韓藥作誌(Korean J. Medicinal Crop Sci.) 20(2) : 101 – 107 (2012)

# 산마늘의 신초와 구근생장에 미치는 질소, 인, 칼륨 및 당의 영향

**정미진* · 송현진* · 심선정* · 도은수** · 장준복** · 양재경* · 윤충원*** · 이철호****
Chandrakant. S. Karigar***** · 최명석*†**

*경상대학교 환경산림과학부, **중부대학교 한방제약학과, ***공주대학교 산림자원학과,
****국립수목원, *****Department of Biochemistry, Bangalore University, Bangalore, 560001

# Influence of Nitrogen, Phosphorous, Potassium and Sucrose on the Shoot and Bulb Growth of *Allium victorialis* var. *platyphyllum*

**Mi Jin Jeong*, Hyun Jin Song*, Seon Jeong Sim*, Eun Soo Doh**, Jun Pok Chang**, Jae Kyung Yang*, Chung Weon Yun***, Cheol Ho Lee****, Chandrakant. S. Karigar***** and Myung Suk Choi*†**

**Division of Environmental Forest Secience, Gyeongsang National University*
*Institute of Agriculture and Life Science, Jinju 660-701, Korea.*
***Department of Herbal Pharmaceutical Science, Joongbu University, Geumsan 312-702, Korea.*
****Department of Forest Resources, Kongju National University, 340-802 Gongju, Korea*
*****Division of Forest Resources Conservation, Korea National Arboretum, Pocheon 487-821, Korea.*
******Department of Biochemistry, Bangalore University, Bangalore 560001, India*

**ABSTRACT : Effects of nitrogen (N), phosphorous (P) and potassium (K) on the shoot and bulb growth of wild garlic (*Allium victorialis* var. *platyphyllum*) were studied by adopting *in vitro* culture. These macronutrients influenced the growth of both the shoot and bulb of garlic depending upon their application doses. A minimum of 3% potassium nitrate ($KNO_3$) as a source of nitrogen was found to be critical for shoot elongation while higher concentrations were inhibitory. Garlic bulb growth was profuse on the usual $KNO_3$ strength and sucrose (7%), followed by $KNO_3$ (9.4 mM) supplement. On providing 41.22 mM ammonium nitrate ($NH_4NO_3$) as nitrogen source highest shoot growth was observed while 82.45 mM $NH_4NO_3$ as a source of nitrogen supported high bulb growth. With regard to potassium a good shoot growth was observed in medium that contained 0.31 mM $KH_2PO_4$ and 3% sucrose, while bulb growth was high on 2.5 mM $KH_2PO_4$ and 7% sucrose. These experiments may thus direct the development of excellent growth conditions for the commercial production of edible wild garlic.**

***Key Words*** **:** *Allium victorialis* var. *platyphyllum*, Sucrose, Macronutrients, *in vitro* Culture

## INTRODUTION

*Allium victorialis* var. *platyphyllum,* wild garlic belongs to the *Liliaceae* family, and it is distributed mainly in Europe and Asia. Their leaves and bulbs have been used not only as wild-edible herbs but also as functional foods for the control of gastritis and heart failures (Moon, 1984). It was reported that the leaves contain carbohydrate (2-3%) and ascorbic acid whereas the bulbs possesses organo-sulfur compounds. This plant species is also known for its anti-cancer (Lee *et al*., 2001), ani-oxidant (Shirataki *et al*., 2001), and anti-atherogenic (Kim *et al*., 2000) activities.

The propagation of wild garlic is difficult, and its cultivation is not technically advanced. As of now wild garlic has been cultivated from seeds and by vegetative propagation (Shigeru *et al*., 2003). As an alternative cultivation method an *in vitro* method of culture would provide an advantage of controlling the environmental factors to a greater extent for enhanced yields. At present the methods that report the rapid propagation of wild garlic through *in vitro* methods are scanty (Ishikawa *et al*., 1997; Park *et al*., 2004).

Mineral nutrients are the basic components of any plant tissue culture medium. Most plant tissue culture media which are in use are still based on the nutrient composition described originally by

†Corresponding author: (Phone) +82-55-772-1856 (E-mail) mschoi@gnu.ac.kr
Received 2012 February 15 / 1st Revised 2012 March 20 / 2nd Revised 2012 March 29 /Accepted 2012 April 3

Murashige and Skoog (1962). MS medium is characterized by the presence of high levels of macronutrients in the form of salts. Thus, a great deal of time and effort has been devoted to identify the optimal concentrations for each of the currently established 14 essential plant nutrients (Marschner, 2003). Macronutrients in the form of $NH_4^+$ and $NO_3^-$ are the dominant nutrients in most of tissue culture formulations. Macronutrient effects are highly dependent both on their individual amounts and also on their proportions. The micronutrients influence a wide range of *in vitro* responses including callus formation, shoot and root organogenesis, embryogenesis, and shoot multiplication (George and Klerk, 2008). However, the requirements of sucrose and macronutrient for shoot and bulb growth of wild garlic has not been reported. Wu *et al* (2010) reported the effects of light, macronutrients strength and sucrose in MS medium on the spore germination and gametophyte development of the endangered fern *Adiantum reniforme* var. *sinense.* The carbon and macronutrients especially, nitrates are essential for the profuse growth of wild garlic. Distribution of biomass among vegetative vine parts is tightly linked to the internal carbon and macronutrients contents of the plant, suggesting that root-to-shoot partitioning is controlled by some aspect of plant C:N ratio (Grechi *et al*., 2007). We thus conducted experiments to determine the influence of macro- nutrients (NPK) and sucrose on the growth of shoot and bulb of wild garlic (*A. victorialis* var. *platyphyllum*).

## MATERIALS AND METHODS

### 1. Plant material and *in vitro* culture methods

Healthy 2-year-old wild garlic, *A. victorialis* var. *platyphyllum*, growing in the Ulleung Island were collected. After the detachment of leaves, garlic bulbs were washed with 0.05% Tween-20 for 10 min followed by three rinses with distilled water and surface sterilized with ethanol (70% v/v) for 3 min. Bulbs were surface-disinfected with NaClO (6% v/v) for 10 min, and rinsed five more times with sterile distilled water.

The sterile bulbs obtained from above step were inoculated to culture bottle containing 50 ㎖ of sterile MS medium of pH 5.7, supplemented with BA (0.44 μM), sucrose (3% w/v) and gerlite (0.4% w/v). The cultures were incubated under a photoperiod of 16 h illumination with light intensity of 25 ㎛ol $m^{-2}$ $s^{-1}$ and 8 h dark at 25 ± 1℃.

### 2. Determination of the effects of macronutrients (NPK and sucrose) wild garlic

Individual wild garlic shoots were cut from the multiple shoot clusters and allowed to proliferate on the basal MS solid medium free from plant growth regulators for 5 weeks. To determine the optimal macronutrient and sucrose levels required for shoot growth, the plantlets were cultured in MS medium supplemented with varying macronutrients (NPK) and sucrose (3 and 7% w/v). The NPK concentrations in the culture medium were regulated by supply of $NH_4NO_3$ and $KNO_3$. The macronutrients were included at various proportions such as 1/4, 1/2, 1 ($NH_4NO_3$; 20.61 mM, $KNO_3$ 18.79 mM and $KH_2PO_4$; 1.25 mM), 2 and 4 into MS medium (Table 1). The medium required for *in vitro* analysis was supplemented with MS basal salts except for NPK nutrients, sucrose (30 and 70 g $l^{-1}$), and 4.0 g $l^{-1}$ gerlite. The pH of the medium was adjusted to 5.7 with 1 N NaOH before autoclaving at 121℃ for 15 min. All cultures were maintained under a 16 h light/8 h dark photoperiod, in a growth chamber fitted with a cool fluorescent light emitting 25 ㎛ol $m^{-2}$ $s^{-1}$ of photosynthetically active radiation (PAR). The shoot and bulb growth was measured at every 7 day interval for a period of 5 weeks. The growth rate of shoot of wild garlic was represented by growth index (GI) as calculated using the equation;

GI = (final shoot length – inoculated shoot length) / inoculated shoot length

**Table 1.** Nutrient composition of medium for the *in vitro* culture of *Allium victorialis*.

| Nutrients | | Strength of MS medium formula | | | | |
|---|---|---|---|---|---|---|
| | | ¼ | ½ | 1 (original) | 2 | 4 |
| $NH_4NO_3$ | ㎎/L | 412.5 | 825 | 1,650 | 3,300 | 6,600 |
| | mM | 5.15 | 10.31 | 20.61 | 41.22 | 82.45 |
| $KH_2PO_4$ | ㎎/L | 42.5 | 85 | 170 | 340 | 680 |
| | mM | 0.31 | 0.62 | 1.25 | 2.50 | 5.00 |
| $KNO_3$ | ㎎/L | 475 | 950 | 1,900 | 3,800 | 7,600 |
| | mM | 4.70 | 9.40 | 18.79 | 37.59 | 75.17 |

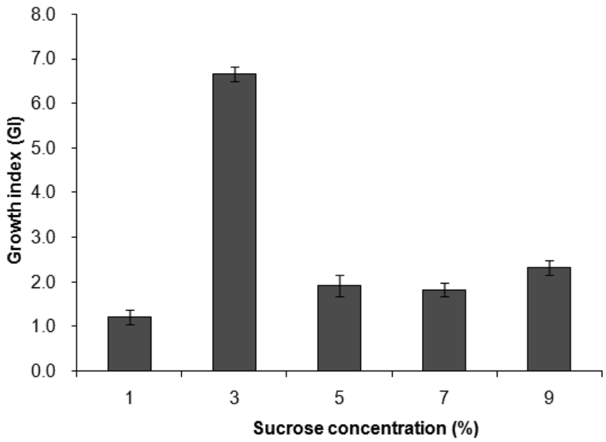


**Fig. 1. Effect of sucrose on the shoot growth of *Allium victorialis*. Shoots were cultured on modified MS medium supplemented with various concentration of sucrose**. The bars indicate standard deviation from the mean of each replicate treatment.

### 3. Statistical analysis

Data are expressed as an average of five separate experiments. The bars indicate standard deviation from the mean of each replicate treatment.

## RESULTS

### 1. Effect of sucrose on shoot growth of wild garlic

When wild garlic was propagated in tissue culture medium the shoot growth was affected depending upon the composition of MS medium. Of the various sucrose concentrations employed a maximum shoot growth were achieved when 3% sucrose was used, while 1% sucrose produced minimum shoot elongation after 5 weeks of culture (Fig. 1).

### 2. Effect of $KNO_3$ on wild garlic shoot and bulb growth

$KNO_3$ influenced the shoot growth of wild garlic in a

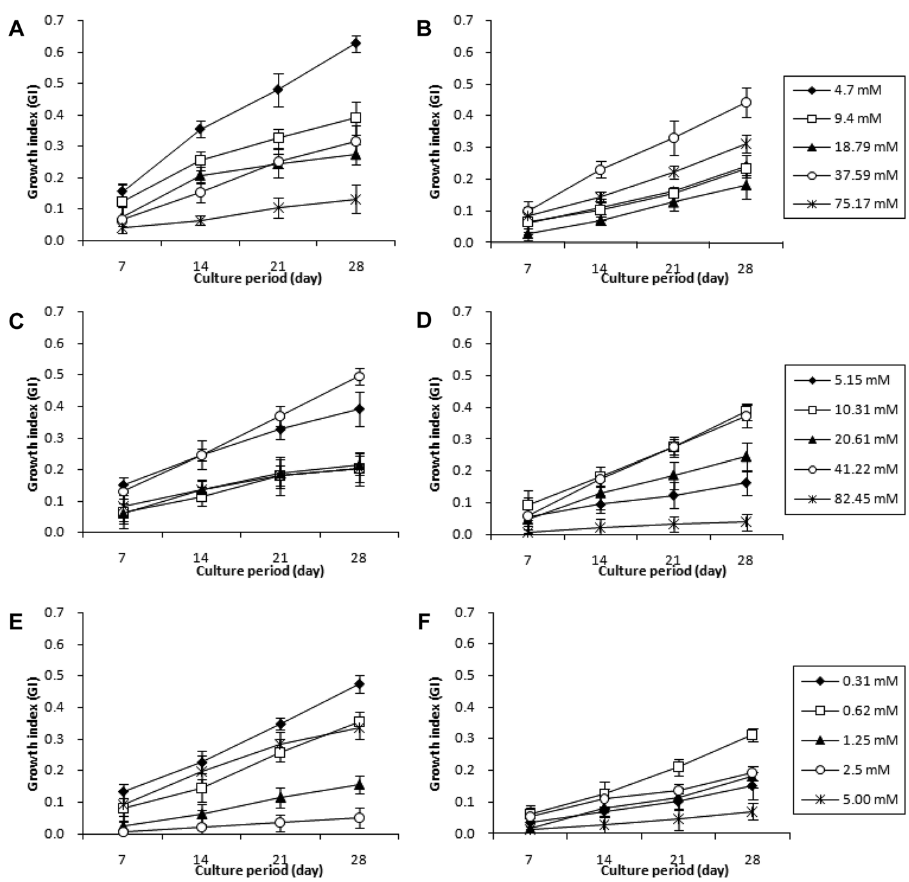


**Fig. 2. Effect of NPK macronutrients on shoot growth of *Allium victorialis* on modified MS solid medium supplemented with 3 and 7% sucrose after five 5 weeks.** $KNO_3$ (A and B), $NH_4NO_3$ (C and D) and $KH_2PO_4$ (E and F) were added at various concentrations into MS basal medium with 3% sucrose (A, C and E) and 7% sucrose (B, D and F) respectively. Data are expressed as an average of five separate experiments. The bars indicate standard deviation from the mean of each replicate treatment.

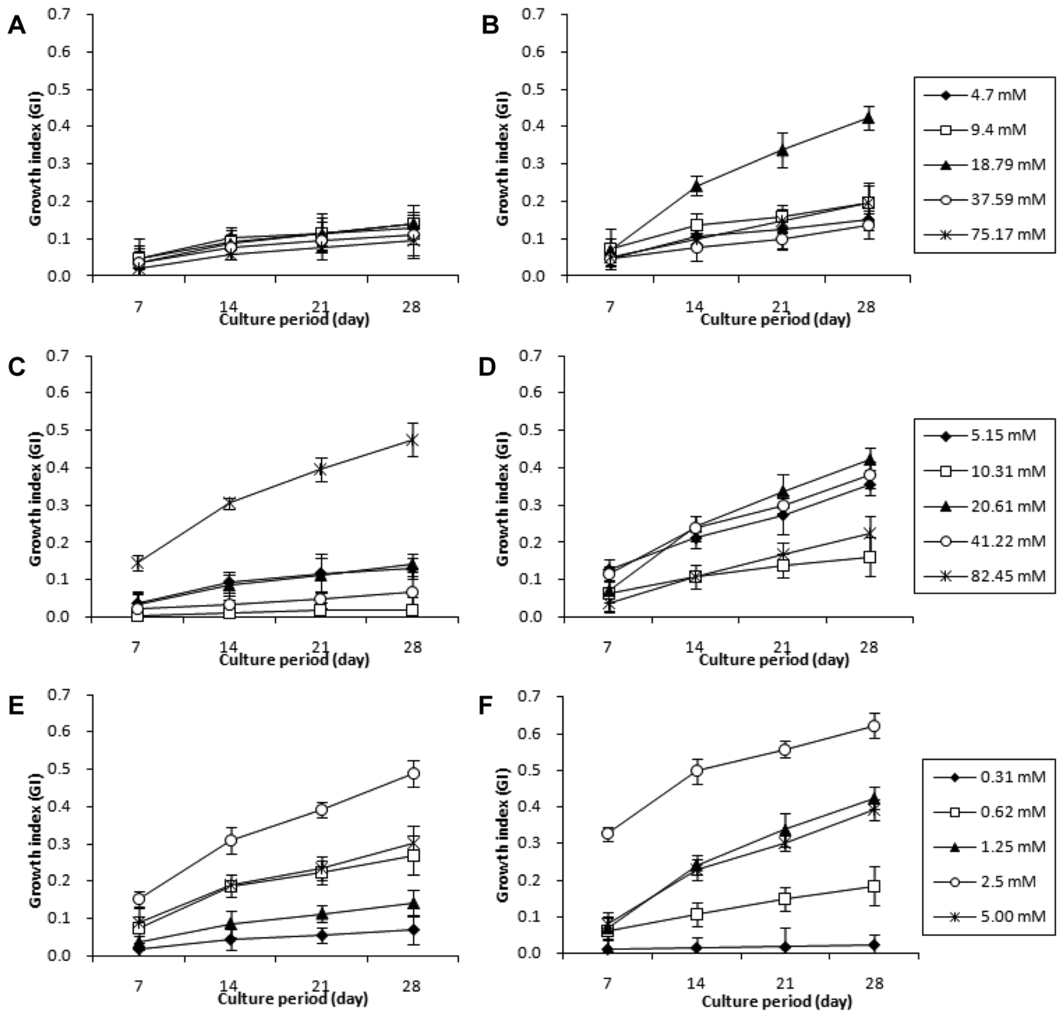


**Fig. 3. Effect of NPK macronutrients on the bulb growth of *Allium victorialis* on modified MS solid medium supplemented with 3 and 7% sucrose after 5 weeks.** $KNO_3$ (A and B), $NH_4NO_3$ (C and D) and $KH_2PO_4$ (E and F) were added at various concentrations into MS basal medium with 3% sucrose (A, C and E) and 7% sucrose (B, D and F) respectively. Data are expressed as an average of five separate experiments. The bars indicate standard deviation from the mean of each replicate treatment.

concentration dependent manner (Fig. 2A and B and Fig. 5). Lower concentrations of $KNO_3$ with sucrose (3%) were found to be critical for shoot elongation, while high concentrations were inhibitory for shoot growth. Highest growth of plantlet was obtained after culturing wild garlic in 4.7 mM of $KNO_3$. However, shoot growth was also found to be more at high $KNO_3$ concentration and sucrose (7%). Among various $KNO_3$ concentrations, the concentration (18.79 mM as in MS medium) showed the best performance with respect to shoot growth.

$KNO_3$ level influenced the growth of wild garlic bulb in a direct way (Fig. 3). Bulb growth on increased supplementation of $KNO_3$ and 3% sucrose was not significantly different. However, bulb growth was more at the original concentration of $KNO_3$ (18.79 mM) with sucrose (7%), followed by 9.4 mM treatment.

### 3. Influence of $NH_4NO_3$ on the growth of shoot and bulb of wild garlic

Shoot growth of wild garlic was dependent on $NH_4NO_3$ supplementation along with 3% sucrose (Fig. 2C and D). A medium containing 41.22 mM and 5.15 mM $NH_4NO_3$ was the best for wild garlic shoot growth. Maximal growth of shoot was obtained on 41.22 mM $NH_4NO_3$ supplement. However, shoot growth was inhibited at 7% sucrose along with $NH_4NO_3$ treatments. When wild garlic was cultured in presence of 41.22 mM and 10.31 mM $NH_4NO_3$, the wild garlic shoot growth appeared higher than those of other treatments.

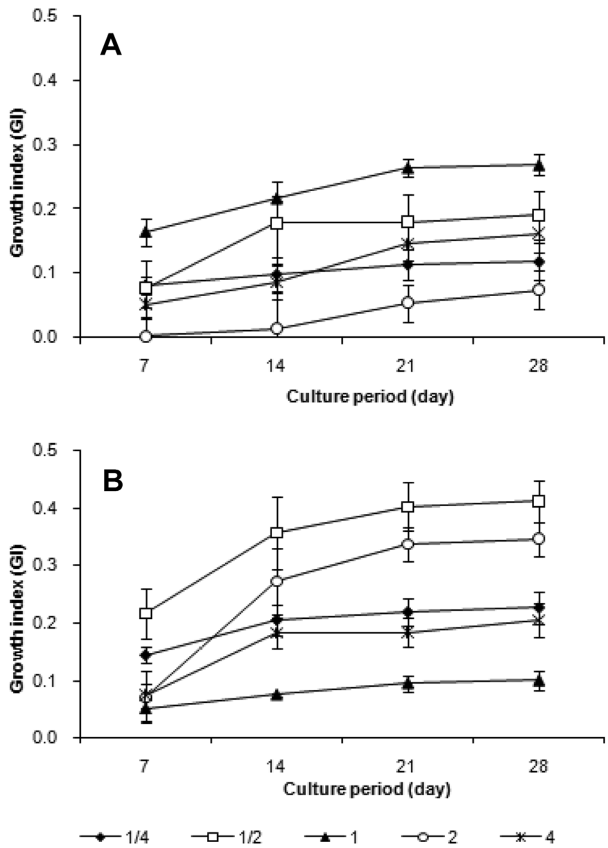


**Fig. 4. Combined effects of sucrose and macronutrients ($NH_4NO_3$ and $KNO_3$) on shoot growth of *Allium victorialis*.** Shoots were cultured on modified MS medium supplemented with 3 and 7% sucrose, and various concentrations of macronutrients. (A) 3% sucrose and $KNO_3$ + $NH_4NO_3$ and (B) 7% sucrose and $KNO_3$ + $NH_4NO_3$. Data are expressed as an average of five separate experiments. The bars indicate standard deviation from the mean of each replicate treatment.

The wild garlic bulb growth was influenced by $NH_4NO_3$. Highest growth of bulb was observed after 82.45 mM $NH_4NO_3$ with 3% sucrose treatments (Fig. 3C and D). However, a high bulb growth was also noted for $NH_4NO_3$ with 7% sucrose treatments. The wild garlic bulb growth was in general high at original concentration of $KNO_3$ (20.61 mM as in MS medium) with 7% sucrose, followed by 41.22 mM.

## 4. Combined effects of nitrogen and sucrose on wild garlic growth

The effects of two sucrose (3 and 7%) strengths and nitrogen macronutrients ($KNO_3$ and $NH_4NO_3$) on the shoot growth of wild garlic were investigated (Fig. 4). At a fixed sucrose (3%) and varying macronutrient treatments, the shoot growth was not significantly different consequent to increase in macronutrients. Among the various treatments, the macronutrients when provided at original concentration showed enhanced shoot growth. However, when wild garlic was cultured with 7% sucrose and macronutrients, the shoot growth depended upon the level of macronutrients. Garlic bulb growth was more at double strength of $KNO_3$ and $NH_4NO_3$ with 7% sucrose, followed by half strength.

## 5. Effect of $KH_2PO_4$ on wild garlic shoot and bulb growth

The concentration of $KH_2PO_4$ influenced the shoot growth in wild garlic (Fig. 2 and 3). The highest shoot growth was observed at 0.31 mM $KH_2PO_4$ in the medium supplemented with 3% sucrose, followed by 0.62 mM of $KH_2PO_4$ (Fig. 2E). However, the garlic shoot growth was high at 0.62 mM $KH_2PO_4$ and sucrose (7%) (Fig. 2C).

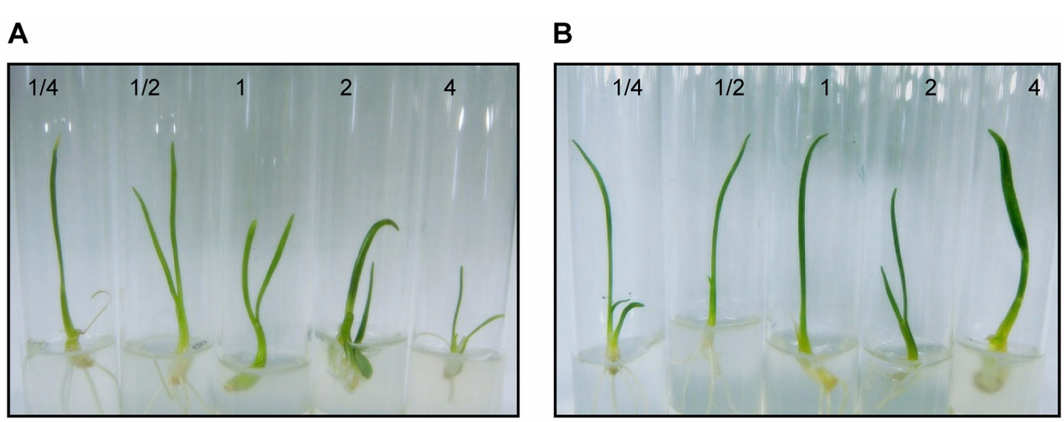


**Fig. 5. Effect of macronutrients ($NH_4NO_3$ and $KNO_3$) and sucrose on shoot growth of *Allium victorialis* on modified MS solid medium after 5 weeks of culture**. These were supplemented with 3% (A) and 7% (B) sucrose and each concentration of macronutrients are in the top panel.

The $KH_2PO_4$ as a source of potassium influenced the garlic bulb growth in a concentration dependent manner (Fig. 3E and F). Bulb growth was noticed to be more on inclusion of 2.5 mM $KH_2PO_4$ and 3% sucrose and at 2.5 mM $KH_2PO_4$ with 7% sucrose, followed by original concentration.

## DISCUSSION

The macronutrients N, P, and K are required essentials for the growth of wild garlic, but this plant is unique and displays an optimum nutrient range and a minimum requirement level. Shoot and bulb growth in wild garlic was significantly influenced by macronutrient composition of the culture medium. The nitrogenous inorganic macronutrients are converted to amino acids and then incorporated into proteins. Macronutrients are typically included in the plant nutrient media as nitrate ion ($NO^{3-}$, oxidized) and/or the ammonium ion ($NH^{4+}$, reduced), in the form of inorganic salts in 25-60 mM range. Ruan *et al*. (2009) reported that increasing N supply significantly raised total biomass production and the yield of young shoots, although this was solely attributed to an increase in the number of young shoots.

Among three macronutrient sources, $KH_2PO_4$ significantly influenced the shoot and bulb growth compared with other macronutrients. Monopotassium phosphate, when used in fertilizer mixtures with urea and ammonium phosphates, minimizes the escape of ammonia by keeping the pH at a relatively low level (Lee *et al*., 2010). Ziv *et al*. (1983) reported that potassium phosphate ($KH_2PO_4$) at 750 ㎎/L were added to the bulbing medium to promote bulblet in *A. ampeloprasum* L. Lee (1966) reported that high phosphate fertilizers have been reported to be successful soil amendments for *Narcissus* bulbs. Also, potassium (K) is a very important nutrient for increasing garlic yields (Castellanos *et al*., 2002). Proper application rates and timing of these nutrients are critical for generating a yield or quality response. As crop yields increase, the demand of K also increases, along with all other nutrients. The amount K applied ranges from 60 to 150 ㎏ $K_2O$/ha (Castellanos *et al*., 2002). The physiological functions of potassium (K) in plants include enzyme activation, osmoregulation, formation of carbohydrates, nucleic acids, and proteins, photosynthesis and enhancement of rooting/early establishment (Fageria *et al*., 1997). Although the amount of potassium required varies widely among different species, in media potassium concentration is generally correlated with that of nitrate and ranges between 20-30 mM.

N, P and K and their optimal concentration needed is different for shoot and bulb growth. When cultured on shoot growth culture media, the shoot growth of plant was enhanced on high concentration of N, P and K. In onion *in vitro* cultures, a ratio of $NO^{3-}$ / $NH^{4+}$ is 29 mM/8 mM (Dunstan and Short, 1977; Kim *et al*., 2007) for optimal yields. Advance garlic clones can be developed by the use of higher levels of macronutrients supplied as nitrate (35 mM $NO^{3-}$ : 8 mM $NH^{4+}$ ratio) and growth regulators (Luciani *et al*., 2001).

Relatively few studies have been conducted on the effect of altering sucrose (or carbohydrate) concentrations on the initiation and growth of bulbous plants *in vitro* (Van Rensberg and Vcelar, 1989; Lee *et al*., 2011). Sucrose concentration in culture medium influenced both shoot and bulb growth of wild garlic. High concentration of sucrose enhanced the bulb growth. However, the same observation was inverse for shoot growth. This result suggests that root-to-shoot partitioning of nutrients is controlled by some aspect of plant C:N balance. A high osmoticum promoted tuberization in the cultivated potato (Barker, 1953) bulblet formation in *Dioscorea* (Asahira and Yazawa, 1979).

The ratio of the macronutrients and carbon sources are important for the wild garlic *in vitro* cultures (Table 2). The relative rates of shoot and root growth are modulated by signals related to carbon and macronutrients status of the plant (Stitt and Scheible, 1998). The distribution of biomass has been related to the ratio of total carbon to total macronutrients (C : N) within plants (Reynolds and Thornley, 1982). Many studies on allocation have also found relationships between levels of starch, soluble sugars, amino acids and root-to-root ratio when growth conditions are altered (Saarinen, 1998).

Cultivation of wild garlic by *in vitro* or by *ex vitro* methods have not been established. Propagation of genetically superior wild garlic is also less studied. In Korea, cultivation of wild garlic on forest land is now in progress for obtaining environmental friendly edible herbs. *In vitro* culture methods also allow monitoring of plant responses at biochemical and physiological level. Thus, it is crucial to establish a relationship between *in vitro* and *ex vivo* responses (Sawwan *et al*., 2000). Thus, these results serve to establish nutrient supply required for cultivation of wild garlic in forest soils. Further the established conditions can be greatly influential in the production of wild garlic of high commercial value.

## ACKNOWLEDGEMENTS

This work was supported by the Forest Science Technology and Development Grant funded by the Korea Forest Service (S120910L140120).

## LITERATURE CITED

**Asahira T and Yazawa S.** (1979). Bulbil formation of *Dioscorea opposita* cultured *in vitro*. Memoirs of the College of Agriculture, Kyoto University. 113:39-51.

**Barker WG.** (1953). A method for the *in vitro* culture of potato tubers. Science (Wash DC). 118:384-385.

**Castellanos JZ, Ojodeagua JL, Méndez F, Alcantar G, Villalobos-Reyes S, Vargas P, Muñoz-Ramos JJ and Lazcano-Ferrat I.** (2002). Potassium requirements for garlic under fertiligation. Better Crops International. 16:11-16.

**Dunstan DI and Short KC.** (1977). Improved growth of tissue cultures of the onion, *Allium cepa*. Plant Physiology. 41:70-72.

**Fageria NK, Baligar VC and Jones CA.** (1997). Growth and mineral nutrition of field crops. Marcel Dekker. New York, USA.

**George EF and de Klerk GJ.** (2008). The components of plant tissue culture media I: macro- and micro-nutrients. In *Plant ropagation by tissue culture* 3rd edition. Edited by: George EF, Hall MA, de Klerk G-J. Dordrecht. The Netherlands. Springer. p.65-113.

**Grechi I, Vivin P, Hilbert G, Milin S, Robert T and Gaudillere J.** (2007). Effect of light and nitrogen supply on internal C : N balance and control of root-to-shoot biomass allocation in grapevine. Environmental and Experimental Botany. 59:139-149.

**Ishikawa T, Masuda K, Harada T, Tamura H and Kanazawa T.** (1997). Micropropagation of *Allium victorialis* L. ssp. platyphyllum Hult. Research Bulletin of the University Farm, Faculty of Agriculture - Hokkaido University. 30:1-7.

**Kim SM, Lee CY, Kim YC, Choi IS, Min KK and Seong JD.** (2007). Effects of organic fertilizers on growth and yield in *Liriope platyphylla* WANG et TANG. Korean Journal of Medicinal Crop Science. 15:148-151.

**Kim TG, Kim SH, Kang SY, Jung KK, Choi DH, Park YB, Ryu JH and Han HM.** (2000). Antiatherogenic effect of the extract of *Allium victorialis* on the experimental atherosclerosis in the rabbit and transgenic mouse. Korean Journal of Pharmaconosy. 31:149-156.

**Lee CH, Lee SW and Lee H.** (2010). Change of soil chemical properties according to cultivation area and cultural year for *Platycodon grandiflorum*. Korean Journal of Medicinal Crop Science. 18:273-279.

**Lee GS.** (1966). Daffodil handbook. (ed.). American Horticultural magazine. Washington, USA. p.45:227.

**Lee KT, Choi JH, Kim DH, Son KH, Kim WB, Kwon SH and Park HJ.** (2001). Medicinal Chemistry & Natural Products Constituents and the Antitumor Principle of *Allium victorialis* var. *platyphyllum.* Archives of Pharmacal Research. 24:44-50.

**Lee SH, Lee SG and Kang HD.** (2011). Conservation of an endangered species of *Lilium cernum* Komarvo. Through *in vitro* Mass-Propagation. Korean Journal of Medicinal Crop Science. 19:9-15.

**Luciani GF, Marinangeli PA and Curvetto NR.** (2001). Increasing nitrate/ammonium ratio for improvementof garlic micropropagation. Scientia Horticulturae. 87:11-20.

**Marschner H.** (2003). Mineral nutrition of higher plants. Academic Press. London, England.

**Moon KS.** (1984). Usage and Components of Herbal Medicine. Scientific Encyclopedia Co., Pyongyang, Korea. 671-672.

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

**Park SY, Lee WY, Ahn JK, Kwon YJ and Park HC.** (2004). High Efficiency Bioreactor Culture System for Mass Proliferation and Bulblet Formation of *Allium victorialis* var. *platyphyllum* Makino. Korean Journal of Plant Biotechnology. 31:127-132

**Reynolds JF and Thornley JHM.** (1982). A shoot:root partitioning model. Annals Botany. 49:585-597.

**Ruan J, Haerdter R and Gerendás J.** (2009). Accumulation of phenolic compounds as influenced by N supply: a case study on catechins in green tea (*Camellia sinensis*). The Proceedings of the International Plant Nutrition Colloquium XVI, UC Davis.

**Saarinen T.** (1998). Internal C/N balance and biomass partitioning of *Carex rostrata* grown at three levels of nitrogen supply. Canadian Journal of Botany. 76:762-768.

**Sawwan J, Shibi RA, Swaidat I and Tahat M.** (2000). Phosphorus regulates osmotic otential and growth of African violet under *in vitro* induced water deficit. Journal of Plant Nutrition. 23:759-771.

**Shigeru M, Tokuro S and Yoshiharu A.** (2003). Research for the cultivation of *Allium victorialis* var. *phyllum*. Agriculture and Horticulture. 78:590-595.

**Shirataki Y, Motohashi N, Tani S, Sunaga K, Sakagami H, Satoh K, Nakashima H, Kanamoto T, Wolfard K and Molnar J.** (2001). Antioxidative activity of *Allium victorialis* L. extracts. Anticancer Research. 21:3331-3339.

**Stitt M and Scheible WR.** (1998). Understanding allocation to shoot and root growth will require molecular information about which compounds acts as signals for the plant nutrient status, and how meristem activity and cellular growth are regulated: Opinion. Plant and Soil. **201**:259-263.

**Van Rensberg JGJ and Vcelar BM**. (1989). The effect of the sucrose concentration on the initiation and growth of adventitious buds from leaf tissue of *Lachenalia*. The South African Journal of Botany. 55:117-121.

**Wu H, Liu, XQ, Ji H and Chen LQ.** (2010). Effects of light, macronutrients, and sucrose on germination and development of the endangered fern *Adiantum reniforme* var. *sinense* (Adiantaceae). Scientia Horticulturae. 125:417-421.

**Ziv M, Hertz N and Biran Y.** (1983). Vegetative reproduction of *Allium ampeloprasum* L. *in vivo* and *in vitro*. Israel Journal of Botany. 32:l-9.