Abstract:

California is a major producer of leaf and head lettuce for the United States, accounting for ~75% of the lettuce produced (USDA, National Agricultural Statistics Service 2006). The successful production of any plant requires the grower to maintain an optimal amount of available nutrients for growth. Over fertilization of a crop increases cost for the grower and increases the chances for groundwater contamination; while under fertilization will cost the grower yield, affecting profitability. Problems with ground water contamination have increased scrutiny of fertilizer practices within the lettuce production regions (Water Quality Control Board). This has led to new regulations in California. The goal of this project is to evaluate lettuce cultivars for genetic variability of nutrient uptake, and to identify genes, which may be associated with controlling nutrient uptake. Lettuce cultivars selected for screening are parents of current lettuce mapping populations. Phenotypic evaluation of lettuce cultivars was conducted utilizing a hydroponic growth system. Lettuce was grown in hydroponic channels using two nutrient concentrations, half label rate and quarter label rate. All samples were analyzed for leaf mass, nitrogen, phosphorus, and potassium. Based on initial results, genetic variability does exist within the current mapping populations, which should allow for more rapid identification of genes associated with nutrient uptake. Based on our current results, the mapping population of Salinas x Valmaine shows the most promise for further study.

Project Title: Identification of Genetic Variability in Lettuce for Nutrient Uptake

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Summary:

As part of an undergraduate research project, two students at Cal Poly San Luis Obispo, with support from the California Leafy Green Research Program, evaluated different lettuce varieties for their ability to absorb fertilizer. The students, Erin Miller and Mitchell Yerxa, two graduating seniors, planted 13 different lettuce varieties in a hydroponic growing system. Plants were grown for 53 days at two different nutrient rates. Plants were harvested and total nitrogen, phosphorous, and potassium were measured. Initial results show that variation is present within lettuce for ability to absorb fertilizer. The next step in the process is to attempt to identify genes which may affect the rate of fertilizer absorption. If genes could be identified, it may be possible to increase fertilizer absorption, reducing the need for high rates of fertilizer application.

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Objectives:

Immediate Objectives

Identify genetic variability within *Lactuca spp.* for efficiency in absorption of nutrients.

Long-range Objectives

1. Isolation of genes responsible for regulating nutrient absorption in lettuce
2. Development of new lettuce lines with elevated nutrient absorption ability

Procedures:

The original intention for the research was to evaluate a broad range of lettuce cultivars, representing two different lettuce species, *L. sativa*, and *L. serriola*. Following discussions with Dr. Richard Michelmore, it was decided that we would focus our attention on parents of current mapping populations within lettuce (Table 1). Phenotypic evaluation of cultivars was done using a greenhouse NFT hydroponic growth system (Figure 1). Prior to placing into the NFT channels, seed was germinated in trays under fluorescent light until radical emergence, and then were transferred to Oasis growing media then placed in a humidome with straight water until plant was large enough to be transferred to NFT channels. Plants in the NFT channels are continuously bathed in a recirculated nutrient solution. Nutrient solutions were based on label rate of General Hydroponics three-part nutrient solutions, FloraGro, FloraMicro, and FloraBloom. To test for variation of nutrient uptake, plants were grown using ½ label rate and ¼ label rate (Table 2). The pH and total amount of solution was checked daily during the growth period in the channels.

Table 1 – Varieties screened, representing mapping populations

CGN141263	La Brilliante	Salinas 88
Diplomat	Margarita	US96UC23
El Dorado	Salad Bowl	Valmaine
Emperor	Salinas	Western Red Leaf
Green Towers		

Figure 1 – NFT Growth Channels in the Greenhouse and Cal Poly San Luis Obispo

Whole plants were harvested at 53 days and roots removed below first leaf. Whole plants where dried in a forced air oven at 60 degrees Celsius for 12 - 24 hours in paper bags and weighed after dried. Samples were ground by a mortar and pestle until particles were of uniform size.

Nitrogen was analyzed by a VarioMax Nitrogen analyzer following a standard protocol. A standard reference material (SRM) was analyzed at the end of each run and to maintain calibration within 80-120% of the known value of the SRM. All samples were loaded into the machine and run, with data saved in a computer document. The nitrogen analyzer provided total nitrogen percentage for leaf material. To help standardize data, total nitrogen uptake was calculated by multiplying total leaf mass by percent nitrogen.

Phosphorus and Potassium were analyzed by inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES) and Flame Atomic Absorption Spectrophotometer (FAAS) respectively following a standard protocol. Each test was ran with a minimum of three standards to maintain a calibration of 90 – 110% of known value.

Table 2 – Total amount of Nitrogen, Phosphorous, and Potassium in the nutrient solution for the ½ and ¼ label rate

	FloraGro	FloraMicro	FloraBloom
Full Label Rate			
Nitrogen	13.98 mL	23.98 mL	0 mL
Phosphorous	6.98 mL	0 mL	17.98 mL
Potassium	41.98 mL	4.8 mL	14.38 mL
1/2 Label Rate			
Nitrogen	6.99 mL	11.99 mL	0 mL
Phosphorous	3.49 mL	0 mL	8.99 mL
Potassium	20.99 mL	2.4 mL	7.19 mL
1/4 Label Rate			
Nitrogen	3.5 mL	5.99 mL	0 mL
Phosphorous	1.75 mL	0 mL	4.49 mL
Potassium	10.49 mL	1.2 mL	3.6 mL

Results and Discussion

Results for the ½ label rate showed varietal differences for nitrogen, phosphorus, and potassium levels (Table 3). Nitrogen uptake values ranged from 0.14 - 2.78 grams with CGN14263 having the lowest nitrogen uptake and Western Red Leaf having the highest nitrogen uptake. Range for total leaf phosphorus was 40.45 – 102.91 mg/L with Margarita having the lowest concentration and Western Red Leaf the highest concentration. Range for total leaf potassium was 269.26 – 671.94 mg/L with Margarita having the lowest concentration and Western Red Leaf the highest concentration.

Table 3 – Nitrogen Uptake, Leaf Phosphorus and Leaf Potassium of the ½ label rate for the 13 varieties tested

Variety	N Uptake (g)	P (mg/L)	K (mg/L)
CGN14263	0.14	60.15	276.25
Diplomat	0.51	53.52	354.28
El Dorado	1.30	50.27	301.72
Emperor	2.07	62.81	363.73
Green Towers	1.18	90.67	351.61
La Brilliante	0.31	58.04	364.31
Margarita	0.67	40.45	269.26
Salad Bowl	0.55	51.84	335.15
Salinas	0.85	71.3	414.13
Salinas 88	0.62	42.27	288.85
US96UC23	0.45	47.72	273.98
Valmaine	0.51	86.38	324.83

Western Red Leaf	2.78	102.91	671.94

Results for the ¼ label rate also showed varietal differences for Nitrogen, phosphorus, and potassium levels (Table 4). Nitrogen uptake values ranged from 0.21 - 2.19 grams with Diplomat having the lowest nitrogen uptake and Western Red Leaf the highest nitrogen uptake. Range for total leaf phosphorus was 46.07 – 101.98 mg/l with Salinas 88 having the lowest concentration and Valmaine the highest concentration. Range for total leaf potassium was 224.56 – 389.12 mg/l with CGN14263 having the lowest concentration and Salad Bowl the highest concentration.

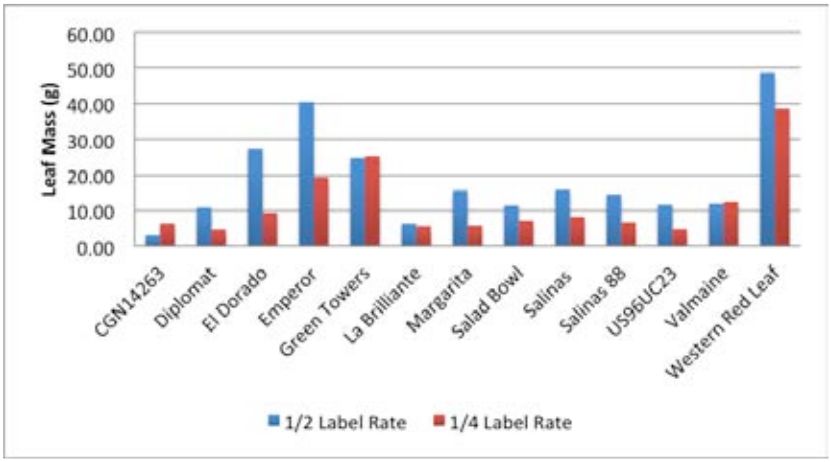
Table 4 – Nitrogen Uptake, Leaf Phosphorus and Leaf Potassium of the ¼ label rate for the 13 varieties tested

Variety	**N Uptake (g)**	**P (mg/L)**	**K (mg/L)**
CGN14263	0.30	57.33	224.56
Diplomat	0.21	52.71	295.26
El Dorado	0.46	53.96	271.51
Emperor	1.10	75.15	342.14
Green Towers	1.15	89.34	302.49
La Brilliante	0.27	67.26	332.85
Margarita	0.28	53.52	278.4
Salad Bowl	0.35	73.01	389.12
Salinas	0.43	57.75	309.26
Salinas 88	0.29	46.07	277.22
US96UC23	0.22	56.27	234.01
Valmaine	0.57	101.98	304.91
Western Red Leaf	2.19	73.24	377.95

Plants grown at the ½ label rate were generally of larger size (Figure 2) and had a higher total leaf Mass (Figure 3) for the varieties tested. Only CGN14263, Green Towers, and Valmaine showed a higher leaf mass in the ¼ label rate then in the ½ label rate.

Figure 2 – Salinas at 53 days growth at ½ and ¼ label rate

Figure 3 – Total dry leaf mass for ½ and ¼ label rate measured in grams for all 13 varieties tested



Two trials have been completed to date. Information reported above represents data taken during the second trial. Trial one was grown at full label rates and ½ label rate. Plants were grown for 53 days and harvested and all material was sent to Fruit Growers Laboratory (FGL). Results from FGL showed that samples had absorbed high to excess amounts of nutrients (Data Not Shown). The first trial was run using an older NFT channel system, which had problems with solution leakage, requiring the addition of high amounts of nutrient solution during the growing period which may have led to the excess amounts of nutrients accumulated within the tissues. Data from this trial were removed and a second trial was immediately started with the lower nutrient solutions. Test 3, which replicate trial 2, have been harvested and are in the process of being analyzed for nutrient content. These results should be available within the next 30 days. Because we only have 1 replication complete our findings are preliminary.

Based on the preliminary results thus far, we are confident that genetic variability exists within lettuce for nutrient absorption. Of the parental lines tested in this trial, we feel the Salinas x Valmaine cross might be of the most interest. At the ½ label rate Salinas and Valmaine show a divergent amount for nitrogen uptake (Figure 4), phosphorus (Figure 5) and potassium (Figure 6), with Salinas having higher levels for nitrogen uptake and potassium, and Valmaine having a higher level of phosphorus. The results from the ¼ label rate are not as clear. Valmaine has a higher nitrogen uptake value then Salinas. In total phosphorous, Valmaine and Salinas show almost no difference. What is of some interest is Valmaine was not affected as much by nutrient level (Figure 7) as Salinas, having a leaf mass of 11.91 and 12.41 grams in the ½ and ¼ label rate respectively. Salinas on the other hand had a large difference in plant size going from 15.81 grams at ½ label rate down to 8.24 grams at ¼ label rate.

Figure 4 – Nitrogen Uptake by Salinas and Valmaine at ½ and ¼ Label Rate

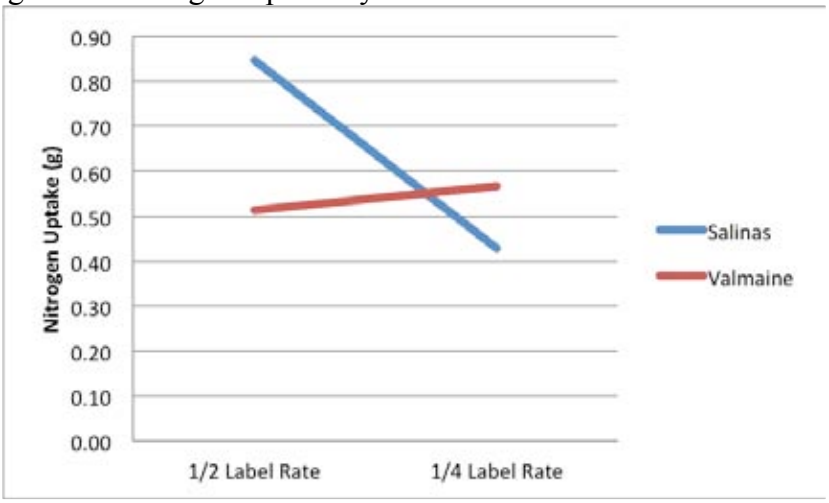


Figure 5 – Total Leaf Phosphorus in Salinas and Valmaine at ½ and ¼ Label Rate

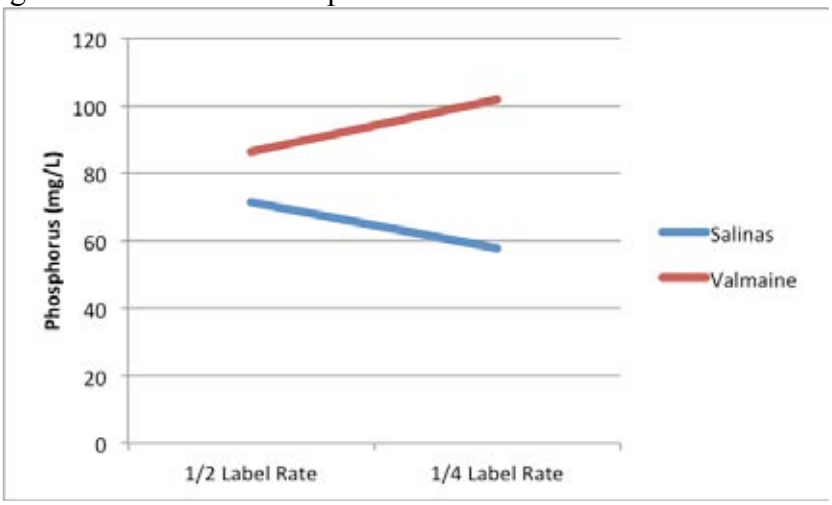


Figure 6 – Total Leaf Potassium in Salinas and Valmaine at ½ and ¼ Label Rate

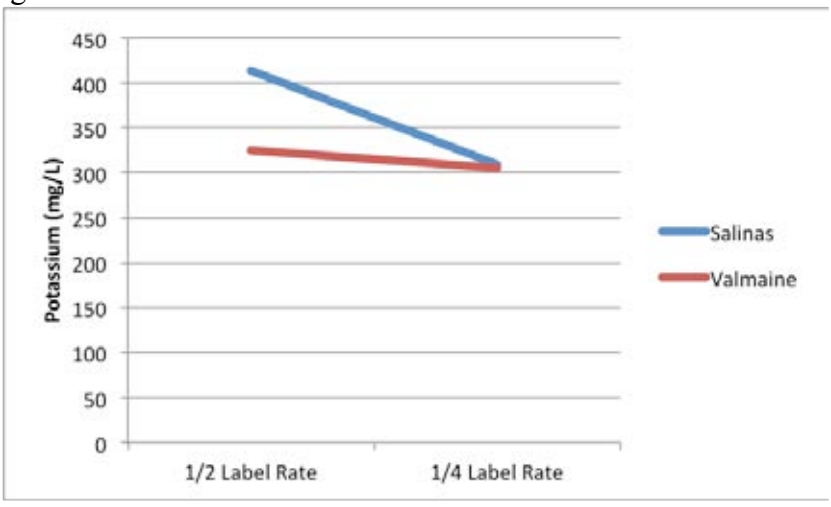


Figure 7 – Valmaine at 53 days growth at ½ and ¼ label rate