Phospholipid and fatty acid turnover in winter rape plants subjected to cold treatment

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Introduction

Changes in the total amount of phospholipids as well in their composition are repeatedly reported in plants subjected to cold. In the winter rape plants, the increased unsaturation of all phospholipid fractions as well as changes in mutual proportions of specific phospholipids were observed as a result of plant exposure to low (5°C) temperature (Smolenska and Kuiper 1977). These observations indicate that either phospholipid biosynthesis or its catabolism are affected by cold. The present work was performed in order to elucidate that problem.

Material and Methods

Winter rape plants were grown in the Hoagland nutrient solution under controlled light and temperature conditions. After 3 weeks of growth at 25°C (day) and 20°C (night) the plants were treated with $1^{-14}C$ sodium acetate solution supplied to each fourth leaf blade or to the root systems. After 6 h treatment, the lipid precursor was washed out from the treated tissues and half of the plants was transferred to cold (5°C) chamber, whereas the other half continued their growth under unchanged temperature conditions. Leaf or root samples were subjected to lipid analysis immediately after isotope treatment of after 2, 4, 8, and 16 days of plant growth under differentiated temperature conditions. Extraction and separation of phospholipids was performed as described earlier (Smoleńska and Kuiper 1977) by a thin layer chromatography on Silica Gel G. Saponification of lipids and methylation of liberated fatty acids with BCl_3 in methanol were done according to Kuiper (1970). The methyl esters of fatty acids were analyzed by TLC. The gels from the areas containing phospholipids or fatty acid methyl esters were scrapped into vials containing 0.5% 2,5-diphenyloxazole and 0,03% 1,4-bis-2(4-methyl-5-phenyl-oxazole) in toluene. The radioactivity of the spots was measured by a liquid scintillation counter. Each experiment was run in triplicates. Standard deviations of the means were calculated and shown in the figures as vertical bars.

Abbreviations: PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PI, phosphatidyl inositol; PX, unidentified phospholipid; PL, phospholipids. Fatty acids are denoted by two numbers, representing the number of carbon atoms in the hydrocarbon chain and the number of double bonds, respectively.

Results and Discussion

It was found that the radioactivity of total lipids as well as phospholipids in the leaves and in the roots decreased in the course of plant growth, independently of the temperature conditions (Fig. 1 and 2). Cold treatment delayed the radioactivity disappearance, especially in the second half of the experiment. On the other hand, a small stimulation of the labelled precursor incorporation into phospholipids was observed in the leaves after 2 days of low temperature treatments (Fig. 2). It coincided with the labelling pattern of PC (Fig. 3), the effect being more consistent in the roots. However, the labelling pattern of PE + PI fraction in the leaves was quite different: there was a marked stimulation of radioactivity incorporation to that PL fraction, observed already after 2 and 4 days of cold treatment (Fig. 3). Since cold ($>0^{o}C$) changes neither the total amount of phospholipids in leaves (Smoleńska and Kuiper 1977) nor the level of their radioactivity (Fig. 2) in comparison to the control, it might be assumed that PE + PI fraction is synthesized on the cost of other phospholipids. Along with the increased radioactivity of PE + PI there was a decrease of a label level in PG + PX fraction. Therefore, one can suppose that in the winter rape plants subjected to cold the fatty acid transfer from PG to PE occurred. Resynthesis of PE from PG was previously proposed from the studies on the frost-induced modifications of phospholipids in the cold-adapted winter rape leaves (Sikorska and Kacperska-Palacz 1980). The studies of Pohl (1973) gave an evidence that in Euglena gracilis the light induced the transfer of fatty acids from PE and PC to PG. The reverse effect may be thus postulated for winter rape leaves subjected to cold. It may be of great importance for the membrane recovery in the frost-thawed tissues (Sikorska and Kacperska 1982).

The data presented in Fig. 4 show general patterns of the radioavtivity changes in fatty acids liberated from lipids. The radioactivity of linolenic acid (18 : 3) was the highest in comparison to the other fatty acids and it increased during the first few days of growth, without regard to the kind of a tissue and the temperature conditions. The radioactivity of other fatty acids decreased in the course of the experiment, the decrease of oleic acid (18 : 1) radioactivity being the most pronounced after 2 or 4 days of the experiment (in the leaves and in the roots, respectively). The observed changes in the labelling patterns of fatty acids are in accordance with the time sequence in biosynthesis of unsaturated fatty acids (Wharfe and Harwood 1978).

The most pronounced effect of cold on fatty acid biosynthesis and turnover was observed for linolenic acid. In the leaves, the low temperature treatment lowered the incorporation of the precursor to the acid and slowed down the radioactivity disappearance (Fig. 4). In the roots the incorporation rate was enhanced by cold during the first four days of the treatment and then its decrease was slown down by low temperature. The effects of cold on the other fatty acid metabolism consisted in slowing down the turnover rate of linolic acid (18 : 2) and saturated fatty acids (18 : 0 and 16 : 0) in the leaves whereas in the roots mainly a catabolism of linolic acid was inhibited. The data indicate that a higher unsaturation degree of the lipids in the cold-treated rape plants may result from two different reactions: a stimulation of desaturase activity in the roots and an inhibition of linolenic acid catabolism both in the roots and leaves.

Conclusions

The performed experiments indicate that the cold-induced modifications of phospholipid content in winter rape plants are due to the inhibition of their turnover rate rather than to the cold-promoted biosynthesis. The conclusion is a full aggreement with our previous studies on ^{32}P incorporation to winter rape lipids (Sikorska and Kacperska 1980). However, in the leaves the low temperature affects differentially the pathways of specific phospholipid synthesis (in the total PL pool), PE being preferentially formed on the cost of PG. This may be an important factor for frost hardening mechanisms operating in winter rape plants.

Acknowledgement

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Figures: see the next pages

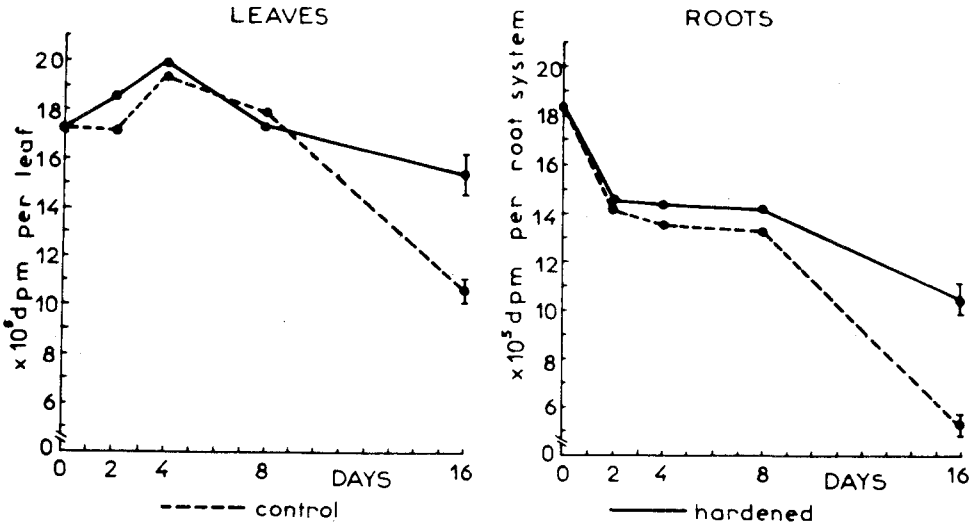

Fig. 1 Changes in total lipid radioactivity during 16 day growth of plants at 25°/20°C (control) or at 5°C (hardened), after 6 h pulse with 1-^{14}C sodium acetate.

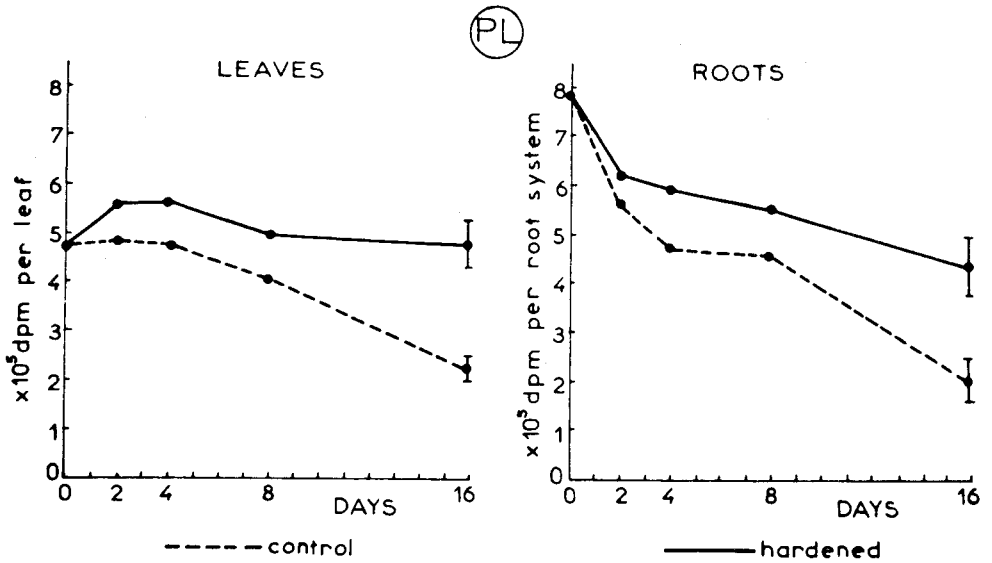

Fig. 2 Changes in total phospholipid radioactivity during 16 day growth of plants at 25°/20°C (control) or at 5°C (hardened), after 6 h pulse with 1-^{14}C sodium acetate.

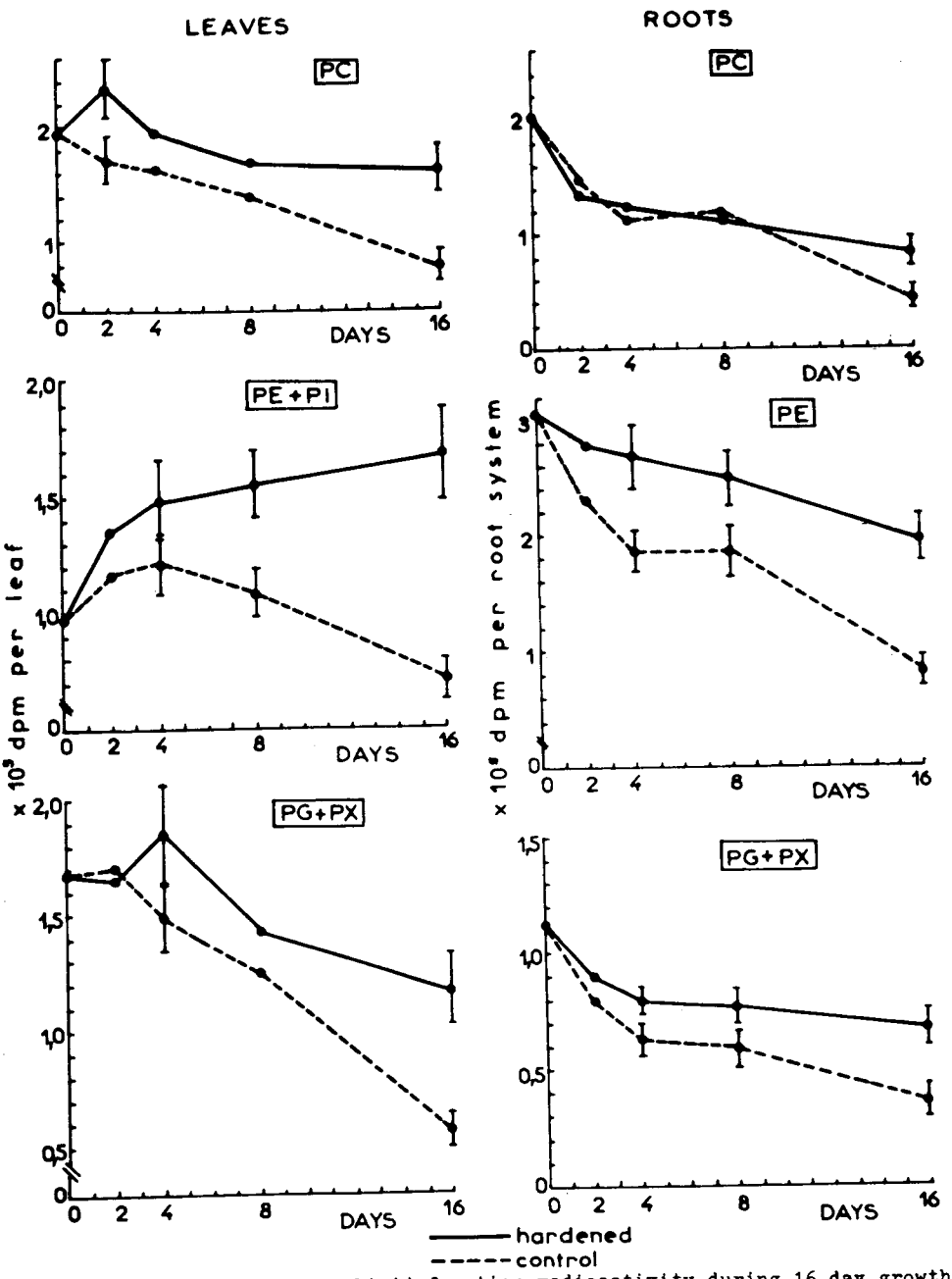

Fig. 3 Changes in phospholipid fraction radioactivity during 16 day growth of plants at 25°/20°C (control) or at 5°C (hardened), after 6 h pulse with 1-^{14}C sodium acetate.

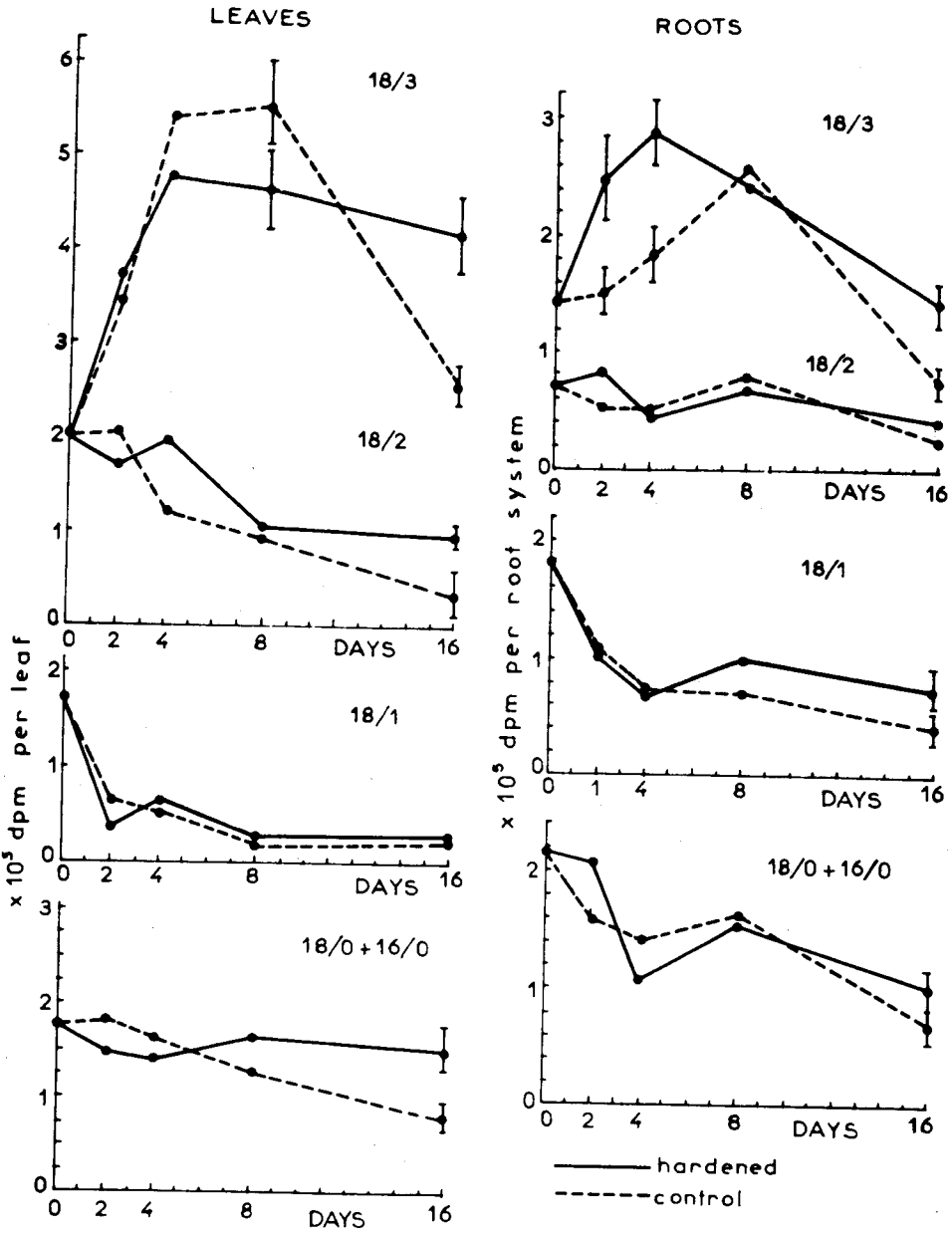

Fig. 4 Changes in fatty acid radioactivity during 16 day growth of plants at 20°/20°C ('control' or at 5°C ('hardened'), after 6 h pulse with 1-^{14}C sodium acetate.