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Introduction

Via-PIXE (Vertical-beam in-air particle-induced X-ray emission) is one of non-destructive analytical techniques that can observe *in situ* elemental distribution of biological samples¹. The elemental compositions of living organs, tissues and cells relate to those physiological statuses. We applied Via-PIXE method to analysis of short-term changes in elemental composition of plant root surface by aluminum stress, which is the major environmental stress factor that decreases crop productivity in acid soils². In our preliminary study, the ratio of potassium to phosphorous (K/P ratio) of alfalfa (*Medicago sativa* L) root-tip was found to be fallen by short-term exposure of root to Al in a solution culture system^{3,4}. We investigated here the relation between K/P ratio and Al stress using two plant species alfalfa and wheat (*Triticum aestivum* L.) which have different susceptibility to Al.

Materials and Methods

Plant material and Al-stressed solution culture system

Young seedlings with 5-mm root(s) of alfalfa (*Medicago sativa* L. natsuwakaba) and wheat (*Triticum aestivum* L. Aoba) were transferred to the Al-stressed (1 mM CaCl₂, 20 μM AlCl₃, pH 4.8) or control (1 mM CaCl₂, pH 4.8) rooting solution. Plants were allowed to grow for 6 h in those solutions at room temperature, followed by Via-PIXE analysis.

Via-PIXE analysis

The details of Via-PIXE system in FNL (Fast Neutron Laboratory, Tohoku University) was described in Iwasaki et al.¹. Energy of accelerated protons was 3 MeV and beam flux was in the range of 200-600 pA. Special attentions for living plant materials were

paid as described in Yokota et al.⁴⁾. Briefly, samples were placed on a ashless-type filter paper (Advantec No. 6, Advantec Toyo, Tokyo) which was moistened with distilled water in order to avoid living samples from drying. According to this method, roots of alfalfa and wheat were kept alive within a measurement. The net X-ray counts corresponding to P and K were estimated using SAPIX program developed by Sera et al.⁵⁾.

Results and discussion

Inhibition of root elongation by Al commonly appears within minutes or hours after exposure of root to Al^{6,7)}. Despite there have been many studies on Al phytotoxicity, its mechanism is still unclear. As one of indexes of physiological status of living cells change in elemental composition is supposed to give information of effects of Al on cells. Then we tried to analyze *in situ* elemental composition of root surface cells treated with Al by Via-PIXE method.

Figure 1 shows Via-PIXE spectra of tip and basal region of alfalfa and wheat root treated with or without Al. Most apparent change by Al was found in relative count of P and K. In alfalfa and wheat, K/P ratios of root-tip or basal region without Al were almost equivalent (Fig. 2). The K/P ratios in Al-treated alfalfa root-tip and basal region were less than the others. Adversely, increase of K/P ratio was observed in the case of Al-treated wheat root (Fig. 2).

Potassium is one of macronutrients for plants and has important roles in growth and development of cells as a regulator of turgor pressure and enzymic activities. Phosphorus concerns almost all of biological processes in cells. The change in K/P ratio suggests the relation between Al and cellular homeostasis, however, we have to know absolute amount of those elements that is expected to show dynamic movement of elements within a root.

In this study, the advantages of Via-PIXE method were reevaluated. Based on data and techniques obtained here, the next approach will be trial of *in situ* mapping of root surface by in-air scanning PIXE method.

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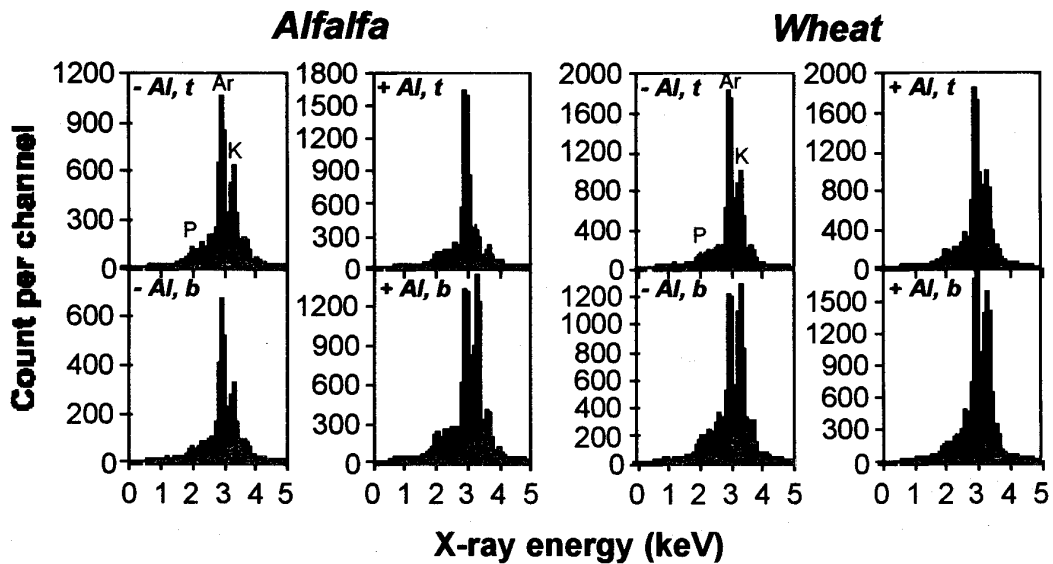


Fig. 1. Via-PIXE spectra of alfalfa root (left panel) and wheat root (right panel) grown with Al (+Al) or without Al (-Al). Both tip region (t) and basal region (b) were analyzed. Peaks corresponding to phosphorus, argon and potassium were indicated as P, Ar and K, respectively. Each spectrum was obtained within 6 min after exposure of root to the 3-MeV proton beam.

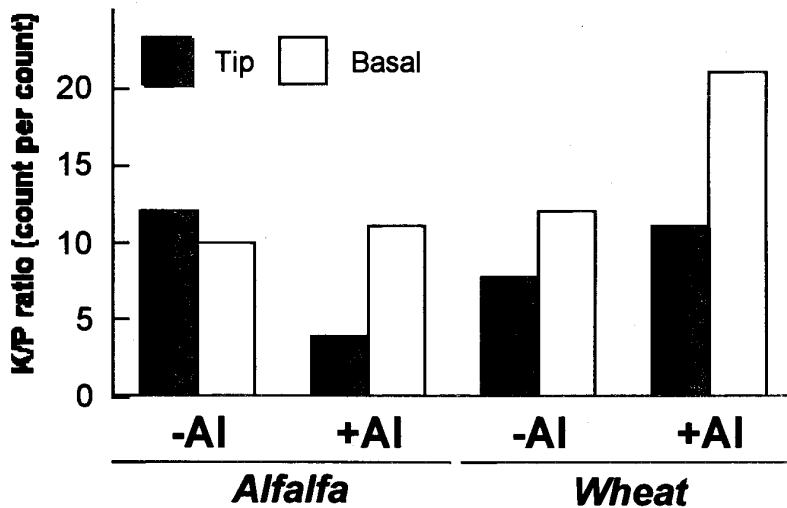


Fig. 2. Effect of Al on the K/P ratio of tip (closed column) or basal (open column) region of alfalfa and wheat root. The K/P ratios (count per count) were calculated using the Via-PIXE data in Fig. 1. For details, see "Materials and methods".