Results and discussion. Nucleoli of interphase nuclei of the paraffin-sectioned and squashed explants frequently contained material that was very refractile under phase contrast microscopy. In the sectioned tissue this refractile nucleolar material appeared at times as filamentous structures and at times as 'granules' depending upon its spatial orientation within the nucleoli. Squash preparations, however, consistently revealed the presence of a definitive filamentous nucleolar component (Figures 1, 2, 3a, 3b) which oftentimes looked like the nucleolonemata described by La Cour[6] (compare Figure 1 with Figure d of Plate II of reference number [6]). Compression of the refractile nucleolar material into few optical planes during squashing was most likely a factor responsible for the more definitive optical resolution of its filamentous nature in the squash preparations than in the sectioned material. Physical displacement of this nucleolar component in the squashed tissue also served to substantiate its filamentous nature since its linear structure was evident regardless of the varied shapes that it assumed during squashing.

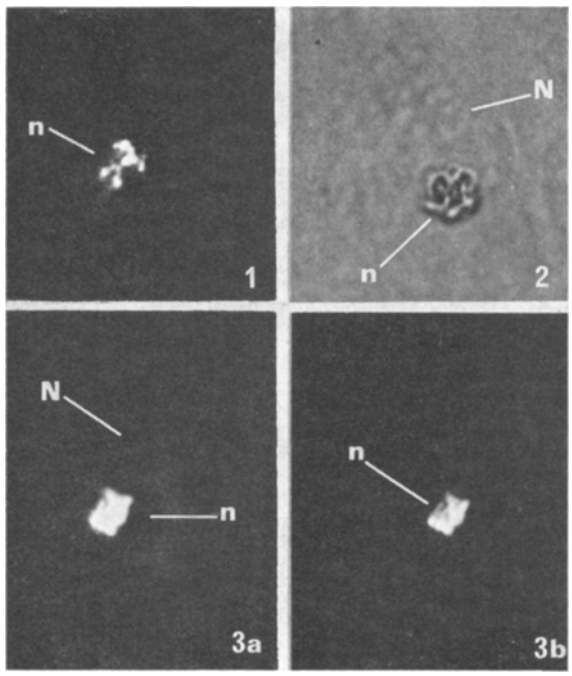

Fig. 1. Unstained squash preparation showing the prominent and highly refractile nucleolonema in the nucleolus. (See Figure d, Plate II of reference number [6].) Phase contrast. × 1350.

Fig. 2. Unstained squash preparation which clearly shows the filamentous nature of the nucleolonema. Bright field microscopy using a Balzers green interference filter (547 nm). × 1350.

Figs. 3a, 3b. Unstained squash preparation showing the highly refractile nucleolonema in the shape of a rectangle. Note that the filamentous nature of the nucleolonema in 3a is obscure and the nucleus and nucleolus are visible whereas in 3b the filamentous nature of the nucleolonema is apparent and the nucleus and nucleolus are no longer visible (see footnote [12] of reference number [14]). Phase contrast. × 1350. N, nucleus; n, nucleolus.

Fabbri[15] has suggested that nucleoli of plant cells contain granules which may give the appearance of being filamentous structures because of optical artifacts. Estable and Sotelo[5] consider nucleolar inclusions such as granules, vacuoles, or networks described by other investigators as being optical misinterpretations of the filamentous nucleolar component they call the nucleolonema. Figures 3a and 3b shown here are of particular interest in that the filamentous nucleolar component has assumed a rectangular appearance following squashing, a shape not likely to be formed from random orientations of granules or vacuoles. The persistent linearity within the refractile nucleolar component irrespective of the overall shape assumed under the physical pressure of squashing likewise would not be expected if this nucleolar component consisted of randomly-situated inclusions such as granules or vacuoles instead of a filamentous structure.

The observations described here do not preclude the existence of various kinds of nucleolar inclusions described by other investigators[3,4]. They do, however, confirm the presence of a filamentous component, the nucleolonema[5–7,16], in nucleoli of interphase nuclei of lemon fruit tissue.

Zusammenfassung. Die Anwesenheit eines Nukleolonemas in den Zwischenphasennukleolen des Zitronenfruchtgewebes wird bestätigt.

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[8] H. A. Kordan and L. Morgenstern, Expl. Cell Res. *28*, 133 (1962).
[9] H. A. Kordan and L. Morgenstern, Expl. Cell Res. *30*, 98 (1963).
[10] H. A. Kordan and R. D. Preston, Nature 216, 1105 (1967).
[11] H. A. Kordan, Experientia *25*, 743 (1969).
[12] H. A. Kordan, Phyton *26*, 31 (1969).
[13] D. A. Johansen, *Plant Microtechnique* (McGraw-Hill Book Co. Inc., New York and London 1940), p. 45.
[14] H. A. Kordan, Experientia *25*, 517 (1969).
[15] F. Fabbri, Caryologia *16*, 715 (1963).
[16] C. Estable, Natn. Cancer Inst. Monograph *23*, 91 (1966).

In vitro Induction of Flowering in *Cucumis sativus* L.

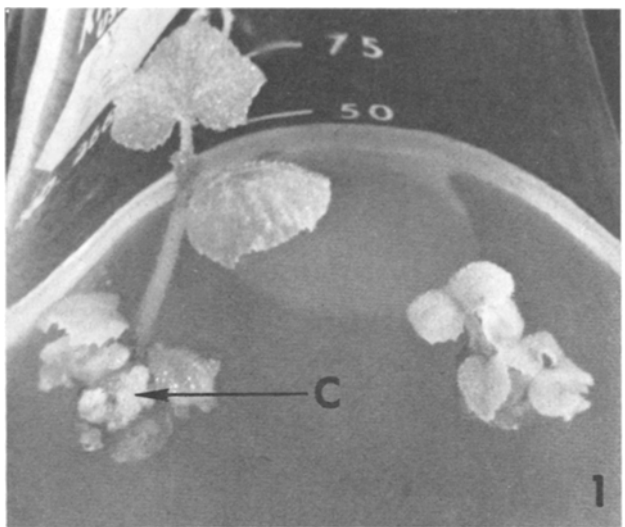
Sex expression in cucumber has been studied by using either whole plants in vivo[1,2] or isolated floral buds in vitro[3]. Recently, we have found that isolated shoot apices of this plant can be grown into plants which produce flowers in vitro. This technique should prove very useful in studying the effects of growth regulating chemicals and environmental factors on sex expression of cucumber.

Seeds of *Cucumis sativus* L. var. 'Long Green Improved', a monoecious variety, were obtained from a commercial seed supply house. They were sterilized in a

[1] C. E. Petersen and F. J. Kribben, Science *131*, 1673 (1960).
[2] D. Atsmon, A. Lang and E. N. Light, Plant Physiol. *43*, 806 (1968).
[3] E. Galun, Y. Jung and A. Lang, Dev. Biol. *6*, 370 (1963).

5.25% sodium hypochlorite solution for 8 min and rinsed 5 times in sterile, glass redistilled water. The seeds were germinated in Petri plates on a sucrose (2.0%) agar (0.8%) medium that was adjusted to pH 5.5 before autoclaving at 121 °C for 20 min. Each plate contained approximately 10 seeds and was placed under continuous fluorescent illumination at 25 ± 2 °C for 5 days to allow germination.

The method of dissecting out the shoot apex was similar to that used by BALL[4]. A microscalpel, consisting of a piece of broken razor blade held in a hemostat, was flame sterilized and used to remove the apex under a dissecting microscope. In this way, only 1 or 2 of the youngest visible leaf primordia remained with the apex. Each excised apex was placed upright on culture medium in 125 ml Erlenmeyer flasks. Our basic nutrient medium was the one used by VASIL[5] for another culture. The culture medium was adjusted to pH 5.5 before autoclaving at 121 °C for 20 min. All inoculated cultures were maintained at 25 ± 2 °C under continuous fluorescent illumination of about 225 foot-candles.



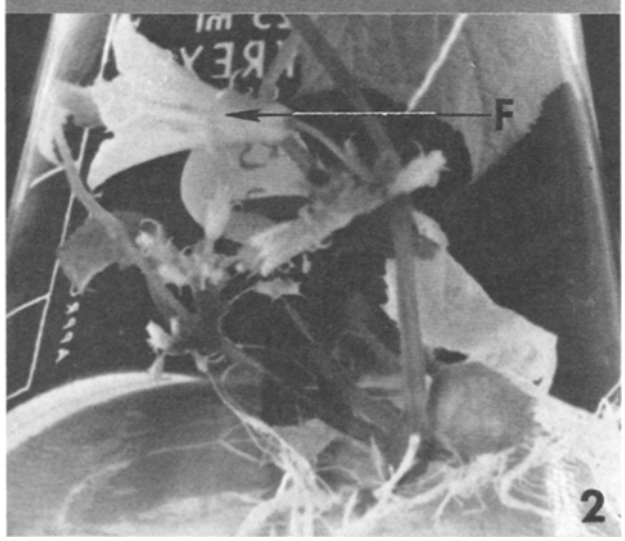


Fig. 1. Young plants of *Cucumis sativus* 2 months after inoculation of shoot apices. Note the callus (C) at the base of the plant. × 1.5.
Fig. 2. Four-month-old culture of *Cucumis sativus* bearing male flowers. One of the male flowers (F) is almost as large as those produced in vivo. × 1.5.

Vegetative shoots developed from the apices and small leaves could be detected within 2 weeks after inoculation. Shoot growth was accompanied by proliferation of a small amount of callus (Figure 1) where the stem base was in contact with the medium. Such callus tissue grew slowly and ceased to enlarge after a few weeks. Subsequently it produced many roots. Root formation by the callus was observed only after several mature leaves had developed on the shoot.

Between 60 and 90 days following inoculation, flower buds were observed in several cultures which contained 10% coconut milk in addition to the basic nutrients. These floral buds developed into apparently normal male flowers (Figure 2) with the characteristic bright yellow color. Pollen produced by these flowers appeared morphologically identical to in vivo pollen samples when examined microscopically. Germination tests were not made.

Flower buds continued to be produced by these cultures for 4 months. At no time during that period have female flowers been observed.

GALUN et al.[3] have observed that monoecious cucumber plants of the York State Pickling variety produce only male flowers on the first 20 nodes when grown under long-day conditions in vivo. As our cultured plants did not exceed 20 nodes in length, in vitro sex expression might follow the same tendency.

The induction of flowering in cultured stem tips has been reported in some plants[6–10]. In many of these reports, the original inoculum included young leaves or cotyledons in addition to the shoot apex. In *Helianthus annuus*, HENRICKSON[8] showed an absolute requirement for some specific amount of cotyledonary tissue to remain attached to the excised stem tip in order to induce flowering in vitro. However, we have observed flowering in cultures of *Cucumis sativus* in which all but the youngest leaf primordia had been removed prior to inoculation.

After having achieved the successful growth of shoot apices and their flowering in vitro, we are now conducting experiments to study the effects of auxins, gibberellins, kinins, photoperiod, and temperature on the expression of sex in cucumber[11].

Zusammenfassung. Erster Bericht über die Blütenbildung einer höheren Pflanze (Schösslinge von *Cucumis sativus* L.) im Reagenzglas und mit synthetischem Nährstoff, welche in der Natur männliche und weibliche Blüten hervorbringt.

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[4] E. BALL, Am. J. Bot. *33*, 301 (1946).
[5] I. K. VASIL, J. exp. Bot. *10*, 399 (1959).
[6] V. RAGHAVAN and W. P. JACOBS, Am. J. Bot. *48*, 751 (1961).
[7] B. BALDEV, Ann. Bot. *26*, 173 (1962).
[8] C. E. HENRICKSON, Plant Physiol. *29*, 536 (1954).
[9] P. R. WHITE, Protoplasma *19*, 97 (1933).
[10] S. LOO, Am. J. Bot. *33*, 295 (1946a).
[11] The authors wish to thank Messrs. K. D. RATLIFF and H. L. SETSER for their help in this project, Dr. M. J. KASPERBAUER for critical suggestions, and the University of Kentucky Research Foundation for financial assistance.