J. Plant Biol. (2010) 53:45–51
DOI 10.1007/s12374-009-9084-0

ORIGINAL RESEARCH

Exogenous Glutamate Inhibits the Root Growth and Increases the Glutamine Content in *Arabidopsis thaliana*

Ta Hee Kim · Eui Cheol Kim · Suk Weon Kim · Hyo Shin Lee · Dong-Woog Choi

Received: 24 June 2009 / Revised: 29 October 2009 / Accepted: 13 November 2009 / Published online: 29 December 2009
© The Botanical Society of Korea 2009

Abstract Previously, our work with ginseng hairy root shows that the tissue of low-branching and slow-growing phenotype contains high level of glutamine. In order to check if the high glutamine concentration inhibits the root growth, we applied exogenous glutamine or glutamate into growth medium and check the root growth of *Arabidopsis*. While glutamine did not affect root growth, over 0.1 mM glutamate inhibited severe root growth. However, when the amino acid solution was adjusted to pH 5.7 and added into medium, *Arabidopsis* seedlings show normal growth pattern on medium containing glutamate or aspartate. These results demonstrated that inhibition of the root growth by high concentration of exogenous glutamate was a result of the low pH toxicity caused by acidic amino acid, although low concentration (0.05 mM) of glutamate has an inhibitory effect on the primary root growth. The application of exogenous glutamine or glutamate increases glutamine concentration within root tissue about 3- to 4-fold. However, concentration of glutamate is not significantly increased. The KO mutant on each of the *Gln1_1*, *Gln1_2*, or *Glu2* gene was little effective on the root growth. These results indicate that high concentration of endogenous glutamine observed in root tissue does not affect root growth.

Keywords Glutamate · Glutamine · *Arabidopsis* · Root growth · pH

T. H. Kim · E. C. Kim · D.-W. Choi
Department of Biology Education, Chonnam National University, Kwangju 500-757, South Korea

S. W. Kim
Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejon 305-333, South Korea

H. S. Lee
Biotechnology division, Korea Forest Research Institute, Suwon 441-350, South Korea

D.-W. Choi (✉)
Kumho Life Science Laboratory, Chonnam National University, Kwangju 500-757, South Korea
e-mail: dwchoi63@chonnam.ac.kr

Introduction

Plant root is responsive to the availability and distribution of nutrients in the soil and proliferate their root to colonize the nutrient-rich region. To capture more mineral nutrients effectively, plants have mechanism for sensing the nutrients in soil and responding the signals to develop the root system (Zhang et al. 1999; Casson and Lindsey 2003; Desnos 2008).

Soil nitrate ions (NO_3^-) are major nitrogen source for plant, and its supplement usually limits plant growth and crop yields (Ericsson 1995; Zhang and Forde 2000). Therefore, plant responds to the presence of the nitrate in soil by developing and growing of the root system. The nitrate absorbed by roots was assimilated into organic nitrogen compounds. The first step of this process is the reduction of nitrate to ammonium by nitrate reductase and nitrite reductase in cytosol and chloroplasts (Taiz and Zeiger 2002). The second step is the assimilation of ammonium into amino acids, glutamine. This reaction is carried out by glutamine synthetase (GS) located in both cytoplasm and chloroplast in most of higher plants. Glutamate synthase catalyzes the conversion of glutamine and 2-oxaloglutarate to two molecules of glutamate in chloroplast. Glutamate and glutamine are amino acids occupying a central position in nitrogen assimilation in plants (Coschigano et al. 1998; Taiz and Zeiger 2002; Forde and Lea 2007). Although nitrate is

the major source of nitrogen for growing plants, the organic form of the nitrogen can contribute to plant nutrition. Amino acid can be a significant organic source of nitrogen since it represents the largest fraction of organic nitrogen in soil. Therefore, it is not difficult to guess that amino acid may affect the growth and development of roots. Amino acids are known to be key factors in higher plant metabolism and development. They can act as signal molecules, control their own metabolism, and express a variety of other genes (Watanabe et al. 1997; Oliveira and Coruzzi 1999; Zhu and Coleman 2001).

Walch-Liu et al. (2006b) report that L-glutamate, an acid amino acid, act as an exogenous signal to modulate root growth and lateral root formation. L-Glutamate inhibits growth of the primary root but does not interfere with lateral root initiation. They also show that nitrate acts as an antagonist of the response of the root to glutamate (Walch-Liu and Forde 2008). A nitrate transporter, NRT1.1, has a direct role in nitrate sensing and is involved in antagonizing L-glutamate affect on root growth inhibition (Walch-Liu and Forde 2008).

Rhizotoxicity in acid soil is considered to be a major environmental stress that limits root growth and plant yield. Low pH causes severe damage to the growing root in plant. When *Arabidopsis thaliana* roots were exposed to a low pH solution, the elongation zone of growing roots lost viability within 1–2 h, but non-growing roots showed no damage under the same treatment (Koyama et al. 2001). The low pH damage was ameliorated by the simultaneous application of calcium, indicating the involvement of a calcium-requiring process in overcoming proton toxicity (Koyama et al. 2001).

Previously, our work shows that ginseng hairy root with low growth rates and few branching phenotype contains high level of glutamine (Jung et al. 2006). In order to understand the relationship of the glutamine content and root growth, we applied exogenous glutamate or glutamine into growth medium and checked root growth as well as glutamine level in *Arabidopsis*.

Materials and Methods

Plant Materials and Chemicals

A. thaliana L. ecotype Columbia_0 was used in this study. Schenk and Hildebrandt (SH) salt and vitamin were from Duchefa (The Netherlands), Murashig and Skoog (MS) salt, phyto-agar, L-glutamine (Gln), L-glutamate (Glu), and other chemicals were purchased from Sigma (USA).

Plant seeds were surface sterilized and germinated and cultured on 50-fold diluted (1/50×) SH medium containing 3% sucrose and 1× SH vitamin in 90-cm Petri dish. SH medium was adjusted to pH 5.7 with 0.1 M NaOH solution, and 1% phyto agar was added for solidification. Amino acids in solution were filter-sterilized and added to the autoclaved growth medium containing phyto agar. To assay amino acid effect such as glutamate and glutamine on root growth, 5-day-old homogeneous seedlings were transferred onto each of 1/50× SH medium containing with amino acids or without. After 7 days, seedlings were harvested and used to assay the amino acids in the roots. Plant seedlings were cultured under white light of approximately 2,500 lx at 23°C, with a day/night cycle of 16/8 h.

Culture Condition

Fifty-fold diluted (1/50×) SH medium containing 3% sucrose and 1× SH vitamin was used as growth medium in 90-cm Petri dish. SH salts were dissolved in Mili-Q water and adjusted to pH 5.7 with 0.1 M NaOH solution, and 1% phyto agar was added for solidification before autocleave. SH vitamin was filter sterilized and added into autoclaved SH medium.

Arabidopsis seeds were surface sterilized with 1% sodium hypochloride solution for 5 min and rinse with autoclaved water three times and keep at 4°C before use. About 50–60 seeds were germinated on the SH agar medium in the growth root with white light of approximately 2,500 lx at 23°C, with a day/night cycle of 16/8 h. To check root growth, 5-day-old homogeneous seedlings were transferred onto solidified SH medium containing amino acid or not in 90-cm Petri dish. Five to ten seedlings were transferred on the new medium, and the Petri dish was stand to vertical root growth. After 8 days, seedlings were pictured, and root weight and length were determined. All the experiments for amino acid assay were performed at least thrice under the same condition.

In order to assay amino acid effect, amino acids were dissolved in water from 0.1 to 2 mM and filter sterilized. A 2× concentration of amino acid was added to the same volume of autoclaved growth medium containing phyto agar. Change in the medium pH by adding acidic amino acid was checked with pH meter before adding phyto agar. To adjust the pH change by the addition of the acidic amino acids, amino acid solution was adjusted to pH 5.7 with 0.1 M NaOH solution at first and then filter sterilized. To test the pH effect on root growth, plant seedlings were cultured on three layers of the filter paper absorbing growth liquid mediums without amino acids adjusted pH 4.0-5.7. To test the ameliorative affect of Ca^{2+} on low pH stress on the root growth, the 2 mM $CaCl_2$ was dissolved with SH salt in water and autoclaved. The SH medium with $CaCl_2$ was mixed with the same volume of double concentration of amino acid solution.

Genetic Analysis of Knockout Mutants

Knockout *Arabidopsis* mutant plants, Salk000459 for *Gln1-1*, Salk145235 for *Gln1-2*, and Salk128455 for *Glu2*, were obtained from *Arabidopsis* Biological Resource Center and Nottingham *Arabidopsis* Stock Centre. Plant seeds were surface sterilized and germinated on solidified MS medium plates containing 50 μg/ml kanamycin. A homozygous transgenic line was isolated by Mendelian test on kanamycin agar plate, and knockout of the target gene by T-DNA insertion was checked by gene-specific PCR analysis. Homozygous transgenic plants were selected and used for further study.

To assay amino acid effect on root growth, 5-day-old homogeneous seedlings were transferred onto solidified SH medium containing 50 μM of glutamine or glutamate in 90-cm Petri dish. After 7 days, seedlings were pictured, and root weights were determined. All the experiments for amino acid assay were performed at least triple under the same condition.

Quantification of Glutamine Contents from ^{1}H-NMR Spectroscopy Data

Contents of glutamine and glutamate were calculated as previous report (Jung et al. 2006). Briefly, to quantify glutamine and glutamate contents from ^{1}H-NMR spectral data, the amino acid solution were prepared with a starting concentration of 5 mg/ml in deuterated solvent mixtures (D2O/CD3OD, 80:20). The solution was then diluted to provide a series of standard solutions with concentrations ranging from 5 to 0.1 mg/ml. To quantify glutamine contents, the sum of glutamine peak areas at 2.136 and 2.448 ppm of ^{1}H-NMR spectra data was calculated. Linear regression analysis was performed through linear fit tool using Origin software (version 6.0). Like glutamine, the contents of glutamate (2.11 and 2.343 ppm) in *Arabidopsis* seedling roots were also examined by taking the sum of ^{1}H-NMR spectral peaks from the standard glutamate solution.

Results and Discussion

Exogenous Glutamate Inhibit the Root Growth

Previously, our work with ginseng hairy root show that high concentration of glutamine is in the tissue of low-branching and slow-growth type hairy root (Jung et al. 2006). It was well-known that NO_3- is the major nitrogen source for plant and affects significantly the development of root system. Glutamine and glutamate are central position occupying in nitrogen assimilation in plant. In an attempt to understand the relation between the root growth and glutamine concentration in plants, we applied exogenous glutamate or glutamine into growth medium and check the root growth of the seedlings grown on the medium. Figure 1 shows that glutamine does not affect the root growth, but the same concentration of glutamate inhibits root growth of *Arabidopsis* (Fig. 1a). For instance, the root growth was inhibited approximately 60% by adding 0.4 mM glutamate, and seedlings were not grown on the medium containing more than 1 mM glutamate (Fig. 1b). These results indicate that exogenous glutamate inhibits the root growth, but glutamine does not affect root growth in *Arabidopsis* seedlings. The inhibition of the root growth was observed in other plants such as tobacco, cucumber, and rice seedlings, but glutamate sensitivity was different in each plant (data not shown).

It was reported that exogenous L-glutamate inhibits primary root growth in *A. thaliana* (Walch-Liu et al. 2006b). They applied 10–1,000 μM of exogenous L-glutamate into 50-fold diluted Gamborg's B5 medium and checked the lengths of the primary and lateral roots. Exogenous L-glutamate elicits complex changes in the root growth, although glutamate sensitivity is different in *Arabidopsis* ecotypes. Other amino acids such as aspartate, glycine, and glutamine were ineffective (Walch-Liu et al. 2006b). Exogenous glutamate is perceived specifically at the primary root tip and inhibits mitotic activity in the root apical meristem but does not interfere with lateral root initiation or outgrowth. These results indicate that cells in the root tip are able to sense extracellular glutamate and to trigger a reduction in the rate of cell production in primary roots or cell expansion in lateral roots. Glutamate is one of the most abundant amino acids in living organism; therefore, glutamate is likely to be most concentrated in the immediate vicinity of decaying organic matter, where it could provide a useful marker for the presence of a locally rich source of nutrients. The glutamate effect on the root architecture could be an effective way of promoting the rapid colonization of a nutrient-rich soil (Filleur et al. 2005).

Exogenous Glutamate Reduce Medium pH

In this study, glutamate solution was filter sterilized and added to the autoclaved growth medium. We guess that the addition of the glutamic acid solution may affect the pH of the growth medium. Figure 2 shows that medium pH was significantly reduced by addition of the glutamic acid solution. When *Arabidopsis* seedlings were transferred onto the medium where pH was adjusted to pH 4.0-4.8 without glutamate, seedling root growth was inhibited significantly at lower than pH 4.5 (data not shown). These results demonstrate that the addition of >0.1 mM concentration of glutamic acid reduces the medium pH, and low

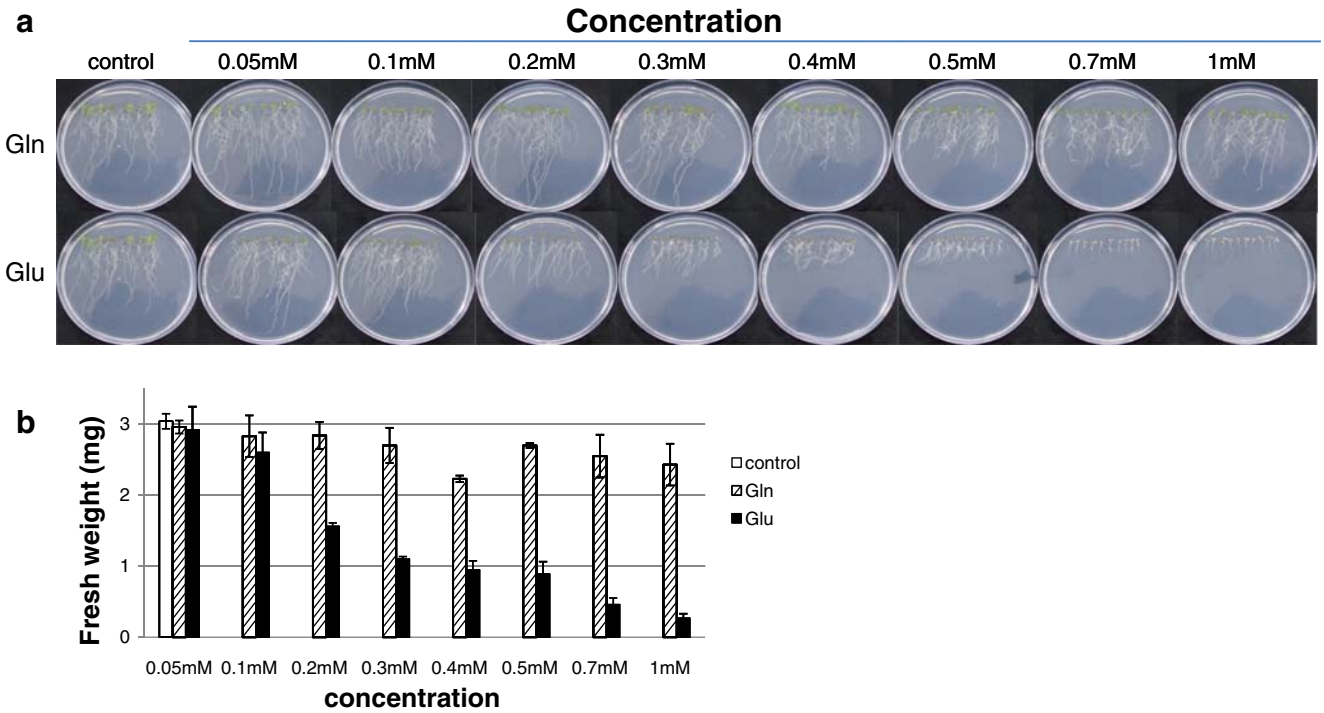

Fig. 1 Effects of glutamine and glutamate on the root growth in *A. thaliana*. **a** Five-day-old seedlings grown on 1/50× SH medium were transferred onto new media containing the different concentration of glutamine (Gln) or glutamate (Glu), and then cultured for 8 days. **b** Fresh weight of the plant roots grown on the media containing glutamine (Gln) or glutamate (Glu) for 8 days. *Error bar* indicates standard deviation

pH inhibits root growth of *Arabidopsis*. The pH is one of the important properties of soil because it affects the growth of plant root and microorganisms. The root growth is generally favored in slightly acidic pH between 5.5 and 6.5 (Taiz and Zeiger 2002) because acidity promotes the weathering of rocks that releases K^+, Mg^+, and Ca^+ and increases the solubility of carbonates, sulfates, and phosphates. However, when soil pH was lower than pH 4.8, plant roots are injured (Fig. 2). The organic matters such as glutamate are major factors lowering the soil pH. The root growth of *Arabidopsis* is severely inhibited by low pH (Koyama et al. 1995).

Base on the results of that glutamate reduces medium pH, we tried to check if the inhibition of the root growth by the addition of exogenous glutamic acid are a result of glutamate or low pH caused by acidic amino acid. In order to answer the question, we designed two experiments. In the first experiment, different concentrations of aspartate, another acidic amino acid, were added into growth medium and check the root growth (Fig. 3). Figure 3a shows that aspartate also inhibits root growth like as glutamate. In the second experiment, the amino acid solution was adjusted to pH 5.7 and filter sterilized and then added into medium. Figure 3b shows that phenotype of *Arabidopsis* seedlings grown on medium contains 0.4 μM of glutamic or aspartic acid solution of pH 5.7. When pH was adjusted, *Arabidopsis* seedlings show normal growth pattern on medium containing glutamate or aspartate (Fig. 3b). These results demonstrated that low pH by acid amino acid such as glutamate and aspartate inhibits the root growth of the *Arabidopsis* seedling.

It is well-known that brief exposure to low pH causes significant damage to the growing root in plant (Fawzy et al. 1954; Koyama et al. 1995; Koyama et al. 2001).

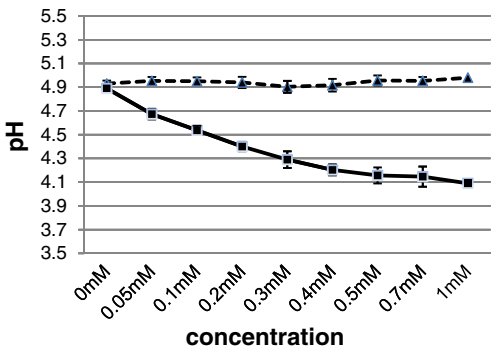

Fig. 2 Application of exogenous glutamic acid reduces medium pH. Medium pH was checked after glutamine or glutamic acid solution was added onto the growth medium. *Dot line* indicates glutamine, and *black line* indicates glutamate. *Error bar* indicates standard deviation

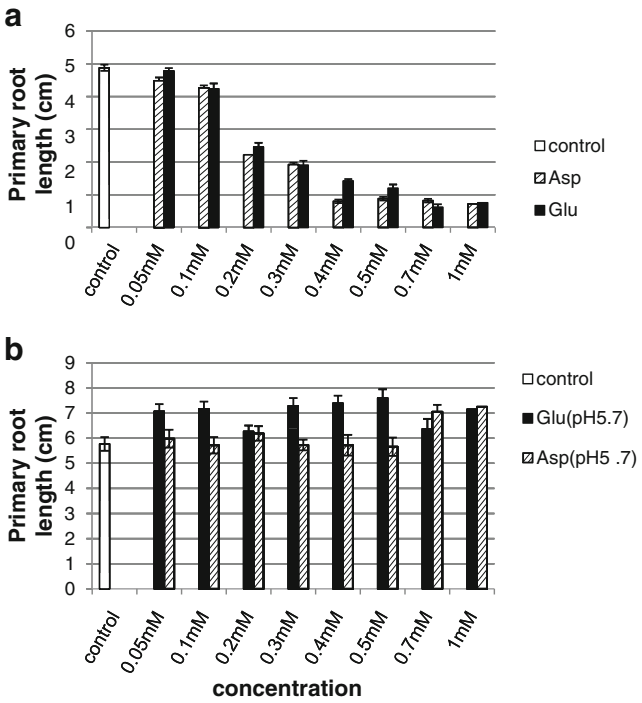

Fig. 3 Effects of glutamate and aspartate on the root growth in *A. thaliana*. **a** Primary root length of the *Arabidopsis* seedlings grown on the growth media containing glutamate (Glu) or aspartate (Asp) for 8 days. Five-day-old seedlings grown on 1/50× SH medium were transferred onto new media containing glutamate or aspartate and then cultured for 8 days. **b** Primary root length of *Arabidopsis* seedlings grown on the growth medium adjusted to pH 5.7. Glutamic and aspartic acid solution was adjusted to pH 5.7 at first and then added into growth medium. Five-day-old seedlings grown on 1/50× SH medium were transferred onto new medium containing the glutamate or aspartate adjusted to pH 5.7. Seedlings were cultured for 8 days, and root length was checked. *Error bar* indicates standard deviation

Koyama et al. (2001) tested the viability of *Arabidopsis* roots exposed to a low pH (4.5) solution and reported that the elongation zone of growing roots lost viability within 1–2 h following exposure to low pH, but non-growing roots showed no damage under the same treatment. The low pH damage was ameliorated by the simultaneous application of calcium or divalent cations. When applied 1 M $CaCl_2$ into growth medium, inhibitory effect of the glutamate and aspartate in the root growth was reduced (data not shown). These results show that inhibition of root growth by high concentration of glutamate is the result of low pH toxicity. Walch-Liu et al. (2006b) have reported that glutamate has an inhibitory effect on the root growth from the data in which root length of the seedlings grown on the medium were applied 10–1,000 μM of exogenous L-glutamate. However, our data demonstrated that inhibition of the root growth by high concentration of glutamate was the result of the low pH toxicity caused by acidic amino acid.

Glutamine Level Was Increased by Application of Exogenous Glutamate or Glutamine

In an attempt to understand the relation between root growth and glutamine or glutamate concentration in the root tissue, we checked the glutamate and glutamine level in the root grown on the medium containing exotic glutamine or glutamate. Figure 4 shows glutamine and glutamate content in seedling roots grown on medium containing 0.4 mM amino acid. Glutamine content was

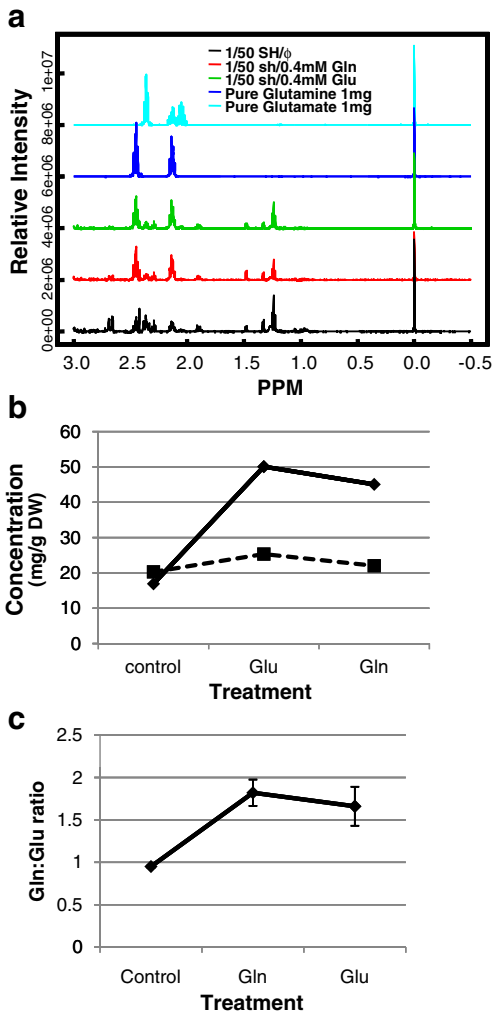

Fig. 4 Glutamine and glutamate content in the root tissue. **a** ^{1}H-NMR spectrum from the root extracts of the *Arabidopsis* seedling grown on the SH growth medium (*top*) containing 0.4 mM of glutamine (*second from top*) or glutamate (*third from top*). Authentic glutamine (*second from bottom*) and glutamate (*bottom*) was used as standard. **b** Glutamine (*dot line*) and glutamate (*linear line*) content was quantified from ^{1}H-NMR spectrum by sum of proton signals from ^{1}H-NMR spectral data as described in "Materials and Methods". **c** Content of the two amino acids, glutamine and glutamate, in root tissue was presented as glutamine to glutamate (Gln/Glu) ratio from Fig. 1b. *Error bar* indicates standard deviation

increased about 3- to 4-fold in the root grown medium containing glutamate, in which growth of seedling roots was about 60% inhibited (Fig. 1a, b). Increase in glutamine content was also observed at seedling root grown medium containing glutamine, which does not affect on the root growth. However, glutamate content did not show significant variation by addition of the both of amino acids, glutamine and glutamate (Fig. 4a). As a result, application of the glutamine or glutamate increased the glutamine to glutamate (Gln/Glu) ratio (Fig. 4b). Variation of amino acid content by exogenous amino acid treatment was also observed in tobacco leaf (Fritz et al. 2006; Scheneidereit et al. 2006). Masclaux-Daubresse et al. (2005) investigated the effect of amino acid addition, including glutamate and glutamine, on endogenous amino acid pools in tobacco leaf disk. The addition of high concentration (100 mM) of glutamine had little effect on the endogenous glutamate concentration, while glutamine content was dramatically increased. When 100 mM glutamate was added, glutamate as well as glutamine concentration increased. Endogenous glutamate content was much more constant, but glutamine concentration increase by amino acid addition. Therefore, Gln/Glu ratio was increased by amino acid addition. Increase in glutamine to other amino acid ratio was observed during the light/dark cycle in tobacco plant (Geiger et al. 1998). These results indicate that high concentration of endogenous glutamine observed in root tissue does not affect on the root growth.

Genetic Analysis of the *gln1_1*, *gln1*_2, and *glu2* Gene

In order to test if the genes involved in biosynthesis of the glutamine and glutamate affected root growth, we check the growth pattern of the seedling root of the *Arabidopsis* KO mutants on each of the *Gln1_1*, *Gln1*_2, or *Glu2* gene. Figure 5a shows the seedling root growth of the mutant plant on the medium containing glutamine or glutamate. The mutant plants *gln1_1*, *gln1_2*, and *glu2* show normal phenotype on the growth medium. The root growths of the three mutants were also similar to those of the control plant, *Arabidopsis* Col_0, on the growth medium containing glutamine or glutamate. The higher plant genome contains several isoenzymes of glutamine synthetase and glutamate synthase. For example, glutamine synthetase (GS) exits distinct isoenzyme in the cyotsol (GS1) and chloroplast (GS2), and five GS1 isoforms including *Gln1_1* and *Gln1_2* are encoded in nuclear genome in *Arabidopsis* (Peterman and Goodman 1991; Ishiyama et al. 2004). There are two different forms of glutamate synthase: ferredoxin-dependent and NADH-dependent glutmate synthase. *Arabidopsis* genome has two Fd-dependent glutamate synthase, *Glu1* and *Glu2* (Coschigano et al. 1998). These results show that the KO mutant on each of the *Gln1_1*, *Gln1_2*, or *Glu2* gene was little effective on the root growth. The presence of isoforms of the glutamine synthase and glutamate synthase may block the observation of visible defects of the *gln1_1*, *gln1_2*, and glu2 mutant.

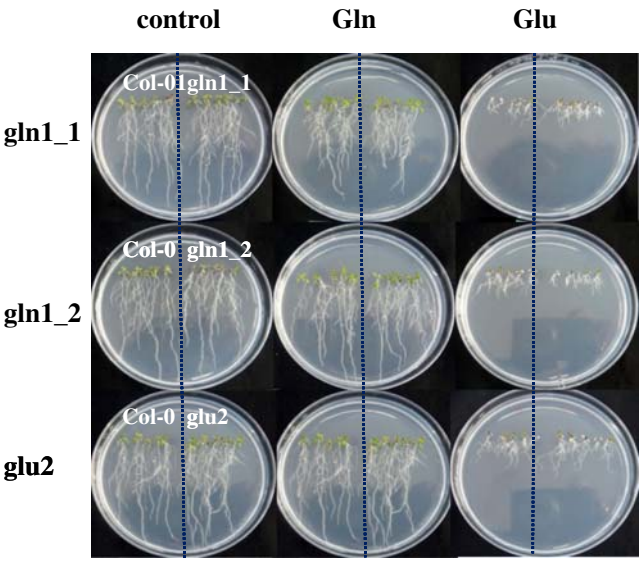

Fig. 5 Phenotypes of the seedlings of the *Arabidopsis* KO mutant of the *Gln1_1*, *Gln1_2*, and *Glu2* gene. Five-day-old seedlings of *Arabidopsis* Col_0 seedlings (*left side*) knockout mutant (*right side*) grown 1/50 SH medium were transferred onto new medium supplemented with 0.4 mM glutamine (Gln) or glutamate (Glu), and then cultured for 7 days

In summary, previously, we observed high concentration of the glutamine in the ginseng hairy root that shows low-branching and slow-growth type. In this study, we applied high concentration of glutamine or glutamate into the growth medium to check the relationship between glutamine and the root growth in *Arabidopsis*. Exogenous glutamine does not have an effect on the root growth, but glutamate severely inhibits the root growth. This study shows that the inhibition of the root growth by glutamate was a result of low pH toxicity caused by acidic amino acid, glutamate. Application of the exogenous glutamate or glutamine dramatically increased glutamine content in root tissue, but glutamate level in root tissue was little variable. These results indicate that inhibitory effect of exogenous glutamate on the root growth is a result of the low pH toxicity rather than plant response to amino acid. The exogenous glutamate and glutamine increase glutamine level in the tissue, but high glutamine concentration has no inhibitory effect on the root growth.

Acknowledgments This study was financially supported by Chonnam National University, 2007

References

Casson SA, Lindsey K (2003) Genes and signaling in root development. New Phytol 158:11–38

Coschigano KT, Melo-Oliveira R, Lim J, Coruzzi GM (1998) *Arabidopsis* gls mutant and distinct Fd-GOGAT genes: implications for photorespiration and primary nitrogen assimilation. Plant Cell 10:741–752

Desnos T (2008) Root branching responses to phosphate and nitrate. Curr Op Plant Sci 11:82–87

Ericsson T (1995) Growth and shoot: root ratio of seedling in relation to nutrient availability. Plant Soil 168–169:205–214

Fawzy H, Overstreet R, Jacobson L (1954) The influence of hydrogen ion concentration on cation absorption by barley roots. Plant Physiol 29:234–237

Filleur S, Walch-Liu P, Gan Y, Forde BG (2005) Nitrate and glutamate sensing by plant roots. Biochem Soc Trans 33:283–286

Forde BG, Lea PJ (2007) Glutamate in plants: metabolism, regulation, and signaling. J Exp Bot 58:2339–2358

Fritz C, Mueller C, Matt P, Feil R, Stitt M (2006) Impact of the C–N status on the amino acid profile in tobacco source leaves. Plant Cell Environ 29:2055–2076

Geiger M, Walch-Liu P, Engels C, Harnecker J, Shulze ED, Ludewig F, Sonnewald U, Scheible WR, Stitt M (1998) Enhanced carbon dioxide leads to a modified diurnal rhythm of nitrate reductase activity in older plants, and a large stimulation of nitrate reductase activity and higher levels of amino acids in young tobacco plants. Plant Cell Environ 21:253–268

Ishiyama K, Inoue E, Watanabe-Takahashi A, Obara M, Yamaya T, Takakashi H (2004) Kinetic properties and ammonium dependent regulation of cytosolic isoenzymes of glutamine synthetase in *Arabidopsis*. J Biol Chem 279:16598–16605

Jung SM, Kim SW, Ban SH, In DS, Jung JD, Chung HJ, Liu JR, Lim YP, Choi DW (2006) Glutamine accumulation inhibits root growth and lateral root formation in ginseng hairy roots. Plant Sci 170:801–807

Koyama H, Toda T, Yokota S, Zuraida D, Hara T (1995) Effects of aluminum and pH on root growth and cell viability in *Arabidopsis thaniana* strain Landsberg in hydroponic culture. Plant Cell Physiol 36:201–205

Koyama H, Toda T, Hara T (2001) Brief exposure to low-pH stress causes irreversible damage to the growing root in *Arabidopsis thaliana*: pectin–Ca interaction may play an important in proton rhizotoxicity. J Exp Bot 52:361–368

Masclaux-Daubresse C, Carrayol E, Valadier MH (2005) The two nitrogen mobilization- and senescence-associated GS1 and GDH genes are controlled by C and N metabolites. Planta 221:580–588

Oliveira IC, Coruzzi GM (1999) Carbon and amino acids reciprocally modulate the expression of glutamine synthetase in *Arabidopsis*. Plant Physiol 121:301–309

Peterman TK, Goodman HM (1991) The glutamine synthetase gene family of *Arabidopsis thaliana*: light-regulation and differential expression in leaves, root and seeds. Mol Gen Gent 230:145–154

Scheneidereit J, Hausler RE, Fiene G, Kaiser WM, Weber APM (2006) Antisense repression reveals a crucial role of the plastidic 2-oxoglutarate/malate translocator DiT1 at the interface between carbon and nitrogen metabolism. Plant J 45:206–224

Taiz L, Zeiger E (2002) Plant physiology, 3rd edn. Sinauer, Sunderland

Walch-Liu P, Forde BG (2008) Nitrate signaling mediated by the NRT1.1 nitrate transporter antagonizes L-glutamate-induced changes in root architecture. Plant J 54:820–828

Walch-Liu P, Ivanov II, Filleur S, Gan Y, Remans T, Forde BG (2006a) Nitrogen regulation of root branching. Ann Bot 97:875–881

Walch-Liu P, Liu LH, Remans T, Tester M, Forde BG (2006b) Evidence that L-glutamate can act as an exogenous signal to modulate root growth and branching in *Arabidopsis thaliana*. Plant Cell Physiol 47:1045–1057

Watanabe A, Takagi N, Hayashi H, Chino M, Watanabe A (1997) Internal Gln/Glu ration as a potential regulatory parameter for the expression of a cytosolic glutamine synthetase of radish in cultured cells. Plant Cell Physiol 38:1000–1006

Zhang H, Forde BG (2000) Regulation of *Arabidopsis* root development by nitrate availability. J Exp Bot 51:51–59

Zhang H, Jennings A, Barlow PW, Forde BG (1999) Dual pathways for regulation of root branching by nitrate. Proc Natl Acad Sci 96:6529–6534

Zhu B, Coleman GD (2001) The poplar bark storage protein gene (*Bspa*) promoter is responsive to photoperiod and nitrogen in transgenic poplar and active in floral tissues, immature seeds and germinating seeds of transgenic tobacco. Plant Mol Biol 46:383–394