Original research article

Intact roots promote shoot regeneration from hypocotyl independent of exogenous plant growth regulators in eggplant *in vitro*

Hideyuki Tanaka, Masahumi Johkan[1], Kazuhiko Mitsukuri[2], Takahiro Tezuka, Hajime Furukawa and Masayuki Oda

Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka 599-8531, Japan
[1] Present address: Graduate School of Horticulture, Chiba University, Matsudo, Chiba 271-8510, Japan
[2] Present address: Faculty of Education, Nara University of Education, Nara 630-8528, Japan

Corresponding author: M. Oda, E. mail: moda@plant.osakafu-u.ac.jp, Phone +81-72-254-9426, Fax +81-72-254-9918

Received on November 21, 2012; Accepted on December 13, 2012

Abstract: Eggplant (*Solanum melongena*) seedlings cultured *in vitro* were excised at the center of the hypocotyl to generate decapitated seedlings with intact roots. This modification of the complete decapitation method (CDM) developed *in vivo* by Harada et al. (2005) allowed *in vitro* culture (CDM *in vitro*; CDM*i*). As controls, rootless hypocotyl segment explants (approximately 1 cm) and cotyledon explants were cultured on media supplemented with 4.4 µM 6-benzyladenin (BA) and 0.2 µM thidiazuron (TDZ), respectively. Cotyledon explants had formed calli 2 weeks after excision but did not develop adventitious buds, despite the use of optimal conditions reported previously for a different eggplant cultivar. Calli formed at the cut ends of hypocotyls 1 week after excision in both CDM*i* and hypocotyl cultures, and adventitious buds regenerated 1 week earlier in CDM*i*. Six weeks after excision, CDM*i* yielded 11.4 adventitious buds per explant, but only 4.1 formed in hypocotyl culture. Moreover, shoots longer than 1 cm developed 2 weeks earlier in CDM*i* than in hypocotyl culture. The number of shoots per explant was 8.1 in CDM*i*, but only 2.4 in hypocotyl culture 6 weeks after cutting. All shoots that developed were rooted on MS medium in CDM*i*, but only 71% of shoots formed roots in hypocotyl culture. These results indicate that intact roots are important for explant shoot regeneration and development, and CDM*i* is a simple and efficient method for obtaining multiple shoots without the need to determine optimal concentrations of plant growth regulators and overcome inhibition of rooting in the obtained shoots.

Keywords: adventitious shoot, complete decapitation method, micropropagation, *Solanum melongena*

Abbreviations: BA, 6-benzyladenin; CDM, complete decapitation method; CDM*i*, CDM *in vitro*; PGR, plant growth regulator; TDZ, thidiazuron.

Introduction

Agrobacterium tumefaciens-mediated genetic transformation is an effective and widely used approach to introduce desirable genes into plants. This transformation method is typically based on *in vitro* culture method. Therefore, to obtain a number of transgenic plants, a high frequency *in vitro* regeneration method is required. *In vitro* regeneration of different explant types, e.g. cotyledon, hypocotyl, leaf and root, from several cultivars of eggplant (*Solanum melongena* L.) has been reported both via embryogenesis (Matsuoka and Hinata 1979, Rao and Singh 1991, Saito and Nishimura 1994, Sharma and Rajam 1995) and organogenesis (Kamat and Rao 1978, Fassuliotis et al. 1981, Allichio et al. 1982). In both techniques, the combination and concentration of plant growth regulators (PGRs) should be determined through complex and empirical processes. However, in eggplant tissue culture, the optimal PGR conditions for regeneration are reported to differ

Tanaka H, Johkan M, Mitsukuri K, Tezuka T, Furukawa H, Oda M 2013 Intact roots promote shoot regeneration from hypocotyl independent of exogenous plant growth regulators in eggplant *in vitro*. Plant Root 7: 5-11. doi:10.3117/plantroot.7.11

depending on the cultivar, growing conditions of the mother plant, explant type and the morphogenetic response that varies within the same explant (Fassuliotis et al, 1981, Sharma and Rajam 1995). Similarly, in strawberry culture, adventitious shoot regeneration differed among cultivars and explant types when a range of explant types from seven cultivars were cultured in the same PGR condition (Passey et al. 2003). These reports indicate that decisions regarding optimal PGR concentrations and combinations for *in vitro* regeneration are difficult, and additional examinations might be required to regenerate plants from an unstudied cultivar in tissue culture using PGRs. In addition, rooting inhibition of shoots derived from explants grown in the presence of thidiazuron (TDZ) (Magioli et al. 1998) or 6-benzyladenin (BA) (Sharma and Rajam 1995) has been reported previously in eggplant culture. To obtain a number of regenerated shoots with high rooting capacity, an efficient *in vitro* method independent of PGRs for shoot regeneration is demanded.

The aim of this study was to establish an efficient *in vitro* method of eggplant culture for adventitious shoot regeneration without PGRs and inhibition of rooting from the regenerated shoot. Recently, the complete decapitation method (CDM) was developed for mass propagation in tomato plants grown in an open field (Harada et al. 2005), and the efficiency of the method was improved by Johkan et al. (2008a, b, c). In this method the main and lateral stems are excised, which enables adventitious shoot regeneration from the cut ends of stems *in vivo*. Tezuka et al. (2011) hypothesized that the promotive effect of CDM on shoot regeneration was due to utilization of endogenous cytokinin synthesized after decapitation. It is generally believed that endogenous cytokinins in higher plants are mainly biosynthesized in the root system and transported via the xylem to the above-ground parts (Davies 2004). From these reports, *in vitro* culture of decapitated seedlings composed of an intact root attached to the remaining shoot axis, i.e., applying CDM to *in vitro* culture (CDM *in vitro*; CDM*i*), has potential to be an efficient *in vitro* method independent of PGRs for mass propagation of eggplant. Pozueta-Romero et al. (2001) developed a similar method independent of PGRs for tomato and bell pepper. In this method, one cotyledon and the apical and axillary meristems are excised and the resulting seedlings, resembling a flamingo bill, are used as explants. Amutha et al. (2009) applied this method to several major dicotyledonous clades. However, this method has the possibility of axillary bud initiation because complete excision of the axillary meristem near the remaining cotyledon is difficult. In *Agrobacterium tumefaciens*-mediated transformation by CDM*i*, we thought that a transformant obtained from an axillary meristem could be a chimera, because the fate of most axillary meristems had been determined at the time of the excision. To reduce the risk of chimera, seedlings should be decapitated at the center of the hypocotyl for complete excision of all meristems. However, the regeneration capacity of seedlings with no leaves and cotyledons were obscured. Therefore, in the present study, we investigated the possible application of the CDM to eggplant culture *in vitro* at the center of hypocotyl, and estimated the efficiency of intact roots on shoot regeneration and rooting of the regenerated shoots compared with rootless hypocotyl and cotyledon segment cultures using PGRs.

Materials and Methods

Plant material

Seeds of eggplant (*Solanum melongena* L. cv. Shisui; Takii & Co., Ltd., Kyoto, Japan) were thoroughly washed in running tapwater, and subsequently surface-sterilized for 10 min in 10% sodium hypochlorite solution containing 1–2 drops of Tween 20. After three rinses with sterile distilled water, the seeds were sown on MS basal medium (Murashige and Skoog 1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. The medium pH was adjusted to 5.7–5.8 prior to the addition of agar, then the medium was autoclaved for 15 min at 121°C. Seedlings were cultured under 16 h photoperiod with fluorescent light at 70 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) at 25°C. The seedlings grown *in vitro* (14–16 days after germination) were used as the source of explants.

Effect of CDMi on adventitious bud and shoot regeneration compared with tissue cultures supplemented with plant growth regulators

The eggplant seedlings grown on MS medium were transferred to fresh MS medium and decapitated at the center of the hypocotyl with the roots remaining intact (CDM*i*). As a control tissue culture, hypocotyl and cotyledon explants were used. Hypocotyl explants, approximately 1 cm long, were placed in a vertical position on MS medium supplemented with 4.4 μM BA. Cotyledon explants, approximately 10 mm × 5 mm, were placed with the abaxial side facing downward on MS medium supplemented with 0.2 μM TDZ. The PGR concentration in cotyledon culture was determined by previous report (Magioli et al. 1998) and the PGR condition in hypocotyl culture was selected by our preliminary experiments on the basis of previous reports (Kamat and Rao 1978,

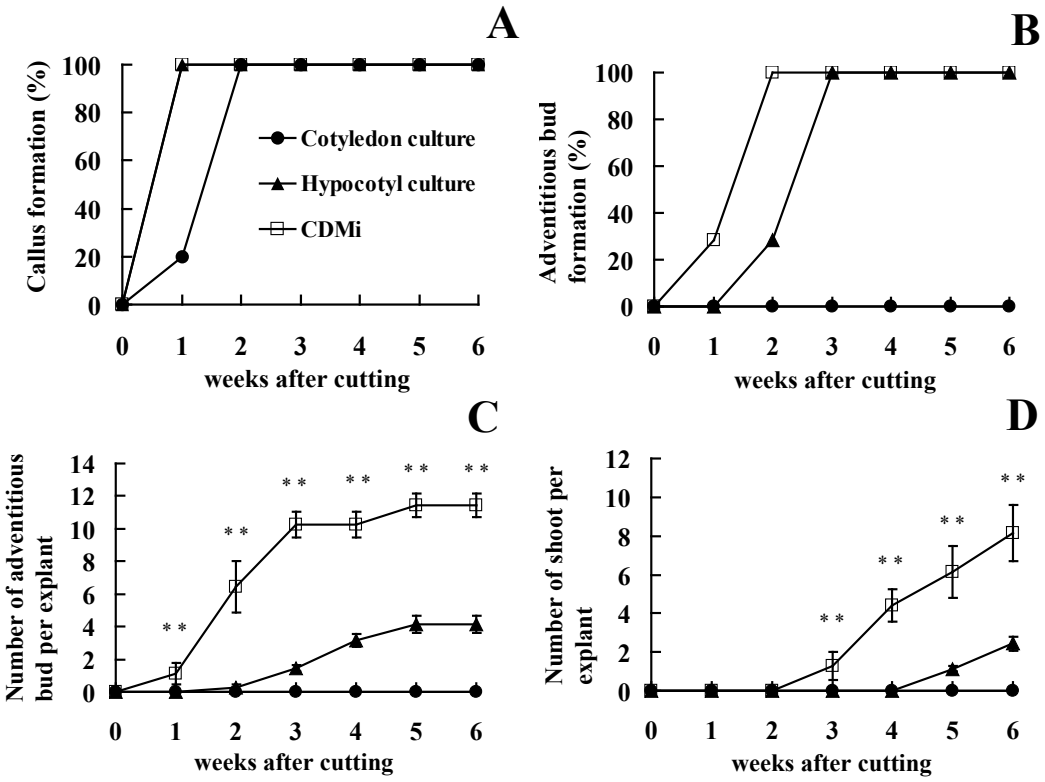


Fig. 1. Callus formation and adventitious bud and shoot regeneration from the cut surface of hypocotyls of eggplant during 6 weeks of culture with the complete decapitation method *in vitro* (CDM*i*). As a control tissue culture, cotyledon and hypocotyl explants were used. The cotyledon explants, approximately 5 mm × 5 mm, were placed with the abaxial side facing downward onto MS medium supplemented with 0.2 μM thidiazuron (●). The hypocotyl explants, approximately 1 cm long, were cultured in a vertical position on MS medium supplemented with 4.4 μM N^6-benzyladenin (▲). In CDM*i*, the eggplant seedlings grown on MS medium were decapitated at the center of the hypocotyl (□). (A) Percentage of explants with callus formation. (B) Percentage of explants with adventitious bud regeneration. (C) Number of adventitious buds regenerated per explant. (D) Number of adventitious shoots regenerated per explant. ** indicates a significant difference between CDM*i* and hypocotyl culture at $P < 0.01$ by Student's *t* test. Error bars represent the SE ($n = 15$).

Matsuoka and Hinata 1979). These explants were cultured under a 16 h photoperiod with fluorescent light at 70 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) at 25°C. The percentage of surviving explants, the percentage of surviving explants with callus and adventitious bud formation, and the number of adventitious buds per explant were determined every week after excision. The regenerated shoots from the cut surface were harvested individually when they had grown to approximately 1 cm in length, and the number of harvested shoots was counted every week after excision.

Rooting

The regenerated shoots, once approximately 1 cm in length, were excised individually and cultured on MS basal medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar under a 16 h photoperiod with fluorescent light at 70 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) at 25°C. The percentage of shoots that formed roots and the number of roots per shoot were determined 4 weeks after excision.

Results and Discussion

Effect of CDMi on adventitious bud and shoot regeneration compared with tissue cultures supplemented with plant growth regulators

All cotyledon explants formed callus at 2 weeks after excision (WAE) and survived until 6 WAE. However, no explants formed adventitious buds and shoots from the callus despite the use of an optimal PGR condition reported for other cultivars (Fig. 1 A, B). This result indicates that the concentration and combination of PGRs for shoot regeneration is highly cultivar-dependent in tissue culture of eggplant, and

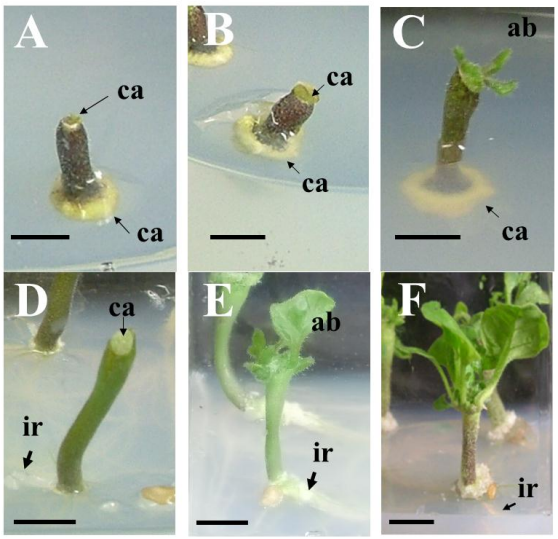


Fig. 2. Regeneration of callus, adventitious buds and shoots from hypocotyls of eggplant with the complete decapitation method *in vitro* (CDM*i*) and hypocotyl culture supplemented with 4.4 μM N^6-benzyladenin. (A) Explant in hypocotyl culture at 1 week after excision (WAE). (B) Explant in hypocotyl culture at 2 WAE. (C) Explant in hypocotyl culture at 3 WAE. (D) Explant in CDM*i* at 1 WAE. (E) Explant in CDM*i* at 2 WAE. (F) Explant in CDM*i* at 3 WAE. ca = callus; ab = adventitious buds; ir = intact root system. Bars = 1 cm.

Fig. 3. Intact roots of 4-weeks-old seedlings with (right) and without (left) decapitation at 2 weeks after sowing. Bar = 1 cm.

extensive experimentation is required to regenerate adventitious buds from an unstudied cultivar with protocols using PGRs.

In both CDM*i* and hypocotyl culture, the apical cut end of hypocotyl explants became enlarged within a few days after excision, and all explants formed callus at 1 WAE and survived until 6 WAE (Fig. 2 A). Although calli formed concurrently in both treatments, adventitious buds regenerated from the callus 1 week earlier in CDM*i* than hypocotyl culture (Fig. 1 A, B and Fig. 2). In CDM*i*, the intact roots of decapitated seedlings drastically developed within 2 WAE compared with those of 4-weeks-old seedlings grown *in vitro* without decapitation (Fig. 3). In hypocotyl culture, callus formed at both the apical and basal end of hypocotyl explants. However, adventitious buds formed only from callus at the apical end (Fig. 2 C). Similar polarity in regeneration response was observed in hypocotyl culture of *Capsicum frutescens* under a 16 h photoperiod (Kumar et al. 2007). At 6 WAE, the number of adventitious buds per explant in CDM*i* was significantly higher than that in hypocotyl culture, namely 11.4 and 4.1, respectively (Fig. 1 C). CDM*i* promotes regeneration of adventitious buds from callus on the cut surface compared with hypocotyl culture in eggplant regardless of the absence of PGRs. The shoots that developed from adventitious buds that grew to 1 cm in length were excised individually. Shoot excision was 2 weeks earlier in CDM*i* than hypocotyl culture (Fig. 1 D). At 6 WAE, the number of shoots obtained per explant in CDM*i* was significantly higher compared with that in hypocotyl culture, namely 8.1 and 2.4, respectively. This result indicates that the presence of intact roots stimulates shoot development and CDM*i* is an efficient method in the absence of PGRs for mass propagation because a number of regenerated shoots are obtained without additional examination to determine the optimal PGR condition.

In plant tissue cultures, organ differentiation is regulated by the relative concentration of PGRs in the culture medium; a relatively higher cytokinin concentration induces bud regeneration, whereas a relatively higher auxin concentration induces root regeneration, and intermediate concentrations induce only callus formation (Thorpe 2007). In the present study, cotyledon explants of 'Shisui' formed only callus and no adventitious buds developed in the treatment with PGR concentrations optimal for adventitious bud regeneration reported previously for a different cultivar. This difference in adventitious bud regeneration between cultivars might be caused by differences in endogenous hormones levels between the cultivars. On the other hand, CDM*i* promoted adventitious bud formation from callus on the cut surface of hypocotyls in the absence of PGRs, and the number of adventitious buds in CDM*i* was higher than that of hypocotyl culture supplemented with exogenous BA at 4.4 μM (Fig. 1 C). This result suggested that the hormonal balance of the explant in

Table 1. Effect of the complete decapitation method *in vitro* on the rooting response of regenerated shoots from explants at 4 weeks after excision

Treatment	Rooting (%)	Number of roots per shoot	Root length (cm)
Hypocotyl culture	63	1.6 ± 0.2	1.8 ± 0.1
CDM*i*	100	2.1 ± 0.2	3.4 ± 0.2
Significance		**	**

Data represent the mean ± SE (n = 16).

** indicates a significant difference at $P < 0.01$ by Student's t test.

CDM*i* was more optimal for adventitious bud regeneration than in hypocotyl culture with 4.4 µM BA. It is generally believed that endogenous cytokinins in higher plants are mainly biosynthesized in the root system and transported via the xylem to the aboveground parts where they regulate growth and development (Van Staden and Davey 1979, Nooden et al. 1990, Davies 2004). From these reports and our present results, we hypothesize that the promotive effect of CDM*i* may be attributed to the presence of the intact root system and utilization of endogenous cytokinin that is biosynthesized by the roots for adventitious bud and shoot regeneration.

In many plant species, growth and development are regulated by hormonal interactions, e.g. the shoot apices repress axillary bud growth and grow dominantly. Auxin, derived from the shoot apex, inhibits the growth of axillary buds, whereas cytokinin, derived from the roots, promotes the growth of axillary buds. Decapitation of *Vicia* plants induces outgrowth of axillary buds, but application of auxin to the stump prevents outgrowth of axillary buds (Thimann and Skoog 1933, 1934). In addition, decapitation in bean and pea leads to transient increases in cytokinin levels in the xylem sap or shoot, and exogenous auxin application partially suppresses these increases (Bangerth 1994, Li et al. 1995). These reports indicate that decapitation, i.e. removal of the site of auxin biosynthesis, accelerates the biosynthesis of endogenous cytokinin in the intact root system, and subsequently axillary buds develop as a result of cytokinin transport through the xylem. In addition, Wightman and Thimann (1980) reported that removal of epicotyl and cotyledons increased the length of primary and lateral roots of pea seedlings. In the present study, the intact roots of decapitated seedlings in CDM*i* drastically developed within 2 WAE compared with those of 4-weeks-old seedlings grown *in vitro* without decapitation (Fig. 3). From these results, the well-developed intact roots synthesized endogenous cytokinin after excision, and the synthesized cytokinin would be used to regeneration adventitious buds. In fact, the presence of intact roots is important for shoot regeneration.

In addition, shoots longer than 1 cm developed at a higher frequency in CDM*i* compared with hypocotyl culture supplemented with 4.4 µM BA (Fig. 1 D). In plant tissue culture, media of different composition are used depending on the stage of regeneration, e.g. callus-induction medium, shoot-induction medium, shoot-elongation medium and root-induction medium. In root culture of eggplant, Franklin et al. (2004) reported that elongation of regenerated shoots was not synchronous in cultures of cytokinin-containing media; in some cultures few shoots elongated, whereas others remained diminutive. When root explants cultured on initial media were subcultured on PGR-free medium, elongation of the shoots was promoted (Franklin et al. 2004). This report and our present results indicate that the intact root system of explants in CDM*i* may regulate the levels of biosynthesis and transport of cytokinin, and optimize the endogenous hormone balance depending on the stage of regeneration, i.e. shoot induction or shoot elongation. In eggplant tissue culture, optimal concentrations and combinations of PGRs in the culture medium are reported to differ markedly depending on the cultivar and explant type (Fassuliotis et al. 1981, Allichio et al. 1982). In the present study, cotyledon explants of 'Shisui' formed no adventitious buds under the optimal PGR condition reported for a different cultivar. In contrast, explants with an intact root system in CDM*i* may self-regulate the endogenous hormone balance to regenerate adventitious shoots and elongate the regenerated shoots, so CDM*i* is an efficient and simple method for production of multiple shoots without need for extensive subculturing.

Rooting

The excised shoots were placed on MS basal medium for rooting. All excised shoots in CDM*i* had formed roots at 4 WAE, whereas 63% of the control shoots had formed roots (Table 1).

Successful rooting of regenerated shoots is a prerequisite for micropropagation. In root explant cultures of eggplant, shoots obtained from media supplemented with TDZ, which promotes shoot formation, did not form roots even after being transferred to root-induction medium containing auxin (Franklin et al. 2004). Similar inhibition of rooting has been reported in shoots derived from explants cultured with TDZ (Magioli et al. 1998) and BA (Sharma and Rajam 1995). Magioli et al. (1998) achieved a 70% rooting efficiency when calli were maintained on PGR-free media before excision of the

shoots for a period of 2 weeks after adventitious bud induction by TDZ. From these observations, we hypothesize that excess exogenous cytokinins are not suitable for micropropagation because they inhibit rooting of regenerated shoots. In the present study, rooting of excised shoots was not inhibited in CDM*i*, whereas hypocotyl culture in the presence of BA inhibited rooting. Kuroha et al. (2002) reported that endogenous cytokinin negatively regulates adventitious root formation on cucumber hypocotyls. These results support the above hypothesis, and indicate that the intact root system in CDM*i* regulates the synthesis and transport of endogenous cytokinins depending on the stage of regeneration, i.e., induces them during shoot regeneration and suppresses them during shoot elongation. In contrast, regenerated shoots from hypocotyl culture continued to be supplied with BA from their medium. Therefore, the inhibition of rooting from these shoots might be due to increase in the endogenous BA level.

In the present study, CDM*i* formed adventitious bud and shoot formation in the absence of PGRs. This promotive effect of CDM*i* might be attributed to the presence of intact root systems that biosynthesized endogenous cytokinin. The utilization of endogenous cytokinin enabled all excised shoots to develop roots, although exogenous cytokinin treatment inhibited rooting in hypocotyl culture. It is concluded that CDM with intact roots is a simple and efficient method for mass propagation of eggplant *in vitro* without the need for complicated experimentation to determine the optimal concentration and combination of PGRs required. In addition, CDM*i* has potential to be an efficient *in vitro* transformation method.

Acknowledgments

The research was financially supported by the Sasakawa Scientific Research Grant from the Japan Science Society.

References

Allichio R, Del Grosso E, Boschieri E 1982 Tissue cultures and plant regeneration from different explants in six cultivars of *Solanum melongena*. Experientia 38: 499-450.

Amutha S, Kathiravan K, Singer S, Jashi L, Shomer I, Steinitz B, Gaba V 2009 Adventitious shoot formation in decapitated dicotyledonous seedlings starts with regeneration of abnormal leaves from cells not located in a shoot apical meristem. In Vitro Cell Dev. Biol.—Plant 45: 758-768.

Bangerth F 1994 Response of cytokinin concentration in the xylem exudate of bean (*Phaseolus Vulgaris* L.) plants to decapitation and auxin treatment, and relationship to apical dominance. Planta 194: 439-442.

Davies PJ ed. 2004 Plant Hormones: Biosynthesis, Signal Transduction, Action! Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 1-15.

Fassuliotis G, Nelson BV, Bhatt DP 1981 Organogenesis in tissue culture of *Solanum melongena* cv. Florida Market. Plant Science Letters 22: 119-125.

Franklin G, Sheeba CJ, Sita GL 2004 Regeneration of eggplant (*Solanum melongena* L.) from root explants. In Vitro Cell Dev. Biol.—Plant 40: 188-191.

Harada M, Oda M, Mori G, Ikeda H 2005 Mass regeneration of shoots from cut surfaces of stems in tomato stock plants. J. Jpn. Soc. Hort. Sci. 74: 479-481.

Johkan M, Mori G, Imahori Y, Mitsukuri K, Yamasaki S, Mishiba K, Morikawa T, Oda M 2008a Shading the cut stems of tomato plants promotes in vivo shoot regeneration via control of the phenolic metabolism. Environ. Control Biol. 46: 203-209.

Johkan M, Mori G, Mitsukuri K, Mishiba K, Morikawa T, Imahori Y, Oda M 2008b Effect of ascorbic acid on in vivo organogenesis in tomato plants. J. Hortic. Sci. Biotechnol. 83: 624-628.

Johkan M, Mori G, Mitsukuri K, Mishiba K, Morikawa T, Oda M 2008c In vivo shoot regeneration promoted by shading the cut surface of the stem in tomato plants. HortScience 43: 220-222.

Kamat MG, Rao PS 1978 Vegetative multiplication of eggplants (*Solanum melongena*) using tissue culture techniques. Plant Science Letters 13: 57-65.

Kumar V, Sharma A, Prasad BCN, Gururaj HB, Giridhar P, Ravishankar GA 2007 Direct shoot bud induction and plant regeneration in Capsicum frutescens Mill.: influence of polyamines and polarity. Acta Physiol. Plant. 29: 11-18.

Li CJ, Guevera E, Herrera J, Bangerth F 1995 Effect of apex excision and replacement by 1-naphthylacetic acid on cytokinin concentration and apical dominance in pea plants. Physiol. Plant. 94: 465-469.

Magioli C, Rocha APM, De Oliveira DE, Mansur E 1998 Efficient shoot organogenesis of eggplant (*Solanum melongena* L.) induced by thidiazuron. Plant Cell Reports 17: 661-663.

Matsuoka H, Hinata K 1979 NAA-induced organogenesis and embryogenesis in hypocotyl callus of *Solanum melongena*. J. Exp. Bot. 30: 363-370.

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

Nooden LD, Singh S, Letham DS 1990 Correlation of xylem sap cytokinin levels with monocarpic senescence in soybean. Plant Physiol. 93: 33-39.

Passey AJ, Barrett KJ, James DJ 2003 Adventitious shoot regeneration from seven commercial strawberry cultivars (*Fragaria* × *ananassa* Duch.) using a range of explant types. Plant Cell Reports 21: 397-401.

Pozueta-Romero J, Houlne G, Canas L, Schantz R, Chamarro J 2001 Enhanced regeneration of tomato and pepper seedling explants for Agrobacterium-mediated transformation. Plant Cell Tissue Organ Cult. 67: 173-180.

Rao PVL, Singh B 1991 Plantlet regeneration from encapsulated somatic embryos of hybrid *Solanum melongena* L. Plant Cell Reports 10: 7-11.

Saito T, Nishimura S 1994 Improved culture conditions for somatic embryogenesis using an aseptic ventilative filter in eggplant (*Solanum melongena* L.). Plant Science 102: 205-211.

Sharma P, Rajam MV 1995 Genotype, explant and position

effects on organogenesis and embryogenesis in eggplant (*Solanum melongena* L.). J. Exp. Bot. 46: 135-141.

Tezuka T, Harada M, Johkan M, Yamasaki S, Tanaka H, Oda M 2011 Effects of auxin and cytokinin on in vivo adventitious shoot regeneration from decapitated tomato plants. HortScience 16: 1661-1665.

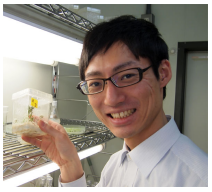
Thimann KV, Skoog F 1933 Studies on the growth hormone of plants. III. The inhibiting action of the growth substance on bud development. Proc. Natl. Acad. Sci. USA 19: 714-716.

Thimann KV, Skoog F 1934 On the inhibition of bud development and other functions of growth substance in *Vicia faba*. Proc. R. Soc. London Ser. B. 114: 317-339.

Thorpe TA 2007 History of plant tissue culture. Mol. Biotechnol. 37: 169-180.

Van Staden J, Davey J.E 1979 The synthesis, transport and metabolism of endogenous cytokinins. Plant Cell Environ. 2: 93-106.

Wightman F, Thimann KV 1980 Hormonal factors controlling the initiation and development of lateral roots. I. Sources of primordial-inducing substances in the primary root of pea seedlings. Physiol. Plant. 49: 13-20.



Mr. Hideyuki Tanaka is a doctoral student in Osaka Prefecture University. He is interested in the role of endogenous auxin and cytokinin in regeneration via both embryogenesis and organogenesis.