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LIPID CHANGES IN BARLEY SEEDLINGS SUBJECTED TO WATER AND COLD STRESS

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Key Word Index—*Hordeum vulgare*; Gramineae barley; betaine; galactolipids, fatty acids, phospolipids, proline, sugars; stress.

Abstract—Barley seedlings were subjected to water and cold stress, and the composition of lipid fractions and the accumulation of several low molecular weight metabolites in leaves determined. Proline, glycine-betaine and the total polar lipid content increased in seedlings under water stress. Proline and sucrose content decreased to 50% after 48 hr of cold treatment. The fatty acid composition and unsaturation ratio of phosphatidyl choline, phosphatidyl inositol, phosphatidyl ethanolamine, phosphatidyl glycerol, monogalactosyl diacylglycerol and digalactosyl diacylglycerol were determined in seedlings under both stress treatments. The content of linolenic acid increased in stressed seedlings but was significantly higher in cold stressed plants.

INTRODUCTION

Water availability and temperature are major factors that limit plant growth [1, 2]. These factors induce a variety of biochemical changes that may help the plant to survive under water or temperature stress. For example, barley and other plants under water stress accumulate proline, glycine-betaine, carbohydrates and other compounds [3]. Also, membrane lipid composition may change under water deficit or chilling [4, 5]. Membrane lipids are important because they provide a matrix for proteins and maintain cellular compartmentation. The membrane-fluidity theory is based on the fact that membranes of resistant plants are often more rich in unsaturated fatty acids than those from sensitive plants [6]. An increase in phospholipid unsaturation has been related to membrane fluidity at low temperatures [7]. Thus, lipid changes that occur during plant acclimation may have profound physiological implications. In this paper we report on the changes in lipid composition in barley seedlings as a response to water and cold stress.

RESULTS

Low M_r solutes and stress treatments

With the exceptions of sucrose and total sugars, the other analysed metabolites increased in barley plants subjected to water stress conditions (Table 1), proline and betaine concentrations increased significantly. Under cold stress proline decreased but betaine content was unchanged. Glucose was the only metabolite that increased significantly in cold stressed seedlings ($P<0.05$, t-test). A significant decrease was also observed in sucrose and total lipids in cold stressed seedlings.

Stress and polar lipids

The concentrations of individual fractions of phospholipids and galactolipids in seedlings under both stress treatments are shown in Table 2. The majority of polar lipid factions increased under water stress. The only fraction that remained unchanged was PI. The percentage of PC in the total polar lipids was higher in stressed seedlings than in that unstressed seedlings (9.1 and 2.4%, respectively); the ratio of PC to PE was 0.65 and 0.25 in stressed and control plants, respectively Other minor differences between stressed and control seedlings were observed in other polar lipids. In cold stressed seedlings the percentage of PC was higher than that in control seedlings, (7.5 and 5.7%, respectively); the PC to PE ratio was 1.16 and 0.44. In addition, cold stressed seedlings showed an increase in the amounts of MGDG, DGDG and a decrease in PE. These results demonstrated that the lipid composition of barley seedlings was affected differently by water and cold stress

Fatty acid composition of lipid fractions

Small changes in fatty acid composition were detected in seedlings subjected to water and cold stress. In plants under water stress a significant increase was observed in 16:1 (Table 3). In addition, the proportions of 18:1 and 18:3 were also higher in stressed than that in unstressed plants; 18:1 decreased in stressed plants.

Cold stressed seedlings showed an increased content of 18:2 and 18:3, whilst 18:1 was unchanged compared to unstressed controls (Table 4). In individual polar lipid fractions, there was an increase in the proportion of 18:3 in PC, PG, MGDG, and DGDG. The relative increase of 18:3 was greatest in PC. In PI and PE a decrease in the relative content of 18:3 was found. The accumulation of 18:3 in phospholipids at low temperature has already been reported in wheat [8].

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Table 1. Accumulation of low M_r solutes and polar lipids in barley seedlings under water and cold stress

Type of stress	Water potential (MPa)	Water content (%)	Proline	Betaine	TSS	Sucr	Glc	TPL
			(mmol kg^{-1} dry wt)					
Water								
Non-stressed	−0 29	91.1	13±1	19±2	12±1	41	309	45 6
Stressed	−0 86	89 4	37±4	59±2	13±3	28	447	67 3
Cold								
Non-stressed								
0 hr	−0 28	90 4	14±2	18±2	14±1	37	216	52 8
48 hr	−0 28	90 4	14±3	16±3	12±3	40	201	60 0
stressed								
12 hr	−0 20	90 9	8±1	16±2	11±2	33	276	63 5
48 hr	−0 20	89 4	8±1	18±2	11±1	27	370	74 2

Ten-day-old seedlings were subjected to water stress for seven days or cold treatment (4°, dark) for 48 hr TSS total soluble sugars, Sucr· sucrose, Glc glucose, TPL: total polar lipid.

Table 2 Concentration of individual polar lipid in water and cold stressed barley seedlings

Type of stress*	MGDG	DGDG	PC	PI	PE	PG
			(mmol kg^{-1} dry wt)			
Water						
Non-stressed	19 4±1 7	18±0 5	1 1±0 1	1 6±0 1	4 4±0 5	1 1±0 0
Stressed	25 6±1 8	22±0 3	6 1±0 7	1 5±0 1	9 3±0 2	2 8±0 2
Cold						
Non-stressed						
0 hr	14 4±0 4	24 2±1 8	3 0±0 3	5 4±0.6	3.6±0.1	2 2±0 1
48 hr	20 6±0 2	22 6±0 0	3 4±0 4	0 5±0.1	7 7±0 2	5 2±0 8
stressed						
12 hr	22 5±0 2	18 5±0 8	5 2±0 2	3 4±0 3	10 6±0 3	3 3±0 3
48 hr	26 6±2 2	34 1±4 0	5 6±1 7	1 0±0 1	4 8±0 7	2 1±0.8

*Ten-day-old seedlings were subjected to water stress for seven days and to cold (4°, dark) The level of stress is shown in Table 1 MGDG monogalactosyl diacylglycerols, DGDG digalactosyl diacylglycerols, PC· phosphatidyl choline, PI phosphatidyl inositol, PE phosphatidyl ethanolamine, PG phosphatidyl glycerol

The unsaturation ratio of the fatty acids in the total polar lipid fractions showed a slight increase in cold stressed seedlings (Table 4). In plants subjected to water stress the unsaturation ratio of PC and PG was increased, whilst other polar lipid fractions showed little change. A similar pattern was observed in cold stressed seedlings, with the exception of PI and PE, all polar lipids showed an increase. The unsaturation ratios of all polar lipids were higher in such seedlings.

DISCUSSION

Plants are affected by several types of stress. The physiological responses, however, depend on the species and on the nature of stress The accumulation of proline, betaine and some sugars by water stressed seedlings appears to be an adaptative metabolic response to drought [9, 10]. Our results agree with those of other authors [11, 12]. It is also known that proline accumulates in plants under cold stress [13] In our work, however, we found a 57% decrease in proline content It is possible that inhibited photosynthesis (treatments were made in the dark) did not provide the necessary carbohydrate for proline accumulation According to Stewart [14], the role of carbohydrates in proline accumulation is to supply precursors for proline synthesis, but sucrose content was drastically reduced after 48 hr of cold stress in our experiments (Table 1) A decrease in proline and sugar content in leaves of several plants kept in the dark during cold hardening has been reported [15, 16] On the

Table 3. Polar lipid and fatty acid composition of unstressed and water stressed barley seedlings

Treatment	PL	Fatty acids (mol %) 16:0	16.1	18.0	18:1	18.2	18:3	U.R.	TPL
Control	PC	39.1	4.0	26.5	22.1	5.6	2.9	0.5	2.4
	PI	34.8	3.1	20.7	31 8	6.1	3.6	0 5	3.5
	PE	41.6	4.2	23.4	19.7	6.4	4.7	0.5	9 6
	PG	45.8	0	26.0	16.7	5.0	6.6	0.4	2 4
	MGDG	31.6	0.3	22.2	15.7	3.7	26.5	0 9	42.5
	DGDG	31.7	4.5	19 5	31 1	4.8	8 4	0 9	39.6
	Total	37.4	2.7	23	22.8	5.3	8.8	0 6	100
Stressed	PC	36.4	6.9	18.6	28.6	7.1	2 4	0 7	9.1
	PI	40.6	10 4	29.6	16.3	1.3	1.8	0.3	2.2
	PE	41.4	6.2	30.1	14.5	4 5	3 3	0.3	13.8
	PG	31.0	6.2	24.4	32.4	4.1	1.9	0 7	4 2
	MGDG	35 1	0	25 0	14 5	3 0	26 4	0.8	38 0
	DGDG	32.9	6.3	21.7	13.0	9.6	16 5	0.7	32 7
	Total	34.8	6.6	27.4	16 7	5.0	9.5	0.6	100

Ten-day-old seedlings were subjected to water stress for seven days (see Table 1). The numbers (% total) at the bottom of each column were calculated as the Σ mol % of every acid/total mol % PI: polar lipid, U.R. unsaturation ratio (18:1 + 18.2 + 18:3/16 0 + 18·0), TPL: total polar lipid. Other abbreviations as Table 2

Table 4 Polar lipid fatty acid composition of unstressed and cold stressed barley seedlings

Treatment	PL	Fatty acids (mol %) 16 0	16 1	18 0	18 1	18:2	18·3	U R	TPL
Control	PC	41	5 7	28 3	12 0	10 7	2 3	0 4	5.7
	PI	44	0	34.4	12 6	5.4	3 9	0 3	0.8
	PE	39 3	4 9	20 6	7.8	13 2	14.2	0 6	12.8
	PG	51 6	0	28 3	8.2	3.6	8.3	0.3	8 7
	MGDG	21.5	0	12.9	6 1	3.4	56 1	1 9	34 3
	DGDG	38	0	14.8	8.5	5 6	33 2	0 9	37.7
	Total	39 2	1 8	23 2	9.2	6 9	19 7	0 7	100
Stressed	PC	49	0	13 5	9 7	15 6	12 3	0 6	7 5
	PI	46 5	0	34 8	13.2	4.2	1.3	0 2	1.3
	PE	44 8	4 7	19 1	8 1	12.3	11 0	0.5	6.5
	PG	43 9	5 2	23 1	10 1	5.8	11 8	0.4	2 8
	MGDG	14 2	0	7.3	5 4	4.6	68.4	3.6	35 8
	DGDG	27.8	0	11.2	7.8	6.3	45 9	1.5	45 9
	Total	37 8	1 6	18.2	9 1	8.1	25 6	1.3	100

Ten-day-old plants were subjected to cold stress (4°, dark) for 48 hr Abbreviations as Table 3.

other hand proline accumulation has been observed in dark cold acclimated leaves of the woody evergreen *Notofagus dombeyi* [17].

The effects of water or cold stress on the lipids of barley membranes were different. The proportion of unsaturated fatty acids such as 16:1 and 18:3 was higher in water stressed than in unstressed seedlings, but in cold stressed seedlings only 18:3 increased. In both stress treatments the increase of this acid was similar. The accumulation of unsaturated fatty acids due to environmental stresses have been reported in several species [18]. In mitochondria from osmotic stressed wheat shoots an increase in PC content with respect to unstressed controls was observed [18] although in such stressed mitochondria the fatty acid composition did not change. De la Roche *et al.* [19] and Willemot *et al.* [20] reported an increase in fatty acids in winter wheat under cold hardening conditions. Similar results have been found in other species [21–23]. However, no relation between fatty acid unsaturation and hardening have been demonstrated in other species [24–27] and it has been proposed that accumulation of 18:3 is not a prerequisite for cold hardening of winter wheat germination in the dark [28]. Although no clear role for accumulation of 18:3 is known, it is difficult to accept that an increase in unsaturation of fatty acids at low temperature does not have some benefit to the plant.

From the results described herein it appears that although the involvement of fatty acid unsaturation in cold acclimatation has been widely reported and discussed, it should not be generalized. Perhaps,

increased unsaturation is an additional mechanism to prevent damage of biomembranes at low temperatures The close relationship between the increase of 18 3 and 18 2 and the decrease in 18:1 and 16·0 in some lipid fractions (PC, MGDG and DGDG) of barley seedlings suggests that desaturation of these acids is stimulated during water stress and cold hardening

Recently, attention has been given to the low temperature induced decrease of *trans*-16·1 of PG in relation to frost resistance of plants [29]. Huner *et al* [30] predicted the frost resistance of Lennox wheat on the basis of analyses of this particular fatty acid Their prediction was later confirmed as a determinant of frost resistance. Other plants, such as spinach and pea do not show changes in 16 1 of PC. Our results show that 16·1 content increased significantly in plants subjected to water stress, while in plants under cold stress there was no change The possible physiological role of *trans*-16·1 accumulation in the stress tolerance of plants remains to be established

EXPERIMENTAL

Growth conditions. Barley seeds were sown in pots filled with vermiculite Seedlings were grown under a light intensity of 67 5 μE sec^{-1} m^{-2} and irrigation with nutrient soln at 27° ± 1 Ten-day-old seedlings were subjected to both H_2O and temp stress Pots containing 25 seedlings each were irrigated (shoot H_2O potential of −0 29 MPa) for 10 days and then subjected to each stress treatment For water stress treatments seedlings were irrigated to obtain a H_2O potential of −0 86 MPa during 7 days For cold stress, seedlings were placed at 4° ± 1 for 48 hr in the dark

Lipid extraction All operations were carried out at 0° under N_2. All solvents were deairated and kept under N_2 at 4°. Leaves (3 g fr wt), were homogenized for 1–2 min in a Sorvall omnimixer under N_2 using $CHCl_3$–MeOH (2 1) Complete extraction of lipids and purification of the $CHCl_3$ phases were performed following the method of ref [31]

Analysis of lipids Portions of purified lipid samples were depigmented by TLC on silica gel–kieselguhr (1·4) using petrol–*iso*-PrOH–H_2O (400 48 1) [32] Sepn and detection of lipids was carried out by TLC on silica gel 60 H as previously described in ref [22] Galactolipids were estimated by determination of galactose residues following acid hydrolysis with H_2SO_4 [33] Phospholipids were estimated according to ref [34] Fatty acids were determined as Me esters by GC as described in ref [35] Each analysis was run at least twice

Analysis of compound Proline was determined by the method of ref. [36], glycine-betaine by a colorimetric method described for quaternary ammonium compounds [37] Sugars were extd by overnight submersion of dry tissues in 80% EtOH with periodic shaking Total sol sugars were determined by reacting 0.1 ml of the EtOH extract with 3 ml of freshly prepd anthrone reagent at 100° for 10 min and measuring the *A* at 625 nm Sucrose was determined by the method of ref [38] Glucose was determined using a Sigma kit

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