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EFFECTS OF WATER STRESS AND SALINITY ON STEROLS IN *ZEA MAYS* SHOOTS

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Key Word Index—*Zea mays*; Gramineae; maize; stress; polyethylene glycol; sodium chloride; phytosterols; cholesterol; campesterol; stigmasterol; sitosterol.

Abstract—Seven-day-old seedlings of maize (*Zea mays* cv. Summer II) were subjected to water and salinity stress with polyethylene glycol (M_r 4000) and sodium chloride to achieve final concentrations of 13 and 17% PEG and 0.8 and 0.16 F sodium chloride, respectively. The water potential values of stressing solutions were −0.4 and −0.8 MPa. The leaf water potential and stomatal response, as well as the dry weight and height of shoots, were compared at the two levels of stress. Leakage of ionic solutes and potassium efflux were used to assess membrane integrity. Qualitative and quantitative changes in free and total sterols were investigated. Sitosterol and stigmasterol, followed by campesterol and a small amount of cholesterol, were the major sterols present in the control and in the treated shoots. After 72 hr of stress imposition there was a reduction in free and total sterols in both treated shoots as compared with the control. In spite of the stress-induced decrease in the sterol content, the sitosterol to stigmasterol ratio and the cholesterol plus campesterol to stigmasterol plus sitosterol ratio did not show any significant difference, suggesting the relative abilities of shoots to regulate membrane permeability by maintaining their 'more planar' to 'less planar' sterol ratio.

INTRODUCTION

Salt and water stresses are important problems in agriculture, limiting crop growth and production in many parts of the world. Many experiments have been performed to investigate the effects of salt or water stresses on the various aspects of plant growth. However, in order to provide a better understanding of plant responses to these two stresses, osmotic effects must be separated from specific ion effects. Attempts to do this might involve comparisons between iso-osmotic solutions of salt and polyethylene glycol (PEG).

Although some authors have compared the effects of sodium chloride and PEG on nitrogen metabolism, NH_4^+ and NO_3^- absorption [1, 2], and other physiological parameters [3], there is little available information on the effect of these two stresses on sterol metabolism and composition of plant tissues. It is now generally accepted that the first deteriorative change during stress injury is an alteration in the structure and function of cell membranes, which results in an impaired ability to retain solutes. The precise nature of this damage is, however, far from understood [4].

It has been shown that sterols influence structural and functional properties of biological membranes of which they are important constituents [5]. Qualitative and/or quantitative differences in sterol composition, particularly in free sterols, whether age, species [6], temperature [7], water deficit or salinity [8] induced, may reflect the relative abilities of higher plants to regulate membrane permeability to ions and to tolerate, or to adapt to, an environmental stress or may be attributed to stress induced reactions.

From published studies no clear picture emerges, since the effect on sterols of these stresses seems to be dependent not only on the species and the growth stage at which the stress was imposed, but also on the stress severity. There was indeed no apparent effect when the stress was applied at some stages or at a certain intensity, but a reduction or an increase resulted when applied at others [9–14].

Recently, we found that maize seedlings, submitted to the highest concentrations of sodium chloride and PEG, iso-osmotic solution, at which their growth was not reduced ($\Psi = -0.2$ MPa), responded by increasing the levels of free sterols in their shoots and by maintaining constant their 'more planar' to 'less planar' free sterol ratios [8].

Based on these results a further study was undertaken to follow the behaviour of *Zea mays* seedlings subjected to salt and PEG stress which was more severe than in the previous experiment, followed by growth reduction. In this way, we tried to discover whether the total and free sterol composition was again equally affected by limiting water conditions and by salt stress. In addition, so as to achieve a good understanding of the strategies of this plant under adverse conditions, we have taken into account increasing salt and PEG stresses.

RESULTS

In maize seedlings exposed for 72 hours to sodium chloride and PEG iso-osmotic treatments the leaf water potential decreased to below that of the control in the salt treated seedlings and even more so in PEG treated seedlings. At −0.4 MPa in the growth media the leaf water potential had reached −0.69 MPa with PEG and −0.57 MPa with NaCl, at −0.8 MPa in the growth media the leaf water potential in PEG was −0.73 MPa

Table 1. Changes in leaf water potential (Ψ_w)*, shoot dry weight,† shoot fresh weight to dry weight and root to shoot ratios† of *Zea mays* seedlings subjected to sodium chloride and PEG increasing iso-osmotic treatments for 72 hr

Growth solution	MPa Ψ	MPa Ψ_w	Dry wt mg/plant	Fr.wt: Dry wt.	Root:shoot
Control	−0.03	−0.37 ± 0.01	88.8b	19.9c	0.45a
Nacl	−0.40	−0.57 ± 0.03	75.7a	12.5b	0.56b
PEG	−0.40	−0.69 ± 0.02	75.3a	12.3b	0.50b
NaCl	−0.80	−0.64 ± 0.02	69.6a	10.6a	0.51b
PEG	−0.80	−0.73 ± 0.01	86.9b	10.7a	0.49ab

*Results are the means of three replicates of 10 plants each ± s.e.

†Results are the means of three replicates of 30 plants each. For comparisons among means the analysis of variance was used.

Means with letters in common are not significantly different at the $P = 0.01$.

and with sodium chloride treatment it was −0.64 MPa (Table 1).

Maize showed a marked reduction in plant height and dry weight when the water potential was −0.4 MPa.

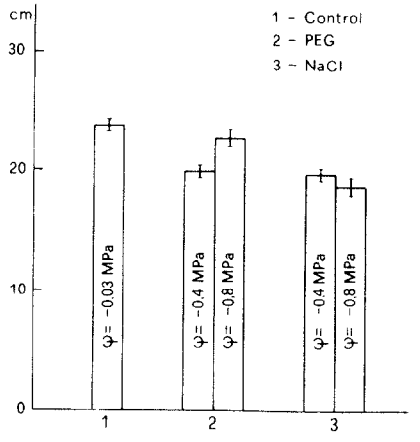

Fig. 1. Effect of PEG and sodium chloride treatments on the height of *Zea mays* shoots.

However, a water potential of −0.8 MPa led to a significantly reduced height and dry weight only in sodium chloride treated shoots (Fig. 1, Table 1).

The fresh weight/dry weight ratio of the shoots had decreased significantly in both sodium chloride and PEG treatments and this ratio had also changed between −0.4 and −0.8 MPa growth media. On the contrary, the root/shoot dry weight ratios increased significantly after these treatments (Table 1).

The stomatal conductance, which decreased when compared with the control during the three days of stress imposition, reached the lowest values for the most severe treatments of added PEG and sodium chloride after 72 hr (−0.8 MPa) without any difference between treatments. In the treatments at −0.4 MPa the values of stomatal conductance in PEG treated shoots were slightly lower than those obtained with sodium chloride (Fig. 2).

Total electrolyte leakage and potassium efflux in the shoots of seedlings grown at −0.4 MPa followed a similar time profile in both stressed seedlings, higher than in the control, while the PEG-treated shoots of seedlings grown at −0.8 MPa had the same electrolyte leakage and potassium efflux as the control. Sodium chloride treated shoots showed a higher electrolyte leakage and potassium efflux than the control (Figs 3 and 4).

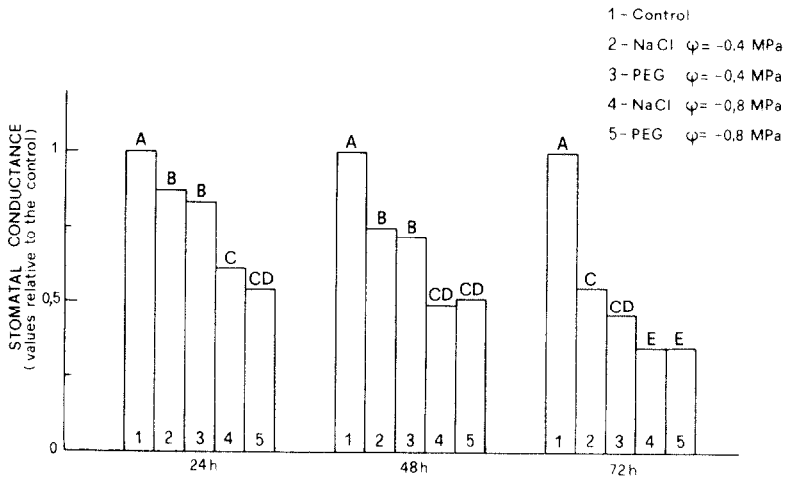

Fig. 2. Stomatal conductance of *Zea mays* shoots as a function of added sodium chloride and PEG over the experimental period. For comparisons among means the analysis of variance was used. Letters in common are not significantly different at the $P = 0.01$.

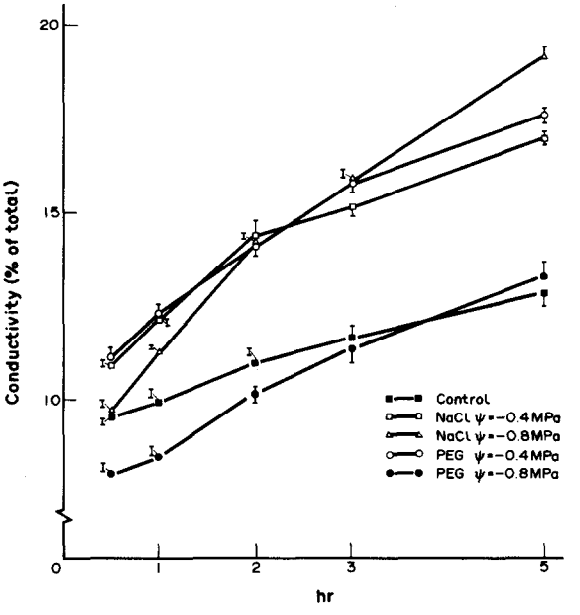

Fig. 3. Cellular leakage from control and stressed *Zea mays* shoots as a percentage of total conductivity. Each value is the mean from three replicates of three different experiments. Standard errors are calculated for each mean.

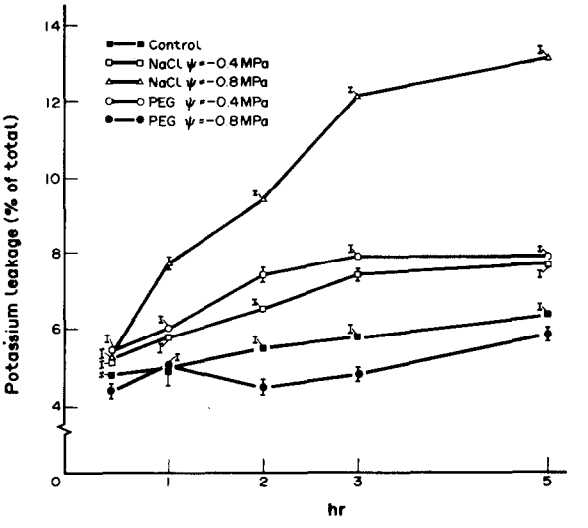

Fig. 4. Time profile of K^+ concentration in leachate of control and stressed leaf discs of *Zea mays*. The relative amount of K^+ is expressed as percentage of the maximum concentration measured in the leachate after killing the samples by immersion in liquid nitrogen. Each value is the mean from three replicates ± s.e.

The main changes related to sodium chloride treatments were an increase of Na^+ and a decrease of K^+ contents in maize shoots, showing at −0.4 MPa, a Na^+ to K^+ ratio of 1.8×10^{-1} against a Na^+ to K^+ ratio of 1.8×10^{-3} in the control and in PEG treated shoots, and at −0.8 MPa a ratio of 2.1×10^{-1} in sodium chloride and of 1.6×10^{-3} in PEG treated shoots.

Maize shoots treated with PEG and sodium chloride did not exhibit significant changes in total lipid and phospholipid contents (F. Navari-Izzo, unpublished data) measured on a dry weight basis, either between the various treatments or dependent upon the severity of the imposed stresses. The free and total sterol contents (Table 2) declined with PEG treatment and even more so with sodium chloride treatment, but they did not change with the increase in osmotic potential of stressing growth media.

The major free sterols in untreated maize shoots were sitosterol (62%), stigmasterol (21%) and campesterol (16%), accompanied by a small amount of cholesterol (less than 1%). This percentage composition changed very little in total sterol upon stressing (calculated from Tables 2–4). Furthermore, although the sterols decreased upon stress, the sitosterol to stigmasterol ratio showed the same behaviour in all treated shoots and in the control, and there were no significant differences in the ratio of 'more planar' (cholesterol plus campesterol) to 'less planar' (stigmasterol plus sitosterol) sterols of the stressed shoots as compared to the untreated ones (Tables 3 and 4).

The molar ratio of free sterols to phospholipids (Table 5) in the shoots of maize grown in saline medium at −0.4 MPa was reduced by 40% and at −0.8 MPa by 50%, while this ratio in shoots grown in PEG medium had decreased only by 20 and 10%, respectively, without any significant difference when compared with the control.

DISCUSSION

Leaf water potentials obtained with sodium chloride solutions were higher than those obtained with iso-

Table 2. Free and total sterol content (mg/100 g dry weight) of *Zea mays* shoots subjected to sodium chloride and PEG increasing iso-osmotic treatments

Growth solution (MPa)	Free				Total			
	Control	NaCl	PEG	Means	Control	NaCl	PEG	Means
−0.03	176.0c			176.0b	231.8c			231.8b
−0.40		94.0a	139.8b	116.9a		151.5a	204.5b	178.0a
−0.80		93.5a	148.4c	120.9a		132.5a	203.3b	167.9a
Means		121.2a	154.7b			171.9a	213.2b	

For comparisons among means the two way analysis of variance was used. The significance of the letters is the same as in Table 1.

Table 3. Free sterol composition (mg/100 g dry weight) of *Zea mays* shoots subjected to sodium chloride and PEG increasing iso-osmotic treatments

Growth solution (MPa) Ψ	Cholesterol				Campesterol				Stigmasterol				Sitosterol			
	Control	NaCl	PEG	Means	Control	NaCl	PEG	Means	Control	NaCl	PEG	Means	Control	NaCl	PEG	Means
−0.03	1.1d			1.1b	28.5c			28.5b	37.8b			37.8b	108.6c			108.6b
−0.04		0.5ab	0.8bc	0.6a		15.7a	22.2b	18.9a		23.6a	32.6b	28.1a		54.2a	84.2b	69.2a
−0.80		0.5ab	0.9cb	0.7a		14.4a	24.7b	19.5a		22.3a	36.3b	29.1a		56.3a	86.5b	71.4a
Means		0.7a	0.9b			19.5a	25.1b			27.9a	35.6b			73.0a	93.1b	

For comparisons among means the two way analysis of variance was used. The significance of the letters is the same as in Table 1.

Table 4. Total sterol composition (mg/100 g dry weight) of *Zea mays* shoots subjected to sodium chloride and PEG increasing iso-osmotic treatments

Growth solution (MPa) Ψ	Cholesterol				Campesterol				Stigmasterol				Sitosterol			
	Control	NaCl	PEG	Means	Control	NaCl	PEG	Means	Control	NaCl	PEG	Means	Control	NaCl	PEG	Means
−0.03	1.9bc			1.9b	41.8d			41.8c	45.2b			45.2b	142.9c			142.9c
−0.40		1.8bc	2.2c	2.0b		27.0b	35.0c	31.0b		32.3a	41.1b	36.7a		90.4a	126.2b	108.3b
−0.80		1.1a	1.4ab	1.2a		21.5a	34.8c	28.1a		29.1a	44.8b	36.9a		80.8a	122.3b	101.5a
Means		1.6a	1.8b			30.1a	37.2b			35.5a	43.7b			104.7a	130.5b	

For comparisons among means the two way analysis of variance was used. The significance of the letters is the same as in Table 1.

Table 5. Free sterol: phospholipid molar ratio of *Zea mays* shoots subjected to sodium chloride and PEG iso-osmotic treatments

Growth solution (MPa)	Control	NaCl	PEG	Means
−0.03	0.20b			0.20b
−0.40		0.12a	0.16ab	0.14a
−0.80		0.10a	0.18b	0.14a
Means		0.14a	0.18b	

For comparisons among means the two way analysis of variance was used. The significance of the letters is the same as in Table 1.

osmotic PEG solutions (Table 1). This may logically be due to ion uptake from the sodium chloride solutions, which process should allow better osmotic adjustment than a supposed less permeating neutral solute such as PEG. One of the consequences of the fact that the leaf water potentials reached with sodium chloride stress were higher than those obtained with PEG would be that leaf diffusion resistance might be more affected by PEG than by sodium chloride. This is not true in our experiments (Fig. 2), since we did not find any difference between the two treatments. Although Sanchez-Diaz *et al.* [4] investigating three different legumes and Soldatini and Giannini [15] examining maize found a lower stomatal conductance in PEG treated plants, the results of our experiment agree with most of the literature data [16–19].

The fresh weight/dry weight ratio (Table 1), already used as a measure of the water content and indirectly as a measure of stress intensity [20], decreased with the degree of severity of the added sodium chloride or PEG in the same manner thus showing the same stress was achieved at the same water potential. An increase in root/shoot ratio with rising salinity has already been observed [21]. This agrees with our experiments, where root growth was rapid under salt conditions at −0.4 and −0.8 MPa, since under these conditions osmotic adjustment does in fact maintain growth of root tissue (Table 1). This increase appears to be a significant feature in the adjustment of a plant to salinity. A similar favourable root/shoot ratio was also observed by Sharp and Davis [22] in maize plants grown under limited water availability, even though plants growing in drying soil exhibited this rise on the days four and five of the drying cycle. Our data on maize grown in PEG at −0.4 MPa showed the same increase in the root/shoot ratio. However, when the water potential was increased to −0.8 MPa the root/shoot dry weight ratio did not change compared with the control, showing that in this situation a constant leaf growth rate may be sustained in spite of the decrease in leaf water potential. In such conditions the differences might be due to the different age of the maize and/or to the different water stress imposed and their degree. Virtually no difference in shoot fresh weight gain was observed with sodium chloride or PEG treatment, although it was lower than in the control. However, while maize grown at −0.4 MPa did not exhibit any differences in plant height and dry weight production between treatments, maize grown at −0.8 MPa accumulated significantly more dry matter in shoots from PEG than from sodium chloride treated and were the same as the control (Table 1, Fig. 1).

The disparity in plant growth in such conditions (−0.8 MPa) in PEG and in salt may reflect PEG absorption or rather a difference in the molecular weight of the principal solutes used for the osmotic adjustment. In addition, under saline conditions, growth may be further reduced by metabolic limitations which might be caused by ion toxicity due to the accumulation of Na^+ and Cl^- used for osmotic adjustment [23]. This reveals how the plant adapts itself to such a situation.

The reduction in plant height and dry weight of maize with salinity increase has been already observed by Yeo [24] and Patel and Vora [25]. Assuming that the greater is the injury of the living tissue, the greater the efflux of ions from the cells [26], the total electrolyte leakage and potassium efflux were used as an index of membrane integrity, as leakage from tissue has been explained in terms of changes which take place in the structure of membranes [27]. A common feature is that the major leakage of solute is a transient phenomenon, often lasting only a few minutes after the addition of water, but a prolonged loss of solute is characteristic of irreversibly damaged tissue. Both electrolyte leakage and potassium efflux in the shoots of seedlings grown at −0.4 MPa, with PEG and sodium chloride, followed a similar time profile in stressed seedlings which was higher than in the control (Figs 3 and 4), showing that both stresses seem to have caused the same changes and damages in membranes.

At −0.8 MPa sodium chloride treated shoots increased even more their electrolyte leakage and potassium efflux resulting in a higher loss of semi-permeability properties of membrane integrity, while PEG treated shoots did not show great differences when compared with the control.

It follows, as already suggested by our results on root/shoot dry weight ratio and shoot dry weight increase, that PEG, at a concentration which gives a water potential of the growth medium of −0.8 MPa, must interact with plant material and must travel in the maize apoplast in quantities sufficient to affect membrane permeability. There are indeed several reports that PEGs of substantial mean M_r do in fact enter plant tissues [28–31] even if with a rather low permeation.

The sterol level and composition agree well with those reported by ourselves [8] and other authors [32, 33], in the present experiment the free sterol content was higher than observed previously [8] because of the higher dry matter content due to the use of Hoagland's growth solution (Tables 2–4).

Some authors [10, 11, 13] found that water stress and salinity can increase the sterol level in low sensitivity species. We found previously that maize shoots in seedlings exposed to sodium chloride and PEG at −0.2 MPa of the growth medium, increased their free sterol content without reducing their dry matter or height as a consequence of an adaptative process to low levels of imposed stresses [8].

The data of the present experiments, which show a decrease on the dry weight basis in free and total sterol with both treatments, are not surprising, because it is a consequence of the dry matter decrease acquired with stress imposition [8]. However, on a plant basis no decrease has been observed with PEG (−0.8 MPa) treated shoots, where no decrease in dry matter content was observed.

Erdei *et al.* [11] and Douglas and Walker [14] found a free sterol decrease with increasing salinity in salt-sensitive species, and maize is a moderately salt-sensitive species [34].

Upon stressing the sterol content changed but not its composition, thus maintaining the 'more planar' to 'less planar' ratio, already considered as an index of membrane properties [8, 14]. This constant ratio contributes to the control of membrane permeability and to reducing stress effects.

In addition, although Douglas and Walker [14] observed a salt induced reduction in sitosterol to stigmasterol ratio, our data (Tables 3 and 4) do not show any variations in this ratio, showing that no irreversible modifications occurred with PEG or with sodium chloride.

Although all forms of sterols appear to be localized in membranes, it has been demonstrated that only free sterols play an important role in the permeability of plant membranes. We believe that, since a large proportion of free sterol is incorporated in the phospholipid bilayers of various membrane structures, a reduced molar ratio of free sterol to phospholipids might alter the physical architecture and permeability of membranes. This ratio, already considered as an index of membrane selectivity and organization [7, 14], was the lowest in sodium chloride treated shoots (Table 5). This suggested that, as already pointed out from total electrolyte leakage, potassium efflux and other physiological parameters, the injuries caused by ionic effects are greater than those caused by the decrease of the osmotic potential in the growth medium, at least using PEG 4000 as osmotic agent.

It has therefore been suggested that PEG at high concentrations reduces the polarity of an aqueous medium, which results in rearrangements of polar and hydrophobic groups of membrane components [35] and in stabilization of membrane lipid structures [36]. More detailed information about physical and biochemical changes due to salt and water stress could be obtained from studies on isolated membranes.

EXPERIMENTAL

Plant material. Maize seeds (*Zea mays* cv. Summer II) were soaked for 2 hr in running tap water and were germinated on moist filter paper in petri dishes at 25° for 72 hr. Uniform seedlings were transplanted and grown, as previously described [8] at 21° and 15° day/night, a 16 hr photoperiod, 85% relative humidity and a light intensity of 18.000 lux, in half-strength aerated Hoagland's soln, renewed every 3 days. 6-day-old seedlings were subjected to stress by placing them in the nutrient soln medium to which NaCl or PEG 4000 were added to achieve a final concentration of NaCl 0.8 and 0.16 F or 13 and 17% PEG, respectively, and an osmotic potential of −0.4 and −0.8 MPa as determined by a freezing point osmometer (Roebling micro-osmometer, Vogel). The basic nutrient soln, used as a control, had an osmotic potential of −0.03 MPa. Plant materials (three replicates of 150 seedlings each) were collected after a 72 hr period from both treatments and the control. Roots and shoots were separated, weighed and immediately frozen in liquid N_2 and freeze-dried.The shoot height was recorded and samples were taken for dry weight measurements. Leaf water potentials were measured with a pressure chamber [37], stomatal behaviour was estimated by measuring leaf resistance with a diffusion porometer (Mod. VPIC Cayuga Development U.S.A.).

Leakage. A solute leakage technique was used to assess membrane integrity at the end of stress imposition [26]. A total of 50 leaf discs with a 1 cm diameter was sampled and then submerged in 50 ml of distilled H_2O. The leaf discs were shaken (at 21°) for 0.5, 1, 2, 3 and 5 hr and at each time, electrical conductance of the effusate was measured with a Metrohm 660 conductometer. The relative amount of leakage was expressed as percentage of the maximum conductivity, measured after submerging the discs for 5 min into liquid N_2 after the fifth conductivity measurement. The discs were placed in the same vial containing the previous effusate and shaken for 1 hr.

A Perkin–Elmer mod. 373 atomic absorption spectrophotometer was used to measure K^- content of effusate and K^+ and Na^+ total content of shoots.

Lipid extraction and sterol analysis. Lipids were extracted according to Navari-Izzo *et al.* [38] with slight modifications. In order to avoid enzymatic lipid degradation, shoots were boiled in *i*-PrOH for 5 min and the tissue residue was homogenized in liquid N_2 with $CHCl_3$–MeOH (2:1) containing butylhydroxytoluol, and further extracted for 24 hr in a Soxhlet apparatus. The combined lipid extracts were then washed according to Folch *et al.* [39] and filtered to remove proteins with a medium grade sintered glass funnel. The extract was brought to a known volume, aliquots were taken to determine the total lipid amounts and total phospholipids [40]. The remainder of the extract was taken to dryness under red. pres. in N_2 at low temp.

The total lipid extract was eluted on a sililic acid column according to Rouser *et al.* [40]. Free and esterified sterols were eluted with $CHCl_3$ together with mono, di, and tryglycerides. To separate free from esterified sterols the dried lipid extract was redissolved in *n*-hexane and applied to a silica gel column and eluted as previously described [42]. Total sterols were extracted and hydrolysed according to Izzo and Navari-Izzo [42] and pptn of free and total sterols was as previously reported [7, 42]. Individual sterols were identified by GLC, cholestane was the internal standard, and for their quantitation, corrections were made for differences in detector response. The Perkin–Elmer gas-chromatograph Sigma I was equipped with a capillary column Permaphase DMS Perkin–Elmer (25 m × 0.32 mm). The operating conditions were: column temp. 230°, flash heater and detector 280°, N_2 was the carrier gas at 115 KPa.

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REFERENCES

1. Senaratna, T., Mc Kersie, B. D. and Stinson, R. H. (1984) *Plant Physiol.* **76**, 759.
2. Frota, J. N. E. and Tucker, T. C. (1978a) *Soil Sci. Soc. Am. J.* **42**, 743.
3. Frota, J. N. E. and Tucker, T. C. (1978b) *Soil Sci. Soc. Am. J.* **42**, 753.
4. Sanchez-Diaz, M., Aparicio-Tejo, P., Gonzales-Murua, C. and Pena, J. I. (1982) *Physiol. Plant.* **54**, 361.
5. Horvath, I., Vigh, L., Farkas, T., Horvath, L. I. and Dudits, D. (1981) *Planta* **153**, 476.
6. Grunwald, C. (1980) in *Encyclopedia of Plant Physiology New Series* Vol. 8 (Bell, E. A. and Charlwood, B. V., eds), pp 221–256. Springer, Berlin.
7. Izzo, R., Navari-Izzo, F. and Bottazzi, F. (1984) *Phytochemistry* **23**, 2479.
8. Navari-Izzo, F., Izzo, R., Bottazzi, F. and Guidi, M. (1985) *Agrochimica* **29**, 154.
9. Kuiper, D. and Kuiper, P. J. C. (1978) *Physiol. Plant.* **44**, 81.

10. Stuiver, C. E. E., Kuiper, P. J. C. and Marschner, H. (1978) *Physiol. Plant.* **42**, 124.
11. Erdei, L., Stuiver, C. E. E. and Kuiper, P. J. C. (1980) *Physiol. Plant.* **49**, 315.
12. Stuiver, C. E. E., Kuiper, P. J. C., Marschner, H. and Kylin, A. (1981) *Physiol. Plant.* **52**, 77.
13. Douglas, T. J. and Paleg, L. G. (1981) *J. Exp. Botany* **32**, 499.
14. Douglas, T. J. and Walker, R. R. (1983) *Physiol. Plant.* **58**, 69.
15. Soldatini, G. F. and Giannini, A. (1985) *Agrochimica* **29**, 74.
16. Hsiao, T. C. (1973) *Annu. Rev. Plant Physiol.*, **24**, 519.
17. Coudret, A., Ferard, G. and Lascève, G. (1982) *Physiol. Veg.* **20**, 711.
18. Hanson, A. D. and Hitz, W. D. (1982) *Annu. Rev. Plant Physiol.* **33**, 163.
19. Heath, R. L., Furbank, R. T. and Walker, D. A. (1985) *Plant Physiol.* **78**, 627.
20. Liljenberg, C., Karunen, P. and Ekman, R. (1985) *Physiol. Plant.* **63**, 253.
21. Turner, N. C. and Begg, J. E. (1981) *Plant Soil* **58**, 97.
22. Sharp, R. E. and Davies, W. J. (1979) *Planta* **147**, 43.
23. Greenway, H. and Munns, R. (1980) *Annu. Rev. Plant Physiol.* **31**, 149.
24. Yeo, A. R. (1983) *Physiol. Plant.* **58**, 214.
25. Patel, J. A. and Vora, A. B. (1985) *J. Exp. Botany* **36**, 49.
26. Palta, J. P., Levitt, J. and Stadelmann, E. J. (1977) *Plant Physiol.* **60**, 393.
27. Simon, E. W. (1974) *New Phytol.* **73**, 377.
28. Lagerwerff, J. V., Ogata, G. and Eagle, H. E. (1961) *Science* **133**, 1485.
29. Lawlor, D. W. (1970) *New Phytol.* **69**, 501.
30. Carpita, N., Sabularse, D., Montezinos, D. and Delmer, D. P. (1979) *Science* **205**, 1144.
31. Yeo, A. R. and Flowers, T. J. (1984) *Plant Physiol.* **75**, 298.
32. Kemp, R. J., Goad, L. J. and Mercer, E. I. (1967) *Phytochemistry* **6**, 1906.
33. Hartmann, M. A., Normand, G. and Benveniste, P. (1975) *Plant Sci. Letters* **5**, 287.
34. Maas, E. V. and Hoffmann, G. J. (1977) *J. Irrig. Drainage Div. Am. Soc. Civ. Eng.*, **103**, 115.
35. Arnold, K., Pratsch, L. and Gawrisch, K. (1983) *Biochim. Biophys. Acta* **728**, 121.
36. Tilcock, C. P. S. and Fisher, D. (1979) *Biochim. Biophys. Acta* **577**, 53.
37. Scholander, P. F., Hammel, H. T., Bradstreet, E. D. and Hemmingsen, E. (1965) *Science* **148**, 339.
38. Navari-Izzo, F., Izzo, R. and Paradossi, C. (1977) *Riv. Ital. Sostanze Grasse* **54**, 22.
39. Folch, J., Lees, M. and Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497.
40. Rouser, G., Fleischer, S. and Yamato, A. (1970) *Lipids* **5**, 494.
41. Rouser, G., Kritchevshy, G., and Simon, G. (1967) *Lipids* **2**, 37.
42. Izzo, R. and Navari-Izzo, F. (1981) *Plant Physiol.* **67**, 1073.