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Establishment of Efficient Regeneration System Through *In Vitro* Culture of Lettuce (*Lactuca sativa*)

Young-Sook Kim and Tae-Ho Kwon
Institute for Molecular Biology and Genetics, Chonbuk National University.
Chonbuk, Chonju, 560-756, Korea.

ABSTRACT

An efficient regeneration system was established by using *in vitro* plantlets of germinated seedlings from different cultivars of lettuce (*Lactuca sativa* cv. Chongchima, Chongchuckmyun, Jeokchima, Jeokchuckmyun). Shoot formation were observed from all cultivars on MS medium supplemented with 0.1 mg/L NAA and 0.5 mg/L BA. In all cultivars, when cotyledon was cultured, the number of shoot per explant was more greater than that hypocotyl and leaf disc were cultured. Shoot formation rate (91.7%) was high in a cotyledon culture of cultivar, Chongchukmyun. The growth of multiple shoots derived from the cultivar, Chongchukmyun, was most effective on medium containing 0.5 mg/L BA and 1.0 mg/L GA_3. When shoots were transferred on MS medium without plant growth regulators, roots were effectively differentiated. Rooted plantlets were acclimated on pots for further propagation.

Key words : lettuce, multiple shoot, plant regeneration

INTRODUCTION

Lettuce (*Lactuca sativa*) belongs to a member of the Compositae, which is one of the major vegetable in the world. It is also amenable to manipulated in tissue culture and to transform genes. Depending on genotypes of lettuces, shoot regeneration through tissue culture was reported by Doerschug and Miller (1967). The effect of light and temperature on shoot regeneration from the cotyledon explants of lettuce was investgated (Koevarny *et al.* 1978) and the effect of auxins on an adventitious root formation has been reported (Kang *et al.*, 1996). In addition, interspecific somatic hybrids between cultivated lettuce (*Lactuca sativa*) and a wild species *L. virosa* were produced by protoplast electrofusion (Matsumoto, 1991) and the transformation using *Agrobacterium tumefaciens* was attempted (Richard *et al.*, 1987; Choi *et al.*, 1994). Although there are many reports on *in vitro* regeneration of lettuce, regenerability is, however, genotype-specific and culture condition have yet to be optimized. These requirements prompt us to investigate the effects of plant growth regulators, cultivars and sources of explants on an efficient regeneration of lettuce. Therefore the present study was conducted to establish an efficient regeneration system of currently commercial different cultivars of lettuce as a preliminary step for gene manipulation in lettuce.

Corresponding author: **Young-Sook Kim,** Institute for Molecular Biology and Genetics, Chonbuk National University, Chonju, 560-756, Korea.

MATERIALS AND METHODS

The seeds of *Lactuca sativa* cv. Chongchima, Chongchuckmyun, Jeokchima, Jeokchuckmyun were first sterilized in 70% ethyl alcohol for 10 sec, in 2% sodium hypochlorite solution for 10 min, and then rinsed three or four times with sterile distilled water. They were germinated on MS medium (Murashige and Skoog, 1962) without plant growth regulators at 25±1℃ in the light. After 7-10 days, the explants from cotyledons (5×5 mm) and hypocotyls (5 mm in length) were inoculated on MS media supplemented with 0.1 mg/L NAA in combination with BA, kinetin and zeatin (0.5 or 1.0 mg/L). From 15d old sterile seedlings, leaf discs (5×5 mm) were excised and cultured on the same medium that cotyledon and hypocotyl explants were cultured. The culture was maintained under irradiance of 60 μ $Em^{-2}s^{-1}$ provided by cool-white fluroescence with 16/8 light cycle at 25 ±1℃. In order to investigate the effects of GA_3 on growth of multiple shoots, multiple shoots induced from cotyledon explants were transferred on MS medium containing 0.5 mg/L BA plus GA_3 (0.1, 0.3 or 5 mg/L). All media were supplemented with 8 g/L

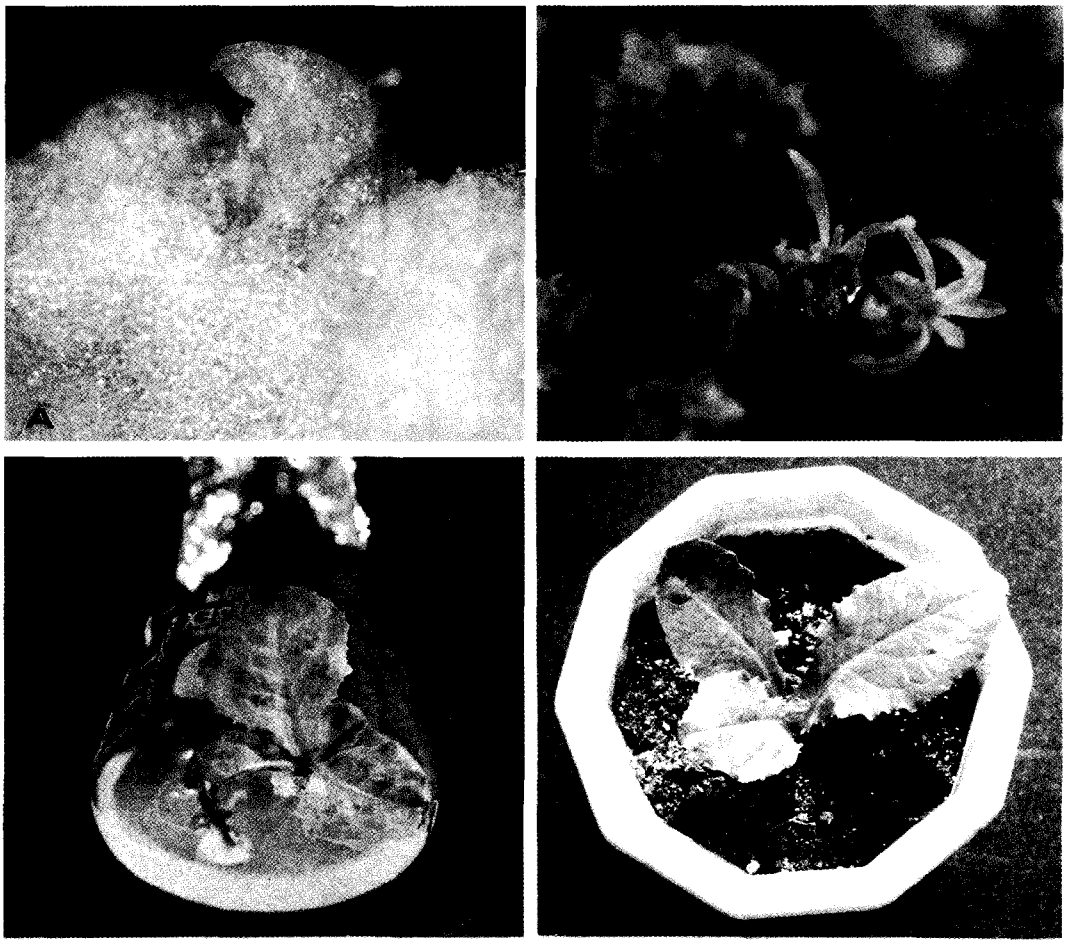

Fig. 1. Plant regeneration from *in vitro* culture of *Lactuca sativa*.
A. shoot primodia on surface of callus. B. Normal shoot differenciated on MS medium containing 0.1mg/L NAA and 0.5mg/L BA. C. Growth of multiple shoots on medium with 0.5mg/L BA and 1.0mg/L GA_3. D. Plant transplanted in the pot.

agar and 30 g/L sucrose, adjusted to pH 5.8 prior to adding agar, autoclaved at 121℃ for 15 minutes and dispensed into petridish. After 6 weeks of culture the frequency of shoot regeneration was investigated. In order for rooting, regenerated shoots were transferred on MS medium without plant growth regulators. Rooted plantlets were acclimated in pots containing a mixture of vermiculite and peatmoss (1:1).

RESULTS

After 10 days of culture, callus and/or shoot primodia with callus were easily induced from the cutting edges of cotyledon explants on the all MS media supplemented with 0.1 mg/L NAA in combination with BA, kinetin and zeatin (0.5 or 1.0 mg/L) except hormone free medium. The calli grew slowly and some of them began to produce yellowish and compact calli or turned brown.

Shoot primodia were appeared on surface of yellowish and compact callus after 2-4 weeks of culture (Fig. 1A). They grew vigorously and showed normal shoot growth (Fig. 1B). Shoot formation rate and number of shoots per explant were examined after 6 weeks of culture as in Figures 2, 3, and 4.

When cotyledon explants of four lettuce cultivars

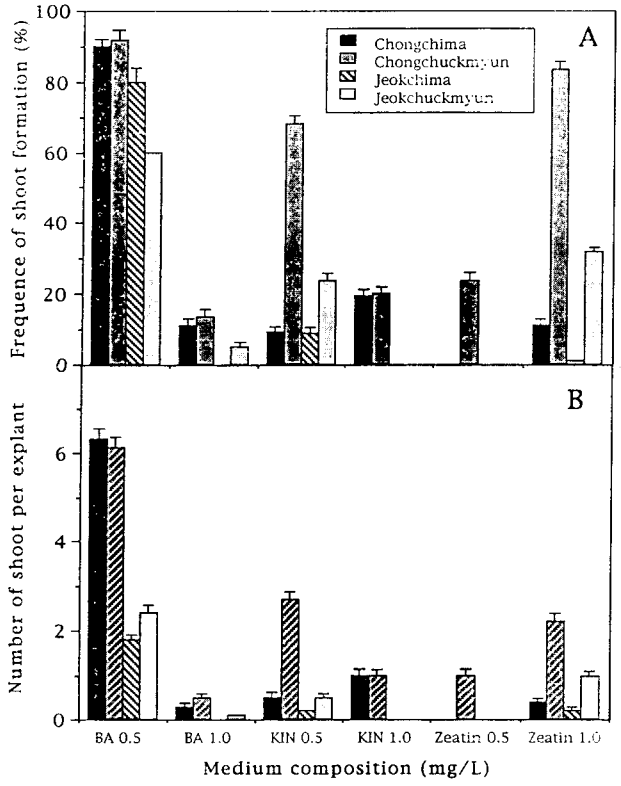

Fig. 2. Effects of cytokinins on shoot formation from cotyledon culture of four cultivars of *Lactuca sativa* on MS medium containing 0.1mg/L NAA after 6 weeks of culture.
A: Frequence of shoot formation, B: Number of shoot per explant. The vertical bars indicate standard error calculated from three replicates.

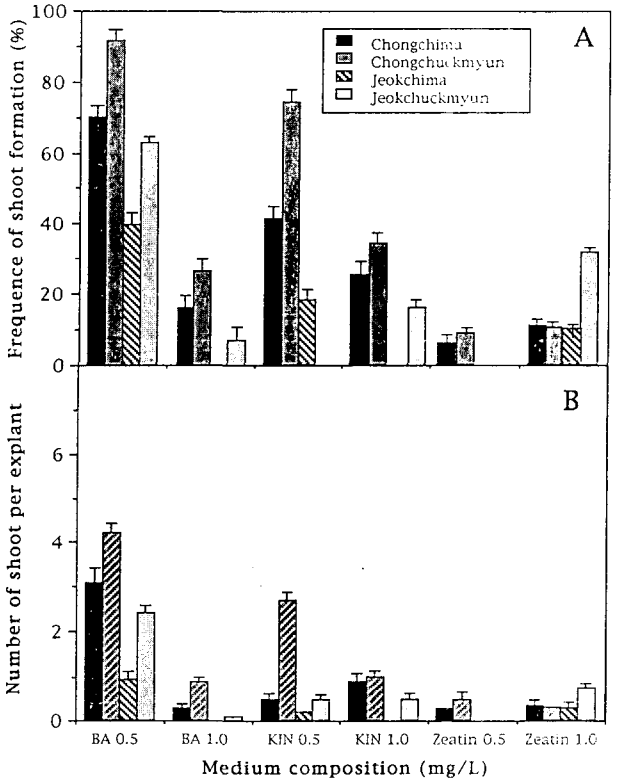

Fig. 3. Effects of cytokinins on shoot formation from hypocotyl culture of four cultivars of *Lactuca sativa* on MS medium containing 0.1mg/L NAA after 6 weeks of culture.
A: Frequence of shoot formation, B: Number of shoot per explant. The vertical bars indicate standard error calculated from three replicates.

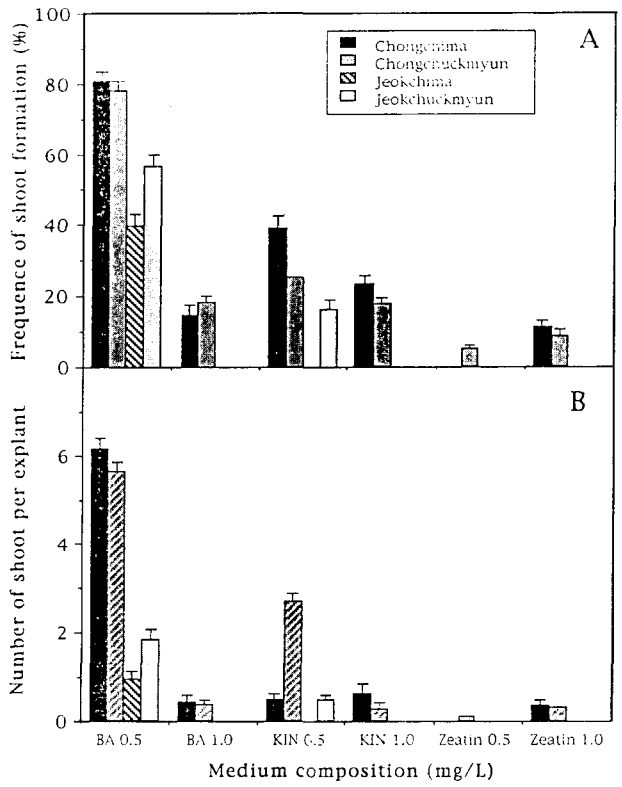

Fig. 4. Effects of cytokinins on shoot formation from leaf disc culture of four cultivars of *Lactuca sativa* on MS medium containing 0.1mg/L NAA after 6 weeks of culture.
A: Frequence of shoot formation, B: Number of shoot per explant. The vertical bars indicate standard error calculated from three replicates.

(Chongchima, Chongchuckmyun, Jeokchima, Jeokchuckmyun) were cultured on MS media with 0.1 mg/L NAA and 0.5 mg/L BA, shoot formation rate was higher than that of other MS media containing NAA in combination with kinetin and zeatin (Fig 2A). Shoot formation rate of cultivars, Chongchima, Chongchuckmyun, Jeokchima and Jeokchuckmyun were appeared to be 90.0%, 91.7%, 80.0% and 60.0%, respectively. On MS medium with 0.1 mg/L NAA and 0.5 mg/L BA, the number of shoots per explant was more greater than that of media with NAA, kinetin and zeatin and they were appeared to be 6.3, 6.1, 1.8 and 2.4 for cultivars, Chong-chima, Chongchuckmyun, Jeokchima and Jeokchuckmyun, respectively (Fig. 1B). In shoot formation, all the cultivars showed a considerably high efficiency on media containing 0.1 mg/L NAA and 0.5 mg /L BA.

When hypocotyl explants were cultured on the same media that cotyledon explants were cultured, they swelled and started to form callus at the basal part of wounded tissue after 1 week of culture. Calli formed on the segments were compact or friable in appearance. Friable calli failed to produce the shoots. Similar to cotyledon culture, a higher shoot formation rate as well as a larger number of shoots per explant were observed on media with 0.1 mg/L NAA and 0.5 mg/L BA (Fig. 3A). However the frequency of shoot formation from hypocotyl explants was lower than that of cotyledon explants. As in Figure 3, shoot formation rates of four cultivars, Chongchima, Chongchuckmyun, Jeokchima and Jeokchuckmyun were 68.8%, 88.2%, 36.4% and 63.6%, respectively.

The numbers of shoots per hypocotyl explant were 2.5, 4.1, 0.7 and 1.3, for these cultivars of Chongchima, Chongchuckmyun, Jeokchima and Jeokchuckmyun, respectively (Fig. 3B). When leaf discs of lettuce cultivars were cultured, results were similar to that of cotyledon and hypocotyl culture (Fig. 4A, 4B). We therefore sugge-

Table 1. Growth response of GA_3 treatment to multiple shoots derived from *Lactuca sativa* cv. Chongchuckmyun after 4 weeks in culture

Growth regulators(mg/L)		Leaf width(mm)	Leaf length(mm)	Remark
BA	GA_3			
0.5	0.1	0.08±0.02a	8.50±0.76	abnormal growth
0.5	0.3	1.73±0.43	6.75±1.14	normal growth
0.5	0.5	1.78±0.62	9.25±1.31	normal growth

[a]Mean value±SE

st that the optimal medium for shoot regeneration in lettuce is MS medium supplemented with 0.1 mg/L NAA and 0.5 mg/L BA and plant regeneration can be accomplished effectively by using cotyledon of cultivar, Chongchuckmyun

In order to elucidate the effect of GA_3 on the growth of multiple shoots, multiple shoots induced from cv. Chongchukmyeon were transferred onto MS basal media with 0.5 mg/L BA and 0.1, 0.3, or 0.5 mg/L GA_3 (Table 1). Multiple shoots were grown normaly on media with 0.5 mg/L BA plus 0.3 or 0.5 mg/L GA_3. Both of length and width of regenerated leaves showed normal growth on MS medium containing 0.5 mg/L BA and 0.5 mg/L GA_3 (Fig. 1C). Abnormal growth was observed on MS medium with 0.5 mg/L BA and 0.1 mg/L GA_3. When shoots were transferred on MS medium without plant growth regulators, roots were effectively differentiated. Rooted plantlets were accliminated on pot with vermiculite and peatmoss (1:1) (Fig. 1D).

DISCUSSION

From explants (cotyledon, hypocotyl, leaf disc) of *Lactuca sativa* cv. Chongchima, Chongchuckmyun, Jeokchima, Jeokchuckmyun, calli and/or shoot primodia with calli were induced on MS medium supplemented with NAA (0.1 mg/L) in combination with BA, kinetin and zeatin (0.5 or 1.0 mg/L) except medium without plant growth regulators. Plant growth regulators were required for induction of callus and/or shoot primodia with callus based on the facts that no callus was obtained on MS medium without plant growth regulators. Shoot formation was strongly affected by the composition and concentration of plant growth regulators, the cultivars used and the source of explants. Choi *et al.*, (1994) have reported that the combination of NAA and kinetin was more effective for shoot formation of lettuce. Also Chung *et al.*, (1998) have reported the effect of NAA and 2ip(isopentenyl Adenine) for shoot regeneration.

In this experiments, shoot formation of all cultivars was effectively responded to MS medium with 0.1 mg/L NAA and 0.5 mg/L BA. It may be attribute to different genotype of donor plant tested. Especially the cultivar, Chongchuckmyun, showed the highest efficiency of shoot formation, and cotyledon explants of all the cultivars were more effective than explants of other sources in plant regeneration. These results were in keeping with Kang and Hall (1996)'s conclusion that shoot formation from explant was affected strongly by the source of explants and the concentration of plant growth regulators. Kang *et al.*(1996) reported that the auxin affected on adventitious root formation of microcalli derived from cotyledon of lettuce cultivars.

In this study, the highest number of shoots was produced on MS medium supplemented with 0.1 mg/L NAA and 0.5 mg/L BA.

In general, it is known that the level of auxin and cytokinin affects callus induction and plant regeneration *in vitro* culture. When cotyledon explants of lettuce were cultured, advenititous root formation occurred on MS mediun containing auxin (Kang *et al.*, 1996) and the formation of shoot was effective when the explants were cultured on MS medium supplemented with 0.1 mg/L NAA and 1.0 mg/L kinetin in lettuce (Kim and Eun, 1992). Regeneration efficiency was very much dependent on cultivars used and *Lactuca sativa* cv. Chongchukmyun was superior to other cultivars in cell suspension culture (Alconero, 1983). In this study, plant regeneration from cvs. Chongchukmyun and Chongchima was superior to cvs. Jeokchukmyun and Jeokchima.

GA is the "effector" in the growth of shoots in some other higher plants (Phinney, 1984) and it probably behaves as an effector in shoot growth of apple (Looney *et al.*, 1988). In *Dysosma pleiantha*, transfer of immature seed-derived embryoids onto MS medium containing 1.0 mg/L BA plus 1.0 mg/L GA_3 gave rise to plantlets, which were grown to full size normal plants and the combination of 1.0 mg/L BA and 1.0 mg/L GA_3 proved to be most efficient in supporting embryoid germination and subsequent normal plantlet

development (Chuang and Chang, 1987). Sikdar *et al.* (1987) suggested that calli grown on medium containing 0.5 mg/L NAA, 4.0 mg/L BA and 0.1 mg/L GA_3 produced the maximum numbers of plantlets, and the regeneration frequency of plants was decreased to one-third in an absence of GA_3. The growth of multiple shoots were most effective on MS medium with 0.5 mg/L BA and 0.5 mg/L GA_3 in this study. The yield of regeneranted plants obtained by this procedure was higher than in other reports(Kim and Eun, 1992; Chung *et al.*, 1998).

In conclusion, the system for shoot regeneration was developed and the regeneration frequency was increased by the GA_3 treatment. Therefore, our results suggest that this regeneration system may be an effective method for gene manipulation in lettuce.

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