

Crop water use efficiency (WUE) has long been considered a target for genetic improvement because of its potential to reduce water inputs and increase drought tolerance. Carbon isotope discrimination (Δ) is a well-established surrogate measurement of WUE that involves assessment of a plant's ability to discriminate between different carbon isotopes during photosynthetic CO₂ assimilation. To genetically dissect the Δ trait in alfalfa (*Medicago sativa* L.), a first generation backcross (BC₁) mapping population was previously generated by mating a high yielding and high Δ genotype of *M. sativa* subsp. *sativa* var. Chilean with a low yielding and low Δ genotype of *M. sativa* subsp. *falcata* cv. 'Wisfal'. A single F₁ plant was then backcrossed to the Chilean parent. Half-sib families derived from 96 BC₁ individuals were evaluated in a replicated field trial near Las Cruces, NM for shoot biomass production under drought stress during two forage regrowth periods in each of three years (2005, 2006 and 2007). Due to the high cost of Δ phenotyping, and given that forage yield and Δ have been previously shown to be positively associated in field grown alfalfa, shoot biomass data from the two drought stressed harvest in 2005 were initially used to identify 29 families that possessed extreme forage yield phenotypes. It was hypothesized that these families would also possess more extreme phenotypes for Δ . Shoot tissues were subsequently sampled from these families for Δ determination during one drought stressed forage regrowth period in 2006 and 2007. Analysis of variance detected significant variation for Δ among the 29 families in both years of the study. Using a previously developed genetic map derived from 334 simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers, analysis of the DNA marker data and Δ data from the subset of 29 families was conducted using interval mapping. Nine regions of the alfalfa genome were identified as influencing Δ , and each region was referred to as a quantitative trait locus (QTL). Four QTL that increased Δ and three QTL that decreased Δ were derived from the low Δ Wisfal parent. Two QTL that decreased Δ were derived from the high Δ Chilean parent. Three Δ QTL co-localized with shoot biomass QTL and one Δ QTL co-localized with a root biomass QTL that were previously detected in the complete mapping population (n=96). In each of these four cases, the direction of the Δ effect was positively correlated with shoot or root biomass effects. This evidence suggests that in many cases, Δ and shoot or root biomass production may be influenced by the same genetic factors. Selection for high Δ per se, or markers associated with high Δ QTL, could potentially be used to increase alfalfa shoot biomass production under drought stress.