GENETIC AND ENVIRONMENTAL FACTORS AFFECTING THE FATTY ACID COMPOSITION OF POLAR AND NON-POLAR LIPIDS OF COTTONSEED T.D. Borth, D.R. Krieg, and G. Jividen Texas Tech University and Cotton Incorporated Lubbock, TX and Raleigh, NC

Abstract

An experiment was set up to determine the genetic and environmental factors affecting the fatty acid composition of polar and non-polar lipids of cottonseed. Five varieties including 2 High Plains stripper types, 2 Delta picker types, and 1 Delta stripper type were chosen to evaluate the genetic variability. White flowers were tagged once a week over a four week blooming period. Developing bolls were collected every 10 days after tagging until the fruit were 50 days old. Differential extraction using hexane/ether and chloroform/methanol was used to separate the non-polar fraction from the polar fraction. The fatty acids from each fraction were methylated to form methyl esters which were analyzed by gas/liquid chromatography to define the fatty acid composition. Boll age (maturity) was the major factor affecting the fatty acid composition of non-polar and polar lipids. Week of bloom was also a significant factor. Fruit developing late in the bloom period are subject to cool temperatures in late September and October which affects their growth rate and chemical composition. Fatty acid composition of the lipid fractions differed among the varieties during the embryogenesis period (0-20 day) but not during the oil storage period (20-50 day).

Introduction

Cotton (*Gossypium hirsutum* L.) is native to the semi-arid tropics of Southern Mexico and Central America. During its evolution, it has rarely been exposed to temperatures below 20° C. The optimum temperature for seed germination is in excess of 30° C, and the absolute minimum temperature is around 12° C (Wanjura,1972).

The current hypothesis on cold tolerance of plants is based on the fatty acid composition of the lipid components. The fatty acid composition of cotton seed lipids causes phase transition around $15^{\circ}C(59^{\circ}F)$. When temperatures drop below this point during germination and seedling establishment, significant disruptions in lipid metabolism occur. The oil which is the primary source of carbon and energy for the emerging seedling tends to solidify making it unavailable for hydrolysis and subsequent metabolism. The phospholipid component of the membranes tend to crystallize causing disruption of the transport functions, loss of cellular integrity and loss of solutes. Depending upon the intensity and duration of the cool temperature exposure, the extent of damage may vary from simply extending the emergence time to loss of vigor and even seedling death.

The fatty acids in membrane lipids are of the same composition as those in storage oil. Therefore, the more unsaturated the fatty acid composition the lower the transition temperature which allows membranes to function properly and storage oil to be metabolized at cooler temperatures. Previous research in our program indicated that cold tolerance increased with degree of saturation of the fatty acids(Speed 1995). Increased concentration of saturated fatty acids (palmitic and stearic) is an indication of maturity in cotton seed. The more mature seed have a higher degree of saturated fatty acids (palmitic and stearic). Mature seed have a higher cold tolerance than immature seed. This result is contrary to the current working hypothesis that the more unsaturated fatty acids in the lipid fraction, the greater the cold tolerance, although it is logical that more mature seed would have a higher cold tolerance. To better understand these conflicting situations we needed to be know when and how polar and non-polar lipids were deposited in cottonseed and their fatty acid compositions.

Material and Methods

White flowers were tagged once a week over a four week blooming period. Developing fruit were collected at 10 day intervals for 50 days from each tagging. Five different varieties were chosen to compare genetic differences. Two Delta varieties (DPL 5409 and DPL 50), two Texas high plains varieties (HS 200 and HS 26), and one high plains adapted delta variety (DPL 2156). Seed were removed from the fruit, and the non-polar and polar fractions were separated by a differential extraction technique using hexane/ether and chloroform/methanol. The fatty acids were defined by analyzing the methyl esters using gas/liquid chromatography, and statistical differences were determined.

Results and Discussion

Boll age (Fig 1) was the main factor affecting the fatty acid composition of lipids in cottonseed. Seed development can be separated into two stages, embryogenesis and oil deposition(cotyledon development). Embryogenesis occurs within the first 20 days after pollination. Beginning about 20 DAP oil storage occurs in the cotyledons. Palmitic acid (C_{16}^{0}) initially comprised about 25-27% of the total fatty acid composition during embryogenesis and then declined due to dilution during cotyledon development. Oleic acid (C_{18}^{-1}) comprised 11-15% of the total fatty acids during embryogenesis. Linoleic acid (C_{18}^2) initially comprised only 15-18% of the total fatty acids, but was the primary fatty acid comprising over 50% of the total fatty acid composition of the oil when the seed matured. Linolenic acid (C_{18}^{3}) is a polyunsaturated fatty acid and comprised 40-45% of the total fatty acids during earlyoembryogenesis.

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All biochemistry books state that fatty acids are synthesized as a saturated molecule (high in energy), and then a desaturase enzyme oxidizes the C-C bonds (removing stored energy) in the fatty acid. Later a saturase enzyme has to come back and resaturate the molecule(Hitchcock 1971). It is somewhat difficult to understand why any plant would "waste" this energy. An explanation for the decline of linolenic acid is that it was saturated into C_{18}^2 and/or it was diluted during the second phase of seed development. Mature cottonseed contains approximately 22-25 % palmitic acid, 1-3% stearic acid (not shown), 17-18% oleic acid, 50-55% linoleic acid, and 0-<1% linolenic acid. (Bailey 1966).

Fatty acid composition of developing bolls was also affected by week of bloom. On the Texas High Plains, bolls developing in September and October are exposed to cooler night temperatures than those that developed in July and August. This temperature difference had the opposite effect on the unsaturated fatty acid $C_{18}^{\ 3}$ as it did on the saturated fatty acid C_{16}^{0} (Fig 2 and 3). C_{18}^{3} was higher in concentration while C_{16}^{0} was generally lower in concentration at later weeks of bloom. This response was mainly due to temperatures under which the bolls developed. As bolls developed from later blooms, they developed under cooler conditions. The cooler temperatures caused slower growth rates, and therefore gave us the observed results in concentrations of C_{18}^{-3} and C_{16}^{-0} . Again, this was due to slowed fatty acid saturation/desaturation and their respective deposition rates. The variability in week of bloom bar heights (Figs 2 and 3) of $C_{16}^{\ \ 0}$ and $C_{18}^{\ \ 3}$ indicates the interaction between boll age and week of bloom.

Varieties have significant differences in fatty acid composition and deposition during early embryogenesis (10-20days). After 20 days, when the seeds begin rapid deposition of storage oils there are some variety differences. But, by the time the seed matures, there are no significant differences in fatty acid composition among varieties (Fig 4).

Summary

Boll age (maturity) was the major factor affecting the fatty acid composition of polar and non-polar lipids. Immature seed were high in linolenic acid and palmitic acid. As the seed matured, linolenic acid was saturated which resulted in an increase in concentration of oleic acid and linoleic acid. Week of bloom had an effect on fatty acid desaturation/saturation and deposition rates due to temperatures under which development occurred. The bolls produced from later blooms developed under cooler conditions than those from the first week of bloom, so growth rates and again maturities were different. Varieties showed differences in fatty acid composition during early embryogenesis, but at maturity there were no significant variety differences.

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Figure 1. Boll age effects on total fatty acid composition.



Figure 2. Week of bloom effects on palmitic acid (C_{16}^{0}) .



Figure 3. Week of bloom effects on linolenic acid (C_{18}^{3}) .



Figure 4. Variety effects on palmitic acid (C_{16}^{0}) .