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Electric and structural studies of hormone interaction with chloroplast envelope membranes isolated from vegetative and generative rape

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Summary

The electric and structural properties of envelope membranes of chloroplasts obtained from vegetative and generative plants of rape and the effect of hormone (IAA, GA_3 and zearalenone) treatment were determined by zeta potential and small-angle X-ray scattering (SAXS) methods. Chloroplasts were isolated from leaves cut off from the vegetative (before cooling) and generative apical parts of plants. The lipid composition of chloroplast envelope membranes were analyzed by chromatographic techniques. Envelopes from generative plants contained higher levels of digalactosyldiacylglycerol (DGDG) and smaller amounts of phospholipids (PLs) in comparison to those obtained from vegetative ones. Moreover, envelopes of generative plants were characterized by higher fractions of unsaturated fatty acids.

The zeta potential changes caused by hormone treatment were higher for chloroplasts isolated from vegetative plants in comparison to chloroplasts isolated from generative ones. An especially strong effect was observed for chloroplasts treated with IAA.

The thickness of bilayers of untreated chloroplasts from vegetative plants were larger by 0.4 nm when comparing to the thickness of layers obtained from generative ones. The effect of hormones (GA_3 and zearalenone) was detected only for vegetative chloroplasts.

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Both applied methods indicated differences in the properties of untreated and hormone-treated chloroplasts obtained from vegetative and generative plants.

Introduction

The transformation of plants from vegetative to generative stage during development is associated with several physiological events. The light and temperature requirements necessary for the flowering induction of winter plants indicate that chloroplasts and their membranes can play an essential role in generative development (Possingham, 1980; Miyamura et al., 1987; Masle, 2000; Samala et al., 1998).

Chloroplast membranes can be divided into two functional parts: envelope and thylakoid membranes. The first are formed of two achlorophyllous membranes, which separate the cytosol from the plastid compartments. The thylakoid membranes contain chlorophyll and take a key role in photosynthesis. Chloroplast envelopes and thylakoids are composed of proteins and lipids with galactolipids: monogalactosyldiacylglycerol (MGDG) and digalactosyl-diacylglycerol (DGDG) (Moreau et al., 1998) as the dominant components. Therefore, the composition of chloroplast membranes is different from the composition of other membranes containing phospholipids (PLs) as a major lipid fraction. Both MGDG and DGDG are uncharged. In a different way from DGDG and from the majority of membrane lipids which form bilayers (Simidjiev et al., 1998; Lee, 2000) MGDG build-up non-bilayer structures. A negative charge of chloroplast membranes is related to the presence of sulfo-quinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG). In addition, zwitterionic phosphatidyl choline (PC) is located in the envelope membranes (Moreau et al., 1998).

The unusual chemical composition of chloroplast lipids is responsible for their unusual packing and the formation of specific domains which can be important for interaction between lipids and membrane proteins (Zaitsev et al., 1999) as well as with other molecules adsorbed from the water phase (Shao et al., 1999; Tazi et al., 1999). It has been suggested that cell surface galactolipids serve as recognition sites for counterpart lectins and cell adhesion receptors (Hakomort and Igarashi, 1995). High levels of polyunsaturated galactolipids in chloroplast membranes have been correlated with the maintenance of membrane fluidity and with the prevention of chilling injuries (Welburn, 1997; Mannock and McElhaney, 2004). Thus, modifications of the chloroplast membrane composition related to the maintenance of optimal membrane functions seem to be important also during the generative development of winter plants (which needs periodic chilling for flowering induction).

The reorganization of membrane lipids occurring during generative development can affect the formation of specific domains important to hormone interactions. Hormones, especially GA_3 and IAA, play a significant role during generative development. Endogenous GA_3 is essential in the photoperiodic control of grass flowering (Junttila et al., 1997). Moreover, transport of IAA from apex to roots is necessary for bud formation (Lomax et al., 1995). As it was presented in the literature (Kulaeva et al., 2000; Hole and Dodge, 1972; Misra and Biswal, 1980; Chaudhry and Hussain, 2001), hormones participate in the control of chlorophyll synthesis and its degradation during chloroplast aging (in vivo and in vitro).

The aim of the paper was to determine the electric and structural properties of chloroplast envelope membranes isolated from vegetative and generative winter rape and their changes caused by interaction with hormones of anionic type: GA_3, IAA and non-ionic zearalenone (Meng et al., 1996). The studies are concentrated on the properties of envelope membranes of intact chloroplasts. Chloroplasts were isolated from vegetative and generative winter rape leaves which have a different morphological shape in these developmental stages. Comparison of electric and structural changes of chloroplasts obtained from generative and vegetative plants seems to be important in explaining the role of these organelles in the mechanism of flowering induction.

Material and methods

Plant material

Seedlings of *Brassica napus* L. var. *oleifera* cv. Górczański (winter) and cv. Mlochowski (spring) were grown for 80 days in a controlled environment under a 16-h photoperiod (irradiance of $250\,\mu mol\,m^{-2}\,s^{-1}$) and at day/night temperatures of 20/17 °C. Then, the plants were cooled to 5/2 °C