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LIPIDS, AMINO ACIDS, SUGARS, HARDINESS AND GROWTH OF *FESTUCA ARUNDINACEA*

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Key Word Index—*Festuca arundinacea*; Gramineae; tall fescue; frost hardiness; adaptation to growth temperature; lipids; fatty acids; amino acids; sugars.

Abstract—Tall fescue (*Festuca arundinacea* Schreb. cv S.170) plants were grown in environments differing only in temperature: 6/4, 16/14 or 21/19°. The content of total and individual sugars and amino acids in leaf laminae and roots did not relate closely to the hardiness of the organ. The unsaturation of lipid fatty acids alone was clearly unrelated to hardiness because the difference in unsaturation was greater in the neutral lipids, glycolipids and phospholipids from roots than from leaves but only the latter differed in hardiness. Total amounts of lipids could have been related to hardiness but phospholipids and glycolipids in the roots were not. At least some of these changes may be related to adaptation of growth to temperature.

INTRODUCTION

The amounts of sugars and free amino acids often increase during hardening of plants to frost and these and membrane properties or amounts have all, at various times, been proposed as possible factors involved in this hardening [1–3]. Sugars and some free amino acids can protect a model system against damage due to freezing [4]. There is ample evidence for higher amounts of membranes or membrane components in hardened plants [3, 5–10] and artificial enhancement of phosphatidylcholine can increase hardiness [11]. It has also been suggested that the increase in unsaturation of fatty acids which often occurs when plants are grown at low temperatures may be involved in hardening to frost but other experiments suggest this is not a factor [3].

Changes in amounts of these various constituents of plants during hardening could be no more than a generalized response to low temperature or they might reflect the possibly stressful conditions which may be involved in hardening (e.g. amino acids, especially proline, often accumulate in stressed plants). Alternatively, they could be part of a mechanism for plant adaptation to growth at the low temperature often used to harden the plants, rather than to frost hardiness itself and this has been suggested for phospholipid and fatty acid changes [13]. It is intrinsically difficult to critically test all of these possibilities in a single experiment. In the experiment described here, tall fescue plants were obtained with different hardiness to frost by growing them in different temperature environments. This gave plants with leaf laminae which differed markedly in hardiness whereas the roots differed little [14]. Fatty acids of different lipid classes (neutral lipids, glycolipids, phospholipids), sugars and free amino acids were measured in the leaf laminae and roots.

RESULTS

Hardiness to frost is summarized in Table 1. The main point of using this material was that, in response to low growth temperature, the leaf laminae hardened by at least 6° whereas the hardiness of the roots was as great in plants grown at 6/4° as in plants grown at 16/14°. Thus in the different parts of the plant, contrasting hardening responses occurred to the low growth temperature. Table 1 also shows the slow growth expected of the plants from the lowest temperature [15]. Temperature mainly affected the quantity of shoot material and the lowest temperature did not give the lowest weight of roots (Table 1) [16]. The results also show that the plants adapted to grow at 6/4° in the sense that their growth at 6/4° after cutting back was faster when they had previously been grown at 6/4° as compared with plants grown at 16/14° or 21/19°.

Total amounts of sugars and the relative amounts of different sugars were not closely related to hardiness at least in the laminae (cf. Tables 1 and 2). Roots of plants grown at 21/19° had a very low total sugar content and a relatively low proportion of sucrose, and these roots grew least (Table 1). Growth temperature affected the proportion of different sugars in the laminae much less than in the roots. The total amounts of free amino acids and the relative amounts of different amino acids were not obviously related to hardiness (Table 2). Again, the results suggest an effect of growth temperature on metabolism, rather-than a role for amino acids in hardening. For example, the relative amount of δ-*N*-acetyl ornithine was high only when the total free amino acid content was high. Proline content was relatively high in plants from 6/4° but it was never the predominant free amino acid (Table 2). As for sugars, total free amino acids were relatively low in roots from plants grown

Table 1. Frost hardiness,* fresh and dry matter and growth of plants grown for 2 months in different temperature environments

Day/night growth temp. (°)	6/4	16/14	21/19
Highest temp. (°) at which significant damage occurred (and approx. LT$_{50}$ in brackets)			
Laminae	−10(−14)	−4(−7)	−3(−4)
Roots	−3(−4)	−3(−4)	−1(−3)
Remainder	−6	−5	−3
Dry wt ± s.e. (mg/tiller)			
Laminae	45 ± 11	167 ± 27	200 ± 27
Roots	41 ± 19	49 ± 16	34 ± 3
Remainder	46 ± 5	105 ± 10	159 ± 32
Increase in plant height 5 days after cutting to a height of 60 mm, ± s.e. (mm)			
Grown at 16/14° immediately before cutting	9.6 ± 1.4	73.2 ± 5.0	107.1 ± 5.9
Grown at 6/4° immediately before cutting	26.6 ± 1.0	—	—
Grown at 21/19° immediately before cutting	8.6 ± 0.5	—	—
Dry wt/unit fr. wt. (g/g × 10^2)			
Laminae	18.4	20.4	20.6
Roots	12.4	10.3	13.0
Remainder	12.8	15.4	14.0

*From data of Pearce [14].

at 21/19°. Plants of *F. arundinacea* cvs S.170 and SYN 1 grown in the field had higher total sugar and free amino acid levels during the early winter compared to other times of year but neither correlated closely with hardiness [17]. Again, proline content was higher in winter but it was never the predominant free amino acid [17].

In the roots, the content of total lipids (measured as fatty acids) was unrelated to growth temperature or hardiness (Table 3). Total lipids in the leaf laminae and leaf sheath increased with hardiness and with reduction in growth temperature. The relative amounts of neutral lipids, glycolipids and phospholipids in the laminae were unaffected by growth temperature, whereas in the roots, their relative amounts were greatly affected (Table 3). In the roots, too, the amounts of glycolipids and phospholipids were higher from plants grown at 6/4° compared to 16/14°, and thus appeared unrelated to hardiness. It was not possible to accurately measure the total amount of lipid fatty acids present in the shoot apex. The ratio of linolenic acid (18 : 3) to linoleic acid (18 : 2) was always highest in the leaf laminae, leaf sheaths and roots of plants grown at 6/4° (Table 4). In the laminae, which hardened in response to low growth temperature, this involved a relatively small change in the amounts of 18 : 3 and 18 : 2 considered individually and the extent of change was similar in phospholipids, glycolipids and neutral lipids. The roots from plants grown at 6/4° or 16/14° were of similar hardiness to each other but the difference in relative amounts of 18 : 3 and 18 : 2 was much larger than in the laminae. Thus unsaturation of fatty acids in the roots was affected by growth temperature but was unrelated to hardiness. Growth temperature did not significantly affect the relative amounts of 18 : 3 and 18 : 2 in the shoot apices (Table 4).

The hardiness of the trimmed tillers ('remainder' in the tables) was greater when the plants were grown at 6/4° compared to 21/19°. Hardiness of this part probably depends upon the hardiness of the shoot apices and the survival of the leaf sheaths is also a factor which can affect the apparent hardiness [18]. Thus, the amount of lipids, rather than their unsaturation, may again be related to hardiness. It can also be argued that lipid content and lipid unsaturation are related to growth adaptation or are a fortuitous result of growth at low temperature. In

Table 2. Sugars and free amino acids in leaf laminae and roots from plants grown in different temperature environments

	Leaf laminae			Roots		
Day/night growth temp. (°)	6/4	16/14	21/19	6/4	16/14	21/19
Total sugars/unit fr. wt (μmol/g)	64.2	82.5	30.5	27.5	33.7	3.2
Relative content of different sugars (mol/mol × 10^2)						
Fructose	8.0	22.0	17.0	41.0	16.0	31.0
Glucose	15.0	17.0	25.0	34.0	21.0	40.0
Sucrose	60.0	55.0	47.0	16.0	53.0	9.0
Others	17.0	6.0	11.0	9.0	10.0	20.0
Total free amino acids/unit fr. wt (μmol/g)	48.9	6.4	11.6	32.1	46.2	4.0
Relative content of different free amino acids (mol/mol × 10^2)						
δ-*N*-acetyl orinthine	40.0	tr	tr	67.0	83.0	tr
Serine	tr	17.0	35.0	tr	tr	22.0
Proline	8.0	6.0	0.0	5.0	1.0	0.0
Threonine	0.0	6.0	4.0	0.0	0.0	8.0
Glutamic acid + glutamine	24.0	42.0	18.0	13.0	5.0	9.0
Glycine	13.0	1.0	2.0	1.0	0.0	7.0
Alanine	5.0	1.0	9.0	3.0	3.0	7.0
Others	10.0	27.0	32.0	11.0	8.0	47.0

tr = trace.

particular, the relatively high content of phospholipids in roots grown at 6/4° compared to 16/14°, suggests that phospholipid content is not inevitably linked to hardiness. It was the plants growing at 6/4°, and adapted to it (Table 1), which had the highest total lipid or phospholipid and glycolipid contents (Table 3) and the highest lipid unsaturation (Table 4).

These various conclusions are for sugars, free amino acids and fatty acids per unit fresh matter. This seems a reasonable form in which to express the data because of ideas sometimes expressed about how they could be involved in hardening. The same conclusions are reached if the data are expressed per unit dry matter. Growth temperature had some effect on the dry matter content of the plants (Table 1).

DISCUSSION

The experimental principle used was similar to that of Smolénska and Kuiper [12], who measured fatty acids and lipids in roots and leaves of winter rape grown at 5° or 20/25°. Under these conditions the leaves hardened and the roots did not harden, as in the tall fescue used here. They found that lipids were higher in amount and more unsaturated in the roots as

Table 3. Lipids measured as fatty acids in leaf laminae, leaf sheaths and roots from plants grown in different temperature environments

	Leaf laminae			Roots			Leaf sheaths	
Day/night growth temp. (°)	6/4	16/14	21/19	6/4	16/14	21/19	6/4	21/19
Total lipid fatty acids/unit fr. wt (μg/g)	774 ± 12	706 ± 24	687 ± 21	178 ± 7	216 ± 17	165 ± 10	559 ± 67	372 ± 45
Relative content of fatty acids in lipid classes (g/g × 10^2)								
Neutral lipids	4	4	—	23	57	—	—	—
Glycolipids	67	68	—	32	9	—	—	—
Phospholipids	29	28	—	45	34	—	—	—

Table 4. Relative amount of different fatty acids in lipids in leaf laminae, leaf sheaths, shoot apices and roots from plants grown in different temperature environments

Lipid fraction	Day/night temp. (°)	Relative amounts of fatty acids (mol/mol × 10^2) 16 : 0	16 : 1	18 : 1	18 :2	18 : 3	20 : 0	18 : 3/18 : 2
Leaf laminae								
Total	21/19	13	2	3	14	68	0	4.86
	16/14	14	1	2	11	71	1	6.45
	6/4	11	4	3	7	73	2	10.40
Neutral lipid	16/14	14	18	8	13	36	11	2.77
	6/4	16	26	26	7	23	2	3.29
Glycolipid	16/14	8	1	1	5	85	tr	17.00
	6/4	5	2	1	2	88	2	44.00
Phospholipid	16/14	27	1	5	25	41	1	1.64
	6/4	25	5	3	20	46	1	2.30
Roots								
Total	21/19	37	0	22	36	5	0	0.14
	16/14	19	0	29	37	12	3	0.32
	6/4	32	0	10	14	25	19	1.79
Neutral lipid	16/14	9	0	45	41	4	1	0.10
	6/4	41	0	25	14	6	14	0.43
Glycolipid	16/14	28	0	19	10	15	28	1.50
	6/4	25	0	4	2	24	45	12.00
Phospholipid	16/14	31	tr	6	37	25	1	0.68
	6/4	28	0	4	24	33	11	1.38
Leaf sheaths								
Total	21/19	24	0	20	25	31	0	1.24
	6/4	23	0	9	19	49	0	2.58
Shoot apices								
Total	21/19	27	0	23	25	25	0	1.00
	6/4	27	0	22	24	27	0	1.33

tr = trace.

well as in the leaves of plants grown at the lower temperature. Taking their results [12] with those reported here, it seems that increased amounts of phospholipid and glycolipid are, alone, insufficient to cause an increase in hardiness and that lipid unsaturation also does not alone explain hardiness. Similar conclusions are here justified for sugar and free amino acid content and composition.

With the present data it is possible to consider whether the several different plant components measured, work together to increase hardiness. For example, the hardiest material, the leaf laminae from plants grown at 6/4°, had both the highest content of free amino acids and of total lipids and phospholipids and the second highest content of sugars. Roots from the same plants were less hardy than the laminae and no hardier than the roots from plants grown at 16/14°. These roots had a high content of free amino acids but their phospholipid content was much less than in the laminae. A difficulty in trying to interpret the data in this way is the uncertainty that exists about how these different components might contribute to hardiness. This is particularly problematic for consideration of the role of the enhanced amounts of total lipids or phospholipids which seem to be a frequent feature of plants hardened to frost [3, 5–10, 12]. This has been envisaged as a possible protection against frost-induced membrane depletion [19–21], at least so far as damage to the plasmalemma is concerned. Cell size could also be an important factor to consider (amount of plasmalemma compared to the surface area of the protoplast). The actual changes in amounts of lipids were much less in the present work and in that of Smolénska and Kuiper [12] than in the case of hardening black locust bark [3] and this paralleled a much larger change in hardiness in the latter compared to the others.

A number of studies of lipid changes during hardening have concentrated on the phospholipids. Sikorska and Kacperska-Palacz [22] found in rape that phospholipids did not increase when the plants were hardened at low positive temperatures, in contrast to a hardening treatment which included exposure to sub-zero temperatures. The results given here for roots shows that phospholipid content was different in roots of similar hardiness. Probably, total membrane lipids are important, not just phospholipids and glycolipids.

When a plant is grown at a low temperature, it may not only harden but its metabolism may also be affected and may adapt to growth at that low temperature. These processes of hardening and adaptation of growth proceed together and in the present type of experiment are difficult to separate. Thus, types and amounts of sugars and amino acids reflect changes in the relative rates of their production and consumption. At present, no theory of metabolic response to temperature explains why such diverse groups as sugars, free amino acids and lipids all increase during growth at low temperature. Therefore, possibly they should be regarded as positive steps in either hardening or growth adaptation rather than fortuitous results of the effect of low temperature on metabolism.

EXPERIMENTAL

Tillers of *F. arundinacea* Schreb. cv S.170 were taken from plants established in the field and planted singly in 4 in. pots containing Vermiculite. They were grown for 1 month in a controlled environment: 12 hr day, 110 μmol/m^2/sec (400–700 nm) photon flux density, 3 mm Hg vapour pressure deficit and 16/14° day/night temp. Then the plants were cut to 60 mm high and transferred to controlled environments differing from the previous one only in temp.: 6/4°, 16/14° or 21/19°. The plants were watered with 'Long Ashton' nutrient soln previously brought to the appropriate temp. After 2 months measurements of growth, hardiness and content of lipids, sugars and free amino acids were made. The leaf sheaths were taken between 1 and 3 cm above the shoot apex and excluded the 2 outermost leaf sheaths. The shoot apices comprised the apical 1 mm. The hardiness measurements have already been described [14].

Lipids were extracted with hot *iso*-PrOH followed by $CHCl_3$–MeOH. The different lipid classes were obtained by CC on acid-washed Florisil and the solvent series: $CHCl_3$ (EtOH-free), $CHCl_3$–Me_2CO (1 : 1), Me_2CO, $CHCl_3$–MeOH (9 : 1 and 1 : 1), MeOH. The acyl lipids were hydrolysed and the fatty acids methylated by refluxing with MeOH–toluene–conc. H_2SO_4 (150 : 75 : 7.5). The methyl esters were analysed by GC-FID using 5% PEGA at 160° (N_2, 50 ml/min; inj. 160°, det. 210°). Standards were pure samples of palmitic acid (16 : 0), margaric acid (17 : 0), oleic acid (18 : 1) and linoleic acid (18 : 2). Butylated hydroxytoluene was included in solvents as an antioxidant. For each fatty acid as a % by wt of total fatty acids the s.d. = ± 1, calculated as a transformed value.

TLC on Si gel G using $CHCl_3$–MeOH–33% NH_4OH–H_2O (65 : 35 : 5 : 3.5) and $CHCl_3$–MeOH–Me_2CO–HOAc–H_2O (6 : 8 : 2 : 2 : 1) and suitable standards were used to obtain a tentative identification of the main glycolipids and phospholipids present and to check the above fractionation. In the leaf laminae these appeared to be monogalactosyldiglyceride, digalactosyldiglyceride, phosphatidylcholine and phosphatidylethanolamine and there were several minor phospholipids. The same phospholipids were present in the roots.

Free amino acids and sugars were extracted with hot 80% EtOH. The different amino acids were analysed using a Technicon TSM automatic amino acid analyser and the s.d. for each was 5% of μmol/g. Sugars were dissolved in pyridine and TMS derivatives obtained using HMDS and TMCS and analysed using GC-FID, 2% SE52 140–290° (4°/min; N_2, 50 ml/min; inj. 140°, det. 310°). The s.d. was 12% of μmol/g. Suitable standard amino acids and sugars were used. Total amino acids and sugars were obtained by summing individual values. Measurements were also made with ninhydrin and anthrone to confirm the relative values of totals.

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