

# What Have We Learned from 20 Years of Breeding for Alfalfa Root System Architecture?

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The root system architecture (RSA) of alfalfa affects biomass production by influencing water and nutrient uptake and responses to biotic and abiotic stresses. Breeding for root traits and RSA has been conducted over the past 20 years by scientists with USDA-ARS Plant Science Research Unit to develop selection methods that has resulted in development of unique germplasm. Four cycles of divergent selection for branched or tap rooted plants showed that the branch-rooted plants had a significantly greater number of tertiary roots and significantly longer tertiary roots relative to the tap-rooted plants when phenotyped at 14 days after germination. The most informative RSA trait was secondary roots > 2.5 cm, which can differentiate the branched- from the tap-rooted plants and can be used for parental and progeny selection. This parameter distinguishing phenotypes was confirmed with two machine learning algorithms, Random Forest and Gradient Boosting Machines. Plants selected as seedlings for the branch-rooted or tap-rooted phenotypes were used in crossing blocks that resulted in a genetic gain of 10%, consistent with the previous selection strategy that utilized manual root scoring to phenotype 22-week-old field grown plants. Heritability analysis of various root architecture parameters from selected seedlings showed that tertiary root length and number were highly heritable, with  $h = 0.74$  and  $0.79$ , respectively. RhizoVison image segmentation and machine learning models successfully predicted RSA types (branched, taproot, and intermediate types) with a prediction accuracy greater than 97% and probability greater than 95%. Computer vision and convolution neural network deep learning using images as direct input had 80% prediction accuracy due to the small number of images for training the model. More efforts are needed to address the low correlation between field phenotyping of adult plants and greenhouse phenotyping of 14-day-old seedlings. To accelerate selection and breeding, genome-wide genotyping with the DArTSeq and Targeted Genotyping markers, developed in partnership with Breeding Insight will be used for genomic prediction and will be discussed for RSA breeding.

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