Short Communication

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Shoot Regeneration from Explants of Seedstalk Developed *in vitro* in Chinese Cabbage

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Abstract

A convenient method for producing shoot regeneration has been developed for the Chinese cabbage (*Brassica campestris* L.). Internodes of seedstalks which were developed from the sterilized seeds by low temperature treatment *in vitro* were used to induce shoot regeneration. The shoot was stably induced when the seedstalk internode sections were incubated in MS medium containing 1 mgl^{-1} naphthaleneacetic acid (NAA) and 5 mgl^{-1} benzyladenine (BA), though the frequency was about 16%. Similar results were also obtained from the explants grown in a greenhouse as well as in a plant box. These results suggest that this culture system may be available for micropropagetion, genetic transformation and mutation breeding.

Breeding methods with cell and tissue culture and genetic transformation assist in the production of vegetables resistant to pathogens and insects. Chinese cabbage (*Brassica campestris* L.) is one of the most popular crops for pickled, fried and boiled vegetables in China, Korea and Japan. In the culture systems of the Chinese cabbage, microspore culture and haploid methods of breeding have been advanced for making a commercial variety (Minato *et al.* 1988). Although regeneration from cultured tissue (Hachey *et al.*, 1991; Choi *et al.*, 1996; Takasaki *et al.*, 1996) and protoplasts (Yamagishi *et al.* 1988) have been reported, it is difficult to obtain regenerated plants without an artisan technique. In *B. napus*, high-frequency plant regeneration has been shown in the experiments using thin layer explants isolated from the internodes of the stems (Klimaszewska and Keller 1985). However, little is known about a plant regeneration method with stable differentiation in Chinese cabbage. In this report, we describe a convenient method for producing shoot regeneration from the explants of seedstalks and the materials developed *in vitro* in the Chinese cabbage.

Seeds of Chinese cabbage, Musou (Takii Seed), CR Shinrei (Nagano Prefecture Seed Production and Plant Biotechnology Research Center) and raising line, M81 (Nagano Vegetable and Ornamental Crops Experimental Station) were used in this study.

The seeds were sterilized in 1% active chlorine of sodium hypochloride solution for 10 min and rinsed three times with sterile distilled water. Seeds were placed on 50 ml of double strength MS (Murashige and Skoog 1962) solid medium (pH 5.8) containing 3% sucrose and 0.8% agar in a plant box (6×6×10 cm, Verde Corp.). After incubation for 1-8 weeks at 4°C in the darkness, the seeds were germinated. The cultures were maintained at 25°C with a 14 h/day photoperiod under fluorescent light of 3000 lux and the bolting was observed during 2-5 weeks. When the seedstalk was grown for 2 weeks after initial bolting in the plant box, the internodes were cut into pieces approximately 3-5 mm and were placed on shoot-inducing MS solid medium (pH 5.8) containing 0-5 mgl^{-1} NAA, 5 mgl^{-1} BA, 3% sucrose and 0.7% agar.

The seedstalks developed in the greenhouse for the current study were grown as follows. Seeds were sown on sterilized soil in plastic pots (12 cm in diameter). After setting for 7 weeks at 4°C in the darkness, they germinated and plants were grown in the greenhouse. The seedstalks of the plants were sterilized in 1% sodium hypochloride solution, cut into pieces and placed on the shoot-inducing medium containing 1 mgl^{-1} NAA and 5 mgl^{-1} BA.

The explants of seedstalk internodes from both sources were incubated at 25°C for 14 h/day photoperiod under fluorescent light (3000 lux). The number of explants producing callus, green callus and shoots was scored after 4 weeks of culture.

The time dependency on the bolting *in vitro* in Chinese cabbage under low temperature conditions is shown in **Fig. 1**. After low temperature treatment (1-8 weeks), the plant boxes moved into the incubator at 25°C for 5 weeks and the ratio of bolting was calculated. The bolting was first observed in the plant boxes which had been incubated at 4°C for 4 weeks. The ratio of bolting reached 100% over 7 weeks of incubation. **Fig. 2** shows the seedstalk of

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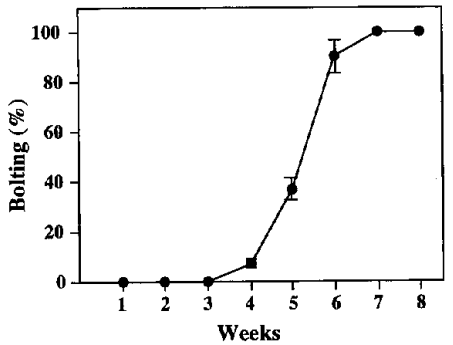

Fig. 1 Bolting efficiency *in vitro* (in a plant box) after low temperature treatment in Chinese cabbage (M81).
The seeds of Chinese cabbages were exposed to low temperature (4°C) for 1-8 weeks and bolting was observed after further incubation for 5 weeks at 25°C. Ten seeds were tested for each of three replicates. Bars represent standard errors.

Fig. 2 Seedstalk of Chinese cabbage (M81) developed in a plant box for 7 weeks after the treatment of the seeds for 6 weeks at 4°C.

Chinese cabbage developed for 7 weeks after the treatment of the seeds for 6 weeks at 4°C. The length of the seedstalks was 5-7 cm, and the largest one extended spirally to 25-cm in length. We used seedstalks of 5-25 cm long in subsequent experiments.

We examined the effects of NAA on the formation of callus, green callus and shoot from the seedstalk explants in the medium containing 5 mgl^{-1} BA (**Table 1**). Callus, green callus and shoots were observed in wide concentrations of NAA. The frequency of shoot regeneration was low compared with those of callus and green callus. Shoot regeneration frequency was 16% on MS medium containing 1 mgl^{-1} NAA and 5 mgl^{-1} BA. There was no formation of callus, green callus and shoots in the hormone-free medium, and the efficiency of shoot regeneration was little affected when BA (0.1-10 mgl^{-1}) was added to the medium containing 1 mgl^{-1} NAA (data not shown). In *B. napus*, the shoot regeneration from the

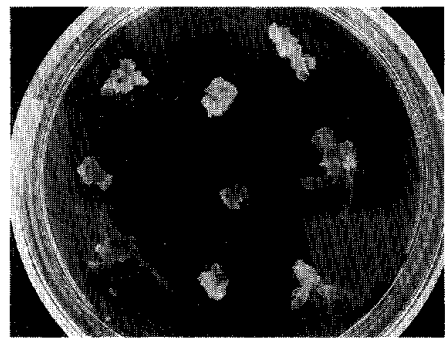

Fig. 3 Shoot regenerated from explant of seedstalk (M81) developed in a plant box.
The explants of seedstalk were grown on MS medium containing 1 mgl^{-1} NAA and 5 mgl^{-1} BA for 4 weeks.

Table 1. Effect of NAA concentrations on the culture of explants from Chinese cabbage (M81) seedstalks developed in a plant box.

NAA (mgl^{-1})	Callus(%)	Green callus (%)	Shoot(%)
0	92.0±1.9	28.0±2.5	9.0±1.5
0.1	91.5±3.0	45.5±4.5	7.0±1.5
0.5	87.0±1.5	86.5±2.2	6.0±2.1
1.0	87.5±1.7	87.5±1.7	16.0±2.1
2.0	84.0±2.1	81.0±4.3	4.5±1.5
5.0	92.5±2.0	85.5±3.6	0

Fifty explants were placed on MS media containing 5 mgl^{-1} BA with four replications. Values are the means ± standard errors of differentiation frequency.

seedstalk was reported to reach 63% (Klimaszewska and Keller 1985). The difference may be due to the difference in species or genotypes.

Shoot regeneration was observed after incubation of the seedstalk explants for 4 weeks on the medium (**Fig. 3**). The number of regenerated shoots was 1-5 shoots per seedstalk explant. Histological studies suggested that shoots are developed directly from the explant tissue, not from callus and green callus (data not shown).

The shoot regeneration frequency from the explants of seedstalk grown in the plant box and greenhouse was compared using M81, Musou and CR Shinrei (**Table 2**). High levels of shoot regeneration were obtained when seedstalks for 1-2 weeks after bolting were used. The efficiency was decreased with increasing incubation time after bolting. Alterable values were observed in the greenhouse especially for the initial three weeks. The shoot regeneration frequency in a plant box was slightly higher than that in the greenhouse.

High frequency (up to 70-100%) of shoot regeneration from cotyledon explant has been reported in *B. campestris* (Hachey *et al.*, 1991; Takasaki *et al.*, 1996). However, there was very little shoot regener-

Table 2. Shoot regeneration efficiency from seedstalk explants after bolting.

Cultivar	Habitat	Shoot regeneration (%)					
		After bolting (weeK)					
		1	2	3	4	5	6
M81	Plant box	26.7±3.3	23.3±8.8	10.0±5.8	16.7±8.8	0	0
	Greenhouse	12.5±6.3	10.0±4.1	7.5±4.8	0	0	0
Musou	Plant box	53.3±8.8	56.7±8.8	50.0±5.8	16.7±3.3	13.3±8.8	6.7±3.3
	Greenhouse	60.0±9.1	32.5±8.5	25.0±8.7	0	0	0
CR Shinrei	Plant box	33.3±8.8	23.3±8.8	20.0±5.8	0	0	0
	Greenhouse	5.0±2.7	40.0±9.1	5.0±2.9	7.5±4.8	7.5±4.8	5.0±2.9

The test plants were grown *in vitro* (plant box) or in a greenhouse. Ten explants were prepared by cutting seedstalk at different incubation times after bolting and placed on MS media containing 1 mgl^{-1} NAA and 5 mgl^{-1} BA with three in plant box and four in greenhouse replications. Values are the means±standard errors of shoot regeneration.

ation from cotyledon explant in M81 and CR Shinrei (data not shown). Thus, it is necessary to develop the method for a shoot regeneration system. The present results provide a shoot regeneration method from seedstalk explants in the Chinese cabbage. This method using *in vitro* bolting, which can be performed in the laboratory without a greenhouse or biotron, is available for reducing contamination and saving space. Furthermore, this system may contribute to the micropropagation and the production of transgenic plants and mutants in Chinese cabbage.

Shoot regeneration efficiency from protoplast and cultured tissue has been improved by the addition of substances other than plant growth regulators to the medium (Chi *et al.*, 1990), pretreatment of tissues (Terada 1987), and the use of tissue having high differentiation potency (Chokyu and Imoto 1993). Chi and Pua (1989) have mentioned that, in *B. campestris*, the efficiency of shoot regeneration from cotyledon and hypocotyl was enhanced by adding $AgNO_3$, Ag_2SO_4, aminoethoxyvinylglycine, aminooxyacetic acid or 2,4-dinitrophenol. Putrescine, a group of polyamines, has been reported to promote differentiation of adventitious buds from explants in some vegetables (Tanimoto and Harada 1991). Recently, shoot induction from cotyledonary explants of Chinese cabbage has also been improved in the presence of $AgNO_3$ (Zhang *et al.*, 1998). Addition of ethylene inhibitors and organic compounds may increase the rate of shoot regeneration. To improve the rate of the shoot regeneration in the Chinese cabbage, the characteristics underlying such a regeneration system should be extensively studied.

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