The Organic Acid Content of the Liquid Endosperm of Cotton

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Abstract

The mature cotton seed does not contain endosperm, however, during early seed development the cotton seed possesses healthy endosperm and nucellar tissues that provide nutrition to the developing embryo. For about the first 12 days of development, the endosperm is liquid and coenocytic (multinucleate).As the young embryo grows larger and begins to elongate within the seed the endosperm becomes cellularized. The objectives of this project was to determine if the organic acid content of the liquid endosperm of cotton was related to the "hollow seed" phenomenon of South Carolina Seed Rot and to determine if the organic acid content of the liquid endosperms of *G. hirsutum, G. barbadense*, and *G. arboreum* differed. For the first objective the endosperm of individual symptom-less seeds and seeds with incipient symptoms was collected with a 10 µL Hamilton syringe and diluted 1:20. For the second objective, the endosperms were collected and pooled from a boll (undiluted). After filtering, 2 µL of each was assayed for organic acids with a Waters HPLC. Total organic acid content in the endosperm did not appear to be related to incipient symptoms of SC seed rot. Malic acid was the most abundant organic acid in the liquid endosperm of all cottons and an accession of *G. arboreum* contained the most of the three species examined.

Introduction

The mature cotton seed does not contain endosperm but the new seed obtains its energy for germination from oils and proteins stored in the cotyledons. However, during early seed development the cotton seed possesses healthy endosperm and nucellar tissues that provide nutrition to the developing embryo (Fig 1). For the first 10-12 days following fertilization the endosperm of cotton is liquid and coenocytic [multinucleate, this resulting from rapid nuclear division (karyokinesis) without cell division (cytokinesis) of the 3X = 6N endosperm nucleus following fertilization. This liquid bathes the young zygote (fertilized egg) that will become the embryo (Fig. 2). As the young grows larger and begins to elongate with in the seed the endosperm becomes cellularized (Fig.3) by forming a cell wall around each formerly free nucleus.

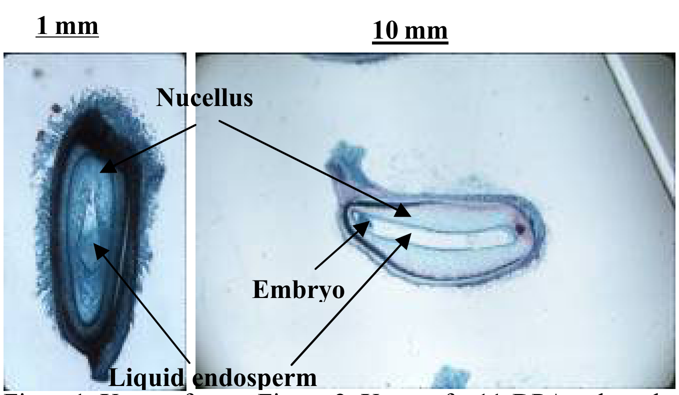

Figure1. X-sec of a 2-DPA ovule with nucellus and start of liquid endosperm.

Figure 2. X-sec of a 11-DPA cultured seed with liquid endosperm and young embryo.

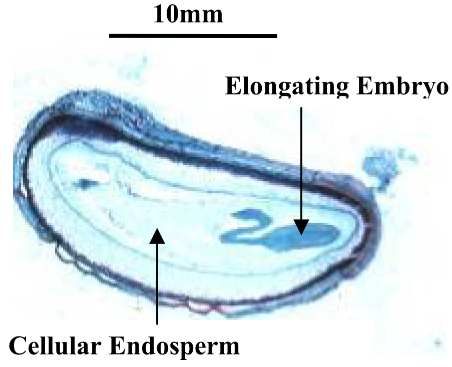

Figure 3. X-sec of a 15-DPA cultured seed with cellular endosperm and an elongating embryo.

Mauney (1961) found that Ca+2 malate were essential for very young cotton embryos to grow normally. This implied that the liquid endosperm probably contains a significant quantity of this organic acid. To our knowledge the organic acid content of the liquid endosperm of cotton has not been determined.

Hypotheses

- The South Carolina Seed Rot phenomenon is related to abortion of the endosperm during early seed development resulting in the classical "Hallow seed".
- Different cultivated species of *Gossypium* have different levels of organic acids in their liquid endosperm.

Objectives

1. Determine if the organic acid content of the liquid endosperm is adversely affected in cotton seeds showing the initial stage of seed rot.
2. Determine if differences exist in the organic acid contents of the liquid endosperms of G. *arboreum, G. hirsutum* and G. *barbadense*.

Materials and Methods

For the first objective small bolls judged to be less than two weeks of age were harvested in September from *G. hirsutum* Acala Maxxa growing in the field at Florence, SC. The developing bolls were taken to the laboratory, opened, and each seed examined for early symptoms of boll rot (Fig. 4).

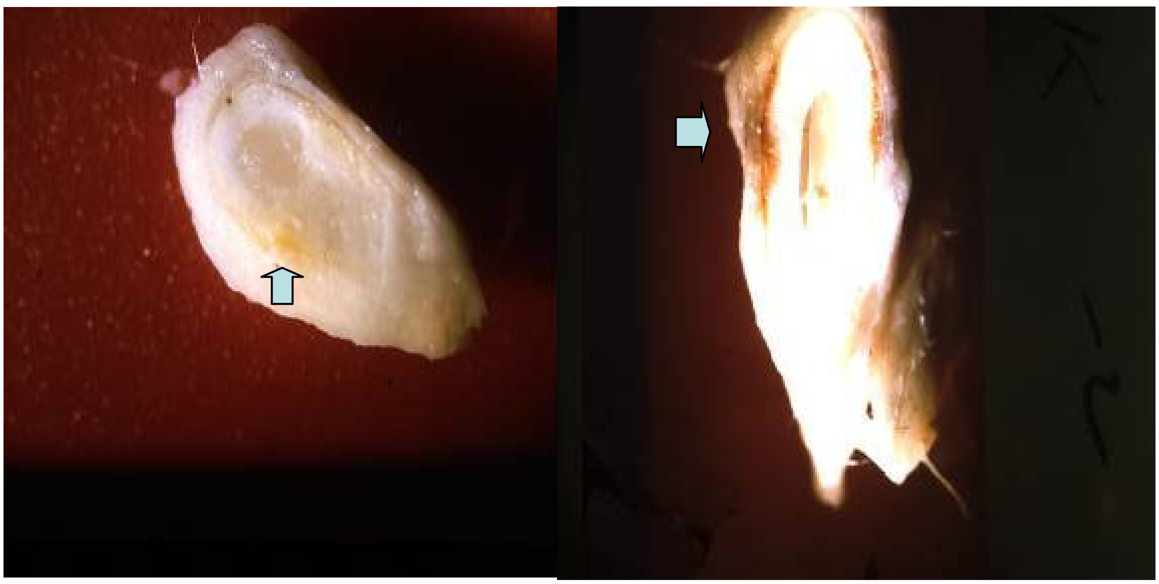

Figure 4. Developing Acala seeds opened to show incipient boll rot symptoms (Arrows). The exterior of the seed looks normal except for a slight discoloration of the seed coat opposite the internal tissue proliferation. Note the seed on the left has cellularized endosperm whereas the seed on the right is hollow.

The seeds of bolls without symptoms were used as controls. The liquid endosperm was collected from individual seeds with a 10 µL Hamilton syringe. With the plunger completely depressed, the needle of the syringe was inserted into the seed cavity and the liquid removed from the seed by negative pressure generated by pulling the plunger part way back. The extracted liquid was stored frozen until assayed. For the second objective all plants were field grown in Fayetteville, Arkansas. All flowers on selected rows were tagged on one day during late August. All tagged bolls were harvested from two accessions of G. arboreum (A2-long lint from India and A2 *Thielaviopsis* resistant from John Gannaway) at 10 days while *G. barbadense* and *G. hirsutum* were harvested at 12 days of age. The bolls were chilled prior to endosperm extraction and the extract was frozen until assayed. For this objective the extracts from the seeds of a boll were pooled. Organic acid were assayed on a Waters Alliance HPLC with a Restek Ultra Aqueous C18 250x2.1 mm column. The organic acid was separated 99:1 mixture of 50mM KH_2PO_4 (pH 2.5): acetonitrile with a flow rate of 0.2ml/mintue. The organic acids were detected with a 996 Photo Diode Array detector. Sample size was 2µl. For objective1 the samples were diluted 1 to 20 µl before injection.

Results and Discussion

The organic acids measured are part of the TCA cycle. Malic acid is the most abundant organic acid in the liquid endosperm of *Gossypium*, and probably represents a pool distinct from the organic acids of the TCA cycle. This high malate concentration confirms the observation of Mauney (1961), since the young embryo is bathed in the liquid endosperm during early development. The decrease in malic acid supports the suggestion that it functions in processes that do not involved in mitochondria such as serving as an osmoticum.

Table1. Effect of SC seed rot on the major organic acids of *G. hirsutum*

	OA	OAA	MA	CA	SA	FA
Normal	1.08	0.15	9.81	0.46	0.97	0.19
	1.36	0.20	7.52	0.49	1.91	0.22
	2.96	0.31	10.59	0.60	0.93	0.13
	1.44	0.31	7.58	0.36	1.45	0.16
	1.67	0.27	10.00	0.70	1.65	0.15
	3.39	1.80	9.49	0.70	1.03	0.16
	0.38	0.18	12.18	0.79	2.50	0.13
	2.34	0.32	12.01	1.08	2.21	0.13
Ave	1.83	0.44	9.90	0.65	1.58	0.16
SCSR	1.73	0.20	9.38	1.10	1.39	0.16
	1.25	0.21	6.88	0.57	0.74	0.17
	1.22	0.16	2.69	0.61	0.93	0.18
	4.38	0.19	9.21	0.92	1.17	0.30
	2.52	0.16	9.96	0.72	1.16	0.33
	1.97	0.27	10.98	0.42	1.55	0.14
Ave	2.18	0.20	8.18	0.72	1.16	0.21

Table 2. Major organic acids of *G. hirsutum, G. barbadense,* and *G. arboreum.*

	OA	MA	CA	SA	FA
AD1	0.51	5.22	0.31	0.20	0.023
AD1	0.38	4.36	0.35	0.08	0.012
AD2	0.49	3.06	0.42	0.30	0.013
A2	0.43	6.20	0.44	0.23	0.013
A2	0.24	4.93	0.12		0.009

Conclusions

When one looks only malate it would appear that the first hypothesis is correct, that is, a decrease in organic acid is associated with the hollow seed phenomena of SC seed rot. However, an increase occurs in the organic acids fumerate and succinate in seed with incipient symptoms. Thus, the observed decrease may be due to an effect rather than the cause of the hollow seed phenomena.

Concerning the second hypothesis the three cultivated species appeared to differ only in the amount of malate in the endosperm. Of the three species examined, *G. arboreum* contained the most malate.