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Research Article

Rapid micropropagation of wild garlic (*Allium victorialis* var. *platyphyllum*) by the scooping method

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Abstract Wild garlic (*Allium victorialis* var. *platyphyllum*, AVVP) is a nontimber forest product used as an edible and medicinal vegetable. AVVP is usually propagated form offspring bulbs but it takes a long time to harvest. Using tissue culture technology could overcome this problem. This study investigated the optimal conditions for shoot multiplication, root growth, and plant growth by scooping AVVP bulbs. AVVP bulbs harvested from Ulleung Island, Korea, the main producer of AVVP, were surface-sterilized and used for in vitro propagation. Shoot multiplication was performed by the scooping method. More than five multiple shoots were induced from scooped tissue in Quoirin and Lepoivre (QL) medium containing plant growth regulators (PGRs); the maximum number of multiple shoots were induced from scooped tissue in QL medium containing 0.45 μM thidiazuron (TDZ) after 16 weeks of culture. Roots were induced directly at the base of the shoots in all treatments. In vitro rooting depended on the type of PGRs, and the best root-inducing treatment was QL medium containing 9.84 μM indole-3-butyric acid (IBA). Plants with in vitro roots were transferred to pots containing artificial soil and successfully acclimatized for 4 weeks. The acclimatized plants showed a survival rate of 80% after 20 weeks and gradually promoted growth depending on the acclimatization period. The results of this study will be of great help to AVVP dissemination through sustainable mass propagation.

Keywords *Allium victorialis* var. *platyphyllum*, thidiazuron, scooped bulb, in vitro, micropropagation

Introduction

Wild garlic (*Allium victorialis* var. *platyphyllum*, AVVP) is a perennial bulbous plant of 6-8 cm width and, on average, has 2-3 leaves. AVVP is distributed mainly in the northern hemisphere, and in Korea is found primarily in the Jiri-, Seorak- and Odae-mountains at over 1,000 m (Hong et al. 2010). *Allium* species such as onion and garlic are used as spices, vegetables, and medicinal plants. AVVP has been used as an edible wild herb and is found to influence lowering of cholesterol, antiatherogenic effect, antimutagenic and cytotoxic effects (Kim et al. 2014).

AVVP is a non-timber forest products (NTFPs) grown in Korea by planting seeds and bulbs in the fall. The plant is exposed to cold during the winter, and then new leaves start to sprout from early spring. AVVP has 2-3 very few leaves and is usually harvested the following April, leaving only one leaf. Therefore, AVVP is a very precious wild herb, and good seedlings are very important. Propagating AVVP conventionally from offspring bulb takes a long time to harvest (Choi et al. 2006). The non-availability of seed bulbs limits the large-scale propagation of AVVP. The propagation from seed and bulb is inefficient, laborious and time-consuming (Maesato et al. 1994). AVVP is carried out by two propagation methods, one by tillering and the other by adventitious buds (Ohnuki-Tierney 1981).

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Tissue culture technology of *Allium* has been used not only for bulbous plants but also for many other plants as an alternative to conventional methods for rapid mass production of homogeneous plant material. Tissue culture techniques have been successfully applied for the rapid propagation of *Allium* species such as *A. cepa* (Kamstaityte and Stanys 2004), *A. sativum* (Ayabe and Sumi 1998), and *A. chinense* (Xu et al. 2008). There are studies on the effects of nitrogen, phosphorus, potassium and sucrose on shoots and bulb growth (Jeong et al. 2012), and on the effects of medium and sugar (Lee and Jeong 2018) on Korean wild garlic.

Most bulbous plants have meager growth rates. Corm and comel, used to propagate bulbous plants, grow so slowly that it is challenging to meet the demand for seedlings by conventional methods (Memon 2012). The research for the multiplication of bulbous plants was focused on plant growth regulators (PGRs) as cytokinins. Shoot propagation from bulblets is applied by chipping, scooping, scaling and scoring (Park et al. 1998). The scooping method combined with PGRs may affect the shoot multiplication of wild garlic.

There have been few reports of rapid mass proliferation of AVVP through tissue culture. Therefore, this study investigated the shoot multiplication efficiency by the scooping method of AVVP and the optimal conditions of the culture medium, carbon source, and PGRs on shoot growth.

Materials and Methods

Plant material and *in vitro* culture

Seeds from a 4-year-old healthy AVVP of Ulleung Island were collected from the plant growing in the Forest Education Extension Center of Gyeongsang National University. The seeds were washed in running tap water for 120 min, and washed with 0.05% Tween-20 for 10 min, followed by ethanol (70% v/v) for 3 min and sterilized with NaClO (5% v/v) for 9 min. Then, the seeds were rinsed 5 times with sterile distilled water and wiped dry with sterile filter paper. Seeds were transferred to the culture bottle containing 20 ml of sterile MS (Murashige and Skoog 1962) medium. The medium was supplemented with 0.23 μM GA_3 (Duchefa, Haarlem, Netherlands) and 30 g L^{-1} sucrose and adjusted to pH 5.7 before the addition of the 0.38% (w/v) gerlite (Duchefa, Haarlem, Netherlands) followed by autoclaving at 121 °C for 15 min. These cultures were carried out at 25±1°C with a photoperiod of 16 hrs of light with 37 μmol $m^{-2}s^{-1}$ (about 3,000 lux)/8 hrs of the dark for 12 weeks.

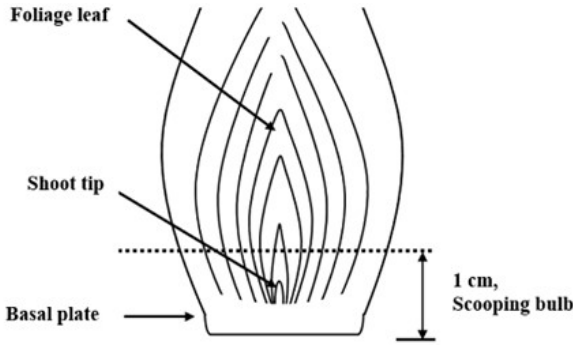

Fig. 1 Scooped bulb of AVVP. AVVP: *Allium victorialis* var. *platyphyllum*

Cultivation of *in vitro* plants

As a culture medium for wild garlic, Quoirin and Lepoivre (Quoirin and Lepoivre 1977; QL) medium identified through previous studies was used, and sucrose (3% w/v) was used as a carbon source (Lee and Jeong 2018). Shoot growth was measured for 5 weeks of culture.

Shoot multiplication through scooping of bulb

Shoot multiplication induction was performed in two stages using a basal bulb and a scooping bulb. First, 3-month-old bulblets were cultured on a shoot multiplication medium with various cytokinins. Secondly, a small portion of the base of the cultured bulbs (3-month-old) was cut to obtain scooped bulbs (10-15 mm) (Fig. 1). The scooped bulbs were placed on solid medium in petri dishes for shoot development. The number of explants from bulblets, number of bulblets formed, and their growth was recorded after 16 weeks of culture. Shoot multiplication was performed using the same medium until bulblets produced from each bulb scale were obtained.

To determine optimal PGRs on shoot multiplication, explants of two types (basal bulb and scooping bulb) were cultured on QL basal salts and vitamins with 3% (w/v) sucrose and 0.38% (w/v) gerlite. The basal medium was supplemented with various PGRs: 0.45, 4.54, 9.08 and 22.7 μM thidiazuron (TDZ) (Sigma, St. Louis, MO, USA), 0.49, 4.92, 9.84 and 24.6 μM 2-isopentenyladenine (2ip) (Sigma, St. Louis, MO, USA) and 0.46, 4.56, 9.12 and 22.8 μM zeatin (Sigma, St. Louis, MO, USA) at alone or in combination with 0.54 μM 1-naphthaleneacetic acid

Table 1 Multiplication from two explants from an AVVP* bulb

Type of explant	Shoots per explant, mean ± SD**	Shoot clump mass (mg), mean ± SD
Basal bulb	3.1 ± 0.45[b]	1.71 ± 0.24[b]
Scooped bulb	18.3 ± 0.9[a]	3.54 ± 0.35[a]

*AVVP: *Allium victorialis* var. *platyphyllum*.
**SD: standard deviation.
All experiments were performed in five replications. The values represent the mean ±SD, and a *t*-test ($P \leq 0.05$) was performed.

(NAA) (Sigma, St. Louis, MO, USA). Individual shoots were cut from multiple shoot clusters propagated through culture in basal QL solid medium without PGRs for 8 weeks. All media were adjusted to pH 5.7 before autoclaving (121°C for 15 min), and *in vitro* cultures were maintained under 16 h light/8 h dark light in a culture room equipped with a cool fluorescent lamp emitting 25 μmol m^{-2} s^{-1} of photosynthetic activating radiation (PAR).

Histological study for observing growth and differentiation of bulb

The bulb's morphology, growth, and differentiation were observed with a stereoscopic microscope (Olympus). Samples were fixed in formaldehyde alcohol acetic acid (FAA) (ethanol: distilled water: acetic acid: formalin, 10:7:2:1 v/v/v/v). After 3 days, fixative was removed, and samples were dehydrated in an alcohol series (80-90-100-100%, 1 h in each), and cleared in a mixture of absolute alcohol and xylene series (3:1, 1:1, 1:3 v/v, 30 min in each), and submerged in xylene (twice for 1 h each). Samples were embedded in Paraplast® (Leica Microsyshoots, 60°C). Tissues were cut to 10 mm thick on a rotary microtome (Microtec CUT 4060), stained with haematoxylin, safranin-fast green and observed under a light microscope and documented using a digital camera (Microscope Eyepiece Camera, AM4023X Dino-Eye).

Shoot elongation and *in vitro* rooting

For shoot elongation, multiplied plants were collected and transferred to QL basal medium supplemented with sucrose (3% w/v). The elongated shoots were cut (5 cm long) and inoculated into a test-tube containing 10 ml solid culture media. The *in vitro* rooting of the shoots was carried out with QL medium supplemented with various concentrations of two-auxins; 2.85, 5.71 and 11.42 μM indole-3-acetic acid (IAA) and 2.46, 4.92 and 9.84 μM indole-3-butyric acid (IBA). After 5 weeks of culture under 3000 lux illumination, the root number and growth were measured.
Acclimatization of *in vitro* plantlets

Rooted plantlets were transferred from the culture vessels, washed gently under running tap water, and transplanted to pots containing autoclaved peatmoss, sand and vermiculite mixture (1:1:1, w/w/w). The pots were placed in a growth chamber maintained at 25±1°C with fluorescent lighting for 4 weeks before they were transferred to soil in the greenhouse. The survival rate was measured after acclimatization for 5 months.

Statistical analysis

Data were subjected to statistical analysis by using the SPSS software. Means were compared using one-way analysis of variance (ANOVA) and Duncan's multiple-range test (DMRT) at a 0.05 level of probability.

Results

Effect of explant types on shoot multiplication

Two types of explants from the bulb of AVVP showed a difference (Table 1). No multiple shoots were observed when basal bulb was cultured under the PGRs conditions within 3 months. However, multiple shoots had formed on the scooped bulb, which produced an average of 18.3 bulblets per explant. However, the basal bulb produced 3.1 multiple shoots. The weight of the multiplied shoot clump was 3.54 mg when the scooped bulb was used and 1.71 mg when the basal bulb was used. Therefore, it is judged that it is good to scoop the bulb for shoot multiplication of AVVP.

Effect of PGRs on shoot multiplication

Multiple shoots developed from the scooped bulb of AVVP were cultured on an QL medium containing various combinations of PGRs. After 8 weeks, adventitious shoot buds had changed on each explant. The PGR effects were monitored by scoring the number of differentiating shoots

Table 2 Effect of various PGRs* on shoot multiplication of AVVP** cultures

PGR Concentration (μM)		Shoots	Diameter of bulblet (mm)	Roots	Root length (mm)	Existing shoot length (mm)	Remarks
Cytokinins	Auxin (NAA***)						
TDZ ****	0.45	0.0	5.33^{a}	6.09^{cdefg}	1.67^{d}	31.04^{defg}	29.24^{abcd}	Shoot cluster formation
	4.54	0.0	1.33^{a}	6.14^{defg}	0.67^{d}	9.42^{i}	31.68^{abcd}	Shoot cluster inside bulb formation
	9.08	0.0	-	10.16^{a}	3.33^{cd}	10.01^{i}	41.95^{ab}	Bulb enlargement
	22.7	0.0	1.00^{a}	9.79^{ab}	1.67^{d}	17.57^{hi}	39.39^{ab}	Bulb enlargement
	0.45	0.54	4.00^{a}	7.23^{cdefg}	3.33^{cd}	39.83^{bcde}	17.25^{cd}	Shoot cluster formation
	4.54	0.54	0.67^{a}	7.75^{cdefg}	-	-	17.92^{d}	Shoot cluster inside bulb formation
	9.08	0.54	1.50^{a}	7.93^{cdefg}	1.00^{d}	8.86^{i}	22.76^{bcd}	Shoot cluster inside bulb formation
	22.7	0.54	-	8.61^{abc}	-	-	41.66^{ab}	Bulb enlargement
Zeatin	0.46	0.0	0.33^{a}	5.69^{cdefg}	4.33^{bcd}	42.34^{abcd}	38.92^{ab}	
	4.56	0.0	-	6.49^{cdefg}	5.33^{bcd}	25.67^{efg}	38.53^{abc}	
	9.12	0.0	-	6.11^{defg}	2.33^{cd}	19.24^{ghi}	41.19^{ab}	
	22.8	0.0	3.00^{a}	6.81^{cdefg}	1.67^{d}	16.38^{hi}	43.85^{ab}	Shoot formation
	0.46	0.54	-	5.98^{fg}	4.33^{bcd}	41.52^{abcd}	23.18^{abcd}	
	4.56	0.54	-	5.87^{fg}	2.00^{d}	16.32^{ghi}	34.33^{abcd}	
	9.12	0.54	5.00^{a}	8.20^{abcd}	-	-	34.41^{abcd}	Shoot formation
	22.8	0.54	-	7.17^{cdefg}	-	-	34.18^{abcd}	
2ip	0.49	0.0	-	5.89^{fg}	9.00^{ab}	62.31^{a}	44.57^{a}	Root apical tissue formation
	4.92	0.0	-	5.79^{fg}	11.33^{a}	53.96^{abc}	32.05^{abcd}	Root apical tissue growth
	9.84	0.0	0.50^{a}	6.21^{defg}	10.33^{a}	65.67^{ab}	25.38^{abcd}	
	24.6	0.0	-	6.55^{cdefg}	3.33^{cd}	50.52^{cd}	34.30^{abcd}	
	0.49	0.54	-	6.09^{defg}	5.00^{bcd}	25.24^{bcd}	34.67^{abcd}	Circular root apex
	4.92	0.54	-	6.87^{cdefg}	10.33^{a}	45.70^{a}	27.49^{abcd}	Growing of root apex
	9.84	0.54	-	7.17^{cdefg}	7.33^{abc}	47.87^{abc}	28.43^{abcd}	
	24.6	0.54	4.00^{a}	8.11^{bcde}	4.00^{cd}	53.97^{cd}	32.60^{abcd}	Formation of shoot cluster

*PGR: plant growth regulator.
**AVVP: *Allium victorialis* var. *platyphyllum*.
***NAA: 1-naphthaleneacetic acid.
****TDZ: thidiazuron.
Scooped bulbs were cultured for 16 weeks. All experiments were performed in five replications. The values represent the mean, and Duncan's multiple-range test ($P \leq 0.05$) was performed.

and observing the morphological changes in the explants during *in vitro* culture (Table 2, Fig. 2 and 5). The formation of bulblet 16 weeks after culture in the various combinations and concentrations of PGRs was investigated (Table 2).

The PGR treatment affected the number of shoots of AVVP, the number of bulbs and roots, and the length of shoots. First, multiple shoots showed the most significant difference between the types of PGRs. As for the morphological changes, the TDZ supplement tended to promote shoot multiplication and bulb enlargement. No significance was found between the single and mixed treatment of NAA, which is an auxin, in TDZ. 5.33 multiple shoots were induced in the 0.45 μM TDZ single treatment.

The explants were cultured on media in the absence of NAA, and after 8 weeks, multiple shoot was first observed on a medium containing 0.45 μM TDZ. After 16 weeks, primordia-like thick and white projections began to be observed on the hypertrophic bulb surface. Additions of 0.54 μM NAA combined with 0.45 μM TDZ produced a shoot cluster with a few adventitious shoots of which the tips were green after 14 weeks of culture. In other combinations of NAA and TDZ, *in vitro* shoot clusters forming inside the bulb were observed (Fig. 2A).

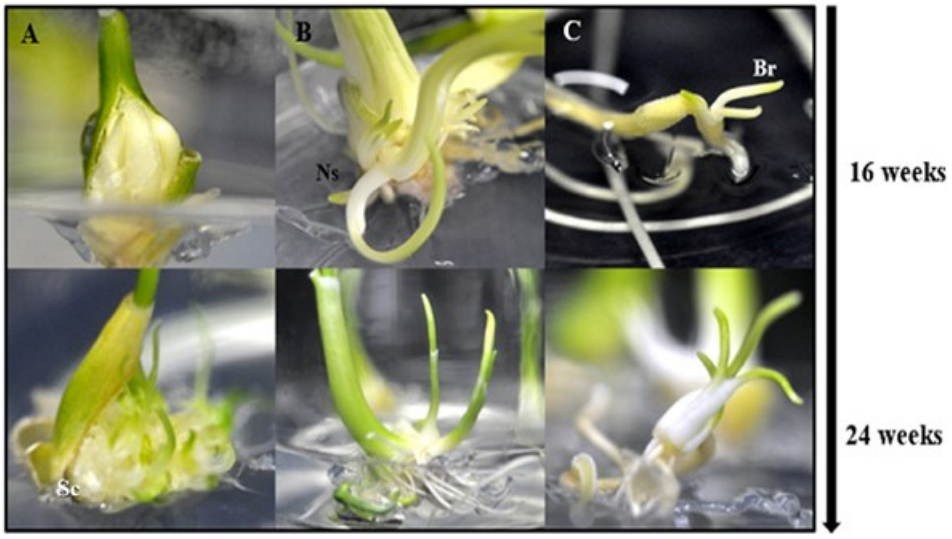

Fig. 2 Effect of various PGRs on shoot multiplication in AVVP after 16 and 24 weeks of culture. (A) Shoot cluster (Sc) development on QL medium supplemented with 0.45 μM TDZ and 0.54 μM NAA, (B) morphologically normal shoot (Ns) development on QL medium supplemented with 22.8 μM zeatin, and (C) budding root-derived shoot (Br) on QL medium supplemented with 4.92 μM 2ip and 0.54 μM. AVVP: *Allium victorialis* var. *platyphyllum*; QL: Quoirin and Lepoivre; NAA: 1-naphthaleneacetic acid; PGR: plant growth regulator; TDZ: thidiazuron

Interestingly, on a medium containing 9.08 and 22.7 μM TDZ, scooped bulb showed a sign of enlargement 8 weeks after culture. TDZ at 9.08 μM resulted in a greater average diameter of *in vitro* bulblet (Table 2).

The multiplied shoots were developed after 10 weeks in a medium containing 9.12 μM of zeatin and 0.54 μM NAA. Shoots appeared healthy and produced an average number of 5.0 shoots (Table 2).

Adventitious shoot formation from the scooped bulb appeared by 24.6 μM 2ip and 0.54 μM NAA. The 2ip supplement tended to promote root formation and growth; however, no shoot induction occurred on low levels of 2ip.

On the other hand, the root apex was changed in media containing low levels of 2ip. Initially, yellow circular root apex was induced on a medium containing 4.92 μM 2ip and 0.54 μM NAA. From 12 weeks, the root apex appeared callus form and gradually turned to shoot after 16 weeks of following culture (Fig. 2C).

The bulb's diameter also differed according to the type and concentration of the PGRs (Table 2). The diameter of the bulb increased as the concentration of the PGRs increased. The best PGRs for bulb size were TDZ. The 9.08 μM TDZ treatment was the best at 10.16 mm. Treatments suitable for bulb growing were the 22.7 μM TDZ and 0.54 μM NAA mixed treatment group, the 9.12 μM zeatin and 0.54 μM NAA mixed treatment group, and the 24.6 μM 2ip and 0.54 μM NAA mixed treatment group, all of which showed a growth of more than 8 mm.

The number of roots also differed according to the PGRs. The best PGR for rooting induction was 2ip. The number of roots increased as the concentration was lowered. Root length also differed according to the type and concentration of the PGRs. 2ip treatment showed good root length growth. The shoot length also differed according to the growth type and concentration. A good PGR for shoot elongation was zeatin.

The relationship between growth characteristics and PGRs for the reproduction of AVVP cultures was investigated (Table 3). The PGRs that had the most influence on shoot length were TDZ, followed by zeatin and 2ip. The most influential PGRs on bulb diameter growth was zeatin, followed by 2ip and TDZ. The PGRs that had the most influence on the number of roots were 2ip, and zeatin and TDZ came after it. The PGRs most influential on root length was zeatin, followed by TDZ and 2ip. TDZ affected the external shoot length the most, and zeatin and 2ip were less effective on it.

Histological study for observing growth and differentiation of bulb

The developmental patterns of adventitious shoots derived from scooped bulbs were observed by microscope. Incubation of scooped bulbs on QL medium supplemented with 9.08 μM TDZ caused enormous bulbs (Fig. 3A). The enlarged bulblet formed shoots over the bulb surface (Fig. 3B), and they later developed into shoots (Fig. 3C).

In the morphological observations of shoots after 16 to

Table 3 Relationship between growth characteristics and PGRs* on the propagation of AVVP** cultures

Growth characteristics	PGRs	Sum of squares	df	Mean squares	*F*	*P*-value
Shoot numbers	TDZ***	86.958	7	12.423	4.969	0.004
	Zeatin	44.000	7	6.286	1.640	0.195
	2ip	18.292	7	2.613	0.965	0.488
Diameter of bulblet (mm)	TDZ	42.354	7	6.051	3.603	0.016
	Zeatin	64.801	7	9.257	1.840	0.148
	2ip	43.633	7	6.233	1.353	0.290
Root numbers	TDZ	37.833	7	5.405	1.730	0.172
	Zeatin	84.667	7	12.095	1.948	0.128
	2ip	234.958	7	33.565	3.678	0.015
Root length (mm)	TDZ	8490.362	7	1212.909	14.028	0.000
	Zeatin	10818.597	7	1545.514	80.093	0.000
	2ip	8321.363	7	1188.766	11.204	0.000
Existing shoot length (mm)	TDZ	2382.604	7	340.372	4.968	0.006
	Zeatin	829.258	7	118.465	1.922	0.132
	2ip	236.424	7	33.775	0.129	0.995

*PGR: plant growth regulator.
**AVVP: *Allium victorialis* var. *platyphyllum*.
***TDZ: thidiazuron.
All experiments were conducted in five replications, and Duncan's multiple-range test ($P \leq 0.05$) was performed.

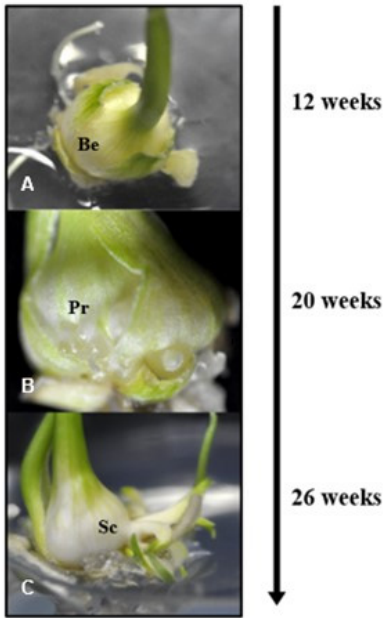

Fig. 3 Effect of TDZ on shoot multiplication from scooped bulbs of AVVP at different developmental stages cultured on QL medium supplemented with 9.08 μM TDZ. (A) Bulb enlargement (Be), (B) primordia-like shoot (Pr), and (C) shoot cluster (Sc). AVVP: *Allium victorialis* var. *platyphyllum*; QL: Quoirin and Lepoivre; TDZ: thidiazuron.

17 weeks of culture, a meristematic tissue with a procambium was formed (Fig. 4A). The microscopic observation of 20-weeks-old bulbs showed elongated

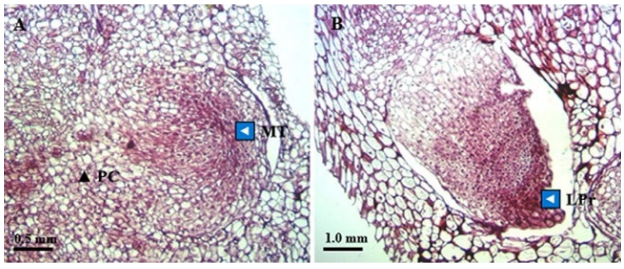

Fig. 4 Histology of scooped bulb development in AVVP and developmental stages of scooped bulbs on QL medium supplemented with 9.08 μM TDZ. (A) Meristematic tissue (MT) and procambium (PC) after 16 weeks (scale bar = 500 μm) and (B) leaf primordium (LPr) after 20 weeks (scale bar = 1.0 mm). AVVP: *Allium victorialis* var. *platyphyllum*; QL: Quoirin and Lepoivre; TDZ: thidiazuron.

structures of continuous meristematic tissue within the meristem (Fig. 4B). Consequently, treatments of bulblets with TDZ increased the frequency of primordia or shoot after 20 weeks (data not shown).

Shoot elongation and *in vitro* rooting and acclimatization

Roots were induced directly at the base of the shoots in all treatments. Mixed treatment of IBA and IAA promoted *in vitro* rooting of wild garlic rather than single treatment (Table 4). In particular, the growth of roots and bulbs was promoted in the high concentration treatment group among

Table 4 Effect of IAA* and IBA** concentration on root induction, shoot induction, and bulb growth

Concentration of PGRs*** (μM)		Roots, mean ± SD****	Plant length (cm), mean ± SD	Leaf diameter (cm), mean ± SD	Bulb length (cm), mean ± SD	Bulb diameter (cm), mean ± SD
IBA	IAA					
0	0	3.00 ± 1.10^{c}	0.24 ± 0.03^{ab}	0.30 ± 0.02^{d}	0.18 ± 0.02^{c}	0.29 ± 0.02^{c}
0	11.42	4.60 ± 1.62^{bc}	0.15 ± 0.02^{c}	1.33 ± 0.16^{b}	0.31 ± 0.03^{a}	0.35 ±0.04^{b}
9.84	0	6.40 ± 2.06^{ab}	0.25 ± 0.02^{ab}	0.20 ± 0.02^{d}	0.13 ± 0.02^{d}	0.06 ±0.01^{e}
4.92	5.71	4.00 ± 0.89^{c}	0.21 ± 0.04^{b}	1.01 ± 0.02^{c}	0.25 ± 0.02^{b}	0.21 ±0.03^{d}
9.84	11.42	7.20 ± 1.47^{a}	0.28 ± 0.03^{a}	1.47 ± 0.08^{a}	0.34 ± 0.03^{a}	0.40 ± 0.05^{a}

*IAA: indole-3-acetic acid.
**IBA: indole-3-butyric acid.
***PGR: plant growth regulator.
****SD: Standard deviation.
Shoots were cultured on Quoirin and Lepoivre (QL) medium supplemented with various concentrations of IAA and IBA and 3% sucrose. All experiments were conducted in five replications. The values represent the mean ±SD. Means with the same letter within columns are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

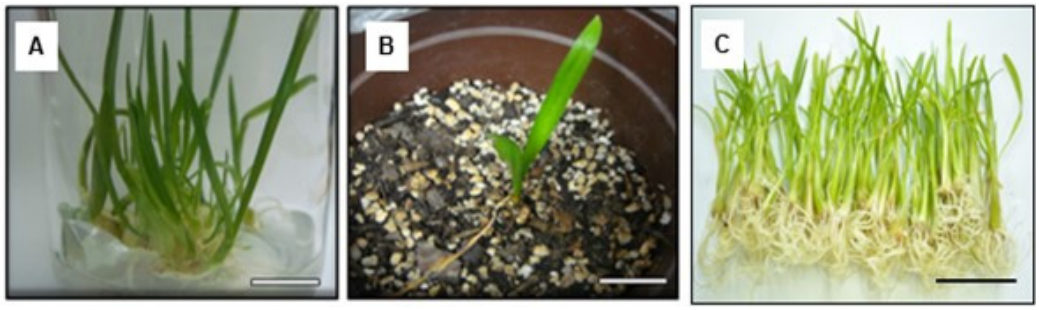

Fig. 5 In vitro cultures of AVVP. (A) Shoot growth on QL medium after 20 weeks (scale bar = 1.5 cm), (B) acclimatized plantlet in pot (scale bar = 1.5 cm), and (C) plants produced through ex vitro acclimatization. AVVP: *Allium victorialis* var. *platyphyllum*; QL: Quoirin and Lepoivre

the mixed treatment groups. Among the treatment groups, 9.84 μM IBA and 11.42 μM IAA treatment increased not only the number of roots but also the size of the bulb.

These rooted plantlets were successfully transferred to pots for 4 weeks of hardening process (Fig. 5). The survival rates of the resultant plantlets gradually decreased during acclimatization period to reach 80% after 5 months (Fig. 5B). However, growth of plantlet gradually increased with acclimatization period (Fig. 5C). The growth of bulb was also increased with acclimatization period.

Discussion

The concentration of exogenous cytokinins appears to be a significant factor influencing shoot proliferation. TDZ was effective for shoot induction and proliferation, with a shoot incidence of 100% at all concentrations. Interestingly, the addition of TDZ (9.08 μM) to the medium induced primordia-like structures. These primordia appeared irregular, and the bulbs excavated were shown to form shoot clusters at low TDZ treatment. Murthy et al. (1998) reported that treatment with very low concentrations of TDZ promoted differentiation in central regions of tissues. However, low concentrations of TDZ caused shoot wilt and poor elongation in long-term culture (Lu 1993).

2ip treatment of tissue cultures led to more significant root development than other PGR treatments. The root number and growth were directly proportional to 2ip treatment concentrations. The low levels of 2ip led to regeneration of the root tip. Zheng et al. (2003) reported that a critical component of the garlic regeneration system from root tip explants is a low concentration of 2ip in the culture medium. However, there is no previous report describing the use of 2ip for *in vitro* culture of AVVP. 2ip is not widely used for rooting, so further research on it is required.

The scooping method (a disc bulb of approximately 1 cm thick) culture for the propagation of AVVP was explored. The bulb scooping method was suitable for the induction of multiple shoots of AVVP. Some plants, known as tunicate or scaly bulbs, require stimulation in

the form of scooping, scoring, and sectioning to induce new plantlets (Kumar et al. 2010). Each explant produced from scooping was more productive in shoot formation with resultant production of a cluster (Ayabe and Sumi 1998). Multiple shoots formed from the basal part, and hiding shoot grew up because the scooped bulb included the shoot tip and foliage leaf.

Scooping and scoring methods are used to remove apical dominance and to encourage bulblet formation. In scooping, the entire basal plate of the mature bulb is scooped out with a special sharp and curved scalpel to destroy the main shoot. Adventitious bulblets will develop from the base of the exposed bulb scales.

Proliferation studies of tunicate bulbs such as garlic by scooping are scarce. Garlic is mainly cultured in meristem for virus-free production and mass propagation (Nam et al. 2002). This meristem culture has a disadvantage because the cultivation efficiency is lowered. After all, one meristem is always extracted from one scale (Kwon et al. 2018). Scooping and scoring are used for commercial mass propagation of Hyacinth and Scilla (Saniewski 1977). Scooping and scoring methods eliminate apical dominance and encourage bulb formation (Alejandro 2016).

Most bulbous plants have low multiplication rates. Its causes are poor new shoot formation due to inferior exogenous regeneration or small axillary branching and low relative growth rate (De Klerk 2012). Like onions, garlic, daffodils and tulips, tunicate bulbs have outer bulb scales that are dry and membranous. This covering or tunic protects the bulbs from drying out and mechanical injury. The fleshy scales are in successive concentric layers or thin layers to provide a rigid structure to the bulb. There are not many studies on bulb proliferation using the scooping method. The differentiation of multiple shoots in scooped AVVP bulbs is due to various PGRs. Saniewski (1977) reported that ethrel and morphactin treatments effectively differentiated hyacinthic bulbs. However, future studies on PGR and AVVP bulb proliferation mechanisms should be performed.

Mixed treatment with high concentration of IBA and IAA was found to promote *in vitro* rooting of wild garlic. A previous study by Lee and Jeong (2018) also reported that the concentration of IBA and IAA affected the root formation time and bulb growth. The combined treatment of high concentration of IAA and IBA promoted rooting and bulb growth than the single treatments. This was found to be better than the treatment group with the highest *in vitro* rooting rate (9.84 μM IBA or 11.42 μM IAA) in the study of Lee and Jeong (2018). IAA and IBA are PGRs that are not well treated simultaneously in plant *in vitro* culture. Paulos et al. (2015) reported that 0.5 mg l^{-1} IAA+1.5 mg l^{-1} IBA treatment increased the rapid induction of roots and the number of rooting in Bavana *in vitro*.

In this study, a rapid *in vitro* propagation system of seedlings for Korean wild garlic was established. This *in vitro* micropropagation method by scooping and PGRs can achieve rapid growth. Regenerated plants grown in greenhouses showed no detectable morphological changes compared to donor plants. This study can be used to develop strategies for large-scale breeding of elite Korean wild garlic.

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