

Project Title: Selecting for improved water and nitrogen uptake by focusing on root characteristics.

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Abstract

Water and nitrogen use efficiency (WUE, NUE, respectively) are intricately linked through physiological and metabolic pathways, and we hypothesize that both can be improved through breeding. Several traits can be targeted to improve WUE and NUE, including root traits. To aid in dissecting the physiological and genetic components that modulate WUE and NUE, mapping populations and genetic lines from genetically diverse sources that segregate based on N uptake and assimilation were developed. Leaf N concentration was used as a proxy for N uptake and assimilation, and genotypes that comprised the phenotypic extremes of leaf N concentration were identified and used to explore the effects of N on root length and biomass. Assessing root characteristics of segregating populations under field conditions is challenging, and ground penetrating radar (GPR) was employed to determine if this technology could be used to detect root length and biomass. Plants were grown under two water and nitrogen (N) treatments, a non-limiting treatment consisting of 280 kg N/ha and water replaced at 130% ETo, and a limiting-treatment of 140kg N/ha and 65% ETo. At market maturity GPR was used to scan for root length and biomass. The GPR technology detected root length, but was not sensitive enough to capture root biomass accurately. A second experiment was performed using the same genotypes as the field study. The plants were grown in a peat-based medium to control soil and N heterogeneity and plants received either the non-limiting or the non-limiting N; all plants received the non-limiting water treatment. Substantial range in root length was observed among the genotypes, but no treatment or phenotype differences were detected. Similarly, the range in root biomass was substantial, and genotypes that displayed the low leaf N concentration had higher root biomass compared to the genotypes that had high leaf N concentration phenotype. The data indicate a genetic basis for N uptake and assimilation, and that it is linked to root response to N levels.

Objectives:

The amount of water available for California agriculture is decreasing due to competing demands and long-term drought. The consensus conclusion of ~ 30 climate models is that less water will be available in the western U.S. in the coming decades. Agriculture must adapt by becoming more efficient with water through conservation, cultural methods and adapting germplasm to this likely scenario.

Lettuce cultivars are shallow-rooted and require frequent irrigations of high-quality water and fairly large amounts of nitrogen fertilizers to support leaf growth. Historically, lettuce

cultivars were developed during a period when water was comparatively plentiful and there was relatively little concern about nitrogen fertilizers. Today, however, the potential of nitrates from nitrogen fertilizers leaching into groundwater is well known and data have shown that nitrogen fertilizers are the largest anthropogenic emitter of nitrous oxide (N₂O), a potent greenhouse gas (GHG). Taken together, there is sufficient incentive to develop lettuce cultivars that require a reduced amount of water and nitrogen applied during crop production.

Water and nitrogen use efficiency (WUE and NUE, respectively) are complex traits modulated by physiological, morphological and genetic factors. At a fundamental level, both WUE and NUE depend on the uptake of water and N by the root system and transport to the leaves. There, water is used both for photosynthesis and transpiration whereas N is assimilated into organic forms, largely to support photosynthesis. Using a recombinant inbred line (RIL) derived from a cross between cultivars Diplomat and Margarita (iceberg and butter type, respectively), multiple quantitative trait loci (QTL) were associated with nitrogen- and water use efficiency (NUE and WUE, respectively). These QTL map to regions with genes associated with metabolic traits. The objective of this project is to develop germplasm with improved NUE and WUE by selecting for root traits, specifically genotypes that have greater root biomass and deeper roots. We hypothesize plants with greater deeper roots and biomass will have greater water and N uptake relative to plants with shallow roots and biomass. We speculate that these plants should have greater WUE and NUE simply due to increased surface area and interception of water and N.

Procedures:

Commercial cultivars are generally shallow-rooted and are derived from a narrow genetic base and likely do not contain the range of root traits that are potentially useful to improve NUE and WUE. We sought to improve WUE and NUE by screening a diverse collection of cultivars, sexually compatible *Lactuca* species, and high NUE lines from the Diplomat x Margarita RIL population and examine root length, root biomass and correlate these measures to leaf N concentration as a proxy of N uptake and assimilation.

To accomplish this, we previously developed plant populations that segregate for rooting depth, WUE and NUE. Plants were screened in a common nursery for N uptake and assimilation, and the best performing genotypes were used to create several F₂ populations. Of these, seven F₂ populations (~1600 genotypes) were evaluated under low N and low water, and the top 2.5% genotypes were advanced to a second round of screening. The genotypes with the highest leaf N concentration were designated as high N phenotypes, and those genotypes with the lowest leaf N concentration were designated as low N phenotypes.

Experiments were supported with funds from the Leafy Greens Research Board and these funds were leveraged as match for a grant from the California State University Agricultural Research Institute (ARI) to expand the work that could be performed in support of the project objectives.

In the first of two experiments, genotypes comprising the phenotypic extremes of leaf N concentration distribution (i.e., high leaf N concentration and low leaf N concentration) from

the above screening trial were planted in a field located at the Yuma Ag Center and grown under high N/high water (280 kg/ha / 130% ETo) and low N/low water (140 kg/ha/65%ETo). At approximately market maturity, a ground penetrating radar (GPR) unit was mounted on a cart and pushed through the furrows to scan rooting depth and biomass of individual plants. The antenna was positioned perpendicular to the ground to enable scanning of the side of the bed to profile the lettuce roots. The objective of this experiment was to determine if GPR could be used as a high-throughput method to scan for rooting depth and root biomass in trials conducted in the field under commercial production conditions.

To establish the range of rooting depth and biomass among the genetic lines and determine the treatment response a second experiment was conducted under semi-controlled conditions using the same genotypes and water/N treatments as experiment one. The controlled conditions of this experiment are more reliable and could then be used as a comparison to the GPR approach used in experiment 1.

For experiment 2, high leaf N / low leaf N genotypes were germinated and grown in 11.36 L containers filled with a commercially available peat-based growing medium. The plants were grown in a field located at Cal Poly Pomona and water and N fertilizer added through a pressure compensated drip system. The plants, having either high N and low N phenotypes, were grown under high N/high water (280 kg/ha / 130% ETo) and low N/low water (140 kg/ha/65%ETo). The genotypes, water and N treatments were identical in both experiments. At approximately market maturity, the plants were harvested and the soil was carefully washed from the roots. The root length was measured and the entire root were collected, oven dried and root dry weight, as a proxy for root biomass, was determined.

Results and Discussion:

Experiment 1: The raw data from ground penetrating radar identified roots from all plants among the furrow rows. That is, for each plant a root image was detected. Next, the raw data was processed to determine root length and root biomass. To verify the GPR data, plants were randomly selected and removed from the beds with a shovel, the soil gently (but not completely) removed from the roots and photographed. The GPR appeared to detect larger roots but many of the smaller roots were missed. These results suggest that, as configured, GPR may be useful to detect root length, especially those plants with larger taproots, but not root biomass.

In general, root systems with greater surface area will have greater uptake of water, and specifically, secondary roots would be more beneficial in this regard than primary or large taproots. Since minerals (N fertilizer for example), are taken up with water, greater water uptake is correlated with greater N uptake. If root biomass can be shifted further below the soil surface, plants with longer root lengths would theoretically be more desirable than shallow rooted plants since water evaporates more quickly near the surface. Water uptake depends on plant transpiration, and deeper-rooted plants would also maintain higher water potentials than shallow rooted plants and the deeper rooted plants would experience less stress due to water deficits.

Our overall conclusion is that as configured, the GPR is likely to be useful for detecting plants with longer root length but would be of limited use for high-throughput screening if the goal is to detect root biomass. In our next field trial, the GPR unit will be reconfigured with a more sensitive antenna with the expectation that it will be more sensitive to smaller diameter roots. In addition, we are working with the GPR manufacturer to modify the processing algorithms to increase sensitivity that might improve detection of smaller diameter roots.

Experiment 2. Previously we observed that soil heterogeneity in field studies is a significant problem that obscures both the physiological response and genetic signals in mapping populations of lettuce. In this study, lettuce genotypes characterized as having either high or low leaf N concentration were grown in containers filled with peat-based media. The media is structurally homogenous, contains minimal available N and the microbial communities that modulate N cycles are expected to be relatively uniform across the experiment.

The concentration of N in leaves was used as a proxy for uptake and assimilation of N. To determine the effect of applied N on root length, high and low N accumulators (i.e., those that have low leaf N concentration, or high leaf N concentration, respectively) were provided N fertilizers at non-limiting rates (equivalent to 280 kg N/ha) or N levels that limit growth (140 kg N/ha). Water was provided to all plants at rates that would not limit growth, transpiration or N uptake (130% ETo). At approximately market maturity, root lengths did not differ significantly between the high and low N accumulator phenotypes when N was provided at non-limiting rates (N100 treatment; Prob > F = 0.4565) or at the lower N rate treatment (N50; Prob > F = 0.5081; Figure 1). At both N levels, the range in root length of the low N accumulator genotypes was greater than that observed in low N accumulators, and the longest roots were observed in the low N accumulator genotypes for both N treatments (Figure 1).

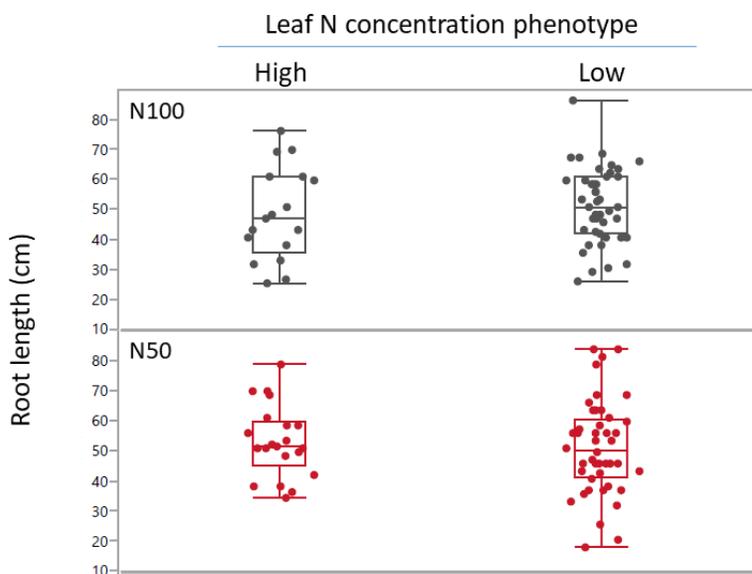


Figure 1. Box plot distribution of root length of genotypes that exhibit a high or low leaf N concentration phenotype. Plants were grown in a peat-based medium to control N availability. N was supplied to a final concentration of 280 kg N/ha (non-limiting treatment) or 140 kg N/ha (growth-limiting treatment). Root length was determined at market maturity. n=21 genotypes for the High N concentration and 43 genotypes for the Low N concentration genotypes.

As a proxy for root biomass, root dry weight was obtained on plants at market maturity to determine the effects of applied N. At the high N treatment rate (N100), genotypes that exhibited low leaf N concentration had significantly greater biomass compared to genotypes that exhibited high leaf N concentration (Prob > F = 0.0005; Figure 2). Similarly, genotypes that were categorized as exhibiting low leaf N concentration had significantly higher root biomass than those genotypes categorized as exhibiting high leaf N concentration under the low N treatment (N50; Prob > F = 0.0005; Figure 2). The population mean of genotypes that exhibited low leaf N concentration was 2.4 or 2.2 times greater than the population mean of high leaf N accumulating genotypes, at the high (N100) and low (N50) N treatment levels, respectively (Figure 2). Similar to that observed for root length, at both N treatment levels, the range in root biomass of the low N accumulator genotypes was greater than that observed in low N accumulators, and the greatest biomass were observed in the low N accumulator genotypes for both N treatments.

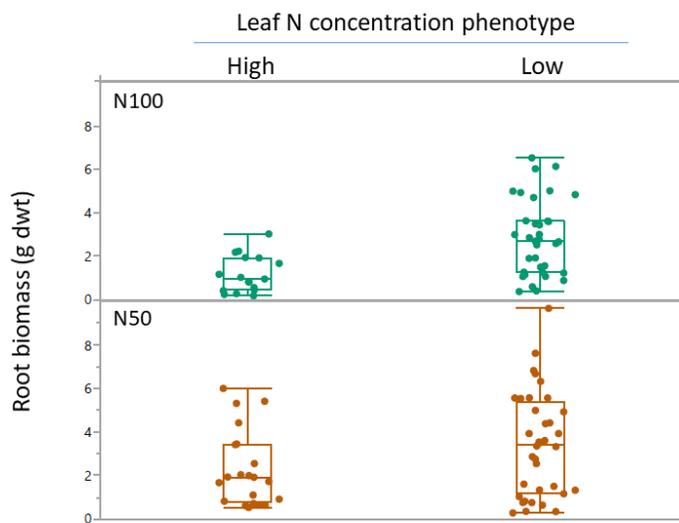


Figure 2. Box plot distribution of root biomass of genotypes that exhibit a high or low leaf N concentration phenotype. Plants were grown in a peat-based medium to control N availability. N was supplied to a final concentration of 280 kg N/ha (non-limiting treatment) or 140 kg N/ha (growth-limiting treatment). Root length was determined at market maturity. n=21 genotypes for the High N concentration and 43 genotypes for the Low N concentration genotypes.

In summary, the substantial variation observed in root biomass and root length indicates that these traits can be improved through breeding. The results indicate that low leaf N phenotypes and root biomass are linked, and respond to low N levels by producing much greater root biomass compared to high leaf N accumulating phenotypes. Further study is warranted to determine the genetic basis for this, and determine how this discovery can be exploited to improved NUE and WUE in lettuce breeding programs.