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Rapid screening for Al-tolerant and -sensitive cultivars in barley (*Hordeum vulgare* L.)

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Objectives

Aluminum (Al) toxicity is the major factor limiting plant growth in the acid soils that comprise large agricultural areas. In particular, inhibition of root growth in the root apex caused by an Al-induced impairment of cell division and elongation is a well-known early and dramatic symptom of Al phytotoxicity. A rapid and reliable screening is needed to discriminate sensitive and resistant genotypes. Here, we report a rapid screening method in nutrient solution based on root growth elongation rate(GEA), Al content and Hematoxylin staining(HS) method as a useful approach macroscopically detecting Al accumulation in root apex.

Materials and Methods

Five-day-old seedlings were transferred to $CaCl_2$ solution (pH 4.5) with 20μM Al treatment. The primary root length of seedlings was measured with a ruler at 24h. In a parallel experiment, seedlings were washed in double-distilled water (DDW) for 5 min, stained with a 0.1% (w/v) aqueous solution of hematoxylin for 10 min, washed in DDW and photographed using a light microscope. Also, the roots were washed three times with DDW, and the root tips (0-10 mm) were excised using a razor blade and transferred to 1.5-mL Eppendorf tubes each containing 1 mL of 2 M HCl for 48 h. The Al content in the HCl digest of root tissue was determined by an atomic absorption spectrophotometer after dilution.

Results and Discussion

First of all root growth measurement at 20 μM Al treatment after 24 h (Fig. 1) was used to select Al tolerant and sensitive in 65 cultivars and we have selected 6 cultivars. Three parameters, GEA (Fig. 2), HS (Fig. 3) and Al content (Table 1), were used to confirm Al tolerant and sensitive in barley seedlings. The phenotypes correlation between GEA and HS for 6 cultivars showed a negative trend (Fig. 2 and 3). However, in the tolerant cultivars there was a small difference of GEA and less HS compare to sensitive cultivars. In agreement with growth pattern after 24 h Al treatments, higher Al accumulations were found in sensitive cultivars (Table 1). Our results lead us to conclude that GEA and HS are a suitable approach to the rapid screening of barley seedlings without destroying the root but Al content also need to confirm it. Now we have selected representative cultivars one for tolerant and the other for sensitive. Further experiments will be done to elucidate Al-tolerant and -sensitive mechanisms using selected two cultivars above.

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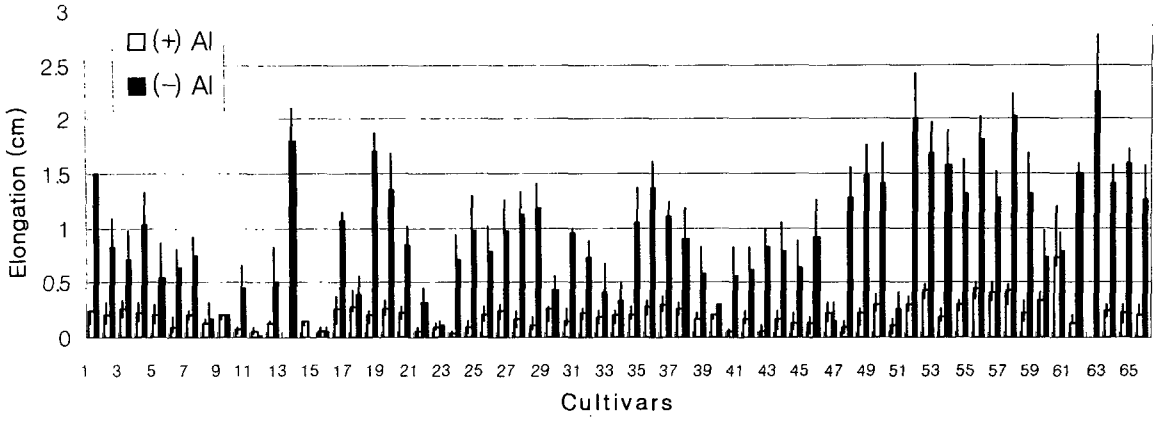

Fig. 1. Effects of 20μM Al supply on the root elongation rate of barley roots after 24 h.

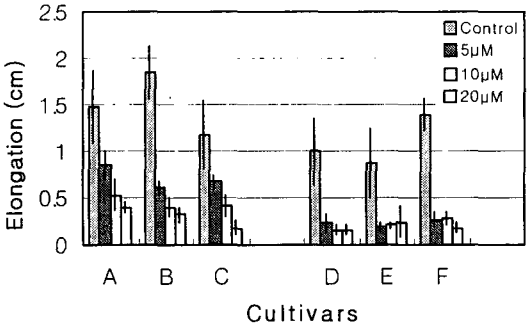

Fig. 2. Effects of Al (0, 5, 10, 20μM) supply on the root elongation rate of selected 6 barley cultivar roots after 24 h. A, B, C are tolerant and D, E and F are sensitive.

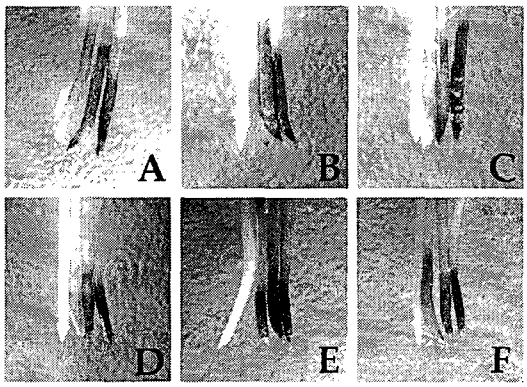

Fig. 3. Hematoxylin-stained roots of selected 6 barley cultivar roots. The plants were grown in the presence or absence of Al (0, 5, 10, 20μM) for 24 h. A, B, C are tolerant and D, E and F are sensitive.

Table 1. Effect of 20μM Al on the Al contents at 10 mm root tip of barley after 24 h Al treatment. Values are mean ± SE.

Cultivars		Al content (nmol/root apex)
Tolerant	A	55.7 ± 2.12
	B	**47.1 ± 1.01**
	C	62.2 ± 3.56
Sensitive	D	**64.9 ± 4.84**
	E	63.5 ± 3.93
	F	63.0 ± 2.67