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In vitro Tissue Culture of *Aloe arborescens* Mill

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ABSTRACT

Aloe *in vitro* culture was attempted to induce callus and regeneration ability from different explant sources onto MS medium with 0.5mg/ *l* NAA plus 1.0mg/ *l* BA. Anthers that no developed any callus and plant regeneration, while only four out of 274 filament explants induced calli at cut edge without regenerated plants. Twenty ovary explants regenerated four direct plantlets without via callus from the base of epidermal tissues. Regenerated plants on the root tip gave 2n=14 of chromosome numbers.

Key words: *Aloe arborescens* Mill, tissue culture, anther, filament, ovary, chromosome.

INTRODUCTION

Aloe is a native north Africa and an important plant(Sapre, 1974)which valued of numerous medicinal and cosmetic preparation with components the juice from leaves(Natalia et al., 1990; Meyer and Staden, 1991), it has been used widely today in Korea(Yu et al., 1997).

Aloe is vegetatively propagated in its natural state(Meyer and Staden, 1991) due to a widespread male sterility(Keijzer and Cresti. 1987). Clonal propagation of selected elite genotype is highly desirable since male sterility in it. In many crops, micropropagation by tissue culture techniques can be increasingly used for large-scale production *in vitro* which will be overcame to propagate problems. However, reports on micropropagation of Aloe are not frequent in the literature.

The objective of this work was to determine the callus induction and organogenic response of different explants in *Aloe arborenscenes* Mill.

MATERIALS AND METHODS

Closed buds were collected pot grown plants when the petals were between 15 and 20mm. The buds were surface-sterilized by immersion in 75% ethanol for 30sec. and then 50%(v/v) sodium hypochlorite(NaClO) solution for 10min. and rinsed three times with sterilized distilled water.

Petal from sterilized bud aseptically removed and separated anther, filament and ovary, respectively. Anthers were observed in aceto-carmine and inoculated at late uninucleate stage. And filaments were cut 0.5mm in length and ovary separated from stigma.

For the medium, the callus induction medium was consist of Murashige and Skoog(Murashige and Skoog, 1962) medium supplemented with 0.5mg/ *l* Naphthalene acetic acid :NAA) + 1.0mg/ *l* 6-Benzylamino purine: BA) and 3% sucrose and 0.5% phytagel(Sigma). Plant regeneration was on MS basic medium without any growth regulators. The pH was adjusted to 5.7 and autoclaved at 121° C for 15min. Explants were cultured in petri dish (87x100 mm) containing 20ml of culture medium and maintained in the dark at 27° C for 4 weeks and subcultured with same components as initial medium. After that, calli from explants were transferred to MS hormone-free medium in petri dishes with 16hours/day of cool-white fluorescent light at an intensity of 3000 lux and allowed to regenerate plants.

Regenerated plants from explants of which root tips for chromosome check were carefully excised and pretreated

in 0.1% colchicine solution at room temperature for 24 hours. The root tips were fixed in acetic-alcohol(1:3) for 24 hours and stored in 70% alcohol until use(Marks, 1973). This tissue were hydrolyzed with 1N HCl at 600C for 8-12min. Squash were made in Fuchsin acid solution for staining the chromosome.

RESULTS AND DISCUSSION

The successful establishment of Aloe *in vitro* culture was found to be influenced the source of the explant materials. To test for their ability to induce callus and regeneration ability, excised materials such as anther, filament and ovary were cultured onto MS medium with 0.5mg/ *l* NAA plus 1.0mg/ *l* BA(Table 1).

The materials on the medium turned brown gradually in the dark culture. In general, callus was induced from the explants within 40 days. All of inoculated anthers that no developed any callus and plant regeneration, only produced a brown exudation and died. The most vigorous callus growth was observed on the filament culture. Out of 274 explants, only four induced callus at cut edge. At this stage, the fresh calli were yellow which gradually turned to be brown as they became old(Fig. 1A). It was observed that the use of BAP and 2,4-D caused browning and callus formation or, at lower BAP concentration, the developed of some primary explants(Natalia et al., 1990). Occasionally, small quantities of compact yellowish callus were formed from some filament bases. But no plant regeneration when the calli were transferred to MS hormone free medium. Since the callus was of non-nodular derived from filament and succeeded subculture, pieces of callus were transferred to hormone free medium. The frequency of shoot formation from the calli was failed with hormone free medium.

When culturing ovary in the dark, 4 plants out of 20 ovary explants regenerated direct plantlets without via callus from the base of epidermal tissues onto MS medium with 0.5mg/ *l* NAA plus 1.0mg/ *l* BA. Some of ovary explants were induced only root(Fig. 1B) without callus induction or plant regeneration. Attempt to regenerate plants were successful in ovary culture only. *In vitro* produced rootless shoots with a length of 2 to 3cm were transferred onto MS medium without regulators and roots were induced within 25 to 30days(Fig. 1C). After that we transferred it into pot and allowed to grow(Fig. 1D).

Regenerated plants on root tip gave 2n=14 of chromosome number(Fig. 1E). It is considered that regenerated plants were originated from somatic cells in ovary, not ovules from haploidal cells.

As it was reported, Aloe genus has been receiving little attention, for example, shoot regeneration by meristem culture only was reported for *Aloe arborenscenes* Mill(Yu et al., 1994 ; 1997). Different regenerate abilities have been observed in several plant tissues culture, but causes of this phenomenon are still unclear(Sanchez et al., 1988). *In vitro* culture very low morphologic ability was correlated with variation in nuclear DNA content occurring through in *Aloe barbadensis*(Sanchez et al., 1988).

The explants from ovary as an explants in this paper, direct organogenesis has led to a reduction in genetic variability in cultured *in vitro*. It might be useful way to introduce new genetic viability. In the present study, the use of ovary resulted in a significantly regenerated plants without genetic variability. Thus the advantage of plant regeneration directly can be exploited without the genetic viability.

Table 1. Tissue culture response of *Aloe arborenscenes* Mill on MS medium containing with 0.5mg/ *l* NAA and 1.0mg/ *l* BA

Explant	No. of inoculated	No. of inoculated callus (%)	Plant regeneration (%)
Anther	132	0(0)	0(0)
Filament	274	4(1.5)	0(0)
Ovary	20	7(35.0)	4(20.0)

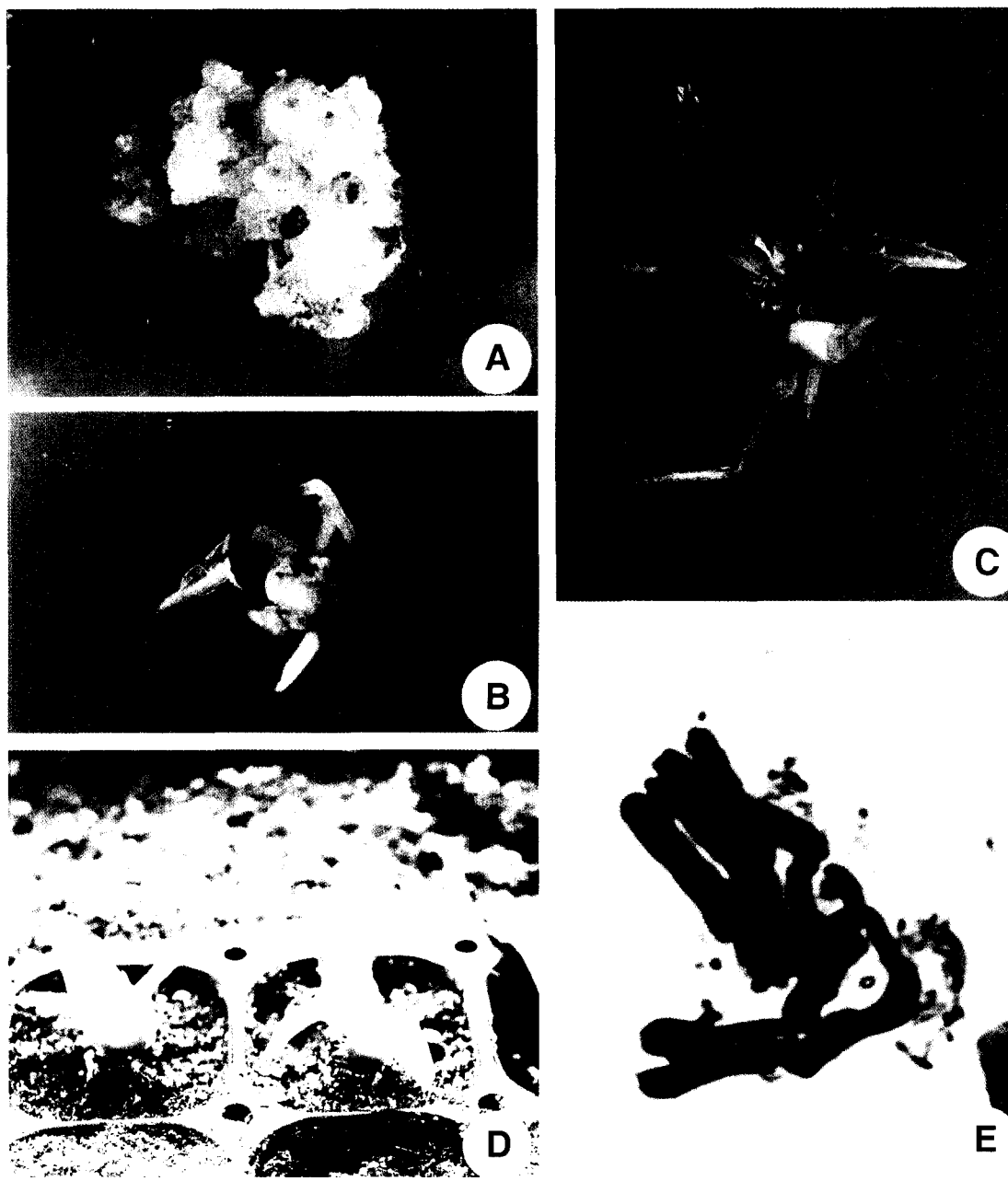

Fig. 1A-E. *In vitro* tissue culture of *Aloe arborenscenes* Mill. Callus induction from filament(A). Only root development(B), plant regeneration(C) and growth into pot(D) from ovary culture. Chromosome on the root tip of regenerated plant(E)

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