Korean J. Plant Res. 32(3):237-243(2019)
https://doi.org/10.7732/kjpr.2019.32.3.237

Print ISSN 1226-3591
Online ISSN 2287-8203

Original Research Article

Optimized Shoot Induction and Histological Study of *in vitro* Cultured Korean Soybean Cultivars

Vipada Kantayos[1] and Chang-Hyu Bae[2]*
[1]Post-doc and [2]Professor, Department of Well-being Resources, Sunchon National University, Suncheon 57922, Korea

Abstract - Soybean is the one of recalcitrant legume species for shoot induction. Shoot regeneration via direct organogenesis was investigated in five soybean cultivars, ‘Dawon’, ‘Pungsan’, ‘Daewon’, ‘Taekwang’ and ‘Chongdoo 1’ by using cotyledonary node explants. Out of 5 soybean cultivars, an efficient shoot regeneration condition was developed in the two soybean cultivars, ‘Dawon’ and ‘Pungsan’. When various kinds of plant growth regulators with different concentration were estimated, the optimum medium condition for shoot induction in both soybean cultivars was MS + B5 vitamin supplemented with BA at concentration 2 ㎎/L. In addition, shoot formation efficiency was increased with 97.09% and 93.88% by the pretreatment of BA onto the explants before *in vitro* culture in both cultivars. Shoot induction in ‘Dawon’ cultivar was originated from epidermal tissue and sub-epidermal layers when histological changes were investigated under shoot regeneration after culturing cotyledonary node segments on shoot induction medium for 0 to 21 days. Especially, cell dedifferentiation was observed from parenchyma cells to meristematic cell in 3-day cultured segments.

Key words − Cotyledonary node, Histological study, *in vitro* culture, Shoot induction, Soybean cultivar

Introduction

Soybean [*Glycine max* (L.) Merr.] is well known as a recalcitrant plant in *in vitro* regeneration. It plays a potential in worldwide for human and animal feed as well as vegetable oil source. Five Korean soybean cultivars, Dawon, Pungsan, Daewon, Taekwang and Chongdoo 1 were one of recommended in Korean elite cultivars. Regeneration by organogenesis is a critical step for plant tissue propagation. It has been reported that regeneration efficiency is affected by genotype, explant source, age and size of explant, plant growth regulators (concentration and kind of plant growth regulators), basal medium content and culture conditions (light and temperature) (Raza *et al*., 2017; Franklin *et al*., 2004; Sairam *et al*., 2003; Yildiz *et al.*, 2002; Bailey *et al*., 1993). In legume plants, many previous researches have been used different explants for shoot regeneration (Zhang *et al*., 2014; Hong *et al*., 2006; Yan *et al*., 2000; Franklin *et al*., 1993; Yue-Sheng *et al*., 1990). MS medium (Murashige and Skoog, 1962) is the most frequently used in plant tissue culture while B5 medium is commonly used in some approaches or shoot induction. Mariashibu *et al*. (2013) indicated that nutrition requirement as a medium composition plays an important role for plant regeneration. Cytokinins are generally used for shoot induction. One of them is benzyladenine (BA) which is commonly used either alone or in the combination with a low concentration of other cytokinins; kinetin/BA or thidiazuron (Choi *et al*., 2019; Ma and Wu, 2008; Franklin *et al*., 2004). Many studies have been reported that MS media supplemented with BA 2 to 3 ㎎/L promoted multiple shoot formation from cotyledonary node (Kim *et al*., 2016; Ma and Wu, 2008; Jackson and Hobbs, 1990).

Furthermore, histological study is important in *in vitro* plant regeneration in order to figure out the origins of the plant during organogenesis (Dibax *et al*., 2013). However, few studies have been researched on the connection between histological and kinds of plant growth regulators during shoot regeneration (Wang *et al*., 2015). The observations were executed to understand the process of adventitious shoot formation during *in vitro* morphogeneis from explants.

*Corresponding author. E-mail : chbae@scnu.ac.kr
Tel. +82-61-750-3214

In this study, we investigated the optimum types of explant, and the optimum concentration and kinds of plant growth regulator (PGR) for plant regeneration in five Korean soybean cultivars. Also, we measured that the pretreatment of BA onto cotyledonary node explant before *in vitro* culture affect shoot regeneration. For understanding the organogenesis process morphologically that is originated from the region of the cotyledonary node in legumes, the histological changes were investigated by using the cotyledonary node explants in a Korean elite soybean cultivar.

Materials and Methods

Plant materials and culture media

Five Korean elite soybean cultivars (Dawon, Pungsan, Daewon, Taekwang and Chongdoo 1) were used for plant materials in this experiment. After the surface sterilization with ethanol 70%, 30s to 1 min approximately, dry seeds were soaked 1% sodium hypochlorite solution for 15 minutes followed by washing with autoclaved distilled water. After germination, cotyledon, cotyledonary node and hypocotyl of 7-10 days grown *in vitro* seedling (Fig. 1), were used as explant. Plant segments were cultured on shoot induction media, MS basal medium supplemented with B5 vitamin with concentration of 6-benzyladenine (BA) at 2 ㎎/L (MSBA2) which was suggested by the previous studied (Kim *et al*., 2016).

Media, culture condition and shoot regeneration frequency

MS media supplemented with B5 vitamin were used as basal media for organogenesis (Raveendar *et al*., 2009). And the MS medium was reinforced with 30 g/L sucrose, 8 g/L agar and the pH of the medium was regulated to 5.7 ± 0.5 after adding plant growth regulators. The medium was autoclaved at 121℃, for 20 min and poured in a culture plates. All the culture media were kept in a culture room at 26 ± 2℃ under 16-h photoperiod provided by cool-white fluorescent lamps at 25 ㎛ol/㎡/sec.

To estimate the optimal shooting condition, basal medium was supplemented with single of BA or kinetin with concentration (0, 1, 2, 4 ㎎/L) and the combinations of BA and kinetin (1, 2 ㎎/L).

The number of shoots and the shoot regeneration frequency were recorded after 21 days in culture. The frequency of shoot regeneration was calculated as follows:

$$\text{Shoot regeneration ratio (\%)} = \frac{\text{Number of explants regenerated into shoots}}{\text{Total number of cultured explants}} \times 100$$

Treatment of plant growth regulators for shoot multiplication

Regeneration via direct organogenesis was investigated in the five soybean cultivars, 'Dawon', 'Pungsan', 'Daewon', 'Taekwang' and 'Chongdoo 1'. After germination 7 to 10 days (when cotyledon turns green), cotyledonary nodes of the five soybean cultivars were used as explant. The segments (about 1 ㎝ in length) of soybean was transferred to shoot induction media containing MS medium supplemented with B5 vitamin as a basal media, supplemented with various plant growth regulators at different concentration including BA (0, 1, 2, 4 ㎎/L), kinetin (0, 1, 2, 4 ㎎/L), a combination BA and kinetin. The frequency of shoot regeneration was calculated as mention above.

Cotyledon (A) | Cotyledonary node (B) | Hypocotyl (C)

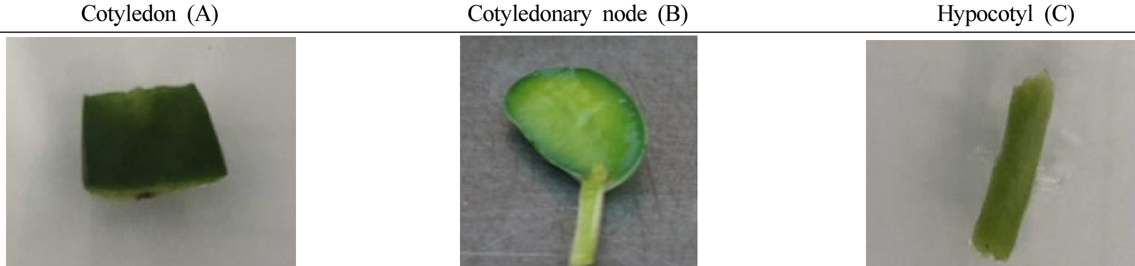

Fig. 1. Types of explant used in this experiment. Cotyledon (A), cotyledonary node (B) and hypocotyl (C) from *Glycine max* L. cv. 'Dawon'.

Fig. 2. Two kinds of cotyledonary node segments. Explants with cotyledonary node (A) and half-split cotyledonary node (B) from *Glycine max* L. cv. 'Dawon', respectively.

Pretreatment of BA and selection of explant types

Two types of cotyledonary node explants of the five soybean cultivars including *Glycine max* L. cv. 'Dawon' were prepared by a scalpel to remove any excess hypocotyl remaining 0.5 ㎝ in length (Fig. 2-A) and half-cotyledons were split off (Fig. 2-B). All the explants were dipped in 200 ㎎/L of BA solution for 1 min pretreatment and then *in vitro* cultured on shoot induction media supplemented with 2 ㎎/L BA. After 3 weeks, the shoot number and the shoot regeneration ratio were measured with three replications.

Histological observation on shoot induction

Cotyledonary node explants of *Glycine max* L. cv. 'Dawon' were *in vitro* cultured on the shoot induction MS media containing B5 vitamin supplemented with 2 ㎎/L BA. The cotyledonary node specimens were selected in 0, 3, 6, 9, 12, 15, 18 and 21 days, and fixed in a mixture of ethanol, formaldehyde and acetic acid for 48 hours. Then, the specimens were trimmed and dehydrated by immersing the specimens in a series concentration of alcohol (70~100%) to remove the water and the formalin from the specimens. After then the specimens were infiltrated and embedded into glycol methacrylate (JB-4 Embedding Kit; Poly Sciences, Warrington, PA, USA). Serial sections, in 5 ㎛ thick, were cut by an ultra-microtome (MT-990 Type S, RMC Boeckeler, AZ, USA), mounted on slides, and stained by periodic acid-Schiff reaction method using hematoxylin (Sigma Co.) as a counter-strain. After rinse slides with tap water, kept the slides warm until dried on a hot plate at 35℃ for 10 minutes. The slides were mounted coverslip onto the section on slide glass with Permount (Fischer Chemical, Pittsburgh, PA, USA) in a week. Finally, the histological investigation of shoot differtiation was observed in a light microscope (Nikon E200, Nikon, Tokyo, Japan).

Results and Discussion

Shoot induction by explant types

In order to check optimized explant type, three kinds of explants including cotyledon, cotyledonary node and hypocotyl of soybean cultivar 'Dawon' were cultured on shoot induction media for 21 days as shown in Fig. 1. Only the cotyledonary node segments gave a response for shoot induction (Fig. 3-B). While both of cotyledon (Fig. 3-A) and hypocotyl (Fig. 3-C) segments did not showed the efficient shoot regeneration, but calli were produced at the surface of cutting region of the explant in the hypocotyl segment (Fig. 3-C, arrow point). Many previous researchers have been reported that different explants were used for shoot formation in *in vitro* culture of legume plants. A whole cotyledonary node of soybean has been used as an explant (Zhang *et al*., 2014), while the other tissue types such as young leaves (Yue-Sheng *et al*., 1990), hypocotyl (Franklin *et al*., 1993), immature embryo (Yan *et al*., 2000), axillary bud (Hong *et*

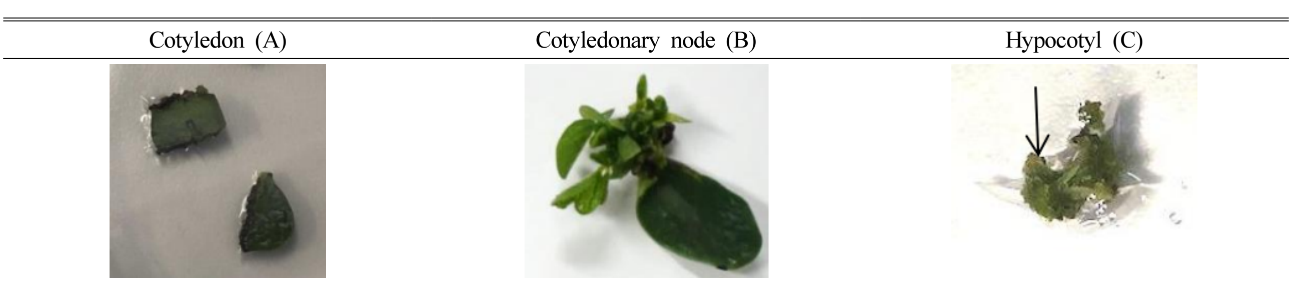

Fig. 3. Response of three-kind explants on shoot induction media (MS+BA 2 ㎎/L) in *Glycine max* L. cv. 'Dawon'. Cotyledon segment (A), Cotyledonary node segment (B) and Hypocotyl segment (C, arrow point showed callus induced from the segment).

al., 2006) have been used. The cotyledonary node explant was the best explant in this experiments and the result was consistent with the previous reports (Kim *et al*., 2016; Sairam *et al*., 2003).

The optimal concentration of plant growth regulators (PGRs) for shoot formation

The cotyledonary node explants yielded shoots on shoot formation frequency with 66.67-100% when treated with BA or kinetin or a combination of BA-kinetin (Table 1). Media containing 2.0 ㎎/L of BA showed the best frequency for multiple shoot induction. The highest average number of shoots/explant was 2.12 in soybean cultivar ‘Pungsan’, followed by ‘Dawon’ (1.48 shoots/explant), ‘Taekwang’ (1.32 shoots/explant), ‘Chongdoo 1’ (1.30 shoots/explant) and ‘Daewon’ (1.25 shoots/explant), respectively. The range of shoot numbers varied from 1 to 9 shoots in soybean cultivar ‘Pungsan’, while that of ‘Dawon’ was varied from 1 to 4 shoots. In addition, individual explant showed different response on shoot regeneration ability, even in the same culture conditions. The synergistic effect by a combination of BA-kinetin was not shown in our results, while Franklin *et al*. (2004) reported a synergistic effect in the presence of BA and TDZ. Moreover, genotype was an important factor in terms of the *in vitro* regeneration efficiency (Raza *et al*., 2017; Kim *et al*., 2016; Franklin *et al*., 2004; Sairam *et al*., 2003; Bailey *et al*., 1993).

Effect of pretreatment and non-pretreatment of BA into explants on shoot induction

As shown in Table 2, pretreatment by a high concentration of BA (200 ㎎/L) before *in vitro* culture onto the explants accelerated the shoot number per explants compared with non-pretreated control. Both of half-split cotyledonary node and cotyledonary node explants showed high frequency of shoots. In the pretreatment condition, the percentage of shoot induction ranged on 88-97% with the maximum 97.09% in half-split cotyledonary node of soybean cultivar ‘Dawon’. While in the non-pretreatment, the percentage of shoot induction ranged on 83-92%. By the pretreatment of BA onto both half-split cotyledonary node and cotyledonary node explants, ‘Dawon’ achieved the highest number of shoots per

Table 1. Shoot induction from cotyledonary node explant of *Glycine max* L. 5 cultivars on shoot induction media supplemented with different concentration and kinds of plant growth regulators after 3 weeks

PGRs[z] (㎎/L)		No. of shoot per explant					Shoot regeneration frequency (%)				
BA	Kinetin	A[y]	B[y]	C[y]	D[y]	E[y]	A[y]	B[y]	C[y]	D[y]	E[y]
0	0	0.13^{ex}	0.13^{ex}	0.00	0.07	0.00	8.70 (2/23)[w]	13.30 (2/15)	0.00 (0/20)	6.67 (1/15)	0
1	0	1.16^{bcd}	1.59^{bc}	0.95^{nsx}	1.09^{nsx}	0.97^{abcx}	97.78 (44/45)	92.41 (134/145)	80.00 (16/20)	90.91 (20/22)	86.67 (26/30)
2	0	1.48^{a}	2.12^{a}	1.25	1.32	1.30^{a}	89.39 (118/132)	96.11 (173/180)	60.00 (18/30)	83.33 (25/30)	90.00 (18/20)
4	0	1.40^{ab}	1.00^{d}	0.92	0.95	1.10^{ab}	98.11 (52/53)	87.50 (63/72)	88.00 (22/25)	90.48 (19/21)	95.00 (19/20)
0	1	1.00^{cd}	1.03^{d}	0.95	0.90	1.05^{abc}	93.33 (28/30)	80.00 (24/30)	85.00 (17/20)	80.00 (16/20)	100.00 (20/20)
0	2	1.03^{cd}	0.97^{d}	0.85	1.16	0.80^{bc}	100.00 (30/30)	76.67 (23/30)	77.78 (21/27)	92.00 (23/25)	80.00 (16/20)
0	4	1.03^{cd}	1.20^{cd}	0.95	0.90	0.75^{bc}	83.33 (25/30)	80.00 (24/30)	90.00 (18/20)	82.61 (19/23)	70.00 (14/20)
1	1	1.37^{abc}	1.40^{cd}	1.10	1.35	1.05^{abc}	96.67 (29/30)	86.67 (26/30)	85.00 (17/20)	95.00 (19/20)	90.00 (18/20)
1	2	1.33^{d}	1.55^{ab}	1.10	1.35	0.70^{c}	90.00 (27/30)	96.67 (29/30)	90.00 (18/20)	80.00 (16/20)	75.00 (15/20)
2	1	0.97^{abc}	1.93^{bc}	1.20	1.30	0.85^{bc}	96.67 (29/30)	94.44 (34/36)	85.00 (17/20)	90.00 (18/20)	85.00 (17/20)
2	2	0.93^{d}	1.00^{d}	0.96	1.04	0.96^{abc}	83.33 (25/30)	13.30 (2/15)	92.00 (23/25)	82.61 (19/23)	78.26 (18/23)

[z]PGRs: Plant Growth Regulators.

[y]A-E: Soybean cultivars follow by A: Dawon, B: Pungsan, C: Daewon, D: Taekwang, E: Chongdoo 1.

[x]Means with the same letters within the column are not significantly different by DMRT ($p < 0.05$). ns: not significant.

[w]Numbers in the parentheses mean (A/B) = Number of explants regenerated in to shoots (A) per total number of cultured explants (B).

Table 2. Effect of pretreatment with high concentration of BA on percentage of shoot induction in half-split cotyledonary node and cotyledonary node explants after 21 days cultures in soybean cultivars (*Glycine max* L.)[z]

Cultivars	Half-split cotyledonary node		Cotyledonary node		Hypocotyl	
	PT[z]	NT[x]	PT[z]	NT[x]	PT[z]	NT[x]
Dawon	97.09	93.90	95.88	90.77	0	0
Pungsan	93.88	89.09	95.92	92.73	0	0
Daewon	90.00	88.89	90.74	90.74	0	0
Taekwang	92.59	83.33	90.00	93.33	0	0
Chongdoo 1	91.84	90.57	88.68	91.11	0	0

[z]After 1 min pretreatment with BA 200 ㎎/L solution, explants were cultured *in vitro* on shoot induction media supplemented with BA 2 ㎎/L.
[y]PT: Pretreatment.
[x]NT: Non-pretreatment.

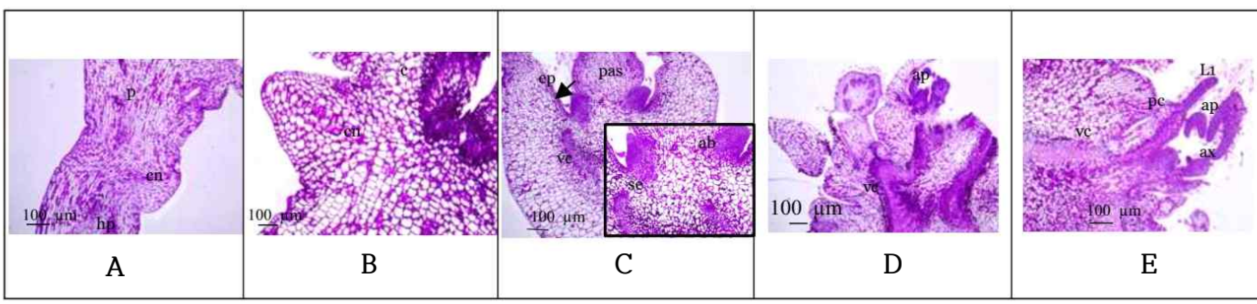

Fig. 4. Histological analysis of the shoot induction process in *Glycine max* L. cv. 'Dawon'. The cotyledonary nodes were cultured for 0 to 21 days on the basal MS with B5 vitamin and 2 ㎎/L BA. A: 0 day, B: 3-day, C: 6-day, D: 12-day, E: 21-day. Abbreviations; ab: axillary bud, ap: apical meristem, ax: axillary meristem, c: cotyledon area, cn: cotyledonary node, ep: epidermal cells, hp: hypocotyl area, L1: leaf primordia, p: parenchyma, pas: primary axillary shoot, pc: procambium, se: sub-epidermal cell, vc: vascular cambium.

explant followed by the soybean cultivar 'Pungsan', 'Taekwang', 'Daewon' and 'Chongdoo 1', respectively.

The pretreatment of explants with plant growth regulators is the general technique to improve the induction of shoot (Thomas, 2007). In our experiment, pretreatment by the BA (200 ㎎/L) on the surface of half-split cotyledonary node and cotyledonary node explants promoted shoot regeneration compared with non-pretreated tissues. In cotyledonary node of cowpea, pretreatment with 3 ㎎/L BA gave the best response in terms of shoot number and shoot length (Tie *et al*., 2013). In this experiment, half-split cotyledonary node showed the best number of shoot, while percentage of shoot induction was not significantly different. However, Compton and Gray (1993) indicated that explant cutting types could influence the shoot formation and cotyledon based explant of watermelon showed higher percentage of explant producing shoot compared with cotyledon cut in half longitudinally.

Histological study on shoot formation

Our histological investigation gave evidence for direct formation of organogenesis structures at cotyledonary node region in soybean (Fig. 4). The results correlated with the cell formation and differentiation that occurred during shoot regeneration. During 3-6 days of culture on shoot induction media containing BA 2 ㎎/L, the meristematic tissue around cotyledonary node was dividing in both of 'Dawon' (Fig. 4) and 'Pungsan' (not shown data) cultivars. Epidermis and sub-epidermis cells (cortical cells layers) became competent and dedifferentiated from parenchyma cells to compact globular meristemoids. Meristemoids were formed both anticlinal and periclinal division to the plane. Cell initiated from outer epidermal cells and cortical cells and then forms afterward. During 9 to 21 days vascular cells expanded, and ground tissues were gradually differentiated by changed to thin wall cells. Shoot bud had usual dome-shaped meristems with two lateral leaf-primordia which are connected with the vascular system. Main vascular tissue of mother plant was expanded to link with pro-vascular (new shoot) cambium. For shoot differentiation more time is needed in soybean shoot formation than that of pea (Kantayos, 2019; Kantayos and Bae, 2019).

In conclusion, cotyledonary node explant of the soybean cultivar was selected and used as explant for estimating the optimal shoot differentiation conditions. MS as a basal media supplemented with B5 vitamin were used as shoot induction media. Also, the optimum PGR condition for the cultivar ‘Dawon’ was BA 2 ㎎/L. Even though cut cotyledon did not show any shoot induction response at wounding point, both of half-split cotyledonary node and cotyledonary node showed the multiple shooting. Especially, the pretreatment of BA onto cotyledonary node explants be *in vitro* culture, promoted shoot induction in 21 days culture.

Acknowledgement

This paper was supported by Sunchon National University Research Fund in 2016. We are very grateful to Prof. Cho, Hyun-Wook (Department of Biology, Sunchon National University, South Korea) for his valuable assist of the providing necessary facilities for histological study part and thanks to KGSP scholarship that give me an opportunity to study in Korea. This paper is a part of Ph.D. thesis of Kantayos (2019).

References

Bailey, M.A., H.R. Boerma and W.A. Parrott. 1993. Genotype effects on proliferative embryogenesis and plant regeneration of soybean. In Vitro Cell. Dev. Biol. 29P:102-108.

Choi, H., J.C. Yang, S.H. Ryu, S.M. Yoon, S.Y. Kim and S.Y. Lee. 2019. *In vitro* multiplication of *Hosta* Tratt. species native to Korea by shoot-tip culture. Korean J. Plant Res. 32:53-62 (in Korean).

Compton, M.E. and D.J Gray. 1993. Somatic embryogenesis and plant regeneration from immature cotyledons of watermelon. Plant Cell Rep. 12:61-65.

Dibax, R., G.B. Alcantara, M.P. Machado, J.C.B. Filho and R.A. Oliveira. 2013. Protocol optimization and histological analysis of *in vitro* plant regeneration of RB92579 and RB 93509 sugarcane cultivars. Ciência Rural, Santa Maria 43(1): 49-54.

Franklin, C.I., T.N. Trieu, B.G. Cassidy, R.A. Dixon and R.S. Nelson. 1993. Genetic transformation of green bean callus via *Agrobacterium* mediated DNA transfer. Plant Cell Rep. 12:74-79.

Franklin, G., L. Carpenter, E. Davis, C.S. Reddy, D. Al-Abed, W. Abou Alaiwi, M. Parani, B. Smith, S.L. Goldman and R.V. Sairam. 2004. Factors influencing regeneration of soybean from mature and immature cotyledons. Plant Growth Regul. 43:73-79.

Hong, H.P., H. Zhang, P. Olhoft, S. Hill, H. Wiley, E. Toren, H. Hillebrand, T. Jones and M. Cheng. 2006. Organogenic callus as the target for plant regeneration and transformation via *Agrobacterium* in soybean (*Glycine max* (L.) Merr.). In Vitro Dev-Pl. 43(6):558-568.

Jackson, J.A. and S.L.A. Hobbs. 1990. Rapid multiple shoot production from cotyledonary node explants of pea (*Pisum sativum* L.). In Vitro Cell Dev-Pl. 26:825-835.

Kantayos, V. 2019. Characterization of plantlet regeneration and genetic transformation of legume plants. School of Plant Production Science, Ph.D. Thesis, Sunchon National Univ., Korea (in Korean).

_______________, and C.-H. Bae. 2019. Optimization of shoot induction, histological study and genetic stability of *in vitro* cultured *Pisum sativum* cv. ‘Sparkle’. Korean J. Plant Res. 32:19-28.

Kim, D-g., V. Kantayos, D.-K. Kim, H.-G. Park, H.-H. Kim, E.-S. Rha, S.-C. Lee and C.-H. Bae. 2016. Plant regeneration by *in vitro* tissue culture in Korean soybean (*Glycine max* L.). Korean J. Plant Res. 29:143-153 (in Korean).

Ma, X.-H. and T.-L. Wu. 2008. Rapid and efficient regeneration in soybean [(*Glycine max* (L.)] Merrill from whole cotyledonary node explants. Acta Physiol Plant 30:209-216.

Mariashibu, T.S., V.R. Anbazhagan, S-Y. Jiang, A. Ganapathi and S. Ramachandran. 2013. A comprehensive survey of international soybean research-genetics, physiology, agronomy and nitrogen relationships. Chapter-20: *In vitro* regeneration and genetic transformation of soybean: Current Status and Future Prospects. *Intec Open*, http://dx.doi.org/10.5772/

54268.

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

Raveendar, S., A. Premkumar, S. Sasikumar, S. Ignacimuthu and P. Agastian. 2009. Development of a rapid, highly efficient system of organogenesis in cowpea *Vigna unguiculata* (L.) Walp. S. Afr. J. Bot. 75:17-21.

Raza, G., M.B. Singh and P.L. Bhalla. 2017. *In vitro* plant regeneration from commercial cultivars of soybean. Bio. Med. Res. Int. 2017, Article ID 7379693, 1-9.

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

Thomas, T.D. 2007. Pretreatment in thidiazuron improves the *in vitro* shoot induction from leaves in *Curculigo orchioides* Gaertn, an endangered medicinal plant. Acta Physiol Plant 29:455-461.

Tie, M., Q. Luo, Y. Zhu and H. Li. 2013. Effect of 6-BA on the plant regeneration via organogenesis from cotyledonary node of cowpea (*Vigna unguiculata* L. Walp). J. Agric. Sci. 5(5), doi: 10.5539/jas.v5n5p1

Wang, H., M. Li, Y. Yang, J. Dong and W. Jin. 2015. Histological and endogenous plant growth regulators changes associated with adventitious shoot regeneration from *in vitro* leaf explants of strawberry (*Fragaria* × *ananassa* cv. 'Honeoye'). Plant Cell Tiss. Org. Cult. 23(3):479-488.

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

Yildiz, M., S. Özcan and C. ER. 2002. The effect of different explant sources on adventitious shoot regeneration in flax (*Linum usitatissimum* L.). Turk J. Biol. 26:37-40.

Yue-Sheng, Y., K. Wada and Y. Futsuhara. 1990. Comparative studies of organogenesis and plant regeneration in various soybean explants. Plant Sci. 72(1):101-108.

Zhang, F., C. Chen, H. Ge, J. Liu, Y. Luo, K. Liu, L. Chen, K. Xu, Y. Zhang, G. Tan and C. Li. 2014. Efficient soybean regeneration and *Agrobacterium*-mediated transformation using a whole cotyledonary node as an explant. Biotechnol. Appl. Biochem. 61(5):620-625.

(Received 6 June 2019 ; Revised 13 June 2019 ; Accepted 13 June 2019)