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OPTIMIZATION OF SOYBEAN (*GLYCINE MAX* L.) REGENERATION FOR KOREAN CULTIVARS

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Abstract

Tissue culture could provide key insights into the development of transgenic plants, production of good cultivars and secondary metabolites, conservation of endangered plants, and safeguarding of germplasms. In this study, the effects of shoot induction media, explants, cultivars, and phytohormone concentrations on the regeneration efficiency of Korean soybean cultivars were evaluated. Restricted dormancy and poor germination may affect regeneration, depending on the type of germination medium or initiation of phytohormone treatment. Therefore, we analyzed the effects of different germination media containing plant growth regulators, i.e., 6-benzyladenine (BAP), gibberellic acid 3 (GA_3), and naphthalene acetic acid (NAA), prior to investigating the influences of explant types, media with or without vitamins, cultivars, and different phytohormones (BAP and GA_3). A high frequency of germination was observed in Murashige and Skooge (MS) medium with vitamins supplemented with 1 mg L^{-1} BAP and 0.25 mg L^{-1} GA_3. Cotyledonary node explants and Gamborg B5 with vitamins supplemented with 1 mg L^{-1} BAP and 0.17 mg L^{-1} GA_3 in callus induction medium (CIM) and 1 mg L^{-1} BAP in shoot induction medium (SIM) were found to be the most efficient conditions for induction of soybean regeneration, both in callus development and shoot regeneration. Two Korean soybean cultivars, cv. Daepung and Nampung, showed similar development of shoot regeneration efficiency, but significantly different shoot induction times. Therefore, the protocol reported here may be used for further development of regeneration efficiency and can be employed for efficient transformation in soybeans.

Key words: Soybean regeneration; Korean cultivars; Plant growth regulators; BAP; Vitamins

Introduction

The soybean (*Glycine max* L.) is a species of legume native to East Asia, including Korea and Northern China, and has been widely grown worldwide for various uses (Liu *et al*., 2008). It is one of the most important crops in the world in terms of area yields and production values and serve as a major food crop and a raw source of nutrition (Anon., 2011). Soybean proteins have been used as components of fermented and non-fermented soy foods, such as tofu, soymilk, soy yogurts, and soy cheese. Hence, soybeans may provide both nutritional and health benefits (Messina, 1999).

Despite its importance, soybean productivity is problematic because of the susceptibility and sensitivity of the plant to biotic or abiotic stresses. Among the abiotic stresses affecting soybeans, drought stress is the most detrimental, affecting all stages of plant growth and consequently reducing yields and leading to poor seed quality (Manavalan *et al*., 2009). Thus, genetic engineering of soybeans has become an important research topic, with the goal of improving the quantity and quality of soybeans (Wang & Xu, 2008). Although transformation efficiency is low, soybeans have been successfully genetically modified using agrobacterium and a regeneration process (*Zia et al*., 2010); some examples include development of transgenic soybeans harboring resistance against *Septoria glycines* (Song *et al*., 1994) and *Soybean mosaic virus* (Furutani *et al*., 2007). However, transformation efficiency is still limited due to low regeneration efficiency. Therefore, the improvement of soybean regeneration will be the key to facilitating the development of transgenic plants or genetic engineering.

Soybean regeneration has been achieved via organogenesis (Kim *et al*., 2001; Sairam *et al*., 2003; Shan *et al*., 2005; Joyner *et al*., 2010) and embryogenesis (Meurer *et al*., 2001; *Zia et al*., 2010). However, a greater understanding of the influences of various conditions, including phytohormone concentrations, explant and media types, and genotypes, is critical for the achievement of successful regeneration. The application of plant growth regulators, including auxins, cytokinins, and gibberellins, is required for shoot induction as well as shoot and root differentiation (Srejović & Nešković, 1985; Overvoorde *et al*., 2005; Teale *et al*., 2006). Additionally, cell competence, differentiation, and morphogenesis are dependent on the particular type of medium and the most suitable concentrations of phytohormones. For example, callus induction and shoot initiation of soybeans regenerated from cotyledonary nodes are achieved by the presence of Murashige and Skoog (MS) medium modified with different concentrations and types of phytohormones, such as 6-benzyladenine (BAP), 2, 4-dichlorophenoxyacetic acid (2,4-D), and gibberellic acid 3 (GA_3) (Kim *et al*., 2009). Because totipotency may depend on the type of explant, many types of explants, such as hypocotyls (Tripathi & Tiwari, 2003; Park *et al*., 2004; Wang & Xu, 2008), leaves (Wright *et al*., 1987) cotyledons (Joyner *et al*., 2010), and cotyledonary nodes (Liu *et al*., 2010), have been evaluated for shoot initiation capacity. The cotyledonary node is thought to be the most efficient explant type for induction of efficient shoot production in a short time period. However, regeneration efficiency may also depend

on the type of cultivar or genotype due to physiological variability (Park *et al*., 2004). Finally, germination efficiency may play an important role in improving regeneration, but may be restricted due to dormancy and poor germination of seeds. However, efficient germination can be achieved using optimal media with added plant growth regulators, such as abscisic acid (ABA), GA_3, and BAP, which have shown to have important roles in the regulation of seed germination (Moradi & Otroshy, 2012).

Although soybean regeneration protocols have been developed, limited numbers of cultivars were tested in these protocols (Yan *et al*., 2000). In addition, many countries throughout the world have developed different cultivars that are grown under many different climatic conditions. Thus, it is important to test and develop suitable protocols for soybean regeneration using each country's own superior cultivars, which are widely cultivated within the given country. To our knowledge, the cultivars "Nampung" and "Daepung" are considered to be good cultivars in the Republic of Korea (South Korea); however, no studies have been performed to standardize regeneration protocols using these two cultivars.

Therefore, the objectives of this study were to evaluate the various protocols that have been used for soybean germination and growth in order to optimize the growth conditions, including types of germination media, concentrations of BAP and GA_3, types of media with or without vitamins, and types of explants and cultivars.

Materials and Methods

Plant material and growth conditions: Two Korean soybean cultivars, cv. Daepung and Nampung, were used in this study for optimization of soybean regeneration. Seeds were sterilized with 70% ethanol for 2 min. After rinsing with sterile water, the seeds were surface-sterilized in 2% bleach solution (made up by diluting household Clorox bleach containing 4% sodium hypochlorite) for 15 min and rinsed thoroughly with sterile water. Subsequently, sterilized seeds were dried and treated with benomyl powder (4 mg g^{-1} seeds) before germinating on germination medium containing 2 mg L^{-1} agrimycin (commercial bactericide).

After excision, explants were immediately cultured on callus induction medium (CIM) for 10 days and then subsequently transferred to shoot induction medium (SIM) for 2-4 weeks, following by culture in shoot elongation medium (SEM) for 2-4 weeks. Plantlets were then excised to culture on rooting medium, and rooting plantlets were established on soil. All cultures were placed in a growth chamber maintained with consistent temperatures of 25 ± 1°C, relative humidity of 70%, 200 µmol m^{-2} s^{-1} photosynthetically active radiation, and a 16/8-h day/night period.

Effects of plant growth regulators on soybean germination: To confirm the effects of plant growth regulators and the presence of vitamins on seed germination, MS medium with and without vitamins and media supplemented with different phytohormones were used (Table 1). Media were adjusted to pH 5.8 and autoclaved at 121°C for 20 min. Phytohormones were added into the medium when the temperature dropped to 50-60°C.

Effects of different types of medium on callus and shoot induction: To study the influence of types of media on callus and shoot induction, MS medium with vitamins (MS + Vitamin), MS medium without vitamins (MS), Gamborg B5 medium with vitamins (GAM + Vitamin), and Gamborg B5 medium without vitamins (GAM) were analyzed (Table 2). The compositions of the media used for regeneration processes were the same as media shown in Table 2. Two Korean soybean cultivars, cv. Daepung and Nampung, were used to determine the effects of genotypes on callus induction and shoot regeneration.

Effects of different types of explants on soybean regeneration: All explants were obtained from 10-day-old seedlings. Cotyledons containing nodes were used to test the effects of phytohormones. Explants (cotyledons, cotyledonary nodes, hypocotyls, and roots) were obtained by the dissection of the two cotyledons, and then epicotyls with first leaves and axillary buds were removed, leaving only cotyledonary nodes next to hypocotyls. Then, 3-5 mm of the hypocotyl, containing the cotyledonary nodes, was dissected, 3 mm of hypocotyls were removed, and 3-5 mm of the remaining hypocotyl was excised. Finally, 1 mm of the apical meristem was removed, and about 3 mm of the apical meristem was dissected.

Table 1. List of compositions used in germination media for this study. All phytohormones were filter sterilized before use.

		Germination media						
Compositions	**Unit per liter**	**Water**	**MS^S**	**MS^l**	**MS^S**	**MS^S NAA**	**MS^S BAP**	**MS^S BAP GA_3**
		20	-	-	-	-	-	-
MS[a]	g	-	4.3	-	-	-	-	-
MS + Vitamins[b]	g	-	-	4.4	4.4	4.4	4.4	4.4
Sucrose	g	-	30	30	30	30	30	30
pH		5.8	5.8	5.8	5.8	5.8	5.8	5.8
Agar	g	-	7	-	7	7	7	7
NAA*	mg	-	-	-	-	1	-	-
BAP*	mg	-	-	-	-	-	1	1
GA_3*	mg	-	-	-	-	-	-	0.25

[a]Murashige and Skooge medium without vitamins, [b]Murashige and Skooge medium with vitamins, [s]Solid medium, [l]Liquid medium

*Phytohormones (naphthalene acetic acid [NAA], 6-benzyladenine [BAP], gibberellic acid 3 [GA_3]) were added to the medium after autoclaving.

Table 2. List of compositions used in regeneration media for this study. All phytohormones were filter sterilized before use.

Components	Unit per liter	Callus induction medium	Shoot induction medium	Shoot elongation medium	Rooting medium
MS[a]	g	-	-	-	4.4
GAM[b]	g	3.19	3.19	3.19	-
Sucrose	g	30	30	30	20
MES	g	3.9	0.59	0.59	0.59
pH	-	5.4	5.7	5.7	5.7
Agar	g	7	7	7	8
BAP*	mg	1	1	-	-
GA_3*	mg	0.17	-	0.17	0.17
IAA*	mg	-	-	0.1	-
Zeatin R*	mg	-	-	1	-
IBA*	mg	-	-	-	1

[a]Murashige and Skooge medium with vitamins, [b]Gamborge B5 medium with vitamins, *Phytohormones (6-benzyladenine [BAP], gibberellic acid 3 [GA_3], indole-3-acetic acid [IAA], indole-3-butyric acid [IBA], zeatin riboside [Zeatin R]) were added to the medium after autoclaving

Results and Discussion

Effects of plant growth regulators on soybean germination: First, we evaluated the effects of different types of media on germination and characteristic of seedlings. Similar higher sprouting rates and breaking seed dormancies were observed for MS solid medium modified with 1 mg L^{-1} NAA, 1 mg L^{-1} BAP, or 1 mg L^{-1} BAP with 0.25 mg L^{-1} GA_3 (Figs. 1 and 2). The highest frequency of germinated seeds with typical growth, rapid germination, enlarged cotyledons, green chlorophyll, healthy thick hypocotyls, and thick typical roots was observed for soybeans grown in 1 mg L^{-1} BAP with 0.25 mg L^{-1} GA_3 (Fig. 2). Reduced germination efficiency was found in liquid medium compared to solid medium, indicating that solidification affected germination efficiency (Fig. 1). Additionally, MS with vitamins was always found to elicit efficient germination compared to medium containing no vitamins (Fig. 2). These data suggested that treatment of seeds with plant growth regulators is a possible method for enhancing germination in soybeans (Moradi & Otroshy, 2012).

Effects of different types of medium on callus and shoot induction: Phytohormones have been used to initiate cell division and differentiation. Changes in hormonal composition may affect plant regeneration (Srejović & Nešković, 1985; Overvoorde *et al.*, 2005; Teale *et al.*, 2006). From the observations, callus biomass decreased as the GA_3 concentration was increased. In contrast, as BAP concentration was increased, callus biomass critically increased (data not shown). These results suggested that BAP had an important role in initiating callus and shoot induction.

Next, we analyzed the effects of different types of media on callus induction and shoot regeneration when cotyledonary nodes explants were used. The absence of vitamins resulted in color changes, shoot reduction, and death of explant tissues (Fig. 3). In addition, high callus biomass with green emerged plantlets was always observed in medium containing vitamins (data not shown). The frequency of emerging shoots was also higher in GAM + vitamin than in MS + vitamin medium (Fig. 4A, B). Hence, GAM + vitamin medium served as an excellent medium for regeneration. In addition, high frequencies of emerging shoots were always found in medium containing vitamins for both MS and GAM medium (Fig. 4A and B). Thus, vitamins are likely one of the parameters affecting soybean regeneration, as reported by Shimasaki & Fukumoto (1998).

Effects of different types of explants on soybean regeneration: The cotyledonary node has been used successfully in both soybean regeneration and transformation (Park *et al.*, 2004; Paz *et al.*, 2004; Liu *et al.*, 2010) as well as for regeneration and transformation in other plants (Jeyakumar & Jayabalan, 2002; Siddique & Anis, 2006; Dang & Wei, 2009; Zhang *et al.*, 2011). In this study, shoot regeneration was observed only in cotyledonary node explants (Fig. 5J), indicating that the cotyledonary node may have morphogenetic potential and a good source for shoot regeneration (Fig. 5). Consistent with this, Park *et al.* (2004) reported that plant regeneration varied with the type of explant and that cotyledonary nodes were more effective for shoot initiation than hypocotyl explants.

The most effective cotyledonary node explants were then used to induce calluses and shoots on the most effective medium (GAM + vitamins modified with BAP [1 mg L^{-1}] and GA_3 [0.17 mg L^{-1}]), as observed from previous experiments. As a result, the increasing rate of callus biomass was less varied between Nampung and Daepung cultivars (data not shown). However, rapid induction of shoots with green calluses was observed for the Daepung cultivar within 2 weeks, while fewer green-colored calluses and emerging shoots were observed for the Nampung cultivar within 3 to 4 weeks (Figs. 6 and 7). These results suggested that different regeneration times were required for different cultivars, consistent with the observations reported by Graybosch *et al*. (1987) using three soybean cultivars.

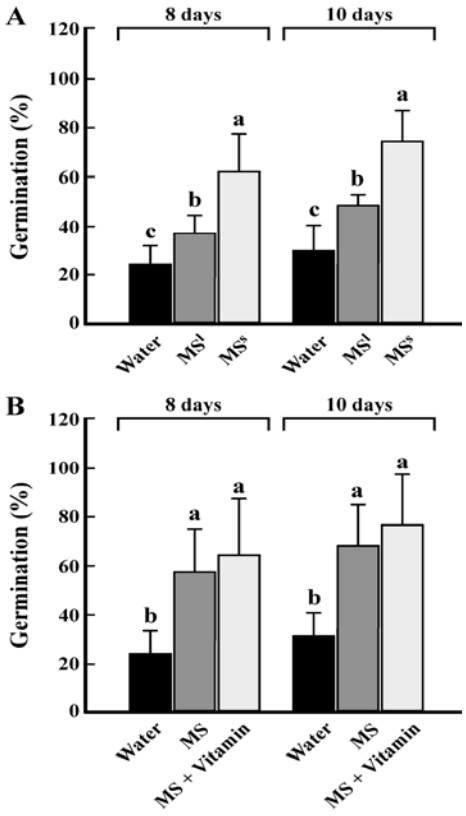

Fig. 1. Effects of the type of medium (A) and addition of vitamins (B) on soybean (cv. Daepung) germination in a growth chamber. Germination was measured at 8 and 10 days after seeding. Comparison of liquid (MS^l) and solidified (MS^s) types of Murashige and Skooge medium (A) and solidified (MS^s) MS medium ± vitamins (B). Vertical bars represent means ± standard deviations (SDs). Means denoted by the same letter are not significantly different at the 5% level according to Duncan's multiple range tests.

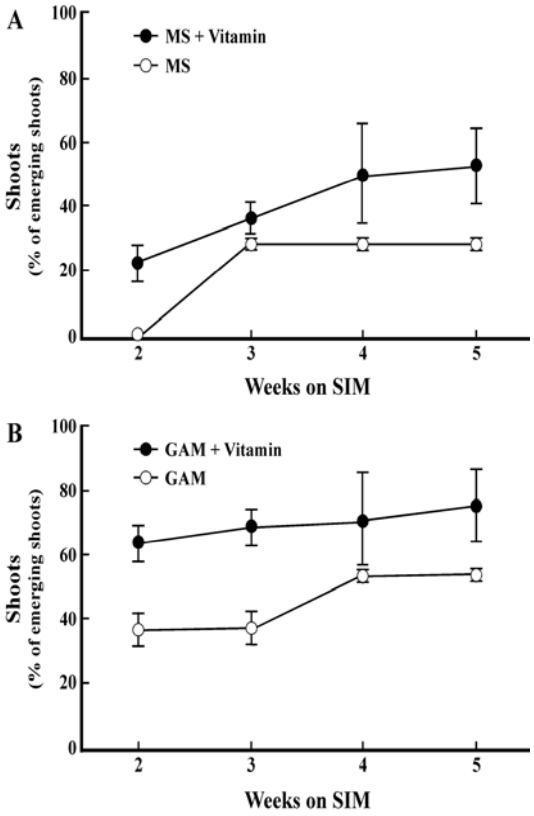

Fig. 4. Effects of different types of medium on the number of shoots regenerated from cotyledonary node explants derived from 10-day-old seedlings of soybeans (cv. Daepung) on shoot induction medium (SIM). Murashige and Skooge (MS) medium with or without vitamins (A) and Gamborg B5 (GAM) medium with or without vitamins (B) on SIM. The number of shoots was evaluated at 2, 3, 4, and 5 weeks after culture. Values represent means ± standard deviations (SDs).

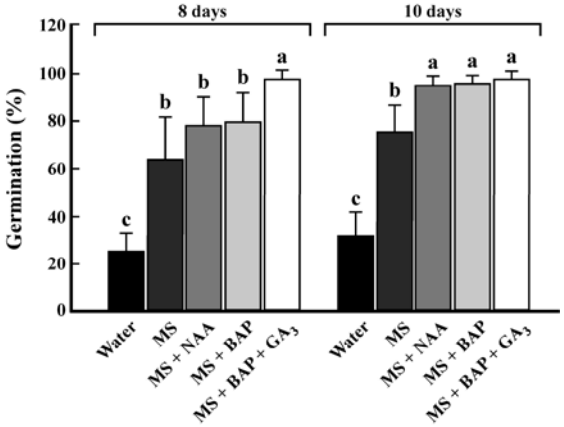

Fig. 2. Effects of plant growth regulators on soybean (cv. Daepung) germination in a growth chamber. Germination was measured at 8 and 10 days after seeding. MS, Murashige and Skooge medium; NAA, naphthalene acetic acid; BAP, 6-benzyladenine; GA_3, gibberellic acid 3. Vertical bars represent means ± standard deviations (SDs). Means denoted by the same letter are not significantly different at the 5% level according to Duncan's multiple range tests.

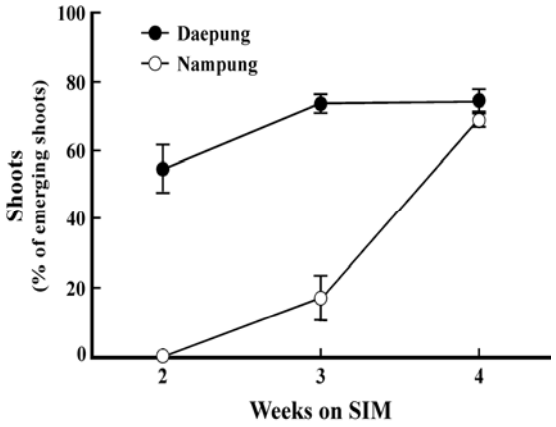

Fig. 7. Effects of two soybean cultivars (cv. Daepung and Nampung) on the number of shoots regenerated from cotyledonary node explants derived from 10-day-old seedlings on shoot induction medium (SIM). The number of shoots was evaluated at 2, 3, and 4 weeks after culture. Values represent means ± standard deviations (SDs).

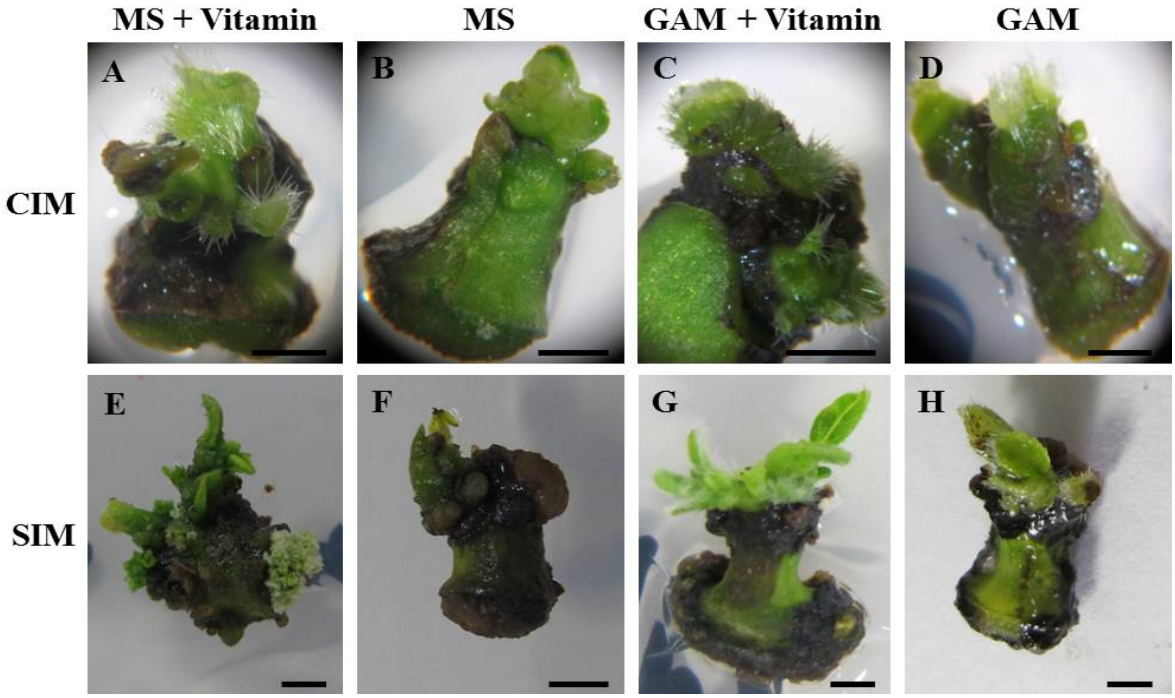

Fig. 3. Effects of different types of medium on callus and shoot induction regenerated from cotyledonary node explants derived from 10-day-old seedlings of soybeans (cv. Daepung) on callus induction medium (CIM) after 10 days of culture and on shoot induction medium (SIM) after 2 weeks of culture. Murashige and Skooge (MS) with vitamins on CIM (A) and SIM (E); MS without vitamins on CIM (B) and SIM (F); Gamborg B5 (GAM) with vitamins on CIM (C) and SIM (G); GAM without vitamins on CIM (D) and SIM (H). Bars represent 2 mm.

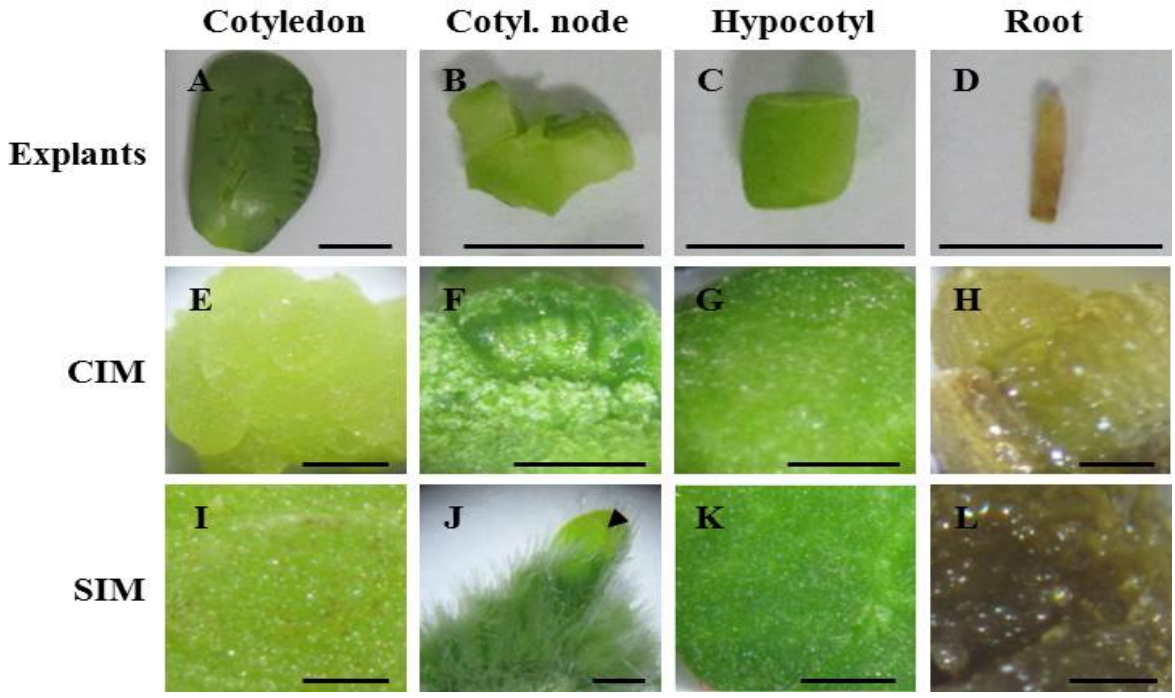

Fig. 5. Development of calluses and shoot buds regenerated from various explants derived from 10-day-old seedlings of soybeans (cv. Daepung) on callus induction medium (CIM) and shoot induction medium (SIM) after 10 days of culture. Explants (A, cotyledon; B, cotyledonary node; C, hypocotyl; D, root) prepared from seedlings. Callus induction of whole cotyledon (E), cotyledonary node (F), hypocotyl (G), and root (H) on CIM. Shoot induction of whole cotyledon (I), cotyledonary node (J, black arrow indicates the new shoot bud), hypocotyl (K), and root (L) on SIM. Bars for A, B, C, and D represent 5 mm, and other bars indicate 0.5 mm.

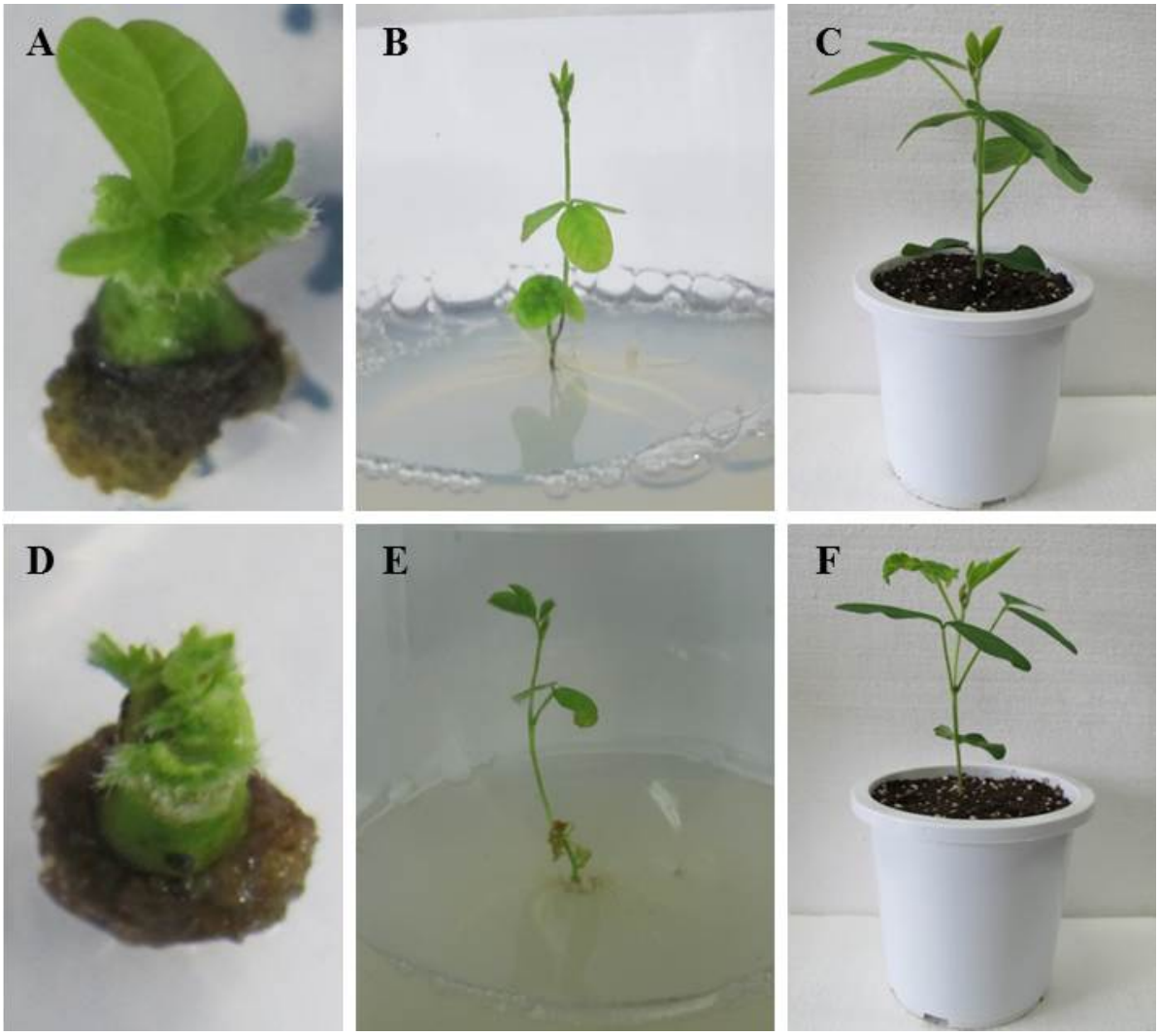

Fig. 6. Regeneration of two soybean cultivars (cv. Daepung and Nampung) from cotyledonary node explants derived from 10-day-old seedlings. Shoot induction (A, Daepung and D, Nampung) on shoot induction medium after 2 weeks of culture, shoot regeneration (B, Daepung and E, Nampung) on regeneration medium, and whole plant regeneration (C, Daepung and F, Nampung) on soil.

Conclusion

In this study, we optimized the protocol for organogenesis regeneration of soybean via cotyledonary nodes. The utility of cotyledonary nodes obtained from 10-day-old seedlings germinated on a medium containing BAP and GA_3 for soybean regeneration on GAM + vitamins medium modified with 1 mg L^{-1} BAP and 0.17 mg L^{-1} GA_3 was the most efficient for soybean regeneration. Therefore, the protocol reported here may be used for further development of soybean transformation systems using *Agrobacterium*-mediated T-DNA transfer.

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References

Anonymous. 2011. FAOSTAT. Food and Agriculture Oranization of the United Nations. Rome, Italy.

Dang, W. and Z.M. Wei. 2009. High frequency plant regeneration from the cotyledonary node of common bean. *Biol. Plantarum*, 53(2): 312-316.

Furutani, N., N. Yamagishi, S. Hidaka, Y. Shizukawa, S. Kanematsu and Y. Kosaka. 2007. Soybean Mosaic Virus resistance in transgenic soybean caused by post-transcriptional gene silencing. *Breed. Sci.*, 57(2): 123-128.

Graybosch, R.A., M.E. Edge and X. Delannay. 1987. Somaclonal variation in soybean plants regenerated from cotyledonary node tissue culture system. *Crop Sci.*, 27(4): 803-806.

Jeyakumar, M. and N. Jayabalan. 2002. *In vitro* plant regeneration from cotyledonary node of *Psoralea corylifolia* L. *Plant Tissue Cult.*, 12(2): 125-129.

Joyner, E.Y., L.S. Boykin and M.A. Lodhi. 2010. Callus induction and organogenesis in soybean [*Glycine max* (L.) Merr.] cv. Pyramid from mature cotyledons and embryos. *Open Plant Sci. J.*, 4(1): 18-21.

Kim, K.H., H.K. Park, M.S. Park and U.D. Yeo. 2001. Effects of auxin and cytokinins on organogenesis of soybean *Glycine max* L. *J. Plant Biotechnol.*, 3(2): 95-100.

Kim, K.H., J.E. Lee, Y.U. Kwon and B.M. Lee. 2009. Influence of antibiotics on shoot regeneration and *Agrobacterium* suppression using cotyledonary node in Korean soybean cultivars. *Korean J. Crop Sci.*, 54(3): 307-313.

Liu, Q.Q., G. Chen, J.Y. Gai, Y.L. Zhu, L.F. Yang, G.P, Wei and C. Wang. 2010. Highly efficient shoot regeneration from cotyledonary nodes of vegetable soybean. *Korean J. Hortic. Sci. Technol.*, 28(2): 307-313.

Liu, X., J. Jin, G Wang and S.J. Herbert. 2008. Soybean yield physiology and development of high-yielding practices in Northeast China. *Field Crop Res.*, 105(3): 157-171.

Manavalan, L.P., S.K. Guttikonda, L.S. Tran and H.T. Nguyen. 2009. Physiological and molecular approaches to improve drought resistance in soybean. *Plant Cell Physiol.*, 50(7): 1260-1276.

Messina, M.J. 1999. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am. J. Clin. Nutr.*, 70(3 Suppl): 439S-450S.

Meurer, C.A., R.D. Dinkins, C.T. Redmon, K.P. McAllister, D.T. Tucker, D.R. Walker, W.A. Parrott, H.N. Trick, J.S. Essig, H.M. Frantz, J.J. Finer and G.B. Collins. 2001. Embryogenic response of multiple soybean [*Glycine max* (L.) Merr.] cultivars across three locations. *In Vitro Cell. Dev. Bio.*, 37(1): 62-67.

Moradi, K. and M. Otroshy. 2012. A combination of chemical scarification and 6-benzylaminopurine (BAP) treatment promote seed germination in *dracocephalum kotschyi* seeds. *Trakia. J. Sci.*, 10(3): 26-29.

Overvoorde, P.J., Y. Okushima, J.M. Alonso, A. Chan, C Chang, J.R. Ecker, B. Hughes, A. Liu, C. Onodera, H. Quach, A. Smith, G. Yu and A. Theologis. 2005. Functional genomic analysis of the AUXIN/INDOLE-3-ACETIC ACID gene family members in *Arabidopsis thaliana*. *Plant Cell*, 17(12): 3282-3300.

Park, H.J., T.R. Kwon, K.H. Kim, T.S. Kim, Y.H. Park and Y.H. Kim. 2004. Effects of explants source, media and growth regulators on shoot regeneration of soybean (*Glycine max* (L.) Merr.) in Vitro. *Korean J. Breed.*, 36(2): 71-75.

Paz, M.M., H. Shou, Z. Guo, Z. Zhang, A.K. Banerjee and K. Wang. 2004. Assessment of conditions affecting *Agrobacterium*-mediated soybean transformation using the cotyledonary node explant. *Euphytica*, 136(2): 167-179.

Sairam, R.V., G. Franklin, R. Hassel, B. Smith, K. Meeker, N. Kashikar, M. Parani, D.A. Abed, S. Ismail, K. Berry and S.L. Goldman. 2003. A study on the effect of genotypes, plant growth regulators and sugars in promoting plant regenerating via organogenesis from soybean cotyledonary nodal callus. *Plant Cell Tiss. Org.*, 75(1): 79-85.

Shan. Z., K. Raemakers, E.N. Tzitzikas, Z. Ma and R.G. Visser. 2005. Development of a highly efficient, repetitive system of organogenesis in soybean (*Glycine max* (L.) Merr). *Plant Cell Rep.*, 24(9): 507-512.

Shimasaki, K. and Y. Fukumoto. 1998. Effects of B vitamins and benzylaminopurine on adventitious shoot formation from hypocotyl segments of Snapdragon (*Antirrhinum majus* L.). *Plant Biotechnol.*, 15(4): 239-240.

Siddique, I. and M. Anis. 2006. Thidiazuron induced high frequency shoot bud formation and plant regeneration from cotyledonary node explants of *Capsicum annuum* L. *Indian J. Biotechnol.*, 5(3): 303-308.

Song, H.S., S.M. Lim and J.M. Widholm. 1994. Selection and regeneration of soybeans resistant to the pathotoxic culture filtrates of *Septoria glycines*. *Phytopathol.*, 84(9): 948-951.

Srejović, V. and M. Nešković. 1985. Effect of gibberellic acid on organogenesis in buckwheat tissue culture. *Biol. Plantarum*, 27(6): 432-437.

Teale, W.D., I.A. Paponov and K. Palme. 2006. Auxin in action: signaling, transport and the control of plant growth and development. *Nat. Rev. Mol. Cell Biol.*, 7(11): 847-859.

Tripathi, M. and S. Tiwari. 2003. Epigenesis and high frequency plant regeneration from soybean (*Glycine max* (L.) Merr.) hypocotyls. *Plant Tissue Cult.*, 13(1): 61-73.

Wang, G. and Y. Xu. 2008. Hypocotyl-based Agrobacterium-mediated transformation of soybean (*Glycine max*) and application for RNA interference. *Plant Cell Rep.*, 27(7): 1177-1184.

Wright, M.S., D.V. Ward, M.A. Hinchee, M.G. Carnes and R.J. Kaufman. 1987. Regeneration of soybean (*Glycine max* L. Merr.) from cultured primary leaf tissue. *Plant Cell Rep.*, 6(2): 83-89.

Yan. B., M.S. Srinivasa Reddy, G.B. Collins and R.D. Dinkins. 2000. *Agrobacteium tumefaciens*-mediated transformation of soybean [*Glycine max* (L.) Merrill.] using immature zygotic cotyledon explants. *Plant Cell Rep.*, 19(11): 1090-1097.

Zhang, H., G. Peng and L. Feishi. 2011. Efficient plant regeneration from cotyledonay node explants of *Cucumis melo* L. *African J. Bioltechnol.*, 10(35): 6757-6761.

Zia, M., Z.F. Rizvi, R.U. Rehman and M.F. Chaudhary. 2010. *Agrobacterium* mediated transformation of soybean (*Glycine max L.*): some conditions standardization. *Pak. J. Bot.*, 42(4): 2269-2279.

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