QTL Mapping of Plant Architectural Traits in Diploid Alfalfa

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Abstract

Presently, breeding Alfalfa (*Medicago sativa* L.) for yield improvement is mostly based on recurrent phenotypic selection; however, national annual biomass yield has remained largely stagnant between 7-8 Mg/ha for the past four decades.[1] Alternative breeding strategies such as molecular marker assisted breeding are being adopted to accelerate breeding, but additional genomic tools connecting phenotype to genotype could further increase genetic gain.

Uncovering the genetic causes of architectural traits, such as pubescence; stem erectness, length, quantity, and thickness; leaf size and quantity; internode length; and maturity are needed because they all potentially affect biomass yield. Single nucleotide polymorphism (SNP) molecular markers are used to form physical and genetic maps of chromosomes to identify regions of DNA associated with phenotypic variation. These regions are called quantitative trait loci (QTL), and this study uses naturally occurring diploid alfalfa which is significantly less genetically complex than agronomic autotetraploid alfalfa. There are two main subspecies of diploid alfalfa, *M. sativa* subsp. *caerulea* and subsp. *falcata* that differ in geographic origin and architectural characteristics making them good parents for linkage mapping studies.[2]

(left) The segregating F2 population UCAL2265-05 planted in a field in Davis, CA with each individual plant flagged. (right) The senior author collecting plant width measurements with a meter stick.

Materials & Methods

Four F2 mapping populations of diploid alfalfa were derived from parents with significant phenotypic variation and unique geographic origins (Table 1). The parents were crossed to create F1 hybrids that were self pollinated to develop the segregating F2 mapping populations. One F2 population "UCAL2265-05" planted in the field in Davis, CA in 2024 in an unreplicated design and has been genotyped using DArTag markers and phenotyped for height and width using a meter stick and for dwarfism visually. Three additional F2 mapping populations are growing in greenhouse and will be phenotyped and genotyped. DArTag markers are an assay of targeted single nucleotide polymorphisms (SNPs), and a 3000 SNP capture set for alfalfa was developed by the Breeding Insight program of the USDA-ARS.[3] QTL analysis was conducted using r/QTL using R.[4]

Table 1. Parental genotypes used for crosses with their accession number, geographical origin, and trait information.

Accession	Name	Subspecies	Geographic Origin	Phenotypic Traits
PI502449	DFL2	*falcata*	Russia	High vigor, large leaves, many erect stems, short internodes, yellow flowers
PI631818	DFL3	*falcata*	Russia	Small leaves, many erect stems, yellow flowers
PI464717	DCA1	*caerulea*	Turkey	Large leaves, many erect stems, long internodes, purple flowers
PI631689	COTL-01	*falcata*	Bulgaria	Medium leaves, mostly erect stems, short internodes, lots flowering, yellow flowers
PI641603	UCAL 2472	*caerulea*	Kazakhstan	Small leaves, prostrate stems, long internodes, purple flowers
PI634106	UCAL 2473	*falcata*	Ukraine	Nonglandular trichomes, semi-erect stems, small leaves, yellow flowers

Table 2. List of F2 populations developed with # of individuals and parent information

F2 population	Individuals Genotyped	Individuals Phenotyped	Female Parent	Male Parent
UCAL2265-05	371	285	DFL2	DCA1
RX01	59	WIP	UCAL2472	COTL-01
RX02	89	WIP	UCAL2472	DFL3
RX03	106	WIP	UCAL2473	UCAL2472

Results

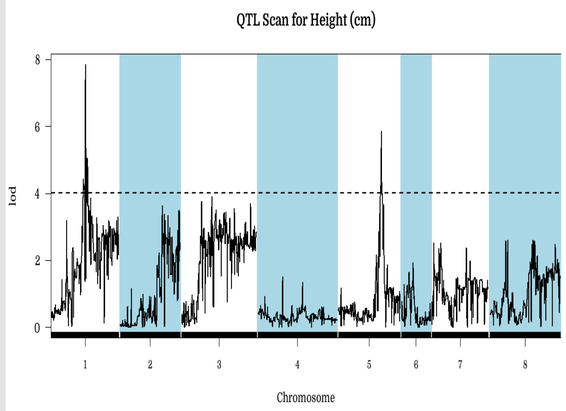

Figure 1. QTL Scan for Height in F2 UCAL2265-05. The permutation significance threshold for α=0.05 is at LOD = 4.01. Two peaks is present at chr 1 pos 577 Mbp [95% CI: 572-578 Mbp] and chr 5 pos 730 Mbp [95% CI: 712-742 Mbp]

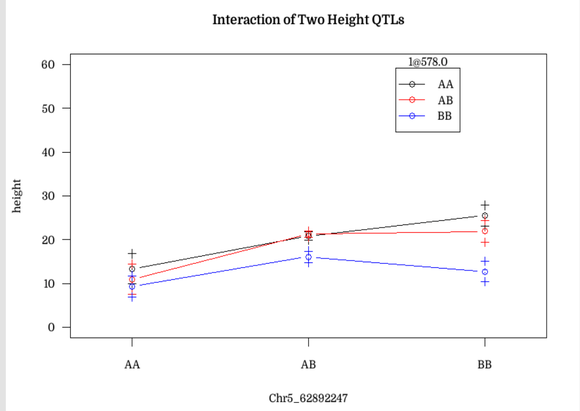

Figure 4. Plot of QTL interactions for height QTLs on chr 1 and 5. The two QTLs together explain 21.1% of height variance. Separately, chr 1 QTL explains 11.0% and chr 5 QTL explains 7.8%.

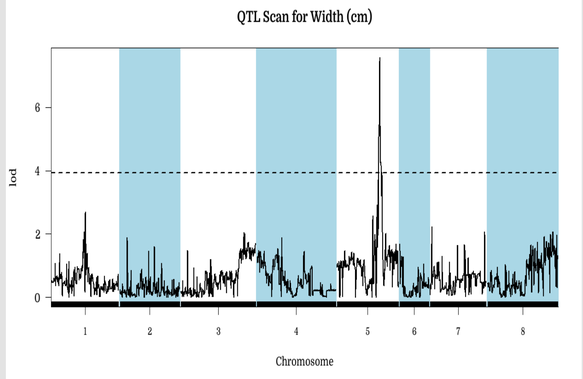

Figure 2. QTL Scan for Height in F2 UCAL2265-05. The permutation significance threshold for α=0.05 is at LOD = 3.93. One peak is present at chr 5 pos 729 Mbp [95% CI: 724-733 Mbp].

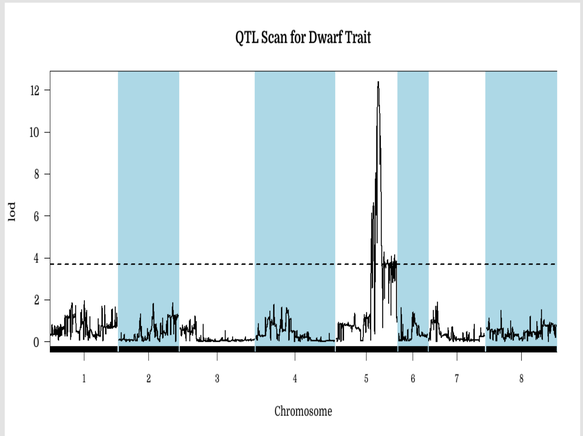

Figure 3. QTL Scan for Dwarfism in F2 UCAL2265-05. The permutation significance threshold for α=0.05 is at LOD = 3.70. One peak is present at chr 5 pos 727 Mbp [95% CI: 712-739 Mbp].

Individuals from RX02 F2 population with varying stem, leaf, and vigor traits. (top) Two individuals with abnormal weak vigor. (middle) Two individuals with prostrate stems. (Bottom) An individual with highly erect stems.

(left) A plant from the UCAL2265-05 F2 population with the dwarf trait (sunglasses for scale). (right) The same dwarf individual next to another dwarf individual among many larger non-dwarf individuals.

(top) Leaves and flowers from RX02 population with segregating sizes and colors. (bottom) Leaves and flowers from RX03 population with segregating sizes and colors.

Discussion

Two major QTLs were identified for height (QTL 1: chr 1 pos 577, QTL 2: chr 5 pos 730) (Fig. 1), and one major QTL was identified for both width (chr 5 pos 729) (Fig. 2) and for dwarfism (chr 5 pos 727) (Fig. 3). Height QTL 1 appears to have a dominance effect; the BB marker genotype has a lower average height than the AA and AB genotypes which are similar (Fig. 4). QTL 2 also appears to have a dominance effect with AA individuals being shorter than BB and AB which are similar. An epistatic interaction between the two height loci exists (Fig. 4); for example, an individual is shorter if it has marker genotypes BB at QTL 1 or AA at QTL 2. The width QTL is also dominant with AA individuals narrower than BB and AB which are similar (Fig. 5).

The dwarfism QTL is in the same location as the width QTL and the height QTL 2 on chr 5. Dwarf alfalfa plants were characterized visually by identifying plants with abnormally small leaf size and vigor. Since these dwarf plants have abnormally low vigor, we expected a QTL controlling dwarfism to also be associated with width and height. The dwarf QTL exhibits significant segregation distortion, as the segregation ratio (14:185:11) for the marker most significantly associated with the trait deviates far from a Mendelian 1:2:1 F2 ratio (χ^2 = 121.99, df = 2, p < 2.2e-16). Because most dwarf individuals have an AA marker genotype, dwarfism is possibly a recessive trait (Table 3).

Next Steps

The next steps include genotyping and phenotyping the RX01, RX02, and RX03 F2 populations growing in greenhouse. Accurate phenotyping methods for traits of interest are being developed. Genotype and phenotype data will subsequently be analyzed to identify QTLs. UCAL2265-05 F2 population will be further phenotyped for more traits.

Architectural traits affect the production of alfalfa. Traits such as leaf size and quantity; stem length, thickness, and quantity; and internode length may influence overall biomass yield. Having identified QTLs for these traits, alfalfa breeders can use marker-assisted selection to accelerate the breeding process for these traits by augmenting costly and long field evaluations with faster and cheaper evaluations through genotyping and selecting for desirable markers. Identified QTLs can lead to further genomic studies to discover candidate genes and their mechanisms for affecting phenotypic variation. Ultimately, the potential value of these genes can be assessed through evaluating transgenic or gene-edited plants.

References

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