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Postprint: Variation Patterns of Membrane Lipids in Tobacco Seeds During Maturation

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Abstract

This study employed a lipidomics approach to systematically investigate the dynamic changes in membrane lipid content and composition during tobacco seed maturation. The results indicated that the content of plastidial membrane lipids, which constitute essential components of chloroplast and thylakoid membranes, and their proportional representation in total membrane lipids exhibited a continuous declining trend throughout the entire seed maturation process. Conversely, the content of extraplastidial membrane lipids, which are crucial components of the cell membrane, decreased significantly during the early maturation stage and remained essentially constant after 21 days post-pollination. The variation pattern of total membrane lipid content paralleled that of plastidial membrane lipids, but stabilized after 29 days post-pollination. Given that storage lipids continuously accumulate during seed maturation and share similar chemical structures with membrane lipids, the reduction in plastidial membrane lipid content may be attributed to the sustained demand for lipid accumulation in seeds and the diminishing requirement for chloroplasts and thylakoids during the maturation process. The relative stability of extraplastidial membrane lipid content after 21 days post-pollination likely reflects the fact that extraplastidial membrane lipids serve as the primary structural components of the cell membrane, which plays vital roles in both seed maturation and subsequent germination; consequently, extraplastidial membrane lipids are only partially converted to storage oils during the early stage of seed maturation.

Full Text

Study on the Changes of Membrane Lipids During Tobacco Seed Maturation

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Abstract: To elucidate the patterns of membrane lipid changes during tobacco seed maturation, we systematically investigated the content and composition of membrane lipids in tobacco seeds at different developmental stages using lipidomics. The results showed that plastidic membrane lipids, which constitute the primary lipid components of chloroplasts and thylakoid membranes, decreased continuously in both absolute content and relative proportion throughout the entire maturation process. In contrast, extraplastidic membrane lipids, the major constituents of cell membranes, declined significantly during early seed maturation but remained essentially stable after 21 days post-pollination. Total membrane lipid content followed a similar trend to plastidic membrane lipids, reaching a stable state after 29 days post-pollination. Since storage oils accumulate continuously during seed maturation and share similar chemical structures with membrane lipids, the reduction in plastidic membrane lipids may be associated with the sustained demand for oil accumulation and the decreasing requirement for chloroplasts and thylakoids. The stabilization of extraplastidic membrane lipids after 21 days post-pollination likely reflects their essential role as primary components of cell membranes, which remain critical for seed maturation and subsequent germination processes. Therefore, we speculate that only a portion of extraplastidic membrane lipids is converted to storage oils during early maturation.

Keywords: tobacco, seeds, maturation, membrane lipids

Introduction

High-quality tobacco seeds are fundamental to tobacco leaf production, and seed quality is directly related to seed maturity (Zhan et al., 2011; Zhang et al., 2012; Shang et al., 2014). Therefore, accurately determining seed maturity to guide harvest timing represents an urgent challenge in tobacco seed production. Previous studies have systematically investigated changes in seed morphology, color, moisture content, soluble sugars, starch, fats, proteins, and hormones during tobacco seed maturation (Shao et al., 2017), while Song et al. (2018) examined the relationship between antioxidant enzyme activity and seed quality. However, research on changes in membrane lipid composition and content during seed maturation remains unreported.

Studies have demonstrated that changes in membrane systems are closely associated with seed quality, longevity, and storability (Yin et al., 1989, 1990; Chen et al., 1995; Song and Lin, 2009). As the primary components of membrane systems, changes in membrane lipid composition and content significantly affect membrane physical properties and phase transition temperatures, thereby influencing seed vigor (Yang and Wang, 2004). Membrane lipids primarily include phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylglycerol (PG), and phospha-

tidic acid (PA), as well as galactolipids including monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) (Song and Lin, 2009). Among these, MGDG, DGDG, and PG are the main lipids comprising chloroplast membranes and are therefore termed plastidic membrane lipids (Dormann and Benning, 2002; Kobayashi et al., 2007). The remaining polar glycerolipids are designated as extraplastidic membrane lipids, which constitute the primary components of plant cell membranes, mitochondrial membranes, and other organelle membranes.

This study systematically analyzed the composition and content changes of membrane lipids in tobacco seeds at different maturation stages through lipidomics. Our objectives were to reveal the patterns of membrane lipid changes during tobacco seed maturation, establish relationships with seed quality, and provide guidance for optimal seed harvest timing.

Materials and Methods

1.1 Materials

The experimental material consisted of seeds from the tobacco cultivar *Nicotiana tabacum* 'Honghuadajinyuan' , provided by Yuxi China Tobacco Seed Co., Ltd.

1.2.1 Seedling Cultivation and Field Planting

Seedlings were cultivated according to GB/T 25241.1-2010 "Technical Regulations for Intensive Tobacco Seedling Cultivation–Part 1: Float Seedling System" and transplanted at 55 days after sowing. Plant spacing was 55 cm × 120 cm, with field management conducted according to GB/T 24308-2009 "Technical Regulations for Tobacco Seed Multiplication."

1.2.2 Seed Production and Preservation

Pollen Collection and Pollination: At early flowering stage, tobacco plants with robust growth and typical cultivar characteristics were selected. Anthers were manually extracted and air-dried for pollen release. Pollen was sieved through a 50-mesh screen and stored at 4°C in sealed containers when moisture content was below 7%. During peak flowering, open flowers and capsules were removed, and a single pollination batch was performed. Young flower buds were removed three days after pollination.

Capsule Harvesting: Capsules were harvested at 7, 21, 25, 29, and 33 days after pollination.

Seed Extraction and Preservation: Seeds were extracted from fresh capsules, pre-chilled in liquid nitrogen for 1 minute, and stored at -80°C.

Freeze-Drying and Weighing: Seeds stored at -80°C were placed in a freeze-dryer and dried at -50°C for 3 days to constant weight. Five 100 mg samples of seeds at each developmental stage were weighed for lipid extraction.

1.2.3 Lipid Extraction and ESI-MS/MS Analysis

Total Membrane Lipid Extraction Procedure:
1) Seeds were fixed in preheated isopropanol (containing 0.01% BHT) at 75°C for 15 minutes to inhibit phospholipase activity.
2) Seeds were transferred to a mortar, ground thoroughly, and moved to a 15 mL centrifuge tube. The mortar was washed with 1 mL chloroform and 1 mL methanol, with washings transferred to the centrifuge tube, followed by addition of 0.8 mL deionized water and thorough mixing.
3) 1.0 mL chloroform and 1.0 mL water were added, mixed thoroughly, and centrifuged for phase separation.
4) The lower phase (containing chloroform and lipids) was aspirated and transferred to a clean centrifuge tube.
5) 1.0 mL chloroform was added to the original tube, mixed and centrifuged, with the lower phase again transferred to the collection tube.
6) Step 5 was repeated.
7) 0.5 mL of 1 $mol \cdot L^{-1}$ potassium chloride was added to the combined extracts, mixed thoroughly, centrifuged, and the upper aqueous layer was discarded.
8) 1 mL deionized water was added to the extract, mixed thoroughly, centrifuged, and the upper aqueous layer was discarded.
9) The extract was dried under nitrogen and dissolved in 1.5 mL chloroform.
10) The chloroform solution was transferred to a 2 mL sample vial, dried under nitrogen, sealed, and stored for analysis.

Instrumental Analysis: Membrane lipid content was determined using electrospray ionization tandem mass spectrometry (ESI-MS/MS) (Welti et al., 2002; Li et al., 2008).

1.3 Statistical Analysis

Data processing and graphing were performed using Microsoft Excel 2017 and Origin 8.0. Significance analysis was conducted using SPSS 16.0.

Results

2.1 Changes in Total Membrane Lipid Content During Seed Maturation

Total membrane lipid content exhibited a decreasing trend throughout seed development, with a rapid initial decline followed by a slower reduction [Figure 1: see original paper]. At 7 days post-pollination, total membrane lipid content was 23.96 $nmol \cdot mg^{-1}$, decreasing sharply to 8.25 $nmol \cdot mg^{-1}$ by 21 days post-pollination. After 21 days, the decline slowed: no significant difference was observed between 21 and 25 days, though both were significantly higher than at 29 and 33 days. No significant difference was detected between 29 and 33 days.

2.2 Changes in Plastidic Membrane Lipids During Seed Maturation

Both the absolute content and percentage of plastidic membrane lipids decreased continuously during seed maturation . Plastidic membrane lipid content was 6.64 nmol · mg ^{1} at 7 days post-pollination, rapidly declining to 1.88 nmol · mg ^{1} by 21 days, after which it decreased slowly and stabilized after 29 days. The proportion of plastidic membrane lipids in total membrane lipids was 27.64%, 24.77%, 19.95%, 13.50%, and 12.90% at 7, 21, 25, 29, and 33 days post-pollination, respectively, indicating that plastidic membrane lipids decreased faster than total membrane lipids.

The contents of DGDG, MGDG, and PG in plastidic membrane lipids all decreased during seed maturation [Figure 2: see original paper]. DGDG and MGDG showed similar patterns, with highest contents at 7 days post-pollination, significantly higher than other stages. Contents at 21 days were second highest, significantly exceeding those at other stages except 25 days, with no significant differences among 25, 29, and 33 days. PG content was highest at 7 days post-pollination, significantly higher than other stages, with no significant changes after 21 days.

2.3 Changes in Extraplastidic Membrane Lipids During Seed Maturation

Total extraplastidic membrane lipid content decreased during seed maturation, showing an initial rapid decline followed by a slower reduction, while its proportion in total membrane lipids increased . At 7 days post-pollination, extraplastidic membrane lipid content was 15.62 nmol · mg ^{1}, accounting for 65.37% of total membrane lipids. By 21 days post-pollination, content had rapidly decreased to 5.35 nmol · mg ^{1}, though its proportion remained relatively stable, indicating that extraplastidic membrane lipids decreased at a similar rate to other membrane lipids during this phase. After 21 days, the decline in extraplastidic membrane lipid content slowed while its proportion in total membrane lipids continuously increased, reaching 80.57% at 33 days post-pollination, suggesting that extraplastidic membrane lipids decreased more slowly than other membrane lipids during this period.

Different extraplastidic membrane lipid classes exhibited distinct patterns during seed maturation [Figure 3: see original paper]. PS content showed no significant changes, while PC, PE, PI, and PA generally decreased. PC, the primary extraplastidic membrane lipid in cell membranes, decreased continuously from 7 to 29 days post-pollination, with a rapid initial decline followed by a slower reduction; no significant difference was observed between 29 and 33 days. PE content decreased continuously, with a rapid initial decline followed by stabilization after 21 days. Compared with PC and PE, PI and PA contents decreased dramatically between 7 and 21 days, followed by fluctuating but non-significant changes.

2.4 Changes in Acyl Chain Length (ACL) and Double Bond Index (DBI) During Seed Maturation

Acyl chain length and double bond index of membrane lipids are two important parameters affecting plant cell membrane systems (Yu and Li, 2014). In this study, the acyl chain lengths of plastidic membrane lipids DGDG, MGDG, and PG did not change significantly with seed maturation [Figure 4: see original paper]. The acyl chain lengths of extraplastidic membrane lipids fluctuated during seed maturation, with PS showing the most pronounced change between 7 and 21 days post-pollination, increasing from 36.92 to 41.66. Other extraplastidic membrane lipids showed no significant changes in acyl chain length. Similarly, the double bond indices of most extraplastidic membrane lipids fluctuated without significant or consistent patterns [Figure 5: see original paper].

2.5 Changes in Lysophospholipids During Tobacco Seed Maturation

Lysophospholipids are a special class of polar lipids present in plants that occur in small quantities and change dramatically with environmental conditions (Li et al., 2011). In this study, LysoPC and LysoPE contents decreased significantly between 7 and 21 days post-pollination, with no significant differences after 21 days. LysoPG showed no consistent pattern across the experimental time points [Figure 6: see original paper].

Discussion

In this study, total membrane lipid content decreased continuously during seed maturation, with a rapid initial decline followed by a slower reduction, particularly between 7 and 23 days post-pollination. This trend contrasts with the accumulation pattern of storage oils (triacylglycerols) during seed maturation (Shao et al., 2017), which increased rapidly between 7 and 23 days post-pollination. Since storage oils and membrane lipids are both glycerolipids with similar structures, we hypothesize that membrane lipids are degraded to generate diacylglycerol (DAG), which is then converted to storage oils by NtDGAT1 and NtDGAT2 for accumulation as nutritional reserves for subsequent physiological activities (Yang et al., 2013; Zhang et al., 2005). Therefore, membrane lipids may serve not only as essential components of cell membranes but also as important precursors for storage oil synthesis in seeds.

Comparison of plastidic and extraplastidic membrane lipid changes revealed that both decreased with seed maturation, but plastidic membrane lipids continued declining until 29 days post-pollination, whereas extraplastidic membrane lipids stabilized after 21 days post-pollination. Moreover, the magnitude of decrease was much greater for plastidic membrane lipids. Plastidic membrane lipids are essential components of chloroplast and thylakoid membranes (Dormann and Benning, 2002). During early seed maturation, tobacco seeds appear yellow-green, indicating abundant chloroplasts and high plastidic membrane lipid content. As seeds matured, their color gradually changed to brown and dark brown,

reflecting chloroplast degradation (Zheng et al., 2015). This transition was also evident from changes in seed coat chlorophyll fluorescence (Shao et al., 2017). Since mature seeds do not require photosynthesis or chloroplasts, the substantial degradation of plastidic membrane lipids during maturation aligns with this functional transition. Extraplastidic membrane lipids are major constituents of cell membranes. Although some degradation occurred during seed maturation, the reduction was much less extensive than that of plastidic membrane lipids, stabilizing around 21 days post-pollination.

Acyl chain length and double bond index of membrane lipids are closely related to membrane fluidity (Yu and Li, 2014). This study found that membrane lipid acyl chain length did not change dramatically during tobacco seed maturation, except for phosphatidylserine, whose acyl chain length increased with seed maturation. Phosphatidylserine is one of the few membrane lipids containing high levels of long-chain fatty acids, and previous research suggests that its acyl chain length may be correlated with plant organ lifespan (Li et al., 2014). The double bond indices of some polar lipids also changed during seed maturation, but these changes were not pronounced or consistent.

In summary, this study systematically investigated the patterns of membrane lipid content and composition during tobacco seed maturation. The results demonstrate that total membrane lipid content decreased continuously with a rapid initial decline followed by stabilization, reaching a stable state at 29 days post-pollination. The reduction in membrane lipids during maturation may contribute to storage oil synthesis. Furthermore, extraplastidic membrane lipid content stabilized after 21 days post-pollination, while plastidic membrane lipids (particularly galactolipids) decreased throughout the entire maturation process, likely reflecting functional transitions during seed development.

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