## The Hidden Half: How Roots Contribute to Alfalfa Productivity

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Roots are often referred to as the "hidden half" of a plant because they are underground. In the case of alfalfa, roots can grow up to 5' a year in deep soils and have been found at an amazing 30' below the surface! That means most of the alfalfa plant is found underground as roots. In most crops, including alfalfa, plant breeders focus on above-ground traits. However, improving root system architecture (RSA) can have a profound impact on plant productivity. For example, combined field and simulation analyses concluded that changes in root architecture were a primary driver of the nearly eightfold increase in U.S. corn yields since the 1930s. In alfalfa, RSA is believed to affect persistence and productivity by influencing symbiotic nitrogen (N) fixation, nutrient uptake and water use efficiency, resistance to frost heaving, winterhardiness, and some pest and pathogen resistance. Yet, we know little about genes controlling root development or how they impact other plant traits.

Root traits are notoriously difficult to study due to the opacity of soil and environmental effects on root development. The most widely used method of study is referred to as "shovel-omics," simply digging up plants at some stage and measuring root traits. Digital images and software to capture numerous traits have been developed that accelerate root analysis and reduce human error. Rhizotrons, that use clear tubes pre-positioned in soil and small cameras inserted into the tubes, can measure root density, root length, and root turnover over multiple seasons. USDA-ARS scientists at St. Paul, MN, followed alfalfa root growth during the establishment year and found more than half of all fine roots in the upper 10" of soil were produced during the first 7 weeks of growth. They measured 8.5 miles of roots per square yard of soil. By the end of the season, almost half of the roots uppermost in the soil had died. This root turnover released an estimated 740 lbs carbon and 54 lbs N/ac.

Alfalfa roots fall into four major classes: taprooted, branch-rooted, rhizomatous, and creeping-rooted. Modern alfalfa genotypes adapted to the Upper Midwest released after 1980 are mostly taprooted with little variation in the number of lateral or fibrous roots. An ARS study on root morphological traits found lateral root number, position of lateral roots, and number of fibrous roots were highly heritable traits and least affected by the environment. However, most alfalfa root traits cannot be measured reliably in the greenhouse or after transplanting to the field. To develop alfalfa germplasm with distinct root morphologies, plants were grown in the field for 20-22 weeks before digging them and measuring root traits. From a base germplasm with good agronomic traits, plants were selected for either few fibrous roots or many fibrous roots and each population intercrossed to form a high-fibrous and low-fibrous population. Each population was then selected for branched roots or taproots (Figure 1).

Selected and unselected populations were evaluated in two field experiments over two years for herbage yield, root morphology, fall dormancy response, root diameter, root branching, and disease resistance. Forage yield of plants selected for fibrous and lateral roots was 7-14% greater in the establishment year and 9-16% greater in the first production year than alfalfa selected for taproot production (Figure 2), suggesting selection for altered root morphology is a viable strategy for enhancing yield

in alfalfa. No changes from the base population were found for the other traits evaluated. Importantly, additional research found root architecture is stable and maintained in different soil types with different P and K availability. A branching root system appears to provide plants with a yield advantage.

Why do the branch-rooted plants have higher biomass yields? Preliminary experiments found branch-rooted plants had a greater number of nodules than the taprooted plants at 3 weeks after inoculation. It is possible that the increased yield observed in branch-rooted plants is due to increased

Figure 1. Development of alfalfa with highly fibrous branch roots and low-fibrous taproots.



**Figure 2.** Cumulative dry matter yield from establishment and first production year at two locations. HF = high fibers, LF = low fibers.



N fixation. Ongoing experiments are measuring number of nodules, N fixation, and herbage biomass over 8 weeks in controlled conditions. Field experiments to measure these traits will also be done.

To improve upon shovel-omics for selecting alfalfa plants with specific root traits, a new method was developed that reduced selection time to 2 weeks compared to 22 weeks in the field. Plants were grown for 2 weeks in a mixture of sand and Turface (a growth medium of calcined clay particles), then gently removed and the root systems digitally scanned

Figure 3. Root systems scanned at two weeks after planting. A, branched root system. B, taproot system.



on a flat-bed scanner. The taprooted and branch-rooted plants could be identified based on total root length, the length of secondary roots arising from the main root, or number of tertiary roots (Figure 3). With this faster selection method, it is possible to do two cycles of selection in a year.

These experiments demonstrated changes to RSA in alfalfa are heritable, stable, and capable of increasing forage yield. How may root architecture affect other important traits? A larger taproot may allow plants to store more carbohydrates for better winter survival. A deep taproot would increase potential access to water resources to improve drought tolerance and reduce competition from shallow-rooted plants such as forage grasses interplanted with alfalfa. More branched roots for increased gas exchange may give plants an advantage in wet soil. Root breeding has the potential to enhance other agronomic traits in alfalfa.

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