

High throughput aluminium toxicity tolerance screening in lentil to expand production area

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Lentil (*Lens culinaris* ssp. *culinaris*) is one of the oldest domesticated crops, and serves as a valuable source of dietary proteins, minerals, fibre and carbohydrates. In Australia, ~50 Mha of the agricultural soils have a surface pH < 5.5, and 12-24 Mha have pH < 4.8. Major grain crops and pulses are affected by acid soils and annual total losses to agriculture is estimated to be AU \$900–1585 million. Lentil is sensitive to soil acidity and its annual production in Australia comes mainly from north-western Victoria and South Australia. In acid soils with pH below 5.5, phytotoxic forms of Aluminium (mainly Al³⁺) become available and inhibit root growth, resulting in reduced yields. Development of lentil varieties with Al toxicity tolerance will be the most efficient and economical way to manage acid soil limitations. The results of this research will enable breeding programmes to use AGG germplasm to produce new, more productive lentil varieties and will enable lentil production to be expanded into other areas of Australia with acid soil conditions. In this study a high-throughput hydroponic screening method was developed for Aluminium (Al) toxicity tolerance screening at the seedling stage for lentil. Diverse germplasm of 111 lines including Focused Identification of Germplasm Strategy (FIGS) lines, local landrace varieties and adapted lines from 18 countries were obtained from Australian Grains Genebank (AGG). Uniform 4-day old seedlings were treated in three-day 5µM Al treatment and relative root growth (RRG%) was assessed. Results showed significant variation for Al toxicity tolerance with 26% of the lines with higher RRG% than the known tolerant line ILL6002 (37.9%). Very tolerant lines AGG70137 and AGG70256 with RRG% greater than 52% were identified in the FIGS set. Histochemical analysis supported the hydroponic results with the tolerant lines AGG70137 and Northfield showing less Al accumulation, plasma membrane damage and lipid peroxidation compared to the sensitive Precoz and AGG70530 lines. Screening of a subset of lines in an acid soil in growth chamber differentiated some tolerant and sensitive lines for all the traits tested based on relative performance (as % limed treatment) but these results were not statistically significant due to the complexity of interactions in a soil-based system. Medium correlation ($r^2 = 0.58$, $p < 0.001$, $n = 15$) obtained with RRG% and number of lateral roots showed the effect of Al toxicity in acid soil is more prominent in sensitive lines than the tolerant lines. This work has developed a high throughput screening method for Al toxicity tolerance for lentil and identified new tolerant germplasm lines. This germplasm will be evaluated with a

Genome Wide Association Study (GWAS) to identify potential markers/genes for AI toxicity tolerance.