Callus Formation and Plant Regeneration from *Nicotiana tabacum* "Mild Cure"

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Abstract

We studied callus formation and plant regeneration from *Nicotiana tabacum* "Mild Cure". Somatic embryogenesis and subsequent formation of plantlets were obtained from callus cultures derived from leaves of *Nicotiana tabacum* "Mild Cure". The callus was induced from leaf explants and grown on Murashige and Skoog's medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) 2 ppm and 3% sucrose. Reduction of 2,4-D concentration during subsequent subcultures resulted in formation of embryoids. On the other hand shoots and plantlets were generated when the auxin-free medium formed the embryoid was transferred to medium supplemented with 3% sucrose.

Introduction

The procedures of plant tissue culture have developed to such a level that any plant species can be regenerated *in vitro* through several methodologies. Recently various cells, tissues and organs from numerous plant species can be cultured successfully to regenerate whole plants [1-4]. The knowledge of plant regeneration have now become powerful tools for agricultural and horticultural research.

Nicotiana tabacum "Mild Cure", commonly known as tobacco, belong to the dicotyledonous family Solanaceae. The leaves of *N. tabacum* "Mild Cure" were used to make the taste of tobacco milder. The parameters of callus formation and plant regeneration from *N. tabacum* "Mild Cure" have not been elucidated yet. In this

paper, we describe parameters for initiating and maintaining callus cultures from leaves, and subsequent regeneration of whole plant in *N. tabacum* "Mild Cure".

Materials and Methods

Whole Plant Material and Induction of Callus: Excised young leaves (0.5—1.0 cm long) of *Nicotiana tabacum* "Mild Cure", collected from one year old plant, cultivated in the field of Niimi-shi in Okayama-ken, were cultured on MS besal medium[5] containing 2,4-dichlorophenoxyacetic acid (2,4-D) 2 ppm, 3% sucrose and 0.7% agar after surface sterilization as follows: the young leaves of *N. tabacum* "Mild Cure" were washed with distilled water for 20 minutes and washed with 70% ethanol solution for 30 seconds, then sterilized with 10% sodium hypochlorite solution for 7 minutes at room temperature. The leaves were washed 3 times with sterile distilled water, 5 minutes each. Furthermore the leaves treated with the above method was placed on the medium described above under aseptic conditions and cultured in the dark at 25°C. After one month of culture on MS medium containing 2,4-D 2 ppm, 3% sucrose and 0.7% agar, the callus formation occurred [Fig. 1 (A)].

The Formation of Tobacco Green Callus: The culture of the callus was maintained in the dark at 25°C and subcultured every 3—4 weeks. For the green callus formation the callus was transferred on 2,4-D free medium (MS, with 5 g/l sucrose and 0.7% agar) at 25°C with constant illumination (approximately 9,000 lux). During 10 days incubation the callus (white callus) was gradually changed to the green callus.

The Plant Regeneration from Tobacco Green Callus: For shoot induction the green callus was transferred on regeneration medium (MS, with 30 g/l sucrose, 0 ppm 2,4-D and 0.7% agar). After one month incubation at 25°C with constant illumination (approximately, 9,000 lux) the shoot occurred from the green callus [Fig. 1 (B)]. Further the shoot was transferred on regeneration medium. After one month incubation under the same condition as described above the root occurred from the shoot [Fig. 1 (C)].

Results and Discussion

The basal media B5 and MS were used in callus initiation. As shown in Table 1 it was found that the basal medium MS supplemented 2,4-D 2 ppm was able to in-

Table 1. Influence of MS and B5 media on callus formation from the young leaves of *Nicotiana tabacum* "Mild Cure" after four weeks of culturing.

Phytohormones		Callusing on different basal media[a)]	
(mg l^{-1})		MS	B5
2,4-D	(2.0)	+++	+
NAA	(2.0)	++	−

[a)] Frequence : − no response, + little, ++ moderate, +++ extensive.

duce callus. No callus was formed if 2,4-D was absent from the medium. The tobacco white callus, rapid growing callus, resulted when 2,4-D (2 mg l^{-1}) was used with mineral salts, microelements and vitamins of MS medium [Fig. 1 (A)], and the cultures were incubated at 25°C in the dark. Cytokinins were not required for callus induction and proliferation. The growth of callus was retarded if 2,4-D as auxin was substituted by α-Naphthaleneacetic acid (NAA).

We clarified the parameter for initiating and maintaining callus obtained from the young leaves of *Nicotiana tabacum* "Mild Cure": the medium for initiating and maintaining the tobacco callus was MS medium supplemented with 2,4-D 2 ppm, 3% sucrose and 0.7% agar.

To form tobacco green callus the white callus was placed on MS medium supplemented with 2,4-D (0, 2, 4 and 6 ppm). After 10 days incubation at 25°C with constant illumination (approximately, 9,000 lux) the green callus occurred on MS medium (2,4-D 0 ppm, sucrose 0.5%). For the green callus formation the effects of sucrose concentration, 0.5, 1, 3 and 5%, were studied. The best sucrose concentration was 0.5 per-cent. When the green callus was placed on MS medium supplemented with 2,4-D (0 ppm) and 3% sucrose, their constitution changed after 3—4 weeks of culturing. They become hard and green with localized dark regions, and with protuberances throughout the tobacco callus surface [Fig. 1 (B)]. Many of these protuberances were transferred on new MS medium (2,4-D 0 ppm, sucrose 3%) and produced the formation of shoots and roots [Fig. 1 (C)].

Thus we revealed the parameter for the regeneration of tobacoo whole plant: the medium for the regeneration of tobacco whole plant was MS medium supplemented

with 2,4-D 0 ppm 3% sucrose and 0.7% agar. On the other hand the methods of the regeneration from the protoplast of tobacco and/or obtained from cell fusion are now in progress.

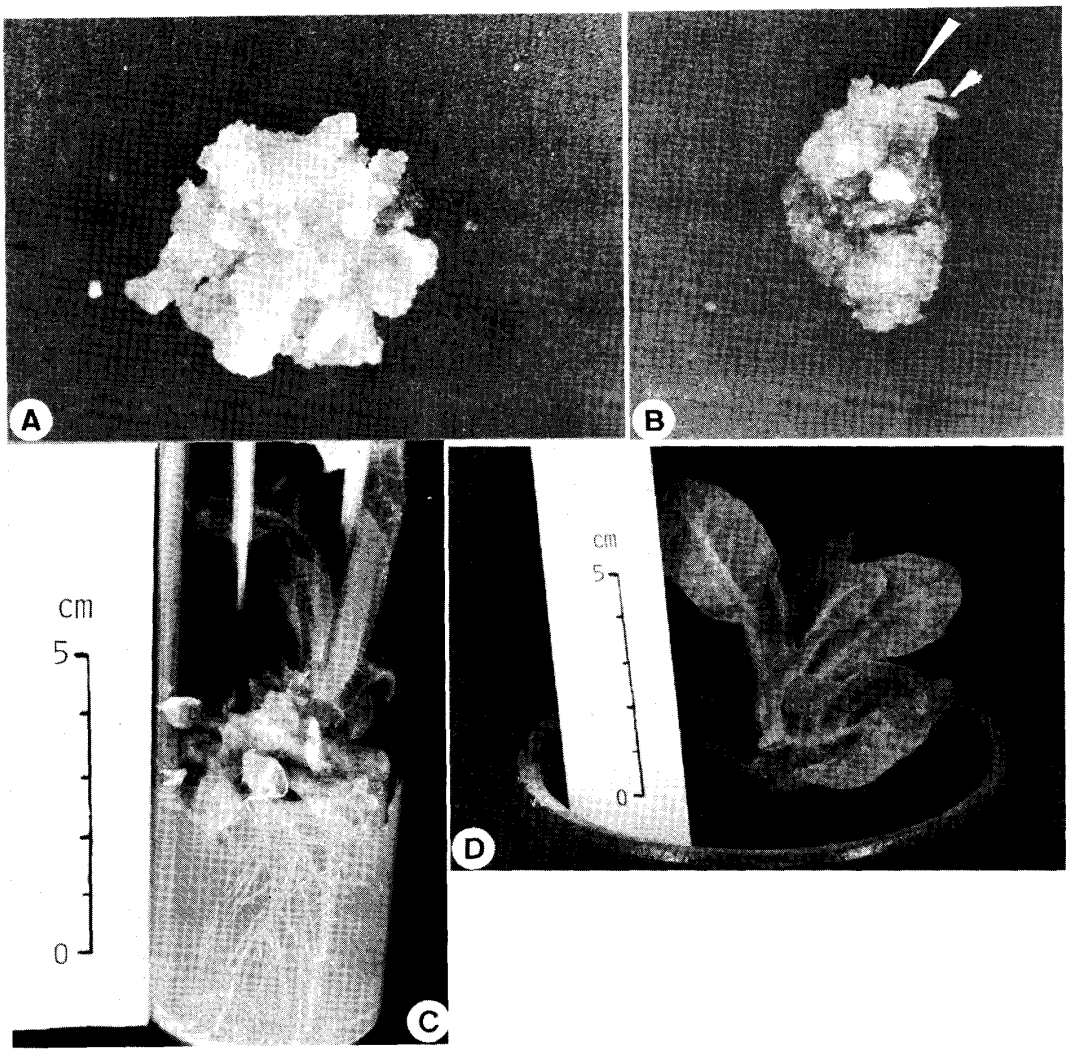

Fig. 1. (A)~(D). Callus induction and plant regeneration from excised young leaves of *Nicotiana tabacum* "Mild Cure". (A), white callus proliferation on MS medium supplemented with 2.0 mg l^{-1} 2,4-D. (B), the tobacco green callus showing apparent somatic embryoids on MS medium (2,4-D 0 ppm). (C), regeneration of plantlet from embryogenic callus on MS medium (2,4-D 0 ppm). (D), a tobacco plantlet after transfer to soil.

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