BIOTECHNOLOGY LETTERS
Volume 16 No.4 (April 1994) pp.397-400
Received as revised 16th February

Callus induction and maintenance of *Zea mays* kernels.

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SUMMARY

Totipotent tissue cultures of maize (*Zea mays* L.) have previously been initiated from various explant tissues. In this paper, we present an alternative source of callus induction.

A callus of maize (G 204 hybrid) was obtained from intact kernels grown on Linsmair and Skoog RM medium supplemented with 20 mg 2,4-dichlorphenoxyacetic acid (2,4-D) per litre. The callus growth was greatest from the first node of the seedling shoot. Occasionally, callus growth was observed from the radicle and coleopticle regions. The callus was easily transferred and maintained on a medium with 2 mg/L 2,4-D. This callus formed numerous roots and leaf-like structures when grown on a medium containing 800 mg/L yeast extract, 30 g/L sucrose and 10 g/L agar.

INTRODUCTION

The ability to develop tissue and cell cultures from cereal crops has increased rapidly in recent years. Plant regeneration from maize tissue has mainly been obtained from callus derived from immature embryos (Duncan et al.,1985). Successful plant regeneration has also been reported from calli initiated from anthers (Ting et al.,1981), glumes (Suprasanna et al.,1986), immature tassels (Rhodes et al.,1986), leaf bases (Chang,1983), mesocotyls (Harm et al.,1976) and seedling segments (Santos et al.,1984). These alternate explant tissues permit selection of specific genotypes via genetic markers or particular plant phenotypes prior to culture initiation.

One of the most critical needs has been to establish callus

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cultures from which fertile plants can be regenerated. This, if it is successful, would provide a direct link between cell culture research and conventional genetic and breeding procedures.

This paper describes the initiation and maintenance of a callus culture from a new explant which is the intact kernel itself.

MATERIALS & METHODS

Dry maize kernels (G 204 hybrid) were sterilized by immersing them in 2.5% Na hypochlorite for 20 mins followed by three subsequent rinsings, in double sterile distilled water. They were then placed onto the media surface. A callus was initiated on modified Linsmaier and Skoog RM medium (Linsmaier & Skoog, 1965).

The culture were incubated at 30 ^{0}C in an incubator (growth room) at a light intensity of 1200 Lux. from cool, white fluorescent lights. The humidity was not controlled, therefore the jars were tightly covered with aluminium foil. Stock cultures were maintained by taking subcultures and transferring them to a fresh medium every thirty days.

In order to produce callus differentiation, 2,4-D concentration was reduced to 2 mg/L and 800 mg/L yeast extract was added.

RESULTS

Preliminary experiments with intact, sterile maize kernels (G 204 hybrid) indicated that no callus was formed on a medium containing 0 to 5 mg/L 2,4-D . However a compact, light- yellow callus was occasionally initiated at the first node of the seedling shoot after incubating kernels for thirty days at 30 ^{0}C on a medium containing 10 to 20 mg/L 2,4-D. The proportion of seedlings which formed a callus under these conditions was high [Fig. 1A].

The incubation of intact, sterile kernels at 30 ^{0}C for thirty days on a medium containing 20 mg 2,4-D/L was the most reliable method of induction of a maize callus. Normal morphological development of these kernels ceased in a few days and after two weeks the nodal region of the shoot began to enlarge visibly.

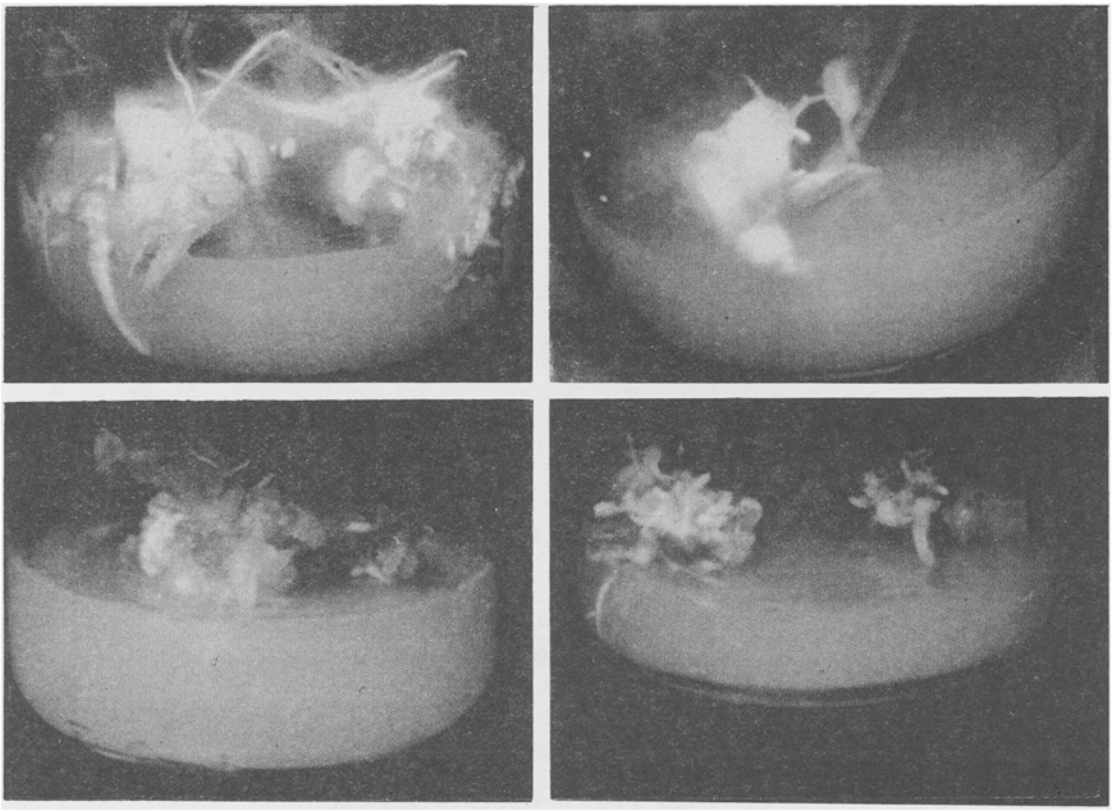

Fig. 1. Callus induction of *Zea mays* kernels.

(A) A compact, light-yellow callus at the first node of the seedling shoot.
(B) An increased growth of vegetative seedling tissue and reduced or prohibited callus formation.
(C) Type II callus [pale yellow, friable and rapidly growing callus].
(D) Formation of roots and leaf-like structures.

Further incubation promoted continued growth of a light-yellow callus around the first node region and occasionally on the seedling radicle and coleoptile. A concentration of 2,4-D below 15 mg/L allowed an increased growth of vegetative seedling tissue and reduced or prohibited callus formation [Fig. 1B]. After thirty days the callus was easily separated from the remaining seedling tissue and subcultured onto a fresh medium containing 2 mg/L 2,4-D. The callus growth rate substantially increased on this concentration [Fig. 1C].

Young maize seedlings contain an active meristem at the region of the first nodal region. This meristem developed in the various media with a high concentration of 2,4-D apparently converting the meristem to callus tissue . The callus formed numerous roots and leaf-like structures when growing on a medium containing 30 g/L sucrose, 10 g/L agar and 800 mg/L yeast extract [Fig. 1D].

DISCUSSION

The induction and maintenance of a particular callus culture, the so-called type II callus, which is white or pale yellow, friable and can be grown directly from *Zea mays* kernels can provide a simple, less time consuming and more economical source for callus and/or cell suspension production compared to other explants previously reported.

ACKNOWLEDGEMENTS

The authors would like to thank L. Peachey for her help in preparing the manuscript.

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