Planta (Berl.) 67, 384—386 (1965)

Short Communication

From the Department of Botany, University of Delhi, Delhi 6, India

PRODUCTION OF ADVENTITIOUS EMBRYOIDS *IN VITRO* FROM STEM CALLUS OF *FOENICULUM VULGARE*

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With 1 Figure in the Text

(Received September 22, 1965)

In the last few years considerable interest has been shown in the differentiation of embryoids and buds *in vitro*. However, in spite of many attempts there are only a few reports of organisation in calli originating from stem. Examplewise, one might mention the investigations on tobacco pith tissue (SKOOG and MILLER, 1957), potato tubers (WURM, 1960) parsley petiole (VASIL et al., 1964) and the petiole and umbellet peduncle of carrot (HALPERIN and WETHERELL, 1964). The present report is concerned with the formation of embryoids in a callus obtained from the stem of *Foeniculum vulgare* MILL.

After sterilisation with chlorine water, segments of the stem were inoculated on White's basal medium (WHITE, 1943). Proliferations occurred from the cells of upper cortical region (Fig. 1a). These were capable of continuous growth on Nitsch's basal medium (NITSCH, 1951) supplemented with coconut milk (CM) and produced a friable yellow callus (Fig. 1b). No differentiation was observed even after ten months. However, when the callus was transferred to a fresh medium (Nitsch's basal or Nitsch's basal + coconut milk) it exhibited a differentiation of not only roots but also embryoids in about three weeks. The embryoids developed into buds having two or three leaf primordia. After four to five weeks, green leafy shoots measuring about 1—2 cm in length could be seen. Besides the coconut milk medium, the callus was also subcultured on Nitsch's basal medium (NB) fortified with β-indoleacetic acid (IAA), kinetin, 2,4-dichlorophenoxyacetic acid (2,4-D) and yeast extract, alone and in combination. The table shows the frequency of formation of buds and roots as well as the general growth response of the callus in the various media. The growth of the callus was greatly enhanced by the addition of 2,4-D. Bud formation occurred in all media, including even the basal medium but was completely inhibited in the presence of 2,4-D. The callus was also grown in suspension cultures on a rotary shaker where also numerous embryoids arose. Many of these developed into leafy shoots.

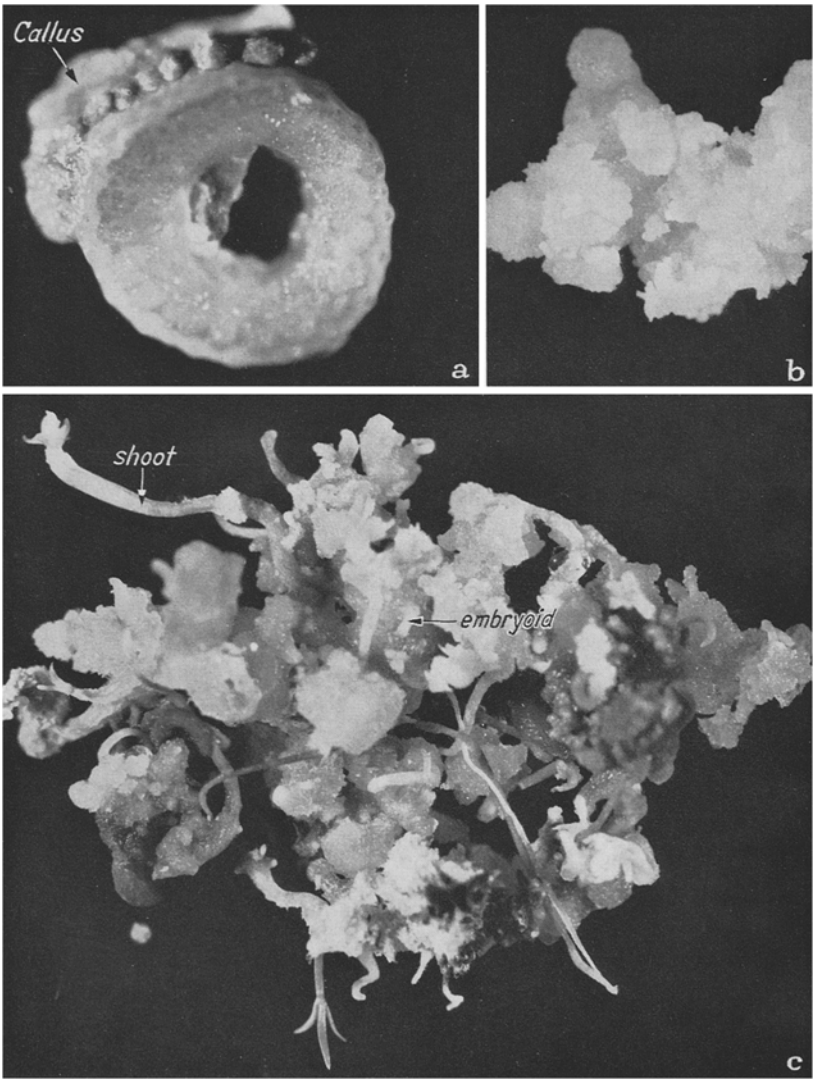

Fig. 1. a A stem segment showing initiation of *callus* on basal medium. × 8. b Subcultured *callus* on NB + CM. × 7. c *Embryoids* and *shoots* arising from the callus on NB. × 3

Squash preparations of the callus were made to determine the mode of origin of the embryoids. These revealed a large number of rounded nodule-like structures consisting of small cells, generally in organic union with larger cells of the callus. We presume, therefore, that these arise as a result of the meristematic activity of some of the peripheral cells of the callus and they become free only later after differentiation.

Table. *The behaviour of callus in different media (number of + signs indicates the degree of response; — indicates no response)*

Medium	Callus growth	Rooting	Bud formation
Nitsch's basal + 2% sucrose (NB)	+	+++	++++
NB + 15% coconut milk (CM)	+++	+	+
NB + 15% CM + 5 ppm 2,4-D	++++	—	—
NB + 15% CM + 10^{-6} M kinetin	+++	+	+
NB + 15% CM + 10^{-6} M kinetin + 5 ppm 2,4-D	++++	—	—
NB + 15% CM + 1000 ppm yeast extract . . .	++	—	+++
NB + 15% CM + 10^{-6} M IAA.	++	++	++
NB + 15% CM + 10^{-6} M kinetin + 10^{-6} M IAA .	++	+	+++

This is unlike carrot root where the callus breaks up into individual cells, each possessing the potentiality of developing into an embryo (STEWARD et al., 1958).

Another interesting point arising from this investigation is the fact that in the stem callus of *Foeniculum* differentiation is achieved even in the basal medium, whereas complex growth substances are needed to achieve the same result in most other calli investigated so far.

We are grateful to Professor P. MAHESHWARI, F.R.S., for his valuable counsel and facilities. Our thanks are also due to Dr. SIPRA GUHA for helpful suggestions.

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