

Aluminum effect on starch, soluble sugar, and phytohormone in roots of *Quercus serrata* Thunb. seedlings

Ubuki Moriyama¹ · Rie Tomioka¹ · Mikiko Kojima² · Hitoshi Sakakibara^{1,2} · Chisato Takenaka¹

Received: 4 November 2014 / Revised: 26 June 2015 / Accepted: 1 July 2015 / Published online: 22 July 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract

Key message Glucose was a key substance as an energy source in the root growth promotion by Al, and ABA may relate to metabolism involved with its process.

Abstract Generally, excess aluminum (Al) ions in soil solution are toxic to many cultivated plant species, but beneficial effects of Al for plant growth have been reported. Previously, we reported stimulation of root growth and nitrate reductase (NR) activity by Al. In this study, we focused on sugars, such as sucrose, glucose, and fructose, as energy sources and also signaling substances to regulate root growth. To understand the mechanism of root growth stimulation by Al, we investigated the change in concentration of sugars and phytohormones, and the activity of NR in roots using *Quercus serrata* seedlings. Ten-week-old *Q. serrata* seedlings were hydroponically cultured with nutrient solution containing 2.5 mM Al (pH 4.0) or 3.25 mM calcium (Ca) (pH 4.0) for 3 and 15 days. The growth of first lateral root and NR activity was stimulated for 3 and 15 days of Al treatment. The concentration of starch and sucrose decreased, while the concentration of glucose increased in the Al-treated roots. The concentration of abscisic acid (ABA) in Al-treated roots increased gradually throughout the experiment. From the present

study, the mechanism of root growth promotion by Al involves a complex signaling network. We suggest that glucose is a key substance as an energy source and a signaling substance to promote root growth induced by Al and ABA may relate to nitrogen (N) and carbon (C) metabolism involved with the signaling network to promote root growth induced by Al.

Keywords Sucrose · Glucose · Abscisic acid · Aluminum · *Quercus serrata*

Introduction

Terrestrial plants survive and grow by absorbing a variety of inorganic elements from the soil and using them for physiological action for individual growth. For each element and each plant species, there is an appropriate concentration range to maintain plant life and growth. Aluminum (Al) is the most abundant metal in the earth's crust and is a component of primary and clay minerals. This means that all terrestrial plants are living in environments with Al present and are exposed to Al ions from the soil solution. Al has not been recognized as an essential element for plant growth. Excess Al ions in soil solution are toxic to cultivated plant species at a soil pH of 5 or lower and can be a critical factor in the growth of cultivated plants (Delhaize and Ryan 1995). Although excess Al ions in soil solution can negatively affect plant growth, there are reports showing the effectiveness of Al for stimulating plant growth within a certain concentration range. The beneficial effects of Al for trees have been reported in *Camellia sinensis* (Tsuji et al. 1994), *Melastoma malabathricum*, *Melastoma cajuputi*, *Acacia mangium* (Watanabe et al. 1998), *Eucalyptus mannifera* (Huang

Communicated by T. Koike and K. Noguchi.

✉ Rie Tomioka
tomikar@agr.nagoya-u.ac.jp

¹ Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

² RIKEN Center for Sustainable Resource Science, Yokohama 230-0045, Japan

and Bechelard 1993), *Quercus acutissima* (Oda and Yamamoto 2002), and so on. Although less than the reports of trees, there are some reports of growth increment by Al in herbaceous plants, such as *Arnica montana*, *Deschampsia flexuosa* (Pegtel 1987), and *Zea mays* (Lindon et al. 1998). Most research on plants showing beneficial effect of Al have been focused on the mechanism of Al tolerance, but very few have approached the mechanism of growth enhancement by Al. We have reported on the beneficial effects of Al for tree seedling growth (Tomioka et al. 2007; Tomioka and Takenaka 2007) and stimulation of root growth (Tomioka et al. 2005, 2012) and have been studying the mechanisms behind these effects using *Quercus serrata* Thunb seedlings.

Quercus serrata is one of the typical deciduous broad-leaved tree species in temperate forest of East Asia and can grow on weak acidic soils such as around pH 4. The surface soil properties of deciduous forest in central Japan is as follow; soil pH ranges from 3.7 to 5.1, and water soluble base cation (K, Ca, Mg) ranges from 14.3 to 927.0 $\mu\text{mol/kg}$, and water soluble Al ranges from 0.1 to 4.7 mmol/kg (Nishina et al. 2009).

It has not been evident that the forest is declined by soil acidification in Japan, because the most of forest soil in Japan are influenced by volcanic substances and have thicker layer of soil and higher ability of acid neutralization than the soil which advanced forest decline such in Europe (Okazaki and Matui 1993). However, acid deposition has been observed in Japan, it is important to establish the diagnosis for condition of tree or forest ecosystem to prevent severe forest decline by soil acidification. Therefore to understand the mechanism of change in root metabolism and action of root to rhizospheric Al will be an essential knowledge for the forest management diagnosis.

We showed that root growth enhancement by Al was related to stimulation of nitrate ion (NO_3^-) uptake and an increase in the number of lateral root primordia (Tomioka et al. 2007). We also observed that development of the root system induced by Al was related to increased nitrate reductase (NR) activity and maintenance of indole-3-acetic acid (IAA) concentrations in roots (Tomioka et al. 2012).

To further understand the mechanism of enhancement of root growth induced by Al, we focused on carbon and nitrogen as these are important elements in living organisms. In actively growing tissue, energy is required to support tissue growth. Metabolic energy in nonphotosynthetic tissue is obtained mainly by respiration, and sucrose and starch are the main substrates in plant tissue. Sucrose and its cleavage products, glucose and fructose, are pivotal molecules for metabolism and regulation of plant growth and development (Smeekens 2000; León and Sheen 2003; Gibson 2005). Pahlsson (1990) reported that starch and total sugar contents in root and shoot were increased by Al

treatment for 31 days without growth reduction in *Fagus sylvatica*. Graham (2002) reported that sucrose content in roots of peach seedlings was increased by 8-week 1-mM Al treatment, but glucose and fructose contents in those roots were decreased. Although there are other reports about Al effect on carbohydrate content in plants, relationship between root growth stimulation by Al and change of carbohydrate content in roots is still unclear. In *Q. serrata*, we observed increase in number of lateral root primordia, uptake of NO_3^- , and activity of NR after 3 days Al treatment started, but photosynthetic activity was not increased by 3-day-Al treatment (Tomioka et al. 2007). So it is considered that energy substrate may increase in roots stimulated growth by Al due to rise of dissimilation of accumulated energy source substance, such as starch and sucrose in roots.

Amino acids, proteins, and nucleotides are important organic nitrogen compounds found in living bodies. Generally, plant roots absorb NO_3^- from the soil and assimilate NO_3^- into nitrogen compounds. The reduction of NO_3^- to nitrite ion (NO_2^-) by NR is the first step in nitrogen assimilation. Previous reports have identified a relationship between NR activity and glucose (Aslam and Oaks 1975; Bortel and Kaiser 1997). Nitrogen metabolism is closely related to Al sensitivity/tolerance in various plant species (Liu and Sukalovic 1998; Foy and Fleming 1982; Mihailovic et al. 2015). In our previous study, NR activity and NO_3^- uptake were stimulated by Al treatment (Tomioka et al. 2007). Therefore we think that NR activity is one of the key factor related to root growth stimulation induced by Al (Tomioka et al. 2012).

Regulation of growth and development in plants are severely affected by phytohormones, such as auxin, cytokinins, and abscisic acid (ABA). It is known that auxin and cytokinin antagonistically act on meristem activity (Moubayidin et al. 2009). A relationship between auxin and cytokinin and nitrogen metabolism has also been reported (Sahulka 1972; Mi et al. 2008). As described above, sugars act as signaling compounds in the whole plant life cycle, and sugar sensing and sugar-induced signal transductions have been revealed to interact with phytohormones such as IAA, cytokinins, ABA, and gibberellins (GA) by comprehensive genetic analysis (Smeekens 2000, Gibson 2005). In root meristem of *Arabidopsis*, glucose regulates auxin transporter and transcription factor of ABA-triggered process expression (Yuan et al. 2014). Moreover, transcriptome data shows that NO_3^- uptake and subsequent process to the absorption are closely related to hormone and carbon signaling pathway (Ruffel et al. 2014). Although strong interaction among metabolism of C and N, signaling pathway of sugar, nitrogenous compounds and hormone, and growth of plant are suggested, comprehensive understanding of mechanism of that has been unknown.

The aim of this study is to understand the process of root growth enhancement by Al, and we investigated how energy source is supplied to support stimulation of NR activity and root growth by Al. In addition, to understand global signaling network (Fig. 1) which promote root growth by Al, quantitative change of soluble sugars, phytohormones by Al treatment were observed for the first step.

Materials and methods

Plant materials and treatment

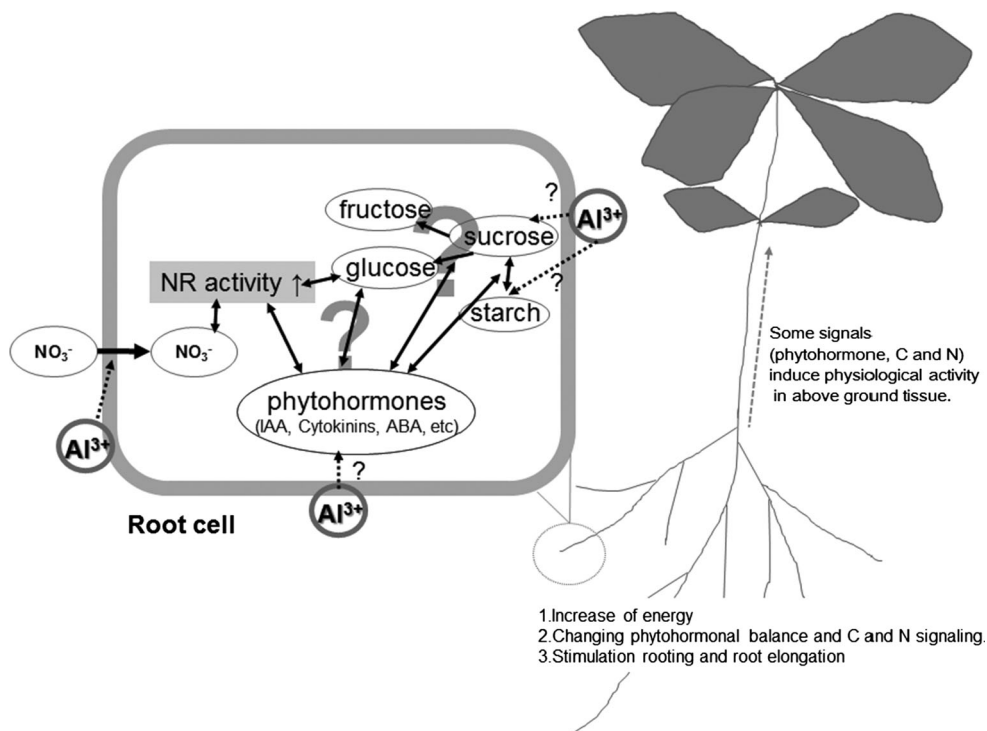
Seeds of *Q. serrata* were collected from a secondary forest at Nagoya University, Japan. Seeds were germinated in siliceous sand. After root germination, seedlings were grown hydroponically in a modified version of the 1/10 Hoagland’s No. 2 nutrient solution, containing 0.6 mM KNO₃, 0.4 mM Ca(NO₃)₂·4H₂O, 0.2 mM MgSO₄·7H₂O, 0.1 mM NH₄H₂PO₄, 45.5 μM MnCl₂·4H₂O, 8.95 μM FeCl₃·6H₂O, 0.4 μM ZnSO₄·7H₂O, 0.15 μM CuSO₄·5H₂O, 2.3 μM H₃BO₃, and 0.25 μM NaMoO₄·2H₂O, with aeration (Tomioka et al. 2012), in a growth chamber at 23 °C with 65 % relative humidity, with a photoperiod of 14/10 h (day/night) and irradiation of 150 μmol m⁻² s⁻¹ for 10 weeks. The nutrient solution was changed once a week and the pH of the solution was adjusted to 4.0 ± 0.1 with 1 N HCl and 1 N NaOH. The reason for setting

nutrient solution pH to 4.0 is that average soil pH of secondary forest in Tokai area in Japan is about 4.0 (Nishina et al. 2009).

After 10 weeks of growth, the 100 seedlings of similar size were used for experiment. As pre-treatment sample, 20 seedlings were sampled. The roots of 80 seedlings were exposed to nutrient solution with 2.5 mM AlCl₃ (pH 4.0) or 3.25 mM calcium chloride (CaCl₂) (pH 4.0). The treatment with 2.5 mM Al in cultured solution resulted in the maximum root biomass in *Q. serrata* seedlings (Tomioka et al. 2005). Ca was used as a control and its concentration was equal in ionic strength to the Al treatment. The ionic strength of nutrient solution affects plant growth and content of inorganic and organic compounds in plant tissue (e.g., Abou-Hadid, et al. 1996; Oh et al. 2014). Therefore it is important to take into consideration of ionic strength of treatment solution in investigation of plant physiological activity. We chose Ca to adjust ionic strength, because NR activity of *Q. serrata* seedling increased in both 1 h Al and Ca treatment in our previous study (Tomioka et al. 2012). The culture medium was replaced with fresh medium every 2 days.

Twenty seedlings in each treatment were randomly sampled after 3 and 15 days treatment started. Right after sampling, the image of whole root system was taken as described below, and 5 cm from the tip of the taproot and the whole tissue of the lateral roots, which have relatively high physiological activity, were collected to use physiological analysis as described below. These procedure were carried under 4 °C. Some collected samples were freshly

Fig. 1 Hypothetical sketch of global signaling network in promotion of root growth enhancement by Al in *Q. serrata*. Broken line indicates direct or indirect effect of Al on a process. A double pointed arrow indicates an interaction between factors via some signals or enzyme activity



used to measure the NR activity, some were frozen in liquid nitrogen and stored at $-80\text{ }^{\circ}\text{C}$ for analysis of phytohormone, and some were dried at $80\text{ }^{\circ}\text{C}$ for 48 h for analysis of carbohydrates.

Root morphogenesis

The images of whole root system of seedling were taken with the scanner and the lengths of the taproot and the 1st lateral roots were measured by LIA 32 software (Yamamoto 2000). The length of the 1st lateral roots was values of the longest three of 1st lateral roots.

Assay of NR activity

To assay NR activity, about 0.5 g freshly prepared root tissue which include lateral root and 5 cm from the tip of the taproot were used, and enzyme fractions were prepared as previously described (Tomioka et al. 2012). NR activity was measured by a modified version of the method of Hageman and Reed (1980) as follows. The reaction mixture (1 mL) containing 23 mM K_3PO_4 (pH 7.5), 4.5 mM KNO_3 , 9 μM flavin adenine dinucleotide, 69 μM nicotinamide adenine dinucleotide, and enzyme fraction was incubated at $30\text{ }^{\circ}\text{C}$ for 30 min in the dark. The reaction was stopped by adding 0.1 mL of 0.5 M zinc acetate. The mixture was then centrifuged at 5000 g for 5 min. An aliquot (0.75 mL) of the supernatant was mixed with 16 μL of 0.15 mM phenazine methosulfate and incubated at $25\text{ }^{\circ}\text{C}$ for 20 min. Then, 0.5 mL of 1 % sulfanilamide (in 1.5 M HCl) and 0.5 mL of 0.02 % *N*-1-naphthyl ethylenediamine dihydrochloride were added to the solution. After incubation at $25\text{ }^{\circ}\text{C}$ for 20 min, absorbance of the sample was measured at 540 nm using a spectrophotometer (model U-3310; Hitachi, Tokyo, Japan). NR activity was calculated on a fresh weight (fw) basis.

Extraction and quantification of carboxylates

The dried root samples were used for carboxylates analysis. Soluble sugars (sucrose, glucose, and fructose) were extracted from 100 mg dried root sample which include lateral root and 5 cm from the tip of the taproot. The samples were immersed in 80 % ethanol at $80\text{ }^{\circ}\text{C}$, homogenized for 3 min, and shaken at $80\text{ }^{\circ}\text{C}$ for 30 min. Mannitol was added to the sample as an internal standard. Samples were centrifuged at 2270 g for 15 min. The pellet was twice re-extracted with 80 % ethanol, the three supernatants were combined, and ethanol was evaporated using a freeze dryer after freezing the sample at $-80\text{ }^{\circ}\text{C}$. The residue was dissolved in ultrapure water and the sugars were purified through a C18 column (Sep-pack C18; Waters, Massachusetts, U.S.A). The soluble sugar concentrations in

purified samples were determined using high-performance liquid chromatography with a refractive index detector (RI-201H, Shodex; Showa Denko, Tokyo, Japan) on a Pb-loaded cation exchange column (SUGAR SP0810, Shodex; Showa Denko) at $80\text{ }^{\circ}\text{C}$. Samples were eluted with ultrapure water at a flow rate of 0.8 mL min^{-1} .

To determine starch content, the residue after soluble sugar extraction was re-suspended in 0.5 M NaOH and extracted at room temperature for 10 min. Then, 0.5 M CH_3COOH was added to the sample and samples were centrifuged at 2270 g for 15 min. The pellet was re-extracted with the same procedure as described above. Two supernatants were combined and the volume was fixed with ultrapure water. Starch content was determined in terms of glucose using the GOPOD method (Miwa et al. 1972) after digestion with glucoamylase and α -amylase.

Extraction and quantification of phytohormones

The frozen root samples were used for phytohormones analysis. Plant hormones such as IAA, cytokinins (*N*⁶-(Δ^2 -isopentenyl)adenine (iP) and *trans*-zeatin (tZ)), and ABA in about 0.5 g root tissues which include lateral root and 5 cm from the tip of the taproot were extracted and quantified using a liquid chromatography-tandem mass chromatography system (AQUITY UPLC System/Quattro Ultima Pt; Waters), as described previously (Kojima et al. 2009).

Results

The length of the taproot was not different between Al and Ca treatments throughout the experimental period. The length of the 1st lateral root in the Al treatment was significantly longer than that of Ca-treated roots for both 3 and 15 days ($p < 0.01$ and 0.05 , respectively) (Table 1).

NR activity of Al-treated roots increased gradually throughout the experimental period. The NR activity of Ca-treated roots slightly decreased from the pre-treatment to 3 days post-treatment and did not become higher than the pre-treatment even at 15 days (Fig. 2).

The concentrations of starch, sucrose, glucose, and fructose in roots are presented in Fig. 3. The starch concentration in Al-treated roots decreased after 3 days and slightly decreased in between 3 and 15 days. In contrast to Al-treated roots, starch concentration increased in Ca-treated roots after 3 days (Fig. 3a). Sucrose concentrations in both Al- and Ca-treated roots decreased after 3 days, and the amounts of sucrose were not significantly different between treatments. After 15 days, the sucrose concentration in Ca-treated roots did not change from the concentration at 3 days, but the concentration in Al-treated roots slightly decreased (Fig. 3b). The glucose concentration in

Table 1 The length of taproot and first lateral root after 3 and 15 days treatment

		Treatment period (days)		
		0	3	15
Taproots (cm)	+Al	20.8 ± 1.6	24.2 ± 1.7	34.4 ± 3.0
	+Ca		21.9 ± 1.6	34.1 ± 2.7
1st lateral roots (cm)	+Al	3.7 ± 0.27	5.0 ± 0.40**	7.3 ± 0.81*
	+Ca		3.3 ± 0.38	4.9 ± 0.60

Values are given as mean ± SE (*n* = 20 for Taproots and *n* = 60 for 1st lateral roots)

The length of the 1st lateral roots was values of the longest three of 1st lateral roots

The asterisks indicate a significant difference between +Al and +Ca at *p* values less than 0.05(*) and less than 0.01(**) according to the *t* test

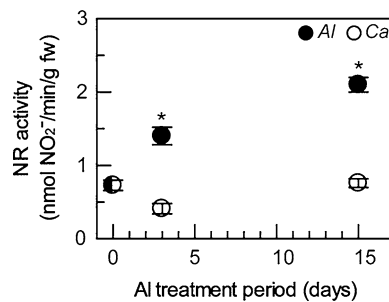
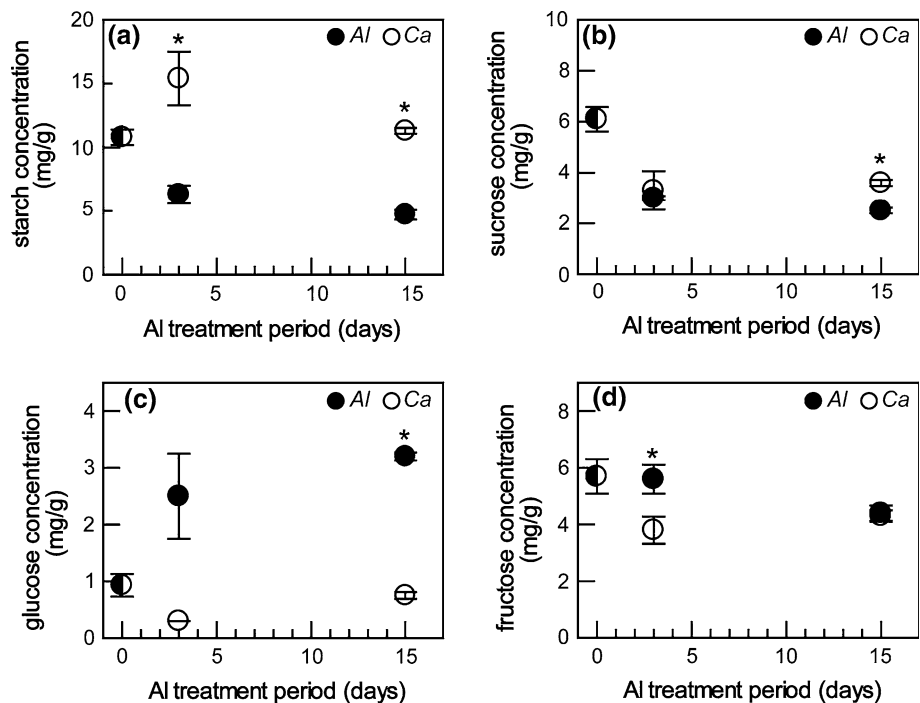


Fig. 2 NR activity in roots of *Q. serrata* treated with 2.5 mM AlCl₃ or 3.25 mM CaCl₂ in modified 1/10 Hogland’s No. 2 (pH 4.0). Vertical bars represent the SE of the mean (*n* = 3). Asterisks indicate significant differences between Al and Ca treatments (*p* < 0.05) according to the *t* test

Al-treated roots increased throughout the experiment, but in Ca-treated roots the glucose concentration decreased after 3 and 15 days (Fig. 3c). The fructose concentration in Al-treated roots was similar before treatment after 3 days and slightly decreased after 15 days. In contrast to Al-treated roots, the fructose concentration in Ca-treated roots decreased after 3 days and stayed the same between 3 days and 15 days (Fig. 3d).

The changes in phytohormone concentrations in roots are shown in Fig. 4. The concentration of cytokinin in Al-treated roots decreased after 3 days and returned to pre-treatment levels after 15 days. The cytokinin concentration in Ca-treated roots stayed the same level of pre-treatment after 3 days and then decreased at 15 days (Fig. 4a). The

Fig. 3 Concentration of carbohydrates, starch (a), sucrose (b), glucose (c), and fructose (d) in roots of *Q. serrata* treated with 2.5 mM AlCl₃ or 3.25 mM CaCl₂ in modified 1/10 Hogland’s No. 2 (pH 4.0). Vertical bars represent the SE of the mean (*n* = 3). Asterisks indicate significant differences between Al and Ca treatments (*p* < 0.05) according to the *t* test



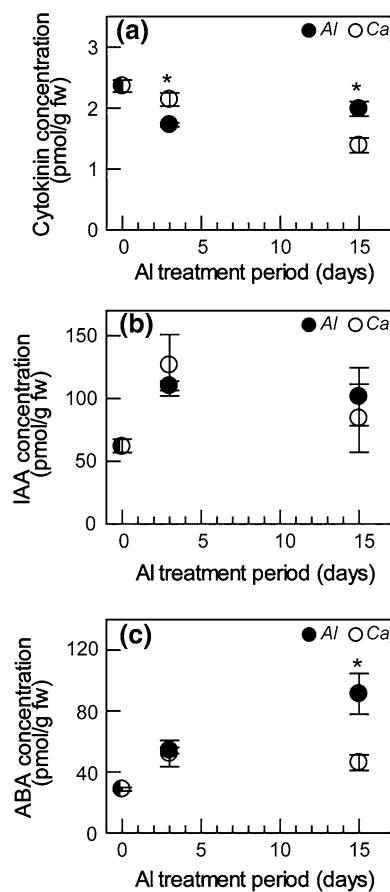


Fig. 4 Concentrations of phytohormones, cytokinin (iP + tZ) (a), IAA (b), and ABA (c) in roots of *Q. serrata* treated with 2.5 mM AlCl_3 or 3.25 mM CaCl_2 in modified 1/10 Hogland's No. 2 (pH 4.0). Vertical bars represent the SE of the mean ($n = 3$). Asterisks indicate significant differences between Al and Ca treatments ($p < 0.05$) according to the t test

IAA concentrations increased in the roots of both treatments after 3 and 15 days, but the changes over time and the difference between Al and Ca treatments were not clear (Fig. 4b). ABA concentration in Al-treated roots increased gradually throughout the experiment. The ABA concentration in Ca-treated roots increased after 3 days and did not change between 3 and 15 days (Fig. 4c).

Discussion

In this study, 1st lateral root elongation was enhanced by Al treatment after 3 and 15 days and the length of that was significantly different to Ca-treated roots (Table 1). NR activity gradually increased in Al-treated roots, but this phenomena was not observed in Ca-treated roots (Fig. 2). We previously reported on the increase in NO_3^- uptake from culture solution for 3–14 days of Al treatment and the enhancement of growth and increased NR activity in lateral roots by Al treatment for 7–14 days in 2-year-old

Q. serrata seedlings (Tomioka et al. 2007). We also observed increased NR activity in roots treated with Al or Ca for 1 h, suggesting increased NO_3^- influx, caused by an increase in positive charge on the cell membrane because of absorption of Al^{3+} or Ca^{2+} (Tomioka et al. 2012). The increased NR activity with Al treatment observed in this study can be because of increased NO_3^- uptake. The increment of NR activity with Ca treatment was not observed in this study (Fig. 2). Although the increase of NR activity with the enhancement of the NO_3^- uptake was expected to Ca-treated roots, that may not have occurred after 3 days of treatment started. Enhancement of NO_3^- uptake might have occurred within a day or 2 days from the start of the treatment in continuous Ca treatment also, but the ongoing metabolic process might be different. The contents of starch, glucose, fructose, cytokinins, and ABA were different between Al and Ca treatments, as discussed below.

In general, sucrose is an energy source for living cells, and sucrose and its cleavage products, glucose and fructose, are pivotal molecules for metabolism and regulation of plant growth and development (Smeekens 2000; León and Sheen 2003; Gibson 2005). To understand the mechanism of enhancement of root growth induced by Al, we investigated the changes in concentrations of starch, sucrose, glucose, and fructose as energy sources and as regulators of plant growth and development. For Al-treated roots, gradual decrease of starch and sucrose was observed, on the other hand, the concentrations of glucose and the NR activity increased after 3 and 15 days in this study (Figs. 2, 3c). In *Hordeum vulgare* seedlings, increased concentrations of reducing sugars, which included glucose, fructose, and others, were observed after Al treatment at a concentration that enhanced plant growth (Abdalla 2008). Although we need to check the enzyme activity involving with starch and sucrose hydrolysis, these results indicate that hydrolysis of starch and sucrose may occur, production of energy may be enhanced and stimulate growth of Al-treated roots as result. The enhancement of NR activity by exogenous glucose treatment was observed in cotton roots (Radin 1974) and corn roots (Aslam and Oaks 1975). Bortel and Kaiser (1997) suggested that NR synthesis appeared to be dependent on sugar availability, based on their experiment using *H. vulgare* roots and glucose. From these results, activation of NR in roots by Al treatment may be as a result of NR synthesis induction by glucose.

There are many reports on interactions between sugar sensing/signaling systems and phytohormones such as auxin, cytokinin, and ABA (Gazzarrini and McCourt 2001; Rolland et al. 2002). In this study, a decrease in sucrose concentrations was observed in roots treated with both Al and Ca (Fig. 3b) and IAA concentrations in both treatments increased over time (Fig. 4b). Interestingly, the concentration of glucose increased in Al-treated roots

(Fig. 3c) in which growth enhancement was observed (Table 1). In Ca-treated roots, the concentration of starch increased (Fig. 3a) but the glucose and fructose concentrations decreased (Fig. 3b–d), and the root growth was lower than for Al-treated seedlings (Table 1). It is important to understand which factors are involved in these differing responses to Al and Ca, given that both treatments resulted in decreased sucrose concentration in the roots, while the Al treatment enhanced NR activity/growth/glucose concentration in roots and the Ca treatment promoted accumulation of starch in roots.

Bhatia and Singh (2000) showed increased accumulation of starch and activation of α -amylase in sorghum grains when treated with 3 mM Ca in culture media. Further work by Bhatia and Singh (2002) showed that IAA increased starch synthesis but ABA decreased starch synthesis in sorghum grains. In tobacco BY2 cells, the proliferation rate of cells treated with auxin was higher than for those treated with cytokinin, and accumulation of starch was only observed for cells treated with cytokinin (Miyazawa et al. 1999). In the present study, the IAA concentration in Ca-treated roots increased over time (Fig. 4b). The ABA concentration in Ca-treated roots slightly increased after 3 days and did not change between 3 and 15 days (Fig. 4c). The cytokinin concentration in Ca-treated roots gradually decreased throughout the experiment (Fig. 4a). It is difficult to explain the accumulation of starch in Ca-treated roots in relation to the change in concentration of endogenous hormones and N-metabolism from our study and previous reports. Therefore further study is needed to understand the process of starch accumulation by analysis of metabolic activity, such as starch synthetase and amylase simultaneously with factors analyzed in this study. From the viewpoint of relationship between nutrient acquisition and expansion of root system, it is possible that *Q. serrata* accumulates starch as an energy source rather than using this for growth in this situation, when the Ca concentration in the rhizosphere is sufficient for maintenance of life and is not inhibitory to growth.

Mishra et al. (2009) suggested that glucose affected the IAA biosynthetic gene family, transport protein, and receptors. In this study, a clear relationship between glucose and IAA concentration in Al-treated roots was not observed, and the difference in IAA concentrations between Al and Ca treatments was not clear. The glucose concentrations observed in this study might not affect the IAA concentration in roots. ABA is known as a stress hormone and an inhibitor of growth, but the study by Cheng et al. (2002) showed ABA was important for cotyledon, leaf, root, stem, and silique development and fertility. There are two types of enzymes (invertase and sucrose synthase) that cleave sucrose. Sucrose cleavage occurs by neutral invertase and sucrose synthase in the

cytoplasm and by acidic invertase in vacuoles or the cell wall. Several studies have reported on the activity of sucrose cleavage enzymes which regulates plant development and is influenced by phytohormones, such as IAA, ABA, and gibberellic acid (GA) (Rotisch and González 2004; Koch 2004). In young chicory roots, exogenous ABA promoted NR activity and neutral invertase activity but reduced acidic invertase activity and sugar contents (Goupil et al. 1998). In maize roots, exogenous ABA enhanced activity of acidic invertase in vacuoles, the activity was correlated with ABA concentration, and the contents of sucrose and hexose (glucose and fructose) were increased at the time and at the concentration of maximum invertase activity (Trouverie et al. 2004). In this study, growth promotion, stimulation of NR activity, and an apparent increase in glucose and ABA concentrations in roots treated with Al were observed (Table 1; Figs. 3c, 4c). The relationship between sugar and phytohormone concentration was not clear from 3-day-Al treatment in this study; however, increase of ABA concentration might be related to glucose concentration and NR activity in 15-day-Al-treated roots. Our future subjects to understand the process of root growth induced by Al in *Q. serrata* are which invertases involve sucrose cleavage and how phytohormones and sugar signaling interact in Al-treated roots.

In this study, we clarified that the stimulation of NR activity in Al-treated roots were related to increase of glucose and ABA concentration in roots. As a conclusion, hypothetical sketch of promotion of root in *Q. serrata* is suggested as following. Al^{3+} bound to plasma membrane and stimulation of NO_3^- uptake occur. Then NR activity is stimulated as a result of increase of NO_3^- concentration in cytoplasm. Direct or indirect effect of Al^{3+} bound to plasma membrane/cell wall results in change of phytohormone concentration/balance in root and stimulation of starch and sucrose hydrolysis. Then increase of glucose concentration and high activity of NR in roots induce proliferation and elongation of lateral roots.

Author contribution statement R. T., I. M. and C. T. contributed the idea and design for the research. I. M., M. K., R. T. and H. S. conducted research. R. T. and I. M. wrote manuscript. The authors declare that they have no conflict of interest.

Acknowledgments We thank Dr Kunio Yamada (Chubu University), Dr Katsuhiko Shiratake and Kayoko Miyashita (Nagoya University), and Dr Takafumi Tezuka for valuable advice and support to analysis of carbohydrates.

References

- Abdalla MM (2008) Physiological aspects of aluminium toxicity on some metabolic and hormonal contents of *Hordum Vulgare* seedlings. Aust J Basic Appl Sci 2(3):549–560

- Abou-Hadid AF, Abd-Elmoniem EM, EL-Shinawy MZ, Abou-El-soud M (1996) Electrical conductivity effect on growth and mineral composition of lettuce plants in hydroponic system. *Acta Hort* 434:59–66
- Aslam M, Oaks A (1975) Effect of glucose on the induction of nitrated reductase in corn roots. *Plant Physiol* 56:634–639
- Bhatia S, Singh R (2000) Calcium-mediated conversion of sucrose to starch in relation to the activities of amylases and sucrose-metabolizing enzymes in sorghum grains raised through liquid culture. *Indian J Biochem Bio* 37(2):135–139
- Bhatia S, Singh R (2002) Phytohormone-mediated transformation of sugars to starch in relation to the activities of amylases, sucrose-metabolising enzymes in sorghum grain. *Plant Growth Regul* 36(2):97–104
- Bortel A, Kaiser WM (1997) Nitrate reductase activation state in barley roots in relation to the energy and carbohydrate status. *Planta* 201:496–501
- Cheng WH, Endo A, Zhou L, Penney J, Chen HC, Arroyo A, Leon P, Nambara E, Asami T, Seo M, Koshiha T, Sheen J (2002) A unique short-chain dehydrogenase/reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. *Plant Cell* 14:2723–2743. doi:10.1105/tpc.006494
- Delhaize E, Ryan PR (1995) Aluminum toxicity and tolerance in plants. *Plant Physiol* 107:315–321
- Foy CD, Fleming AL (1982) Aluminum tolerance of two wheat genotypes related to nitrate reductase activities. *J Plant Nutr* 5:1313–1333
- Gazzarrini S, McCourt P (2001) Genetic interactions between ABA, ethylene and sugar signaling pathways. *Curr Opin Plant Biol* 4:387–391
- Gibson SI (2005) Control of plant development and gene expression by sugar signaling. *Curr Opin Plant Biol* 8:93–102. doi:10.1016/j.pbi.2004.11.003
- Goupil P, Loncle D, Druart N, Bellettre A, Rambour S (1998) Influence of ABA on nitrate reductase activity and carbohydrate metabolism in chicory roots (*Cichorium intybus* L.). *J Exp Bot* 49:1885–1886
- Graham CJ (2002) Nonstructural carbohydrate and prunasin composition of peach seedlings fertilized with different nitrogen sources and aluminum. *Sci Hortic Amsterdam* 94:21–32
- Hageman RH, Reed AJ (1980) Nitrate reductase from higher plants. *Method Enzymol* 69:270–280
- Huang J, Bechelard EP (1993) Effects of aluminum on growth and cation uptake in seedlings of *Eucalyptus mannifera* and *Pinus radiata*. *Plant Soil* 149:121–127
- Koch K (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr Opin Plant Biol* 7:235–246. doi:10.1016/j.pbi.2004.03.014
- Kojima M, Kamada NT, Komatsu H, Takei K, Kuroha T, Mizutani M, Ashikari M, Ueguchi TM, Matsuoka M, Suzuki K, Sakakibara H (2009) Highly sensitive and high-throughput analysis of plant hormones using MS-probe modification and liquid chromatography-tandem mass spectrometry: an application for hormone profiling in *Oryza sativa*. *Plant Cell Physiol* 50(7):1201–1214. doi:10.1093/pcp/pcp057
- León P, Sheen J (2003) Sugar and hormone connections. *Trends Plant Sci* 8(3):110–116. doi:10.1016/S1360-1385(03)00011-6
- Lindon FC, Ramalho JC, Barreiro MG (1998) Aluminium toxicity modulates nitrate to ammonia reduction. *Photosynthetica* 35:213–222
- Liu B, Sukalovic VH (1998) Effect of aluminum on growth and nitrate reductase activities of maize seedlings. *Acta Phytophysiol Sin* 24:347–353
- Mi G, Chen F, Zhang F (2008) Multiple signaling pathways control nitrogen-mediated root elongation in maize. *Plant Signal Behav* 3:1030–1032
- Mihailovic N, Vucinic Z, Sukalovic HT (2015) Ammonium enables aluminum-induced stimulation of nitrogen assimilation in roots of Al-tolerant maize genotypes. *J Plant Nutr* 38:371–383
- Mishra BS, Singh M, Aggrawal P, Laxmi A (2009) Glucose and auxin signaling interaction in controlling *Arabidopsis thaliana* seedlings root growth and development. *PLoS One* 4(2):e4502. doi:10.1371/journal.pone.0004502
- Miwa I, Okuda J, Maeda K, Okuda G (1972) Mutarotase effect on colorimetric determination of blood glucose with β -D-glucose oxidase. *Clin Chim Acta* 37:538–540
- Miyazawa Y, Sakai A, Miyagishima S, Takano H, Kawano S, Kuroiwa T (1999) Auxin and cytokinin have opposite effects on amyloplast development and the expression of starch synthesis genes in cultured bright yellow-2 tobacco cells. *Plant Physiol* 121:461–469
- Moubayidin L, Mambro RD, Sabatini S (2009) Cytokinin-in-Auxin Cross Talk. *Trend. Plant Sci* 14:557–562. doi:10.1016/j.tplants.2009.06.010
- Nishina K, Takenaka C, Ishizuka S (2009) Relationship between N₂O and NO emission potentials and soil properties in Japanese forest soils. *Soil Sci Plant Nutr* 55:203–214
- Oda A, Yamamoto F (2002) Effects of aluminum on growth and biomass allocation of hydroponically cultured *Quercus acutissima*, *Cinnamomum comphora* and *Eucalyptus viminalis* seedlings. *J Tree Health* 6:99–103
- Oh HJ, Park JE, Park YG, Jeong BR (2014) Growth and quality of plug seedlings of three indigenous medicinal plants as affected by ionic strength of the nutrient solution. *Hort Environ Biotechnol* 55(2):63–69
- Okazaki M, Matui K (1993) Environmental soil science. Aasakura Pub Co Ltd, Tokyo
- Påhlsson AB (1990) Influence of aluminium on biomass, nutrients, soluble carbohydrates and phenols in beech (*Fagus sylvatica*). *Physiol Plantarum* 78:79–84
- Pegtel DM (1987) Effect of ionic Al in culture solutions on the growth of *Arnica montana* L. and *Deschampsia flexuosa* (L.) Trin. *Plant Soil* 102:85–92
- Radin JW (1974) Distribution and development of nitrate reductase activity in germinating cotton seedlings. *Plant Physiol* 53:458–463
- Rolland F, Moore B, Sheen J (2002) Sugar sensing and signaling in plants. *Plant Cell* S185–S205. doi:10.1105/tpc.010455
- Rotisch T, González MC (2004) Function and regulation of plant invertases: sweet sensations. *Trend Plant Sci* 9(12):606–613. doi:10.1016/j.tplants.2004.10.009
- Ruffel S, Gojon A, Lejay L (2014) Signal interactions in the regulation of root nitrate uptake. *J Exp Bot* 65(19):5509–5517. doi:10.1093/jxb/eru321
- Sahulka J (1972) The effect of exogenous IAA and kinetin on nitrate reductase, nitrite reductase and glutamate dehydrogenase activities in excised pea roots. *Biol Plantarum* 14:330–336
- Smeeckens S (2000) Sugar-induced signal transduction. *Annu Rev Plant Phys* 51:49–81
- Tomioka R, Takenaka C (2007) Enhancement of root respiration and photosynthesis in *Quercus serrata* Thunb. seedlings by long-term aluminum treatment. *Environ Sci* 14(3):141–148
- Tomioka R, Oda A, Takenaka C (2005) Root growth enhancement by rhizospheric aluminum treatment in *Quercus serrata* Thunb. seedlings. *J Forest Res* 10:319–324. doi:10.1007/s10310-005-0152-0
- Tomioka R, Uchida A, Takenaka C, Tezuka T (2007) Effect of aluminum on nitrate reductase and photosynthetic activities in *Quercus serrata* Seedlings. *Environ Sci* 14(3):157–165
- Tomioka R, Takenaka C, Maeshima M, Tezuka T, Kojima M, Sakakibara H (2012) Stimulation of root growth induced by aluminum in *Quercus serrata* Thunb is related to activity of

- nitrate reductase and maintenance of IAA concentration in roots. *Am J Plant Sci* 3:1619–1624. doi:[10.4236/ajps.2012.311196](https://doi.org/10.4236/ajps.2012.311196)
- Trouverie J, Chateau-Joubert S, Thévenot C, Jacquemot MP, Prioul JL (2004) Regulation of vacuolar invertase by abscisic acid or glucose in leaves and roots from maize plantlets. *Planta* 219:894–905
- Tsuji M, Kuboi T, Konishi S (1994) Stimulatory effects of aluminum on the growth of cultured roots of tea. *Soil Sci Plant Nutr* 40:471–476
- Watanabe T, Osaki M, Tadano T (1998) Effects of nitrogen source and aluminum on growth of tropical tree seedlings adapted to low pH soil. *Soil Sci Plant Nutr* 44:655–666
- Yamamoto K (2000) Estimation of the canopy-cap size using two photographs taken at different heights. *Ecol Res* 15(2):203–208. doi:[10.1046/j.1440-1703.2000.00341.x](https://doi.org/10.1046/j.1440-1703.2000.00341.x)
- Yuan TT, Xu HH, Zhang KX, Guo TT, Lu YT (2014) Glucose inhibits root meristem growth via *ABA INSENSITIVE 5*, which represses PIN1 accumulation and auxin activity in *Arabidopsis*. *Plant Cell Environ* 37:1338–1350