Korean J.Plant Res. 8(3) 286~292(2005)

Plant Regeneration from Turnip (*Brassica rapa* ssp. *rapifera*) Organs

Sarantuya Gendaram[1)] and Chang-Hyu Bae[1), 2)*]
[1)]Research Institution of Agricultural Science, Sunchon National University, Sunchon 540-742, Korea
*[2)]College of Agriculture and Life Science, Sunchon National University Sunchon 540-742, Korea

ABSTRACT

Shoot induction system was developed in the recalcitrant plant species, *Brassica rapa* ssp. *rapifera* by using optimum selection of profit organ, phytohormone combination, seedling age and kind of culture container. Out of *in vitro* cultured leaf segment, petiole, hypocotyl, and cotyledon with petiole, only cotyledon with petiole derived from 4 day-old seedlings induced multiple shoot. The optimum combination of auxin and cytokinin for the multiple shoot induction was MS medium containing 5mg/L BA and 0.5mg/L NAA. The major factors for multiple shoot propagation were part of plant organ, age of seedling, and ratio of auxin and cytokinin. In addition, shoot regeneration was promoted in the 100ml Erlenmeyer flask compared with the 90mm × 20mm Petri-dish. The induced shoots formed roots easy on MS medium containing 0.1mg/L IBA and the whole plants were successfully cultivated in soil.

Key words : BA (N^6-benzyladenine), *Brassica*, cotyledon, hypocotyl, NAA (α-naphthalene acetic acid), multiple shoot, petiole

INTRODUCTION

Turnips (*Brassica rapa* ssp. *rapifera*) are one of the members of *Brassicaceae* (Cruciferous) and one of the edible plants originated from Europe and widely distributed in the world. The turnip is biennial plant, vegetative in the first year with leaves of the rosette, and completes its life cycle in the second year. The turnip produces white, large and round roots that usually weigh up 1.5 to 3 kg. The roots of turnip are possible to eat in salads, pickles, or slices. Because the turnip contains very active biological catalizer in its composition and supplies easy digestible nutrients, it is cultivated for human consumption and animal nutrition in the world (Shebalina, 1974).

The biological characteristic and the productivity of the turnip have been studied in many countries (Shebalina, 1974; Harper and Compton, 1980; Panteleeva, 1986; Jung *et al.*, 1986; Gendaram, 2001). And the callus initiation and regeneration capacities have been investigated in Brassica species (Murata and Orton, 1987; Christey and Sinclair, 1991; Takasaki *et al.*, 1996). However, successful plant regeneration system was very difficult in the turnip plant, except for some of *Brassica* species with specific tissues and growth regulator combination (Christey and Sinclair, 1991; Takasaki *et al.*, 1996; Hara *et al.*, 2001). The

*Corresponding author : **Chang-Hyu Bae,** E-mail chbae@sunchon.ac.kr

plant regeneration was reported in other cruciferous members, such as chinese cabbage *Brassica campestris* L. ssp. *pekinensis* (Zhang *et al*., 1998), oilseed *Brassica campestris* (Hachey *et al*., 1991), horseradish *Armoracia rusticana* (Bae, 2001) and Crambe (Sonntag and Muller, 2000). The reports indicate that plant part, ratio of growth regulators and plant species are important factors for shoot regeneration of the recalcitrant *Brassica* species.

Even though the high frequency shoot regeneration is one pre-requisite for the application of *Agrobacterium*-mediated genetic transformation and/or production of the secondary metabolites in turnip (Pierik, 1987; Murakami *et al*., 1995), there are limited reports on efficient plant regeneration systems in this variety (Esti-Naeris) turnip to date.

Hence, we investigated an efficient regeneration conditions of the turnip by using different organs, seedling age, phytohormone combination and culture container in this study.

MATERIALS AND METHODS

Plant materials and *in vitro* seed germination

The seed of turnip *Brassica rapa* ssp. *rapifera* used for this experiment was provided from Research Institute of Animal Husbandry (Ulaanbaatar, Mongolia). The variety of turnip is Esti-Naeris originated from Russia and rehabilitated well for sharp continental climate of Mongolia. The germination rate of the seed Esti-Naeris was 88% and the tissue culture experiment was carried out at Plant Biotechnology Laboratory (PBL) of Sunchon National University.

Turnip seeds were sterilized by submerging in 70% (v/v) ethanol for 1 min and in 2% of sodium hypochlorite solution for 20 min. Then the seeds were rinsed three times with sterile distilled water. After the surface sterilization, the seeds were placed onto the 90mm × 20mm Petri-dishes (90mm in diameter, 20mm in height) containing 20~25ml hormone-free MS media (Murashige and Skoog, 1962), which were supplemented with 30 g/L sucrose and solidified with 0.8% (w/v) agar. The culture media were adjusted for pH 5.80 prior to autoclave. The culture plates were sealed with 2 layers of Parafilm and incubated at 25 ± 1 ℃. The contamination-free germinating seeds were selected and the seed coats were removed in two days after culture. Then the embryos were transferred to the medium and incubated in the culture room. The cultures were kept at 25 ± 1 ℃ with continuous fluorescent light at 30 μmol/m^{2}s light intensity.

Plant tissue culture of various explants

All explants were excised from the *in vitro* germinated seedlings. Leaf segment (4mm × 4mm), petiole (4mm in length), hypocotyl (7 to 8mm in length) and cotyledon with petiole (5 to 7mm in length) excised from 4 day-old seedlings (the optimal age was determined by pre-experiment) were cultured on the 90 mm × 20mm plastic Petri dishes or 100ml Erlenmeyer flasks. Hypocotyl and cotyledon segment with petiole were cultured on the plastic Petri dishes by 3 replications with 10 explants (Table 1). Another explants including hypocotyl and cotyledon with petiole were cultured on the Erlenmeyer flasks (Table 2, Table 3) with various concentrations of NAA and BA by 3 or 6 replications with 3 explants. The cultures were evaluated after 4 weeks (Table 1, 2, 3) in terms of percentage of shoot regeneration (the number of explants forming shoots/the total number of explants), rooting and callus induction.

In order to test organogenesis efficiency, hypocotyls and cotyledon with petiole explants were cultured with NAA and BA combinations on the 90mm × 20mm Petri-dish container (Table 1).

To determine optimum conditions for organogenesis in the 100mℓ Erlenmeyer flasks, 5 combinations with BA (2, 5, and 10 mg/L) and NAA (0.5, 1.0 mg/L) were

tested using leaf segment, petiole, hypocotyls and cotyledon with petiole explants with the different growth regulator combinations (Table 2, Table 3).

To evaluate root induction from regenerated shoots, the regenerated shoots derived from cotyledon with petiole were cut and cultured on MS medium with 0.1, 1.0, and 1.5 mg/L IBA (Table 4). Explants were evaluated after 3 weeks in culture in terms of percentage of rooting, which was calculated as the number of explants forming roots relative to the total number of explants.

RESULTS

Organogenesis from different organs cultured on Petri dish

In order to evaluate organogenesis ability, explant age and five combinations of growth regulators were examined on the 90mm×20mm plastic Petri dishes by using plant organs described in Table 1. The optimal age of the explants for organogenesis was 4 days seedling after germination rather than 7 days (not shown data). Thus, all of the explants were obtained from 4 day-seedling by the following experiments.

As shown in Table 1, three kinds of response were observed from cotyledon with petiole and hypocotyl segment culture; calli, root and shoot formation. Shoots were induced as well as calli and roots from cotyledon with petiole explants and hypocotyl culture (Fig. 1). Shoot and root formation were higher in cotyledon with petiole than hypocotyl segments. Moreover, multiple shooting was induced in just cotyledon with petiole and the best shoot number/explant was approximately 1.4 per explant. Interestingly, percentage of explant forming callus was increased by the BA concentration from 2 mg/L to 5 or 10 mg/L in cotyledon with petiole (Table 1). Because high volume of the culture container promote organogenesis in general, we test another type of tissue culture container. Thus, the 100 ml Erlenmeyer flasks were adopted by the following experiments.

Table 1. The effect of growth regulator combination on callus induction, shoot and root regeneration from cotyledon with petioles and hypocotyles of *Brassica rapa* ssp. *rapifera* (Esti-Naeris) cultured after 28 days on the Petri dish culture.

Organ	NAA	BA (mg/L)	No. of exp[x]	No. of expc[w]	No. of exps[v]	No. of shoot/ explant	No. of expu[u]
Cop[z]	Control		30	7 (23.3)[t]	5 (16.7)t	1.0	7 (23.3)t
	0.5	2	30	15 (50.0)	4 (13.3)	1.0	1 (3.33)
		5	30	19 (63.3)	11 (36.7)	1.4	0
	1.0	2	30	10 (33.3)	9 (30.0)	1.0	0
		10	30	23(76.6)	17 (56.7)	1.1	10 (33.3)
Hyp[y]	Control		30	0	0	0	4 (13.3)
	0.5	2	30	3 (10.0)	0	0	1 (3.3)
		5	30	8 (26.7)	0	0	2 (6.7)
	1.0	2	30	6 (20.0)	1 (3.3)	1.0	2 (6.7)
		10	30	0	1 (3.3)	1.0	0

[z]Cotyledon with petiole, [y]Hypocotyl, [x]No. of explant cultured, [w]No. of explant forming callus, [v]No. of explant forming shoots, [u]No. of explant forming roots, [t]Numbers in parentheses indicate percentage to the number of explants cultured.

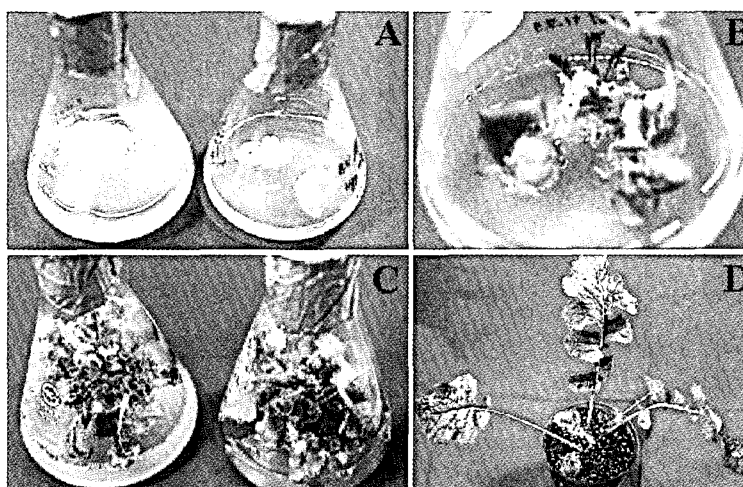

Fig. 1. Profiles of dedifferentiaion (A) and plant regeneration (B, C, D) of turnip (*Brassica rapa* ssp. *rapifera* (Esti-Naeris)). A: Callus induction from petiole segments on MS medium containing BA 0.5mg/L and NAA 5mg/L. B: Shoot regeneration from cotyledon with petioles on MS medium containing 5mg/L BA and 0.5mg/L NAA after 3 weeks in culture. C : Shoot regeneration from cotyledon with petioles on MS medium containing 5mg/L BA and 0.5mg/L NAA after 7 weeks in culture. D : The regenerants derived from shoot of cotyledon with petiole transferred to soil after rooting.

Organogenesis from different organs cultured on Erlenmeyer flask

In order to evaluate shoot regeneration ability by the culture container type, we treated five combinations of growth regulators by using plant organs described in Table 2 and Table 3 on the 100 ml Erlenmeyer flasks. Calli were induced by the treatment of NAA and BA combination except for hormone free MS medium, and root induction from petiole were observed by the treatment of NAA and BA combination except for hormone free MS medium. Shoots were not induced from both the leaf segment and the petiole culture (Table 2). Percentage of callus induction was higher in leaf segment than petiole, but root induction was higher

Table 2. The effect of growth regulator combination on callus induction, shoot and root regeneration from leaf segments and petioles of *Brassica rapa* ssp. *rapifera* (Esti-Naeris) cultured after 28 days on the Erlenmeyer flask culture.

Organ	NAA (mg/L)	BA (mg/L)	No. of explants cultured	No. of explants forming callus	No. of explants forming shoots	No. of explants forming roots
Leaf segment	Control		9	0	0	0
	0.5	2.0	9	7 (77.7)[Z]	0	0
		5.0	9	4 (44.4)	0	0
	1.0	2.0	9	9 (100)	0	1 (11.0)[Z]
		10.0	9	3 (33.3)	0	0
Petiole	Control		9	0	0	0
	0.5	2.0	9	1 (11.1)	0	2 (22.2)
		5.0	9	6 (66.6)	0	2 (22.2)
	1.0	2.0	9	3 (33.3)	0	1 (11.1)
		10.0	9	4 (44.4)	0	2 (22.2)

[Z]Numbers in parentheses indicate percentage of forming callus or roots to the number of cultured explants, respectively.

Table 3. The effect of growth regulator combination on callus induction, shoot and root regeneration from cotyledon with petioles and hypocotyles of *Brassica rapa* ssp. *rapifera* (Esti-Naeris) cultured after 28 days on the Erlenmeyer flask culture.

Organ	NAA BA (mg/L)		No. of exp[x]	No. of expc[w]	No. of exps[v]	No. of shoot/ explant	No. of expu[u]
Cop[z]	Control		9	0	0	0	3 (33.3)[t]
	0.5	2.0	9	8 (88.8)[t]	7 (77.7)[t]	1.7	7 (77.7)
		5.0	9	9 (100.0)	8 (88.8)	1.8	5 (55.5)
	1.0	2.0	9	7 (77.7)	7 (55.5)	1.0	6 (66.6)
		10.0	9	6 (66.6)	7 (77.7)	1.2	0
Hyp[y]	Control		9	0	0	0	4 (44.4)
	0.5	2.0	9	3 (33.3)	0	0	1 (11.1)
		5.0	9	8 (88.8)	0	0	2 (22.2)
	1.0	2.0	9	6 (66.6)	1 (11.1)	1.0	2 (22.2)
		10.0	9	0	1 (11.1)	1.0	0

[z]Cotyledon with petiole, [y]Hypocotyl, [x]No. of explant cultured, [w]No. of explant forming callus, [v]No. of explant forming shoots, [u]No. of explant forming roots, [t]Numbers in parentheses indicate percentage to the number of explants cultured.

in petiole than leaf segment (Table 2). The calli with roots occurred simultaneously and the roots were long with abundant root hairs.

Even though shoots were not induced in the leaf segment and the petiole as shown in Table 2, shoots were induced as well as calli and roots from cotyledon with petiole explants and hypocotyl culture (Table 3). Interestingly, shooting and rooting were higher in cotyledon with petiole than hypocotyl. Out of five tissues shown in Table 2 and Table 3, multiple shooting was induced in just cotyledon with petiole showing 1.0 to 1.8 shoot number per explant (Table 3). And the best shoot number/explant was 4 per explant on MS medium containing 5mg/L of BA and 0.5mg/L of NAA in the cotyledon with petiole (Fig. 1B, C). This result was coincident with the result of Petri dish culture shown in Table 1.

Root regeneration from the regenerants

The regenerated shoots were rooted in the presence of IBA (Table 4) and successfully transferred to soil (Fig. 1D). As shown in Table 4, however, high concentration of IBA reduced in frequency of root

Table 4. Effect of IBA treatment on root formation from the regenerated shoot cuttings derived from cotyledon with petiole of *Brassica rapa* ssp. *rapifera* (Esti-Naeris) after 21 days in culture.

Origin con. used for shooting[z]	MS+IBA 0.1mg/L		MS+IBA 1.0mg/L		MS+IBA 1.5mg/L	
	Rooting (%)	Root length (cm)	Rooting (%)	Root length (cm)	Rooting (%)	Root length (cm)
BN2.05[y]	75.0	4.1	50.0	4.2	50.0	10.0
BN2.1[x]	50.0	3.7	25.0	5.1	50.0	3.9
BN5.05[w]	75.0	7.6	25.0	2.1	25.0	1.0
BN10.1[v]	100.0	5.3	50.0	10.5	25.0	7.5

[z]Source of BA and NAA concentration yielded shoots before IBA treatment. Shoots for root formation were induced from the BA and NAA treatment, respectively. [y]BA 2mg/L+NAA 0.5mg/L, [x]BA 2mg/L+NAA 1.0mg/L, [w]BA 5 mg/L+NAA 0.5mg/L, [v]BA 10mg/L+NAA 1.0mg/L.

regeneration. Adventitious roots were 1.0cm to 10.5cm long with abundant root hairs. Our study clearly demonstrated a method for high frequency shoot regeneration from cultured explants of turnips.

DISCUSSION

Previous investigations confirmed that turnip is very recalcitrant *Crucifera* member for multiple shooting (Murata and Orton, 1987; Takasaki *et al*., 1996). In addition seedling age (Hachey, 1991), profit organ and phytohormone combination (Murakami *et al*., 1995; Hara *et al*., 2001) influence on an efficient shoot regeneration of turnip tissues. In view of the fact, we investigated major factors that play roles in regeneration from the cultured turnip explants. This study showed that hormone combination, profit organ and seedling age of explant are important factor on organogenesis especially, shoot regeneration. Moreover, shoot regeneration was promoted by kind of culture container.

The study of hormone combination for shoot induction has been reported that medium only containing 0.1mg/L BA induced the highest frequency of shoot formation and most important factors were genotype and hormone in other *Brassica* species (Murata and Orton, 1987). And calli were induced most frequently MS medium with 1.0mg/L 2,4-D, whereas NAA induced preferentially roots at a concentration of 2 to 5mg/L. Hachey (1991), Murakami *et al*. (1995) and Takasaki *et al*. (1996) said that BA with NAA treatment is effective for shoot regeneration also. Hence we used BA and NAA combination as growth regulator factor and our result showed a similar tendency in hormone combination and its concentration.

Our study demonstrates that cytokinins has promotive effects on shoot regeneration, only when the 4 day-old cotyledon with petioles were cultured as explants. In addition, all explants did not produce any callus except cotyledon with petioles on MS hormone free medium. The results indicate that regeneration ability vary with cultured organs. This regeneration tendency was showed in the shoot regeneration from cotyledon explants (Takasaki *et al*., 1996) and from 4 day-old cotyledons with petioles (Hachey, 1991). In addition, Murakami *et al*. (1995) studied that shoot regeneration of 32 cultivars of radish varied with genotypes. Low concentration of cytokinins has promotive effects on callus and root initiation in general, however, high concentration of cytokinins promoted callus induction in cotyledon with petiole (Table 1). This result indicates that hormone response varies with genotype.

Pua *et al*. (1995) reported that the rooting of chinese radish from shoot cuttings with 1～5mg/L IAA and IBA treatment was more effective than that with NAA only. In our case, lower concentration of IBA containing media showed good effect for rooting of turnip.

In conclusion the shoot regeneration was the highest in the combination treatment of 0.5, 1.0mg/L NAA with 5, 10mg/L BA group. Our result was confirmed the above parameters, seedling age, profit organ and phytohormone combination to promote shoot regeneration. Thus the major factors for multiple shoot propagation were part of plant organ, age of seedling, and ratio of auxin and cytokinin. In addition, shoot regeneration was promoted in the 100ml Erlenmeyer flask compared with the 90mm × 20mm Petri-dish. Although the tissue culture system described here may facilitate a genetic improvement of turnip var. Esti-Naeris for the genetic engineering approach, the culture optimization to increase the number of regenerated shoots per explants is more needed to enhance the efficiency of the genetic engineering system.

ACKNOWLEDGEMENT

This work was supported by grant (KAERI/CM-

835/2004) to Proton Engineering Frontier Project from The 21st Century Frontier R&D Program of Ministry of Science & Technology (MOST). We are grateful to Jae-Il Lyu for his help in prèparing of the manuscript.

LITERATURE CITED

Bae, C.H. 2001. Effect of carbenicillin on callus induction and regeneration efficiency of tissues of horseradish (*Armoracia rusticana*). Plant Resources 4:53-58.

Christey, M.S. and B.K. Sinclair. 1991. Regeneration of transgenic kale (*Brassica oleracea* var. *acephala*), rape (*B. napus*) and turnip (*B. campestris* var. *rapifera*) plants via *Agrobacterium rhizogenes* mediated transformation. Plant Science 87:161-169.

Gendaram, S. 2001. Elaboration of some technological methods for cultivation of the turnip (*Brassica rapa* ssp. *rapifera*) in the central region of the Mongolia. Ph.D thesis, Mongolian State University of Agriculture, Ulaanbaatar.

Hachey, J.E., K.K. Sharma, and M.M. Moloney. 1991. Efficient shoot regeneration of *Brassica campestris* using cotyledon explants cultured *in vitro*. Plant Cell Rep. 9:549-554.

Hara, M., H. Eto, and T. Kuboi. 2001. Tissue printing for myrosinase activity in roots of turnip and Japanese radish and horseradish: a technique for localizing myrosinases. Plant Science 160:425-431.

Jung, G.A., R.A. Byers, M.T. Panciera, and J.A. Shaffer. 1986. Forage dry matter accumulation and quality of turnip, swede, rape, Chinese cabbage hydrids, and kale in the eastern USA. Agron. J. 78: 245-253.

Murashige, T. and F. Skoog. 1962. Arivised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Murata, M. and T.J. Orton. 1987. Callus initiation and regeneration capacities in *Brassica* species. Plant Cell, Tissue and Organ Culture 11:111-123.

Murakami, T., Y. Ono and Y. Takahata. 1995. Phytohormonal and genotypic factors affecting shoot regeneration from cotyledonary explants of radish (*Raphanus sativus* L.). Plant Tiss. Cult. Lett. 12:321-323.

Panteleeva, N.G. 1986. Biological characterization and Productivity of Fodder root crops in the central zone of the BAM. Ph.D thesis Agricultural University of Irkutsk, Russia.

Pierik, R.L.M. 1987. Preparation and composition of nutrient media. *In* Pierik, R.L.M. (eds.) *In vitro* culture of higher plants. Martinus Nijhoff Publishers, Netherlands. PP. 48-50.

Pua, E.C., G.E. Sim., G.L. Chi and L.F. Kong. 1996. Synergistic effect of ethylene inhibitors and putrescine on shoot regeneration from hypocotyl explants of Chinese radish (*Raphanus sativus* L. var. *Longipinnatus* Bailey) *in vitro*. Plant Cell Rep. 15:685-690.

Shebalina, M.A. 1974. Turnip, rutabaga and swede. *In* Dervesheb. A.N (eds) Kolos, Leningrad. PP. 148-153 (in Russian).

Sonntag, K., and J. Muller. 2000. Study of the *in vitro* culture in *Crambe*. 3rd ISHS International Symposium on Brassicas. 12th Crucifor Genetics Workshop. UK. P. 103.

Takasaki, T., K. Hatakeyama, K. Ojima, M. Watanabe, K. Toriyama and K. Hinata. 1996. Effects of various factors (hormone combinations, genotypes and antibiotics) on shoot regeneration from cotyledon explants in *Brassica rapa* L. Plant Tiss. Cult. Let. 13(2):177-180.

Zhang, F.L., Y. Takahata and J-B. Xu. 1998. Medium and genotype factors influencing shoot regeneration from cotyledonary explants of Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*). Plant Cell Rep. 17:780-786.

(Received Jun. 10, 2005)
(Accepted Sep. 29, 2005)