Research Report

Growth and Quality of Plug Seedlings of Three Indigenous Medicinal Plants as Affected by Ionic Strength of the Nutrient Solution

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Abstract. The objective of this study was to investigate the effect of ionic strength of the nutrient solution on growth and quality of plug seedlings of three indigenous medicinal plant species. Seeds were sown in 200-cell plug trays, containing a commercial medium in a glasshouse. The pH of a nutrient solution supplied was adjusted to 5.96. The electrical conductivities (EC) of the nutrient solutions supplied were 0.43 dS·m⁻¹ (1/4x ionic strength), 0.84 dS·m⁻¹ (1/2x ionic strength), and 1.20 dS·m⁻¹ (1x ionic strength). Growth and quality of *P. frutescens* var. *acuta* Kudo and *A. gigas* Nakai were the greatest in the 1/2x ionic strength, while those of *S. tonkinensis* were the greatest in the 1x ionic strength. Elemental contents in the shoot and root of *P. frutescens* var. *acuta* Kudo were the greatest in the 1/2x ionic strength, while those of *S. tonkinensis* were the least in the 1/2x ionic strength and of *A. gigas* Nakai were the greatest in the 1 storic strength. Effect of ionic strength of the nutrient solution on plant growth was not the same in all species, but slightly different depending on the plant species. Seedlings applied with a solution with a proper ionic strength grew faster and were of better quality.

Additional key words: Angelica gigas Nakai, element content, inorganic nutrition, Perilla frutescens var. acuta Kudo, Sophora tonkinensis

Introduction

Plants containing medicinal properties have been known and used in some forms or others, even by primitive people (Jain and Sakalani, 1991). There are total about 4,200 plant species growing wild in Korea, and about 1,000 of them are cultivated and used as medicinal plants which have medicinal effects on human bodies to some extent (Rural Development Administration, 2011). Total domestic production of medicinal plants is steadily increasing every year. As the cultivation technics advance and the growers' income levels improve, research on natural products progresses to make more plants available for medicinal use.

The *P. frutescens* (Lamiaceae) has been used as an important traditional herbal medicine for treating various diseases including depression, anxiety, tumor, cough, antioxidant, allergy, intoxication, and some intestinal disorders (Makino et al., 2003; Park et al., 2013; Yang et al., 2012) in East Asian countries such as Korea, China, and Japan. Sophora

(Fabaceae) is widespread in warm and dry habitats, including Asia, North and South Americas, and New Zealand (Jana et al., 2013; Lai et al., 2003). Its stems and roots have been commonly used as a traditional drug to treat acute pharyngolaryngeal infections and sore throats (Krishna et al., 2012). The root of *A. gigas* Nakai (Umbelliferae) has been used to treat female afflictions and anemia in traditional oriental herbal medicine since ancient times in Korea (Son et al., 2010).

However, there are some issues we need to resolve in the production and utilization of these valuable resources. Firstly, if the weather condition is not suited for growth of certain medicinal plants, it will be difficult to cultivate the plants or the medicinal property of the plants may not be as good as expected. Another problem is not sufficient supply for increasing demand in Korea. It is necessary to have a mass production system be established to solve such problems as lack of stable supplies, disproportional contents of medicinal properties, and safety issues caused by uncertain sources. Plug seedling production technology was introduced to Korea in the early 1990s and has been widely used in recent years because it saves labor for raising seedlings, facilitates mass production of uniform seedlings, and allows division of production labor (Jeong, 1998, 2000). Plug transplants are seedlings or small propagation plants raised in uniform individual cells called plugs, which are filled with a cohesive medium, and to be transplanted to other growing systems. In plug system usually seeds are sown to plug trays by an automated seeder, and with a few exceptions, only one plant per cell is raised (Jeong, 1998, 2000).

It is necessary to have a plug seedling production technology be established for the medicinal plants to examine proper ionic strength of the nutrient solution. These essential nutrient elements contained within the plug cell are frequently insufficient to sustain plant growth for an extended period (Garton and Widders, 1990). Providing proper mineral nutrition to seedlings in nurseries is essential for optimum performance after transplanting (Bigg and Schalau, 1990). Fertilization during nursery culture has ramifications for seedling morphological and physiological characteristics (Landis, 1985; Rook, 1991) and environmental quality (Brown, 1992; Molitor, 1990). A proposed fertilization scheme (Widders, 1989) for culture of tomato seedlings involves application of moderately low-concentration nutrient solutions. The strength and composition of nutrient solution should be determined by considering nutrient accumulation in the growing medium in addition to EC of a nutrient solution (Noh and Son, 2010).

Due to the excess of basal fertilizers or top dressing, most of the fruits and vegetables grown in soil cultivation are affected (Hwang et al., 2006). Plugs cultivated in plug trays are in practice to produce uniform seedlings. Since many types of plug trays are in use and each plug tray has its own specific properties such as cell size, nutrient requirement (Hwang and Jeong, 2002), an important aspect to be considered is optimal strength of nutrient solution (Choi et al., 1997) for the production of uniform seedlings. The objective of this study was to investigate the effect of ionic strength of the nutrient solution on growth and quality of plug seedlings of indigenous medicinal plant species.

Materials and Methods

Seeds of Perilla frutescens var. acuta Kudo, Sophora tonkinensis, and Angelica gigas Nakai were sown in 200-cell plug trays, containing a commercial medium (Tosilee medium, Shinan Grow Co., Jinju, Korea), on Sept. 16, Sept. 7, and Sept. 21, 2012, respectively. Starting from 22, 31, and 18 days after sowing, respectively, seedlings of Perilla frutescens var. acuta Kudo, Sophora tonkinensis, and Angelica gigas Nakai were fed with a nutrient solution once a day by submerging the plug trays in the nutrient solution for 1-2 minutes. The pH of the nutrient solution supplied was 5.96. The electrical conductivities (EC) of the nutrient solution used were 0.43 (1/4x ionic strength), 0.84 (1/2x ionic strength), and 1.20 (1x ionic strength) dS·m⁻¹, respectively. The nutrient solution used was a multipurpose solution used in the Department of Horticulture, Gyeongsang National University and its composition is shown in Table 1. Plants were grown in a glasshouse with RH and temperature of 70% and 18-25°C, respectively.

Growth data of plug seedlings of *Perilla frutescens* var. *acuta* Kudo, *Sophora tonkinensis*, and *Angelica gigas* Nakai before treatment initiation are shown in Table 2. Growth of

Table 1. Composition of the nutrient solution (1x ionic strength) used in the experiment.

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Chemical	Conc. (mg·L ⁻¹)
Ca(NO ₃) ₂ ·4H ₂ O	436.60
KNO ₃	232.30
Fe-EDTA	15.00
KH₂PO₄	272.00
MgSO₄ · 7H₂O	209.10
NH4NO3	80.00
K₂SO₄	17.40
H ₃ BO ₃	1.40
CuSO₄·5H₂O	0.20
MnSO₄ · 4H₂O	2.10
NaMoO ₄ ·2H ₂ O	0.12
ZnSO ₄ ·7H ₂ O	0.80

Table 2.	Growth	of	plug	seedlings	of	medicinal	plants	before	treatment initiation	on.
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	Plant	Length of	Stem	Le	eaf	Fresh v	vt. (mg)	Dry wt. (mg)	
Scientific names	height (cm)	longest root (cm)	diameter (mm)	Length (cm)	Width (cm)	Shoot	Shoot Root		Root
<i>P. frutescens</i> var. <i>acuta</i> Kudo	1.3 ± 0.06 ^z	4.5 ± 0.20	0.65 ± 0.02	1.4 ± 0.05	1.0 ± 0.07	50.9 ± 2.16	10.7 ± 0.78	4.2 ± 0.13	0.3 ± 0.05
S. tonkinensis	3.0 ± 0.15	4.3 ± 0.19	0.37 ± 0.02	0.8 ± 0.07	0.3 ± 0.04	25.3 ± 1.30	11.2 ± 0.51	5.6 ± 0.34	0.9 ± 0.10
<i>A. gigas</i> Nakai	2.2 ± 0.10	4.0 ± 0.18	-	0.7 ± 0.04	0.7 ± 0.05	41.7 ± 0.78	16.1 ± 0.63	3.4 ± 0.16	0.5 ± 0.06

^zMean ± standard error.

seedlings was measured at one month after treatment initiation. Growth parameters measured were plant height, length of the longest root, stem diameter, leaf length, leaf width, leaf area, internode length, no. of leaves, fresh weight, dry weight, total chlorophyll and anthocyanin contents, root ball formation, and tissue elemental contents. Internode length was measured at the closest node to the top with the full-grown leaf. Dry weight was measured after drying the plant for 72 h in a dry oven (JSOF-150, JSR Micro, Gongju, Korea) at 70°C. Total chlorophyll content was estimated by grinding 10 mg of fresh leaf in 1 mL of 80% acetone and filtered using Whatman #1 filter paper and brought up to 10 mL using the same solution. The absorbance of the solvent was recorded at 663 and 645 nm, and contents of total chlorophyll were calculated according to the method described by Dere et al. (1998). Anthocyanin content was measured using the method described by Fuleki and Francis (1968). Root ball formation was rated on a 1 to 5 scale, where 1 being the worst and 5 being the best. Tissue elemental contents were measured after one gram of ground leaf or root samples was bunt to ashes in a porcelain crucible in microwave furnace (Model LV 5/11B180, Lilienthal, Berman, Germany) for 4 h at 525°C. The ash was dissolved in 5 mL of 20% HCl, followed by 20 mL of hot water, and brought to 100 mL with sterile water. Contents of N, P, K, Ca, Mg, Na, Fe, Mn, Zn, and Si were analyzed by ICP-AES (Optima 4300DV/5300DV, Perkin Elmer Inc., Waltham, MA, USA). Total N concentration was measured by the Kjeldahl method (Kjeltec 2300 Analyzer, Foss Tecator AB, Höganäs, Sweden) (Pieterzyk and Frank, 1979).

The experiment had 10 plants per replication and three replicates laid out in a completely randomized design. Data collected were analyzed for statistical significance with the SAS (Statistical Analysis System, V. 9.1, Cary, NC, USA) program. The experimental results were subjected to an analysis of Duncan's multiple range tests at 5%.

Results and Discussion

Quality characteristics of plug seedling of *P. frutescens* var. acuta Kudo as affected by ionic strength of the nutrient solution are shown in Table 3. Plant height, stem diameter, leaf length, width and area, shoot fresh and dry weights, and total chlorophyll contents were not significantly different among treatments. However, root growth of P. frutescens var. acuta Kudo was the greatest in the 1/4x ionic strength solution. In case of total anthocyanin content, as the nutrient solution concentration decreased, the purple coloration visualized by the naked eyes intensified, although there were no significant statistical differences. Ten days after treatment initiation, leaf showed much darker color in the 1/4x ionic strength solution. This phenomenon lasted throughout the seedling stage. Elemental contents of P. frutescens var. acuta Kudo as affected by the nutrient solution strength are shown in Table 4. It was shown that root tissue contents of inorganic elements except K was the greatest in the 1/2x treatment and therefore, it was concluded that they were absorbed the greatest amount in the 1/2x treatment. The results was similar to that of Abou-Hadid et al. (1996) and Serio et al. (2001) which showed that the EC of the nutrient solution significantly affected lettuce growth, including visual quality and mineral contents in the plant. The Zn in the shoot, and Na, Zn and Si in the root were not detectable. Contents of N, Ca, and Mg in the shoot, and Fe in the root were the greatest in the 1/2xionic strength. The absence of K, although tended to decrease red color, resulted in plants that were not significantly different from the control plants (Hodges and Nozzolillo, 1996). Growth and quality, except anthocyanin content, of P. frutescens var. acuta Kudo was the greatest in the 1/2x ionic strength. These results suggested that anthocyanin content was lower in the 1/2x ionic strength because growth rate was faster in the 1/2x ionic strength than other treatments. Anthocyanin accumulation in vegetative tissues of plants is a well-recognized indicator of environmental stress (Hodges

lonic strength of nutrient	Plant	Length of	Stem				Fresh (m		Dry (m	wt. Ig)	Total _ chlorophyll (μg·mg ⁻¹ SFW)	Anthocyanin (µg · mg ⁻¹ SFW)	Root ball formation (1-5) ^z
solution (c	height (cm)	longest root (cm)	diameter (mm)	Length Width Area (cm) (cm) (cm ²)		Shoot	Root	Shoot	Root				
1/4x	4.3 a ^y	10.2 a	1.3 a	3.1 a	2.7 a	13.8 a	337.8 a	95.9 a	35.9 a	6.2 a	3.50 a	0.25 a	3.2 a
1/2x	4.1 a	7.4 b	1.3 a	3.1 a	2.7 a	14.6 a	350.2 a	61.4 b	32.2 a	4.3 b	4.22 a	0.15 a	1.6 b
1x	3.9 a	8.3 ab	1.3 a	3.3 a	2.8 a	14.2 a	380.0 a	64.8 b	30.6 a	2.8 b	4.86 a	0.09 a	2.6 b
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^z Level of roo	t ball for	mation: 1,	; 2,	; 3,	; 4,	; a	nd 5,						

Table 3. Growth and quality of plug seedlings of P. frutescens var. acuta Kudo as affected by the ionic strength of the nutrient solution.

^yMean separation within columns by Duncan's multiple range test at P = 0.05.

and Nozzolillo, 1996). The higher anthocyanin content of seedlings in response to a deficiency of nitrogen or phosphorus is typical of observations reported in the literature for com (Lawanson et al., 1972). In the present study, when a nutrient solution with a 1/4 ionic strength was supplied, it was observed that N, P, K, Ca, and Mg contents decreased in the shoot as compared to other treatments. The cause of purpling under stress has not been elucidated, but the fact that proteins and anthocyanins share a common substrate, phenylalanine, would permit enhanced anthocyanin synthesis when growth is restricted by nutrient deficiency, since fewer proteins would be expected to be made (Margna, 1977).

Quality characteristics of plug seedlings of *S. tonkinensis* as affected by ionic strength of the nutrient solution are shown in Table 5. Plant height, length of longest root, and root ball formation were not significantly different among treatments. However, stem diameter, leaf area, and fresh and dry weights were the greatest in the 1x ionic strength. Leaf length and width, and total chlorophyll were the greatest in the 1/2x or 1x ionic strength. Nutrient concentrations in leaves can vary by as much as 40% during a diurnal period (Goodall and Gregory, 1947). Elemental contents of *S. tonkinensis* as affected by the ionic strength of the nutrient solution are shown in Table 6. The lowest nutrient content in the root

Table 4. Elemental contents of P. frutescens var. acuta Kudo as affected by the ionic strength of the nutrient solution.

lonic strength of	Tissue -		Macro	-element (%	6 DW)		Micro-element (mg·g ⁻¹ DW)					
nutrient solution	TISSUE .	Ν	Р	К	Ca	Mg	Na	Fe	Mn	Zn	Si	
1/4x		3.21 c ^z	0.59 c	10.62 c	0.83 c	0.60 c	8.6 a	0.10 b	0.02 a	-	0.43 a	
1/2x	Shoot	5.03 a	0.67 b	11.70 b	1.15 a	0.82 a	2.5 c	0.11 a	0.02 a	-	0.39 b	
1x		4.76 b	0.71 a	12.02 a	0.99 b	0.72 b	5.5 b	0.11 a	0.02 a	-	0.21 c	
1/4x		3.55 c	0.44 c	9.26 a	0.45 b	0.48 c	у	0.24 c	0.02 a	-	-	
1/2x	Root	4.48 a	0.65 a	6.07 c	0.54 a	0.65 a	-	0.43 a	0.02 a	-	-	
1x		4.00 b	0.57 b	6.25 b	0.44 c	0.58 b	-	0.33 b	0.02 a	-	-	

^zMean separation within columns by Duncan's multiple range test at P = 0.05. ^yNot detected.

Table	5.	Growth	and	quality	of	plug	seedlings	of	S.	tonkinensis	as	affected b	V	the	ionic	strenath	of	the	nutrient	solution.

lonic strength of nutrient	Plant height	Length of longest root	Stem		Leaf		Fresl (rr	hwt. ng)	Dry (m		Total - chlorophvll	Root ball formation
solution	(cm)	(cm)	(mm)	Length (cm)	Width (cm)	Area (cm²)	Shoot	Root	Shoot	Root	(µg · mg⁻¹ SFW)	-
1/4x	9.3 a ^y	5.6 a	0.49 b	1.6 b	0.5 b	5.73 c	99.1 b	25.1 ab	25.7 b	5.0 ab	7.91 b	3.5 a
1/2x	9.7 a	5.4 a	0.54 ab	1.8 a	0.7 a	7.10 b	115.2 b	19.9 b	19.9 b	4.0 b	10.98 a	3.3 a
1x	10.0 a	6.1 a	0.62 a	1.8 a	0.6 a	8.83 a	146.0 a	33.7 a	38.5 a	6.8 a	11.10 a	2.2 a

^zLevel of root ball formation: 1, ; 2, ; 3, ; 4, ; and 5,

^yMean separation within columns by Duncan's multiple range test at *P* = 0.05.

Table 6. Elemental contents of S. tonkinensis as affected by the ionic strength of the nutrient solution.

lonic strength of	Tissue		Macro-	element ('	% DW)		Micro-element (mg·g ⁻¹ DW)						
nutrient solution	nssue	Ν	Р	К	Ca	Mg	Na	Fe	Mn	Zn	Si		
1/4x		2.82 c ^z	0.62 c	4.06 b	1.00 b	0.39 a	0.22 c	0.32 a	0.04 a	0.03 b	0.20 c		
1/2x	Shoot	3.41 b	0.71 b	4.05 c	1.01 a	0.38 b	0.64 b	0.14 c	0.04 a	0.04 a	0.25 b		
1x		3.78 a	0.73 a	4.21 a	0.99 c	0.37 c	0.72 a	0.15 b	0.03 b	0.03 b	0.35 a		
1/4x		2.06 c	0.62 b	6.47 a	0.32 c	0.45 a	1.46 b	0.31 a	0.03 a	0.21 a	у		
1/2x	Root	2.71 b	0.50 c	3.95 c	0.26 c	0.32 c	0.01 c	0.13 c	0.02 b	0.05 b	-		
1x		3.20 a	0.68 a	6.38 b	0.46 a	0.43 b	3.98 a	0.22 b	0.02 b	0.04 c	-		

²Mean separation within columns by Duncan's multiple range test at P = 0.05. ^yNot detected.

lonic strength of nutrient solution	Plant	Length of	Stem	No. of		Leaf			hwt. 1g)	Dry (m		Total chlorophyll	Root ball formation
	height (cm)	longest root (cm)	diameter (mm)	leaves	Length (cm)	Width (cm)	Area (cm²)	Shoot	Root	Shoot	Root	(µg mgʻ SFW)	(1-5) ^z
1/4x	5.6 a ^y	7.5 a	2.3 a	8.0 a	1.9 a	2.0 a	7.4 a	265.0 a	82.3 a	31.1 a	11.1 a	0.59 a	3.7 a
1/2x	7.1 a	6.3 a	2.6 a	8.3 a	2.1 a	2.1 a	9.9 a	367.5 a	90.4 a	39.2 a	11.5 a	0.79 a	3.3 a
1x	6.3 a	9.3 a	2.6 a	8.0 a	2.1 a	2.3 a	9.1 a	354.7 a	101.5 a	38.1 a	13.1 a	0.86 a	5.0 a
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Table 7. Growth and quality of plug seedlings of A. gigas Nakai as affected by the ionic strength of the nutrient solution.

^zLevel of root ball formation: 1, ; 2, ; 3, ; 4, ; and 5,

^yMean separation within columns by Duncan's multiple range test at *P* = 0.05.

Table 8. Elemental contents of A. gigas Nakai as affected by the ionic strength of the nutrient solution.

lonic strength of	Tissue -		Macro	-element (%	6 DW)			Micro-element (mg·g ⁻¹ DW)						
nutrient solution		Ν	Р	К	Ca	Mg	Na	Fe	Mn	Zn	Si			
1/4x		3.13 c ^z	0.62 c	13.86 b	0.66 c	0.39 c	6.06 c	0.07 b	0.04 a	1.50 b	0.20 c			
1/2x	Shoot	4.04 b	0.71 b	14.09 a	0.67 b	0.40 b	6.63 b	0.06 c	0.03 b	1.79 a	0.25 b			
1x		4.41 a	0.73 a	11.93 c	0.80 a	0.44 a	7.71 a	0.09 a	0.03 b	1.44 c	0.35 a			
1/4x		2.35 c	0.63 c	6.01 c	0.28 c	0.39 c	7.04 a	0.20 a	0.05 a	_y	-			
1/2x	Root	4.02 a	0.73 b	6.38 b	0.31 b	0.44 a	6.90 b	0.16 c	0.05 a	-	-			
1x		3.81 b	0.80 a	7.35 a	0.32 a	0.40 b	5.72 c	0.18 c	0.05 a	-	-			

^ZMean separation within columns by Duncan's multiple range test at P = 0.05. ^yNot detected.

was found in the 1/2x ionic strength. The Si in the root was not detectable.

Quality characteristics of plug seedling of A. gigas Nakai as affected by ionic strength of the nutrient solution are shown in Table 7. All growth parameters were not significantly different among treatments. However, shoot growth was greater in the 1/2x ionic strength than in other treatments, and root growth was greater in the 1x ionic strength than in other treatments. Plant height, leaf area, no. of leaves, and shoot fresh and dry weights were the greatest in the 1/2xionic strength. Length of the longest root, leaf width, root fresh and dry weights, total chlorophyll content, and root ball formation were the greatest in the 1x ionic strength. Elemental contents of A. gigas Nakai as affected by the ionic strength of the nutrient solution are shown in Table 8. The highest nutrient content in the shoot was found in the 1x ionic strength. According to Resh (1981), plants differentially absorb various elements. As a result of the differential uptake of the various elements, the composition of the nutrient solution changes constantly.

In our study, effect of ionic strength of the nutrient solution on plant growth was not the same to all species, but slightly different depending on the plant species. The nutrient uptake differs strongly between crops, not only with respect to the quantity of nutrients absorbed, but also the ratio between the nutrients are different (Sonneveld and Voogt, 2009). Rate of ion absorption depends upon the plant, climatic, and environmental conditions such as light intensity and duration, temperature, and humidity, as well as the type of culture and its stage of development (Schwarz, 1995). Moreover, interactions among nutrients in higher plants occur when the supply of one nutrient affects the absorption, distribution or function of another nutrient (Schwarz, 1995). Thus, interactions among nutrients can induce either deficiencies or toxicities, and can modify growth response (Schwarz, 1995).

Overall growth and quality of the medicinal plants tested in this study were enhanced by increased tissue N content in the shoot. Nitrogen (N) is the major constituent of amino acids and nucleic acids, which play essential roles in plant growth and development (Miller and Houghton, 1945). The concentration of foliar N is an indication of the amount of N in the foliage, not necessarily an indication of the uptake of N by the root system, and is likely to be influenced by the number of shoots the plant produces (Armitage and Tsujita, 1979). In the absence of supplied nitrogen to the soil, the plant degrades the chlorophyll molecule, nitrogen going to regions of active growth, where it performs its functions (Furlani Jr. et al., 1996). The elemental concentration might not be in agreement with some of the earlier reports on medicinal plants (Ozcan and Akgul, 1998). The differences observed might be due to different growth conditions, genetic factors, geographical variations in the level of soil fertility, efficiency of mineral uptake, and the analytical procedure employed (Ozcan and Akgul, 1998).

In conclusions, growth and quality of *P. frutescens* var. *acuta* Kudo and *A. gigas* Nakai were the greatest in the 1/2x ionic strength, while those of *S. tonkinensis* were the greatest in the 1x ionic strength. Hence, seedlings of these medicinal plant species have to be grown a nutrient solution with a proper, but not so strong, ionic strength to ensure fastest growth rate and best quality.

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