

Abstract

Uncertainty about water availability for agriculture due to prolonged droughts and environmental concerns about nitrogen fertilizers has made it necessary to improve the efficiency in which both resources are used by the lettuce industry. A long-term objective of the Still lab is to improve both water use efficiency and nitrogen use efficiency of lettuce through genetic means. To accomplish this goal, our immediate objectives, reported here, were to map as quantitative trait loci, water and nitrogen use efficiency in a recombinant inbred line (RIL) of lettuce. Carbon and nitrogen isotope discrimination were used as proxies of water and nitrogen use efficiency, respectively. The advantage of using isotope discrimination is that it is a measure of the efficiency by which C and N are assimilated over the duration of the crop cycle. Seed from 182 families from the RIL were planted in a field in the low desert in September 2013. To estimate the effects of water and nitrogen on water and nitrogen use efficiency, a control and a low water and nitrogen treatment were applied. The control treatment consisted of supplying water and N through drip irrigation with water replacement at 130% ET and 100% N (225 lbs N/acre) while the low water/ low N treatment consisted of water replacement at 75% ET and 50% N. A broad range of carbon and N isotope discrimination was observed in both water/N treatments. The population mean value for carbon isotope discrimination did not vary between the two water/N treatments, but N isotope discrimination differed by more than two-fold. Harvest weight did not differ between the two water/N treatments. Several quantitative trait loci were mapped for carbon and nitrogen isotope discrimination. Our data indicate a strong relationship between carbon and nitrogen assimilation, and therefore, water and nitrogen use efficiency. These data also indicate a large amount of variation among the genotypes, suggesting that both water and nitrogen use efficiency can be improved through genetic means.

Project Title: Mapping water- and nitrogen-use efficiency in a recombinant inbred line of lettuce.

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Objectives:

Our long-range objectives are to develop and make publically available, genetic and mapping resources by which water use efficiency (WUE) and nitrogen use efficiency (NUE) may be enhanced in lettuce. A parallel long-term goal is to learn what physiological mechanisms and specific genes underlie these traits which, if successful, will provide a direct avenue for improvement of these traits. We anticipate that the genetic resources can be developed within a two-three year time period and the physiological and genetic basis for these traits can be elucidated within a three to five year time period. We define genetic resources as mapping populations, improved genetic lines (but not cultivars) and single-nucleotide markers associated with these traits which, ultimately, will be linked to causative genes.

The objectives for this funding cycle are to quantify WUE and NUE in the Diplomat x Margarita recombinant inbred line (DxM RIL) population grown under reduced water and nitrogen conditions. A second objective is to map WUE and NUE as QTL in the DxM RIL population.

Procedures:

To determine WUE and NUE, the DxM RIL was planted at the Yuma Agricultural Center, whose climate, soil and cultural methods are representative of the low desert production areas of California and southwestern Arizona. The DxM RIL is a highly inbred population created from single seed descent. Seed from 182 F₁₂ families of the DxM RIL population were planted at the Yuma Valley Agriculture Center 17 September 2013 and germinated on 19 September using overhead sprinkle irrigation. An augmented block design was implemented with the parental lines serving as replicated checks across each block and the RIL genetic lines as unreplicated treatments. Each plot was approximately 6.1 m in length and a single RIL family/cultivar was planted in both seed lines of an individual plot. To estimate the effects of limiting water and nitrogen on water and nitrogen use efficiency, a control and a water and nitrogen –limiting treatment were applied: 1) Control: drip irrigation with water replacement at 130% ET and 100% N (225 lbs N/acre); 2) low water/ low N: drip irrigation with water replacement at 75% ET and 50% N.

On 15 and 16 December 2013 the plants had reached market maturity and were evaluated. To determine fresh weight for each genotype, a total of nine plants were harvested from the middle of each plot. The above-ground biomass was weighed and from these nine plants, three were

placed in labeled plastic bags and transported to Cal Poly Pomona for processing. The bagged lettuce were kept in a walk-in cooler and held at 4 °C until processed, which began on 17 December and was completed on 22 December 2013.

WUE and NUE were determined by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope discrimination. The advantage of this approach is that carbon fixation and nitrogen assimilation are integrated over the entire growing season. Leaves from the three plants harvested from the field were oven dried and subsequently pulverized using a ball-mill. Because of the costs associated with the isotope discrimination, and based on previous results that there is minimal between-plant variation of plants from the same plot in isotope composition, an equal amount of dried powdered from each of the biological replications was mixed together to form a single sample representative of each genetic line. The stable isotope data are reported as total C, total N, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Linear contrasts using the GLM procedure of SAS (ver. 9.3) were used to test for differences between the control and low H_2O /nitrogen treatments for carbon and nitrogen compositions, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope discrimination, and fresh weight. Each of these traits were mapped as QTL using the QTL module in SAS JMP Genomics (ver.6.0).

Results and Discussion:

When averaged across all genotypes for the two water/N treatments, the above-ground plant biomass did not differ between the control (130% ETo/100%N) and the low H_2O /N treatment (75%ETo/50%N) plots (Prob > F = 0.6993). These results indicate that under the conditions of this experiment, neither N nor water limited growth. This statement is with respect to the entire D x M population but pairwise comparisons between the two treatments within a genetic line indicated several families differed.

Similarly, the amount of C (dry weight basis) averaged across all genotypes, did not differ (Prob > F = 0.2586) between the two H_2O /N treatments, nor did $\delta^{13}\text{C}$ values (Prob > F = 0.1164).

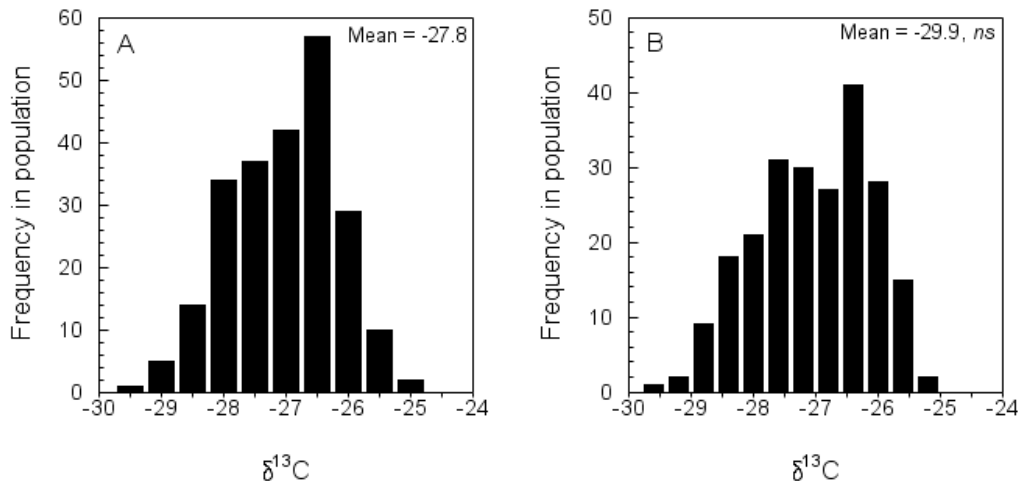


Figure 1. Distribution of $\delta^{13}\text{C}$ discrimination under control (A) and low (B) H_2O /N treatments of the DxM RIL population grown in the low desert. Seed of 182 F_{11} families were planted on 17 September and the field evaluated at market maturity on 19 December 2013. The $\delta^{13}\text{C}$ isotope discrimination values integrate the carbon fixation and nitrogen uptake during this period.

In contrast to that observed for the above ground biomass, C content and $\delta^{13}\text{C}$ isotope discrimination, both N content and $\delta^{15}\text{N}$ isotope discrimination were affected by the $\text{H}_2\text{O}/\text{N}$ regimes. The N content averaged across all genotypes, was significantly (Prob. > F = 0.0249) higher for the control treatment (144.15) than the low $\text{H}_2\text{O}/\text{N}$ treatment (137).

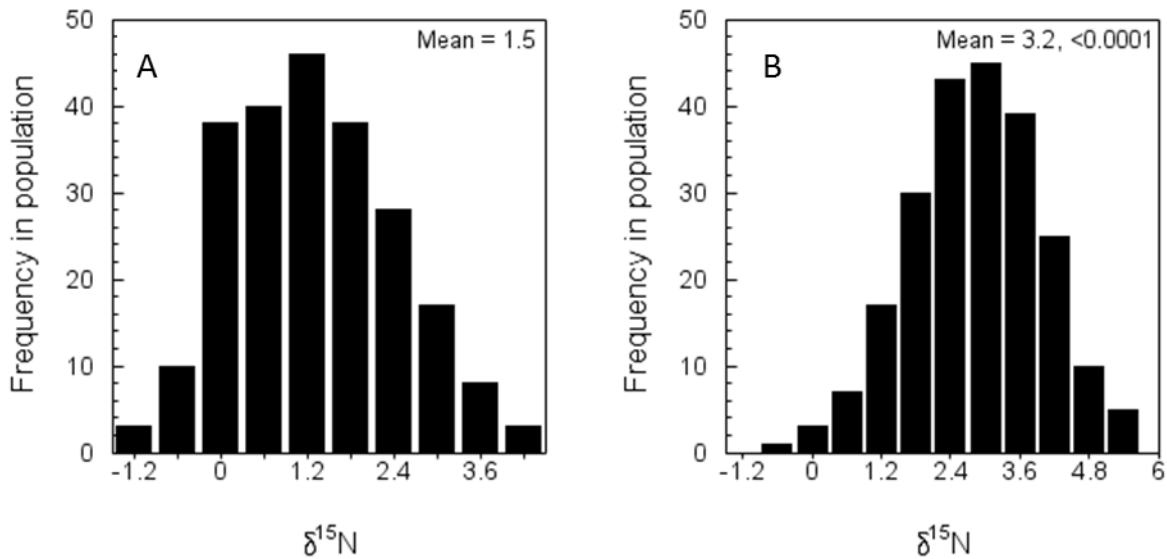


Figure 2. Distribution of $\delta^{15}\text{N}$ discrimination under control (A) and low (B) $\text{H}_2\text{O}/\text{N}$ treatments of the DxM RIL population grown in the low desert. The mean value of $\delta^{15}\text{N}$ isotope discrimination for the DxM RIL population was higher in genotypes under the low $\text{H}_2\text{O}/\text{N}$ treatment (B) (Prob > F, <0.0001) than that measured for the population grown under control $\text{H}_2\text{O}/\text{N}$ conditions. Seed of 182 F_{11} families were planted on 17 September and the field evaluated at market maturity on 19 December 2013. The $\delta^{15}\text{N}$ isotope discrimination values reflect nitrogen uptake and assimilation during this period.

The $\delta^{15}\text{N}$ content of the DxM RIL population under the control $\text{H}_2\text{O}/\text{N}$ treatment ranged from -1.2 to 4.4, whereas the population grown under the low $\text{H}_2\text{O}/\text{N}$ treatment had a greater range and the population mean was higher compared to the control treatment (Prob > F <0.0001; Figure 2). Variation in $\delta^{15}\text{N}$ discrimination values observed across the genotypes provides valuable information because it is indicative of the ability of a given genotype to uptake N from the soil and assimilate the N into leaves. Nitrogen is taken up by the plants as either nitrate (NO_3^-) or ammonium (NH_4^+) by two different mechanisms, depending on N concentration. All genotypes were grown on a field with the same N source applied (ammonia and nitrate) in a field with nutritional profiles that were largely uniform, but regardless are beyond the ability to control in a large field trial. Our study does not provide information about which N form is assimilated, but it does indicate there is wide variability among the genotypes. This indicates that genetic variation in NUE is present in lettuce germplasm. Because there is such wide range within the population, it indicates that large gains in NUE are possible. The $\delta^{15}\text{N}$ discrimination under control (A) and low (B) $\text{H}_2\text{O}/\text{N}$ treatments varied by more than two-fold, and this is a strong indication that water and N regimes strongly influences NUE (Figure 2).

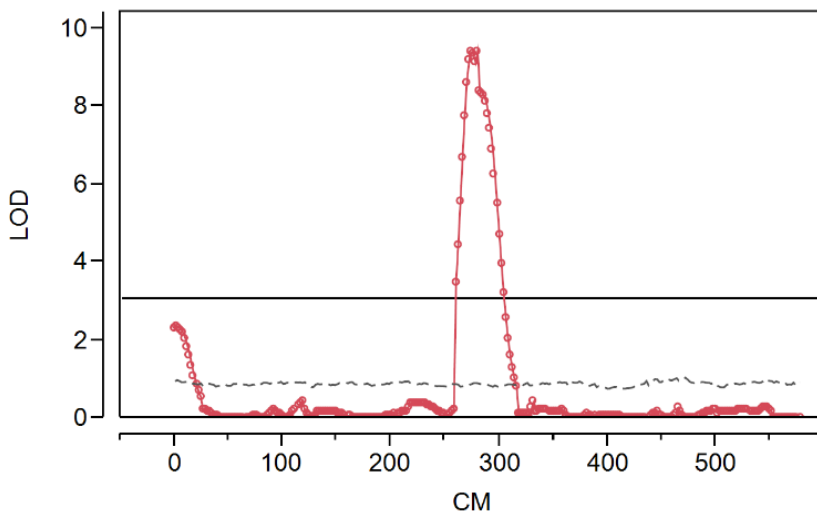


Figure 3. Quantitative trait loci (QTL) for $\delta^{13}\text{C}$ discrimination mapped to linkage group 3 of the DxM RIL population under the low $\text{H}_2\text{O}/\text{N}$ treatment. In addition, QTL for $\delta^{15}\text{N}$ discrimination collocated to this position on linkage group 3. The percent variation explained by the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ discrimination QTL was 24% and 17%, respectively. The additive effect of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ QTL were from allelic effects from Margarita and Diplomat, respectively.

We next sought to map as quantitative trait loci (QTL) the traits measured in this study, ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C and N concentration). Considering the control treatment (130% ETo/100%N), carbon discrimination ($\delta^{13}\text{C}$) mapped to linkage groups (LG) 3 and 6 and $\delta^{15}\text{N}$ mapped to two locations on LG 3 and one location on LG 7. The major QTL for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mapped to LG 3 in approximately the same location. A QTL for N composition mapped to LG 3; C composition did not map. Considering the DxM RIL population grown under the low water/N treatment (75% ETo/50%N), carbon discrimination ($\delta^{13}\text{C}$) mapped to LG 2 (two locations), 3, and 7 and $\delta^{15}\text{N}$ mapped to LG 3 and 8. The major QTL for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mapped to LG 3 in approximately the same location and in the same location as for the control experimental treatment (Fig. 3). No QTL was detected for N composition, but C composition mapped to LG 2.

In summary, our mapping data using C and N isotope discrimination indicate a close relationship between water and nitrogen use efficiency. Carbon fixation is affected by transpiration and therefore, water use efficiency. A close relationship between carbon and nitrogen is also predicted since the majority of leaf nitrogen is used for photosynthesis, either through structural proteins or photosynthetic enzymes. Using other funding, these studies will be extended across additional growing seasons and locations to assess the environmental influence on the expression of NUE and WUE.