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## Somatic embryogenesis and plant regeneration from zygotic embryo explants of onion

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### ABSTRACT

The study was undertaken to examine the effect of two synthetic auxins on somatic embryogenesis and plant regeneration from mature embryo explants of 16 onion cultivars. Cotyledons were removed from the embryos and remaining portions were cultured on MS medium supplemented with 1.0 mg/L 2,4-D and 5.0 mg/L picloram alone or in combination (1.0 mg/L 2,4-D + 2.5 mg/L picloram) to produce embryogenic callus. MS medium supplemented with 5.0 mg/L picloram was found to be the best one for both embryogenic callus induction (85%) and callus diameter (3.8 mm). Of the 16 cultivars studied, Yeoeuijuhwan exhibited the lowest frequency of embryogenic callus induction (50.5%), whereas all the other 15 cultivars showed more than 60% embryogenic callus induction. Scanning electron micrograph (SEM) analysis of embryogenic callus showed all stages of somatic embryos such as globular, scutellar and coleoptilar. Plant regeneration was significantly affected by the composition of embryogenic callus induction medium. The greatest frequency of somatic embryo conversion was obtained from embryogenic callus developed in MS medium with 2,4-D (70.1%) followed by picloram (38.9%) and 2,4-D + picloram (34.5%). The germinated plantlets were further developed on the half-strength MS medium containing 3% sucrose and were acclimatized in the culture room with 98% survival rate.

**Keywords:** *Allium cepa*, auxin, embryogenic callus, *in vitro* propagation, picloram.

### RESUMO

#### Embriogênese somática e regeneração de plantas a partir de explantes de embriões zigóticos de cebola

O estudo foi realizado para examinar o efeito de duas auxinas sintéticas na embriogênese somática e na regeneração de plantas a partir de explantes de embriões maduros de 16 cultivares de cebola. Os cotilédones foram removidos a partir dos embriões e as porções restantes foram cultivadas em meio MS suplementado com 1,0 mg/L de 2,4-D e 5,0 mg/L de picloram sozinho ou em combinação (1,0 mg/L de 2,4-D + 2,5 mg/L de picloram) para produzir calos embriogênicos. Verificou-se que o meio MS suplementado com 5,0 mg/L de picloram foi o melhor tanto para a indução de calo embriogênico (85%) quanto para o diâmetro do calo (3,8 mm). Das 16 cultivares estudadas, Yeoeuijuhwan exibiu a menor frequência de formação de calos embriogênicos (50,5%), ao passo que todas as outras 15 cultivares apresentaram mais de 60% de indução de calos embriogênicos. A análise dos calos por micrografia eletrônica de varredura (SEM) mostrou todas as fases dos embriões somáticos, tais como as fases globular, escutelar e coleoptilar. A regeneração de plantas foi significativamente afetada pela composição do meio de indução de calos embriogênicos. A maior frequência de conversão de embriões somáticos foi obtida a partir do calo embriogênico desenvolvido em meio MS com 2,4-D (70,1%) seguido por picloram (38,9%) e 2,4-D + picloram (34,5%). As plântulas germinadas foram desenvolvidas no meio contendo metade da dose de MS, com 3% de sacarose e foram aclimatizadas em ambiente de cultura com 98% de taxa de sobrevivência.

**Palavras-chave:** *Allium cepa*, auxina, calo embriogênicos, propagação *in vitro*, picloram.

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Onion (*Allium cepa*) is one of the most important vegetable crops in the world. It has been used as an herbal drug in traditional medicine for the treatment of various ailments. Onion is reported to contain dietary fiber, fructooligosaccharides, flavonoids and alk(en)yl cystein sulphoxides (Benítez *et al.*, 2011). Compounds obtained from onion have been reported to possess antiasthmatic, antibiotic, anticarcinogenic, antioxidant,

antiplatelet, and antithrombotic activities (Griffiths *et al.*, 2002; Santas *et al.*, 2010; Albrechtova *et al.*, 2012). Onion has been cultivated worldwide, with an annual production of 85 million tons. The consumption of onion is increasing sharply in recent years. The productivity of onion is adversely affected by various abiotic and biotic factors. The crop improvement through conventional breeding has certain limitations. Onion will not cross readily with other *Allium*

species (Griffiths *et al.*, 2002). Further, it takes two years to produce seeds due its biennial nature.

Plant tissue culture technique has become an important tool for crop improvement. Development of efficient plant regeneration protocol is a prerequisite for genetic transformation studies. Several studies on onion reported plant regeneration from bulb (Hussey & Falavigna, 1980), immature flower bud (Pike & Yoo, 1990), immature

zygotic embryo (Marinangeli *et al.*, 2005), mature flower bud (Luthar & Bohanec, 1999), and mature zygotic embryo (Zheng *et al.*, 2001). However, plant regeneration is highly dependent upon the cultivars studied (Tanikawa *et al.*, 1998). On the other hand, somatic embryogenesis (SE) is considered useful tool for genetic manipulation and large scale propagation. In addition, this process is also considered as a most suitable system for physiological and morphological studies in plant during morphological development, and conservation of desired genotypes through mass propagation (Lelu-Walter *et al.*, 2013). SE is mainly associated with culture conditions, concentration of plant growth regulators in the culture medium and stress factors. The addition of high levels of auxin to the culture medium is often required to induce somatic embryos in most plant species. However, development beyond the globular stage was inhibited by maintaining the same induction medium. Thus, for promoting further growth and development of SES it is necessary to subculture the embryos to the PGR-free medium. SE in onion has been reported previously using embryo (van der Valk *et al.*, 1992; Eady *et al.*, 1998; Zhang *et al.*, 2004), flower bud (Luthar & Bohanec, 1999), radical (Aswath *et al.*, 2006), shoot apex (Ramakrishnan *et al.*, 2013), and seed (Saker, 1997). These studies have demonstrated the influence of culture media, genotype and plant growth regulators on SE. The frequency of SE is mainly affected by genotype (Saker, 1997; Eady *et al.*, 1998). Saker (1997) demonstrated that 2,4-dichlorophenoxyacetic acid (2,4-D) was suitable for the induction of SE in embryo explants of onion. On the other hand, Eady *et al.* (1998) reported the induction of SE from embryo explants of onion cultured on medium with picloram. Thus, in this study we investigated the effect of auxins on somatic embryogenesis and plant regeneration from mature embryo explants of 16 onion cultivars.

## MATERIAL AND METHODS

### Plant material and surface

**sterilization** - Seeds of 16 onion (*Allium cepa*) cultivars (Geumgodeahwang, Geumokhwang, Dabonghwang, Bonganhwang, Chunjudaegeo, Chunjuhwan, Chunjujunggo, Yeoeuijuhwang, Okayhwang, Yangchunhwang, Younganhwang, Seouldaegeo, Spring Gold, Turbo, Manchuhwang, and Higuma) were obtained from Onion Research Institute, Gyeongsangnam-do Agricultural Research and Extension Services, Changnyeong, Korea. The characteristics of onion cultivars are given in Table 1. Seeds were thoroughly washed with distilled water. Then, they were surface sterilized in 70% ethanol for 30 seconds, immersed in 2% sodium hypochlorite for 20 minutes with agitation, washed with sterile distilled water for five times, and stored in sterile distilled water at 4°C. After 24 hours, seeds were rinsed two times with sterile distilled water. Seeds were dissected and mature embryos were isolated.

### Embryogenic callus induction

- Cotyledons were removed from the embryos and remaining portions (Figure 1A) were cultured in 90×15 mm Petri dish containing 25 mL of Murashige & Skoog (MS) medium supplemented with 1.0 mg/L 2,4-D, 5.0 mg/L picloram or 1.0 mg/L 2,4-D + 2.5 mg/L picloram. The medium consisted of MS nutrients and vitamins (Murashige & Skoog, 1962) with 3% sucrose and solidified with 0.8% plant agar (Duchefa Co., Netherlands). The pH of the medium was adjusted to 5.8 before autoclaving at 121°C, for 20 minutes. The cultures were maintained at 25±2°C in darkness and subcultured onto the same fresh medium at 4 weeks. For each treatment, 60 embryos were used and the experiment was repeated three times. The percentage of embryogenic callus induction and diameter of callus were recorded after 8 weeks of culture.

**Scanning electron microscopy (SEM) analysis** - Embryogenic calli (8 weeks old) were fixed in 5% paraformaldehyde, 5% glutaraldehyde and 0.2 M phosphate buffer (pH 7.2) at 4°C. After 4 hours, the fixed samples were washed three times with 0.1 M phosphate buffer solutions for 15

minutes. The samples were fixed again in 2% OsO<sub>4</sub> at 4°C for 2 hours, and 0.2 M phosphate buffer (pH 7.2) for 4 hours. Then, they were washed with 0.1 M phosphate buffer solution for 15 minutes with 3 times, and dehydrated with ethanol series of 15 minutes exposures to 50, 60, 70, 80, and 90%. Finally exposure to 100% ethanol was repeated three times. The samples were critical-point dried and coated with gold before observation. They were analyzed using scanning electron microscope (JSM-5410LV, JEOL, Japan) operating at 15 kV.

**Plant regeneration** - Embryogenic calli obtained from each induction medium were separately transferred to hormone-free MS medium containing 3% sucrose. The cultures were maintained at 25±2°C under a 16 hours photoperiod with light of 45 μmol/m<sup>2</sup>/s. For each experiment, 30 embryogenic calli were used and the experiment was repeated three times. The percentage of plant regeneration was calculated after 8 weeks of culture.

### Plantlet development and acclimatization

- Regenerated plantlets were transferred to half-strength MS medium containing 3% sucrose for further shoot and root development. After 4 weeks, plantlets were removed from the culture, washed thoroughly with distilled water, planted in 10 cm pots containing peat, perlite and vermiculite (1:1:1), kept under culture room conditions, and irrigated two days interval with quarter-strength MS salt solution. Plantlet survival was recorded after 4 weeks.

**Data analysis** - Experiments were set up in a completely randomized design and repeated three times. The percentage of SE induction was determined by counting explants forming embryogenic callus from the total number of the cultured explants. The percentage of plant regeneration was determined by counting the number of embryogenic calli forming plantlets out of the total number of calli cultured. In each treatment, 60 explants or 30 calli were used and the experiment was repeated three times. Data were subjected to analysis of variance (ANOVA) by using SAS program (Release 9.1,

SAS Institute, NC, USA). Differences between the mean values were assessed with Duncan's multiple range test at  $p < 0.05$ . Percentage values were subjected to arcsine transformation before variance analysis (Compton 1994).

## RESULTS AND DISCUSSION

**Effect of auxins on embryogenic callus induction** – The induction of embryogenic callus from various explants of onion has been best achieved by incorporating auxins such as 2,4-D and picloram into the culture medium (Phillips & Luteyn, 1983; van der Valk *et al.*, 1992; Saker, 1997; Eady *et al.*, 1998; Zhang *et al.*, 2004; Aswath *et al.*, 2006; Ramakrishnan *et al.*, 2013). However, the frequency of callus induction was low (33 - 60%). Furthermore, the callus induction is mostly dependent on genotype. In this study, we investigated the effects of 2,4-D and picloram on embryogenic callus induction from zygotic embryo of 16 Korean onion cultivars. The zygotic embryo explants formed callus within 2 weeks of culture on the MS

medium supplemented with 2,4-D and picloram alone or in combination (Figure 1B). After 4 weeks of culture, three morphologically different callus types were observed: compact, friable and transparent types. Yellowish compact and nodular structures were observed after 8 weeks of culture in all studied cultivars (Figure 1C). However, the frequency of embryogenic callus induction and growth of callus varied among the cultivars (Table 2). MS medium supplemented with 5.0 mg/L picloram was found to be best for both embryogenic callus induction (85%) and callus diameter (3.8 mm). Phillips & Luteyn (1983) reported that picloram was superior than 2,4-D for callus induction. Eady *et al.* (1998) also reported that picloram was suitable for callus induction in onion, but the mean frequency of callus induction was low (48%). The major difference between the protocols is use of MS medium instead of MS nutrients with B<sub>5</sub> vitamins. Of the 16 cultivars studied, Yeoeuijuhwan exhibited the lowest frequency of embryogenic callus induction (50.5%), whereas all the other 15 cultivars showed more than 60% embryogenic callus induction in all studied media.

'Geumgodeahwang', 'Dabonghwang', 'Chunjudaego', 'Okayhwang', 'Yangchunhwang', 'Younganhwang', 'Turbo', and 'Higuma' showed more than 90% embryogenic callus induction. The highest callus diameter was observed in 'Geumokhwang' (4.55 mm) and the lowest was in 'Yeoeuijuhwan' (2.31 mm) when the explants were cultured on MS medium supplemented with 5.0 mg/L picloram or 1.0 mg/L 2,4-D and 2.5 mg/L picloram, respectively (Table 2). In the medium containing 2,4-D, the frequency of embryogenic callus induction ranged from 32.6 to 95.3%. In 'Bonganhwan', 'Chunjudaego', 'Yeoeuijuhwan', 'Spring Gold', 'Manchuhwan', and 'Higuma' combination of 2,4-D and picloram enhanced the frequency of embryogenic callus induction than 2,4-D or picloram.

Embryogenic callus induction is dependent on genotype and plant growth regulators. Auxins have been proven to be the most effective for embryogenic callus induction from various explants of onion (Saker, 1997; Eady *et al.*, 1998; Zhang *et al.*, 2004; Aswath *et al.*, 2006; Ramakrishnan *et al.*, 2013).

**SEM analysis of embryogenic callus** - The processes of SE have been documented in onion using 2,4-D (Saker, 1997) and picloram (Eady *et al.*, 1998). However, the morphology of somatic embryos has not been well studied. In this study, the morphology of embryogenic callus (8 weeks old) was examined under microscope. Various stages of somatic embryos were observed on the surface of the embryogenic callus through light microscope (Figure 2A). SEM analysis also confirmed the presence of various stages of somatic embryos such as globular (Figure 2B), scutellar and coleoptilar (Figures 2C, 2D). The callus mediated SE has also been confirmed through SEM analysis in other monocotyledons (Ozias-Akins & Vasil, 1982, 1983; Chaudhury & Qu, 2000; Cabral *et al.*, 2011).

**Plant regeneration from embryogenic callus** - The embryogenic callus showing somatic embryos and plant regeneration were transferred to the hormone-free MS medium containing 3% sucrose. The regeneration potential

**Table 1.** Details of onion cultivars used in this study (detalhes de cultivares de cebola utilizadas neste estudo). Seoul (Korea), Konkuk University, 2014.

Cultivar	Cultivation group		
	Sowing in spring	Sowing in autumn	Ripening stage
Geumgodeahwang	-	o	Mid and late
Geumokhwang	-	o	Mid and late
Dabonghwang	-	o	Mid and late
Bonganhwan	-	o	Mid and late
Chunjudaego	-	o	Late
Chunjuhwan	-	o	Mid and late
Chunjujunggo	-	o	Mid and late
Yeoeuijuhwan	-	o	Mid and late
Okayhwang	-	o	Mid and late
Yangchunhwang	-	o	Extremely early
Younganhwang	-	o	Mid and late
Seouldaego	-	o	Mid and late
Spring Gold	o	-	Early
Turbo	-	o	Mid
Manchuhwan	-	o	Late
Higuma	o	-	Early



of embryogenic callus was significantly affected by the callus induction medium. The plant regeneration frequency ranged from 15.8 to 87.2% (Table 3). ‘Geumgodeahwang’, ‘Geumokhwang’ and ‘Chunjuhwang’ showed more than

80% of plant regeneration, whereas ‘Yeoeuijuhwang’ exhibited low regeneration frequency (56.4%). The highest frequency of plant regeneration was observed in ‘Geumokhwang’ (87.2%). Of the various calli studied, the embryogenic calli induced on MS medium containing 2,4-D had high regenerative potential. Similar result has also been reported in onion (van der Valk *et al.*, 1992; Luthar & Bohanec, 1999). In contrast, somatic embryos of onion produced on MS medium containing low concentration of 2,4-D (1.0 mg/L) failed to develop into plantlets (Saker, 1997). In this study, the greatest frequency of somatic embryo conversion was obtained from callus developed in MS medium with 2,4-D (70.1%) followed by picloram (38.9%) and 2,4-D + picloram (34.5%). 2,4-D has been reported to be the most efficient synthetic auxin for SE in onion (van der Valk *et al.*, 1992; Saker, 1997; Zheng *et al.*, 1998; Luthar & Bohanec, 1999; Ramakrishnan *et al.*, 2013). On the other hand, Eday *et al.* (1998) and Phillips & Luteyn (1983) found that picloram was more efficient for SE than 2,4-D. In most of the studies, somatic embryos of onion converted to plantlets on the culture medium without hormones (van der Valk *et al.*, 1992; Zheng *et al.* 1998). In this study, somatic embryos germinated to plantlets within 8 weeks of culture on the hormone-free MS medium (Figure 3A). However, the plant regeneration frequency was lowest when somatic embryos induced on MS medium containing picloram or combination of 2,4-D and picloram were cultured on MS basal medium (Table 3), and this possibly due to the residual effects of auxins. Activated charcoal (AC) has the potential to absorb some inorganic ions, growth hormones and phenolics. Aboshama (2011) suggested that there was an important role of AC in reducing the inhibitory effect of residual auxins and stimulating embryo conversion. Thus, inclusion of AC to the regeneration medium may increase the plant regeneration frequency.

**Plantlet development and acclimatization** - The germinated plantlets further developed on the half-

**Table 2.** Effects of auxins on embryogenic callus induction (%) and callus growth from zygotic embryos of 16 onion cultivars after 8 weeks of culture (efeito de auxinas na formação de calo embriogênico e de desenvolvimento do calo a partir de embriões zigóticos de 16 cultivares de cebola após 8 semanas de cultura). Seoul (Korea), Konkuk University, 2014.

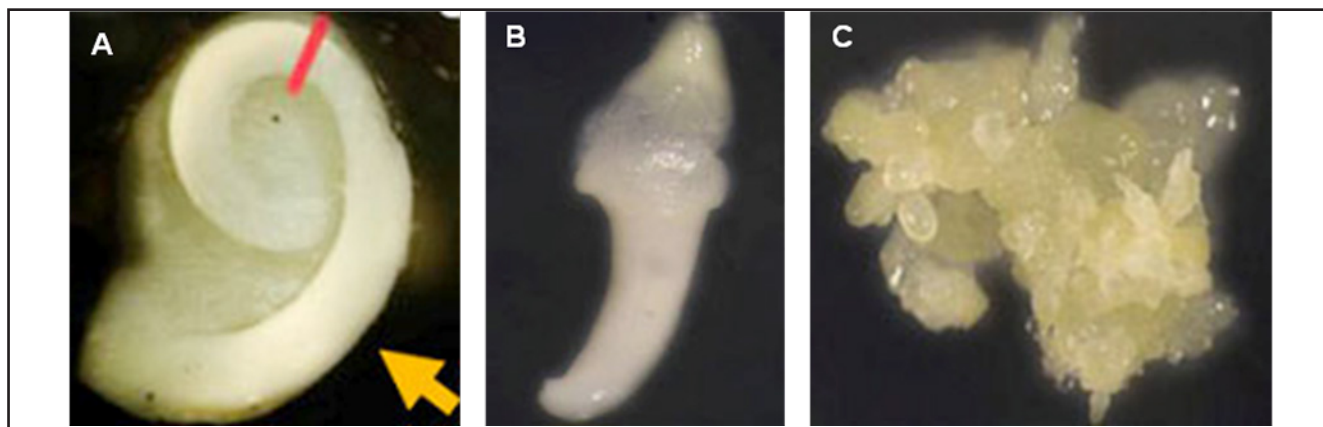
Cultivars	Treatments			Mean
	1	2	3	
Geumgodeahwang	91.6±2.4	94.7±1.3	84.7±1.2	90.3±5.1 ab
Geumokhwang	60.5±1.5	81.1±3.0	65.8±1.7	69.1±10.7 e
Dabonghwang	86.3±3.0	90.5±3.5	83.2±2.6	86.6±3.7 a-d
Bonganhwang	76.8±2.2	87.9±2.1	94.0±2.4	86.2±9.0 a-d
Chunjudaeo	95.3±2.4	96.8±2.2	98.0±1.0	96.7 ±1.3a
Chunjuhwang	89.5±1.5	86.3±1.0	88.3±3.2	88.0±1.6 a-c
Chunjujunggo	74.2±1.7	83.7±1.4	66.8±2.1	74.9 ±8.5de
Yeoeuijuhwang	32.6±1.0	39.5±2.0	50.5±1.4	40.8±9.0 f
Okayhwang	78.4±1.2	93.6±2.4	74.2±3.4	82.0 ±9.1b-d
Yangchunhwang	91.1±2.0	97.9±1.2	86.8±3.7	91.9±5.6 ab
Younganhwang	85.3±3.7	92.6±3.4	90.0±3.9	89.3±3.7 a-c
Seoul daego	74.7±1.2	87.9±3.2	78.9±2.2	80.5±6.7 b-e
Spring Gold	77.9±2.1	67.9±2.2	87.4±2.4	77.7 ±9.8c-e
Turbo	82.4±2.6	90.1±3.0	80.5±2.9	84.3±6.2 a-d
Manchuhwang	76.3±1.7	83.7±1.4	89.0±2.0	83.0 ±6.4b-d
Higuma	84.7±2.4	85.8±3.2	95.8±3.0	88.7±6.1 a-c
Mean	78.6±14.9 b	85.0±14.1 a	82.1±12.6 ab	
	Callus diameter (mm)			
Geumgodeahwang	3.48±0.12	3.83±0.15	3.22±0.13	3.51±0.30 bc
Geumokhwang	3.32±0.12	4.55±0.11	3.13±0.24	3.66 ±0.77b
Dabonghwang	3.05±0.08	4.04±0.13	3.07±0.12	3.38±0.57 cd
Bonganhwang	2.74±0.10	3.36±0.18	2.50±0.11	2.86±0.44 g
Chunjudaeo	3.89±0.18	4.42±0.12	3.64±0.11	3.98 ±0.40a
Chunjuhwang	3.24±0.11	3.71±0.15	2.95±0.13	3.30±0.38 c-e
Chunjujunggo	2.88±0.12	3.36±0.17	2.67±0.08	2.97±0.35 fg
Yeoeuijuhwang	2.38±0.16	2.83±0.28	2.31±0.08	2.50±0.28 h
Okayhwang	2.85±0.20	3.71±0.16	2.81±0.09	3.12 ±0.51d-g
Yangchunhwang	3.42±0.13	4.26±0.16	3.55±0.07	3.74±0.45 ab
Younganhwang	3.06±0.12	3.42±0.23	2.65±0.13	3.04±0.39 e-g
Seoul daego	3.08±0.19	3.65±0.22	2.86±0.15	3.19±0.41 d-f
Spring Gold	2.97±0.11	4.06±0.22	2.97±0.11	3.33±0.62 c-e
Turbo	3.08±0.10	3.62±0.14	2.69±0.18	3.13±0.47 d-g
Manchuhwang	3.55±0.10	4.31±0.25	3.36±0.18	3.74 ±0.50ab
Higuma	3.28±0.12	4.04±0.19	2.77±0.10	3.36±0.63cd
Mean	3.14±0.36 b	3.82±0.46 a	2.94±0.37 c	

Treatment 1= 2,4-D (1.0 mg/L); Treatment 2= Picloram (5.0 mg/L); Treatment 3= 2,4-D (1.0 mg/L) + Picloram (2.5 mg/L). Average values followed by same letters within a column are not significantly different according to Duncan’s multiple range test ( $p < 0.05$ ) (valores médios seguidos de letras iguais na coluna não diferem entre si, pelo teste de Duncan, 5%).

**Table 3.** Plant regeneration frequency of embryogenic callus obtained in MS medium containing auxins cultured on the hormone-free MS medium after 8 weeks of culture (frequência de regeneração de plantas de calo embriogênico obtido em meio MS contendo auxinas quando cultivados em meio MS isento de reguladores, após 8 semanas de cultura). Seoul (Korea), Konkuk University, 2014.

Cultivars	Plant regeneration (%)			
	Treatment 1	Treatment 2	Treatment 3	Mean
Geumgodeahwang	83.8±1.2	20.9±1.0	27.8±1.2	44.1±34.5 b-d
Geumokhwang	87.2±1.0	73.8±2.2	32.9±1.2	64.6±28.3 a
Dabonghwang	69.1±2.4	36.2±0.8	27.4±2.0	44.2±22.0 b-d
Bonganhwang	63.5±3.2	39.0±1.0	50.1±1.8	50.9±12.3 a-d
Chunjudaego	78.4±1.6	45.5±1.4	43.4±1.6	55.8±19.6 a-d
Chunjuhwan	86.7±1.8	40.6±2.8	46.8±2.0	58.0±25.0 a-c
Chunjujunggo	61.6±4.4	37.3±1.6	57.0±1.4	49.7±12.9 a-d
Yeoeuijuhwang	56.4±1.6	16.4±3.0	38.2±1.8	37.0±20.0 d
Okayhwang	68.2±1.0	24.5±3.4	22.4±1.6	38.4±25.9 cd
Yangchunhwang	71.0±3.0	23.7±3.2	34.7±2.2	43.1±24.8 bd
Younganhwang	76.7±2.4	57.8±2.4	45.0±1.0	59.8±15.9 ab
Seouldaego	67.2±2.8	33.4±1.5	15.8±1.3	38.8±26.1 cd
Spring Gold	60.5±3.4	43.0±2.0	30.6±2.2	44.7±15.0 b-d
Turbo	78.6±1.6	56.2±1.6	40.2±2.0	58.3±19.3 a-c
Manchuhwang	62.1±1.0	47.0±2.0	23.3±1.6	44.1±19.6 b-d
Higuma	66.3±2.6	27.6±2.4	16.3±1.4	36.7±26.2 d
Mean	70.1±9.7 a	38.9±15.1 b	34.5±12.1 b	

Treatment 1= 2,4-D (1.0 mg/L); Treatment 2= Picloram (5.0 mg/L); Treatment 3= 2,4-D (1.0 mg/L) + Picloram (2.5 mg/L). Average values followed by same letters within a column are not significantly different according to Duncan's multiple range test ( $p < 0.05$ ) (valores médios seguidos de letras iguais na coluna não diferem entre si, pelo teste de Duncan, 5%).

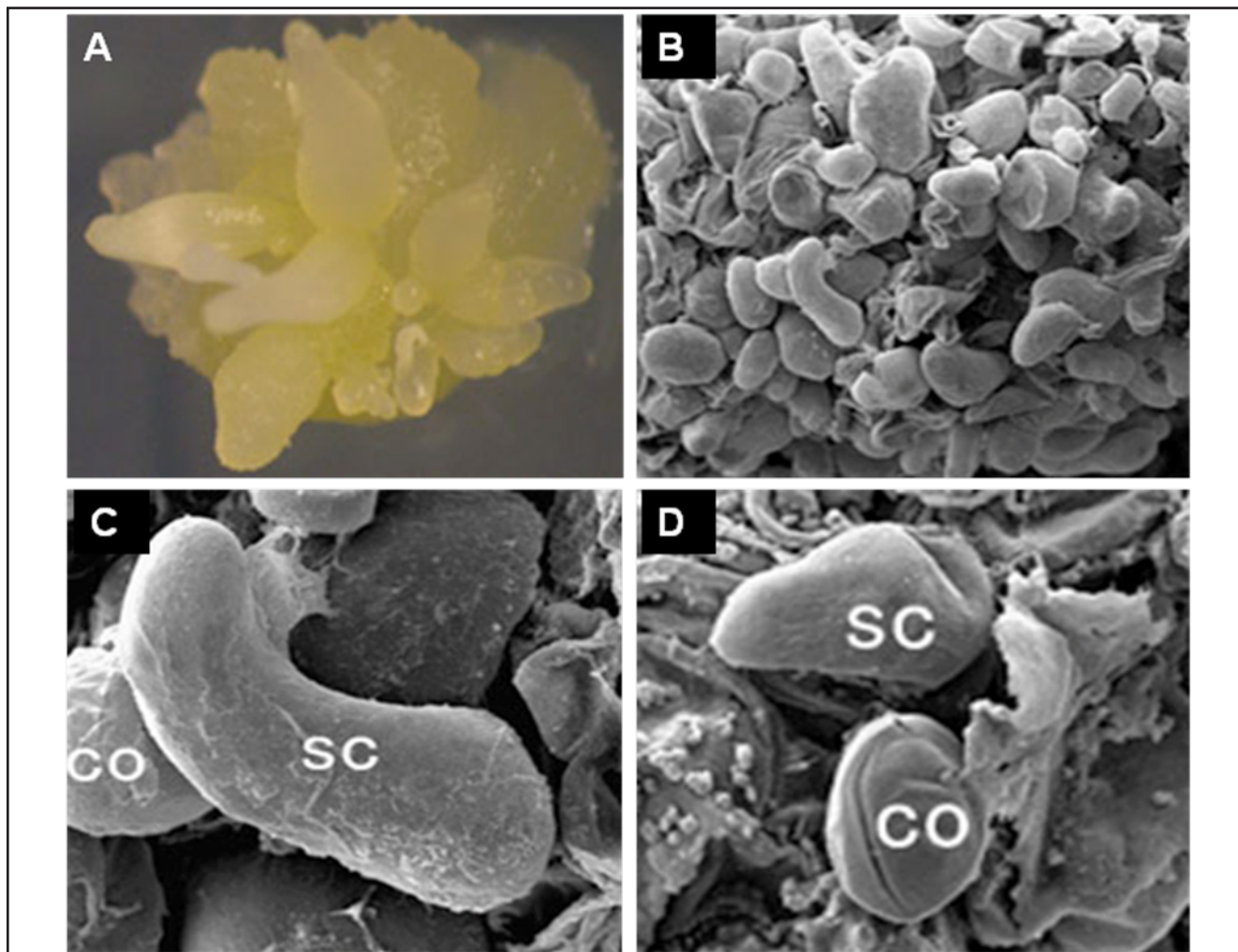


**Figure 1.** Different stages of somatic embryogenesis from zygotic embryo explants of *A. cepa*. (A) mature zygotic embryo (arrow represents the explant); (B) callus initiation after 2 weeks of culture and (C) embryogenic callus with somatic embryos after 8 weeks of culture {fases da embriogênese somática a partir de explantes de embriões zigóticos de *A. cepa*. (A) embrião zigótico maduro (seta representa o explante); (B) iniciação de calo, após 2 semanas de cultura e (C) calo embriogênico com embriões somáticos após 8 semanas de cultura}. Seoul (Korea), Konkuk University, 2014.

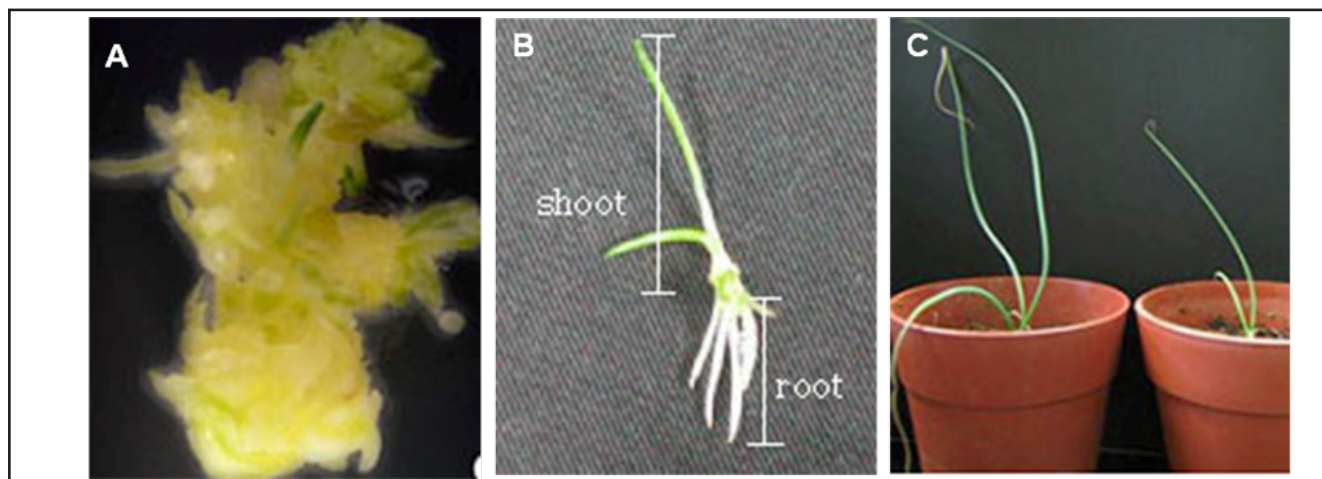
strength MS medium containing 3% sucrose (Figure 3B). After 4 weeks of culture, the regenerated plantlets were transferred to pots containing peat, perlite and vermiculite (1:1:1) and acclimatized in the culture room with 98% survival rate (Figure 3C).

In conclusion, an efficient plant regeneration protocol has been developed for 16 onion cultivars using mature embryo explants. Auxin had a significant effect on embryogenic callus induction and plantlet conversion. Picloram was shown to be the most

effective than picloram + 2,4-D and 2,4-D in the induction of embryogenic callus. The genotypic differences were observed in the frequency of embryogenic callus induction at different media composition. Thus, embryogenic callus induction is



**Figure 2.** Scanning electron micrographs of SE in onion. (A) embryogenic callus with somatic embryos; (B) scanning electron micrograph of embryogenic callus showing various stages of somatic embryos; (C) scutellum (SC) associated with coleoptile (CO) and (D) a well developed coleoptile (CO) and scutellum (SC) {micrografias de exploração eletrônica de SE em cebola. (A) calo embriogênico com embriões somáticos; (B) micrografia eletrônica de varredura de calos embriogênicos mostrando vários estágios de embriões somáticos; (C) escutelo (SC) associado com coleóptilos (CO) e (D) um coleóptilo bem desenvolvido (CO) e escutelo (SC)}. Seoul (Korea), Konkuk University, 2014.



**Figure 3.** Plant regeneration from embryogenic callus of *A. cepa*. (A) germination of somatic embryos on MS medium; (B) somatic embryo derived plantlet cultured on half-strength MS medium after 4 weeks of culture and (C) acclimatized plantlets {regeneração de plantas a partir de calos de *A. cepa*. (A) germinação de embriões somáticos em meio MS contendo metade da dose; (B) plântula obtida de embrião somático cultivada em meio MS após 4 semanas de cultura e (c) plântulas climatizadas}. Seoul (Korea), Konkuk University, 2014.



dependent on genotype and plant growth regulators. The best medium for 'Geumgodeahwang', 'Geumokhwang', 'Dabonghwang', 'Chunjujunggo', 'Okayhwang', 'Yangchunhwang', 'Younganhwang', 'Seouldaego', and 'Turbo' was MS medium with picloram, while the best one for 'Bonganhwang', 'Chunjudaeo', 'Yeoeuijuhwan', 'Spring Gold', 'Manchuhwang', and 'Higuma' was MS medium with 2,4-D + picloram. In 'Chunjuhwan' the highest frequency of embryogenic callus was observed in MS medium with 2,4-D. Light and scanning electron microscopy analysis confirmed the presence of various stages of somatic embryos. Maturation and conversion of somatic embryos were achieved after transfer of embryogenic calli to the PGR-free MS medium. Embryogenic callus obtained from the 2,4-D-medium converted into plantlets at higher frequency than those from picloram- or 2,4-D + picloram-medium. The plant regeneration frequency of embryogenic callus induced on MS medium supplemented with 2,4-D varied from 56.4 to 87.2%. The present study can be used for genetic transformation studies.

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