African Journal of Biotechnology Vol. 12(7), pp. 695-701, 13 February, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1818
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Micropropagation of *Vaccinium bracteatum* Thunb.

Kunyi Han[1#], Hengkang Hu[1#], Shunfu Li[1], Huawei Xv[1], Haiying Lin[2] and Qixiang Zhang[1]*

[1]School of Forestry and Biotechnology, Zhejiang & F University, Lin'an, Zhejiang, 311300, China.
[2]Taizhou Junlin Blueberry Co., Ltd., Linhai 317000, Zhejiang, China.

Accepted 6 September, 2012

***Vaccinium bracteatum* Thunb. is a vigorous plant that is easy to grow, can adapt to various acid soil species and has good resistance to drought. It has fewer pest problems than most other blueberry cultivars, offering an advantage rootstock for various blueberry cultivars to resist pest and disease. However, it is relatively difficult to propagate and the cuttings can be difficult to root. The present study is an investigation into the effects of the types and combination of plant growth regulators on the tissue culture system of *V. bracteatum* Thunb. When nodal explants were cultured on media *without* plant growth regulators (*PGRs*), the shoots did not produce new buds, while all media supplemented with PGRs significantly affected adventitious bud proliferation in woody plant medium (WPM*). Upon addition of PGRs, adventitious buds occurred and significant differences were observed ($P < 0.05$). *The* shoot length and proliferation coefficient in WPM basal medium containing 0 mg L^{-1} KT, 2.0 mg L^{-1} BA, 2.0 mg L^{-1} ZT and 2.0 mg L^{-1} TDZ was much more than other concentrations. The shoot length and proliferation reached 9.64 and 7.66 cm. For rooting, significant variation was observed ($P < 0.05$) between different concentrations of IBA and NAA. In general, the effect of IBA was much better than NAA. The root length gradually increased with increasing concentration of IBA from 0 to 2.0 mg L^{-1} followed by a decrease from 2.0 to 4.0 mg L^{-1}. The highest root length and number was 3.16 and 3.33 cm respectively when the concentration of IBA was 2.0 mg L^{-1}.**

Key words: *Vaccinium bracteatum* Thunb., plant growth regulator, tissue culture, micropropagation.

INTRODUCTION

The genus *Vaccinium* has a circumpolar distribution with species in North America, Europe and Asia. It can double as ornamentals while being grown as a food source. The genus contains several species of economic importance including blueberries (*Vaccinium cyanococcus*) which are native to North America, Changbai Mountain area and Da Xinan Mountain area in China. The natural conditions of south China are suitable for growing of blueberries, especially the highbush cultivars. Plants derived from tissue culture of blueberries grow more vigorously (Debnath, 2009). However, the *in vitro* rooting percentage is very low (Xu et al., 2008). Several authors (Frett and Smagula, 1983; Huang et al., 2004; Erig and Schuch, 2006; Ostrolucká et al., 2007; Zhao et al., 2008; Fira et al., 2008; Tetsumura et al., 2008; Sedlak and Paprstein, 2009; Litwinczud and Prokop, 2010) have reported tissue culture of blueberry cultivars. Most of them are focus *in vitro* micropropagation of highbush blueberries (Huang et al., 2004; Fira et al., 2008; Tetsumura et al., 2008; Sedlak and Paprstein, 2009; Litwinczud and Prokop, 2010). Huang et al. (2004) reported that the modified woody plant medium (WPM) basal media containing ZT were effective to induce shoots formation and propagation of *Vaccinium Corymbosum* L. and *V.* × *Corymbosum*. Fira et al. (2008) considered FeNaEDDHA (Sequestrene 138) providing far better results than FeNaEDTA for *in vitro* propagation of highbush blueberry cultivar blue crop. Tetsumura et al. (2008) demonstrated that WPM was not an ideal basal medium for the micropropagation of some highbush blueberries, because shoots grew better on MW, which is a mixture of equal parts of MS (Murashige and Skoog, 1962) and WPM.

Vaccinium bracteatum Thunb. is member of the genus *Vaccinium* which belongs to Ericaceae. The bushes provide accent to the landscape during all four seasons of the year because it is usually evergreen, erect but some-

*Corresponding author. E-mail: njzhangqixiang@qq.com. Fax: 86-571-63740809.

Co-first author.

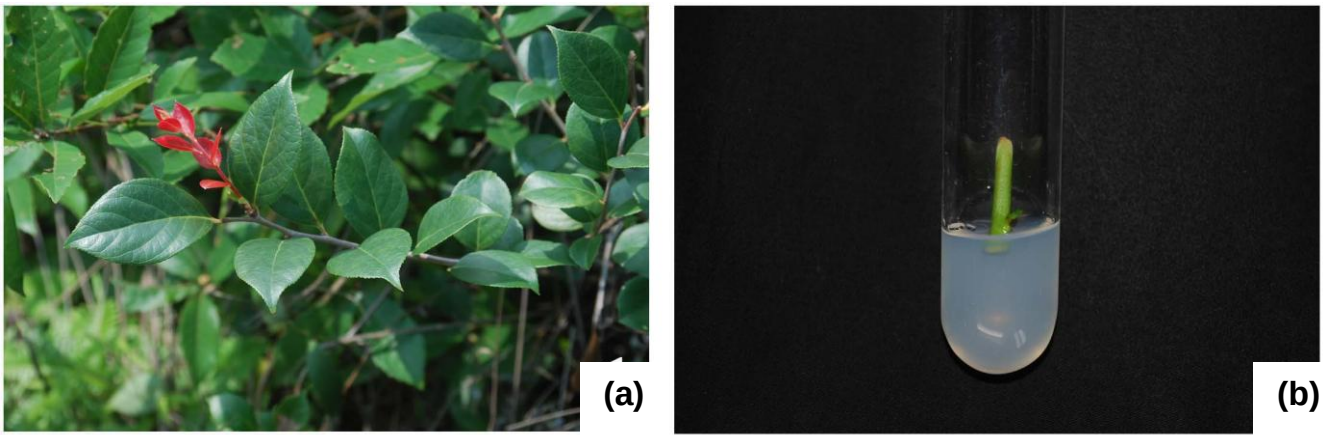

Figure 1. Explant of this project (a) and mother plant (b). After 2 weeks of culture, the axillary buds sprouted.

times prostrate shrubs varying in size from 1 to 4 meters tall with the characters of vigorous, good resistance to drought, easy to grow and adapt to various acid soil types. It has fewer pest problems than most other blueberry cultivars, offering an advantage rootstock for various blueberry cultivars to resist pest and disease. In recent years, reports about *V. bracteatum* Thunb. was focused on physiology and biochemistry field (Yang et al., 2008; Wang et al., 2006). Some authors (Zhou, 2007) reported a series of techniques for transplanting of its test tube shoot and the best transplanting survival rate reached 90%.

The aim of the present research was to optimize the cultural conditions of *V. bracteatum* Thunb. explants, and to determine the influence of 6-benzyladenine (BA), kinetin (KT), zeatin (ZT), thidiazuron (TDZ) concentration on the micropropagated shoots proliferation and indole-3-butyric acid (IBA) and α–naphthalene acetic acid (NAA) concentration on the rooting of micropropagated shoots.

MATERIALS AND METHODS

Plant materials

In April to May, 2010, annual branches were collected from the donor plant (Figure 1a) of *V. bracteatum* Thunb. growing on the campus of Zhejiang A and F University, Zhejiang province, China.

Disinfection of plant materials

The nodal explants of annual branch, taken during their vegetative phase (September to December), were used as primary explants. The sample annual branches (ca. 2 to 3 cm) were washed with tap water, surface-disinfection in 70% ethanol for 30 s and rinsed 3 times with sterile distilled water, then disinfected using 0.525% sodium hypochlorite for 15 min with three drops of Tween 20/500 ml, shaken on a rotary shaker for 20 min and rinsed three times with sterile distilled water. Primary explants were cultured on WPM (Lloyd and McCown, 1980) that was free from growth regulators. A total of 200 primary explants were placed in test tubes (25 × 150 mm, Sigma, St. Louis, MO) each containing 20 ml of medium. After three weeks of culture, the efficiency of each medium combination and biocide treatment was determined by recording the percentage of primary explant survival, contamination, and necrosis.

Culture media and growth conditions

The nodal explants of annual branch (ca. 0.5 to 1 cm) were inoculated *in vitro* in test tubes containing 20 ml medium. The WPM medium or half strength of WPM was supplemented with 3% sucrose and 0.65% agar type A (SIGMA). Plant growth regulators (PGRs) were added to the medium as specified below. The pH of medium was adjusted to 5.2 with 0.1 N NaOH prior to agar addition and autoclaving at 121°C for 15 min. Cultures were maintained at 25 ± 1°C and 16 h photoperiod by cool white fluorescent lamps (Philips, China) at 40 μmol $m^{-2}s^{-1}$.

Indexing for explant contamination

After two weeks of culture in the establishment stage, the axillary buds sprouted (Figure 1b). Then, a month later, when the apical and axillary buds reached the length of 2.0 ~ 2.5 cm, the buds were taken from the shoots, then transferred to Leifert and Waites Test Sterility Medium (Leifert et al., 1989) and made solid with 0. 5% agar type A in order to identify invisible bacterial contamination. A test tube with only substrate (control) was made. The vial was observed to verify the presence of contamination.

Proliferation of micropropagated shoots

The established shoots with negative Leifert and Waites Test results were transplanted into treatments as follows: WPM supplemented with 0.01 mg L^{-1} IBA in each treatment as well as different concentration of 6-BA at 0, 1.0, 2.0, 4.0, 8.0 mg L^{-1}, KT at 0, 1.0, 2.0, 4.0, 8.0 mg L^{-1}, ZT at 0, 1.0, 2.0, 4.0, 8.0 mg L^{-1} and TDZ 0, 1.0, 2.0, 4.0, 8.0 mg L^{-1} in orthogonal experimental design for micropropagated shoots proliferation.

Rooting of micropropagated shoots

Each regenerated shoot was rooted in a test tube (one regenerated shoot per tube) containing 20 ml of 1/2 WPM supplemented with IBA at concentrations of 0, 1.0, 2.0, 4.0 mg L^{-1}, NAA at concentrations of 0, 1.0, 2.0, 4.0 mg L^{-1} respectively for rooting (Table 2).

Hardening of plants and transplanting

In vitro micropropagated plantlets were transferred to an acclimation chamber in culture. After one week, the plantlets were washed gently with tap water to remove traces of agar and nutrients. Plantlets were then transplanted into plastic pots containing a 1:1:1 mixture of peat, perlite, and vermiculite. To retain moisture, the pots and plantlets were covered with a plastic bag; after 2 days, a single cut was made in each bag. After one week, the plastic bag was removed, and the plants were transferred into a greenhouse at 18 ± 2°C, with a photon fluence of 100 µmol m^{-2} s^{-1} and a 16 h photoperiod.

Statistical analysis

An orthogonal experimental design was used in this experiment for studying the shoot proliferation. Also, a complete randomized design was used for rooting. Ten replicates were used for each treatment and experiments were repeated three times. Buds length and proliferation coefficient were recorded at six weeks culture. Rooting percentage, root length and root piece per bud was recorded at eight weeks of culture. Visual observations were performed everyday. Data was analyzed by analysis of variance (ANOVA) using statistical package for the social sciences (SPSS) 16 and linear regression analyses were fitted using Sigma Plot 8.0. The means were compared using Duncan's multiple range test (95% confidence level) and the standard errors of means were calculated.

RESULTS

Explants showed a low presence of contamination (15 ~ 20%) in the three weeks of culture. Healthy buds that appeared free of contamination were transferred individually to fresh culture at the first signs of bud break. However, these buds showed a high percentage of contamination during the phase of proliferation and rooting, reaching 40 ~ 50% of contamination. Such behavior indicated the presence of endophytic pathogens. *V. bracteatum* Thunb. showed a good adaptation to the different stages of *in vitro* culture and an excellent vigor as the following demonstrate.

Indexing for explant contamination

The nodal sections showed negative contamination. Test results were used in subsequent experiments and the phases of proliferation and rooting. Between 90 to 95% of the cultures were positive for contamination.

Proliferation of micropropagated shoots

When cultured on media without PGRs, the shoots did not produce new buds, while all media supplemented with PGRs significantly affected adventitious bud proliferation in WPM medium. The shoot length and proliferation coefficient when BA, KT, ZT and TDZ were applied is given in Figure 2. Upon addition of PGRs, adventitious buds occurred and significant differences were observed ($P < 0.05$). The shoot length and proliferation coefficient in WPM basal medium containing 0 mg·l^{-1} KT, 2.0 mg L^{-1} BA, 2.0 mg L^{-1} ZT and 2.0 mg L^{-1} TDZ was much more than other concentrations. The shoot length and proliferation was up to 9.64 cm and 7.66 (Figures 2 and 3a) while the shoot length and proliferation coefficient in WPM basal medium containing 1.0 mg L^{-1} KT, 1.0 mg L^{-1} BA, 2.0 mg L^{-1} ZT and 4.0 mg L^{-1} TDZ was 7.5 and 1.33 (Figure 3b). For KT, the shoot length gradually increased with increasing concentration of KT from 0 to 1.0 mg L^{-1}. However, the proliferation coefficient was decreased with increasing concentration of KT from 0 to 4.0 mg L^{-1}, with the highest shoot length of 4.74. The proliferation coefficient was decreased with increasing concentration of KT from 0 to 8.0 mg L^{-1}. It is interesting that with the increasing concentration of KT, the terminal bud of shoots withered gradually after 15 days of culture. The effects of ZT concentration on the length and proliferation coefficient of adventitious buds were significant when compared with the control. The shoot length and proliferation coefficient gradually increased with increasing concentration of ZT from 0 to 2.0 mg L^{-1} followed by a decrease from 2.0 to 8.0 mg L^{-1}, which indicated that appropriate concentration of ZT promoted adventitious shoot length and proliferation coefficient. But for BA and TDZ, there were no significant differences between the concentrations of BA and TDZ on bud length. For BA, shoot proliferation coefficient gradually increased with increasing concentration of BA from 0 to 2.0 followed by a decrease from 2.0 to 8.0 mg L^{-1}. The largest shoot proliferation coefficient was 3.4. For TDZ, shoot proliferation coefficient gradually increased with increasing concentration of ZT from 0 to 2.0 followed by a decrease from 2.0 to 8.0 mg L^{-1}. The largest shoot proliferation coefficient was 2.6. With the increases in the concentration of BA and TDZ, callus was induced from the stem gradually and the new shoot was tender. Furthermore, treatment 0 mg L^{-1} KT, 2.0 mg L^{-1} BA, 2.0 mg L^{-1} ZT and 2.0 mg L^{-1} TDZ was found to be the optimum treatment for adventitious bud proliferation (Table 1).

Rooting of micropropagated shoots

The results and analysis of variance showed a statistically significant effect of auxin; the auxin concentration on root number, root length, and rate of rooted shoots. As shown in Table 2, appropriate concentration of IBA and NAA had a positive effect on root proliferation and promotes root growth. Significant variation was observed ($P < 0.05$) between different concentrations of IBA and NAA. In general, the effect of IBA on root growth was much better than that of the NAA. The root length gradually increased with increasing concentration of IBA from 0 to 2.0 mg L^{-1} followed by a decrease from 2.0 to 4.0 mg L^{-1}. The highest root length and number was 3.16 cm and 3.33 respectively when the concentration of IBA was 2.0 mg L^{-1} (Figure 3c). For NAA, the same trend of the root length was found for IBA. As the concentration of NAA was increased, the root number and rooting length first increased and then decreased. When the concentration of NAA was 2.0 mg L^{-1}, the root number was the highest,

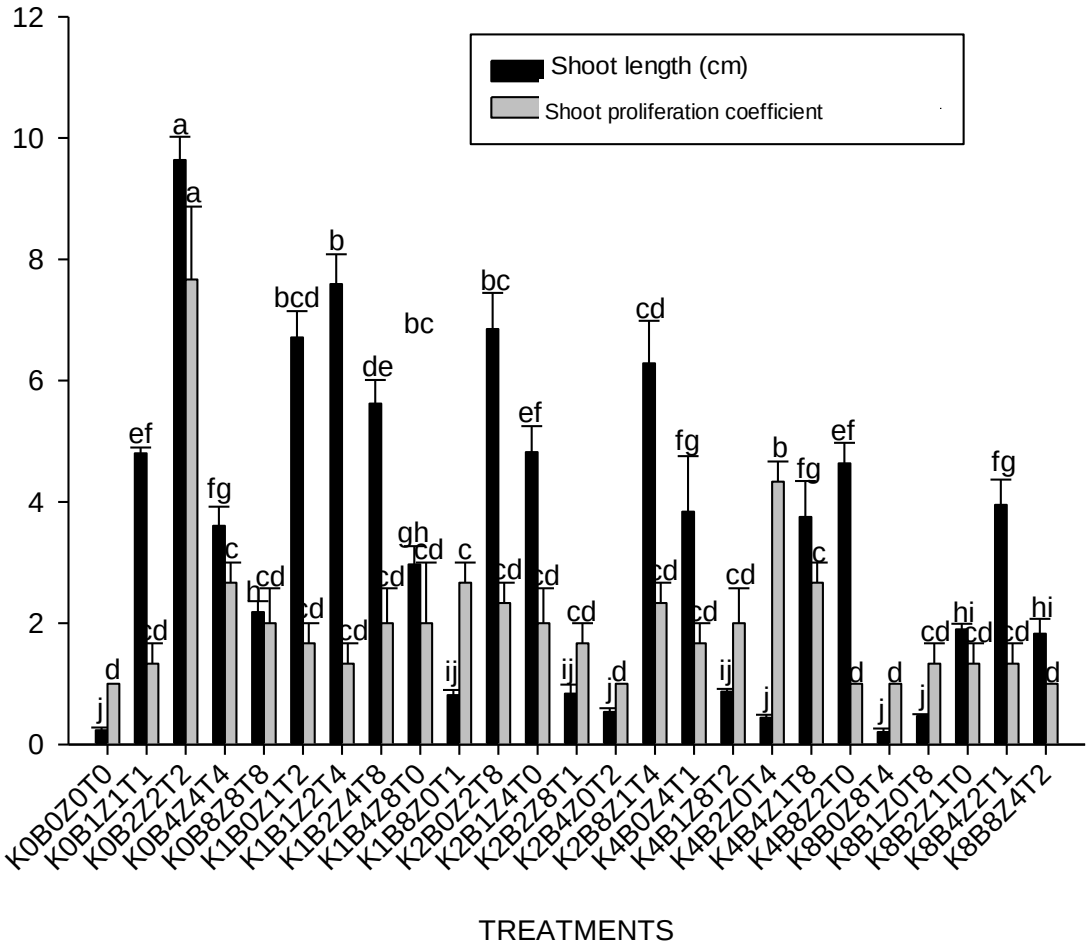

Figure 2. Effects of interaction among BA (B_n), KT (Kn), ZT (Zn) and TDZ (T_n) on shoot proliferation of *V. bracteatum* Thunb. Letters a, b, c and d denote significant different among different treatments and values followed by the same letter are not significantly different. Data are expressed as mean±SD. B0, B1, B2, B4, B8 respectively indicate 0, 1.0, 2.0, 4.0, 8.0 mg L^{-1} BA; K0, K1, K2, K4, K8 respectively indicate of 0, 1.0, 2.0, 4.0, 8.0 mg L^{-1} KT; Z0, Z1, Z2, Z4, Z8 respectively indicate 0, 1.0, 2.0, 4.0, 8.0 mg L^{-1} ZT; T0, T1, T2, T4, T8 respectively indicate 0, 1.0, 2.0, 4.0, 8.0 mg L^{-1} TDZ.

reaching only 1.66, respectively. When 4.0 mg L^{-1} NAA was used, many calli were produced on the basal buds, and browning was produced. The regeneration plants were uniform, grew vigorously when transplanted into the plastic pots (Figure 3d), and showed no morphological abnormalities or variations compared to controls as shown in Figure 4.

DISCUSSION

The selection of PGRs and their proper combination is crucial to obtain a high percentage of axillary buds formation. Axillary bud development is the most applied and reliable system for true-to type *in vitro* propagation, wherein an apical bud or nodal segment harbouring and axillary bud is cultured to proliferate multiple shoots addition, the development of a higher throughput transformation system depends on the availability of efficient and reliable tissue culture system for target tissue production and plant regeneration. Recent progress to improve *V. bracteatum* micropropagation system has been limited. Traditionally, softwood, semi-hardwood, and hardwood cuttings have been used to propagate *V. bracteatum*, but these kinds of propagation are slow and difficult to root (Sheat, 1948). In this study, an effectiveness of micropropagation and plant regeneration from seedlings of *V. bracteatum* was observed and we found that the combination of plant growth regulators have an important influence on plant tissue culture without any intervening callus phase (Debnath, 2007). In Previous studies on genus *Vaccinium* are often observed in micropropagation (Frett and Smagula, 1983; Huang et al., 2004; Erig and Schuch, 2006; Ostrolucká et al., 2007; Zhao et al., 2008; Fira et al., 2008; Tetsumura et al. ,2008; Sedlak and Paprstein, 2009; Litwinczud and Prokop, 2010). Most of

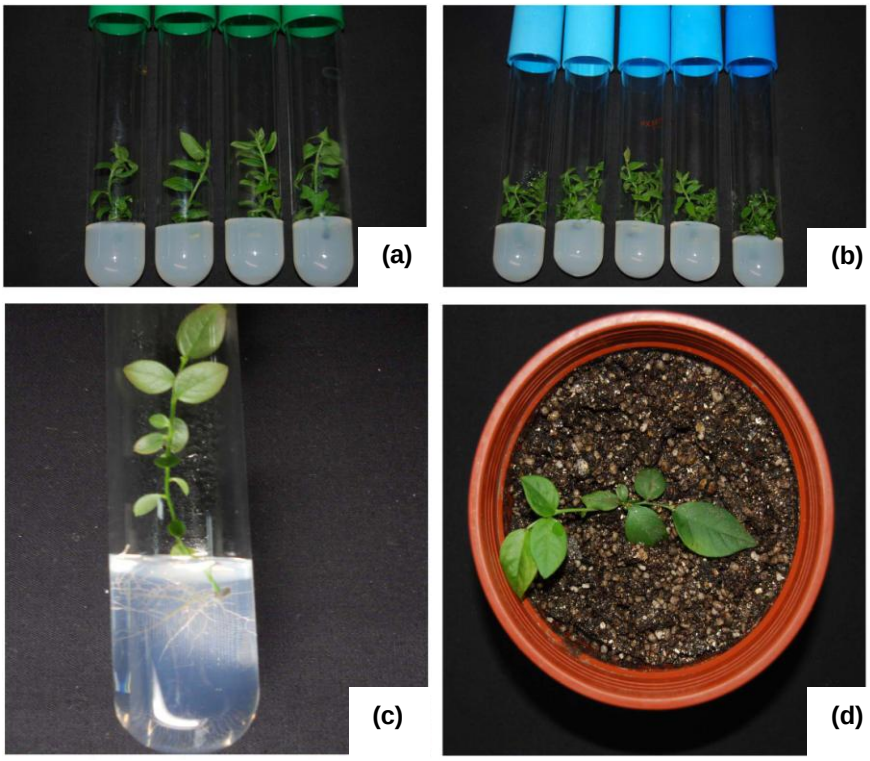

Figure 3. Micropropagation of *V. bracteatum* Thunb (*in vitro*) culture. a, proliferation stage (WPM basal medium containing 0 mg L^{-1} KT, 2.0 mg L^{-1} BA, 2.0 mg L^{-1} ZT and 2.0 mg L^{-1} TDZ); b, proliferation stage (WPM basal medium containing 1.0 mg L^{-1} KT, 1.0 mg·l^{-1} BA, 2.0 mg L^{-1} ZT and 4.0 mg L^{-1} TDZ); c, rooting stage; d, acclimatization stage.

Figure 4. Hardened plants after 6 months transplanted into plastic pots.

Table 1. Effects of BA, KT, ZT and TDZ respectively in orthogonal experimental design on shoot length and shoot proliferation coefficient of *V. bracteatum* Thunb.

Treatment (mg L^{-1})		Shoot length (cm)	Shoot proliferation coefficient
BA	0	4.33 ± 0.99[a]	1.53 ± 0.17[b]
	1.0	3.57± 0.71[a]	1.60 ± 0.19[b]
	2.0	3.31 ± 0.52[a]	3.40 ± 0.68[a]
	4.0	2.62 ± 0.56[a]	1.93 ± 0.27[b]
	8.0	3.03 ± 0.51[a]	1.80 ± 0.22[b]
KT	0	4.07 ± 0.84[ab]	2.93 ± 0.69[a]
	1.0	4.62 ±0.67[a]	1.93 ± 0.25[ab]
	2.0	3.85 ± 0.72[ab]	1.87 ± 0.19[ab]
	4.0	2.65 ± 0.46[b]	2.33 ± 0.33[ab]
	8.0	1.68 ± 0.41[c]	1.20 ± 0.11[b]
ZT	0	0.51 ± 0.08[c]	2.07 ± 0.36[b]
	1.0	6.19 ± 0.54[a]	1.87 ± 0.19[b]
	2.0	4.88 ± 0.57[b]	2.73 ± 0.71[a]
	4.0	3.91 ± 0.36[b]	1.87 ± 0.22[a]
	8.0	1.38 ± 0.27[c]	1.73 ± 0.25[a]
TDZ	0	3.23 ± 0.66[a]	1.467 ± 0.24[b]
	1.0	3.30 ± 0.63[a]	1.73 ± 0.18[b]
	2.0	3.36 ± 0.81[a]	2.67 ± 0.71[a]
	4.0	3.87 ± 0.84[a]	2.33 ± 0.33[ab]
	8.0	3.11 ± 0.47[a]	2.07 ± 0.21[ab]

Letters a, b, c and d denote significant different among different treatments and values followed by the same letter are not significantly different.

Table 2. Effects of IBA and NAA respectively on rooting of *V. bracteatum* Thunb.

Treatment (mg L^{-1})		Root length (cm)	Root number (piece)
IBA	0	0.00 ± 0.00[c]	0.00 ± 0.00[d]
IBA	1.0	0.66 ± 0.33[bc]	0.67 ± 0.32[cd]
IBA	2.0	3.15 ± 0.44[a]	3.33 ± 0.29[a]
IBA	4.0	1.33 ± 0.32[b]	1.32 ± 0.28[bc]
NAA	0	0.00 ± 0.00[c]	0.00 ± 0.00[d]
NAA	1.0	0.18 ± 0.16[c]	0.32 ± 0.31[d]
NAA	2.0	1.33 ± 0.30[b]	1.67 ± 0.29[b]
NAA	4.0	0.67 ± 0.33[bc]	0.66 ± 0.30[cd]

Letters a, b, c and d denote significant different among different treatments and values followed by the same letter are not sighificantly different.

them focus on plant growth regulators and basal medium. The combination of plant growth regulators (2.0 mg L^{-1} BA, 2.0 mg L^{-1} ZT and 2.0 mg L^{-1} TDZ) gave the highest adventitious bud propagation for *V. bracteatum*. Wolfe et al. (1983) showed that WPM produced the best shoot growth of seven media tested for the micropropagation of the “Bluecrup” highbush blueberry. Hence, most researchers have used WPM or modified WPM as a basal medium for blueberry micropropagation while Tetsumura et al. (2008) determined a mixture of equal parts of MS (Murashige and Skoog, 1962) and WPM. In the present study, however, we micropropagated *V. bracteatum*, finding that WPM was an ideal basal medium (data not shown). The auxin (2.0 mg L^{-1} IBA) gave the largest length and number of adventitious root for *V. bracteatum*. This is in agreement with the previous studies on micro-

propagation in lowbush blueberries cultivars (Zhao et al., 2008).

The aim of this study was to increase the number of units in the culture system until the desired number is obtained. In some cases, as in blueberry, organogenic propagation by axillary shoots is recommended in most cultivars (Erig and Schuch, 2006; Tetsumura et al., 2008), while in the case of coffee, orchid and soybean, the use of somatic embryogenesis is more suitable and efficient (Niemenak et al., 2008; Hong et al., 2010; Loganathan et al., 2010). To enhance the morphogenic responses from any explant cultivated in vitro, it is necessary to study and establish the effect of plant growth regulators like auxin and cytokinin interactions. Also, explant management and position onto the culture medium can also play a key role to improve the morphogenic response (Papafotiou and Martini, 2009).

Through this study of tissue culture in *V. bracteatum* Thunb, methods for the induction of adventitious bud and root proliferation, and plant regeneration were established. This efficient plant regeneration system would be helpful for graft improvement of blueberries through future biotechnology research. The potential of *V. bracteatum* Thunb as blueberries stock can be explored further in future research.

ACKNOWLEDGMENTS

The authors acknowledge the Project of Undergraduate Scientific and Technological Innovation of Zhejiang Province（2012）, Venture Project of School of Forestry & Biotechnology, Zhejiang A&F University（2012）, and Project of Forestry Department, Zhejiang province (2012) for the financial support.

REFERENCES

Debnath SC (2007). Strategies to propagate Vaccinium nuclear stocks for the Canadian berry industry. Can. J. Plant Sci. 87:911–922.

Debnath SC (2009). A two-step procedure for adventitious shoot regeneration on excised leaves of lowbush blueberry. *In Vitro* Cell. Dev. Biol. Plant. 45:122-128.

Erig AC, Schuch MW (2006). Factors that affect the *In vitro* multiplication of blueberry trees. Scientia Agraria 7(1):83-88.

Fira A, Clapa D, Badescu C (2008). Aspects regarding the *in vitro* propagation of highbush blueberry cultivar blue crop. Bull. UASVM, Hortic. 65(1):104-109.

Frett JJ, Smagula JM (1983). *In vitro* shoot production of lowbush blueberry. Canadian J. Plant Sci. 63:467-472.

Hong PI, Chen JT, Chang WC (2010). Chang W.C. Shoot development and plant regeneration from protocorm-like bodies of *Zygopetalum mackayi*. *In Vitro* Cell. Dev. Biol. Plant 46:306-311.

Huang WJ, Liu QZ, Kan XZ (2004). *In vitro* micropropagation of high blueberry. J. Anhui Normal University (Natural Science) 27(3):314-317.

Leifert C, Waites WM, Nicholas JR (1989). Bacterial contaminants of micropropagated plant cultures. J. Appl. Microbiol. 67:353-361.

Litwinczud W, Prokop A (2010). The usefulness of dikegulac in propagation of highbush blueberry (*Vaccinium corymbosum* L.) “Herbert”. J. Fruit Ornam. Plant Res. 18(2):85-92.

Lloyd G, McCown B (1980). Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot tip culture. Proceedings of the International Plant Propagators’ Society, 30:421-427.

Loganathan M, Maruthasalam S, Shiu LY, Lien WC, Hsu WH, Lee PF, Yu CW, Lin CH (2010). Regeneration of soybean (*Glycine max* L. Merrill) through direct somatic embryogenesis from the immature embryonic shoot tip. *In Vitro* Cell. Dev. Biol. Plant 46:265-273.

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

Niemenak N, Saare-Surminski K, Rohsius C, Ndoumou DO, Lieberei R (2008). Regeneration of somatic embryos in *Theobroma cacao* L. in temporary immersion bioreactor and analyses of free amino acids in different tissues. Plant Cell Rep. 27:667-676.

Ostrolucká M, Gajdošová A, Libiaková G, Hrubíková K, Bežo M (2007). Protocol for micropropagation of selected *Vaccinium* spp. Protocols Micropropagation Woody Trees Fruits 3:445-455.

Papafotiou M, Martini AN (2009). Effect of position and orientation of leaflet explants with respect to plant growth regulators on micropropagation of *Zamioculcas zamiifolia* Engl.(ZZ). Sci. Hortic. 120(1):115-120.

Sedlak J, Paprstein F (2009). *In vitro* multiplication of highbush blueberry (*Vaccinium corymbosum* L.) cultivars. Acta Horticulturae 810(2):575-580.

Sheat WG (1948). Propagation of trees, shrubs and conifers. Macmillan,

Tetsumura T, Matsumoto Y, Sato M, Honsho C, Yamashita K, Komatsu HM, Sugimoto Y, Kunitake H (2008). Evaluation of basal media for micropropagation of four highbush blueberry cultivars. Scientia Hortic. 119:72-74.

Wang L, Zhang XT, Yao HY (2006). The protective effect of *Vaccinium bracteatum* Thunb. leaves and the extract against light injury of retina. (in Chinese). J. Xi’an Jiaotong University (Medical Sciences) 27(3):284-303.

Wolfe DE, Eck P, Chin CK (1983). Evaluation of seven media for micropropagation of highbush blueberry. HortScience 18:703-705.

Xu H, Hu Y, Yang P, Xia CC, Deng YJ, Hu WS, Yu LN (2008). Preliminary discussion on the technologies of tissue culture and cutting breeding in blueberry seedlings of Anhui province. J. Anhui Agri. Sci. 36(11):4427-4428.

Yang XD, Xiao SM, Xu YS, Han Z, Wu YJ (2008). Analysis of volatile oil from *Vaccinium bracteatum* Thunb. leaves by gas chromatography-mass spectrometry (in Chinese). Biomass Chem. Eng. 42(2):23-26.

Zhao GJ, Wang ZB, Wang D (2008). *In vitro* propagation and Ex rooting of blueberry plantlets. Plant Tissue Cult. Biotech. 18(1):187-195.

Zhou CD (2007). Study on transplanting of tissue culture shoots of *Vaccinium* (in Chinese). Shanxi For. Sci. Technol. 3:7-10.