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AGE DEPENDENT CHANGES IN PHOSPHOLIPIDS AND GALACTOLIPIDS IN PRIMARY BEAN LEAVES (*PHASEOLUS VULGARIS*)

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Key Word Index—*Phaseolus vulgaris*; Leguminosae; phospholipids; galactolipids; leaf development.

Abstract—Primary leaves of *Phaseolus vulgaris* show concomitant changes in phospholipid, galactolipid, chlorophyll and fresh weight during leaf development from 3 to 32 days after planting. Phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol show only small changes on a mole per cent lipid phosphate basis during leaf development. The chloroplast lipids, phosphatidyl glycerol, monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) all show marked increases and decreases which are coincident with chloroplast development. The decline in the leaf content of chloroplast polar lipids and chlorophyll become evident upon reaching maximal leaf size. The molar ratio of galactolipids (MGDG/DGDG), reaches a maximum value of 2.3 in expanding leaves, but steadily declines during senescence to a minimum value of 1.5 at abscission. The declining ratio is caused by a preferential loss of MGDG in the senescing leaves.

INTRODUCTION

The primary bean leaf is frequently used in studies on leaf metabolism, especially those dealing with membrane function, such as ion permeation [1, 2], and chloroplast development [3]. In spite of the wide range of physiological information available on the bean leaf, there are few studies on their phospholipid and galactolipid content [4,5]. In this paper we report the changes in polar lipid composition of the primary bean leaf (*Phaseolus vulgaris* var. 'Pinto') from 3 to 32 days after planting.

RESULTS

Under the growth conditions used in this study, the developing primary leaves were whitish in appearance and still within the cotyledonary cover from day 3 to 5. At day 6 the cotyledons were above soil level. The hypocotyl hook was still present and the pale green leaves were just emerging from in between the cotyledons. By day 8 the cotyledons were pale green and shrivelled, while the primary leaves were dark green and unfolded. The primary leaves continued to expand, reaching maximal fresh weight and chlorophyll content at day 16 (Fig. 1). By day 30 the leaves were chlorotic and abscission occurred by day 32. The amounts of phospholipid and galactolipid in the primary leaves showed similar patterns of change during leaf development as seen in fresh weight and chlorophyll (Fig. 2). A dramatic increase is observed in all these parameters between day 5 and 16, but an abrupt decrease is seen after day 17.

To estimate changes in the polar lipid pattern without considering the net changes in lipid content, the data

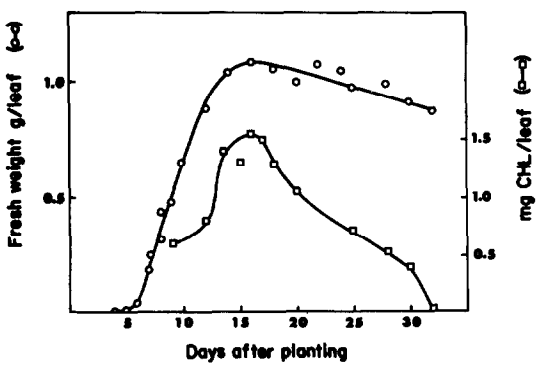

Fig. 1. Age dependent changes in fresh weight and chlorophyll content of primary bean leaves. Sample sizes were from 100–150 leaves for ages less than 6 days, 20 leaves for ages 6–10 days and 4 leaves for ages greater than 15 days.

* Portions of the material presented in this paper were taken from a dissertation submitted to the Graduate Division of the University of California at Riverside in partial fulfillment of the requirements for the Ph.D degree in Biology. Present address: Institute of Photobiology for Cells and Organelles, Brandeis University, Waltham, MA 02154, U.S.A.

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‡ *Abbreviations*: DGDG, digalactosyl diglyceride; MGDG, monogalactosyl diglyceride; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol.

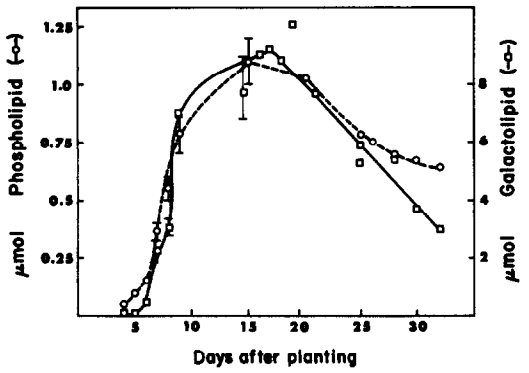

Fig. 2. Age dependent changes in phospholipid and galactolipid levels in primary bean leaves. Sample sizes were as described in figure 1. Bars indicate two standard deviations of 10–15 replicate extractions of leaves.

were expressed as the mol % lipid phosphate or as the molar ratio of galactolipids. The major changes in the phospholipid composition during leaf development were associated with PG, which increased from 10 mol % at day 5 to 29 mol % at day 8, increased at a more gradual rate to 34 mol % by day 20 and finally decreased to 15 mol % by day 32 (Fig. 3). PC was about 50 mol % between days 5 and 8, decreased to 45 mol % by day 20 and then increased again to 50 mol % by day 30. PE steadily decreased from 30 mol % at day 5 to 20 mol % at day 30. PI steadily decreased from 11 mol % to 8 mol % between days 5 and 8, and remained unchanged until day 25 when it increased to 15 mol %.

The MGDG and DGDG content of the primary leaves increased to maximum values of 6 and 3 µmol per leaf, respectively, at day 16 (Fig. 4a). The decline in MGDG between day 16 and 32 appeared to be greater than that occurring in DGDG content. The molar ratios of MGDG/DGDG increased from 1.8 to 2.1 between days 5 and 8, remaining unchanged until after day 16 when the ratio steadily declined to a final value of 1.5 at day 32 (Fig 4b). This preferential loss of MGDG is most likely caused by galactolipase activity which is known

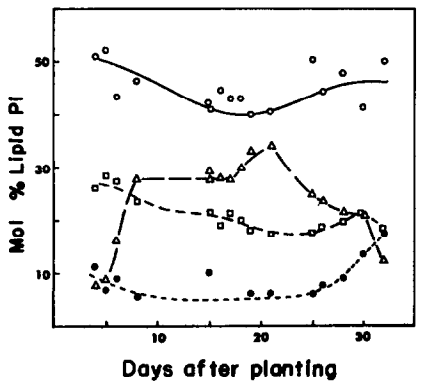

Fig. 3. Age dependent changes in phospholipid composition of primary bean leaves. Symbols: ○, phosphatidyl choline; △, phosphatidyl glycerol; □, phosphatidyl ethanolamine; ●, phosphatidyl inositol.

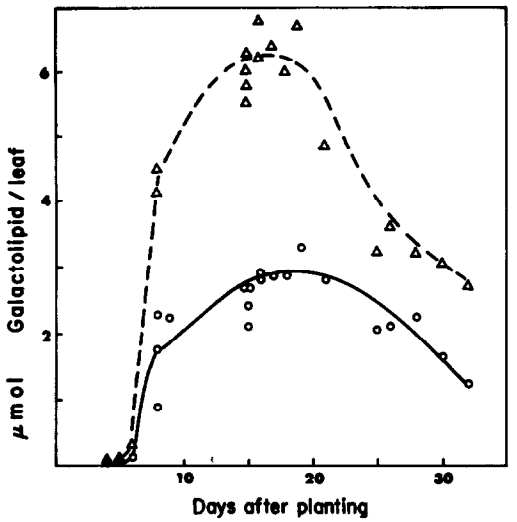

Fig. 4a. Age dependent changes in monogalactosyl diglyceride and digalactosyl diglyceride content of primary bean leaves. Symbols: △, monogalactosyl diglyceride; ○, digalactosyl diglyceride.

to have greater hydrolytic activity with MGDG than DGDG [6,7].

DISCUSSION

Both phospholipid and galactolipid content of the primary leaves increased and decreased concomitantly with changes in fresh weight and chlorophyll during leaf development. These changes are generally consistent with those reported for cucumber leaf development [8]. The major qualitative changes were in compounds associated with the synthesis and degradation of chloroplast membranes, i.e. PG, MGDG and DGDG. These changes correlate well with the ultrastructural changes occurring during bean leaf development [3]. Though the function of PG in plant cells is unclear, it is located in the endoplasmic reticulum and mitochondria as well as the chloroplasts [9,10]. Ferguson and Simon [8] found that PG content declined several days before maximal chlorophyll content had been attained in cucumber leaves. This early loss in PG content in cucumber leaves may be caused by changes in a pool of PG which is not associated

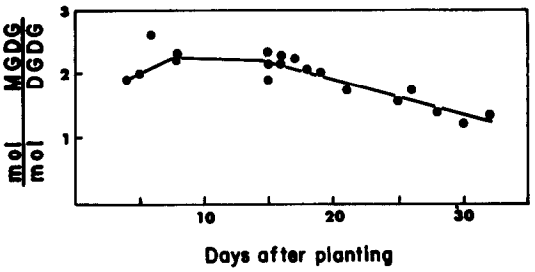

Fig. 4b. Molar ratios of monogalactosyl diglyceride and digalactosyl diglyceride (MGDG/DGDG) during leaf development.

with the chloroplasts. Thus the age dependent changes in PG may vary among species depending on the relative distribution of PG among the membraneous organelles.

EXPERIMENTAL

Plant material. Phaseolus vulgaris var. 'Pinto' (Burpee Seed Co., Riverside, CA U.S.A., Lot #415) seeds were planted in vermiculite in styrofoam cups and watered with deionized water. Cups were placed in a dark germination box at 28° for 3 days and then transferred to an environmental growth chamber. Plants were watered daily with $\frac{1}{8}$ strength nutrient solution. Plants were numbered as to days after planting.

Growth chamber conditions. Light intensity was *ca* 9000–10000 lx, supplied by 12 40-in fluorescent lamps and 8 25-W incandescent bulbs; photoperiod was 12 hr light/12 hr dark; temperature program was 27° during the light and 18° during the dark. Relative humidity was maintained at 70–80%.

Lipid extraction. For samples assayed less than 6 days after planting, *ca* 100–150 leaves were taken for extraction, for 6–10 days, 20 leaves were taken and for older than 15 days, 4 leaves were taken for analysis. Leaves were quickly weighed and extracted in boiling MeOH (25 ml) to inactivate the phospholipases. After cooling, 7 ml $CHCl_3$ and 7 ml H_2O were added to form a monophase [11]. Extraction in monophase soln proceeded overnight at 4° in flasks sealed with several layers of parafilm under N_2. Leaves were re-extracted by grinding in a mortar with additional monophase soln until no detectable chlorophyll was present in the extract. The pooled monophase solutions were separated into organic and aqueous phases by the appropriate additions of $CHCl_3$ and H_2O [11]. The organic phase was concentrated on a rotary evaporator to near dryness and resuspended to a final vol of 2 ml with $CHCl_3$–MeOH (2:1) and stored in Teflon sealed culture tubes sealed under N_2.

TLC. For separation of the polar lipids, the total lipid extracts were separated using two dimensional Si gel TLC according to Nichols and James [12]. The solvent in the first direction consisted of $CHCl_3$–MeOH–7N NH_4OH (130:60:8), and in the second direction $CHCl_3$–MeOH–HOAc–H_2O (170:30:25:6). The polar lipids were identified using indicator sprays. The separation of polar lipids was as described by Ongun and Mudd [13]. The lipids were routinely located by spraying with 0.1% 8-anilino-1-napthalenesulfonate in water and viewed in UV light [14].

Lipid Pi and galactose determinations. Individual spots identified under TLC separation were scrapped into test tubes and analyzed for either Pi or galactose. Lipid Pi was estimated using procedures described by Rouser *et al.* [15]. Lipid galactose was estimated by using a modification of the procedures described by Roughan and Batt [16]. Samples were hydrolyzed using 0.5 ml 2N H_2SO_4 in a boiling H_2O-bath for 1 h. The assay was modified by adding 1 ml 5% (w/v) phenol in H_2O and 4 ml conc H_2SO_4 to the hydrolyzed sample and incubating for 30 min. Tubes were then centrifuged to remove the Si gel and the absorbance of the soln was determined at 480 nm. Not hydrolyzing the galactose moieties from the lipid before assaying resulted in very erratic values. All determinations were done in triplicate. The galactose values varied *ca* 10% and Pi values less than 1% between replicate plates. Chlorophyll content was estimated according to Arnon [17].

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