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Abstract

Drought tolerance in a number of crop species has been correlated with changes in and composition of cell membrane lipids. Since membrane stability is a function of the physical properties of the lipid constituents, recent studies have focused on lipid changes in response to natural and artifically-induced water stress. Such studies have clearly shown that lipid adjustment and composition differs in drought-resistant and -tolerant species.

A difficulty in working with higher plant systems is that vascular plants make structural and functional level adjustments which aid in drought resistance and which affect the process of adjustment at the cellular level. For this reason, in vitro cell culture techniques have many advantages in studying stress responses. Thus, the objectives of this research were to determine the effects of polyethylene glycol (PEG) -induced osmotic stress on the lipid and sterol composition of osmotic tolerant and susceptible species of unicellular algae belonging to the genus Chlorella.

Keywords

drought tolerence, cell membrane lipids, polyethylene glycol

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Comments

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GROWTH AND LIPID CONTENT OF OSMO-TOLERANT AND SENSITIVE ALGAE

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ABSTRACT

Drought tolerance in a number of crop species has been correlated with changes in and composition of cell membrane lipids. Since membrane stability is a function of the physical properties of the lipid constituents, recent studies have focused on lipid changes in response to natural and artifically-induced water stress. Such studies have clearly shown that lipid adjustment and composition differs in drought-resistant and -tolerant species.

A difficulty in working with higher plant systems is that vascular plants make structural and functional level adjustments which aid in drought resistance and which affect the process of adjustment at the cellular level. For this reason, in vitro cell culture techniques have many advantages in studying stress responses. Thus, the objectives of this research were to determine the effects of polyethylene glycol (PEG) -induced osmotic stress on the lipid and sterol composition of osmotic tolerant and susceptible species of unicellular algae belonging to the genus Chlorella.

Chlorella vulgaris Beyerinck (Pratt strain) and Chlorella pyrenoidosa Chick were grown in Knop's medium adjusted osmotically with PEG 4000 (MW 3350) to water potentials of -0.1 (control), -0.5, -1.0, -1.5, and -2.0 MPa. Cultures were grown at 27 C under continuous light (photon flux density, 25 umol $m^{-2}s^{-2}$). Growth was determined by optical density measurements at 678 nm. Lipids were extracted using chloroform:isopropanol (1:1 v/v). The extract was evaporated to dryness, dissolved in $CHCl_3$:MeOH (2:1 v/v) and washed with 0.88% KCl. The bottom $CHCl_3$ layer was recovered as the purified lipid. Major lipid classes were separated using thin layer chromatography (Silica gel 60 G, n-hexane:diethylether:acetic acid, 85:15:1 v/v/v). Lipids were visualized using 0.2% 2,7-dichlorofluorescein in 95 % ethanol and viewed under long wave UV light. Phospholipids were separated using high performance thin layer chromatography (Silica gel 60, acetone:benzene:water, 91:30:8, v/v) and visualized by charring after spraying with 10% $CuSO_4$ in 8% H_3PO_4. Sterols and fatty acids were quantified and identified by gas liquid chromatography as the trimethylsilyl ether and methyl ester derivatives, respectively. Glass columns (2m x 2mm i.d.) packed with 3% SE-30 on 80/100 Gas Chrom Q and 10% DEGS on 80/100 Chromasorb WAW were used to separate sterols and fatty acids, respectively. Identification of unknowns was based on comparisons with Rf or retention time values of known standards.

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The relative growth rate of osmotically stressed C. pyrenoidosa was higher than C. vulgaris regardless of degree of stress. C. pyrenoidosa had considerably higher concentrations of free sterol (FS) but lower concentrations of steryl ester (SE) than C. vulgaris. C. vulgaris exhibited a vastly different sterol composition (8 sterols) than C. pyrenoidosa (3 sterols). In both species FS declined while SE increased with declining water potentials. At -2.0 MPa FS decreased 4.2 fold while SE increased 1.6 fold in C. pyrenoidosa, while in C. vulgaris FS declined 9.2 fold and SE increased 3.5 fold at the same water potential. Polar lipid (PL), triglycerides (TG) and free fatty acids (FFA) declined with decreasing water potentials in C. pyrenoidosa. The same was true for C. vulgaris with the exception that TG increased at -2.0 MPa. C. vulgaris exhibited lower total concentrations of TG (except -2.0 MPa) and FFA and higher concentrations of PL than C. pyrenoidosa at all water potentials.

Phospholipids (phosphatidic acid, PA; phosphatidylcholine, PC; phosphatidylinositol + phosphatidylethanolamine, PI + PE; phosphatidylglycerol, PG; and digalactasyldiglyceride, DGDG) declined in both algal species as water potential decreased. However, C. vulgaris had higher total concentrations of PC, PG, and DGDG than did C. pyrenoidosa. Conversely, C. pyrenoidosa generally had higher concentrations of PA and PI + PE.

Qualitatively, the fatty acid composition of the PL and TG fractions of both algae species were similar. However, they differed in relative abundance. C. pyrenoidosa had higher concentrations of 18:1 in both the PL and TG fractions while C. vulgaris had higher concentrations of 18:2. C. vulgaris exhibited higher concentrations of 16:0 and 18:0 in the TG fraction compared to C. pyrenoidosa. Differences in water potential had little effect on the individual fatty acid components of the PL fraction of either species. However, in the TG fraction of C. vulgaris the saturated fatty acids declined and the unsaturated fatty acids increased as water potential declined. This was not as evident in C. pyrenoidosa.

The current study demonstrated that C. vulgaris possessed higher percentages and a greater diversity of sterols than C, pyrenoidosa. However, the latter produced higher concentrations of FS. In addition, as the organisms were exposed to decreasing osmotic potential the majority of the FS of C. vulgaris appears to be converted to SE which cannot be incorporated into cell membranes. Differences in the sterol composition of the two algae species suggests that a critical level of FS or a specific sterol structural requirement may be important in maintaining stable functional membranes in cells under osmotic stress. Decreasing FS/PL ratios (implies less densely packed membrane bilayers and hence greater permeability) have been correlated with plant resistance to environmental stress. Since the amount of FS in C. vulgaris was considerably lower than in C. pyrenoidosa and declined more drastically as the osmotic potential decreased it is possible that the maintenance of a high molar ratio of FS to PL in C. pyrenoidosa is important in controlling membrane function as osmotic stress increased.