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Independent Control of Fiber Development and Nitrate Reduction in Cultured Cotton Ovules[1]

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ABSTRACT

Several lines of evidence implicate ammonium as an important factor in the growth and development of cotton (*Gossypium hirsutum* L.) ovules cultured *in vitro*. For example, ovules cultured at 28 C require indoleacetic acid (IAA) and either ammonium or gibberellic acid (GA_3) in the medium for fiber development, whereas ovules cultured at 34 C require only IAA. Because of this effect of ammonium supply, it seemed possible that hormones or increased temperature were also promoting the availability of reduced nitrogen by induction of increased nitrate reductase activity in the ovules. This possibility was tested.

***In vivo*, where ovules received mostly reduced nitrogen and very little nitrate, they did not display appreciable nitrate reductase activity even when nitrate was forced into the ovary wall by transpiration. After initiation of culture, nitrate became freely available to ovules and their nitrate reductase activity increased rapidly. Treatment with ammonium, GA_3, IAA, or increased temperature had no effect upon this induction. It is concluded that ammonium, hormone, and temperature effects on fiber development are independent of the availability of reduced nitrogen as a general substrate for growth.**

Ammonium ions strongly influence fiber development during *in vitro* growth of unfertilized ovules of cotton. Fiber initiation begins the morning of anthesis, the time when unfertilized ovules are placed into culture (3). Continued initiation and the subsequent development of fibers are complex processes controlled by hormones, especially auxin (5); ammonium in the growth medium, along with the normally present nitrate, caused a greater percentage of ovules to produce fibers in response to IAA (3). For any one ovule, fiber development was an "all-or-none" process. Increased culture temperature or addition of GA_3 to the medium caused similar developmental shifts (3).

Because GA_3 has been reported to stimulate NR[3] activity (9), and because ammonium itself is an early product of the nitrate assimilation pathway, it seemed possible that the developmental changes induced by these agents could be related to availability of reduced N. Although the standard medium for ovule culture (2, 4) contains N only as nitrate, ovules apparently receive reduced N almost exclusively *in vivo* (8). Isolated ovules supplied with nitrate respond with increased NR activity, and presumably with increased ability to assimilate nitrate (8). The present work was begun to determine whether agents which cause increased fiber formation also cause changes in ovular NR activity.

MATERIALS AND METHODS

Procedures for the greenhouse production of flowers of cotton (*Gossypium hirsutum* L. cv. Acala SJ-2), sterile culture of unfertilized ovules, and measurement of the percentage of ovules forming fiber have been described in detail (1–5).

Assay of NR activity in the ovules was by an *in vivo* procedure extensively modified from Radin and Sell (8). On the day of anthesis, ovules were removed from an adequate number of ovaries (approximately 30 ovules per ovary) and randomized in a flask containing ice-cold solution. That collection solution was

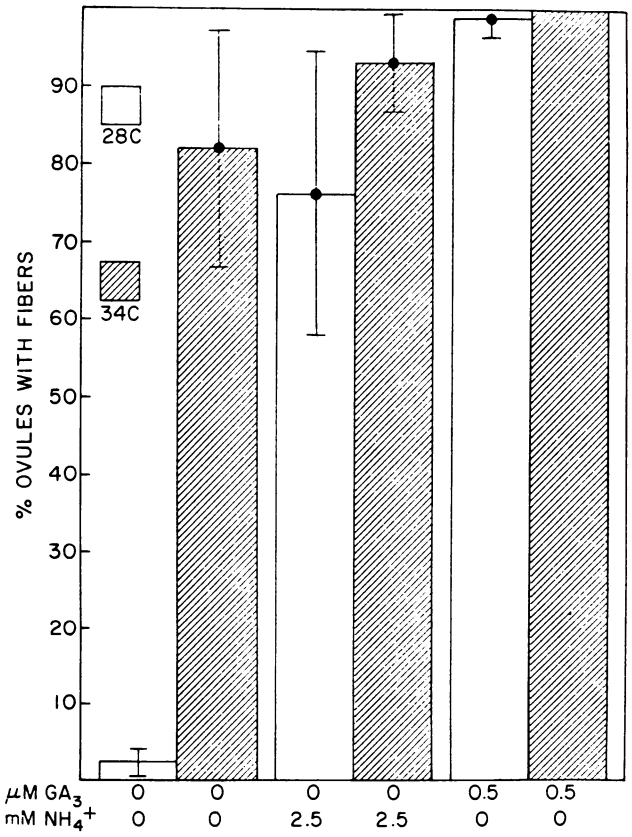

FIG. 1. Percentage of ovules forming fibers at 28 and 34 C in response to 2.5 mM NH_4NO_3 or 0.5 μM GA_3. IAA at 5.0 μM was present in all treatments.

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[3] Abbreviation: NR: nitrate reductase.

modified from the basal culture medium (2) in that glucose and KNO_3 were omitted, $CaCl_2$ was reduced from 3 to 1 mM, and the pH was adjusted to 7.5 with NaOH. For each treatment, randomized ovules were transferred, in batches of 20, to tubes (four to six per treatment) containing 3 ml of assay solution. For cultured material, 20 ovules (all from the same ovary) from each culture flask were placed in each of four to six tubes containing 3 ml of assay solution. The assay solution consisted of basal culture medium (2) modified as above, except that the glucose and 50 mM KNO_3 were retained and 1-propanol was added at a concentration of 1% (v/v). Tissues were infiltrated twice under vacuum, and the vacuum was released with N_2. Assay tubes were immediately placed in a shaker bath at 32 C and incubated for varying periods, depending upon the experiment. Nitrite concentration was determined colorimetrically (7) in aliquots of the assay solution.

In some experiments we attempted to alter *in situ* nitrate availability and NR activity by preculturing floral branches in appropriate media. Floral branches were cut from the plant on the day of anthesis and their bases were immediately placed in vials containing basal culture medium (2) without glucose and ±50 mM KNO_3. Ovary walls and ovules were collected from precultured branches and from intact plants at the same time. Ovary walls were denuded of all other floral parts and assayed, one per tube, in sextuplicate. When ammonium was supplied as 2.5 mM NH_4NO_3 in the basal culture media, the concentration of KNO_3 was reduced to 45 mM.

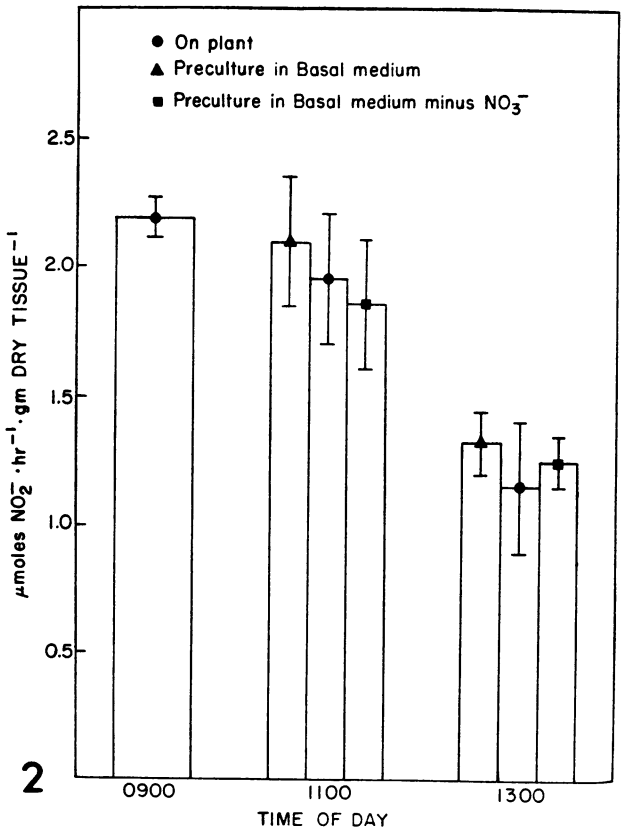
Vertical limits plotted in the figures represent two standard error values above and two below the means which were derived from no less than four values.

RESULTS

Ovules cultured at 34 C were induced to form fibers by treatment with 5 μM IAA (3); little or no response to IAA was observed at 28 C (Fig. 1). The percentage of ovules which responded to IAA at the lower temperature was greatly increased by treatment with either 0.5 μM GA_3 or 2.5 mM ammonium (Fig. 1). Thus, at a temperature which restricted the ability of IAA to induce fiber formation, both GA_3 and ammonium effectively restored this ability. These results agree with earlier data (3).

Treatments which stimulated the formation of fiber were suspected of affecting NR activity as well. Before testing this hypothesis, we first followed NR activity in ovules, and the effect of nitrate upon that activity before and after initiation of culture. Ovules *in vivo* had a low but detectable level of activity. Preculture of the floral branch in nitrate-free medium had little effect upon ovular NR activity, and preculture in nitrate-containing medium caused no significant increase over the controls (Fig. 2). In contrast, preculture in nitrate induced considerable NR activity in ovary walls from the same experiments (Fig. 3). These results indicate that preculture is an effective means of forcing nitrate into the ovary. It is unclear whether ovules *in vivo* are incapable of responding to increasing levels of nitrate or (more likely) whether that nitrate is unavailable to the ovules.

Upon their removal from the ovary, ovules gained the ability to respond to added nitrate. In just the 1st hr after placing ovules in the basal culture medium (50 mM KNO_3 and 120 mM glucose), NR activity increased more than 4-fold (Fig. 4). In ovules cultured without KNO_3, activity also increased, but much less than in the



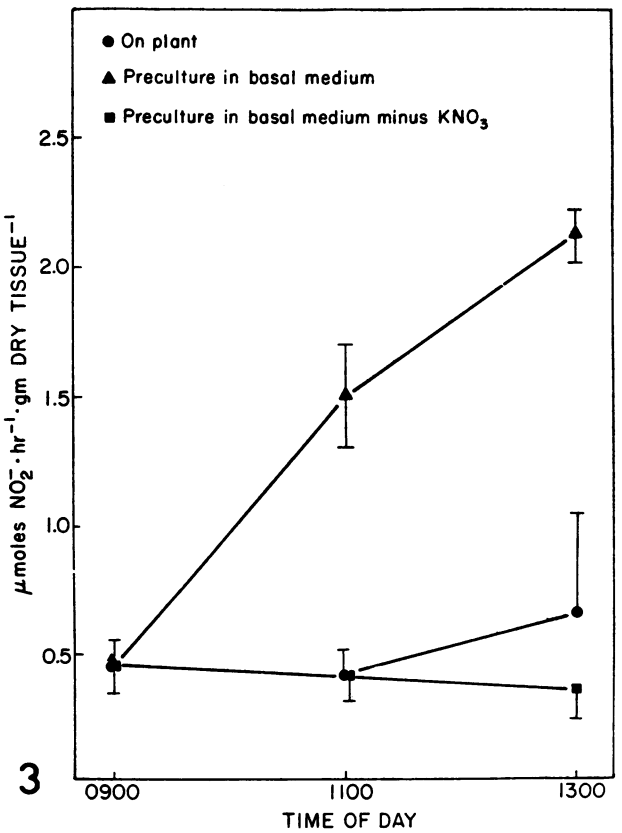
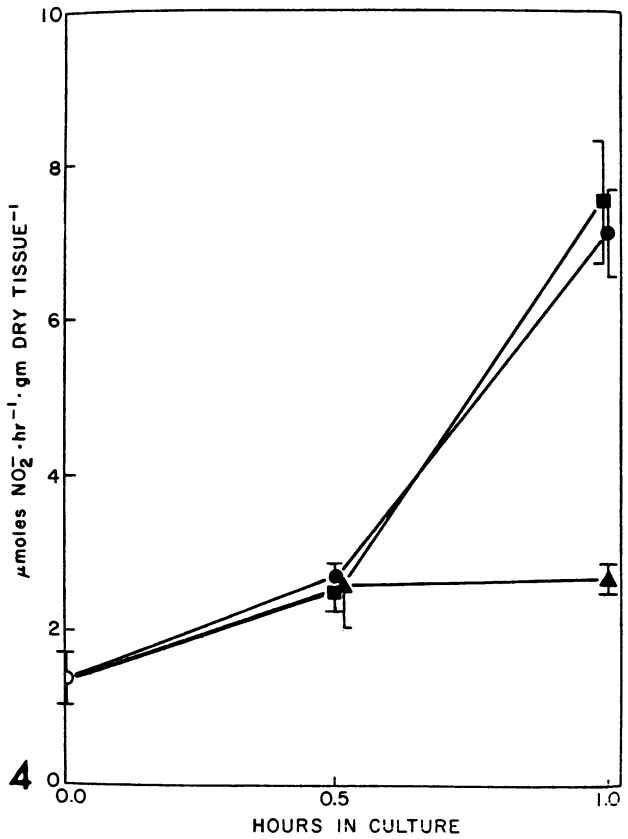


FIG. 2. NR activities from ovules at different times of the day, when floral branches were precultured (beginning at 0900 hr) in basal culture media lacking (■) or containing (▲) 50 mM KNO_3. Enzyme activities in ovules from those treatments are compared with activities in ovules taken directly from parent plants (●).

FIG. 3. NR activity from walls of whole ovaries, at different times of the day when floral branches were precultured (beginning at 0900 hr) in basal culture media lacking (■) or containing (▲) 50 mM KNO_3. Enzyme activities in ovary walls from those treatments are compared to activities in walls of whole ovaries taken directly from parent plants.



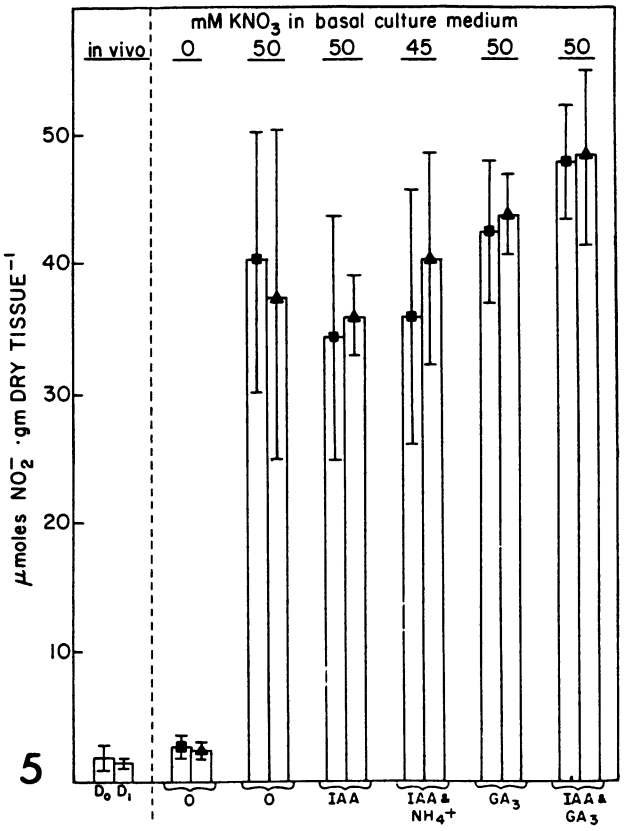


FIG. 4. NR activities in ovules taken from parent plants at 0800 hr (○) cultured in IAA-containing basal culture media (●) or in the same media that included an additional 50 mM glucose (■) or lacked KNO_3 (▲).

FIG. 5. NR activities from on-plant day-of-anthesis (D_0) and 1st-day-postanthesis (D_1) ovules, and from 1st-day-postanthesis ovules placed into various culture media at 28 C (■) and 34 C (▲) on the day of anthesis. Where indicated, IAA, GA_3, and NH_4NO_3 were included at 5.0 μM, 0.5 μM, and 2.5 mM, respectively.

nitrate treatment. Glucose in excess of 120 mM did not further promote NR activity (Fig. 4). Activity continued to increase in the presence of nitrate, and reached approximately a 20-fold increase after 24 hr of culture (compare Figs. 4 and 5).

In ovules cultured for 24 hr, there were no apparent differences in NR activity with IAA, GA_3, or temperature treatments (Fig. 5). Similarly, ammonium at 2.5 mM had no significant effect (Fig. 5). In all cases, activity increased greatly from *in vivo* levels, but apparently the only factor of importance was increased nitrate availability during *in vitro* culture. Even though increased temperature and other treatments increased the channeling of synthetic materials into fiber initiation, the induction of NR activity was seemingly regulated independently of this process.

DISCUSSION

There is little doubt that ammonium is intimately involved in the growth and differentiation of cotton ovules. Addition of relatively small amounts of ammonium (5% of total N of the medium) causes large changes in fiber formation during culture. These changes are similar in many respects to those brought about by increased culture temperature or the presence of GA_3. Stewart and Hsu (10) recently reported that ammonium is essential to the normal development and maturity of embryos of fertilized cultured ovules. Ammonium also is necessary, but not always sufficient, for the production of interspecific hybrid embryos from parents which are normally incompatible (11).

Despite the dramatic effects of IAA, GA_3, and temperature on fiber development, we were unable to find any parallel effects on ovular NR activity. We concluded that their mechanism(s) of action is unrelated to the availability of reduced N as a general substrate for growth. Also arguing against such a mechanism is the qualitative ("all-or-none") nature of the response to ammonium. This ion affects the percentage of ovules forming fiber, but not the extent to which any one ovule produces it (3). Such a phenomenon suggests a relatively specific mechanism of control. If N availability were the only parameter, one might expect a quantitative response to ammonium from each competent ovule. Extensive data (not shown) further indicate that N fertilization, water stress, and other factors which influence N assimilation in the whole plant have no effect upon this response to ammonium.

The specificity of the ammonium effect is difficult to reconcile with any general changes in metabolism; this situation is similar to the ammonium inhibition of flowering in *Lemna* (6). Our results suggest strongly that NR activity of young cotton ovules is determined by nitrate, and not by ammonium or hormone additions to the culture medium. Other possible mechanisms by which ammonium or hormones may regulate fiber development remain untested.

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