Initiation of Sugarbeet (*Beta vulgaris* L.) Callus[1]

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Introduction

The production of haploid plants from *Nicotiana tabacum* (5)[3] and parasexual hybridization by protoplast fusion and plant regeneration (1) have created considerable interest in tissue culture of agronomic crops. Haploid plants have been produced in several genera and tissue cultures were established in other genera (2).

This paper reports the initiation of callus from sugarbeet (*Beta vulgaris* L.) embryos, cytological observations, and notes on callus maintenance.

Materials and Methods

The tested embryos were from sugarbeet line C17 (4). Branches with seedballs in various stages of maturity were collected and cut into short sections, and the ends were dipped in paraffin to reduce water loss during handling. Branches were surface sterilized with 20% Clorox[4] solution for 10 minutes, rinsed in sterilized distilled water, dipped in 70% ethanol, and then rinsed again with sterilized distilled water. Fifteen immature embryos were dissected from ovaries and placed on the medium in 15 x 60 mm sterile, disposable petri dishes sealed with parafilm. The cultures were maintained in the dark in a germinator that fluctuates from 23° to 26°C.

Norstog's barley medium II (6) was modified to a 5 g/l concentration each of agar and sucrose. In 7 days, the ovules developed into seedling plants which were then divided into roots, hypocotyl, and shoot. Callus differentiated from the cotyledons on two embryo shoots within 7 days. In 6 weeks, the callus growth was sufficient to allow it to be removed from the cotyledons. The callus was then divided into several parts to increase the number of cultures and was placed on fresh medium with sucrose at 30 g/l. The embryo tissue

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[3]Numbers in parentheses refer to literature cited.

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from other ovaries was also transferred to this medium and left in the germinator. Callus was later isolated from two other embryo cotyledons.

Callus was also transferred to one medium that received 0.2 mg/l of 2,4D [(2,4-dichlorophenoxy) acetic acid] and another which included 0.2 mg/l of IAA (indole-3-acetic acid) and 5 mg/l of kinetin [6-(furfurylamino) purine]. Growth was measured by weighing the fresh callus on a precision balance.

Chromosome counts and cytological observations were made from smears prepared from small pieces of the callus. Pieces of callus were placed in a saturated solution of monobromonapthalene for 2-3/4 hours at 10°C, hydrolized for 11 minutes in HCl at 60°C and transferred to Feulgen's stain.

Results and Discussion

Good growth occurred on Norstog's medium with 30 g/l sucrose. This medium maintained and increased most of the cultures of callus. Most cultures died when placed on the medium with IAA and kinetin. Weight differences of eight individual callus isolates after 2 weeks in the medium without 2,4-D were 49.3, 44.2, 41.4, 32.8, 9.9, 0.2, and −2.6 mg. With 0.2 mg/l of 2,4-D weight differences were 48.1, 30.8, 6.0, 3.8, 0.0, and −0.2 mg. These results were variable, but more cultures had more growth on the medium without 2,4-D.

Most cells had 18 chromosomes, but cells with 54 chromosomes were observed. Also cells with two and three nuclei were observed, and one cell contained a dividing nucleus with two other nuclei. Nuclear fusion of three nuclei in such a cell would account for the 54-chromosome tissue.

Ingram and Joachim (3) had good results on coconut milk and Murashige and Skoog basal medium but their results were.inconsistent in that all sugarbeet varieties did not react the same. In this study, callus was initiated and maintained on Norstog's medium but differentiation did not occur. Other media and environmental conditions must be investigated to find the optimum conditions for sugarbeet tissue cultures.

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