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# Phosphate-deficient oat replaces a major portion of the plasma membrane phospholipids with the galactolipid digalactosyldiacylglycerol

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**Abstract The plasma membranes of oat normally resemble those of other eukaryotes in containing mainly phospholipids and sterols. We here report the novel finding that the galactolipid digalactosyldiacylglycerol (DGDG) can constitute a substantial proportion of oat plasma membrane lipids, in both shoots and roots. When oat was cultivated under severe phosphate limitation, up to 70% of the plasma membrane phosphoglycerolipids were replaced by DGDG. Our finding not only reflects a far more developed potential for plasticity in plasma membrane lipid composition than often assumed, but also merits interest in the context of the limited phosphate availability in many soils.**



*Key words:* Digalactosyldiacylglycerol; Galactolipid; Membrane lipid; Plasma membrane; Phosphate deficiency; Phospholipid

## 1. Introduction

Phosphorus is an essential nutrient and in a leaf, approximately a third of the organic phosphate resides in membrane phospholipids [1]. Chloroplast membranes, however, predominantly contain non-phosphorus galactolipids [2–4]. The generally presumed exclusive plastid localization of these lipids was recently challenged: *Arabidopsis thaliana* responded to phosphate limitation by exchanging a significant proportion of the leaf phospholipids with the galactolipid digalactosyldiacylglycerol (DGDG) [5]. It was suggested that the activity of a formerly unknown DGDG synthase, DGD2 [6], supplied non-chloroplast membranes with DGDG, to reduce membrane-associated phosphate in order to sustain other phosphate-requiring cellular processes [4–6]. The aim of the present study was to determine whether the replacement of phosphoglycerolipids with galactolipids, shown to occur in the leaf tissue of the annual ruderate *A. thaliana* [4–6], could occur also in a plant of agricultural importance and whether the lipid replacement could be demonstrated to occur in a specific non-chloroplast membrane. We chose to work with the plasma membrane from oat (*Avena sativa* L.) for two reasons: the plasma membrane because of its importance in regulating the flow of components and information between the cell and its surrounding and oat because of its importance as a crop. We found that severe phosphate limitation induced a replacement of a large portion of the plasma membrane glycerophospholipids with DGDG of a non-chloroplast fatty acid composition, in the roots as well as in the shoots.

## 2. Materials and methods

### 2.1. Oat cultivation, isolation and characterization of plasma membrane

Oat was cultivated in a growth chamber on vermiculite in a complete nutrient solution [7] or in a nutrient solution without phosphate (KCl replaced $KH_2PO_4$).

Plasma membranes were isolated from shoots and roots of 2 or 4 week old oat by aqueous polymer two-phase partition [7]. The plasma membrane marker glucan synthase II [8] was used to assess the recovery. The activity was circa 10% lower in the plasma membranes from phosphate-limited plants compared to control plants but was lower to the same extent also in the respective parent 10 000–60 000 × *g* membrane fractions. The recovery of plasma membranes was 10–15% higher from shoots than from roots, but for each tissue, there was no significant difference in recovery between the two sets of growth conditions or the two different plant ages. SDS–PAGE [9] revealed strikingly similar polypeptide patterns for plasma membranes from both tissues and both cultivation regimes. The minute differences were between plasma membranes from shoots compared to those from roots, rather than between different growth conditions. To assess plastid contamination, we assayed the capacity for synthesis of the major plastid lipid monogalactosyldiacylglycerol (MGDG) [10] for the plastid envelope. In the plasma membrane fractions the MGDG-synthase activity was reduced 15–20-fold, to just above the detection level, compared to the parent 10 000–60 000 × *g* membrane fraction. The suspended plasma membrane fractions from both roots and shoots were white. Assuming that the assays (adjusted [10] protein quantification [11], glucan synthase II, SDS–PAGE, MGDG synthase) had not been affected by phosphate limitation, we concluded that in all cases (two plant ages, two tissues, two nutrient solutions) the isolated plasma membrane fractions were essentially of equal and high purity.

### 2.2. Lipid analysis

Lipids were extracted from oat shoot or root tissues [12] and suspended freshly isolated plasma membranes [13] as previously described. The lipid extracts were separated by thin layer chromatography [10] and fatty acid methyl esters were produced from the individual lipids by base-catalyzed methylation in 0.5 M sodium methoxide [14] together with a known amount of diheptadecanoyl-phosphatidylcholine (PC) as internal standard. The fatty acid methyl esters were separated and quantified by gas liquid chromatography [10] and identified through comparison with standard fatty acid methyl esters.

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*Abbreviations:* 16:0, hexadecanoic acid (palmitic acid); 18:2, octadeca-*all cis*-9,12-dienoic acid (linoleic acid); 18:3, octadeca-*all cis*-9,12,15-trienoic acid (linolenic acid); DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine

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Unless otherwise stated, all presented data are mean values ± the range of two samples obtained from independently cultivated plant material.

## 3. Results and discussion

Up to well over 1 week after sowing, the well-fertilized and phosphate-limited oat was morphologically indistinguishable from each other. Between 1 and 2 weeks after sowing, the plants cultivated without phosphate began to show the classical signs of phosphate limitation [15], such as a decrease in shoot growth and an increased root growth (Fig. 1), a darker green leaf color, purple stains on the stems and increased formation of side roots. At 4 weeks of age, the phosphate-limited plants were severely stunted in shoot growth as compared to controls and the stems were purple in color. It should be noted that eventually the phosphate-limited plants were able to produce grains, although the yield was very low (results not shown).

In well-fertilized plants, DGDG is generally considered to be restricted to plastids, where it constitutes 25–30% of the lipids [2,3]. Thus leaves normally have a high DGDG content, reflecting the large chloroplast membrane area in these cells, whereas the DGDG content in roots is low, as plastids make up only a small portion of the membranes in its tissues. In our study, the DGDG proportions of acyl lipids in the well-fertilized oat corresponded with these general findings. In phosphate-limited oat, the proportion of DGDG began to increase in the roots before the plants were 1 week of age, but in the shoots, the increase in DGDG began somewhat later (Fig. 2). The DGDG increase in the roots thus began several days prior to any observable morphological effects. If the increase in DGDG represented lipid replacement in one or several non-plastid membranes, a limited exchange of phospholipids for DGDG did not severely affect membrane function when reflected at the tissue level.

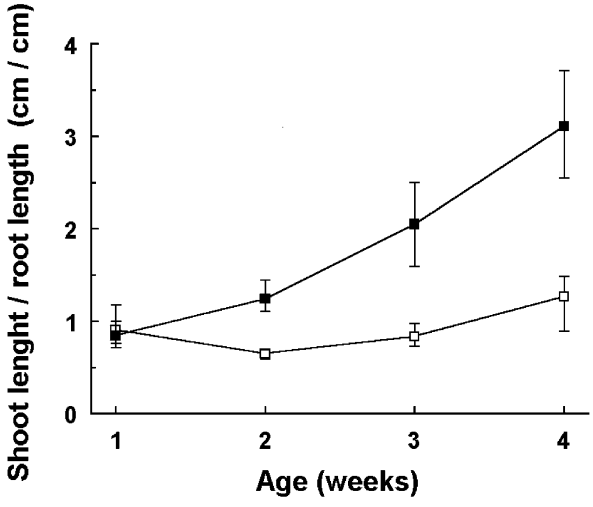


Fig. 1. Growth of oat cultivated with or without phosphate for 4 weeks. The ratio of shoot length to root length (measured from the seed to the tip of the tallest leaf and tip of the main root, respectively) is shown for well-fertilized oat (closed symbols) and phosphate-limited oat (open symbols). The data present mean values ± standard deviations for three independently cultivated plant batches for each of the two growth conditions. For each plant batch, triplicate measurements were made.

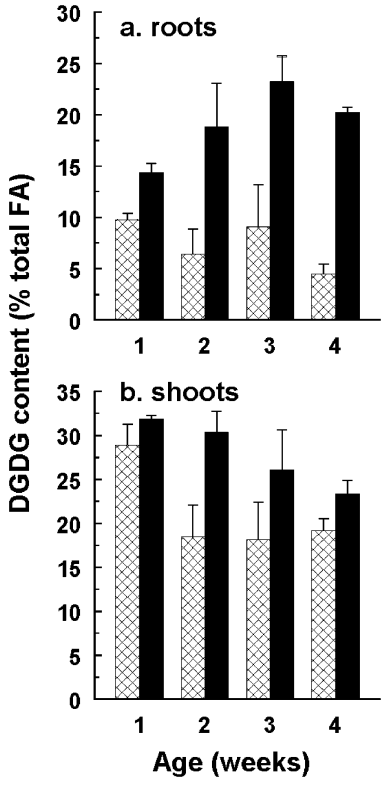


Fig. 2. DGDG content of oat grown with or without phosphate for 4 weeks. The proportion of DGDG of the tissue glycerolipids from well-fertilized (cross-hatched bars) or phosphate-limited (solid bars) oat is shown for roots (a) and shoots (b). Mean values ± standard deviations are shown for three independently cultivated plant batches for each of the two growth conditions.

Our next step was to investigate whether the increased proportion of tissue DGDG could be traced to the plasma membrane lipids. We used plants beginning to exhibit visible stress symptoms (2 weeks old) and plants that had developed clear stress symptoms (4 weeks old). DGDG was present already as a minor constituent in the plasma membranes of control plants but became, with time, the major acyl lipid class of the plasma membranes of severely phosphate-limited oat (Fig. 3). In 2 week old plants cultivated in a phosphate-free medium, DGDG constituted 12% and 29% of the plasma membrane glycerolipids isolated from shoots and roots, respectively (Fig. 3a,b). The proportions of DGDG increased to 46% and 70% in the plasma membranes isolated from shoots and roots of 4 week old phosphate-limited plants (Fig. 3c,d). In the 4 week old plants, the increase in the proportion of DGDG was balanced by decreases in all phospholipid classes, except phosphatidic acid (PA) in the shoot plasma membrane. We are aware of only one previous investigation where the plasma membrane lipid composition was related to phosphate stress, but in this investigation on root plasma membranes of broad bean, only phospholipids were analyzed [16]. On a protein basis, the phospholipid content decreased by 50% [16], and, as in the present case, the decrease occurred in all phospholipid classes.

The fatty acid composition of chloroplast DGDG is usually 10–15% palmitic acid (16:0), 2–5% linoleic acid (18:2) and 80–90% linolenic acid (18:3) [2,3], whereas the fatty acid compo-

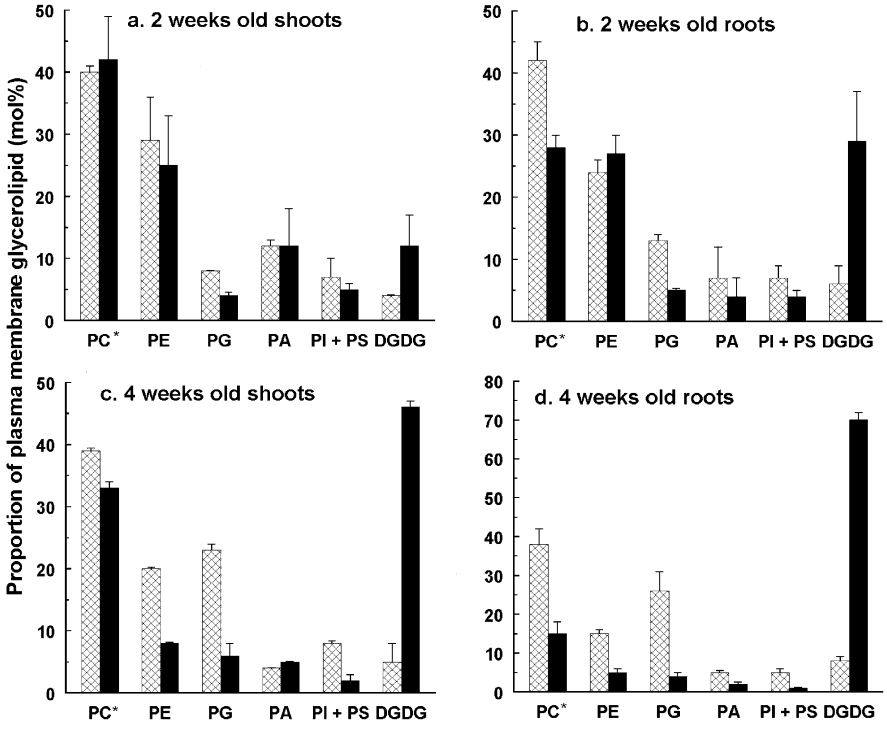


Fig. 3. The glycerolipid composition of plasma membranes isolated from roots and shoots of oat grown with or without phosphate. Plasma membranes were isolated from 2 week old shoots (a) and roots (b) as well as from 4 week old shoots (c) and roots (d) from well-fertilized (cross-hatched bars) or phosphate-limited (solid bars) oat. PC*, PC+sulfoquinovosyldiacylglycerol; PG, phosphatidylglycerol; PI+PS, phosphatidylinositol+phosphatidylserine. The data for MGDG is not shown. It constituted below 1 mol% in all cases except for the root plasma membranes of 4 week old phosphate-limited oat, where it constituted 1.4 ± 0.5 mol%. There were no statistically significant differences between the different plasma membrane fractions concerning this lipid class.

sition of DGDG of non-shoot plastids is markedly less desaturated [17,18]. The chloroplast DGDG fatty acid composition was reflected in DGDG extracted from shoot tissue, which in the 4 week old well-fertilized oat contained 10% 16:0, 3% 18:2 and 87% 18:3 (results not shown). The fatty acid composition of DGDG extracted from the roots of these plants was even less unsaturated than that of non-shoot plastids [18,19] in containing 32% 16:0, 28% 18:2 and 36% 18:3 (results not shown). The DGDG from isolated plasma membranes (Table 1) had a fatty acid composition markedly different from that of chloroplast or root plastid DGDG. The increased proportion of DGDG in plasma membranes isolated from phosphate-limited plants was reflected at the level of tissue DGDG, but to different extents in the shoots and roots. In shoots, only a small decrease in fatty acid desaturation of the extracted shoot DGDG was evident, whereas plasma membrane DGDG apparently contributed significantly to the pool of DGDG in roots (cf. Table 1 and above). The unsaturation level of the DGDG fatty acids of the plasma membranes increased with plant age, to become more closely resembling that of phospholipids [2,7], especially in the root plasma membrane.

It should be pointed out that earlier investigations have reported that isolated plant plasma membranes contained low amounts of DGDG, although its presence was often considered as a contamination with plastid membranes. A few reports, however, have suggested that DGDG could indeed be a minor plasma membrane constituent; the suggestions were based on membrane isolation characteristics [19] or on the clearly different fatty acid compositions of chloroplast and plasma membrane DGDG [7,20]. We suggest that the presence of low levels of DGDG in control plants, in the present

Table 1
The fatty acid composition of DGDG from plasma membranes and whole tissue from oat roots and shoots grown without inorganic phosphate

| Fatty acid | 2 week old plants | | | | 4 week old plants | | | |
|---|---|---|---|---|---|---|---|---|
| | Whole shoots | Shoot plasma membranes | Whole roots | Root plasma membranes | Whole shoots | Shoot plasma membranes | Whole roots | Root plasma membranes |
| 16:0 | 10 ± 1 | 53 ± 2 | 30 ± 9 | 55 ± 3 | 12 ± 1 | 32 ± 1 | 36 ± 5 | 34 ± 1 |
| 18:2 | 5 ± 1 | 19 ± 2 | 42 ± 3 | 30 ± 2 | 4 ± 1 | 19 ± 1 | 38 ± 1 | 45 ± 1 |
| 18:3 | 84 ± 1 | 20 ± 2 | 27 ± 5 | 9 ± 1 | 83 ± 1 | 43 ± 1 | 25 ± 4 | 18 ± 1 |

The balance to 100% is made by predominantly octadecanoic acid and octadeca-*cis*-9-enoic acid.

as well as other [7,19,20] investigations, reflects a basal activity of DGDG synthesis for non-plastidic membranes. Even when optimal cultivation conditions are aimed for, phosphate may become a limiting nutrient, and the lipid replacement mechanism thus initiated.

In phosphate-deficient leaves of *A. thaliana*, the pool of inorganic phosphate was depleted prior to that of organic phosphate, where lipids were affected at an earlier stage than nucleotides and other phosphate esters [1]. We suggest that in our case, the observed phosphate deficiency symptoms may have biochemical explanations in part related to altered plasma membrane lipid composition. We furthermore suggest that at least in the shoots, the plasma membrane was a major target for the lipid replacement mechanism. The proportion of DGDG in the shoot plasma membranes increased to 8–10 times that of the control in the 4 week old plants, but at the shoot tissue level, the DGDG proportion of the lipids only increased about 1.2-fold, reflecting that chloroplast DGDG still strongly dominated the DGDG pool. Furthermore, the plasma membrane DGDG of a non-plastid fatty acid composition had only a minor influence on the fatty acid composition of shoot tissue DGDG. These results suggest an increase in plastidic DGDG or that in shoots, other non-chloroplast membranes besides the plasma membrane did not replace phospholipids with DGDG to any larger extent, or did so with DGDG of a plastid fatty acid composition.

It is well established that within the membrane, the critical entity is not the lipid composition per se, but the capacity for acclimation to the prevailing physiological and environmental conditions, which can be achieved by means of various lipid combinations [21]. For example, when plants are subjected to various forms of stress, the acclimation at the plasma membrane lipid level often includes alterations in the PC to phosphatidylethanolamine (PE) ratio, the sterol to phospholipid ratio and/or alteration in the degree of unsaturation of the individual phospholipids [7,22–26]. To receive its lipid components, a membrane needs to maintain capacity for vesicle fusion and/or for lipid delivery at membrane/membrane contact sites [27]. The appropriate ability of membranes to fuse is partly related to the physical properties of the membrane lipids, especially the inherent tendency to relax into the reversed hexagonal phase [28]. This property relates to the presence of lipids with a cone-shaped molecular geometry [28], which in the plasma membrane are represented by PE and PA. In relation to these findings, it is somewhat surprising that in 4 week old phosphate-limited oat, DGDG could substitute all plasma membrane phospholipid classes to similar extents. There were no clear preferences for or discriminations against any specific phospholipid class or lipid property as shape or charge. For the plasma membrane to maintain a capacity for fusion, it would have been expected that DGDG should replace other phospholipids preferentially over PE or even perhaps that another plastid lipid, the cone-shaped MGDG, should substitute for PE. This was not the case in the 4 week old severely phosphate-limited oat. As both shoots and roots continued to expand also in these plants, the plasma membranes must nevertheless have received membrane material. In addition, as the plants were able to produce grain, the functional properties of the root and shoot plasma membranes must have been maintained to a significant extent. To understand how the functionality of the plasma membrane could be largely maintained when half of its zwitterionic and charged lipids were replaced with an uncharged lipid, there is a need for further investigations into the physical properties of DGDG, including its abilities to interact with other plasma membrane components.

To conclude, our results demonstrate that severe phosphate limitation induced a drastic change in the glycerolipid composition of the plasma membranes of oat shoots and roots, where DGDG of a non-plastid fatty acid composition replaced a large portion of the phosphoglycerolipids. Intriguing subjects for future research include identification as well as localization of the activity that provides the plasma membrane with DGDG, how DGDG is transported to the plasma membrane, to which extent and under which forms of environmental stress oat as well as other plants can function with a partial replacement of phospholipids with DGDG and, not the least, a more detailed investigation into to the role of the lipid replacement mechanism in general phosphate house-holding.

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