Title
Screening Arabidopsis Activation Tagged Lines Based on Tolerance to Low Zn in Hydroponics

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Publication Date
2009-04-02

Peer reviewed

INTRODUCTION

Zn is an essential component of hundreds of transcription factors and enzymes including superoxide dismutases and DNA polymersases (Marshner, 1995). Zn deficiency is a major micronutrient problem; about 30% of world's soils are considered Zn deficient (Hacisalihoglu and Kochian, 2003). It appears that use of Zn fertilizers alone is not sufficient to completely eliminate Zn deficiency problem. Furthermore, Zn fertilizers may not be affordable or available in certain developing countries around the world (Hacisalihoglu and Kochian, 2003). Zn efficiency (ZE) is the ability of plants to maintain high yield under low-Zn conditions. However, limited information is known about the mechanisms underlying ZE in plants such as Arabidopsis.

There is growing evidence that considerable genetic variation exists in plants for tolerance to abiotic stress. Genetic differences for ZE in plant species as well as plant genotype collections have been reported in rice (Bowen, 1987), wheat and tomato (Graham and Rengel, 1993), and common beans (Hacisalihoglu et al., 2004).

Arabidopsis is a well-studied model flowering plant system with extensive genomics and genetic information readily available. Activation tagging with T-DNA vectors causing gain of function phenotypes are excellent resources to identify mutants and genes that are tolerant to environmental stress (Weigel et al., 2000).

Despite the studies on physiological mechanisms, information about genetics of plant ZE is limited. Therefore, the objective of this study was to perform a large scale primary screening of 30,000 Arabidopsis activation tagged lines for tolerance to low Zn, and identification of putative mutants for ZE.

MATERIALS AND METHODS

Seeds of ecotype Col (WT) and 30,000 Arabidopsis (*Arabidopsis thaliana*) activation tagged lines including a pSKI15 vector were obtained from Arabidopsis Biology Resource Center (Columbus, OH).

Hydroponic screening was adapted from Hoekenga et al. (2003) with some modification. Seeds were surface sterilized and vernelized at 4 ºC for 4 d. Small magenta boxes with polycarbonate stands and mesh top were used plant growth (Fig. 1A, 1B). Boxes were filled with a hydroponic solution culture containing the following: 1 mM KNO_3, 1mM $Ca(NO_3)_2$, 0.05 mM $NH_4H_2PO_4$, 0.25 mM $MgSO_4$, 0.1 mM NH_4NO_3, 50 μM KCl, 12.5 μM H_3BO_3, 0.1 μM H_2MoO_4, 0.1 μM $NiSO_4$, 0.4 μM $MnSO_4$, 1.6 μM $CuSO_4$, 96μM $Fe(NO_3)_3$-H_3HEDTA, 0.1 pM $ZnSO_4$-H_3HEDTA and 2 mM MES at pH 6.0. Seeds were planted on the mesh above solution level and plants were grown at 24 ºC (light) for 10 d in a growth room. The best grown healthy seedlings were then isolated and transferred to solid agar (0.7 %) media plates contained 1X Murashige and Skoog basal mixture supplemented with 1% sucrose as carbon source for 3 d (Fig. 1C). Seedlings were then transplanted into 10-cm pots filled with soil. The rescued plants were maintained in the growth room, and were watered, fertilized, and grown to seed set (Fig. 1D).

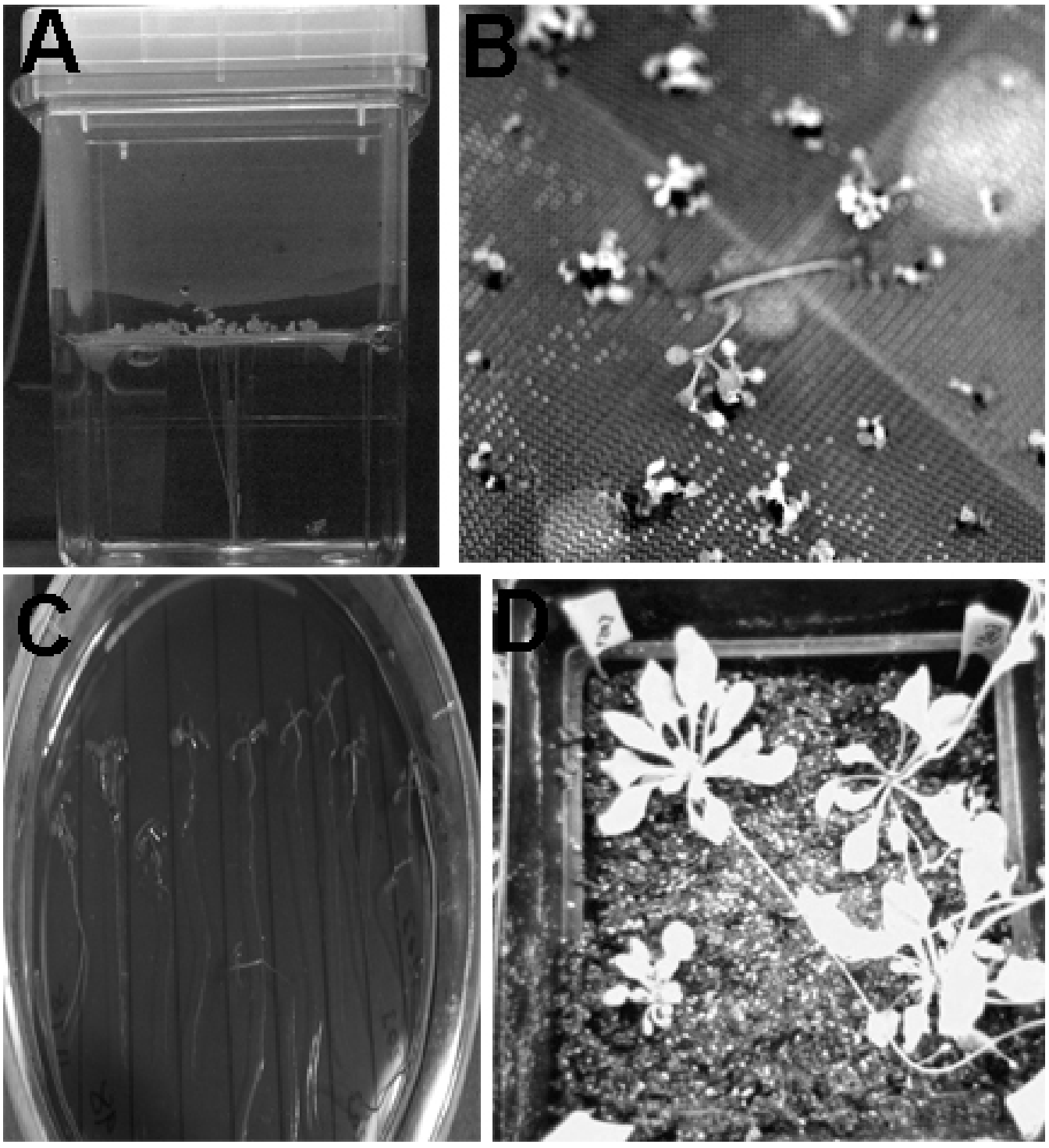

Figure 1. Phenotype of putative ZE mutants identified with hydroponic assay. Seedlings were grown for 10 d in solution culture (A, B), transferred to 0.1% agar media for 5 d (C), and rescued to soil (D) until seed set.

RESULTS AND DISCUSSION

Primary screening was conducted on T2 Arabidopsis lines. Approximately 30,000 T2 lines were screened for enhanced capacity of ZE on our hydroponic assay.

In general, typical Zn deficiency symptoms including stunted growth and leaf chlorosis were observed within 10 d after seed sowing. This was in agreement with previous findings (Hacisalihoglu and Kochian, 2003). Low Zn concentration of 2 pM (as Zn activity) was effective in screening for ZE of Arabidopsis. This is consistent with findings for common bean (Hacisalihoglu et al., 2004) and bread wheat (Hacisalihoglu et al., 2001).

The primary screening of the 30,000 activation tagged lines yielded more than 22 putative Zn efficient mutant seedlings. Next, the putative seedlings were rescued to soil, grown to maturity, and seeds were harvested from individual mutant plants.

In this study, we developed a fast, straightforward and repeatable technique to screen ZE mutants from Arabidopsis. This hydroponic assay saves time and does not require expensive materials or instruments. Our preliminary findings also showed that activation tagged gain of function mutants in Arabidopsis have great potential for improving ZE in plants.

In the future, T3 Arabidopsis progeny obtained from soil grown Arabidopsis seedlings will be re-evaluated as a secondary screening of putative ZE mutants as described in the Materials and Methods section.

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