acid, 3.6 mM cysteine, and 0.6% (wt/wt) polyvinylpyrrolidone (PVP). The buffer-saturated material was homogenized, filtered through a 240-μm mesh nylon cloth, and centrifuged at 10,000 *g* for 15 min. The supernatant was collected and centrifuged at 100,000 *g* for 30 min to yield a microsomal pellet which was resuspended in 3 cm^3 of 330 mM sucrose, 5 mM HEPES-KOH (pH 7.6) (buffer 1). The suspension (3 g) was loaded onto 9 g of phase mixture to yield a 12 g two-phase system containing 6.2% (wt/wt) Dextran T500, 6.2% (wt/wt) polyethylene glycol (PEG) 3350, 3 mM KCl, 5 mM HEPES-KOH (pH 7.6), and 330 mM sucrose. A batch procedure was used to purify the PMs (Larsson et al. 1987). The third upper phase was diluted with buffer 1 plus 4 mM dithiothreitol (DTT) and centrifuged for 30 min at 100,000 *g*. The resulting pellet was resuspended in 1 cm^3 of the same buffer. Aliquots of the resuspended PM pellet were taken for sterol, phospholipid and protein analysis, ATPase assays, and proton-pumping and fluidity measurements. All were stored at −20°C.

Sterol Analysis

Sterols were analyzed using the method described by Burden et al. (1987). Five percent KOH in 80% ethanol (2.5 cm^3) was added to 0.25 cm^3 of resuspended PM along with 10 mm^3 β-cholestanol (0.1 mg cm^3) as an internal standard. The mixture was heated at 80°C for about 3 min, cooled, and 10 cm^3 hexane added, followed by 5 cm^3 water, and shaken. The hexane layer was removed to a tube containing about 0.1 g of sodium sulfate to dry the solvent, and a further 10 cm^3 of hexane added to the original extract. The mixture was shaken and the hexane layer removed and pooled with the first 10 cm^3 hexane solution. The hexane extracts were dried and acetylated for 1 h with 50 mm^3 pyridine and 50 mm^3 acetic anhydride. The solvent was removed under nitrogen and the residue dissolved in ethyl acetate. The sterol acetates were analyzed by gas chromatography (GC) using an SE-52 bonded capillary column (25 m × 0.25 mm ID) with hydrogen (0.75 kg cm^{-1}) as the carrier gas and a temperature program of 120–260°C at 5°C min^{-1}. Sterols were identified by comparing relative retention times with known standards.

Phospholipid Analysis

The method used was that previously described by Cooke et al. (1989). Chloroform:methanol (1:2 vol/vol) (0.75 cm^3) was added to the PM suspension (0.25 cm^3) in an Eppendorf tube and the mixture shaken, after which a further 0.25 cm^3 of chloroform was added, followed by shaking, and centrifugation at 10,000 *g* for 6 min. The chloroform layer was removed and the solvent evaporated under nitrogen. Propan-2-ol:hexane:water (54:40:6) (0.2 cm^3) was added to the residue and the resulting lipid extract stored at −20°C. Phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylcholine (PC) were separated by high-performance liquid chromatography (HPLC) using the method of Patton et al. (1982). The PE, PI, and PC fractions were detected by UV (205 nm) and collected; 25 mm^3 methyl heptadecanoate (0.1 mg cm^{-3}) was added as an internal standard and the sample evaporated to dryness under nitrogen. Sodium methoxide (0.5 N) (2 cm^3) containing 5% (vol/vol) 2,2'-dimethoxypropane was added to the residue and heated at 40°C for 10 min. The solution was cooled, 2 cm^3 of hexane added followed by 1 cm^3 water, and the mixture shaken. The hexane layer was evaporated in a "Speed-vac concentrator" and the residue dissolved in 25 mm^3 of ethyl acetate. The resultant solution was analyzed for fatty acids by GC using an RSL 500 BP bonded capillary column (25 m × 0.25 mm ID), with helium as the carrier gas (0.75 kg cm^{-1}) and a temperature program of 170–200°C at 2°C min^{-1}. The phospholipid content was calculated from the amount of fatty acid found in the PE, PI, and PC fractions.

Fluorescence Polarization Measurements

The fluidity of the PM was determined by steady-state fluorescence polarization using a Perkin-Elmer LS5 luminescence spectrophotometer fitted with a polarization accessory. Determinations were made using the resuspended PM fractions, diluted to give a protein concentration of 300 μg cm^{-3} with an assay medium of 40 mM HEPES-KOH (pH 7), 100 mM KCl, 5 mM $MgSO_4$, and 0.1 mM EGTA. Samples contained 0.5 mm^3 of 1 mM 1,6-diphenyl-1,3,5-hexatriene (DPH) in tetrahydrofuran (THF) and were compared with samples to which only 0.5 mm^3 THF had been added (blanks). The THF was evaporated under nitrogen and samples and blanks incubated in a water bath for 1 h at 20°C. Measurements of the fluorescence emission of samples and blanks at 430 nm with excitation at 360 nm were made at 20°C. The fluorescence polarization (P) was calculated from the following equation:

$$P = \frac{I_{VV} - I_{VH}\,(G)}{I_{VV} + I_{VH}\,(G)}$$

where I is fluorescence intensity and G (the grating correction factor) is I_{HV}/I_{HH}. The subscript V (vertical) and H (horizontal), describe the position of the polarizers in the excitation or emission beams, respectively.

ATPase Assays

The assay medium contained 0.01% Triton X-100, 3 mM $MgSO_4$, 1 mM sodium azide, 0.7 mM sodium molybdate, and 3 mM ATP (Na salt), all in 50 mM MES-TRIS buffer (pH 6.5). The reaction was started by adding 25 mm^3 PM (diluted to give 1–2 μg protein

per assay in a final volume of 112.5 mm^3). After incubation for 30 min at 37°C, the reaction was stopped by adding 250 mm^3 sodium dodecyl sulfate (SDS) (10%). Total inorganic phosphate (P_i) released from ATP hydrolysis was determined by the method of Ames (1966); 300 mm^3 ascorbic acid (10%), ammonium molybdate (0.42%) in sulfuric acid (1 N) was added to the reaction mixture. After 45 min at 37°C the reaction was stopped with ice and the absorbance measured at 820 nm. Samples were compared with blank and standards to which SDS was added before the reaction started.

Proton-Pumping Measurements

Resuspended PM vesicles (enough to give 300 μg of protein), which had undergone three freeze–thaw cycles (from −20–20°C), were added to a cuvette containing 20 mM BTP/MES (pH 6.5), 5 mM TRIS · ATP (pH 6.5), 25 mM potassium nitrate, 1 μM ACMA, and water to make the volume 2 cm^3. The cuvette was placed in a Perkin-Elmer LS5 luminescence spectrophotometer and maintained at 30°C with constant stirring. The reaction was started with the addition of 20 mm^3 5 mM magnesium chloride and the change in fluorescence emission measured at 485 nm with excitation at 415 nm. The change in emission was recorded on a Bryans recorder with a chart speed of 30 cm h^{-1}. The rate of proton-pumping was calculated from the slope. The proton gradient was collapsed with 20 mm^3 calcium chloride (100 mM).

Protein Determinations

The Bio-Rad protein assay reagent was used with thyroglobulin as standard (Bradford 1976).

Data Analysis

All experiments were repeated at least three times and the data analyzed using the unpaired Student's *t*-test. The significance level was fixed at $p < 0.05$.

Results

Shoot height was reduced by 50% after 5 days with ABA treatment, but the fresh and dry weights increased by 50 and 39%, respectively, as compared with untreated plants (Table 1). However, with GA_3 treatment the opposite effect was observed on these parameters, i.e., an increase of 38% in shoot height and a reduction in fresh and dry weights of 17 and 21% (Table 1).

The specific hydrolytic activity of the PM Mg^{2+}-ATPase was reduced by ABA and GA_3 treatment by about 25 and 35%, respectively (Table 2). The PM ATPase proton-pumping activity was also decreased by ABA and GA_3 by about 23% with respect to control plants (Table 2).

Changes were detected in the % composition of the PM sterols in ABA- and GA_3-treated plants compared with controls. GA_3 treatment resulted in a significant reduction from 38.2–34.1% in stigmasterol and an increase from 3.7–7.2% in Δ^5-avenasterol; a trend was observed which suggested an increase in sitosterol. Opposite, but not statistically significant changes in these sterols were observed after 5 days of treatment with ABA. Total sterol content (on a unit protein basis) was not affected by any treatment (Table 3).

Table 1. Growth parameters of rice shoots treated with and without ABA or GA_3 (10^{-5} M).

	Height (mm) N = 4	Fresh wt (mg cm^{-1}) N = 4	Dry wt (mg cm^{-1}) × 10^{-1} N = 4
Control	131.7 ± 11	4.4 ± 0.1	0.60 ± 0.02
ABA (10^{-5} M)	66.3 ± 4 (50)[a]	6.6 ± 0.1 (150)[a]	0.84 ± 0.03 (139)[a]
GA_3 (10^{-5} M)	181.7 ± 6 (138)[a]	3.8 ± 0.1 (86)[a]	0.48 ± 0.01 (79)[a]

Mean values ± SE (% over controls).
[a] Indicates significant differences at the 95% level of confidence or better (unpaired *t* tests), compared with controls.

Phospholipids (PE, PC, and PI) were determined on the basis of their fatty acid composition, and were expressed as total (on a unit protein basis) percentage composition of fatty acid, fatty acid chain length ratio (C_{16}/C_{18}), and fatty acid bond index (BI) (calculated from % 16:1 + % 18:1 + % 18:2 × 2 + % 18:3 × 3) (Tables 4–6). No changes were found in the fatty acid composition, C_{16}/C_{18} ratio, BI, and total amount of PE with either of the treatments (Table 4). With ABA treatment, decreases were observed in the relative composition of the $C_{18.1}$ and $C_{18.3}$ fatty acyl chains of PC (Table 5). GA_3 treatment had no effect on the acyl chain composition of PC, or the total amount (Table 5). However, ABA and GA_3 treatments affected the relative fatty acid composition of PI (Table 6). Both treatments resulted in a significant decrease in the relative amounts of $C_{16.1}$ (from 28.2% for the control to 15.9 and 16.3% for ABA and GA_3, respectively), accompanied by increases in $C_{18.0}$ and $C_{18.3}$. With GA_3, the percentage composition of $C_{18.1}$ was reduced from a control value of 12.6–7.8%, and this treatment resulted in a reduction in the total amount of PI in the PM (by 50% with respect to control) (Table 6).

The results of exogenous hormone treatment on PM order parameter (P), as measured by steady-state fluorescence polarization (Table 7), showed that ABA and GA_3 significantly increased the fluidity by about 7 and 6%, respectively (the lower the P value, the greater the fluidity). This modification was not accompanied by changes in the PE/PC ratio or the mole percentage sterol of total lipid (sterol

Table 2. Mg^{2+}-ATPase hydrolytic activity and rates of proton-pumping from PMs of rice shoots treated with and without ABA or GA_3 (10^{-5} M).

	Total activity (μmol P_i h^{-1} g fw^{-1}) N = 4	Specific activity (μmol P_i h^{-1} mg^{-1} protein) N = 4	Proton-pumping (% quench min^{-1} mg^{-1} protein) N = 2
Control	5.3 ± 0.7	91.1 ± 8.4	7.0 ± 0.15
ABA (10^{-5} M)	2.8 ± 0.4 (53)[a]	67.9 ± 4.6 (75)[a]	5.4 ± 0.1 (77)[a]
GA_3 (10^{-5} M)	4.06 ± 0.5 (77)[a]	59.1 ± 4.5 (65)[a]	5.4 ± 0.3 (77)[a]

Mean values ± SE (% over control).
[a] Indicates significant differences at the 95% level of confidence or better (unpaired *t* tests), compared with controls.

Table 3. Sterol composition (% of total), sitosterol/stigmasterol ratio, campesterol/sitosterol ratio, and total sterol content (μg mg^{-1} protein) of shoot PMs from rice plants treated with and without ABA or GA_3 (10^{-5} M).

	Control	ABA (10^{-5} M)	GA_3 (10^{-5} M)
Cholesterol	2.7 ± 0.6	4.8 ± 2.1	2.4 ± 0.7
Campesterol	22.1 ± 0.7	22.8 ± 0.9	21.0 ± 1
Stigmasterol	38.2 ± 0.57	39.2 ± 1.3	34.1 ± 0.86[a]
Sitosterol	33.4 ± 0.98	30.4 ± 0.78	35.2 ± 2.6
Δ^5-avenasterol	3.7 ± 0.05	2.9 ± 0.3	7.2 ± 1.2[a]
Sitosterol/ stigmasterol	0.88 ± 0.04	0.78 ± 0.03	1.03 ± 0.1
Campesterol/ sitosterol	0.66 ± 0.05	0.75 ± 0.04	0.61 ± 0.07
Total sterols	109.3 ± 15	83.7 ± 6.5	93.7 ± 6.7

Mean values ± SE (N = 3).
[a] Indicates significant differences at the 95% level of confidence or better (unpaired *t* tests), compared with controls.

Table 4. Fatty acids (%) and total amount (μg mg^{-1} protein) in the PE fraction of shoot PMs from rice plants treated with and without ABA or GA_3 (10^{-5} M).

	Control	ABA (10^{-5} M)	GA_3 (10^{-5} M)
$C_{16.0}$	42.3 ± 1.8	39.5 ± 1.2	40.7 ± 0.5
$C_{16.1}$	8.2 ± 1.7	8.4 ± 1.0	6.1 ± 1.2
$C_{18.0}$	5.8 ± 0.2	7.0 ± 0.6	5.7 ± 0.2
$C_{18.1}$	5.7 ± 0.2	6.4 ± 0.98	4.8 ± 0.6
$C_{18.2}$	29.2 ± 2.6	30.8 ± 0.7	34.2 ± 1.1
$C_{18.3}$	8.9 ± 0.8	8.1 ± 0.6	8.4 ± 0.3
Bond index	98.9 ± 6	100.8 ± 3	104.8 ± 1.6
C_{16}/C_{18}	1.04 ± 0.15	0.92 ± 0.05	0.88 ± 0.02
Total PE	156.6 ± 11.9	159.1 ± 3.7	134.4 ± 13.5

Mean values ± SE (N = 3). Bond index and C_{16}/C_{18} ratio are also shown.

plus phospholipid) (Table 7). However, only GA_3 treatment produced plants with a lesser proportion of C_{16} fatty acids, but more C_{18} fatty acids in the PM compared with untreated plants. This was reflected in the overall C_{16}/C_{18} ratio of GA_3-treated plants which was 19% less than the control (Table 7). More unsaturated fatty acids were present in the PM from GA_3-treated plants, and a significant increase (7%) in the overall BI was found in these plants with respect to controls.

Discussion

Both hormones, ABA and GA_3, modified the growth of rice. The decrease in shoot height produced as a result of ABA application is the normal response to this treatment (Addicott and Lyon 1969, Walton 1980). Though total plant fresh and dry weight also decreased in comparison to controls, an increase in fresh and dry weight per unit length was observed (Table 1). On the contrary, with GA_3 treatments, shoot extension growth increased greatly (Table 1). This is a typical effect that has been observed with pea plants (Westerman and Roddick 1982) and *Avena* stem segments (Jusaitis et al. 1981). However, fresh and dry weights per unit length were reduced by GA_3 application (Table 1).

Although applications of GA_3 and ABA produced opposite effects on shoot growth, both hormones had the same effect of reducing the specific hydrolytic and proton-pumping activities of PM Mg^{2+}-ATPase (Table 2). Thus, the hormone effect on ATPase activity does not seem to be a consequence of the growth response.

Reductions in ATPase activity have been observed in plants under stress, viz. nitrogen starved cleavers (Rooney et al. 1990) and freezing injury in wild potato (Iswari and Palta 1989), conditions that also lead to ABA accumulation (Chapin 1990). Though the mechanism by which ATPase activity is affected by stress is not known, it is worth noting

Table 5. Fatty acids (%) and total amount (μg mg^{-1} protein) in the PC fraction of shoot PMs from rice plants treated with and without ABA or GA_3 (10^{-5} M).

	Control	ABA (10^{-5} M)	GA_3 (10^{-5} M)
$C_{16.0}$	34.8 ± 0.8	36.9 ± 1.3	33.2 ± 1.1
$C_{16.1}$	6.4 ± 0.6	8.2 ± 0.3	6.6 ± 0.9
$C_{18.0}$	7.4 ± 0.3	8.3 ± 1.6	6.6 ± 0.5
$C_{18.1}$	8.9 ± 0.4	7.0 ± 0.4[a]	8.3 ± 0.5
$C_{18.2}$	31.0 ± 1.8	30.0 ± 1.8	35.0 ± 1.2
$C_{18.3}$	11.6 ± 0.2	9.6 ± 0.5[a]	10.4 ± 0.4
Bond index	112.0 ± 3	103.8 ± 5	116.0 ± 2
C_{16}/C_{18}	0.70 ± 0.04	0.83 ± 0.05	0.66 ± 0.05
Total PC	228.9 ± 21	228.5 ± 22	191.1 ± 11

Mean values ± SE (N = 3). Bond index and C_{16}/C_{18} ratio are also shown.
[a] Indicates significant differences at the 95% level of confidence or better (unpaired *t* tests), compared with controls.

Table 6. Fatty acids (%) and total amount (μg mg^{-1} protein) in the PI fraction of shoot PMs from rice plants treated with and without ABA or GA_3 (10^{-5} M).

	Control	ABA (10^{-5} M)	GA_3 (10^{-5} M)
$C_{16.0}$	41.1 ± 3	44.4 ± 5	50.1 ± 2
$C_{16.1}$	28.2 ± 4	15.9 ± 0.3[a]	16.3 ± 1.2[a]
$C_{18.0}$	10.0 ± 1.5	15.0 ± 0.7[a]	13.1 ± 0.5
$C_{18.1}$	12.6 ± 1.1	13.0 ± 2.4	7.8 ± 0.8[a]
$C_{18.2}$	8.2 ± 0.3	10.6 ± 2	10.2 ± 1
$C_{18.3}$	ND	1.2 ± 1[a]	2.3 ± 0.2[a]
Bond index	57.2 ± 4.5	53.5 ± 10	51.5 ± 0.3
C_{16}/C_{18}	2.3 ± 0.07	1.5 ± 0.3	2.0 ± 0.01[a]
Total PI	73.1 ± 7	74.5 ± 2.1	36.6 ± 0.9[a]

Mean values ± SE (N = 3). Bond index and C_{16}/C_{18} ratio are also shown.
[a] Indicates significant differences at the 95% level of confidence or better (unpaired *t* tests), compared with controls.

Table 7. Fluidity (P), measured by steady-state fluorescence polarization, PE/PC ratio, mol% of sterols (assumed average M_r of sterols = 390.7 and phospholipids = 750), the overall phospholipid (PE, PC, and PI) bond index, and the C_{16}/C_{18} ratio from PMs of rice shoots treated with and without ABA or GA_3 (10^{-5} M).

	Control	ABA (10^{-5} M)	GA_3 (10^{-5} M)
Fluidity (P)	0.312 ± 0.001	0.290 ± 0.01[a]	0.293 ± 0.01[a]
PE/PC	0.690 ± 0.03	0.710 ± 0.09	0.700 ± 0.04
mol% sterol	31.4 ± 3.7	24.9 ± 1.8	33.3 ± 2.4
Bond index	98.8 ± 1.6	94.3 ± 4	105.4 ± 1.5[a]
C_{16}/C_{18}	1.07 ± 0.04	0.98 ± 0.07	0.87 ± 0.03[a]

Mean values ± SE (N = 3).
[a] Indicates significant differences at the 95% level of confidence or better (unpaired *t* tests), compared with controls.

that the effects we found with exogenous applications of ABA were the same as those reported in stressed plants. On the other hand, the decrease in ATPase activity produced by GA_3 is consistent with the stimulation produced by applications of the GA biosynthesis inhibitor tetcyclasis (Cooke et al. 1989).

The relative stigmasterol content of the PM was reduced by GA_3 application, whereas a large proportional increase in Δ^5-avenasterol was observed, along with a small, but statistically insignificant, increase in sitosterol (Table 3). Jusaitis et al. (1981) also reported a decrease in stigmasterol after in vivo GA_3 treatments in *Avena* stem segments. Our results contrast with the decrease in PM stigmasterol content observed after treatment with GA_3 biosynthesis-inhibiting plant growth retardants on oat (Burden et al. 1987, Cooke et al. 1989), barley, maize, and rice plants (Grossman 1990). These apparently contradictory effects may be explained by the fact that plant growth retardants, which are inhibitors of cytochrome P_{450}-dependent monooxygenases, may also inhibit the Δ^{22}-desaturase, the enzyme responsible for the conversion of sitosterol to stigmasterol (Haughan et al. 1989).

It is interesting to note that the effects of ABA treatment on the relative proportions of Δ^5-avenasterol, sitosterol, and stigmasterol were opposite to those of GA_3 treatment (Table 3). This could indicate hormonal regulation of sterol biosynthesis in rice at the step where Δ^5-avenasterol is converted to sitosterol and stigmasterol.

There was a trend towards a decrease in the sitosterol/stigmasterol ratio with ABA treatment (Table 3) and although this was not statistically significant, such a change seems to be a feature of a variety of stresses, including water stresses in tobacco (Davis 1972), salt stresses in citrus roots (Douglas 1985), chilling in beans (Guye 1987, 1989), and nitrogen starvation in cleavers (Rooney et al. 1990). It has been suggested that this decrease in the sitosterol/stigmasterol ratio represents a stress-sensitive response which affects the stability of the PM (Guye 1987). It was proposed that the more bulky C_{17} side-chain of stigmasterol resulted in a less stable membrane than that produced by the more planar sitosterol. In the present study, ABA treatment made the membrane more fluid (Table 7); however, it could be misleading to attribute this change in membrane fluidity solely to the reduction in the sitosterol/stigmasterol ratio especially in view of the results of GA_3 treatment.

Membrane fluidity was increased following GA_3 treatment; this effect was similar to the ABA treatment, although the effects on sterol composition were quite different. Neither of these hormones af-

fected the PE/PC ratio or the molar sterol composition, only GA_3 affected the overall fatty acid unsaturation and chain length (Table 7). This suggests that there was no common link between a specific change in lipid composition and fluidity in these membranes, since the two treatments produced different perturbations of membrane composition and still had similar effects on fluidity. It has recently been shown that the factors which determine the fluidity of the plant PM are extremely complex, and simple explanations based on specific membrane lipids are not always possible (Cooke et al. 1991). The reduction in Mg^{2+}-ATPase activity found with both hormones provides further evidence that there is no relationship between membrane-bound enzyme activity and membrane fluidity, since, according to that hypothesis, an increase in fluidity should have been accompanied by an increase in enzyme activity. Similarly, an increase in ATPase activity was observed in heavy metal-stressed rice plants, in which sterol composition was altered but membrane fluidity remained unchanged (Ros et al. 1990).

A more direct effect of lipids on ATPase activity could also be considered. In this context it is interesting to note that ABA treatment increased the campesterol/sitosterol ratio in PM vesicle (Table 3). A decrease in this ratio has been correlated with the stimulatory effect of cadmium and nickel in PM ATPase activity from rice shoots (Ros et al. 1990). In that work it was found that in heavy metal-stressed plants the percentage of Δ^5-avenaserol also decreased, whereas the present work showed that GA_3 produced the opposite effect. Phospholipid acyl chain analysis again revealed the opposing effects of heavy metal and GA_3 treatments as GA_3 increased the fatty acid chain lengths and the degree of saturation (Table 7), whereas nickel and cadmium decreased them (Ros et al. 1990).

Treatment with GA_3 also resulted in a reduction of PM PI content and both GA_3 and ABA significantly altered the fatty acid composition of PI (Table 6). These alterations to the PI fraction, a phospholipid involved in cell signal transduction (the PI cycle), may indicate some interaction between PI and GA_3 or ABA which is related to the hormones' modes of action and the PI cycle. It is worth noting in this context, that treatment of celery cell suspension cultures with the GA_3 biosynthesis-inhibiting plant growth retardant, paclobutrazol, resulted in an increase in PM PI content (N. Rasheed et al., unpublished results).

In conclusion, ABA and GA_3 treatments affected the lipid composition, fluidity, and membrane-bound enzyme-ATPase activity of rice shoot PMs. Changes in fluidity produced by the treatments does not seem to be related to specific changes in lipid composition, since each hormone affected it in different ways. On the other hand, we did not find a direct relationship between ATPase activity and membrane fluidity, since the increased fluidity was not reflected in an increase of the ATPase activity. In some instances, the effects of the exogenously applied hormones could be related to the reported actions of stress and xenobiotics. It illustrates the possibility of using experiments such as these to perturb various aspects of plant metabolism and hence help to elucidate the regulatory mechanisms.

Acknowledgments. This study was supported by grants from the Ministerio de Educación y Ciencia to C.M.C. and DGICYT (grant no. PS89/0082) to C.M.C., R.R., and A.S.

References

Ames BN (1966) Assay of inorganic phosphate, total phosphate and phosphatases. Meth Enzymol 8:115–118

Addicott FT, Lyon JL (1969) Physiology of abscisic acid and related substances. Ann Rev Plant Physiol 20:139–164

Barceló J, Cabot C, Poschenrieder CH (1986) Cadmium-induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv. Contender). II. Effect of Cd on endogenous abscisic acid levels. J Plant Physiol 125:27–34

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgramme quantities of protein utilising the principle of protein-dye binding. Anal Biochem 72:248–254

Burden RS, Cooke DT, White PJ, James CS. (1987) Effects of the growth retardant tetcyclasis on the sterol composition of oat (*Avena sativa*). Plant Growth Regul 5:207–217

Carruthers A, Melchior DL (1986) How bilayer lipids affect membrane protein activity. TIBS 11:331–335

Chapin FS III (1990) Effects of nutrient deficiency on plant growth: evidence for a centralised stress-response system. In: Davies WJ, Jeffcoat B (eds) Importance of root to shoot communications in the responses to environmental stress. BSPGR Monograph 21:135–148

Cooke DT, Burden RS, Clarkson DT, James CS (1989) Xenobiotic-induced changes in membrane lipid composition: effects on plasma membrane ATPases. In: Atkin RK, Clifford DR (eds) Mechanisms and regulation of transport processes. BSPGR Monograph 18:41–53

Cooke DT, Burden RS (1990) Lipid modulation of plasma membrane-bound ATPases. Physiol Plant 78:153–159

Cooke DT, Munkonge FM, Burden RS, James CS (1991) Fluidity and lipid composition of oat and rye shoot plasma membrane: effect of sterol perturbation by xenobiotics. Biochim Biophys Acta 1061:156–162

Creelman RA (1989) Abscisic acid physiology and biosynthesis in higher plants. Physiol Plant 75:131–136

Davis DJ (1972) Sterol distribution within green and air cured tobacco. Phytochemistry 11:489–494

Douglas TJ (1985) NaCl effects on 4-desmethylsterol composition of plasma membrane-enriched preparations from citrus roots. Plant Cell Environ 8:687–692

Douglas TJ, Walker RR (1984) Phospholipids, free sterols and adenosine triphosphatase of plasma membrane-enriched

preparations from roots of *Citrus* genotypes differing in chloride exclusion ability. Physiol Plant 62:51–58

Grossman K (1990) Plant growth retardants as tools in physiological research. Physiol Plant 78:640–648

Guye MG (1987) Chilling and age related changes in the free sterol composition of *Phaseolus vulgaris* L. primary leaves. Plant Sci 53:209–213

Guye MG (1989) Phospholipid, sterol composition and ethylene production in relation to choline-induced chill-tolerance in Mung bean (*Vigna radiata* L. Wilcz) during a chill–warm cycle. J Exp Bot 40:369–374

Haughan PA, Burden RS, Lenton JR, Goad LJ (1989) Inhibition of celery growth and sterol biosynthesis by the enantiomers of paclobutrazol. Phytochemistry 28:781–787

Iswari S, Palta JP (1989) Plasma membrane ATPase activity following reversible and irreversible freezing injury. Plant Physiol 90:1088–1095

Jusaitis M, Paleg LG, Aspinall D (1981) Effects of temperature and gibberellic acid on 4-desmethylsterol composition of *Avena sativa* stem segments. Phytochemistry 20:417–423

Larsson C, Widdell S, Kjellbom P (1987) Preparation of high-purity plasma membranes. Meth Enzymol 148:558–568

Patton GM, Fasulo JM, Robins SJ (1982) Separation of phospholipids and individual molecular species of phospholipids by high-performance liquid chromatography. J Lipid Res 23:190–196

Rooney JM, Cooke DT, Clarkson DT, Burden RS, James CS (1990) Plasma membrane lipid composition and ATPase activity in shoots during the development of nutrient stress in *Galium aparine* L. (cleavers). Abstracts of papers and posters on topics relevant to plant biology presented at the 1990 Annual Meeting of the Society for Experimental Biology, J Exp Bot pp 1–7

Ros R, Cooke DT, Burden RS, James CS (1990) Effects of the herbicide MCPA, and the heavy metals, cadmium and nickel on the lipid composition, Mg^{2+}-ATPase activity and fluidity of plasma membranes from rice, *Oryza sativa* (cv. Bahia) shoots. J Exp Bot 41:457–462

Simmonds AC, East JM, Jones OT, Rooney EK, McWhirter J, Lee AG (1982) Annular and non-annular binding sites on the (Ca^{2+}-Mg^{2+})-ATPase. Biochim Biophys Acta 693: 398–406

Walton DC (1980) Biochemistry and physiology of abscisic acid. Ann Rev Plant Physiol 31:453–489

Westerman L, Roddick JG (1982) Sterol composition of dwarf and tall *Pisum sativum* seedlings in relation to gibberellic-acid-enhanced shoot growth. Phytochemistry 21:1567–1572