INDUCTION AND DEVELOPMENT OF SUBMERGED COTTON FIBERS IN VITRO

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Abstract

Cotton ovules grown in culture float on the liquid medium and produce fibers only on the surface exposed to air. For the first time, fibers have been induced from the submerged portion of the ovule. Five media containing different combinations of IAA and GA_3, as well as five different ages of ovules were used to study induction and development of submerged fiber growth. The induction rate was determined by the ratio of the number of ovules producing submerged fibers to the total number of ovules in culture. Hormone combinations and the age of ovules affected the induction of submerged fiber growth. Relatively high levels of IAA (5-20 μM) were required to induce the growth of submerged fibers. In addition, 0 DPA and -1 DPA ovules had more potential to produce submerged fibers than other ages of ovules. Other factors, such as pH of the medium, may also play a role in submerged fiber induction and growth.

Introduction

About three decades ago, a method for growing cotton fibers in ovule culture was first described (Beasley, 1971). Cotton fiber development has been shown to be driven by turgor pressure and mediated by plant hormones (Basra, 1984). Fertilization is critical for fiber growth when the culture medium does not contain exogenous plant hormones (Beasley, 1971). To induce fiber growth from unfertilized cotton ovules, exogenous plant hormones, including IAA and GA, act as an alternative to fertilization (Beasley, 1973). Liquid medium has been shown to be better than agar solidified medium for fiber development. On solidified media, the ovules tend to form callus tissue or fail to grow (Beasley, 1971). It has been observed that epidermal cells on the surface of the cotton ovule contacting the liquid culture medium do not form fibers. Many researchers (Dhindsa et al., 1976; Hof and Saha, 1998; Kloth, 1989; Triplett et al., 1995; Triplett 1998; Triplett and Johnson, 1999; Xie et al., 1993) have used this culture method, and others (Trolinder et al., 1987; Davidonis and Hinojosa, 1994; Seagull, 1986, 1990, 1992; Huang and Xu, 1996; Haigler et al, 1991) have modified it in various ways; however, to our knowledge there are no reports concerning the successful induction and growth of cotton fibers submerged in the culture medium.

Recently, submerged fiber growth was induced and characterization of submerged cotton fibers was described (submitted, Feng and Brown, 1999). The mechanism of submerged fiber growth is not clear. In our preliminary results, the induction of submerged fibers seem to be affected by plant hormone combinations and the age of ovules at induction.

In the present work, we have investigated the effects of five combinations of IAA and GA_3 in MS medium (Murashige and Skoog, 1962) on the induction of submerged fiber growth. Five ages of ovules, from -2 DPA (days post anthesis, i.e. 2 days before anthesis) to 2 DPA (2 days after anthesis) were used in ovule culture. The results confirm that exogenous plant hormones and the age of ovule are factors involved in the induction and development of submerged cotton fibers.

Discussion

Effects of the Age of Cotton Ovules at Induction

Our study showed that 0DPA and 1DPA ovules had more potential to produce submerged cotton fibers than other ages of ovules. Older fibers appeared to be more vulnerable to the osmotic pressure than fibers that had just emerged or were about to emerge. Another possibility is that the older fibers are more sensitive to the low pH of the medium. It has been shown that the acid growth mediated by expansins is required for plant cell elongation apart from turgor pressure (Rayle and Cleland, 1992). The effects of pH of medium on submerged fiber growth are under continuing investigation.

Effects of IAA and GA_3 Combinations

Plant hormones play an important role in fiber cell initiation from cotton ovules, especially in unfertilized ovules. GA and IAA can also induce increased production of fiber growth in vivo (Beasley, 1973). Our study indicated that a low level of IAA (1μM) failed to induce submerged fibers except in -1 DPA ovules. A higher level of IAA (5-20 μM) was required for submerged fiber growth. This suggests that IAA promotes cell elongation and, at the same time, might have some involvement with adaptation of the fiber cell to water stress. However, we observed that the induction rate did not increase when the IAA level was raised from 5μM to 20 μM.

The level of exogenous GA was also important. When we raised the GA level from 0.5 μM to 2.5 μM while maintaining IAA at 10μM, no increase in the induction rate was observed. In our previous study (Feng and Brown, 1999), we observed that raising the GA level to 5-10 μM (with 10 μM IAA) inhibited the growth of submerged fibers. Thus, the role of GA and IAA in submerged fiber growth is still not clear.

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Differences Between the Induction of Submerged Fibers and Air-grown Fibers

The hormone combinations did not show any obvious effects on air-grown fibers, whereas they did have an influence on the production of submerged fibers. This phenomenon might be interpreted on the basis that the submerged portion of the ovule could be more sensitive to IAA and GA_3 in the medium than the surface exposed to air. Almost all of the older ovules, especially fertilized ovules including 2 DPA, 1 DPA, and 0 DPA produced air-grown fibers. However, younger ovules that were unfertilized, including -1 DPA and -2 DPA, produced air-grown fibers at a lower rate. This suggests that there may be factors triggered by fertilization other than plant hormones which induce fiber growth from ovules.

Summary

Optimal hormone combinations induce the growth of fibers on the submerged surface of cultured ovules. The age of the cotton ovules also affected the rate of induction of submerged fiber growth. The contribution of pH may also have important effects on submerged fiber growth and development.

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Table 1. The induction rate (%) of submerged fiber growth related to the exogenous hormone combinations and the age of ovules at induction.

	0.5 µM GA_3 + 1µM IAA	**0.5 µM GA_3 + 5µM IAA**	**0.5 µM GA_3 + 10µM IAA**	**0.5 µM GA_3 + 20µM IAA**	**2.5 µM GA_3 + 10µM IAA**	**avg**
2d	0	15.63	14.29	0	0	5.98
1d	0	46.40	54.17	0	80.47	36.21
0d	0	84.94	88.49	87.50	57.25	63.64
-1d	4.55	53.86	81.00	79.09	56.89	55.08
-2d	0	35.38	1.67	23.40	18.18	15.73
avg	0.91	47.24	47.924	38.00	42.56	35.33

Table 2. The induction rate of air-grown fiber growth related to the exogenous hormone combinations and the age of ovules at induction.

	0.5 µM GA_3 + 1µM IAA	**0.5 µM GA_3 + 5µM IAA**	**0.5 µM GA_3 + 10µM IAA**	**0.5 µM GA_3 + 20µM IAA**	**0.5 µM GA_3 + 10µM IAA**	**avg**
2d	100.00	100.00	100.00	100.00	96.97	99.39
1d	96.97	98.65	100.00	100.00	98.00	98.72
0d	95.83	100.00	97.14	100.00	100.00	98.59
-1d	69.70	81.74	97.92	97.83	83.22	86.08
-2d	43.65	78.13	35.00	54.03	81.82	58.53
avg	81.23	91.70	86.01	90.37	92.00	88.26