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Relationship between growth temperature and the levels of phosphatidylglycerol (PG) molecular species in thylakoid membranes of squash and spinach cotyledons

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Introduction

Thylakoid membranes in higher plants contain four glycerolipids, monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulfoquinovosyl diacylglycerol (SQDG), and phosphatidylglycerol (PG). Changes in fatty acid composition in thylakoid lipids in response to low temperature have been observed to occur in a variety of plants. In general, low temperature induces an increase in linolenic acid level, which is thought to maintain membrane fluidity at low temperatures for the survival of plants under chilling conditions (Vigh et al., 1993; Kodama et al., 1995). However, the mechanism leading to temperature-induced changes in the fatty acid composition of thylakoid membranes has not yet been fully elucidated. If the desaturation of lipids induced by low temperature is the factor to maintain membrane fluidity, the desaturation of PG should be important. PG is the only phospholipid and seems to play an important role in the structural characteristics and functions in the photosynthetic apparatus (Trémolières and Siegenthaler, 1998). PG has been also reported to be related to the susceptivity of plants to low temperature. Of all the thylakoid membrane lipids, only PG contains high levels of saturated molecular species, and the levels are significantly higher in chilling-sensitive plants than those in resistant ones (Murata, 1983; Roughan, 1985).

In this study, we investigated the effect of growth temperature on PG molecular species in thylakoid membranes in spinach and squash cotyledons. The possible mechanism leading to temperature-induced changes in composition of PG molecular species in the thylakoid membranes are also discussed.

Materials and methods

Squash (*Cucurbita pepo*) and spinach (*Spinacia oleracea*) plants were germinated in darkness and grown in a controlled environmental growth chamber with the continuous lights (photon flux density: 300 μmol $m^{-2}s^{-1}$). The temperatures of seed germination and seedling growth were 15°C□ 20°C and 30°C for spinach and 20°C, 30°C and 35°C for squash. Cotyledons were collected during plant growth. Lipid extraction and separation were carried out as previously described (Xu and Siegenthaler, 1997). PG molecular species were separated by HPLC (Xu and Siegenthaler, 1996a). PG was first treated with phospholipase C (Grade II from *B. cereus*, Boehringer Mannhaeim) to produce diacylglycerol which was reacted with 3,5-dinitrobenzoyl chloride (Sigma) to give the dinitrobenzoyl derivatives (DNB-DAG). The

DNB-DAGs were separated by reversed-phase HPLC on a Millipore Waters 600E Multisolvent Delivery System with a Nucleosil 100-5 C_{18} column (250 x 4 mm). The solvent was acetonitrile/2-propanol (80:20, v/v) with a flow rate of 0.5 ml/min. The DNB-DAGs were detected at 254 nm with a Millipore Waters 470 Scanning Fluorescence Detector. The fatty acid distribution at the *sn*-1 and *sn*-2 position of glycerol backbone was determined according to the method described by Chapman and Barber (1987)

Results and discussion

Ten molecular species were found in the thylakoid membranes of both spinach and squash cotyledons. 18:3/16:0, 18:3/16:1(3*t*), 18:2/16:0, 18:2/16:1(3*t*), 18:1/16:0, 18:1/16:1(3*t*), 18:0/16:0, 18:0/16:1(3t), 16:0/16:0 and 16:0/16:1(3t). The level of each molecular species changed during the cotyledon growth. Fig. 1 and Fig. 2A show, as examples, the changes in the PG molecular species during initial 4 growth days. When squash plants geminated in darkness were exposed to light, all molecular species containing 16:1(3*t*) increased at the expense of molecular species containing 16:0 at the *sn*-2 position of glycerol backbone (only the major molecular species shown here). These changes depended on plant species. Under all growth conditions, the increases of 16:1(3*t*) containing molecular species were more rapid in spinach than those in squash. Previously report (Xu and Siengenthaler, 1996b) has shown that the state of chloroplast maturity in squash cotyledons was closely correlated with the content of 16:1(3*t*) in them. Thus, the different increase rates of 16:1(3*t*) during the initial growth period of plants reflect that these two plants have different rates of chloroplast maturity. As shown in Fig. 1 and Fig. 2A, as examples, when squash plants were grown at different temperatures and spinach plants were grown at 15 and 20°C, the molecular species composition of PG displayed a changed phase during certain time and then remained constant.

However, when spinach plants were grown at 30°C, 16:1(3*t*) containing molecular species increased rapidly and reached maximal values after only 9 h illumination, and then decreased (Fig. 2B). It has been reported that these molecular species play a crucial role in the biogenesis and the trimerization of LHCII (Trémolières and Siegenthaler, 1998). Our results suggest that the process could be affected by high levels of 18:3 containing molecular species at a relatively high temperature. The *sn*-2 position of the glycerol backbone in PG was occupied always by 16:0 or 16:1(3*t*) and these two fatty acids have the similar phase transition temperature (Bishop and kenrick 1987). Therefore, the physical property of a molecular species mainly depends on the desaturation degree of the fatty acids at the *sn*-1 position of the glycerol backbone. The effects of growth

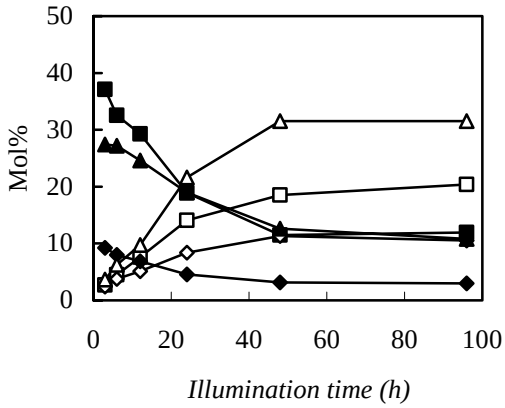

Fig. 1. Changes in the composition of major phosphatidylglycerol molecular species in squash cotyledons during growth. After seed germination in darkness at 20°C, plants were exposed to continuous light (350 µmol $m^{-1}s^{-2}$) at the same temperature. ■18:3/16:0; □18:3/16:1(3*t*); ♦ 18:0/16:0; ◊ 18:0/16:1(3*t*); ▲ 16:0/16:0; Δ 16:0/16:1(3*t*)

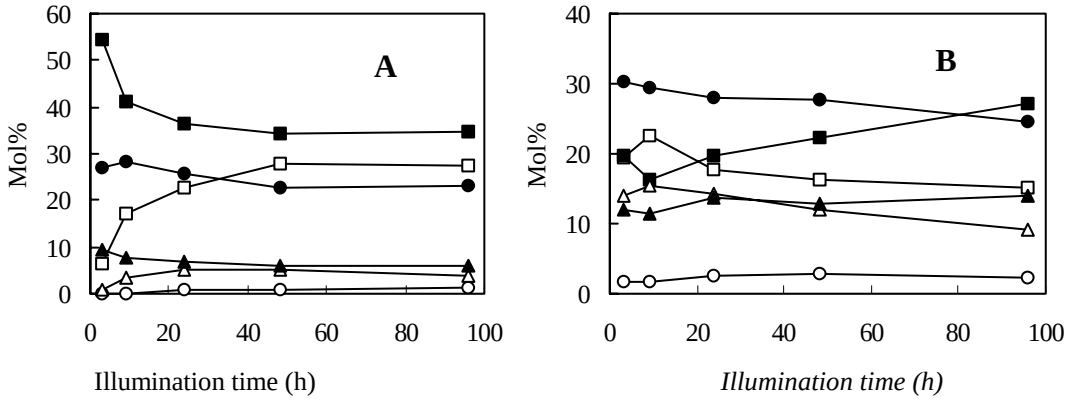

Fig. 2. Changes in the composition of major phosphatidylglycerol molecular species in spinach cotyledons during growth. Plants were exposed to continuous light (350 μmol $m^{-2}s^{-1}$) at 15°C (**A**) and 30°C (**B**) after germination in darkness at the same temperature of growth under light. ■ 18:3/16:0; □ 18:3/16:1(3*t*); ● 18:2/16:0; O 18:2/16:1(3*t*); ▲ 16:0/16:0; Δ 16:0/16:1(3*t*)

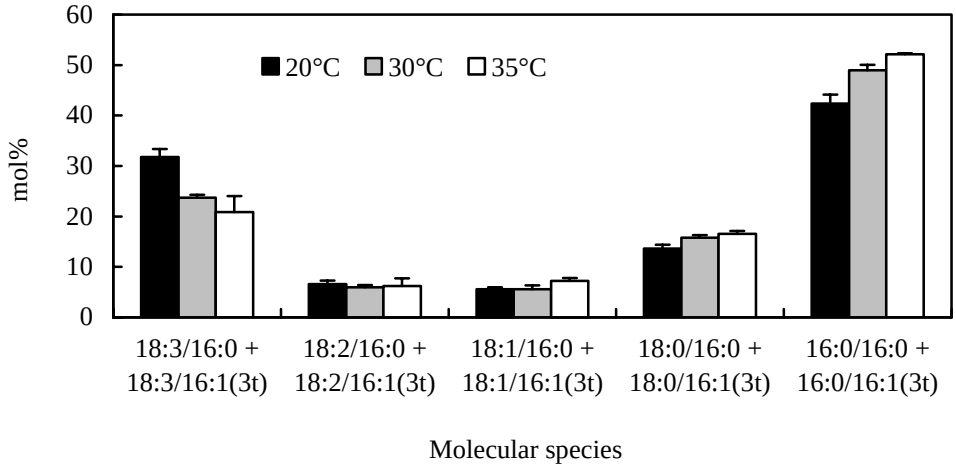

Fig. 3. The effects of growth temperatures on the composition of major molecular species containing the same fatty acid at the *sn*-1 position of glycerol backbone of phosphatidylglycerol in squash cotyledons. Data are expressed as means ± SE of 3 analysis (24, 48 and 96h of illuminated plants).

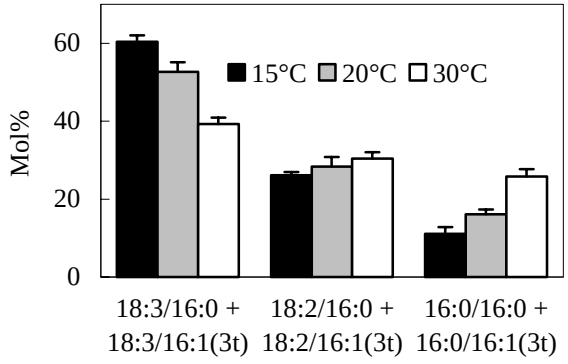

Fig. 4. The effects of temperatures on the composition of major molecular species containing the same fatty acid at the *sn*-1 position of glycerol backbone of phosphatidylglycerol in spinach cotyledons. Data are expressed as means ± SE of 5 analysis (6, 9, 24, 48 and 96h of illuminated plants).

temperature on such molecular species were investigated. As shown in Fig. 3 and Fig. 4, of all the molecular species, only the level of molecular species which contain 18:3 and 16:0 at the

sn-1 position of glycerol backbone affected greatly by growth temperature. The others were barely changed. In the cotyledons of both plants, increasing growth temperatures induced a decrease in the 18:3 containing molecular species and a concomitant increase in the molecular species containing 16:0 at the *sn*-1 position of glycerol backbone. These results clearly showed that both spinach and squash are able to adjust their PG molecular species composition to adapt the changes of growth temperature. Glycerol-3-phosphate acyltransferase in chloroplasts catalyzes the transfer of the acyl group of acyl-ACP to the *sn*-1 position of glycerol-3-phasphate and this enzyme plays a critical role in the regulation of the level of the molecular species containing 18 and 16-carbon fatty acids at the *sn*-1 position of glycerol backbone (Gombos and Murata, 1998). Our results showed that the temperature alter mainly the level of the molecular species containing 18:3 and 16:0 at the *sn*-1 position of glycerol backbone, indicating that the temperature can affect the selectivity of glycerol-3-phosphate acyltransferase for 18 or 16 carbon fatty acids as a substrate. Moreover, lowing temperature induced an increase in the level of molecular species containing 18:3 at the *sn*-1 position of glycerol backbone, but the level of molecular species containing 18:1 and 18:2 hardly changed, suggesting that plants desaturase activities also affected by changing temperatures.

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