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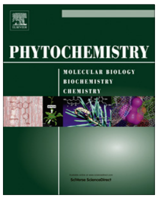

Differential changes in galactolipid and phospholipid species in soybean leaves and roots under nitrogen deficiency and after nodulation

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ABSTRACT

The availability of nitrogen (N) to plants has a profound impact on carbohydrate and protein metabolism, but little is known about its effect on membrane lipid species. This study examines the changes in galactolipid and phospholipid species in soybean as affected by the availability of N, either supplied to soil or obtained through *Bradyrhizobium japonicum* nodulation. When N was limited in soil, the content of galactolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacyglycerol (DGDG), decreased drastically in leaves, while a smaller decrease of DGDG was observed in roots. In both leaves and roots, the overall content of different phospholipid classes was largely unchanged by N limitation, although some individual phospholipid molecular species did display significant changes. Nodulation with *Bradyrhizobium* of soybean grown in N-deficient soil resulted in a large increase in levels of plastidic lipid classes, MGDG, DGDG, and phosphatidylglycerol, along with smaller increases in non-plastidic phospholipids in leaves. Nodulation also led to higher levels of phospholipids in roots without changes in root levels of MGDG and DGDG. Overall, N availability alters lipid content more in leaves than roots and more in galactolipids than phospholipids. Increased N availability leads to increased galactolipid accumulation in leaves, regardless of whether N is supplied from the soil or symbiotic fixation.

1. Introduction

Nitrogen (N) is an essential element of key macromolecules such as proteins, nucleic acids, some lipids, and chlorophylls (Crawford and Forde, 2002; Peng et al., 2007), and N availability is critical for optimal growth and development of plants. In nature, numerous factors, including soil erosion, water leaching, plant growth, and microbial consumption, lead to N deficiency (Good et al., 2004). N deficiency also leads to alterations in root architecture, especially in lateral root initiation (Little et al., 2005; Orsel et al., 2006; Remans et al., 2006), and up-regulation of genes responsible for nitrate transporters for N uptake (Guo et al., 2001). These responses enhance N acquisition (Lawlor, 2002; Zheng, 2009). Meanwhile, N deprivation induces the catabolic process of vacuolar autophagy and increases N remobilization from older to younger leaves and reproductive organs (Hanaoka et al., 2002; Ono et al., 1996; Peng et al., 2007; Remans et al., 2006). The synthesis of amino acids, proteins, and nucleic acids are suppressed under N-limiting conditions (Scheible et al., 2004; Wang et al., 2003). It has been well documented that N deprivation decreases crop yield, particularly by decreasing photosynthesis (Boussadia et al., 2010; Zhao et al., 2003).

While N availability is known to impact carbohydrate and protein metabolism, its effect on lipid metabolism and membrane lipid remodeling remains poorly understood. N and phosphorus are components of the head group of the major glycerophospholipids, phosphatidylcholine (PC; **1**–**20**) and phosphatidylethanolamine (PE; **21**–**43**), as well as phosphatidylserine (PS; **44**–**69**). Membrane lipid remodeling during phosphate starvation in plants leads to decreases in phospholipids and increases in galactolipids, particularly digalactosyldiacylglycerol (DGDG; **70**–**85**) (Gaude et al., 2008; Härtel et al., 1997; Li et al., 2006). This decrease in phospholipids allows phosphorus to be used for other critical cell functions and also makes diacylglycerol (DAG) available for galactolipid biosynthesis. In contrast, the level of galactolipids was decreased in N-deprived *Arabidopsis* seedlings (Gaude et al., 2007). The hydrolysis of phospholipids, such as PC to phosphatidic acid (PA; **86**–**97**) by phospholipase Dε, has been implicated in *Arabidopsis* response to N availability, leading to an increase in root surface

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Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PLD, phospholipase D; PS, phosphatidylserine.

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area and improved N uptake and utilization (Hong et al., 2009). However, little is known about how membrane lipid species change in response to N availability.

This study was undertaken to analyze the changes in molecular species of membrane phospholipids and galactolipids in soybean under N-deficient and N-sufficient conditions. Each membrane glycerolipid class is composed of various molecular species with varied fatty acid chain length and degree of unsaturation. To date, a detailed analysis of lipid molecular species alterations due to N limitation in a crop species has not been reported. The present study used soybean because leguminous plants have the ability to form nodules that fix nitrogen (N_2) to ammonia through symbiotic association with *Rhizobiaceae* bacteria. Use of a leguminous species, therefore, offers an opportunity to analyze the effect of symbiotic N fixation on membrane lipid changes.

2. Results

2.1. N deficiency decreases galactolipid more than phospholipid content

Soybean seedlings were grown in a mixture of vermiculite and perlite containing 5 or 0.5 mM N supplied as a combination of nitrate and ammonium. Compared with seedlings at 5 mM N, soybean seedlings grown at 0.5 mM N for 15 days displayed overt symptoms of N deficiency. The growth of the above ground portion of the seedlings was inhibited, with leaves turning yellow (Fig. 1A). The average leaf area was 50% smaller than that of seedlings grown at 5 mM N (Fig. 1B), while the number of leaves was reduced by 20% (Fig. 1C). The older leaves of N-deficient seedlings had 60% and the younger leaves 30% of the chlorophyll content of seedlings with sufficient N (Fig. 1D). At 5 mM N, soybean seedlings grew and developed normally; 5 mM N is referred to as the N-sufficient condition throughout the text.

Lipids from leaves and roots grown under these conditions were quantitatively profiled using electrospray ionization triple quadrupole mass spectrometry (ESI-MS/MS). All the compounds analyzed are numbered and presented in Supplemental Tables 1 and 2. Representative structures of each lipid class are shown in Supplemental Fig. 1 and modified from Devaiah et al. (2006). The photosynthetic membranes of plants are rich in the non-nitrogenous galactolipids, MGDG (**98**–**113**) and DGDG (**70**–**85**). Under N-sufficient conditions, the mass spectral signals from MGDG (**98**–**113**) and DGDG (**70**–**85**) (normalized mass spectral signal/mg dry mass) in leaves were approximately fivefold greater than those from total phospholipids (Fig. 2; Supplemental Table 1). On the other hand, phospholipids are the major lipids in roots, and the mass spectral signals from root phospholipids were approximately ten-fold greater than those of galactolipids (Fig. 2; Supplemental Table 1). When soybean plants were deprived of N, the ratio of galactolipids to phospholipids in leaves decreased twofold due primarily to a decline in the galactolipids MGDG (**98**–**113**) and DGDG (**70**–**85**) (Fig. 2; Supplemental Table 1). In roots, the ratio of galactolipids to phospholipids was also decreased, but the decrease was smaller than that in leaves. The decrease in roots came primarily from a 39% decrease in DGDG (**70**–**85**) whereas the content of MGDG (**98**–**113**) and phospholipids were not significantly changed (Fig. 2). These results show that N availability alters lipid content more in leaves than roots, with greater changes in galactolipids than phospholipids.

2.2. Polar glycerolipid species change differentially in N-deficient leaves and roots

MGDG (**98**–**113**) is the most abundant glycerolipid in leaves and the highly polyunsaturated 36:6 (di18:3)-MGDG (**104**) accounts for more than 90% of MGDG (**98**–**113**) whereas DGDG (**70**–**85**) is composed mostly of 36:6 (**76**)- and 34:3-species (**73**) (Fig. 3A;

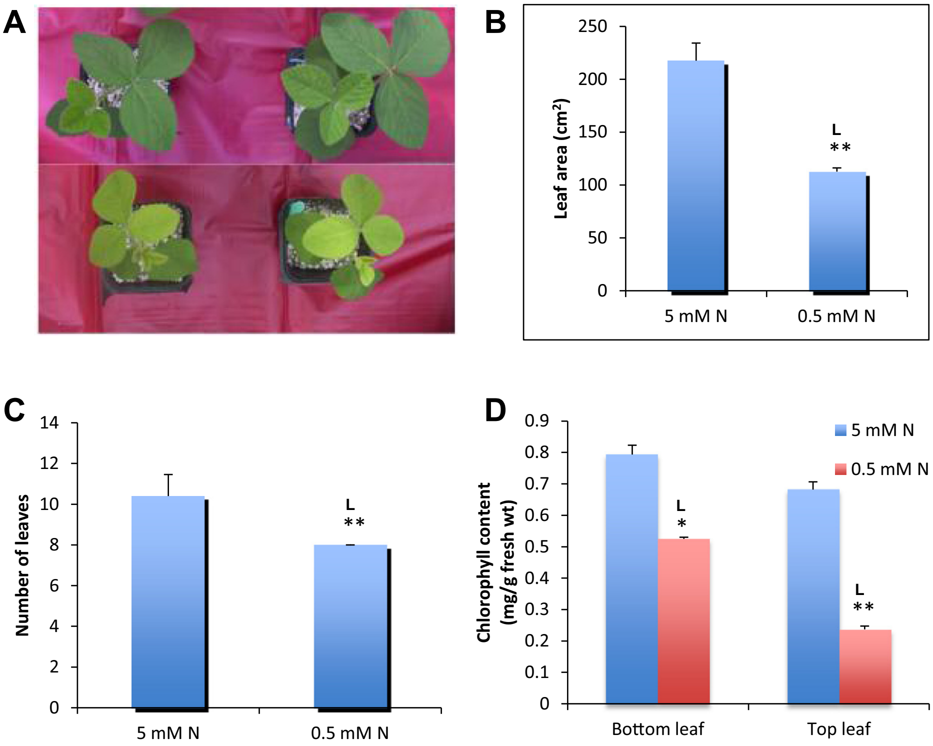

Fig. 1. Effect of nitrogen starvation on soybean growth. (a) Soybean seedlings exposed to sufficient nitrogen (5 mM; top panel) and deficient nitrogen (0.5 mM; bottom panel). (b–d) Growth parameters as affected by nitrogen starvation. Surface sterilized soybean seeds were germinated on vermiculite and perlite mix (1:3) and grown for 15 days with 5 mM and 0.5 mM nitrogen. Leaf area (B), number of leaves (c) and chlorophyll content (d) were estimated. Values are means ± SE ($n = 5$) from one representative of three independent experiments. Nitrogen was varied by varying levels of exogenously supplied ammonium nitrate.

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