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High Frequency Shoot Regeneration from Leaf Explants of Cucumber

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Abstract

Leaf explants of the cucumber (*Cucumis sativus* L.) were cultured on Murashige and Skoog's (MS) medium supplemented with various concentrations of α-naphthalene acetic acid (NAA) and 6-benzylaminopurine (BAP). Direct shoot orgnogenesis as well as callus formantion with somatic embryos and multiple shoots was observed from leaf explants of cvs. Shinhukjinju and Chungjang. The highest frequency of shoot formation 80% was observed on MS medium supplemented with NAA/BAP (5.0 μM/2.5 μM), with explants forming 3-7 shoots. Shoots formation occured within 3 to 4 weeks. Only one subculture of calli was required for plant regeneration on normal growth regulator-free medium. Plantlets transferred to soil developed into plants of normal appearance, which flowered and set fruits.

Introduction

High frequency of plant regeneration from cultured cells or tissues is important for successful application of tissue culture technology to plant breeding.

Cucumber (*Cucumis sativus* L.) is an important vegetable crop widely cultivated all around the world. Only a limited success has been obtained in regenerating cucumber from variety of explants, e.g., cotyledons (Trulson and Shahin, 1986; Ziv and Gadasi, 1986; Kim et al., 1988; Chee, 1990), primary leaves (Chee and Tricoli, 1988), and hypocotyl segments (Rajasekaran et al., 1983; Ziv and Gadasi., 1986; Chee, 1990). Several phenomena including abnormal embryo development (Ziv and Gadasi, 1986), poor differentiation of callus into shoot, low survival rates of regenerated plants in soil, and changes in ploidy level (Wehner and Locy, 1981; Kim et al., 1988) have been reported.

The purpose of this study was to establish an efficient and reproducible method for regeneration of cucumber (*Cucumis sativus* L. cvs. Shinhukjinju and Chungjang). The successful regeneration of cucumber is a limiting step for transformation. For plant regeneration and transformation of cucumber have been successfully achieved (Chee, 1990). Here we study combinations of growth regulators to the basal medium, as well as the developmental stage of the leaf used as explant source for improved regeneration rates of cucumber genotypes.

Materials and Methods

Plant materials

Commercial F_1 hybrid seeds of cucumber (*Cucumis sativus* L. Shinhukjinju and Chungjang from Heung Nong Seed Co., Seoul, Korea) were used. Seeds were surface-sterilized by immersion in 95% ethanol for 30 sec, followed by soaking in 3% sodium hypochlorite for 5 min and rinsed three times with sterile distilled water. Seeds were placed in petri dishes containing MS medium (Murashige and Skoog, 1962), and germinated in the dark at 26℃ for 96 hours.

When the radicles emerged, the seeds were transf-

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Table 1. Influence of growth regulator combinations on callus and shoot formation of *Cucumis sativus* cv. Shinhukjinju

Growth regulator combination and concentration(μM)		Callus and shoot formation from the leaf	
		Callus formation (%)	Shoot formation (%)
NAA/BAP	2.5/0.1	100	0
	2.5/2.5	100	74
	2.5/5.0	100	13
	5.0/0.1	100	0
	5.0/2.5	100	80
	5.0/5.0	100	17

erred to culture vessels containing 100 mL of full strength MS basal medium in addition of 20 g/L sucrose. The medium was adjusted to pH 5.8 before the addition of 0.8% agar, and the mixture was then autoclaved for 15 min. Plantlets were cultured at a 21/25℃ night/day temperature regime with a 16 hours per day photoperiod provided by cool-white fluorescent lamps with 6000 Lux. The in vitro-grown seedlings were used as explant sources. Explants for culture were excised under sterilized conditions from the first and second normal foliage leaves of 20 to 25 days old seedlings.

Medium and culture condition

The basal medium consisted of MS medium supplemented with sucrose (30 g/L), myo-inositol (100 mg/L), and Bacto-agar (0.8 g/L). This medium was supplimented with several NAA and BAP concentration as described in Table 1. All plant growth regulators were added to the medium before autoclaving. Six to eight explants (5 × 5 mm) were cut from each leaf avoiding the midrib and transferred to the medium. Each dish containing up to 10 explants were placed in a growth chamber at 25℃ ± 2℃ with a 16 h photoperiod using cool white fluorescent lamps of 3000 Lux. After two weeks, the dishes were transferred in a growth chamber with 6000 Lux light intensity.

Plant regeneration

After 4 weeks of culture, embryo-like structure and shoot primodia appeared. They were subcultured onto growth regulator-free MS medium. After two weeks of subculture, calli were rated for frequency of regeneration (embryo or shoot formation) and the data were expressed as a percentage on the total number of explants tested. Subsequently, embryos or shoots were transferred to culture vessels containing growth regulator-free MS medium to allow shoot elongation and root proliferation. All tests were repeated at least four times.

After rooted plantlets reached a height of 10-15cm, they were transferred onto soil. They were acclimatized in the growth chamber for 10 days and then moved to greenhouse.

Results and Discussion

Callus or shoot formation from cucumber leaf explants depended on the concentration of growth regulators. The highest regeneration efficiency from all the combinations was obtained on MS medium supplemented with 5.0μM NAA and 2.5μM BAP (Table 1). During the first two weeks of culture, explants slightly expanded and callus formation began at the edge of the explant. Calli could be classified in two types depending on thir appearance; compact green embryogenic and white friable. After 2-3 weeks of culture, only the formed nodules, immature embryos and shoot buds (Figure 1A and B). These structures required subsequent subcultures on the same medium for the development of shoots. On occassion the direct shoot formation (Figure 1C) was observed even on callus forming explants.

Direct regeneration from somatic tissue is a rare phenomenon and has only been described in a few species such as *Daucus carota* L. (McWilliam et al., 1974), *Ranunculus sceleratus* L. (Konar and Nataraja, 1965), *Lycopersicum* (Young et al., 1987), and *Nicotiana tabacum* L. (Stolarz et al., 1991). Regeneration through embryogenesis and shoot formation in cucumber was previously noted in hypocotyl and cotyledon explants (Wehner and Locy, 1981; Kim et al., 1988). However, only a very low frequency of regeneration was obtained from cotyledons, and all plantlets explants were tetraploid, and none could be acclimated to soil (Kim et al., 1988).

The two cultivars (Shinhukjinju and Chungjang) tested in this study were different from each other in many morphological characteristics. Nevertheless, re-

Table 2. Number of shoots per explant and percentage of regeneration observed for two genotypes.

Genotypes	Growth regulator (μM)		Leaf age	Number of explants with shoots/total	% Regeneration	Number of shoots/explant
Shinhukjinju	NAA/BAP	5.0/2.5	First leaf	9/30	30	1 ~ 3
			Second leaf	23/30	77	3 ~ 7
Chungjang	NAA/BAP	5.0/2.5	First leaf	7/30	23	1 ~ 2
			Second leaf	13/30	43	1 ~ 3

generation was obtained both genotypes tested. Cultivar Shinhukjinju exhibited a relatively high level of direct shoot formation compared to Chungjang. The highest regeneration rate obtained for Shinhukjinju was 77% yielding 3-7 shoots. For Chungjang it was 43% yielding 1-3 shoots (Table 2). Our results were consistent with earlier findings demonstrating that success in shoot regeneration was genotype dependent (Wehner and Locy, 1981; Kim et al., 1988).

Different developmental stage within the leaf influenced regeneration, but had no influence on callus formation (data not shown). Frequency of shoot regeneration declined with increased leaf age. Second leaf explants from 25 days old seedlings regenerated well, whereas first leaf (older leaf) explants regenerated poorly (Table 2). In general, younger tissue has been found to be more responsive in tissue culture than older tissue (Brown and Thorpe, 1986). Approximately after 4-5 weeks of culture, well-developed direct shoots were transferred to growth regulator-free MS medium where they gave normal leaf and root development (Figure 2A and B). Plantlets recovered directly from medium supplemented with growth regulators were successfully transferred to soil in the greenhouse with survival frequencies of up to 90%. Regenerated plants of both genotypes had normal appearance (Figure 2C) and most of them set flowers and fruits.

In the present study we developed a highly efficient system for regeneration from cucumber leaf

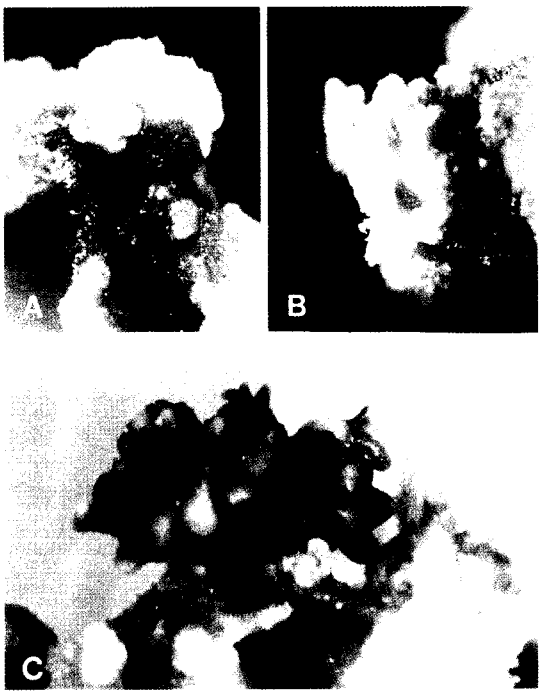

Figure 1. Regeneration of *Cucumis sativus* cv. Shinhukjinju leaf explants on MS medium containing NAA/BAP at 5.0 μM/2.5 μM. Nodules formation after three weeks of culture (A). Immature embryo after four weeks of culture (B). Direct shoot formation after four weeks of culture (C).

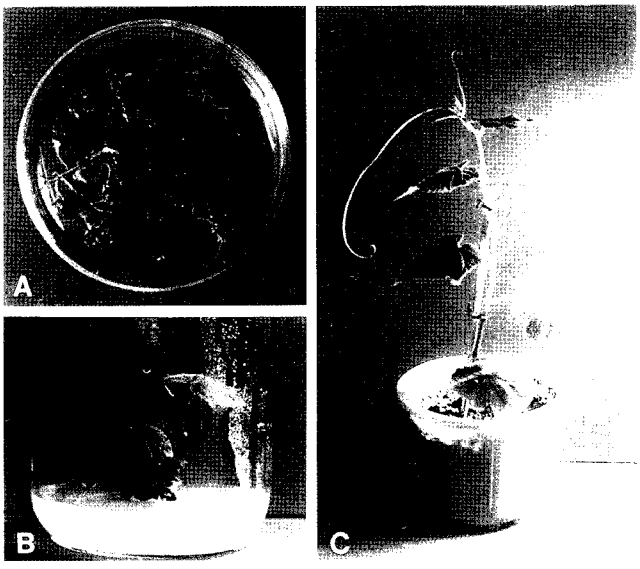

Figure 2. Plant regeneration of *Cucumis sativus* cv. Shinhukjinju subcultured on MS medium without growth regulator (A and B). Normal plantlet transferred to soil and grown in the greenhouse (C).

explants including direct shoot formation. In summary, the most successful treatment consisted in the culture of second foliage leaf explants on MS medium supplemented with 5.0 μM NAA and 2.5 μM BAP.

Using the rapid and efficient method for regeneration as described above, shoot production from cucumber leaf tissue was considerably more than that from cotyledon as reported earlier. The use of leaves instead of cotyledons as an explant source mean that more can be obtained from one individual plant. We are currently testing the usefulness of this system for cucumber transformation by an *Agrobacterium tumefacience* based vector.

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