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Improved Micropropagation of Root Chicory, Cichorium *intybus* L. var. *sativus*.

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ABSTRACT

The establishment of an efficient protocol for plant regeneration and micropropagation from leaf explant cultures of Chicory, *Cichorium intybus* L. var. *sativus*. is reported. Callus formation rate appeared 100% from explant in all growth regulators, but calli formed in the prensence of naphthaleneacetic acid (NAA) were appeared very compact and non-embryogenic state. The regenerated shoots were obtained from leaf explant cultures on solid MS medium containing different concentrations of cytokinins and auxin. The highest number of shoots (5.7) per explant and shoot growth (2.8cm) was obtained on MS medium containing 0.1 mg BAP L^{-1} and 0.1 mg NAA L^{-1}. Indole acetic acid was the most suitable auxin for root formation among three auxins tested. 2,4-D had no effect on shoot and root formation.

Key words : Micropropagation, Root chicory, Auxin, Cytokinin

INTRODUCTION

Among chicory varieties, *Cichorium intybus* L. var. *sativus* is bred and cultivated for its roots that are still used as a coffee substitute but more and more for its storage polyfructan, inulin. Inulin is a food ingredient that can be converted into fructose syrup upon chemical or enzymatic hydrolysis. Chicory is a perennial plant native to Europe. Chicory roots contain at least thirty percent inulin, sesquiterpene lactone composed of lactucin, 8-deoxylactucin, lactucopicrin, 11β,13-dihydrolactucin, 11β,13-dihydro-8-deoxylactucin, 11β,13-dihydrolactucopicrin (Angeline *et al.*, 1996) and other fructo-oligo-saccharides. A recent critical review examined the composition and physiological effects of inulin and oligofructose from chicory root. Inulin and oligofructose, unlike sugars and starches, are not digested in the upper gastrointestinal tract and they do not affect blood sugar or insulin levels. Inulin and oligofructose are fermented by beneficial colonic Bifidobacteria and selectively stimulate their growth. Researchers concluded that inulin and oligofructose are valid forms of dietary fiber, that is, saccharides of plant origin showing resistance to digestion and absorption in

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the small intestine, and fermentation in the colon to produce short-chain fatty acids that are absorbed and metabolized in various parts of the body. They also induce a bulking effect characteristic of dietary fibers. Based on scientific studies, inulin increases mineral absorption during digestion, boosts bifidobacteria within the digestive tract and eliminates pathogens. Inulin also stimulates the immune system, suppresses abnormal growth, is beneficial for kidney health, improves blood sugar control and reduces serum cholesterol (Kim and Shin, 1996)

Plant cell and organ culture system offers a viable alternative for not only the production of secondary metabolites (Sahal and Knuth, 1990; RavWankar and Venkataramaa, 1990), but also development of transgenic plant through hairy root system and regulator factor integration such as MADS box gene (Lim *et al.*, 2003) for flowering control in chicory

Chicory (*Cichorium intybus* L.) is an excellent material for genetic transformation because of its great capacity to regenerate shoots from explants (Mohamed-Yassen and Splitstoesser, 1991 and 1995; Park and Lim, 1999; Vermeulen *et al.*, 1992). However, most of earlier studies were limited to specific varieties, such as the witloof type and there is little information available on the plant regeneration of root type chicory (*C. intybus* L. var. *sativus*). To establish a stable and high efficient regeneration system is necessary.

MATERIALS AND METHODS

Young leaves of root chicory were taken from greenhouse grown plants. Explants were surface-sterilized by a 30 sec immersion in 70% (v/v) ethanol and for 10 min in aqueous solution of 1% (v/v) sodium hypochlorite containing a few drops of tween 20. After five washes in sterilized water, leaves were cut aseptically at the ends, into sections of approximately 5 × 5 mm^2 in size. Explants were placed on medium in the test tube (10 × 150 mm). Test tube contained approximately 10 ml of culture medium. Only one explants were cultured in each test tube. The basal medium consisted of salts and vitamins of Murashige and Skoog (MS) medium (1962) containing 3% of sucrose and solidified with 0.25% (w/v) gelrite. The pH of medium was adjusted to 5.8 before adding gelrite. The media were sterilized by autoclaving at 1.1 kg cm^{-2} (121℃) for 15 min. For shoot regeneration from leaf explants, the MS medium containing different combinations and concentrations (0.1, 1, 2 mg L^{-1}) of BAP (6-benzylaminopurine), TDZ (thidiazuron), 2,4-D (2,4-dichlorophenoxyactetic acid) and NAA (1-naphthalene-acetic acid). The chemicals were purchased from Sigma (USA) and Duchefa (Netherlands).

Regenerated shoots (1 cm) were transferred to MS medium supplemented with different concentrations (0.0, 0.1, 0.5, and 1.0 mg L^{-1}) of IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), and NAA for root induction. Cultures were maintained at 25℃ in a growth chamber with a 16-h photoperiod under standard cool white fluorescent tubes (35 mol s^{-1} m^{-2}) for 45 days. Data collected included callus formation, shoot regenration and rooting of regenerated shoot being measured upon 45days after initiation of cultivation.

RESULTS AND DISCUSSIONS

Callus formation

Effect of auxin and cytokinin treatment on callus formation from explants was examined in terms of types and concentration of the phytohormone (Table 1). Callus formation rate appeared 100% from explant in each all growth regulator and concentration, however, calli formed in the prensence of naphthaleneacetic acid were very compact and non-embryogenic in all combinations of plant growth regulators tested (data not shown). TDZ and 2,4-D was the most suitable cytokinin and auxin for callus formation among the four growth

Table 1. Effect of the different concentration of cytokinins (BAP, TDZ) and NAA on shoot regeneration from leaf explants of *Cichorium intybus* L. var. sativus after 45 days on MS medium.

Plant growth regulator (mg L^{-1})			Shoot induction	Mean number of	Shoot length
BAP	TDZ	NAA	rate (%)	shoot/Explant ±SE	±SE (cm)
0.1			27	2.7±0.2	0.9±0.4
2			45	4.3±0.5	0.7±0.3
	0.1		22	2.3±0.4	0.3±0.1
	2		34	3.2±0.4	0.2±0.1
0.1		0.1	85	5.7±0.7	2.8±0.3
2		0.1	40	4.7±0.3	1.0±0.8
	0.1	0.1	57	4.2±0.7	0.7±0.2
	2	0.1	56	3.2±0.5	1.1±0.4

Each value is the mean standard error of three repeated experiments with 30 explants used in each treatment.

regulators tested. Thidiazuron has recently received considerable attention as a potent regulator of in vitro propagation systems and as an effective stimulus for the development of adventitious shoots and somatic embryos in a wide variety of plants (Huetteman and Preece, 1993; Lu, 1993). Thidiazuron is a substitute phenylurea, and although the biochemical action of the phenylureas is not completely understood, are believed to function in the regulation of purine cytokinin metabolism or act directly as cytokinins or in concert with cytokinins (Mok and Mok, 1985)

Establishment of shoot regeneration

A simple and effective protocol has been developed for in vitro plant regeneration and micropropagation of root chicory. Regenerated shoots were obtained from leaf explant cultures on MS medium containing different combinations and concentrations of cytokinin (BAP and TDZ) and NAA (Table 1). BAP was marginally more effective than TDZ and addition of BAP or TDZ alone was less effective than a combination of NAA (0.1 mg L^{-1}) for shoot regeneration after 4 weeks of culture. The highest number of shoots (5.7) per explant and shoot growth (2.8cm) was obtained on MS medium containing 0.1 mg BAP L^{-1} and 0.1 mg NAA L^{-1} (Fig. 2A).

The multiple form of shoots were observed on the medium containing 0.1 mg TDZ L^{-1} and 0.1 mg NAA L^{-1}, but they were weak and vitrified. It has been reported that weak and vitrified multiple shoots were observed on the medium containing 0.2 mg TDZ L^{-1} and 1.0 mg IAA L^{-1} (Lim *et al.*, 2003). Mohamed-Yassen and Splitstoesser (1991) developed a method for regenerating witloof chicory using thidiazuron (TDZ). Later, Vermeulen et al. (1992) reported that BAP was a more appropriate cytokinin for chicory regeneration. In this study, plant growth regulators, BAP with NAA showed efficient regeneration rates from leaf explants of root type chicory. Other researchers (Pieron *et al.* 1992) reproted that nodules were induced on leaves by IBA and BA in the medium in chicory.

Rooting of regenerated plants

Regenerated shoots (0.5 cm) were normal and could be easily rooted. Roots formed on shoots within 2-4 weeks on all media tested (MS medium with different concentrations [0.0, 0.1, 0.5, and 1.0 mg L^{-1}] of IAA , IBA, and NAA). IAA was the most suitable auxin for root formation among the three auxins tested. One mg L^{-1} treatmment of IBA and NAA induced callus with

Table 2. Effect of the auxins on rooting of regenerated shoots from *Cichorium intybus* L. var. *sativus* after 45 days on MS medium.

Auxin (mg L^{-1})		Rate of root formation (%)	Mean number of root/explant ±SE	Root length ±SE (cm)
IAA	0.1	84	7.8±0.8	2.7±0.3
	0.5	74	6.2±0.9	2.1±0.8
	1	77	7.1±0.4	1.7±0.4
IBA	0.1	76	5.4±0.7	1.5±0.4
	0.5	82	6.3±0.8	1.5±0.7
	1	79	6.1±0.9	1.8±0.4
NAA	0.1	74	4.8±0.5	1.0±0.3
	0.5	70	5.2±0.3	1.0±0.4
	1.0	75	4.3±0.5	0.8±0.4

Each value is the mean standard error of three repeated experiments with 30 explants used in each treatment.

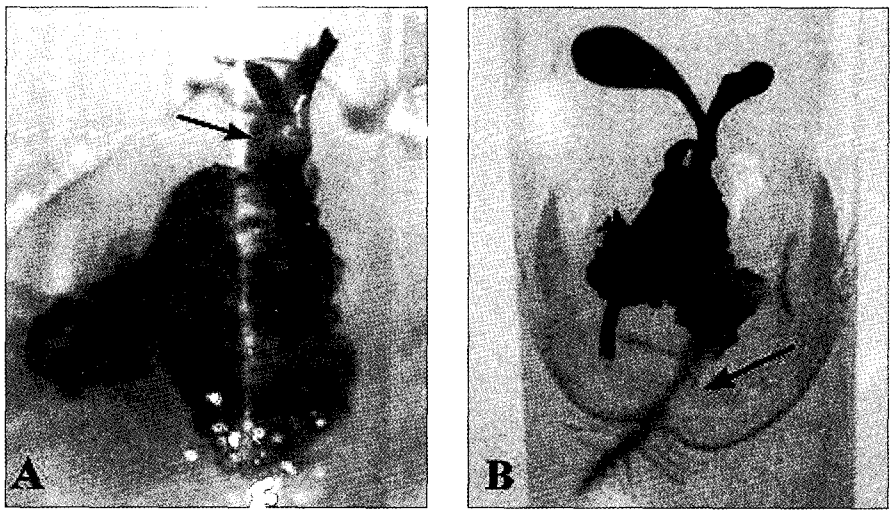

Fig. 1. Shoot elongation (A) and root formation (B) from regenerated shoots on MS medium supplemented growth regulators, auxin and cytokinin in *Cichorium intybus* L. var. sativus.

root and inhibited root induction. The best rooting response with respect to the percentage of shoots producing roots (84%), the number of roots per shoot (7.8) and the average root length (2.7 cm) was achieved with 0.1 mg l^{-1} IAA after 5 weeks of culture (Table 2, Fig. 2B). Plantlets successfully grew and produced roots in rooting medium. 2,4-D had no effect on shoot and root formations (data not shown).

Previously, Park and Lim, (1999) reported that plantlets were obtained from leaf and petiole of four genotypes in root-type chicory, and shoot regeneration rates varied from 20% to 95%, depending on genotype

and regenerated shoots vitrified when the culture period was extended. In this study, tissue culture system of root-type chicory was optimized not only to improve regeneration rates but also to reduce the vitrification of regenerated plantlets.

Further investigation of chicory tissue culture will eventually provide knowledge and propagation system by modified culture condition such as glucose concnetration, light condition or effect of polyamine induced root growth and production of coumarin (Bals *et al.*, 1999). We remain hopeful that the plant regeneration protocol will create opportunity for obtaining transgenic plants.

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