Fermentation in Cotton (Gossypium hirsutum) Seeds

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ABSTRACT

Ethanol and acetaldehyde production by cotton seeds subjected to anoxic stress imposed by CO_2 or N_2 gas was quantified during the imbibition phase. Fermentation capacity was low in dry seeds and quickly increased during the first few hours of imbibition. In hydrated seeds, ethanol and acetaldehyde excretion following anoxic stress followed a linear trend in time. Ethanol excretion exceeded that of acetaldehyde by an order of magnitude. Similar rates of production were observed whether anoxic was imposed by either CO_2 or N_2 gas. Excreted ethanol and acetaldehyde were rapidly metabolized following alleviation of anoxic stress.

INTRODUCTION

Among the many stresses faced by germinating cotton seeds is low oxygen or anoxic stress. In wetter climates, cotton seeds frequently encounter brief periods of anoxic stress following flooding due to heavy rainfall. In the desert Southwest, anoxic stress may be induced similarly following heavy irrigation. Excessive capping, a cultural practice in which soil is ridged up over the planted furrow to conserve moisture, may also deprive cotton seeds of sufficient oxygen. Unlike animals, many plant seeds can tolerant being deprived of oxygen for extended periods by shifting their carbon metabolism to fermentation, in which ethanol is synthesized from pyruvate. Despite the essential importance of ethanolic fermentation in seed survival under anoxic conditions, this pathway has not received extensive investigation in cotton seeds. The objective of this research was to characterize ethanol fermentation in cotton seeds subjected to sudden anoxic stress.

METHODS AND MATERIALS

All seeds were from a single commercial lot of Deltapine 90 produced in Arizona in 1985 and donated by the Delta Pine & Land, Co. For the experiment concerning the development of fermentation capacity, five unimbibed cotton seeds were placed in small flasks containing moistened filter paper. After set intervals, replicate flasks were flushed with pure CO_2 gas and sealed with solid rubber septums. Sealed flasks were incubated for 3 hours at 28°C.

In all other experiments, we selected seeds of uniform root growth of 2 to 5 mm after imbibition at 28°C for 22-24 hours in moistened rolls of paper toweling. Five of these imbibed cotton seeds were placed in small flasks as above. The flasks were flushed with either N_2 or CO_2 gas and sealed with a solid rubber septum. All flasks were incubated at 28°C either in light or darkness for up to 4.5 hours. Anoxic stress was alleviated in some experiments by the addition of oxygen to 20% of the total flask volume.

The ethanol and acetaldehyde which partitioned into the head-space during treatment was quantified by gas-liquid-chromatography. Ethanol and acetaldehyde content of the imbibition solution was estimated from partition equations derived from solutions of known concentration in similarly sealed flasks. Analysis of variance was performed using a completely randomized design replicated two or more times.

RESULTS AND DISCUSSION

Cotton seed fermentation capacity in response to anoxia induced by CO_2 develops rapidly as seed tissue is hydrated (Table 1). Dry seeds have little ability to ferment, but the capacity quickly develops within the first 16 h of imbibition. Ethanol-forming capacity increases rapidly up to 16 h following imbibition onset and then subsequently declines slightly over the next 16 h. Acetaldehyde-forming capacity increases linearly during the first 32 h of imbibition. The initial lag in fermentation capacity probably reflects the hydration and activation of enzymes and organelles associated with aerobic respiration.

Cotton seeds, imbibed in air for 24 hours, rapidly synthesized ethanol and acetaldehyde following sudden anoxic stress imposed by CO_2 gas. Accumulation of both compounds in sealed vials followed a linear trend with time. After 4 hours of anoxic stress, about 2,000 nmoles seed^{-1} of ethanol and 40 nmoles seed^{-1} acetaldehyde had been excreted. Excretion of ethanol and acetaldehyde of cotton seeds in air under similar experimental conditions fell below about 100 and 5 nmoles seed^{-1}, respectively. Similar rates of production were observed when anoxic conditions were imposed by N_2 gas. Ethanol and acetaldehyde excretion was the same whether anoxia was imposed in light or darkness. Following alleviation of anoxic stress, excreted ethanol and acetaldehyde were rapidly metabolized.

Table 1. Ethanol and acetaldehyde production of cotton seeds subjected to anoxic stress at various stages of early imbibition. Cotton seed were imbibed at 28°C in air. At various times, five seeds were transferred to sealed flasks containing pure CO_2 gas. After 3 h, accumulated ethanol and acetaldehyde were quantified by GLC of head-space samples.

Time from imbibition onset	Ethanol	Acetaldehyde
h	nmoles seed^{-1}	
0	47 a	1.2 a
4	320 a	1.4 a
8	959 b	4.6 a
12	1184 b	7.8 ab
16	1350 b	9.7 ab
20	1135 b	8.5 ab
24	1025 b	14.4 ab
28	863 b	10.6 ab
32	1019 b	22.6 b
L.S.D.$_{0.05}$	334	10.6