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

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Abstract:

Lettuce production may be impacted by the availability of water and reductions in applied nitrogen (N) fertilizers. To address both issues, our lab has developed and screened lettuce mapping populations to select genotypes with better water and nitrogen use efficiency (WUE, NUE, respectively). Our lab is developing genetic maps and identifying quantitative trait loci associated with WUE and NUE. We hypothesize that both WUE and NUE can be improved by increasing root biomass, simply because a greater volume of soil is mined for water and N. In trials this year, a newly created mapping population consisting of 279 F7 families of the D221 x W28 recombinant inbred line was grown under a limited N treatment (N50) and a non-limited N treatment (N100). At market maturity, root and leaf biomass, root length, and leaf N and C concentration were evaluated. Based on population averages, leaf biomass decreased, and root biomass increased in response to limited N. Leaf N concentration decreased by only 12%, in response to a 50% decrease in N supply. Seven F7 families were identified that increased their root biomass by almost two-fold under limited N, and they will be further evaluated in the field for release as genetic lines to improve root biomass under limited N.

Objectives

Objective 1. Determine root length and root biomass of a F7 recombinant inbred line (RIL) when grown under limiting and non-limiting N.

Objective 2. Determine leaf N concentration of the lettuce F7 RIL population when grown under limited and non-limiting N.

Objective 3. Determine relationship between leaf N concentration and root biomass of genotypes grown in Objective 1 and 2.

Objective 4. Identify quantitative trait loci (QTL) associated with root biomass under non-limiting and limiting N. Identify genetic lines that will be used for backcrossing.

Procedures:

A newly constructed F7 RIL population was used to examine root architecture and its relationship with leaf biomass and leaf N concentration, two key components of nitrogen use efficiency (NUE). To understand the physiological and genetic underpinnings of root architecture, root biomass, leaf biomass, and leaf N concentration, a newly developed F7 RIL population was examined. Previously, we showed that plant growth rates and photosynthesis are positively related to leaf N concentration. Genotypes with high leaf N concentration had higher photosynthetic rates and higher growth rates compared to genotypes with lower leaf N concentration.

The RIL population, named D221 x W28, consisted of 279 F7 families derived from a cross between D221, a *Lactuca sativa* selection from the Diplomat x Margarita RIL population, and 'W28', a primitive *L. sativa* accession (Figure 1).

Two-week old seedlings were transplanted into 20 L grow bags, each filled with the same water-saturated weight and volume of a high porosity peat-based media. Plants grown under the non-limiting treatment (N100) and limiting N treatment (N50) received the equivalent of 280 kg/ha N and 140 kg/ha N, respectively. Nitrogen was applied in four equal quantities beginning 4 weeks after planting, and every 5 days thereafter. Plants were watered with a domestic water source (i.e., minimal additional N) and fertilized via drip irrigation. Both limited and non-limited N treatments received the same volume of water and irrigation frequency. Watermark soil moisture sensors were used to monitor water potential of the potting media over the duration of the experiment. Plants were watered frequently to ensure leaf and root growth were not influenced, positively or negatively, as water deficits could induce morphological and physiological changes.

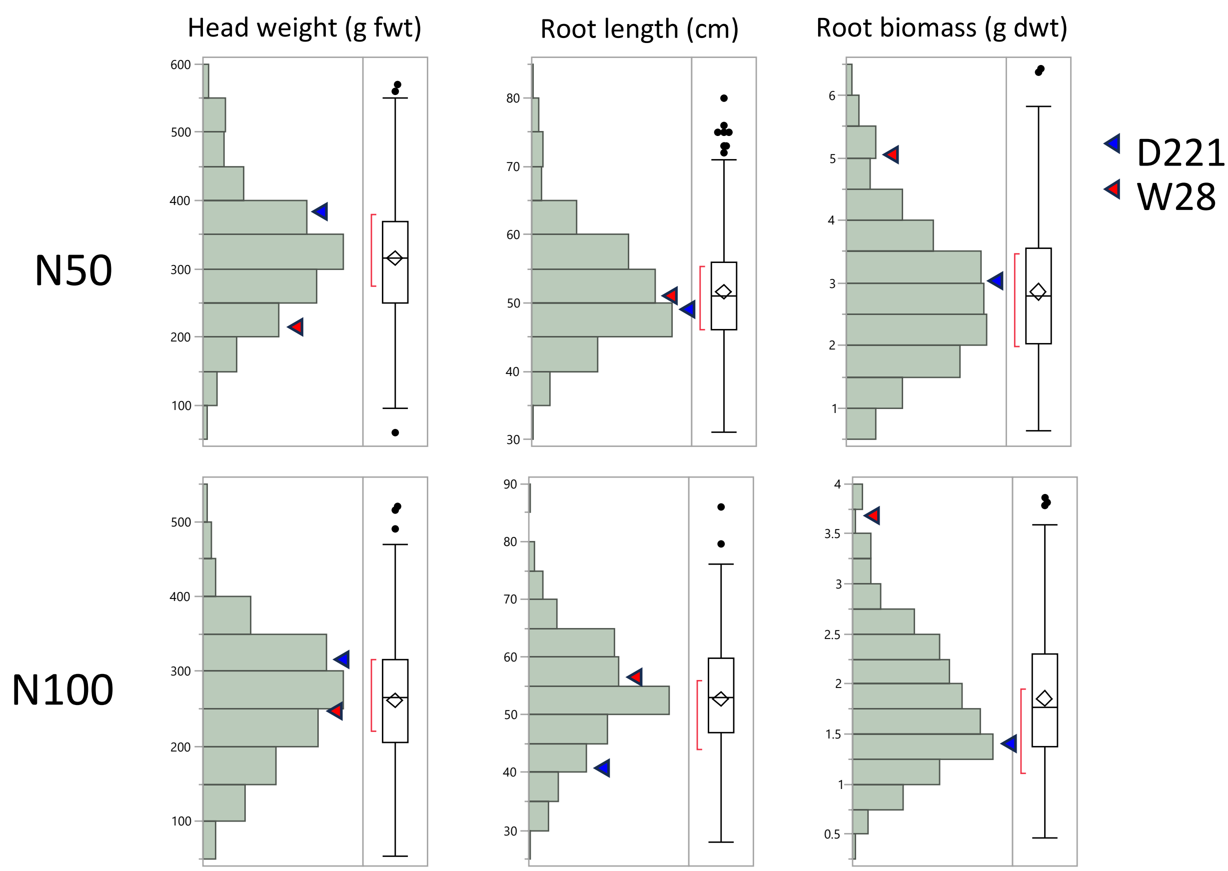
D221 W28

Figure 1. Parental lines D221 and W28 were used as parents in the D221 x W28 F7 recombinant inbred line (RIL) population. D221, a selection from the Diplomat x Margarita RIL, was previously characterized as a low root biomass, low leaf N concentration phenotype with high root growth plasticity in response to N. W28, a primitive *L. sativa* accession, was previously characterized as a high root biomass, high leaf N concentration phenotype with high photosynthetic rates, but relatively low root growth plasticity in response to N.

Plants were harvested at market maturity, approximately seven weeks after transplanting. At harvest, each plant was removed from their 20L grow bag and cut at ground level to separate the roots from the leaves (or head). For each individual plant, the root biomass and roots were tagged to retain genotype information during processing. The fresh weight of the leaves was obtained in the field immediately upon harvesting. To obtain root length and biomass, the roots of each individual plant were gently washed, and the longest root was identified and measured.

Once washed, the roots were transferred to trays, lightly dried and photographed for down-stream analyses of root architecture. Those data will not be presented herein. To obtain dry weight, the roots were chopped into 5-10 mm segments, placed into envelopes and oven dried at 65 °C for 48 h, and then weighed. To determine leaf C and N concentration, leaf discs were excised from freshly harvested plants, placed in envelopes and dried at 65 °C for 48 h. The oven-dried leaves and roots were pulverized to a fine powder in a ball mill, and ~50 mg of

Figure 2. Frequency distribution of head weight, root length and root biomass of the D221 x W 28 F7 recombinant inbred line grown under limited N (N50) or non-limited N conditions (N100). Plants were harvested and evaluated seven weeks after transplanting, which is approximately equivalent to market maturity. N = 279 F7 families for the N50 treatment; N = 277 F7 families for the N100 treatment. Parental values of D221 and W28 are indicated within the figures with blue and red arrowheads, respectively.



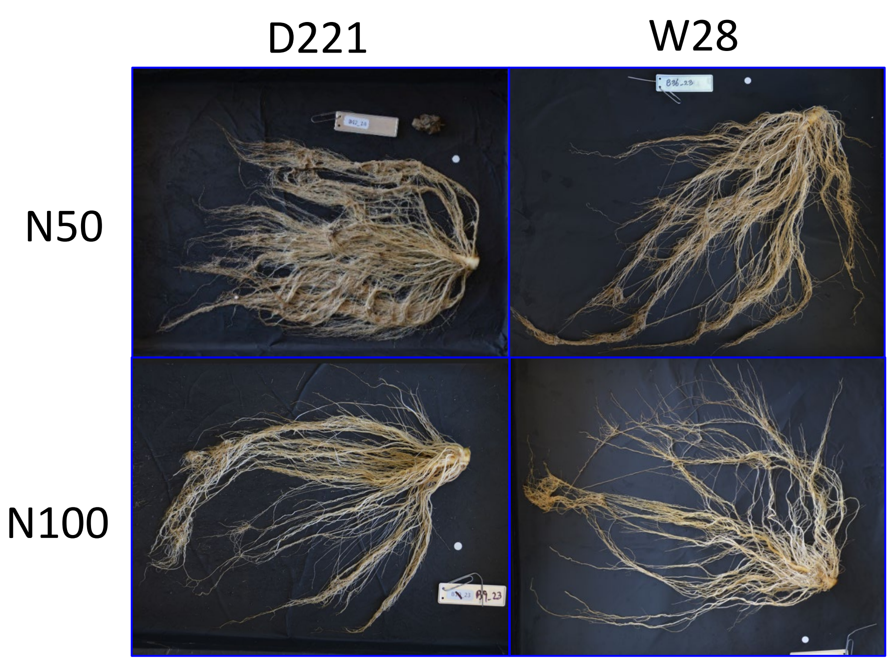
powder was transferred to sample capsules and held in a desiccator until C and N concentration analysis was performed using an Elementar vario Max cube instrument.

Results and Discussion

Head weight, Root length, and root biomass

Head weight of the D221 x W28 F7 families was broadly distributed and ranged from 60 to 570 g, and from 55 to 520 g for plants grown under the limited N treatment (N50) and non-limited N treatment (N100), respectively (Figure 2). As expected, the head weight of plants grown under the N100 treatment was significantly greater than those grown under the N100 treatment (μ = 315 and 262 g fwt, respectively; Prob > F <0.0001; Figure 2). The root length of plants grown under the two N treatments (N50 or N100) did not differ (Prob > F = 0.1161) and ranged between 30 to 80 cm (μ = 52 cm) for plants grown under the N50 treatment and between 28 and 86 cm (μ = 58 cm) for those grown under the N100 treatment (Figure 2). In response to limited N, the root biomass of the F7 families grown under the N50 treatment was significantly higher than the population when grown under the N100 treatment, with an average of 2.9 and 1.9 g dwt for the N50 and N100 treatments, respectively (Figure 2; Prob. > F < 0.0001).

Figure 3. Root architecture of parental lines D221 and W28 grown under limited N treatment (N50) or a non-limited N treatment (N100). Root architecture of each F7 family from the D221 x W28 recombinant inbred line population was evaluated by harvesting plants at market maturity, gently washing roots to remove potting media, and photographing. Each photograph will be evaluated using a software platform to derive root biomass, root area, length, width, projection area volume, spatial distribution and orientation. and multiple other attributes. These root attributes will be used as a trait for mapping, and each will be evaluated for its contribution to both water and nitrogen use efficiency.



The distributions of head weight, root length and root biomass closely approximated a normal distribution, and in each trait, transgressive segregation was observed. One strategy to improve N uptake and assimilation (NUA), is to select for genotypes that increase their root biomass, which serves to increase the volume of soil roots will contact, and thus, increase NUA. In the D221 x W28 population, when grown under the N50 treatment, root biomass increased by 1.55 times that measured when grown under N100.

Plasticity in root growth is a desirable feature. We previously reported that in general, plants will increase root biomass as N becomes limited, and that trend was widely observed in other lettuce RIL populations we previously reported. However, and importantly, it is not a universal response, and many lettuce genotypes did increase root biomass under the N50 treatment. This too was observed in other RIL lettuce populations our lab has evaluated. When the parental line D221 was grown under the N50 treatment, root biomass increased by 2.2-fold over that measured under the N100 treatment, thus confirming substantial plasticity to soil N availability. Many root architecture traits will be evaluated for the population using photographs taken at time of harvest (Figure 3). The same trend of increased root biomass under N50 versus N100 was observed for parental line W28, but only a 1.4-fold increase was observed. The 2.2-fold increase in D221 confirms results from earlier trials regarding plasticity in response to limited N.

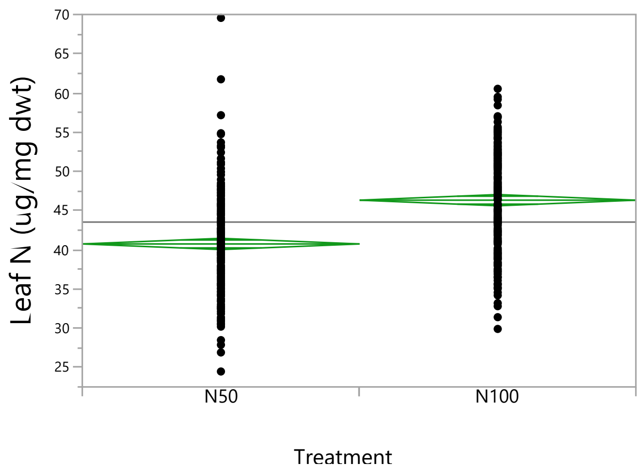
Under the N50 treatment, seven genotypes were observed within the 97.5 percentile of the population, corresponding to a root biomass value of 5.46 g dwt, a 1.96-fold increase over the median value (Figure 2). These seven genotypes will be further evaluated in the field and are candidates for release as genetic lines to improve root biomass under limited N.

Leaf Nitrogen, Leaf Carbon and C:N ratios

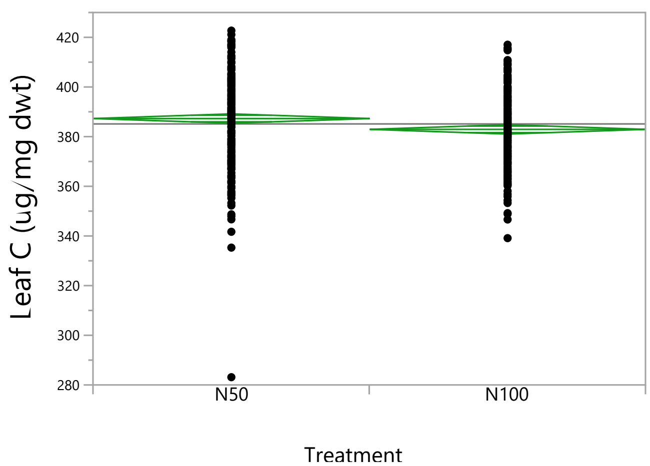
Nitrogen is required in relatively high amounts in a plant to support photosynthesis, particularly for the generation/regeneration of Rubisco and as components and building blocks of the light-harvesting complex found in leaves. We previously reported that genotypes from other lettuce RIL populations characterized as having high leaf N concentration phenotypes had higher photosynthetic rates (i.e., carbon assimilation rates) compared to those exhibiting a low leaf N concentration phenotype. Additionally, genotypes exhibiting high leaf N concentration phenotype have greater growth rates than the low leaf N concentration phenotypes. Thus, selecting for high leaf N concentration would enable higher growth rates, and identifying genotypes that maintain the highest leaf N concentrations under limited N is a strategy our lab has adopted.

In the D221 x W28 RIL, the population average of leaf N concentration ranged from 29.9 to 60.5, and 24.5 to 69.6 µg N/mg leaf dry weight under the N100 and N50 N treatments, respectively (Figure 4). Population mean values were 46.3 and 40.8 µg N/mg leaf dry weight under the N100 and N50 N treatments, respectively (Figure 4; Prob > F <0.0001). It is important to note that despite decreasing N supply by 50%, leaf N concentration decreased by only 12% in response. The 97.5 percentile value for N50 was 53.8, which is 132% above the population average for the limited N treatment. This suggests that these genotype exhibit an uncommon ability for NUA under a limited N supply.

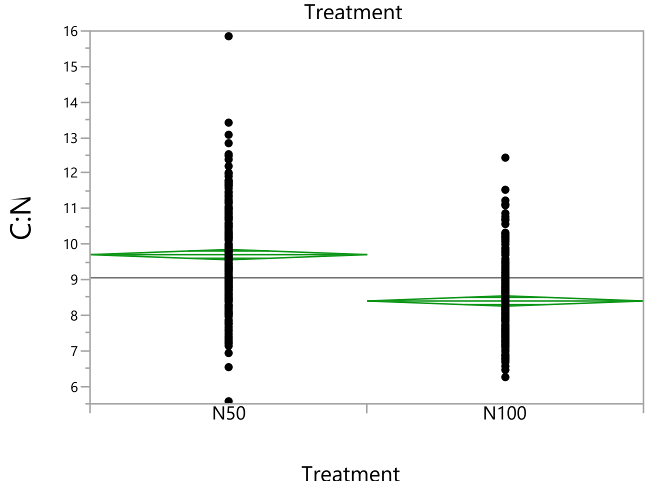
Figure 4. Leaf N concentration (A), leaf C concentration (B) and C:N ratios (C) of plants grown under limited N (N50) or non-limited N (N100).



Panel A. Leaf N concentration of the D221 x W28 RIL population in response to limited N (N50) or non-limiting N (N100) treatments. Plants grown under the non-limiting N treatment (N100) had higher leaf N concentration than plants grown under the limited N (N50) treatment Prob > F <0.0001; For N50, n=279 F7 families; for N100, n=277 families.



Panel B. Leaf Concentration of the D221 x W28 RIL population in response to limited N (N50) or non-limiting N (N100) treatments. Plants grown under the limited N treatment (N50) had higher leaf C concentration than plants grown under the non-limited N (N100) treatment. Prob > F = 0.0014; For N50, n=279 F7 families; for N100, n=277 families.



Panel C. Carbon:Nitrogen (C:N) ratios of the D221 x W28 RIL population in response to limited N (N50) or non-limiting N (N100) treatments. Plants grown under the limited N treatment (N50) had higher leaf C/N ratios than plants grown under the non-limited N (N100) treatment. Prob > F < 0.0001; For N50, n=279 F7 families; for N100, n=277 families.

Nitrogen assimilation is tightly coupled and coordinated with C metabolism though several metabolic pathways and signaling molecules. The integrated C/N network influences plant growth, including root versus shoot (i.e., in lettuce, leaves), root architecture, the transition from vegetative to flowering, respiration and photosynthesis. Biomass production is directly related to N assimilation, which is dependent on the C fixed during photosynthesis which serve as C skeletons used in N assimilation. Thus, tracking C and C:N ratios provides an insight into the efficiency of N assimilation as well as providing an indication of the impact of inorganic N supplies. In general, higher C:N ratios suggest N-limited growth.

Leaf C concentration ranged from 339 to 417, and from 283 to 423 μg C/mg leaf dry weight, for the D221 x W28 population grown under N100 and N50 treatments, respectively (Figure 4). Population mean values were higher in plants grown under limited N, with 383 and 387 μg N/mg leaf dry weight under the N100 and N50 N treatments, respectively (Figure 4; Prob > F 0.0014).

C:N ratios ranged from 6.3 to 12.4, and from 5.6 to 15.6, for the D221 x W28 population grown under N100 and N50 treatments, respectively (Figure 4). Population C:N mean was higher in plants grown under limited N, with values of 8.4 and 9.7 observed for plants grown under the N100 and N50 N treatments, respectively (Figure 4; Prob > F < 0.0001). The 97.5 percentile C:N value for population grown under the N50 treatment was 12.5, compared to 10.8 for those grown under the N100 treatment. We can speculate that genotypes exhibiting a lower C:N ratio under limited N might be under less N-induced stress, compared to those with higher ratios. Thus, selecting for low C:N ratio phenotypes may offer an additional method to improve NUE in lettuce, and a method that is relatively straight-forward and inexpensive.