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STEROLS IN HARDENING WINTER WHEAT

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Key Word Index—*Triticum aestivum*; Graminae; winter wheat; phytosterols; low temperature; frost hardening; sitosterol; campesterol; stigmasterol; cholesterol.

Abstract—The main sterols in winter wheat crowns and roots were sitosterol and campesterol, with significant amounts of stigmasterol and traces of cholesterol. The main groups of sterol-containing lipids were free sterols, steryl glucosides, steryl esters and esterified steryl glucosides. Sterol analysis within each group showed little difference between them. Steryl esters were relatively rich in cholesterol and poor in stigmasterol. Free sterols were rich in stigmasterol. Low temperature caused an increase in sterol content but had little effect on sterol composition and sterol to lipid P ratio. There was some increase in steryl esters and some decrease in free sterols. Cholesterol and stigmasterol decreased in the steryl ester and free sterol fractions, respectively. There was little evidence for involvement of sterols in winter wheat frost hardening.

INTRODUCTION

Through their condensing effect on phospholipid films [1–3], sterols influence biophysical characteristics of cell membranes, e.g. permeability [4] and temperature of phase transitions [3].

It has been suggested repeatedly that changes in sterol content of membranes may be a factor in low temperature acclimation of plants [5, 6]. Since changes in the degree of unsaturation of fatty acids do not seem to be involved in frost hardening of winter wheat [7–9], it became increasingly interesting to investigate whether changes in sterols were involved.

Davis and Finkner [6] found little effect of temperature on free sterol content and free sterol profile of winter wheat roots, except for a slight shift towards sitosterol. de la Roche [7] found a significant increase in the ratio of sitosterol to stigmasterol in a total membrane fraction of winter wheat seedlings grown at 1°. This shift occurred to the same extent in 4 cultivars differing in frost-hardening potential. No change in sterol to lipid P ratio was observed. Grenier *et al.* [10] found no increase in ^{14}C-incorporation into free sterols of alfalfa roots during hardening. Sterols were, however, increasingly esterified.

We extended these observations by studying the effects of low temperature on total sterol profile, proportions in groups of sterol-containing lipids, sterol profile within each group, and sterol to lipid P ratio in the crowns and roots of young winter wheat plants of the hardy cultivar Kharkov during frost hardening at 1°.

RESULTS AND DISCUSSION

The main sterols of 12-day-old winter wheat crowns and roots were sitosterol and campesterol, with significant amounts of stigmasterol and some cholesterol (Table 1). The data agree with those obtained by Davis and Finkner [6] with 7-day-old plants and by de la Roche (private communication) with free sterols from a total membrane fraction.

Low temperature treatment had little influence on total sterol composition (Table 1). We did not observe the shift towards sitosterol described by Davis and Finkner [6] and by de la Roche [7]. Cholesterol did

Table 1. Levels of sterols and phosphorus in total lipids in winter wheat crowns and roots during hardening at 1° (means of 5 samples ±s.d.)

Hardening (days)	Sterols (% of total sterols) Chol*	Camp	Stig	Sito	Total sterols (μg/g fr. wt)	Lipid P (μg/g fr. wt)	Molar ratio† sterol/P
0	0.4±0.1	28.8±0.3	6.3±0.7	64.8±0.7	245.6±12.3	48.82±3.44	0.376
7	0.3±0.1	29.5±0.3	5.2±0.3	65.1±0.3	267.1±6.4	61.27±3.00	0.326
14	0.4±0.1	29.0±0.2	4.7±0.3	65.9±0.6	249.2±3.6	51.80±2.98	0.359
28	0.5±0.1	28.3±0.4	5.0±0.4	66.3±0.5	345.9±15.9	68.24±2.03	0.379

* Chol: cholesterol; Camp: campesterol; Stig: stigmasterol; Sito: sitosterol.

† Sterol MW was averaged at 415.

Table 2. Groups of sterol-containing lipids in winter wheat crowns and roots during hardening at 1° (percentage of total sterols; means of 5 samples ±s.d.)

Hardening (days)	Steryl glucosides	Esterified steryl glucosides	Free sterols	Steryl esters
0	32.2±5.3	10.4±1.2	46.5±5.3	11.0±1.4
7	28.9±2.9	9.4±1.4	48.9±2.2	12.9±1.3
14	32.7±3.4	10.9±0.4	39.9±6.1	16.5±1.6
28	34.1±7.1	10.8±1.3	40.8±6.4	14.4±0.4

not disappear upon hardening [6]. Sterol content was compared before and after low temperature exposure in this work, while the above authors compared plants grown at two temperatures. The changes observed by them may be more related to development than to low temperature [11]. Molar ratio of sterol to phospholipids was not affected by temperature, in accordance to de la Roche's results [7]. The parallel increase in sterol and phospholipid content upon hardening may reflect an increase in membrane material [12] or, less specifically, in percentage dry weight of the tissues [13].

The main groups of sterol-containing lipids detected after separation by TLC were, in order of increasing R_fs: steryl glucosides (SG, including the origin and 2 bands near the origin), esterified steryl glucosides (ESG), free sterols (FS) and steryl esters (SE, including the solvent front) (Table 2). These groups correspond to the 4 main groups described in the literature [14, 15]. Except for a lower content in steryl esters, the percentages of the groups compare with those published for barley roots [4].

Little change was observed in the proportions of these groups during hardening (Table 2). There was a trend towards an increase in steryl esters, a decrease in free sterols, and a temporary decrease in steryl glucosides followed by an increase beyond the initial percentage. The increase in steryl esters concurs with the results of Grenier *et al.* [10] with hardening alfalfa roots.

Sterol analysis within each group showed little difference between them (Table 3). Steryl esters were relatively rich in cholesterol but poor in stigmasterol. The level of stigmasterol was higher in the free sterols than in the other groups. Enrichment of steryl esters in cholesterol has been reported previously [4, 15].

Upon hardening the cholesterol content of steryl esters decreased significantly with no concommittant increase in stigmasterol (Table 3). Stigmasterol decreased in the free sterol fraction. But for these relatively minor changes, sterol profiles were quite stable during 4 weeks of hardening at 1°.

The molar ratio of total sterol to lipid P (0.38) shown in Table 1 corresponds closely to that obtained for free sterols in a total membrane fraction (0.14) by de la Roche [7], free sterols representing 46% of total sterols (Table 2). This result suggests that most sterols are localized in the cell membranes.

Only free cholesterol is considered to act as membrane stabilizer [4]. Our results do not indicate any involvement of this sterol in frost hardening of winter wheat. No interaction between frost resistance and levels of other sterols or of groups of sterol-containing lipids could be shown. There is therefore little evidence for sterol involvement in frost hardening, even through other mechanisms than membrane organiza-

Table 3. Sterol profile within groups of sterol-containing lipids in winter wheat crowns and roots during hardening at 1° (percentage of sterols in the group; means of 5 samples ±s.d.)

Hardening (days)	Sterol–lipid group*	Cholesterol	Campesterol	Stigmasterol	Sitosterol
0	SG	0.5±0.1	26.7±0.6	5.6±0.3	67.2±0.5
	ESG	0.6±0.1	28.4±0.6	3.0±0.1	68.6±0.7
	FS	0.5±0.1	27.7±0.4	7.8±0.3	64.0±0.3
	SE	2.9±0.6	28.7±0.8	1.2±0.4	71.9±1.4
7	SG	0.4±0.1	27.8±0.5	5.0±0.3	66.9±0.6
	ESG	0.6±0.1	30.0±1.0	2.4±0.5	67.1±0.9
	FS	0.6±0.1	28.5±0.7	6.6±0.4	67.5±0.4
	SE	2.2±0.5	33.5±0.8	1.3±0.4	63.5±0.5
14	SG	0.6±0.1	25.4±0.3	4.7±0.4	67.3±0.4
	ESG	0.6±0.1	31.5±0.3	2.5±0.1	66.0±0.3
	FS	0.7±0.2	28.4±0.2	6.0±0.2	65.2±0.3
	SE	1.7±0.4	33.8±1.4	1.3±0.4	63.8±0.8
28	SG	0.4±0.1	26.5±0.2	4.3±0.4	68.9±0.5
	ESG	0.8±0.2	30.6±2.5	2.9±1.3	65.7±1.2
	FS	0.5±0.1	26.7±0.6	5.3±0.5	67.6±0.9
	SE	1.8±0.1	32.3±0.5	1.2±0.2	64.6±0.2

*Abbreviations. SG: steryl glucosides, ESG: esterified steryl glucosides, FS: free sterols, SE: steryl esters.

tion. However, the data presented are from bulk analyses. Analysis of purified membrane fractions, in particular of the plasmalemma, may show otherwise.

EXPERIMENTAL

Plant material. *Triticum aestivum* cv Kharkov 22MC was grown in 10 cm pots on sand–vermiculite (1 : 1) at 20° day and 15° night temps., with 16 hr photoperiods (240 μE), at 60% relative humidity. Plants were watered with Hoagland No. 2 soln. Twelve-day-old plants were transferred to a growth cabinet for frost hardening at 1°, with 8 hr photoperiods (135 μE) [12].

Lipid extraction and sterol analysis. After various times of hardening, lipids were extracted from 5 samples (10 g fr. wt) with boiling EtOH, followed by Me_2CO and $CHCl_3$–MeOH (2 : 1). The lipid extract was purified by the method of Bligh and Dyer [16]. An aliquot of the $CHCl_3$ phase (0.5 g fr. wt) was used for total sterol analysis. The extract was evapd to dryness under N_2 and subjected to acid hydrolysis in 4.5 ml HCl (2.5% in MeOH) at 90° for 2 hr, followed by alkaline hydrolysis, after addition of 0.8 ml 7 N NaOH, at 90° for 1.5 hr. Sterols were extracted from the hydrolysate into petrol and determined by GLC of the TMSi derivatives (columns of 210 × 0.4 cm, containing 5% OV-101 on Gas-Chrom Q 100–120 mesh; oven temp. at 265°; helium flow rate of 60 ml/min) [17]. A reference sample was run daily to establish the relative wt responses. An internal standard of cholestane was added before initial homogenization of the tissue. Lipid P was determined by the method of Allen [18]. A second aliquot (3 g fr. wt) of the purified lipid extract was used to separate the sterol-containing groups by TLC on 1 mm Si gel G in Et_2O–C_6H_6–95%EtOH–HOAc (40:48:4:0.5) [15]. Compounds were detected by I_2 vapors. Sterols were detected by $SbCl_3$ (saturated soln in glacial HOAc) on a reference mixture of cholesteryl palmitate, sitosterol and potato tuber extract rich in SG and ESG. Sterols in each group were determined by GLC after acid and alkaline hydrolysis on the gel. Cholestane was added before hydrolysis, except for the SE band, which included, at the solvent front, cholestane initially added before homogenization. All fractions were subjected to both acid and alkaline hydrolysis for better quantitative comparison between the fractions, and to hydrolyse methyl esters formed during acid hydrolysis. All experiments were duplicated with similar results.

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