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High Temperature Tolerance in Relation to Changes in Lipids in Mutant Wheat

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Abstract

Radiation-induced high temperature-tolerant spring wheat mutant WH 147M and its mother cultivar WH 147 (sensitive) were grown in phytotrons at day/night temperature of 37°C/18°C (stress) and 20°C/ 12°C (normal) for 12 h each and fluorescent light (5.34 J/cm2/h) for 12 h per day. Grains of WH 147M had A considerably higher content of phospholipids than WH 147. In leaf samples of both the genotypes polar lipids constituted 80 to 90 per cent of the total lipids. But galacto-, phospho- and neutral lipid content was higher in leaf samples of heat-stressed plants of both genotypes than the normal ones. Similarly, increase of monogalactosyl diglycerides (MGDG) predominated over that of digalactosyl diglycerides (UGDG) under normal temperature. The mutant showed a higher content of galatolipid-bound linolenic acid and especially phospholipid-bound trans-Δ-3-hexfldecenoic acid than the heat sensitive WH 147. Following heat stress, increase in these parameters was less pronounced in the mutant than in WH 147. These observations indicate the role of membrane lipids in adaptation to heat stress.

1 Introduction

High temperature stress, an important yield--limiting factor in wheat, is known to affect several membrane-associated processes, like photosynthesis, respiration, protein synthesis etc. (KAUR et a1. 1988, SHANAHAN, 1990). Lipids, being important constituents of biomembranes (lipoprotein complexes), are responsible for maintaining the membrane integrity. Changes in structure and composition of lipids in response to heat shock have been observed in wheat (YANG et al. 1984). Whether these changes are consequences of or causes of adaptation to heat stress is still uncertain. In this context, comparative analysis of mutants differing mainly in thermotolerance would elucidate the role of lipid metabolism in adaptation to heat stress (KUNST et al. 1989). The present study was, therefore, planned to lead to further understanding of high temperature stress-induced changes in various lipid components and lipid bound fatty acids in a spring wheat mutant and its mother variety.

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2 Materials and Methods

Gamma ray induced, high temperature-tolerant spring wheat mutant WH 147M selfed up to M8 generation, (BEHL et al, 1986) and its mother cultivar WH 147 (sensitive) were grown in pots filled with sterilised sand and organic manure. Soon after seeding, 20 pots of each genotype containing four plants each were placed in phytotrons maintaining 37°C/18°C (stress) and 20°C/ 12°C (normal) day/night temperature for 12 h each and with fluorescent light (5.34 J cm^2/h) for 12 h per day.

Lipids were extracted in chloroform-methanol mixture (2:1 v/v) according to BLIGH and DYER (1959) using grain and leaf samples (from six weeks old plants). Before extraction, leaf samples were frozen in liquid nitrogen and ground in a mortar. Lipids were extracted at the chloroform phase. After separating the proteins, the evaporated lipids were again put into solution and used for thin layer chromatography according to POHL et al (1970). The individual bands were extracted for quantitative determination of lipid components, such as acyl esters, phosphate, galactose, glycerol and free and bound fatty acids as described by HEISE and JACOBI (1973).

3 Results and Discussion

Storage lipids: Analysis of grain samples for important storage lipid components (Table 1) revealed that the mutant contained considerably higher contents of phospholipids than WH 147. However, the relative amount of fatty acids in various lipid fractions did not differ significantly in both the genotypes (data not given).

Leaf lipids: Lipid components extracted from leaf samples (Table 2) revealed a slightly higher quality of total lipid bound fatty acids in the mutant than in WH 147 under normal temperature. Following high temperature stress, both the genotypes showed an increase in total lipid bound fatty acids - relatively more pronounced in WH 147. Although galactolipids were comparable in both the genotypes under the same conditions, WH 147M showed a lower increase in these lipids than WH 147 under heat stress. Moreover, the ratio of monogalactosyl diglycerides (MGDG) to digalactosyl diglycerides (DGDG) increased from 1.3 under normal conditions to 1.7 under stress temperature. The fatty acid composition of individual lipid components revealed that trans-Δ - 3- hexadecenoic acid (trans-Δ - C16:1) in phospholipids increased in both the genotypes, four fold in WH 147 and two fold in WH 147M following heat stress. Similarly the heat stress resulted in the production of increased quantities of linolenic acid (C18:3) in galacto- and phospholipids in both the genotypes, which was lower in the mutant variety.

Changes in proportion of membrane components of cells induced by high temperature stress are expected to be reflected in qualitative and quantitative changes in membrane associated dynamic lipid classes (KUIPER 1984). Although composition of storage lipids is not necessarily related to that of membrane lipids, nevertheless, their chemical and physical nature may influence lipid metabolism at different temperatures. Thus, differences in phospholipid content in grains of the mutant and cultivar WH 147 prompted our interest to know about changes in leaf membrane lipids under normal and high temperatures. Several workers observed a correlation between thermal stability and changes in membrane lipid composition, particularly a decreased level of fatty acid desaturation (RAISON et al. 1982, LYNCH and THOMPSON 1984, KUNST et al. 1989). Such lipid changes influence membrane lipid fluidity vis-à-vis spatial relationships and interactions between lipid-enzyme complexes (BARBER et al, 1984).

In the present study the major and consistently quantitative changes under high temperature stress were an enhanced MGDG/DGDG-ratio and an increased production of trans-Δ 3-C16:1 residues of phospholipids and of linolenie acid (C18:3) in galaeto and phospho lipids of both the genotypes. Although both the genotypes reacted to changes in temperature, the mutant exhibited such changes to a considerably lower degree. Changes in MGDG/ MGDG-ratio may be either due to increased synthesis of MGDG as compared to DGDG, or to a different hydrolysis by galactolipase of both galactolipids (WINTERMANS et al. 1987). The high desaturation degree of chloroplast lipids found in a wide variety of species suggests its important role in maintaining chloroplast structure and function (YANG et al. 1984, BROWSE et al. 1989). Moreover, An increase in trans-Δ 3-C16:1 is related to membrane appression (KHAN et al. 1979, KUNST et al. 1989). Changes in its content alter the ratio of appressed to exposed membranes in chloroplasts. It is possible that altered lipid composition and synthesis of specific membrane proteins confer increased thermal stability upon chloroplast membranes of the mutant (HUNER et al. 1989). Synthesis of heat shock proteins (BEHL et al, 1991) and high rates of photosynthesis (KAUR et al. 1988) in WH 147M under high temperature stress further lend support to this view. It is, therefore, suggested that simultaneous measurement of heat stress mediated changes in lipids, proteins and ultrastructure and functions of chloroplast membranes would be useful in elucidating cause and effect relationship determining heat tolerance.

4 Zusammenfassung

Toleranz gegen hohe Temperaturen und veränderte Lipide in einer Weizenmutate

Eine durch Strahlung induzierte Mutante mit Toleranz gegen hohe Temperatur von Sommerweizen WH 147M und die Ausgangssorte WH 147 (hitzeempfindlich) wuchsen im Phytotron bei einer Tag/Nacht-Temperatur von 37/18 ° C (Stress) und 20/12 ° C (normal zu je 12 h unter Leuchtstofflampen (5,34 J/cm^2 / h) mit täglich 12 h Licht. Die Körner von WH 147 M wiesen beträchtlich mehr Phospholopide auf als WH 147. In den Blättern beider Genotypen stellten polare Lipide 80...90 % der Gesamtlipide. Jedoch waren die Gehalte an Galakto-, Phospho- und Neutrallipiden in den Blättern der hitzegestreßten Pflanzen beider Genotypen erhöht gegenüber normal. Die Zunahme der Monogalactosyl-Diglyceride (DGDG) übertraf die der Digalactosyl-Diglyceride (DGDG) bei normaler Temperatur. Gegenüber der hitzeempfindlichen Sorte WH 147 hatte die Mutante einen höheren Gehalt an galactolipidgebundener Linoleasäure. Die

Zunahmen dieser Verbindungen verliefen nach dem Hitzestress bei der Mutante langsamer als in WH 147. Diese Beobachtungen belegen die Funktion der Membranlipide in der Anpassung an Hitzestress.

Tolerancia contra temperaturas altas y lipidos cambiados en una mutacion de trigo.

Resumen

Una mutadon de trigo de verano WH 147 M con toierancia contra temperaturas altas, inducida por rayos, y ia variedad madre WH 147 (sensible al calor) crecieron en ei fltotrono bajo temperaturas variadas entre dia y noche 37 /18 C (stress) y20/1 2 C (norma) y bajo lamparas fluorescentes (5,34 JIcm Ih), dando una duracion del dia de luz igual a 12 horas. Los granos de WH 147 M contenian notablemente mas fosfo-lipidos que los de WH 147. En las hojas de ambos genotipos los lipidos polares constituieron 80.. .90 % de los lipidos totales. No obstante, ei contenido de galacto- y fos-fo-lipidos y de los lipidos neutrales en las hojas de plantas affectadas por ei calor en ambos genotipos era elevado en comparicion con ei de plantas no affectadas. Bajo temperaturas normales ei aumento de los monogalactosii-digliceridos (DGOG) supero ei mismo de los digalactosildigliceridos (DGDG). En comparicion con la variedad WH 147, succeptible al calor, ei mutante contenia mas acido linolea ligado a galactolipidos. Despues del effecto de calor ei contenido de dichas sustancias aumento mas lentamente en ei mutante que en la variedad WH 147. Esas observaciones comprueban la funcion de los lipidos en la membrana para la adaptacion al effecto de calor.

Tolérance aux fortes températures et transformations des lipides d'une variété mutante de blé.

Résumé

Une variété mutante de blé de printemps WH147M, induite par radiation, tolérante aux fortes températures, et la variété initiale WH147 (sensible à la chaleur) poussaient sous des températures de 37°C le jour et 18°C la nuit (condition de stress) et 20°C / 12°C (condition normale) chacune sous des lampes fluorescentes (5,34 J/cm2/h), pendant 12 heures d'éclairage. Les grains du blé WH147M renfermaient considérablement plus de Phospholipides que ceux de la variété non traitée. Les lipides polaires extraits des feuilles des 2 génotypes représentaient 80 à 90% de la quantité totale des lipides. Cependant, la quantité de lipides galactosiques, phosphoriques et neutres extraite des feuilles des plantes des 2 génotypes soumis au stress de la chaleur était supérieure à la normale. L'augmentation du taux de MGDG, sous température normale, était supérieure à celle du DGDG. La quantité de lipides galactosiques associés à l'acide linolique de la variété mutante comparée à la variété WH147 (stress) est plus élevée.On observe chez la variété mutante que l'augmentation de ces composés se manifeste plus lentement que chez la variété WH147. Ces observations justifient alors la fonction d'adaptation des lipides membraneux au stress à la chaleur.

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