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

Project Investigator
David W Still, CSU Agricultural Research Institute, and Department of Plant Sciences, Cal Poly Pomona, Pomona California.

Cooperating Personnel
Youngsook You, Department of Plant Science, Cal Poly Pomona, Pomona California

Abstract
Lettuce cultivars were bred to perform with high N and water inputs, and new cultivars will need to perform with less N and water. New regulations seek to limit N amounts and reduce nutrient runoff. To meet these requirements growers will need to use less N fertilizer. In addition, climate change has reduced agricultural water availability, and growers will likely need to produce their crop with less water. Selecting for increased rooting depth and root biomass can improve both water and N use efficiency (WUE, NUE, respectively). Leaf N concentration (LNC) is an integration of N uptake and assimilation over the growing season, and we hypothesized that genotypes with high LNC would also have greater root depth and root biomass under low N. Genotypes with high or low LNC (HLNC and LLNC, respectively) were grown under non-limiting and limited N (N100 = 225 lbs N/acre; N50 = 112 lbs N/acre) with water replaced at 130% ETo. Plants were grown in peat-based media to control water and N uniformity and harvested at market maturity. Root and leaf N concentration, root length, and root biomass was determined. Three independent trials were run. We observed that HLNC genotypes had ~22% higher LNC than LLNC genotypes, and 2.1 to 3.0-fold higher root biomass than HLNC genotypes when grown at the lower N level (Prob > F 0.001). We identified genotypes with high root biomass under both N100 and N50 treatments, and highly plastic genotypes which were able to increase their root biomass when grown under N50 conditions. Genetic variation existed in LNC, root biomass and root length. Root biomass, but not root length, exhibited plasticity in response to low N.

Objective:
Select for improved rooting depth.
Deliverables:
Deliverable 1. Identification of genotypes that have demonstrated deep rooting traits
Deliverable 2. Identification of genotypes that have superior N uptake and assimilation
Deliverable 3. Determine the relationship between root length and N uptake and assimilation
Deliverable 4. Evaluation of ground penetrating radar for high throughput screening of germplasm for root biomass and rooting depth.

Procedures (1 April 2020 – 31 March 2021)
Nitrogen use efficiency (NUE) is composed of N uptake and assimilation of N into organic compounds in the leaf to support photosynthesis. For most plant species, about 70% of the N found in leaves is used for photosynthesis, and higher leaf N concentration is directly correlated to higher growth and yields. Leaf N concentration (LNC) is an integration of N uptake and

assimilation, and was used as a proxy for uptake and assimilation of applied N. We hypothesized that high leaf N concentration (HLNC) phenotypes would have higher root biomass compared to low leaf N concentration (LLNC) phenotypes. It would be reasonable to speculate that greater root biomass will result in greater water and N uptake, simply because of an increased root-soil interface. If true, LNC can be used as an easily measured trait to indirectly select for increased root biomass. Further, plants with greater root biomass would have the potential to mine the soil for water, particularly at the lower depths where there is typically a greater volume of available water. Plants with greater root biomass and rooting depth would be less susceptible to the negative effects associated with water deficits and could require less-frequent irrigation.

To test the hypothesis that HLNC is correlated with greater root biomass (deliverables 1,2 and 3), a mapping population was screened and LNC was determined. HLNC and LLNC phenotypes from and the phenotypic extremes were identified and used in three independently replicated experiments. HLNC and LLNC genotypes were grown under non-limiting N (hereafter, N100 treatment), equivalent to 225 lbs N/acre, and limited N (hereafter N50 treatment), equivalent to 112 lbs N/acre. For both the N100 and N50 treatments, plants were watered was replaced at 130% of ETo after 1.25" of ET accumulation. Plants were grown in 11.4 L (3 gallon) containers filled with a high-porosity peat-based growing medium. A peet-based medium was used to reduce variation in soil bulk density, and to increase uniformity in N and water availability. Plants received four proportionate applications of fertilizer beginning 28 days after planting and every 7 days thereafter.

At experimental end (12 weeks after planting), plants were harvested and leaf fresh weight was recorded to obtain above ground biomass, and root length and root biomass for each plant was determined. Soil was carefully washed from roots and oven dried for 72 hours at 60 °C to obtain biomass on a dry weight basis. Leaves and roots were harvested separately, oven dried and processed to obtain N and C concentration using a vario Max cube C/N analyzer. Three independent experimental trials were run and each experiment resulted with similar trends and conclusions relative to N treatments and phenotypes.

Results and Discussion

A wide variety of genetic materials were screened, including a mapping population. All materials were grown in the field under N100 and N50 treatments and LNC was determined. We defined HLNC and LLNC phenotypes as those comprising the bottom 10% (LLNC) or top 10% (HLNC) of the distribution of LNC when grown under limited N (i.e., N50) (You, Stroud and Still, manuscript in preparation). In these experiments, genotypes that exhibited a HLNC phenotype had higher rates of carbon assimilation and biomass accumulation compared to LLNC phenotypes when grown under N50 (Medina and Still, manuscript in preparation). From these experiments we established that when grown under low N, uptake and assimilation of N is greater in HLNC phenotypes than LLNC. Our work also established a highly significant relationship between LNC and C assimilation (R^2= 0.67, Prob > F < 0.0001). Taken together, selecting genotypes that exhibit HLNC when grown under low N should improve NUE due to higher biomass gain per unit of leaf N.

In general, plants alter their biomass based on environmental parameters, especially those related to water availability, but also in accordance with the abundance of mineral elements. Under water deficits, root biomass is increased relative to leaf biomass, and under low N, plants tend to allocate a greater proportion of their biomass to the root system, serving to increase the root:shoot biomass ratio. Thus, we hypothesized that genotypes exhibiting a HLNC phenotype would have greater root biomass than genotypes with LLNC phenotypes.

Leaf and root N and C concentrations

Four LLNC genotypes (52, 148_1, 187_2, and 236) and three HLNC genotypes (9, 92, and 227_3) were used to examine the relationship between LNC, root length and root biomass. HLNC and LLNC genotypes had an average of 15% and 18% higher LNC when grown under N100 than when grown under low N (N50; Figure 1A). Comparing HLNC to LLNC genotypes grown under the N100 treatment, HLNC genotypes had 19% higher LNC than LLNC genotypes; when grown under low N (N50), HLNC genotypes had 22% higher LNC than LLNC genotypes (Figure 1 A). In all treatments and phenotypes, roots had had less than half (range 42-45%) the N concentration of leaves (Figure 1B). Similar to the trends observed in leaves, HLNC genotypes had higher N concentration than LLNC genotypes when grown at N100 and N50 (Figure 1B). Nitrogen concentration in leaves was significantly affected by LNC phenotype and N treatment, but root:leaf N ratios were not affected (Figure 2).

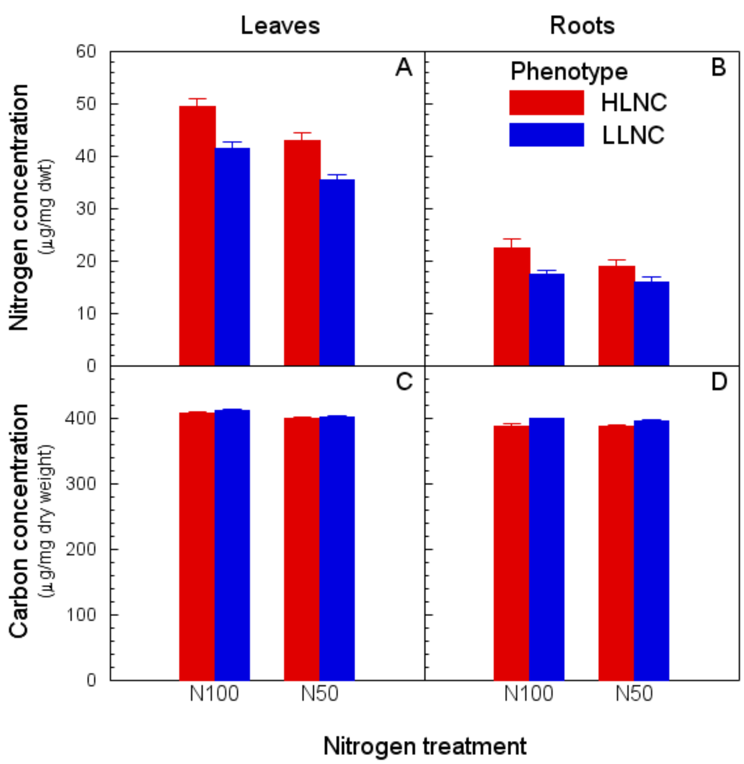

Figure 1. Nitrogen (A, B) and carbon (C, D) concentration of leaves (A, C) and roots (B, D) of high leaf nitrogen concentration (HLNC, red bars) and low leaf nitrogen concentration (LLNC, blue bars) of plants grown under two nitrogen treatments. Data represent the averaged value of three independent experiments. HLNC genotypes included 9, 92, and 227_3; LLNC genotypes included 52, 148_1, 187_2, and 236. N100 = 250 lbs N/acre; N50 = 125 lbs N/acre.

Carbon and N metabolism in plants are tightly coordinated to optimize growth. Leaf N is used to directly support photosynthesis, and the C-skeletons from photosynthesis are used for ammonium assimilation during amino acid biosynthesis. In response to environmental cues, biotic stresses, and elemental status, plant will alter C and N metabolism to optimize growth and ensure completion of their life cycle. For example, plants have mechanisms to sense the status of root N and respond to a variety of environmental, biotic and elemental stresses. For example, by increasing C supply, N uptake and assimilation can be enhanced. And vice-versa: decreases in N uptake reduce C assimilation.

In our studies with lettuce, C concentration of both leaves and roots was higher in LLNC genotypes than HLNC genotypes when grown under N100 or N50 (Figure 1 C, D). In particular, N treatment significantly affected leaf C (see Figure 2: N100 v N50, Leaf C; $HLNC_{N100}$ v $HLNC_{N50}$; $LLNC_{N100}$ v $LLNC_{N50}$). Considering root C, HLNC genotypes were significantly different than LLNC, regardless of N treatment (see Figure 2, HLNC v LLNC; $HLNC_{N100}$ v $LLNC_{N100}$; $HLNC_{N50}$ v $LLNC_{N50}$). In addition, leaf carbon:nitrogen ratios were significant for LNC phenotype and N treatment (see Leaf C:N column, Figure 2). Taken together, these results suggest that C metabolism, likely through a leaf-root feedback mechanism, differs between the two phenotypes and has a genetic basis that can be exploited to improve NUE.

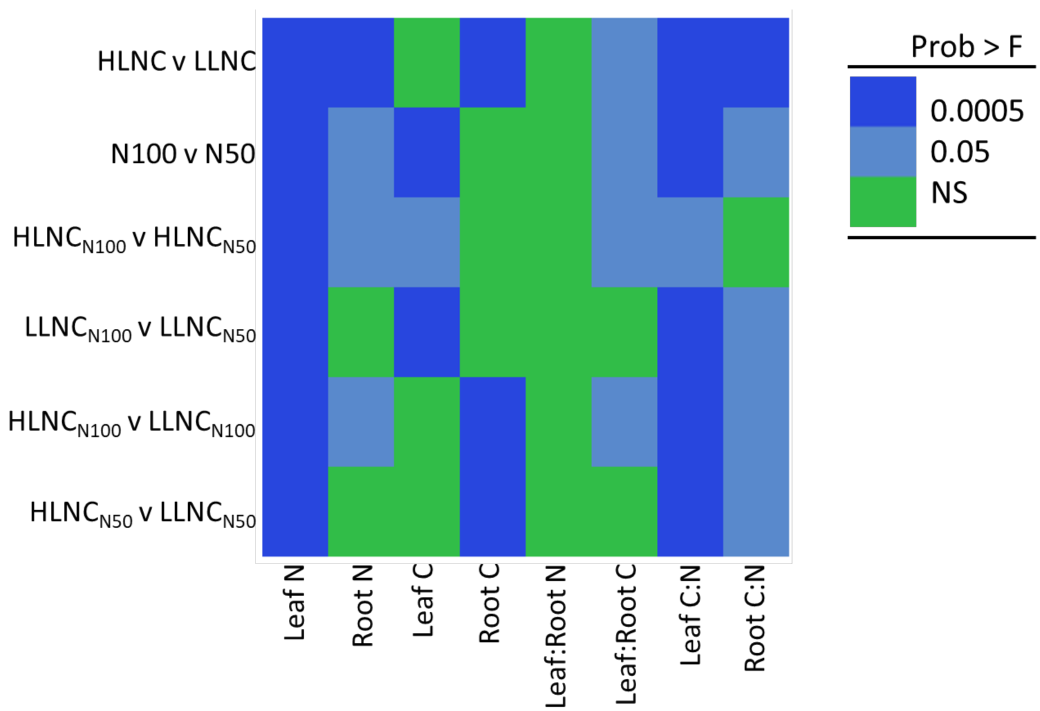

Figure 2. Heat map displaying significance levels between phenotypes (HLNC and LLNC) and nitrogen treatments (N100 and N50), and leaf nitrogen (Leaf N), root nitrogen (Root N), leaf carbon (Leaf C), root carbon (Root C), the ratio of leaf N concentration to root N concentration (Leaf:Root N), the ratio of leaf C concentration to root C concentration (Leaf:Root C), the carbon to nitrogen ratio of leaves (Leaf C:N), and the carbon to nitrogen ratio of roots (Root

C:N). Linear contrasts were performed from the pooled observations of three independent experiments. For any comparison, dark blue squares indicate significance at the 0.0005 level, light blue squares indicate significance at the 0.05 level, and green squares indicate the linear contrast comparison was not significant (i.e., Prob > F > 0.05).

Relationships between LNC, root length and root biomass

At market maturity (12 weeks after planting), the plants were harvested, and the roots were separated from the leaves at the crown at soil level. While the mean root length of LLNC was higher than HLNC genotypes when grown under N100 and N50 conditions, the variation among genotypes and within genotypes was relatively high, and consequently no statistical difference was observed between the N treatments (Prob > F = 0.79) or by phenotype (HLNC v. LLNC, Prob > F = 0.3069) (Figure 3 A).

In contrast, root biomass was affected by both N treatment and by LNC phenotype. Compared to HLNC genotypes, LLNC genotypes had higher root biomass when both N100 and N50 were averaged together, (µAll Prob > F = 0.0497; Figure 1B). Averaged across both N treatments and the three experiments, root biomass of LLNC phenotypes was 2.3 times greater than HLNC genotypes (µAll Probability > F < 0.0001; Figure 3 B). Root biomass was significantly higher in LLNC genotypes than HLNC genotypes both when grown in N100 (Prob > F = 0.0013) and when grown in N50 (Prob F < 0.0001).

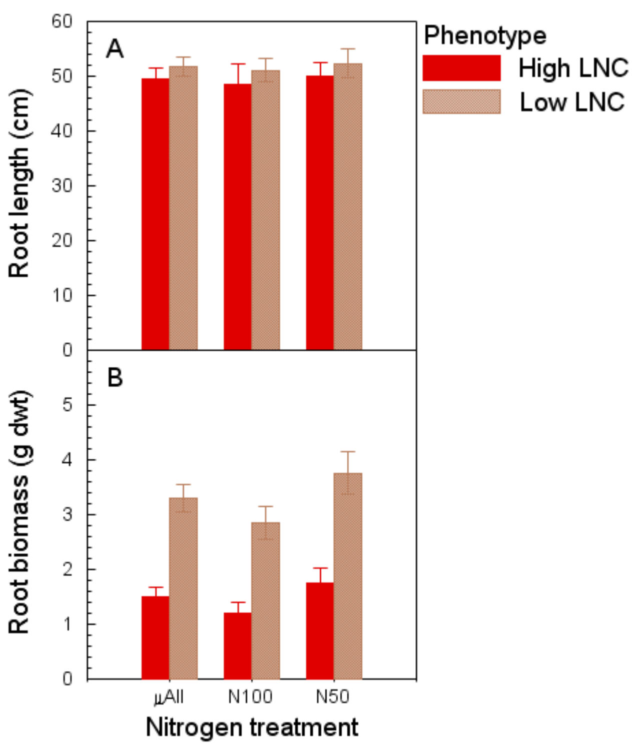

Figure 3. Root length (A) and root biomass (B) of genotypes exhibiting high leaf nitrogen concentration (High LNC, red bars) or low leaf nitrogen concentration (Low LNC, brown bars). In each of three independently replicated experiments, three representative plants were harvested, root length was measured and roots oven dried. Three HLNC and four LLNC genotypes were used. The data represents the averaged results of three experiments. For the N100 treatment, N=52; for the N50 treatment, N=57; for the HLNC phenotype, N= 38; for the LLNC phenotype, N=71. µAll represents the root length and root biomass average of both the N100 and N50 treatments and across all three experiments.

Root length was not affected by N treatment (Prob > F = 0.5357) but varied by genotype (Prob > F 0.0001). When averaged across N100 and N50 treatments, root length ranged from 64 to 42 cm for genotypes 227_3 and 9, respectively (Figure 4 A). These results indicate that genetic variation exists in root length, and while appreciable variation within a genotype was observed, root length does not appear to respond to N treatment. Instead, in agreement with expectations reported in the literature that plants allocate a greater portion of their biomass to roots in response to low N, lettuce plants grown in the N50 treatment had higher root biomass than when grown in N100 (Prob > F = 0.0497). It is important to note, however, that that the ability to respond to low N varied considerably. Appreciable genetic variation in root biomass was observed within a phenotypic class (i.e., HLNC and LLNC) in the response to low N. The genotypes with the highest overall root biomass, irrespective of N treatment, included LLNC genotypes 52, 187_2, and 236, and a single HLNCA genotype. 227_3 (Figure 4 B). The genotypes for which the greatest biomass was observed included 236, 52,187_2, grown under N50, and genotype 52, grown under the N100 treatment (Figure 4B). The greatest root biomass was observed in genotype 236 grown under N50 (4.9 g dwt), and the smallest biomass was genotype 9, grown under N100 (0.9 g dwt), a more than 5.4-fold difference in root biomass.

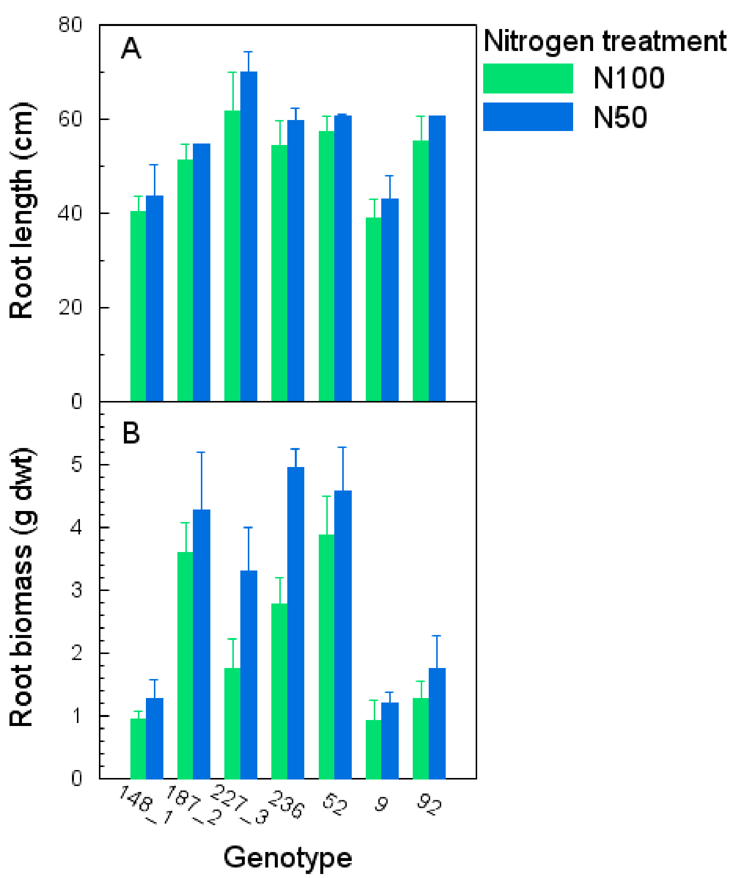

Figure 4. Root length (A) and root biomass (B) of lettuce genotypes grown under non-limiting N (N100) and limited N (N50). The data for each genotype represent averages of three plants per N treatment and three independently replicated experiments. HLNC genotypes included 9, 92, and 227_3; LLNC genotypes included 52, 148_1, 187_2, and 236. N100 = 250 lbs N/acre; N50 = 125 lbs N/acre.

The differential physiological or morphological response by plants to an external factor is called plasticity. The ratio of root biomass of plants grown under N50 vs N100 (N50:N100) can be used as a simple index reflecting the plasticity of root biomass in response to soil N concentration. In these experiments, genotype 227_3 and 236, classified as HLNC and LLNC phenotypes, respectively, had N50:N100 root biomass ratios of 1.9 and 1.8, respectively, indicating a high degree of plasticity, suggesting these genotypes are sensitive to soil N concentration. Conversely, genotype 187_2, with a LLNC phenotype, had the greatest root biomass regardless of N treatment. Genotypes 52 and 187_2, both LLNC phenotypes, had N50:N100 root biomass ratios of 1.2, and high root biomass, and exhibited the lowest plasticity of any of the genotypes used in these experiments.

Conclusions

From the perspective of breeding lettuce to improve NUE, two conclusions can be reached. First, selection based on HLNC genotypes will support the greatest NUE. This conclusion is based on observations of these genetic materials (and other genetic materials) of C assimilation measured through photosynthesis (data not presented) and higher rates of biomass accumulation per unit of leaf N. HLNC genotypes, on average, have 22% greater LNC than LLNC phenotypes when grown under limited N (i.e., N50) and 19% higher LNC than LLNC when grown under non-limiting N (i.e., N100). These results have been consistent when these genotypes were grown in the field and under conditions where soil, water and mineral nutrition were uniform and carefully controlled, as in the experiments presented herein.

Appreciable and exploitable genetic variation exists in root biomass. Plasticity was observed in root biomass but not root length in response to soil N, specifically nitrate levels. These observations and conclusions were consistent across three independently replicated experiments. In these experiments, HLNC genotypes had 22% higher LNC than LLNC genotypes. Further, in other experiments not presented here, LNC of HLNC genotypes were about 22% higher than commercial cultivars when grown under N50. As growers must comply with new N discharge targets and limits, these data suggest that in addition to higher NUE, HLNC plants would allow greater contribution of "R", the amount of N removed from the field through harvest. In addition, selecting plants with higher root biomass will likewise contribute to "R" by sequestrating N in root biomass, due to up to a 5.4-fold increase in root biomass observed in these experiments. Ideally, both HLNC and high root biomass traits can be optimized in a single genotype. We are working to combine and optimize both traits in a single plant and develop breeding lines that can be used by the lettuce breeding community to improve NUE and WUE of their materials.