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Lipids in Grape Roots in Relation to Chloride Transport[1]

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Abstract. **A comparison was made between the lipids of the roots of 5 grape rootstocks which differ markedly in the extent to which they permit chloride accumulation in leaves. Monogalactose diglyceride concentration was directly related to chloride accumulation in the leaves of the 5 rootstocks. Phosphatidylcholine and phosphatidylethanolamine were inversely related to chloride accumulation. The variety with the highest chloride accumulation contained an unusually small amount of sterols. A striking negative correlation between content of lignoceric acid and chloride accumulation was observed. The lignoceric acid concentration ranged from 11.9 % in the rootstock with the lowest chloride accumulation to 0.8 % in the rootstock with the highest chloride accumulation. This fatty acid was found mainly in the phosphatidylcholine and the phosphatidylethanolamine lipid fractions.**

Bernstein, Clark, and Ehlig (personal commun.) observed that different grape rootstocks provide markedly different chloride transport to the leaves. When the roots of plants growing outdoors in sand cultures were exposed to 25 meq/l Cl in the nutrient solution, the most salt-sensitive rootstock, Cardinal, accumulated 19.8 meq/Cl/100 g dry weight of leaves. Leaves on Thompson Seedless, Dog Ridge, 1613-3, and Salt Creek rootstocks accumulated, respectively, 8.9, 7.0, 2.6, and 1.4 meq Cl/100 g dry weight. There was a 15-fold difference in Cl accumulation in leaves between the extremes, Cardinal and Salt Creek. Differences between scions were small, compared with differences between rootstocks. Bernstein, *et al.* express differences between the rootstocks by a chloride accumulation ratio defined as (meq Cl/100 g dry wt of leaves)/(meq Cl/liter in nutrient solution).

Elzam *et al.* (5), studying chloride absorption by barley roots, distinguished between a high affinity transport system operating below 0.1 meq/l Cl and a number of low affinity transport systems operating above this concentration. According to Luttge and Laties (11), transport to the shoot of corn seedlings is either by the high affinity mechanism operating at low concentrations, or *via* diffusion across the plasma membrane, at higher concentrations. Torii and Laties (15) also contend that chloride movement across the plasma membrane proceeds by diffusion at such high concentrations.

For ordinary diffusion, a linear relation between transport across the membrane and concentration should be expected. Taking into consideration the view of cell membranes composed of a bimolecular lipid layer surrounded by protein, ordinary diffusion of ions across such a membrane is not likely. Another possibility is that Cl-ions are exchanged against other anions across the lipid layer of the plasma membrane. The observed saturation of Cl uptake at very high Cl concentration would be explained by the limited number of sites in the lipid layer available for anion exchange. Exchange as postulated here should operate equally in both directions. Elzam and Epstein (6) showed that ^{36}Cl absorbed by barley roots exchanged out at a very low rate, compared with the rate of absorption. This experiment however was carried out below 0.5 meq/l Cl, where the high affinity transport system is operating. No experiments, done at higher chloride concentration, are reported.

Preliminary experiments showed differences between the anion exchange characteristics of lipids extracted from the roots of the 2 most extreme grape rootstocks, Cardinal and Salt Creek. I therefore studied the composition of lipids and fatty acids of the roots of the 5 rootstocks and report the results here.

Materials and Methods

Extraction. Healthy-looking grape roots were cut from rootstocks growing in aerated nutrient solution in the greenhouse. All rootstocks were harvested at the same time to avoid seasonal effects on lipid composition. The roots were washed with tap water and distilled water, and minced. The root samples were then frozen and freeze-dried. The dry roots were stored below 0° in a nitrogen atmos-

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phere. Samples of the dried roots were extracted twice with chloroform/methanol (2/1 v/v). Sufficient solvent was added to completely submerge the roots. Each extract was gently shaken for 2 hours at room temperature, and then filtered through sintered glass. To the combined extracts, 0.9 % NaCl in H_2O (w/v) was added, equivalent to one-fifth of the extract volume. After shaking, the lower phase (chloroform) was separated and washed again with a trace of saline solution. After separation, the chloroform phase, containing the lipids, was dried with an air stream at 5°. The last traces of solvents were removed over paraffin and $CaCl_2$ in a vacuum desiccator at 5°. Lipids were stored below 0° under nitrogen.

Separation. Lipids were separated on a silicic acid chromatography column. Ten g of silicic acid of chromatography grade were gently tapped to a final column height of 10 cm. The diameter of the column was 1.5 cm. The silicic acid was washed with 100 ml of hexane. The lipid sample, not exceeding 200 mg was triturated in 10 ml of hexane and brought on the column. Different lipid classes were eluted with hexane/ethyl ether mixtures (neutral lipids) and chloroform/methanol mixtures (charged lipids) following directions of Hirsch and Ahrens (9), Barron and Hanahan (2), and Hanahan, *et al.* (8). Some modification proved to be necessary. The following elution schedule was found to be satisfactory for grape root lipids: hydrocarbons, 175 ml hexane/ethyl ether (99/1 v/v); triglycerides, 175 ml hexane/ethyl ether (96/4 v/v); sterols, 325 hexane/ethyl ether (92/8 v/v); diglycerides, 125 ml hexane/ethyl ether (75/25 v/v); monoglycerides, 150 ml ethyl ether; glycolipids, fraction 1, 100 ml chloroform/methanol (95/5 v/v); glycolipids, fraction 2, 125 ml chloroform/methanol (90/10 v/v); phospholipids, fraction 1, 100 ml chloroform/methanol (75/25 v/v); and phospholipids, fraction 2, 100 ml chloroform/methanol (50/50 v/v). Figure 1 gives the elution peaks of the lipid classes. Hydrocarbons, triglycerides, and monoglycerides of Cardinal root lipid contained 2 peaks. Subsequent analysis showed that this was caused by differences in chain length and in the degree of saturation of the hydrocarbon and fatty acid molecules. Some of the charged lipid classes exhibited tailing of the peaks, *e.g.*, the second fraction of the phospholipids in figure 1. In further experiments, tailing was reduced by decreasing the solvent flow rate to less than 2 ml per minute. The column should not be allowed to run dry. For separation of charged lipids of different sources, solvent modifications are usually required. Recovery varied between 85 and 92 %.

Identification. The following chemicals were brought on the column and eluted with the above solvent mixtures: paraffin, carotene, tristearin, triolein, trilinolein, cholesterol, distearin, monostearin, monolein, and lecithin. Elution peak values of these chemicals corresponded closely with the values of the lipids of figure 1. Lipids with unsaturated fatty acids eluted slightly later than the corresponding lipids with saturated fatty acids. Lecithin eluted as the second fraction of the phospholipids. Sterols were identified by the Liebermann-Burchard reaction.

Samples of the charged lipid fractions were analyzed by thin-layer chromatography, using silica gel G as the medium. Solvents used were chloroform/methanol/7N NH_4OH (65/30/4 v/v) and chloroform/methanol/acetic acid/H_2O (170/25/25/6 v/v). Spots were made visible by charring (13). Monogalactose diglyceride was the only glycolipid detected in the 2 glycolipid fractions. The R_F-values were in agreement with those reported (13). Glycolipids were deacylated (16) and the water-soluble deacylated lipids were refluxed for 4 hours with excess 6 N HCl. The solution was neutralized and dried in vacuum. Pyridine was added to the dry sample, and the sugars present were silylated (14) and ana-

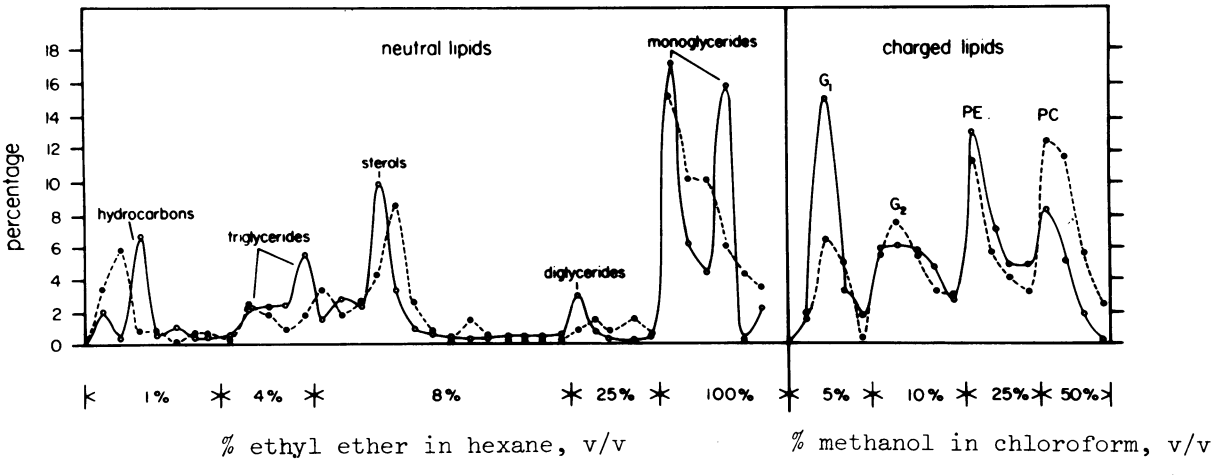

FIG. 1. Separation of lipid classes from Salt Creek (●) and Cardinal (○) rootstocks. Lipids are expressed as percent of neutral lipids or percent of charged lipids of the extracted roots. G_1, G_2 = monogalactose diglyceride fractions 1 and 2; PE = phosphatidylethanolamine; PC = phospatidylcholine.

lyzed by gas chromatography using a SE30 column. Comparison with samples of known silylated sugars revealed that galactose was the only sugar present in my samples. R_F-values of fractions 1 and 2 of the phospholipids agreed with those reported for phosphatidylethanolamine and phosphatidylcholine, respectively (3). In addition the R_F-value of fraction 2 agreed with that of a commercial sample of phosphatidylcholine. The water-soluble deacylated lipids of fractions 1 and 2 were analyzed on Whatman No. 1 paper using phenol/water (3/1 v/v) as a solvent. The observed R_F-values were similar to those reported for phosphatidylethanolamine and phosphatidylcholine (3). In some samples of fraction 1 of the phospholipids, a trace of phosphatidyl inositol was observed.

Fatty Acid Analysis. Numbers following fatty acids indicate number of C-atoms and number of double bonds, respectively. Neutral lipids were saponified with methanolic KOH as described by James (10). Charged lipids were deacylated according to Benson *et al.* (4). The separated fatty acids were methylated with methanolic H_2SO_4, following James' directions (10) or with methanolic BF_3 (12). Samples of the methyl esters were analyzed on a gas chromatograph, using a hydrogen flame ionization detector. Two columns were used, one composed of 15 % diethylene glycol as a stationary phase on Chromosorb W as a support (1), and the other of 3 % Apiezon L on Chromosorb P (2). Most fatty acid methyl esters present in the root lipids could be identified on both columns with known fatty acid methyl esters. The exceptions were palmitoleic acid (16:1) and hexadecatrienoic acid (16:3). When analyzed on the Apiezon-L column, the retention time of these 2 fatty acid esters was identical to that recorded (10). The relation between the logarithm of the retention time and the number of C-atoms of the saturated fatty acids was linear. Parallel curves for fatty acids with 1 or 3 double bonds showed retention times for fatty acids with 16 C-atoms, identical to the unidentified peaks in some of my samples.

Results and Discussion

The lipid content of the grape roots varied between 0.2 and 0.3 % based on fresh weight but there were no significant differences between the contents of the varieties studied. The lipid compositions of the roots of the 5 varieties are given in table I. As regards the neutral lipids, sterols and monoglycerides were predominant. Variations among the rootstocks did not seem significant, except that the sterol fraction of the variety with the highest chloride accumulation, Cardinal, was much smaller (8.7 %) than those of the other varieties (15.6–22.4 %). Cardinal differed from the other rootstocks in another way. Its charged/neutral lipid ratio was 0.56, compared with values ranging from 0.37 to 0.44 for the other rootstocks. The ion exchange capacity of charged lipids may be expected to be much higher than that of neutral lipids and certainly much higher than that of sterols. Thus, the relatively large proportion of charged lipids of Cardinal may be related to its high chloride accumulation rate.

A distinct correlation between chloride transport to the leaves of the 5 rootstocks and several of the charged lipids was observed (table I). The chloride accumulation ratio was correlated with an increase in the percentage of monogalactose diglyceride fractions 1 and 2 and a decrease in the percentage of the phosphatidylcholine fraction. A less pronounced correlation with decreasing percentage of the phosphatidylethanolamine fraction was observed.

In summary, a definite correlation between the composition of the charged lipids and the chloride accumulation in the leaves of the grape rootstocks was observed. While phosphatidylcholine and, to a lesser extent, phosphatidylethanolamine were the dominant charged lipids of the roots of the rootstock with the lowest chloride accumulation, Salt Creek, the 2 varieties with the highest chloride accumulation, Thompson Seedless and Cardinal, were characterized by large amounts of monogalactose diglyceride in the root lipids. The higher chloride

Table I. *Lipid Composition of the Roots of the Five Grape Rootstocks, Salt Creek (SC), 1613-3, Dog Ridge (DR), Thompson Seedless (TS), and Cardinal (Card), Expressed as Percentage of Total Lipids*

The data on chloride accumulation ratios are from Bernstein, *et al.* (private commun.) and are expressed as (meq Cl/100 g dry wt of leaves)/(meq Cl/l in nutrient solution).

Variety	SC	1613-3	DR	TS	Card
Chloride accumulation ratio	0.06	0.11	0.28	0.39	0.86
Ratio, charged lipids/neutral lipids	0.430	0.438	0.366	0.437	0.564
Hydrocarbons	9.5	8.3	7.5	9.7	9.2
Triglycerides	10.0	7.8	10.8	7.3	4.8
Sterols	19.4	15.6	20.7	22.4	8.7
Diglycerides	1.8	2.6	3.3	3.6	1.9
Monoglycerides	16.3	21.9	21.1	13.3	19.0
Monogalactose diglyceride					
fraction 1	3.5	12.5	11.7	23.0	22.8
fraction 2	6.0	9.9	11.3	10.9	16.2
Phosphatidylethanolamine	12.7	6.8	6.6	6.1	11.4
Phosphatidylcholine	20.8	14.6	7.0	3.7	6.0

accumulation ratio of Cardinal as compared with Thompson Seedless might be correlated with the unusually small amount of neutral lipids, and especially of sterols, present in the roots of Cardinal. Studies on the lipid composition of the mitochondria of the root cells may give information on the location of these lipids in the root cells.

The fatty acid composition of the root lipids of all varieties was determined (table II). Palmitic (16:0), oleic (18:1), linoleic (18:2), linolenic (18:3), and lignoceric acid (24:0) were the most important fatty acids present. An unusual amount of palmitic acid was observed in the Cardinal roots, 36 %, as compared with 23 to 28 % in the roots of other varieties. There was a tendency for the varieties with high chloride accumulation, Thompson Seedless and Cardinal, to contain relatively larger amounts of polyunsaturated fatty acids. Lignoceric acid showed a strikingly negative correlation with the chloride transport of the grapes in all fatty acid determinations, ranging from 11.9 % in Salt Creek to 0.8 % in Cardinal. The difference in lignoceric acid content of Dog Ridge and 1613-3 was small compared with the difference in Cl sensitivity, but in 1613-3, there occurred another long-chain saturated fatty acid, behenic acid (22:0), which was

Table II. *Fatty Acid Composition of the Roots of the Five Grape Varieties Expressed as Percentage of Total Fatty Acid*

The numbers following fatty acids represent number of C-atoms and number of double bonds, respectively. For key to varieties and definition of the chloride accumulation ratio, see table I.

Variety		SC	1613-3	DR	TS	Card
Fatty acid	Chloride accumulation ratio	0.06	0.11	0.28	0.39	0.86
Lauric	(12:0)	7.5	0.5	6.4	2.3	4.3
Myristic	(14:0)	6.1	1.0	6.4	3.4	4.3
Palmitic	(16:0)	26.5	23.4	27.8	22.9	35.9
Stearic	(18:0)	8.0	14.4	12.5	6.2	7.2
Oleic	(18:1)	9.4	16.1	23.7	4.6	11.6
Linoleic	(18:2)	21.0	18.8	13.6	43.3	22.6
Linolenic	(18:3)	9.6	14.0	5.0	15.4	12.8
Behenic	(22:0)	...	5.0	0.3	0.4	0.5
Lignoceric	(24:0)	11.9	6.8	4.3	1.5	0.8

Table III. *Fatty Acid Composition of the Lipids of the Roots of 1613-3, Dog Ridge, and Cardinal Varieties Expressed as Percentage of Total Lipid of Each Class*

The numbers following fatty acids represent number of C-atoms and number of double bonds, respectively. Lipid classes are monoglycerides (M), diglycerides (D), triglycerides (T), monogalactose diglyceride fractions 1 and 2 (G_1 and G_2), phosphatidylethanolamine (PE), and phosphatidylcholine (PC).

Fatty acid	12:0	14:0	16:0	18:0	18:1	18:2	18:3	22:0	24:0
Lipid class				1613-3					
M	3	6	30	15	17	19	7	...	3
D	4	7	27	15	18	19	7	...	3
T	2	4	24	8	16	31	13	...	2
G_1	4	5	32	8	9	33	9	...	...
G_2	2	4	27	9	13	32	13	...	...
PE	1	3	26	6	9	27	9	13	6
PC	...	3	21	13	11	12	...	14	26
				Dog Ridge					
M	11	19	26	19	14	8	3	...	...
D	1	2	21	24	24	17	10	...	1
T	2	5	30	13	19	19	10	...	2
G_1	2	4	33	17	19	16	6	...	3
G_2	2	4	30	16	19	18	9	...	2
PE	...	2	29	6	9	33	7	...	14
PC	1	3	26	13	14	19	5	...	19
				Cardinal					
M	3	3	40	13	17	21	3	...	...
D	5	8	32	22	24	7	2	...	...
T	2	4	20	9	16	29	17	3	...
G_1	3	4	25	8	11	30	12	4	3
G_2	3	5	29	8	10	33	8	2	2
PE	8	5	49	12	12	11	3	...	...
PC	...	1	18	4	15	35	12	...	15

practically absent in all the other varieties (see table II). Thus, a definite correlation was observed between chloride accumulation and the amount of long-chain saturated fatty acids (22:0 and 24:0). One wonders if these fatty acids are located in the phosphatidylcholine and the phosphatidylethanolamine fractions, lipid classes which show the same correlation with chloride accumulation. Fatty acid analysis of the lipid classes of 1613-3, Dog Ridge, and Cardinal is presented in table III. This table shows that the long-chain saturated fatty acids (22:0 and 24:0) are found in the phosphatidylcholine and the phosphatidylethanolamine fractions of the lipids of the grapes studied. Detectable amounts of these fatty acids were also observed in the monogalactose diglyceride fraction of the Cardinal lipids. Two fatty acids not mentioned in table III, palmitoleic acid (16:1) and hexadecatrienoic acid (16:3), were found in small quantity in the monogalactose diglyceride fraction of the 3 grape varieties. The latter acid was also obtained from the monogalactose diglyceride of spinach chloroplasts (1).

Finally, the fatty acid analysis of the grape lipids showed that the observed relation between the amount of long-chain saturated fatty acids and chloride tolerance depended completely on the observed correlation between the phosphatidylcholine and the phosphatidylethanolamine fractions and chloride tolerance. It should be interesting to determine if the observation that some fatty acids may be restricted to specific lipids also holds for other instances where differences in fatty acid compositions are observed, *e.g.*, in plants which differ in low-temperature hardiness (7).

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