Rapid phenotyping of alfalfa root system architecture

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Root system architecture (RSA) influences the capacity of an alfalfa plant for symbiotic nitrogen fixation, nutrient uptake and water use efficiency, resistance to frost heaving, winterhardiness, and some pest and pathogen resistance. However, 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. Additionally, past methods to phenotype alfalfa RSA required a minimum of 20 weeks of field cultivation to complete one cycle of selection. The objective of this study was to develop a rapid, streamlined process to phenotype alfalfa root systems and thereby select representative candidates with more robust and homozygous highly branched or tap-root characteristics. 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 UMN 3234, germplasm from three cycles of selection for tap roots. Seedlings were grown in 7.5 x 35 cm cone-tainers containing a 1:1 sand:turface mixture in a growth chamber with a 16 h photoperiod at 24°C. Plants received 0.25X Hoagland's nutrient solution daily. After 14 days, roots were removed from the soil, 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. In contrast, the root parameters associated with the length of the primary root and the length and number of the secondary roots did not differ between the two plant lines. Applying a slight stress, either drought or nutrient (N), augmented the expression of the tap-rooted phenotype as shown by an increase in the percentage of plants that had a distal taproot fragment greater than 3 cm. This suggests that the phenotypic plasticity of these traits may be a response to their growth environment. When comparing root nodule numbers and the location of nodulation, the results show that the tap-rooted line had fewer nodules relative to the branch-rooted line. For both plant lines the majority of nodules were located on secondary roots. This result is intriguing because total secondary root length and number at 14 days did not vary between the two plant lines. This suggests that the lower nodule number associated with the tap-rooted line may be caused by a trait other than root availability. Individual plants that showed the most distinct phenotypes are being genotyped using genotyping-by-sequencing analysis to identify DNA markers associated with these distinct root phenotype characteristics and were used to create cycle 4 selected populations.