ALUMINUM TOLERANCE QTL IN DIPLOID ALFALFA

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ABSTRACT
Aluminum (Al) toxicity associated with acid soils greatly inhibits alfalfa (*Medicago sativa* L.) productivity throughout much of the world's major grassland areas. In this paper, we report the identification of quantitative trait loci (QTL) controlling aluminum tolerance in diploid alfalfa (*Medicago sativa* L). An in vitro callus growth bioassay was used to select aluminum tolerant and aluminum sensitive parents, and to screen an F2 population for aluminum tolerance. Fifty-eight cDNA probes were mapped to nine linkage groups, and the F2 genotypic classes were contrasted with means from the callus growth bioassay using ANOVA. We also used Mapmaker-QTL to identify markers associated with aluminum tolerance. Four markers, UGAc044, UGAc053, UGAc141, and UGAc782, were found to be associated with aluminum tolerance. UGAc044 had the greatest effect, accounting for 15% (LOD 2.3) of the variation in aluminum tolerance.

KEYWORDS
Aluminum (Al) tolerance, alfalfa, marker-assisted selection, quantitative trait loci (QTL), restriction fragment length polymorphism (RFLP)

INTRODUCTION
Acid soils, with its accompanying Al toxicity, are found on nearly every continent and is a major factor limiting the productivity of cultivated alfalfa. At low pH, Al becomes soluble and available to plants, resulting in inhibition of root elongation and reduced plant growth. Surface liming, typically used to overcome this problem, is expensive and does not affect the pH of the subsurface soil. The breeding of Al tolerant alfalfa would overcome this problem. Conventional breeding methods, however, have made little progress in introducing Al tolerance into cultivated alfalfa. The objective of this study was to map quantitative trait loci (QTL) associated with Al tolerance in diploid alfalfa, and identify RFLP markers associated with these loci. These RFLP markers can then be used to aid in the introgression of this trait into cultivated, tetraploid alfalfa.

METHODS
Thirty-eight P.I.s were screened for Al tolerance with a callus growth bioassay as described by (Dall'Agnol et al., 1996), using leaves rather than petioles. Methods for DNA extraction, Southern blotting, and hybridization were performed as previously described (Brummer et al., 1993). Briefly, genomic DNA was extracted from freeze-dried tissue using a CTAB extraction buffer and was digested using 3 restriction enzymes: EcoRI, EcoRV, and HindIII. Digested DNA was separated by electrophoresis in 1.2% agarose gels, and blotted onto nylon membranes (GeneScreen Plus[DuPont]). DNA probes were from a cDNA library (Lambda Zap II) made from alfalfa seedlings. Cloned inserts were PCR amplified, and hexamer-labelled with 32P-dCTP and 32P-dATP. Hybridization was overnight at 65° C, followed by 1 wash with 2X SSC +0.1% SDS and 2 washes with 1X SSC + 0.1% SDS , 20 minutes each at 65° C. The membranes were then exposed to Kodak X-OMat film, using 1 intensifying screen, for 7 days at -80° C.

To identify probes putatively linked with Al tolerance two methods were employed: one-way analysis of variance, and interval mapping. In one-way analysis of variance, the marker genotype was used as the predictor variable and the Al tissue culture ratio score as the response variable. A significant association between a DNA marker and Al tolerance response was declared if the probability was equal or less than 0.05. The coefficient of determination (R2) was used as a measure of the magnitude of the marker association. Means obtained from averaging over the allelic classes were used to indicate the direction of allele effects (originating from parents). Multi-point linkage analysis was performed on an IBM-PC Pentium using MAPMAKER-EXP (Lander et al., 1987). Linkage data was used to assign markers to linkage groups using the Kosambi function and a LOD of 3.0. Using the linkage groups created in MAPMAKER-EXP, interval mapping was performed with MAPMAKER-QTL (Lincoln et al., 1993) to identify putative QTL. A QTL was declared whenever the LOD score exceeded 2.0.

RESULTS AND DISCUSSION
Genotype 724-25 (selected from PI 464724) was chosen as the Al tolerant parent, and one of the parental genotypes which gave rise to the mapping population described previously by Brummer et al (1993), genotype 440501-2, was used as the Al sensitive parent. These parents were crossed to produce several F1 hybrids. These F1s were confirmed to be hybrids by screening them with a set of RFLP probes polymorphic between the two parents. Three of the confirmed F1 hybrids were chosen at random and selfed to form a population of 104 F2s.

Al tolerance was normally distributed in the F2 population, as measured by the callus growth bioassay. The mean of the population was 0.63 with a standard deviation of 0.13. The tolerant parent (724-25) had an Al tolerance score of 0.72 while the sensitive parent had a score of 0.48. The 3 F1s had scores of 0.55, 0.61, and 0.65, respectively. Transgressive F2s had scores as high as 0.98 and as low as 0.38 .

RFLP markers used to construct the current diploid alfalfa map were used to probe Southern blots of the F2 population. A total of 146 markers were screened with 58 showing polymorphisms that could be scored and mapped.. Four markers were found to be associated with Al tolerance. Markers UGAc044 and UGAc053 are on linkage group 4 of the map by Brummer et al. (1993), and UGAc141 is on linkage group 5. Marker UGAc782, added after 1993, also occurs on linkage group 4 (Figure 1). UGAc044 accounted for 15% of the variation found, UGAc053 accounted for 10%, and UGAc782 and UGAc141 accounted for 8% each.

The use of these markers for marker-assisted selection will be the basis of further research in this area. The first step will be to show that the relationship seen between these markers and Al tolerance in the callus growth bioassay can also be seen in a soil test. Once this has been established, these markers can be used to move Al tolerance from the diploid to the tetraploid level either by colchicine doubling of chromosomes, or by making 2x-4x crosses. Plants carrying favorable alleles can then be identified at the seedling level with a DNA test, thereby reducing the number of plants that will require further testing in a field setting, resulting in a savings of both time and money, as well as the creation of Al tolerant cultivars.

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Figure 1

Linkage groups from the map of Brummer et al 1993. Markers in bold are associated with aluminum tolerance. Four new markers have been added to group 5 and two new markers have been added to group 4 since 1993.