Fermentation as an Estimator of Cotton (Gossypium hirsutum) Seed Vigor

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ABSTRACT

Anoxic-induced fermentation was evaluated as a potential cotton seed vigor test. Seed samples from a single seed lot were subjected to accelerated aging for different durations to create five classes of seeds on the basis of vigor. The ethanol and acetaldehyde excreted from seeds from each class during brief periods of anoxia was quantified by gas-liquid-chromatography. Ethanol and acetaldehyde production during anoxia was negatively correlated with standard germination test results of all seed samples receiving accelerated aging. The fermentation capacity of hydrated cotton seeds remained intact at imbibition temperatures, which significantly reduced radicle growth.

INTRODUCTION

The standard germination test of cotton seed, conducted under optimal growth conditions, frequently correlates poorly with cotton seed performance under stressful field conditions. The difference in seed performance between ideal and stressful conditions is frequently attributed to differences in seed vigor. Seed technologists have proposed several tests to measure the vigor of cotton seed, but none have been universally accepted by the seed industry. Most of the vigor tests that have been proposed are based on growth responses which take considerable time to develop. More rapid tests will, of necessity, be based on physiological responses to stress that precede significant seed growth. Fermentation capacity in cotton seed is fully developed within the first day of imbibition and may represent a physiological stress response that reflects seed vigor. The objective of this study was to correlate ethanol and acetaldehyde production induced by anoxia with cotton seed vigor.

MATERIALS AND METHODS

All seeds were from a single commercial lot of Deltapine 90 produced in Arizona in 1986 and donated by the Delta Pine & Land, Co. Samples from this seed lot were subjected to accelerated aging at 46°C and 100% relative humidity for up to 216 hours and then air-dried. A standard germination test of 4 days at 30°C in rolled paper towels was then performed on each artificially aged seed lot sample. Fifty unimbibed cotton seeds from each of the artificially aged seed lot samples were placed in flasks containing moistened filter paper and imbibed in air for 24 hours at 28°C. All flasks were then flushed with pure CO_2 gas and sealed with solid rubber septums. Sealed flasks were incubated for 2 hours at 28°C.

In an experiment to determine the effect of high imbibition temperatures on fermentation in cotton seeds, we selected seeds of uniform root growth of 1 to 5 mm after imbibition at 30, 35, and 40°C for 24 hours in moistened rolls of paper toweling. Five of these imbibed cotton seeds were placed in small flasks containing moistened filter paper. All flasks were then flushed with pure CO_2 gas, sealed with solid rubber septums, and incubated for 2 hours at 28°C.

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 for both experiments was performed using a completely randomized design replicated four times.

RESULTS AND DISCUSSION

Lengthy exposures of cotton seed to accelerated aging increasingly reduced cotton seed quality in terms of germination. Short exposures were slightly stimulatory. Germination of untreated seed averaged 78% and increased to 89%, following a 48-hour exposure to accelerated aging. Exposures in excess of 48 hours reduced germination percentage dramatically. No cotton seed germinated following 216 hours of accelerated aging.

Ethanol production following sudden anoxic stress was highly correlated ($R^2 = 0.84$, $P > 0.001$) with standard germination test results of artificially aged cotton seeds. Untreated seeds excreted about 1640 nmoles $seed^{-1}$ during the first 2 hours following anoxic stress. Cotton seeds exposed to 48 hours of accelerated aging, had slightly higher levels of ethanol production which averaged about 1,900 nmoles $seed^{-1}$ in 2 hours. Like germination percentage, ethanol production was significantly decreased by exposures of accelerated aging greater than 48 hours. After 216 hours of accelerated aging exposure, cotton seed excreted, on average, about 280 nmoles $seed^{-1}$ during 2 hours of anoxia.

Acetaldehyde production during anoxia was also correlated ($R^2 = 0.63$, $P > 0.001$) with standard germination test results of artificially aged cotton seeds. Untreated seeds excreted on average about 18 nmoles $seed^{-1}$ of acetaldehyde during the first 2 hours of anoxia. Seeds receiving up to 96 hours of accelerated aging had similar levels of acetaldehyde excretion. Acetaldehyde excretion during anoxia declined sharply thereafter in seeds exposed to longer periods of accelerated aging.

The fermentation capacity in cotton seeds is robust, remaining largely intact at imbibition temperatures which inhibit growth (Table I). At 40°C, cotton radicle growth was only about 62% of that obtained at 35°C, but ethanol excretion following 2 hours of anoxia was unaffected. This was an interesting observation, since selection of the same morphological stage from populations which had been imbibed at different temperatures would be expected to bias the sample in terms of seed vigor.

Differences in seed vigor would be expected, because as the temperature of imbibition increased, a increasing proportion of the seed population would have developed beyond the selection criteria. Thus, at lower temperatures of imbibition, only the fastest growing seeds would be eligible for selection. At higher temperatures, these fast growing seeds would have grown too much to be considered for selection. Since ethanol production was similar regardless of the imbibition temperature, this could be interpreted to mean that anoxic-induced fermentation is not directly related to vigor differences that affect germination rate.

Unlike ethanol, acetaldehyde excretion during anoxia was reduced at the higher temperature (Table 1). The biological significance of this is not clear since ethanol production was not affected.

These results suggest that anoxia-induced fermentation during early imbibition in cotton seeds correlates well with seed vigor differences induced artificially. Further study is necessary to determine if this relationship can be utilized to predict seed performance under stressful field conditions.

Table 1. Effect of imbibition temperature on mean cotton radicle length after 24 h and on acetaldehyde and ethanol production of a selected subsample of cotton seeds subjected to CO_2 gas for 2 h. Radicles of selected subsample seeds were 2 to 5 mm in length. Five seeds, imbibed for 24 h at 28°C in air, were sealed in a flask flushed with CO_2 gas. Headspace samples from each of four replicate flasks were separated by GLC following incubation for 2 h at 28°C.

Imbibition temperature	Population mean radicle length	Selected subsample	
		Acetaldehyde	Ethanol
°C	mm	nmoles·seed^{-1}	
30	1.5 a	19.7 a	1560 a
35	4.7 b	22.2 a	1450 a
40	2.9 c	12.7 b	1460 a
$LSD_{0.05}$	1.0	5.0	280