

PLANTING SEED

Can Cotton (*Gossypium hirsutum*) Seed Vigor be Assessed in the Absence of Growth?

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ABSTRACT

Although seed vigor is ultimately expressed in terms of growth, it is not clear if metabolic processes unlinked to growth can also estimate vigor. The objective of this study was to determine if the relationship between seed vigor and ethanol metabolism differed depending on whether seed growth was presence or absence. For individual imbibed cotton seed, ethanol assimilation in air and ethanol biosynthesis in N₂ gas was correlated to seed vigor as measured by cool test performance. Seed growth in N₂ gas was prevented by the addition of polyethylene glycol. Results were inconclusive, because seed performance (radicle growth) during cool testing was not significantly correlated to either ethanol biosynthesis or ethanol assimilation. It was concluded that ethanol metabolism both in the absence and presence of seed growth has limited usefulness as a metabolic marker of cotton seed vigor.

INTRODUCTION

Since cotton seed vigor is ultimately expressed in terms of growth, the question arises whether all cotton seed vigor tests must be based on a growth response. The problem is important because there continues to be interest in identifying metabolic markers that predict seed performance before significant seed growth has occurred. That hypothesis may be tested by identifying a metabolic process that can be experimentally linked and unlinked to growth during the early stages of imbibition. An example of such a process is the role of the enzyme alcohol dehydrogenase (ADH) in the biosynthesis and assimilation of ethanol. During low oxygen stress, seed growth slows while ethanol is synthesized from acetaldehyde by ADH. When the stress is removed and oxygen is restored, growth resumes its normal rate and ethanol is assimilated. ADH also catalyzes that reaction, except in the reverse direction. In this study, we attempted to correlate the reversible *in vivo* activity of ADH with cool test performance to determine if cotton seed vigor assessment must be linked to seed growth.

MATERIALS AND METHODS

A single, high-vigor cotton seed lot of Deltapine 90 (1986 harvest) was used for all experiments. Prior to experiments, all seeds were stored over a saturated solution of LiCl to bring their moisture content to about 11%. Prior to treatment, all seeds were imbibed at 28°C for 28 hours in rolls of paper toweling. Seeds were identified by numbering their placement in the roll.

To measure ethanol assimilation, 50 seeds were incubated individually in air at 28°C for 2 hours in test tubes containing 50 μ L of 64 mM ethanol. To measure ethanol biosynthesis, 50 seeds were incubated individually in N₂ gas at 28°C for 2 hours in tubes containing 50 μ L of 4 mM ethanol with or without polyethylene glycol 6000

N₂ gas at 28°C for 2 hours in tubes containing 50 uL of 4 mM ethanol with or without polyethylene glycol 6000 (PEG, 0.8 g mL water⁻¹). Cotton seed radicle growth rate is reduced, but not entirely stopped under N₂ gas; radicle growth is prevented by exposure to both PEG and N₂ gas (Lehle and Zegeer, 1989). Ethanol excreted into (biosynthesis) and taken up from (assimilation) the imbibition solution of individual seed was quantified by gas-liquid chromatography on a 1.8 by 32 mm column of Porapak N. Ethanol was detected by flame ionization. After treatment, seeds were placed back in the germination roll and cool tested for 6 days at 19°C. Cool-test results were expressed in terms of radicle length.

RESULTS AND DISCUSSION

In air, tested cotton seeds showed rapid assimilation of ethanol and vigorous growth during cool testing. An average of 18.7 nmoles of ethanol per uL incubation solution was assimilated under the conditions of the study. Mean radicle length of these same seeds following cool testing was 60.9 mm. The cool-test results of individual seeds, however, were not significantly ($P=0.16$) correlated their assimilation of ethanol (Figure 1).

As expected, cotton seeds produced ethanol under N₂ gas. An average of 8.9 nmoles of ethanol per uL incubation solution was produced during the 2 hour incubation. Mean radicle length of the same seeds following cool testing was 50.3 mm. That was slightly less than the average for seeds which had assimilated ethanol in air. The results are consistent with our previous observations that cotton seeds readily ferment ethanol when subjected to anoxic stress (Lehle and Ahmed, 1988). Anoxic stress induced ethanol biosynthesis was not, however, significantly ($P=0.89$) correlated with cool-test results (Figure 2). That is contrary to our previous findings which suggested a strong correlation between seed vigor and ethanol production in cotton seeds (Lehle, 1988). The results of the two studies are not, however, directly comparable for several reasons. In the earlier study, different vigor levels were created by artificial aging. Seeds subjected to such treatment may not respond the same as naturally-aged seed. Also in the earlier study, ethanol biosynthesis was compared to warm-germination performance rather than cool-test performance. The earlier study also assayed the ethanol production of a pooled sample of 50 seeds, whereas in the present study, each seed was assayed individually. Finally, in the earlier study, ethanol biosynthesis was induced by CO₂ gas, whereas, in the present study, N₂ gas was used for this purpose.

Cotton seeds also produced ethanol in N₂ gas in the presence of PEG. Under those conditions, we have shown that seed growth is totally prevented. An average of 12.9 nmoles of ethanol per uL incubation solution was produced during the 2-hour treatment. Mean radicle length of these same seeds following cool testing was 25.3 mm. That indicates that the combination of water and oxygen stress was substantially more inhibitory to cool-test performance than was oxygen stress alone. As before, ethanol biosynthesis by seeds exposed to both PEG and N₂ gas was not significantly ($P=0.6$) correlated with cool-test performance (Figure 3). No conclusion concerning whether seed vigor must be assessed with a growth response was possible, because neither ethanol biosynthesis nor ethanol assimilation was correlated to cool-test performance. It was concluded that ethanol metabolism both in the presence and absence of seed growth has limited usefulness as a metabolic marker of cotton seed vigor.

ACKNOWLEDGMENT

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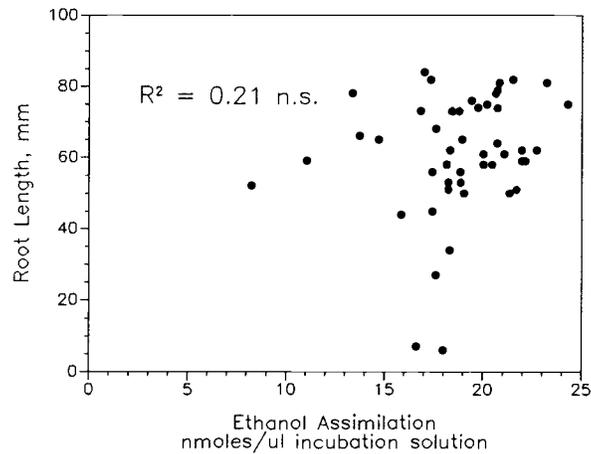


Figure 1. Relationship between cool test results and ethanol assimilation of individual cotton seeds incubated in air for 2 hours.

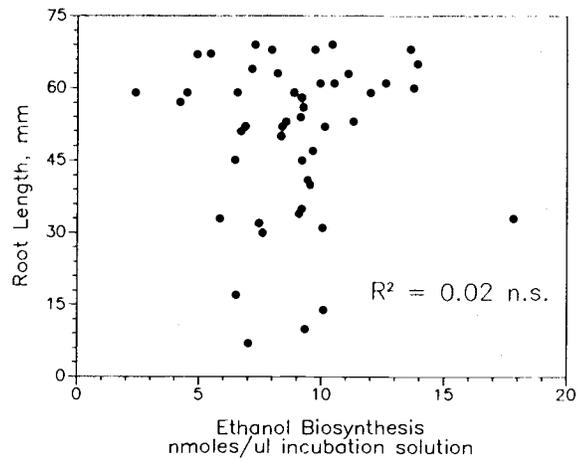


Figure 2. Relationship between cool test results and ethanol biosynthesis of imbibed cotton seeds incubated in N_2 gas for 2 hours.

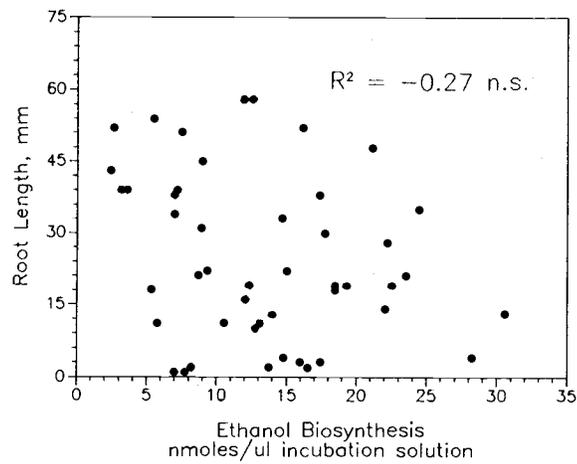


Figure 3. Relationship between cool test results and ethanol biosynthesis of imbibed cotton seeds incubated in N_2 gas and PEG for 2 hours.