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Ikeda, Motoki
Laboratory of Plant Nutrition, Department of Agricultural Chemistry, Faculty of Agriculture, Kyushu University

Yamanishi, Tomoharu
Laboratory of Plant Nutrition, Department of Agricultural Chemistry, Faculty of Agriculture, Kyushu University

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Accumulation of Nitrogen Supplied as Ammonium in the Root Tips of Aluminum-Stressed Wheat Cultivars Differing in Aluminum Sensitivity

Motoki Ikeda and Tomoharu Yamanishi

Laboratory of Plant Nutrition, Department of Agricultural Chemistry, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan

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Ammonium labeled with ^{15}N was supplied for two days to two wheat (*Triticum aestivum* L.) cultivars treated with Al. At a low Al concentration, root elongation was greatly inhibited by treatment for 24 hours in cultivar Chikushikomugi, while in cultivar Shirosanjyaku, significant inhibition of root elongation required treatment for 48 hours. Decreases in pH values of nutrient solution were greater in Shirosanjyaku than Chikushikomugi and smaller at a higher Al concentration in both the varieties. These results indicate that uptake of ammonium was more strongly inhibited by Al in Chikushikomugi than Shirosanjyaku. Accumulation of newly absorbed nitrogen (^{15}N) was decreased at a low Al concentration in the apical part of root tip (0–12 mm) of Chikushikomugi. In Shirosanjyaku treated with a low concentration of Al, accumulation of ^{15}N increased rather than inhibited. At a high Al concentration, accumulation of ^{15}N slightly decreased in the root tip of Shirosanjyaku. Accumulation of ^{15}N in four segments dissected from a root tip was differently affected between Shirosanjyaku and Chikushikomugi. It is considered that Shirosanjyaku is able to maintain active nitrogen metabolism in the root tip under Al-stress conditions compared to Chikushikomugi.

INTRODUCTION

The most adverse factor related to growth inhibition of plants in acid soils is aluminum (Al) toxicity (Marschner, 1995). The distinct and rapid effect of Al appears as the inhibition of root elongation (Barcelo *et al.*, 1996). Differential Al tolerance of wheat cultivars has been widely demonstrated (Polle *et al.*, 1978). Tolerant wheat cultivars are able to excrete malate to chelate toxic Al species in low pH media and thereby to protect root apices from Al toxicity (Delhaize *et al.*, 1993). On the other hand, it is well known that Al affects uptake and transport of essential mineral elements such as K, Ca, Mg and P in crop plants including wheat (Taylor and Foy, 1985; Ohki, 1985; Huang and Grunes, 1992). With regard to uptake of N, relationships between varietal differences in Al tolerance and N sources were extensively investigated in soybean (Klotz and Horst, 1988), wheat (Taylor, 1988), and sorghum (Galvez and Clark, 1991) because nitrate and ammonium are able to differentialy change solution pH after uptake of them and thereby to change the concentrations of toxic Al species in culture solution. However, the preference of individual varieties to ammonium or nitrate did not always connect sensitivity to Al of those plants. When Al was added to culture solution, uptake of nitrate was inhibited in sorghum (Keltjens, 1988), maize (Durieux *et al.*, 1993) and wheat (de Andrade *et al.*, 1996), but it was enhanced in an Al-sensitive barley cultivar (Nichol *et al.*, 1993). In soybean, short-term exposure to Al inhibited the uptake of $^{15}NO_3^-$ to a similar extent between two cultivars with different Al-tolerance (Lazof *et al.*, 1994).

When plants were supplied with ammonium in nutrient media, uptake of ammonium was inhibited by Al in barley (Nichol *et al.*, 1993) and rice (Hai *et al.*, 1989) but it was not affected in sorghum (Keltjens, 1988), wheat (de Andrade *et al.*, 1996) and maize (Calba and Jaillard, 1997). Inhibition of absorption of inorganic nitrogen by Al might lead to less accumulaton of nitrogen in roots and shoots. Although nitrogen is required at sufficient amounts for maintenance of vital metabolic activity in roots, little work dealt with nitrogen accumulation under Al-stress conditions in root tips, which are the primary site of action for Al toxicity. In the present study we investigated accumulation of nitrogen supplied as ammonium in simple nutrient solution in the root tip sections of two wheat varieties that have different sensitivity to Al and had been imposed by Al stress.

MATERIALS AND METHODS

Seeds of two cultivars of wheat (*Triticum aestivum* L.), Al-sensitive cultivar Chikushikomugi (CK) and Al-tolerant one Shirosanjyaku (SS), were sterilized in NaClO solution (1% active chlorine) for 60 min, washed with deionized water, and placed in a petri dish at 20 °C in the dark for two days. Sixteen germinating seedlings were transplanted onto the plastic mesh sheet of a floating raft, and were cultured in a 5.5 L vat containing aerated 0.2 mM $CaCl_2$ (pH 4.5) solution in a growth chamber (20 °C, 70% relative humidity, 250 μmol m^{-2} s^{-1} photon flux density) of the Biotron Institute, Kyushu University. For treating wheat plants with Al, parts of the rafts were transferred into 0.2 mM $CaCl_2$ solution (pH 4.5) containing 10 μM and 100 μM $AlCl_3$, and plants on the rafts were grown for one day, two days and three days prior to the supply of ^{15}N-ammonium. Control plants were cultured in 0.2 mM $CaCl_2$ solution (pH 4.5) for six days.

Eight days after germination, all plants on rafts were washed with deionized water and then with 0.2 mM $CaCl_2$ solution three times. Those plants were grown in 500 mL of aerated solution of 0.2 mM $CaCl_2$ solution (pH 4.5) containing 1 mM $(^{15}NH_4)_2SO_4$ (30.5 atom % ^{15}N) for two days. Harvested roots were washed with running water and then deionized water to well remove ammonium from the root surface. Then, the length of the longest primary root of each plant was measured with a rule, and the pH of each nutrient solution was measured by the glass electrode method. Plants were frozen, lyophilized and stored in a desiccater. The longest primary root of each plant was dissected with a razor at 2, 6, 9 and 12 mm from the apex of the root.

Nitrogen content of the root segment was quantified by the method of Heberer *et al.* (1985). Briefly, the segment was digested in H_2SO_4 with sequential additions of 30% H_2O_2. An aliquot of the digest was used for colorimetric determination of NH_4-N by the indophenol method. For ^{15}N analysis, nitrogen contained in the root segment was gassified to N_2 with both CaO granules and CuO wires in a vacuum discharge glass tube by Dumas' method (Kumazawa and Goleb, 1969). Abundance of ^{15}N was measured by emission spectrometry with a NIA-1 analyzer (Jasco Co., Hachioji, Japan).

RESULTS

Aluminum treatment caused the inhibition of root elongation in two wheat varieties (Table 1). The higher concentration of Al and the longer treatment period was, the

Table 1. Effects of Al concentration and treatment period on root elongation of wheat cultivars differing in Al tolerance and medium-pH decreases due to ammonium uptake.

Cultivar	Al concentration (μM)	Treatment period (h)	Root length (mm)	pH decrease
Chikushi-komugi	0	0	104 a	0.98
	10	24	85 b	0.87
	10	48	82 b	0.83
	10	72	77 c	0.67
	100	24	85 b	0.52
	100	48	77 c	0.46
	100	72	58 d	0.53
Shiro-sanjyaku	0	0	83 a	1.01
	10	24	75 ab	0.89
	10	48	68 bc	0.82
	10	72	63 cd	0.76
	100	24	71 bc	0.71
	100	48	67 bc	0.71
	100	72	56 d	0.59

Means followed by the same letter in each cultivar are not significantly different at the 5% level according to Duncan's multiple range test.

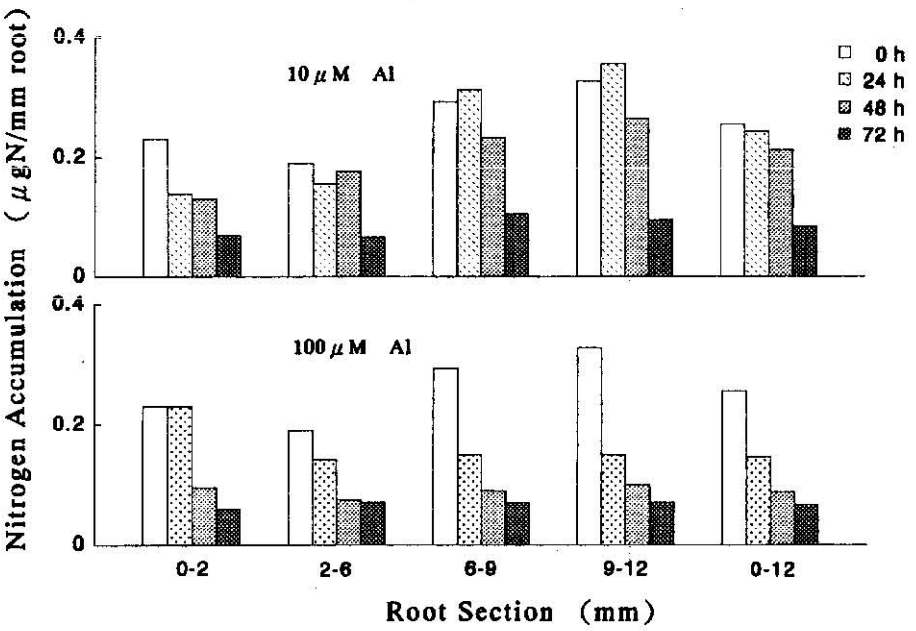

Fig. 1. Effect of Al concentration and treatment period on the accumulation of nitrogen supplied as ammonium in the root tip of Al-sensitive wheat cultivar, Chikushikomugi.

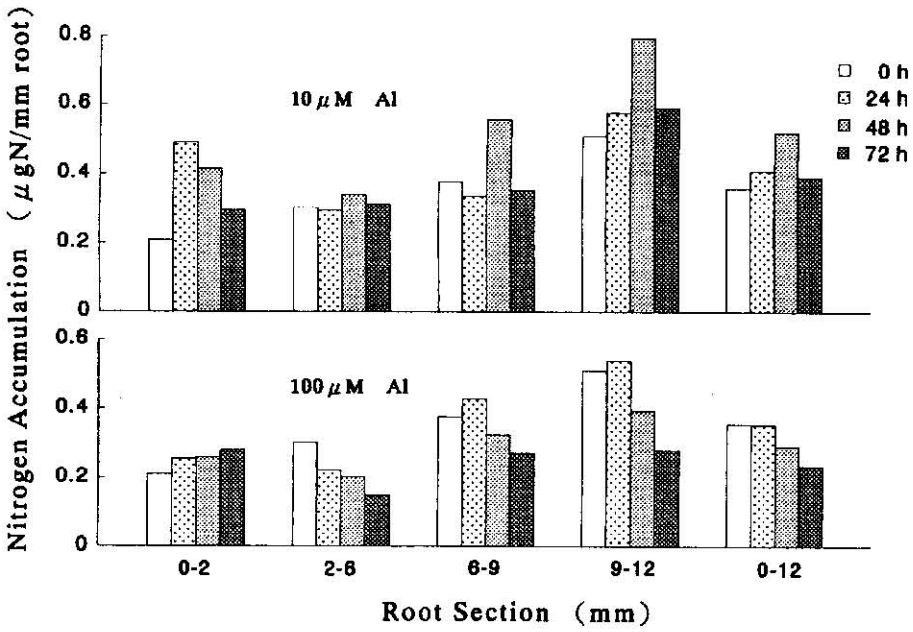

Fig. 2. Effect of Al concentration and treatment period on the accumulation of nitrogen supplied as ammonium in the root tip of Al-tolerant wheat cultivar, Shirosanjyaku.

shorter root length was. Root elongation was inhibited more greatly by Al treatment in CK plants than SS plants. Decreases in pH values of nutrient solution due to ammonium uptake became smaller by treatment with higher concentrations of Al and for longer periods (Table 1). In Al-stressed plants, pH values more greatly decreased in SS plants than CK plants.

Accumulation of nitrogen (^{15}N) absorbed as ammonium in each section of the tips of primary roots was shown in Figs. 1 and 2. Accumulation of ^{15}N per unit root length was basipetally larger in the root tips of control plants of both the cultivars, partly depending on the diameter of each section. Slightly larger amounts of ^{15}N were present in each section of SS plants than that of CK plants.

In CK plants treated with 10μM Al, accumulation of ^{15}N in the apical section (0–2 mm) was suppressed even by treatment of 24 h and greatly suppressed by treatment of 72 h while that in basal sections was only slightly affected by treatment of 24 h and 48 h but greatly decreased in all the sections of roots treated for 72 h. When CK plants had been treated with 100μM Al for longer treatment periods the severe suppression of accumulation of ^{15}N arose in all the sections.

When SS plants had been treated with 10μM Al, accumulation of ^{15}N was not suppressed but increased or almost equal in all the sections. In SS plants treated with 100μM Al, accumulation of ^{15}N always increased in the apical section and decreased in

other sections of roots treated for 48 h and 72 h.

When plants were treated with 100 μM Al for 72 h, accumulation of ^{15}N decreased only by 34% in the whole root section (0–12 mm) of SS plants while it did by 74% in that of CK plants (Figs. 1 and 2).

DISCUSSION

It is confirmed from results on the inhibition of root elongation that cultivar SS is more tolerant to Al than cultivar CK (de Andrade *et al.*, 1996). The pH changes directly reflect uptake of ammonium by plants from ammonium media (Breteler, 1973). Therefore it is considered that uptake of ammonium by whole plants might be reduced by the treatment with Al and the extent of its inhibition might be greater in CK plants than SS plants. At the same time, the total accumulation of newly absorbed nitrogen in a root tip was more greatly inhibited in CK plants than SS plants. In contrast, de Andrade *et al.* (1996) reported that the cumulative uptake of ammonium for 12 days was little affected even in the presence of 120 μM Al in the same varieties as examined in the present study. This might result from that de Andrade *et al.* (1996) used 15–day–old wheat plants, which might be less injured by Al and/or absorb all of added ammonium from the nutrient solution during each culture period since ammonium absoption was not completely inhibited by Al.

Because in the present experiment wheat plants were treated with Al and thereafter supplied with ^{15}N–labeled ammonium in nutrient media without Al, an interaction between NH_4^+ and Al in nutrient media can be ruled out in the present experiment. As shown by limited root elongation due to Al stress, less root mass may be responsible for reduced absorption of ammonium from nutrient media. However, it cannot be excluded that functions for absorption of ammonium also might be hampered by prior exposure of roots to Al. In rice plants a sensitive variety took up less ammonium and acidified less the culture solution containing Al than a resistant one did (Hai *et al.*, 1989).

Aluminum stress differentialy affected accumulation of newly absorbed nitrogen in each position of wheat root tips, indicating that nitrogen metabolism in those tissues might be differently disturbed in different positions of a root by the prior Al treatment. The root tip of CK plants was more fragile in nitrogen metabolism than that of SS plants. Reduced accumulation of ^{15}N in the root tip of CK plants might be basically due to strong suppression of ammonium uptake compared to SS plants (Table 1), and partly to inhibition of cell activity caused by Al stress (Marschner, 1995). On the other hand, in SS plants weak Al stress could rather enhance accumulation of newly absorbed nitrogen in the root tip even though ammonium uptake by whole plants was suppressed to a lower extent. At the present time we have no evidence for explanation of this result, but it can be pointed that morphological changes, i.e. swelling of the root tip or inhibited translocation of nitrogen will be responsible for such unexpected accumulation of newly absorbed nitrogen in the root tip of SS plants.

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