A *Medicago Truncatula* Population Segregating for Aluminum Tolerance

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Introduction Aluminium (Al) toxicity, manifested in inhibition of root elongation and reduced plant growth, is a major cause of poor crop yields on acid soils, which comprise up to 40% of the world's arable land. Al toxicity associated with acid soils has been a major obstacle in alfalfa (*Medicago sativa*) production in the USA, as well as in tropical areas of the world. Recent molecular marker mapping studies indicate that the genomes of *M. truncatula* and *M. sativa* are highly similar (Choi *et al*., 2004). Thus, *M. truncatula* could be used as a source of genes that could be used to improve Al tolerance of cultivated alfalfa. The objective of this study is to identify QTL for Al tolerance in *M. truncatula*, using *M. truncatula* EST-SSR markers and a population from a cross between the Al sensitive Jemalong A17 and an Al tolerant USDA plant introduction, PI 566890 (Sledge *et al*., 2004), with the long term goal of cloning Al tolerance genes to improve cultivated alfalfa for Al tolerance.

Materials and methods Jemalong A17 was crossed to PI 566890 using a dissecting microscope. Flowers were opened at an early bud stage, vacuum emasculated by attaching a micropipette to a vacuum hose, and pollen was applied from another flower, using a pair of fine forceps. The flower was then placed into a 50 ml plastic centrifuge tube with a few drops of water to prevent desiccation, and the tube was stopped with cotton. After 5-7 days, the developing seed pod was removed from the centrifuge tube and allowed to develop. Seed pods were harvested when completely dried and brown in colour. An F_1 individual was obtained, and was allowed to self-pollinate to produce the F_2 mapping population. Development of EST-SSR markers from the *M. truncatula* EST-databases has been previously described (Eujayl *et al*., 2003). Forward and reverse primers were custom synthesized with the additional 18 nucleotides from the M13 universal primer appended to the 5' end of the forward primer. PCR reactions were prepared according to the protocol of Schuelke (2000) with the following modifications. The total reaction volume of 10µl contained 20ng of template DNA, 2.5mM $MgCl_2$, 1X PCR buffer II (Perkin-Elmer), 0.15mM dNTPs, 1 pmol of each reverse and M13 universal primer, 0.1pmol of the forward primer, and 0.5 U Ampli Taq Gold DNA polymerase (Perkin Elmer). The M13 universal primer was labeled either with blue (6-FAM), green (VIC), yellow (NED) or red (PET) fluorescent tags. PCR reactions were run in a 384 well plate format, and PCR products with different fluorescent labels and with different fragment sizes were pooled for detection. PCR products (1.6µl) were combined with 10µl of deionized formamide and 0.5µl of GeneScan-500 LIZ internal size standard and analyzed on the ABI3730 Capillary Genetic Analyzer (PE Applied Biosystems). The SSR fragments were visualized and scored using GeneMapper software (PE Applied Biosystems). A linkage map is being constructed using JoinMap 3.0 software (Van Ooijen, 2001). The population was analyzed as a cross-pollinated (CP) population, using the Kosambi mapping function. Expected segregation ratios of 1:2:1 were verified by χ^2 analysis.

Results A hydroponics study was used to identify Al tolerant and Al sensitive genotypes of *M. truncatula*. Jemalong A17 was selected as the sensitive parent, and PI 566890 as the tolerant parent. Two genotypes from these accessions were crossed, and the hybrid nature of the F_1 was confirmed with EST-SSR markers. Preliminary results indicate that approximately 30% of all primers tested in the population are polymorphic, making this a suitable mapping population. Linkage groups are being constructed.

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