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Phosphatidylcholine Synthesis as Affected by Heat Stress Applied to the Cotton Seedling Roots

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Abstract: The experiment was carried out to study the effect of temperature on (Me-^{14}C) choline uptake in cotton roots and its incorporation into phosphatidylcholine. The results suggest that (Me-^{14}C) uptake and incorporation increased with increased germination time. Temperature above 35°C had significant effect on (Me-^{14}C) choline uptake and incorporation. Thus, as the temperature increased above 35°C, the rate of synthesis of phosphatidylcholine decreased. At 50°C, however, more than 80% of the phosphatidylcholine synthesis was inhibited.

Key words: Cotton, synthesis, phosphatidylcholine, heat stress

Introduction

Cotton is one of the most important crops grown commercially in about 60 developing countries between 47°N and 35°S (Beilorai *et al.*, 1983), such as Pakistan, India, China, Egypt, Mexico and Peru etc. According to the United Nations Food and Agriculture Organization's statistics, the world's planted area in cotton is about 33x10^6 ha, which is about 23% of the worldwide cultivated arable land (FAO, 1978). The summer growing season starts from the month of April and ends in September. In summer the temperature often exceeds 45°C and the diurnal variations may be as much as 20°C.

Cotton is a warm-season crop growing in subtropical and tropical countries and requiring a temperature range of 20-30°C from germination to harvest (Abdel Magid and Osman, 1977). Sethar (1993) reported that changes in temperature above 35°C can affect the germination and seedling growth. Raison *et al.* (1980) reported that membranes are primarily involved in injury due to high temperature. Quinn (1988) reported that changes in growth temperature are associated with changes in membrane lipids. It is well established fact that the physical phase of lipids mainly phospholipids play an important role in the structure and functions of membranes.

Phosphatidylcholine is the most abundant phospholipid in nature (Ansel and Spanner, 1982). This phospholipid is thought to be present in all the membranes and comprises more than 45% of the total phospholipid fraction of non-photosynthetic membranes of plants, including cotton (Rakhimov *et al.*, 1986), wheat (Vakharia, 1986) and runner beans (Kates, 1960). It has also been shown that (Me-^{14}C) choline is incorporated exclusively into phosphatidylcholine (Mirbahar, 1981). It was for this reason that the uptake of (Me-^{14}C) choline into cotton roots and its incorporation into phosphatidylcholine was measured to study the effect of temperature on the synthesis of phosphatidylcholine.

Materials and Methods

The experiment was carried out at School of Biological Sciences, University of Wales, U.K. in 1994. Fifty cotton seeds were delinted and sown in rolled filter paper as method adopted by Sheikh *et al.* (1996). After 48 hour germination periods, the seeds were harvested and 25 longest and healthy seedlings (av. root length 51mm) were taken and their roots were detached from the seeds using a scalpel. For the measurement of (Me-^{14}C) choline uptake by cotton roots and its incorporation into phosphatidylcholine, the roots were placed in 100 cm^3 of 50 mM tris-maleate buffer, pH 7.2 containing 50 μg mc^{-3} chloramphenicol and 1 μCi ((Me-^{14}C) choline (specific activity 55 mCi mmo1^{-1}). The tissues were incubated for 30 and 60 minutes in a reciprocating water bath operating at 175 oscillations per minute. After incubation, the roots were washed three times with distilled water and then incubated for 5 minutes in 100 cm^3, 100mM choline chloride to remove radioactive material from the free spaces of the cell walls. Their lipids were then extracted by hot, water- saturated butanol by the method of Sheikh *et al.* (1996). The crude lipid extract was purified by chromatography on columns of Sephadex G-25.

Uptake of radioactivity into the tissue was based on the radioactivity in an aliquot taken from the crude lipid extract. Incorporation of radioactivity into phosphatidylcholine was determined as the radioactivity contained in the purified total lipids from Sephadex G-25 chromatography.

For this determination, the lipid samples were transferred to scintillation vials, dried under nitrogen and dissolved in 5 cm^3 of scintillation fluid (6 cm^3 plastic vials inserted in 22 cm ^{3} glass scintillation vials were used). The scintillation liquid used was Cocktail. T. Radioactivity was counted in a Beckman spectrometer, Raw counting data were converted from counts per minute (cpm) to disintegration per minute (dpm) by the spectrometer using the Crompten end effect (Mirbahar, 1981). All the data were analyzed statistically using Systat software program.

Results and Discussion

The results in Fig. 1A reveal that the uptake and incorporation of (Me-^{14}C) at 25°C control increased linearly with increasing incubation time. Increasing the incubation temperature increased the uptake rate but the relationship between uptake and temperature was rather random.

In contrast to the picture obtained for uptake, incorporation of (Me-^{14}C) choline into phosphatidylcholine was strongly affected by temperature (Fig. 1B). The roots incubated at 30 and 35°C were not significantly affected ($P>0.07$) as compared with (25°C) control. The incorporation was significantly decreased at 40°C, however, ($P<0.06$) above 40°C, the effect became very large ($P<0.001$). At 50°C, incorporation was reduced by 88% at 60 minute incubation period. This suggests that as temperature increased above 30°C, the incorporation was decreased progressively.

In order to get more accurate assessment of the effect of temperature on the incorporation alone, it was decided to calculate uptake/ incorporation ratios. These values are presented in Fig. 2. Thus, as the temperature increased above 30°C the incorporation/uptake ratio decreased progressively. The results suggest that temperature above 35°C had significant effect on (Me-^{14}C) choline uptake and incorporation. Thus, as the temperature increased above 35°C, the rate of synthesis of phosphatidylcholine decreased. At 50°C, however, more than 80% of the phosphatidylcholine synthesis was inhibited. The present effects of high temperature could be due to the direct effect of temperature on the biosynthetic enzyme systems involved. Membrane-

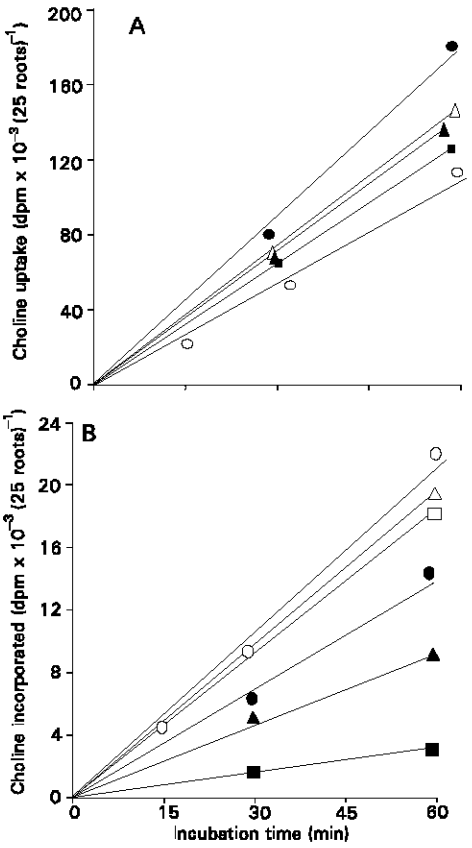

Fig. 1: Effect of heat-stress on uptake and incorporation of (Me-^{14}C) choline. A) Uptake (B) Incorporation. ○ 25°C, ▵ 30°C, □ 35°C, ● 40°C; ■ 50°C; ▲ 45°C;

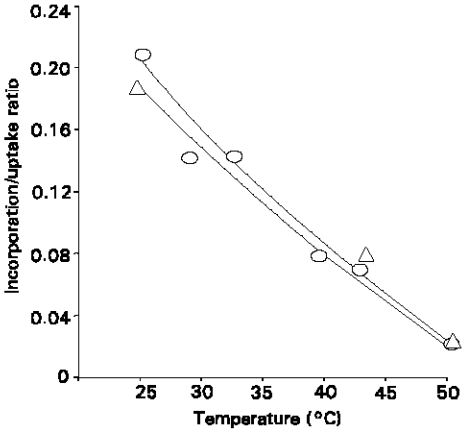

Fig. 2: Effect of heat-stress on (Me-^{14}C) choline incorporation/ uptake ratios. ▵ 30 min, ○ 60 min.

bound enzymes might be expected to be especially sensitive to temperature change due to their dependence on the correct degree of fluidity in the membrane. Lin *et al.* (1984) reported that high temperature reduces the rate of normal protein synthesis and induces the synthesis of heat-shock proteins. Key *et al.* (1981) found that soybean seedlings shifting from a normal growth temperature of 28°C to 40°C, similarly showed dramatic changes in protein synthesis including the synthesis of heat-shock proteins. There have been only a few studies of the effects of high temperature on lipids, and the literature available on the subject supporting our observations (Benzioni *et al.*, 1973). Thus, it has been reported that high temperature inhibits the synthesis of phosphatidylcholine. Benzioni and Itai (1973); Tischner *et al.*, (1978) and Sheikh *et al.* (1996) reported that in tobacco, *Synchronous chlorella,* and cotton roots, reduction in phospholipids biosynthesis occurred at high temperatures. Lin (1990) reported that exposure of *Phaseoulus vulgaris* seeds to 100% relative humidity at 45°C for 1-4 days reduced the total phospholipids due to the degradation of phosphatidylcholine.

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