

## **Root traits to enhance nutrient and water use in alfalfa**

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Selection for yield in alfalfa has focused on aboveground plant traits, largely ignoring the potential contribution of the root system to improve yield due to enhanced water and nutrient acquisition. Root system architecture (RSA) of alfalfa affects persistence and productivity by means of influencing the capacity of the plant for symbiotic nitrogen fixation, nutrient uptake and water use efficiency, resistance to frost heaving, winter hardiness, and some pest and pathogen resistance. In alfalfa, we currently lack a basic understanding of root system development, spatial distribution of root nodules, and the effect of root nodules on root growth and RSA. Without such basic phenotypic information we will be unable to take advantage of the growing genomic data available for alfalfa improvement. The objectives of this project were to: (1) Utilize 2D and 3D imaging systems to characterize root system architecture traits in alfalfa; (2) Identify genetic loci associated with root system architecture traits in alfalfa; and (3) Develop and implement education and outreach programs to transfer information from the research to alfalfa producers, the alfalfa seed industry, crop consultants, and state forage educators.

Various growing conditions and soil mixes were tested to identify treatments that promoted the expression of these two diverse root system characteristics using UMN3233, germplasm resulting from three cycles of selection for branch roots, and UMN3234, germplasm from three cycles of selection for tap roots. Roots were removed from the soil mix, washed gently, scanned, and root parameters measured using the WinRHIZO software. The results show that the strongest indicator of phenotypic divergence between the highly branched and tap-rooted alfalfa lines was associated with the length and number of the tertiary roots. These two parameters, regardless of experimental treatment, were the greatest indicator of differences between these two plant lines. Plants could be selected after 14 days of growth and were used to create cycle 4 selected populations. Candidate genes and transcription factors associated with root growth and development were mined from the literature and used for primer development. A subset of plants contrasting for root traits (strong tap root vs. fibrous network of roots) were genotyped using the primers to identify single nucleotide polymorphisms (SNP) that were assayed with a high-resolution melting (HRM) approach. Ongoing efforts include digital phenotyping of cycle 3 and cycle 4 plants under field conditions, genotyping-by-sequencing of selected plants and association of DNA markers with plant phenotype, and development of mapping populations generated from crosses between individuals with tapped and branched roots. The segregating populations will be phenotyped and genotyped to identify and/or validate QTL associated with root traits.

The outcomes of this project will facilitate ‘root breeding’ approaches aimed at modifying RSA to increase the absorptive capacity of roots for water and nutrients to increase alfalfa productivity, persistence, and resilience to environmental stresses.