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Abstract

In acidic soils aluminum toxicity is a major threat for plant growth. In this study, we report the establishment of a novel aluminum stress test for potato based on a liquid in vitro culture system. This stress test system was applied to classify several potato genotypes according to their growth and morphology as well as nutrient concentrations in shoots. Five potato genotypes ('Bintje', 'Desiree', 'Eurobravo', 'Maxi', and 'Piról') and one wild species (*Solanum commersonii*) were first rooted in liquid MS medium, before half of the cultures were submitted to 10 days of aluminum stress by MS medium containing 2.5 mM AlCl_3 at pH 3.05. The other half served as control plants. Dry masses of roots and shoots mostly showed reductions in response to the AlCl_3 treatment. However, the extent of alteration depended on the genotype allowing their differentiation. Impaired nutrient uptake was demonstrated by reduced calcium, magnesium, manganese and zink concentrations in Al-treated shoots of most genotypes, in part contradicting results from previous ex vitro studies.

1. Introduction

Aluminum, making up 8.1% of the earth's crust, is the third most abundant element. Large deposits are found in countries such as India, in the eastern and western regions of the USA, and in Brazil (McFaul et al. 2000). Under neutral soil pH conditions (pH 5-7), aluminum ions are stably bound in the soil and thus unavailable to plants. However, when the soil becomes more acidic (pH below 5), these ions become soluble and toxic to plants. Nearly half of the world's agricultural land is classified as acidic (Kochian et al. 2004). The Al^{3+} ion is particularly toxic to plants (Foy 1988; Parker et al. 1988; Kochian 1995). In acidic soils, aluminum is the primary limiting factor for crop growth, though iron and manganese toxicity may also occur (Marschner 1991).

Plant mechanisms for coping with aluminum in the soil are commonly divided into two strategies: aluminum accumulation and aluminum avoidance. Potato (*Solanum tuberosum*) was categorized as an aluminum avoider (Poschenrieder et al. 2008). In both strategies, the root is the key organ due to its direct contact with aluminum in the soil (Barceló and Poschenrieder 2002). Among avoiders, differences in aluminum uptake can be observed, with more sensitive species tending to absorb more aluminum than tolerant ones (Tabaldi et al. 2007). Another resistance mechanism involves increasing the pH in the rhizosphere, which has been documented in aluminum-tolerant plants (Yang et al. 2011). In a hydroponic system using two wheat cultivars (tolerant vs. sensitive), the tolerant cultivar showed a significantly stronger pH elevation (Yang et al. 2011).

Aluminum toxicity mainly manifests through alterations in the root system, especially through reduced root length (Poschenrieder et al. 2008). Nutrient uptake is also impaired (Tabaldi et al. 2007; Lazarević et al. 2012), with calcium and magnesium uptake being particularly affected (Lazarević et al. 2012). Additionally, reduced levels of zinc and sodium were observed, while manganese and potassium levels increased. These nutrient content changes were found in potato leaves following treatment with $1.85 \text{ cmol}(+) \text{ kg}^{-1}$ aluminum in soil for 49 days (Lazarević et al. 2014).

In this study, we employed a recently developed liquid in vitro culture system (Wellpott et al. 2024) to establish a new aluminium stress test. This stress test system was applied to classify several potato genotypes according to their growth and morphology as well as nutrient concentrations in shoots.

2. Data, Methods and Procedure

2.1. Plant material

Five potato genotypes ('Bintje', 'Desiree', 'Eurobravo', 'Maxi', and 'Piról') and one wild species (*Solanum commersonii*) were selected to examine their differential response to aluminum stress in vitro. All genotypes were available as established in vitro shoot cultures and were maintained on MS (Murashige and Skoog 1962) medium containing 30 g L^{-1} sucrose at pH 5.80. Salts, vitamins, and Plant agar (7.5 g L^{-1}) were supplied by Duchefa (Haarlem, The Netherlands). The cultures were subcultured every three weeks for three months under controlled in vitro conditions ($18 \pm 2 \text{ }^\circ\text{C}$; 16/8 h light/dark cycle; PPFD-PAR: $35 \pm 8 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$).

2.2. Application of aluminum stress

Apical shoot tips after 24 days of growth being at least 1.5 cm in length were used for the stress experiment. Rooting took place in liquid MS medium for 11 days. Aluminum chloride (AlCl_3 ; Sigma-Aldrich, Darmstadt, Germany) was then introduced by replacing the medium with MS medium containing 2.5 mM AlCl_3 , adjusted to a pH of 3.05. Control plants received MS medium without AlCl_3 with the pH value at 5.80. The stress treatment lasted ten days and included 4 replicates (vessels) with 5 shoots each. The whole experiment was carried out with two repetitions. Subsequently, shoot length, and fresh and dry mass of both shoots and roots were measured. For dry mass analysis, shoots were freeze-dried for 48 hours using a Christ Alpha 1-4 LSC freeze-dryer (Christ, Osterode am Harz, Germany). Roots were dried in an oven at $70 \text{ }^\circ\text{C}$ for 48 hours.

2.3. Nutrient analysis

Freeze-dried shoot samples were further analyzed for nutrient content. Samples were ashed overnight at $480 \text{ }^\circ\text{C}$. One ml of HNO_3 and 9 ml of deionized water were added to the ash, and the mixture was filtered using blue band filter paper. The supernatant of 4 replicates (vessels) was analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES; Varian 725-ES ICP, Varian, Ontario, Canada).

2.4. Statistical analysis

All data were analyzed using R version 4.4.2 within RStudio 2024.12.0 (Posit Software, PBC). Graphs were generated using the R package 'ggplot2' (Wickham 2016). Data were transformed using the 'bestNormalize' package (Peterson 2021) to approximate normality prior to statistical analysis. Three-way analysis of variance (ANOVA) was performed including genotype, treatment and repetition as fixed effects and their interactions, followed by a Tukey post hoc test ($p \leq 0.05$) using the 'multcomp' package (Hothorn et al. 2008).

3. Results and Discussion

3.1. Shoot and root dry mass were negatively affected by aluminum

Dry masses of roots and shoots of all analysed potato genotypes showed significant reductions in response to the $AlCl_3$ treatment (Fig. 1). In the first repetition of the experiment,

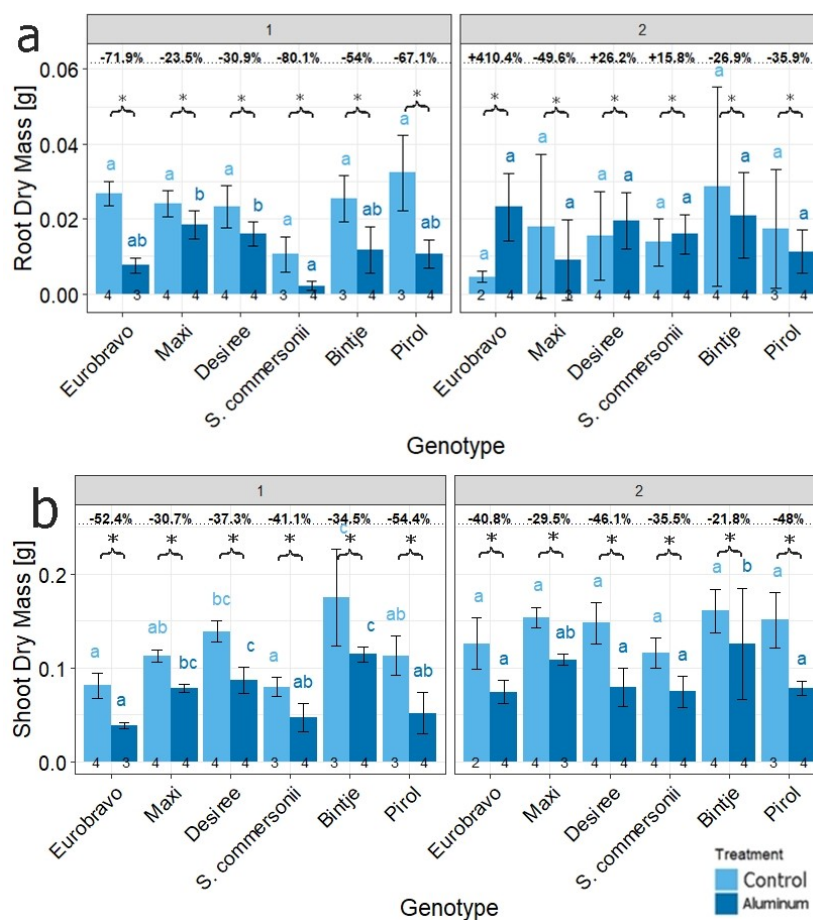


Figure 1. Root (a) and shoot dry masses (b) of five potato genotypes and one wild species with and without the addition of 2.5 mM $AlCl_3$ after 10 days of in vitro culture, shown are two repetitions [1. rep and 2. rep]. Means \pm SD of n replicates (n) indicated on the x-axis. Percentages above the bars indicate the alteration between control and treatment. Light and dark blue letters indicate significant differences within the control and treatment groups, respectively, and asterisks indicate significant differences between treatment and control within the respective genotype. Tukey test at $p < 0.05$, square-root-transformed data.

the root dry mass responded in a genotype-specific way, when comparing among the AlCl_3 -treated plants, as also reported for root length in a hydroponic system by Lazarevic et al. (2014). Genotype-dependent differences were likewise observed in the shoot dry mass analysis across both repetitions (Fig. 1b).

Comparison of root and shoot dry mass analyses revealed that some genotypes, such as 'Eurobravo' and 'Desiree', responded differently between repetitions, whereas the shoot dry mass analysis showed consistent behavior in all genotypes across both repetitions (Fig.1). The root dry mass assessed in this study might be used as a substitute for root length as indicators of aluminum stress. Differences in n number resulted from contamination of vessels during the duration of the experiment. Due to difficulties in the handling of the delicate roots and due to the very low masses being prone to weighing errors, in some cases larger variations occurred as expressed by the high standard deviations.

3.2 Nutrient analysis

Two of four analysed nutrients (calcium and magnesium, Fig. 2a+b) have previously been described to be affected by aluminum stress in potatoes: Lazarevic et al. (2012) reported a decrease of calcium and magnesium content in roots and shoots due to aluminum stress with higher concentrations in roots than in shoots. Our data also proved a decrease in shoots (Fig. 2a+b) which in most cases was significant.

Manganese toxicity was presumed to result from elevated uptake due to aluminum-induced soil acidification (Marscher 1991). We could show a reduced concentration of manganese in most of the AlCl_3 -treated in vitro shoots, therefore a toxicity seems unlikely (Fig. 2c). Only few genotypes displayed significant changes in zinc concentration after the treatment (Fig. 2d). Thus, the postulated reduction of zinc concentrations due to aluminium stress (Lazarevic et al. 2014) could not be verified in this in vitro culture system. This might be due to the different concentrations of AlCl_3 , length of the treatment and different genotypes. Lazarevic et al. (2014) tested twelve genotypes in a hydroponic system with a treatment duration of three days with 25 and 50 $\mu\text{mol AlCl}_3$.

All genotypes showed a significant reduction of the shoot calcium concentration due to the AlCl_3 -treatment with the exception of 'Picol' in the second repetition (Fig. 2 a).

For magnesium, manganese and zinc 'Eurobravo' showed over both repetitions contrary reactions, but also 'Maxi' in manganese and 'Bintje' in zinc showed contrary reactions.

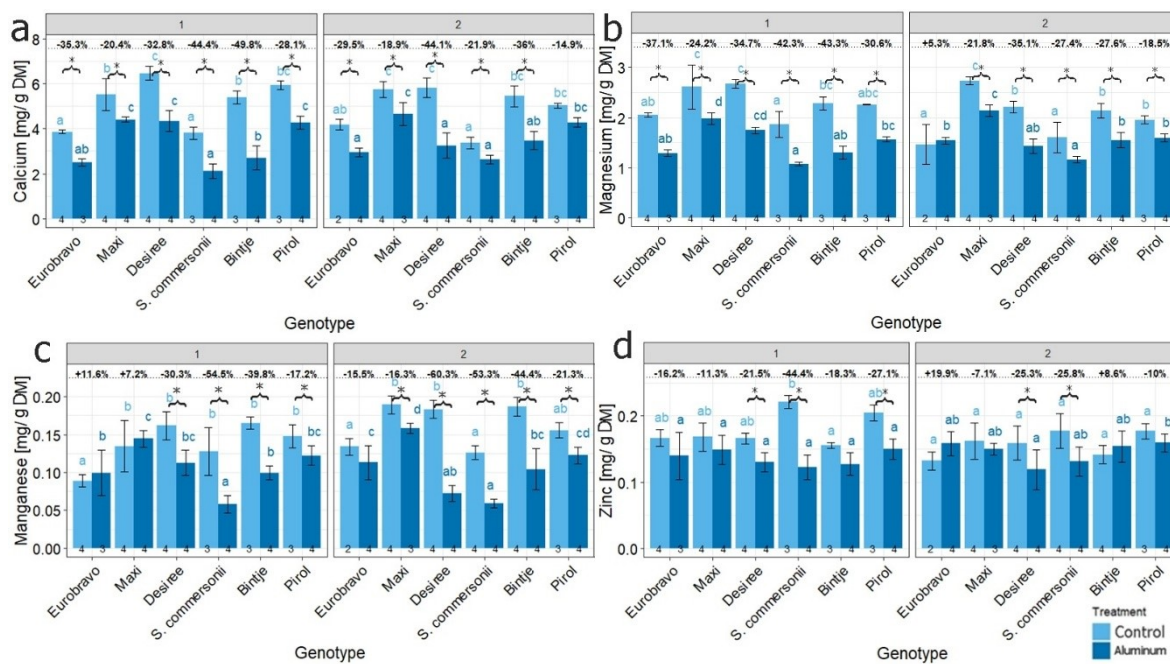


Figure 2. Nutrient concentrations in shoots of five potato genotypes and one wild species after 10 days of in vitro cultivation with and without the addition of 2.5 mM AlCl_3 , shown for two repetitions [1. rep and 2. rep]. Means \pm SD with replicates (n) indicated on the x-axis.

Light blue bars = control, dark blue bars = AlCl_3 treatment. Percentages above the bars indicate the alteration between control and treatment. Light and dark blue letters indicate significant differences within the control and treatment groups, respectively, and asterisks indicate significant differences between treatment and control within the respective genotype. Tukey test at $p < 0.05$. (a) calcium concentration with orderNorm-transformed data, (b) magnesium concentration with square-root-transformed data, (c) manganese concentration with exponentially-transformed data, (d) zinc concentration with orderNorm-transformed data.

4. Conclusions

Most previously published aluminum stress tests took place in hydroponic cultures (Tabaldi et al. 2007; Lazarevic et al. 2012; Lazarevic et al. 2014), whereas here we demonstrated that the liquid in vitro system developed by Wellpott et al. (2024) for osmotic stress could also be used for aluminum stress tests. The in liquid in vitro system allows aluminum stress tests under pathogen-free and highly standardized conditions with the possibility to apply aluminum at a specific time point. The reduced root length used as a biomarker in hydroponics might be interchangeable with the root mass, since we could show genotype-dependent reductions. However, this would need a comparison of both test systems involving the same genotypes. Furthermore, manganese and zinc could be useful for screening of different genotypes for tolerance to aluminum stress. Both nutrients exhibited variation across the genotypes, which may aid in identifying genotypes with higher or lower sensitivity. The changes in the nutrients magnesium and manganese suggest 'Maxi' to be more tolerant and 'Bintje' to be more sensitive to aluminum application in our test system. However, further analysis will be needed to evaluate the findings and should include more genotypes for which ex vitro tolerance or sensitivity has been described.

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