

Callus Initiation from Cucumber (Cucumis sativus L.) Fruits

H. Abd. Aziz, B. H. McCown, and R. L. Lower, Department of Horticulture, University of Wisconsin, Madison, Wisconsin 53706.

A tissue culture system may be useful in rescuing potentially-resistant fruits from tests using Rhizoctonia solani and Pythium aphanidermatum. A screening program with subsequent callus initiation and plantlet regeneration from the selected tissues has been proposed. Callus initiation from fruit tissues of some plants has been reported in the literature (1,2,3,4, 5,6), but not from cucumber fruits.

Prior to experimentation with fruit tissue, cucumber shoot cultures were established using shoot meristems of non-embryonic origin. Callus was initiated from interstem pieces taken from shoot cultures and subsequent plantlet regeneration was attempted. Shoots were easily cultured, but exuberant callus growth was difficult to achieve. Root regeneration was easily obtained, but subsequent shoot regeneration was difficult. Greenish nodules from this callus were postulated as potential sites for plantlet regeneration. Following this preliminary work, callus initiation from fruit tissues was explored.

Fruit tissue may be more recalcitrant than stem tissue, thus a strong auxin such as 2,4-dichlorophenoxy acetic acid (2,4-D) may be needed. Slices of cucumber fruits of 4 different ages (3,5,7, and 14 days after pollination) were placed onto solidified Murashige and Skoog medium supplemented with 0.1 μM benzyladenine (BA), 1 μM naphthalene acetic acid (NAA), and one of the following concentrations of 2,4-D: 0, 0.01, 0.1 or 1 μM . Two slices were placed in each culture jar and each treatment was replicated 5 times. Cultures were grown under continuous fluorescent light at 27°C - 30°C. The ability of each explant to form callus was determined at the end of the fourth week of growth.

Less vigorous callus initiation was observed on fruit tissue as compared to that of stem internode tissue. The youngest tissue (3 days after pollination) produced the greatest growth (Table 1). Whitish-green gel-like callus occasionally formed at the peripheral layer and at cut surfaces. This callus was subcultured onto MS medium supplemented with 0.1 μM BA and 1 μM NAA and greenish nodule formation was observed. No callus was initiated in 7 and 14 day old tissues. On these explants, fleshy tissues turned dark brown, and browning of the epidermal layer was observed. Very low or no 2,4-D resulted in more callus growth than higher levels of 2,4-D.

Our results showed that the best callus growth was initiated from younger tissues. Sommer et al. (6) showed that peach mesocarp cells retained the ability of cell division after ripening. The ability of mature fruit tissue to form callus has also been reported by others in apple (1), avocado (5), citron (3) and peach (2). Whether the same is true for an herbaceous annual, such as cucumber, is not fully known. However, our results indicate that mature fruits cannot be induced to form callus and, subsequently, shoots.

Table 1. Effects of fruit age and 2,4-D concentration on callus initiation from cucumber fruit tissues after 4 weeks in culture^Z.

2,4-D Conc (uM)	Fruit Age After Pollination (Days)			
	3	5	7	14
0	1.0 ± 0.71	0.8 ± 0.84	0	0
0.01	1.4 ± 0.89	0.6 ± 0.55	0	0
0.1	0.8 ± 0.84	0.6 ± 0.55	0	0
1	0.6 ± 0.55	0.2 ± 0.45	0	0

^ZResults are the mean number of explants per jar with callus formation + S.E. for 5 replicates. F-test at 5% level shows no significant differences among concentrations used.

Besides the physiological stage of growth of the explants, the growing medium also plays an important part in callus initiation. This includes the nutrients as well as the hormones added to the medium. According to Schroeder (4), fruit tissue can be established in vitro if proper nutritional conditions are provided.

Results from our experiment showed that callus initiation from fruit tissue is possible, but age is a major obstacle in mature fruits. More detailed research should be conducted to determine if viable tissue which is capable of mitosis exists. Pedicel and epidermal layers are 2 good candidates. Greenish nodules may be the site of plantlet regeneration and further work on the developmental biology of such nodules is warranted.

Literature Cited

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