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Genetic mapping in tetraploid alfalfa: Results and prospects

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Introduction Among the difficulties of improving forages is their perennial nature, which necessarily requires long selection cycles to fully evaluate genotypes. Further, traits of particular importance—yield and winter hardiness—are difficult to assess on single plants, necessitating evaluation of progeny, which is both time consuming and expensive. Because of this, yield of many forages, and particularly alfalfa, has not improved substantially over the past 25 years (Riday and Brummer, 2002). Winter hardiness often has a negative correlation with autumn growth, although some evidence suggests this is not always true (Brummer *et al.*, 2000). One way to overcome some of these limitations may be through the use of genetic markers to help select desirable genotypes. The objective of this experiment was to test the hypothesis that quantitative trait loci (QTL) for complex agronomic traits could be identified in a segregating tetraploid alfalfa population.

Materials and methods A segregating F_1 alfalfa population consisting of 200 genotypes was developed by crossing an elite genotype ABI408 with a semi-improved genotype WISFAL-6 (Brummer *et al.*, 2000). The population was clonally propagated and planted into field trials at Ames and Nashua, IA between 1998 and 2002. Biomass yield, autumn plant height, winter survival, root mass, and a suite of physiological constituents of roots (sugars, fatty acids, starch, protein, and total nonstructural carbohydrates) were measured (Robins *et al.*, 2005; Alarcón Zúñiga, *et al.*, 2004). Genetic maps based on SSR and RFLP markers were constructed of both parents of the mapping population using the computer program TetraploidMap (Hackett *et al.*, 2003). Phenotypic data were used to map QTL based on single factor analysis of variance, and multiple regression models were built based on significant markers.

Results QTL were identified on all eight linkage groups of alfalfa, and at least one QTL was identified for each trait. Partial R^2 values for particular QTL, based on multiple regression models, ranged from 4 to 39%. A substantial proportion of the population variation could be explained by marker-trait associations for many traits averaged across environments, including biomass yield (36%), winter survival (33%), root soluble protein concentration (63%), root linoleic acid concentration (47%) and autumn plant height (69%). Although several regions of the genome included QTL for multiple traits, Linkage Group 7 (which corresponds to LG 7 in *Medicago truncatula*) is particularly noteworthy. It contains a gene associated with cold tolerance (MsaciB) and that locus is either directly associated or linked to a marker that is associated with biomass yield, autumn plant height, winter survival, and root concentrations of glucose, sucrose, linoleic acid, starch, and soluble protein.

Discussion Genetic mapping of complex traits is possible in complex autotetraploid populations. That this would be the case is not intuitively obvious, because in order for a marker to have a significant association with a trait, it needs to overcome the effects of other alleles within a given parent as well as those effects of alleles from the other parent. Dissecting the physiological basis of traits like winter survival may be possible by successively evaluating its various components. Genome locations with QTL for multiple traits, particularly those, such as linoleic acid and glucose, that are implicated in the same overarching trait (e.g., cold tolerance), are areas warranting further investigation. In particular the region on LG 7 is a prime candidate for loci controlling many important traits. How this information can be used productively in an alfalfa breeding program needs further investigation.

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