Annual Leafy Greens Research Report

For the Period April 1, 2017 to March 31, 2018

Project Title: Developing Baby Leaf Spinach with Lower Cadmium Uptake

Project Investigator(s): E. Charles Brummer; ecbrummer@ucdavis.edu; 530-574-6133
Allen Van Deynze; avandeynze@ucdavis.edu; 530-754-6444
UC Davis, One Shields Ave., Davis, CA 95616

Cooperating Personnel: Rachel Greenhut, Tim Hartz, Richard Smith, Juliana Osorio Marin

Abstract
The coastal ranges of Central California have areas with high cadmium (Cd) in bedrock and agricultural soils. Spinach is an agronomically important crop on the Central Coast and a known hyper-accumulator of Cd (Majmundar 1980, Alia et al. 2015). Because Cd accumulation can cause health risks, understanding the genetic mechanisms by which spinach accumulates Cd so that new varieties with reduced Cd content can be developed is an important goal for spinach production. Our long-range objective is to develop superior cultivars with reduced Cd uptake. Spinach germplasm was screened using high Cd field soil from the Central Coast. Screened spinach accessions were selected from international germplasm collections to cover a wide range of genetic diversity, including 615 accessions from 42 countries. Cadmium content of each trial entry was determined by Inductively Coupled Plasma Atomic Emission Spectrometry. Observed Cd content varied widely among accessions. Progress was made in the identification of candidate Cd regulation genes and marker development for a low Cd phenotype in spinach. Genetic marker assays were developed and used to genotype screened germplasm accessions for seven SNPs across three candidate genes. These genes were previously targeted for breeding low Cd accumulating bread wheat and rice. Three markers identified were associated with Cd content, but only a small portion of Cd content variation was explained by these markers. Low and high Cd accumulating accessions with consistent performance across environments were identified and will serve as a resource for the UC Davis spinach breeding program.

Objectives:
Our long-range objective of this project is to develop superior spinach cultivars that have reduced cadmium (Cd) uptake. Our immediate objectives are as follows:

1. Evaluating an additional 150 spinach accessions from international genebanks, including commercial cultivars, for Cd uptake in a soil-based greenhouse assay to complement our screening from the previous year.
2. Genotyping germplasm used in Objective 1 for DNA sequence diversity in candidate genes associated with low cadmium accumulation in leaves and confirm relationship between alleles at these genes and the low Cd phenotype.
3. Validate 20 low accumulating accessions in 2 replicated field trials on high CD soils in the Central Coast.
4. Developing hybrids between low Cd lines and 3-5 breeding populations as a first step to incorporate the trait into cultivars and to enable genetic mapping of genes associated with Cd uptake.

Procedures:

Objective 1

Field soil with ~3.0 ppm total Cd was used for the germplasm screening for three greenhouse trials: Fall 2016, Spring 2017, and Fall 2017. Seaside and Unipack 12 were used as controls in all trials, randomized within blocks. Four-inch diameter by 4.5-inch tall square pots were filled with prepared soil and 16 seeds were sown in each pot and covered with ¼ inch of vermiculite and ½ tsp D45 slow release fertilizer was applied to the surface. Border pots sown with a commercial cultivar were placed around all greenhouse bench edges to reduce edge effects. Pots were watered as needed, thinned to a maximum of 6 seeds per pot after germination, and grown for 5 weeks. Leaf discs were collected from each plant (up to 6 per pot), pooled by entry into microtubes (70 mg each), lyophilized in a freeze dryer, and stored for DNA extraction and genotyping. Duplicate tissue samples were collected of each entry. At 5 weeks post sowing, leaves with petioles were harvested, dried in a drying oven at 60C for one week, and sent to the UC Davis Analytical Lab for cadmium analysis. The first two trials evaluated a total of 594 accessions and cultivars. The third trial in Fall 2017 reevaluated the lowest 80 and highest 10 accessions.

Analysis of variance of observed Cd from combined trials was completed by fitting a linear mixed-effects model with entry and blocking factors (trial, environment, bench, and block) as random effects, and mean observed Cd of each check by bench within each trial as covariates. Covariates were calculated as such to reduce the effect of widely varying check plots in the model. To avoid circular analysis (i.e., 'double dipping'), check plots used to calculate covariates were excluded from the model with the exception of one plot per check per trial, selected at random. Insignificant blocking factor terms were dropped from the model, leaving environment and entry as random effects in the final model. Best linear unbiased prediction (BLUP) estimates were calculated for each screened accession and the grand mean Cd of combined trials was added to BLUPs to obtain BLUP-adjusted Cd content of each accession.

Objective 2.

DNA extractions from the 625 screened accessions were done using the NucleoSpin® 96 Plant II kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). DNA was sent to LGC Genomics LLC (Beverly, MA) for SNP assay development using KASP markers and subsequent genotyping of seven SNP loci on all accessions. Some accessions did not have either genotypic or phenotypic data, so 569 accessions were used in the analysis to determine a relationship between alleles at these genes and the low Cd phenotype. The BLUP-adjusted Cd concentrations of each accession and the SNP marker genotypes, together with the country of origin were analyzed using the computer program TASSEL (http://www.maizegenetics.net/tassel).

Results and Discussion:

The results of Objectives 1 and 2 constitute a MS thesis completed by graduate student Rachel Greenhut (Greenhut 2018).

Objective 1.
Evaluation of a large number of accessions necessitated using unreplicated trials (with replicated check entries) conducted over several trials. The mean Cd concentrations differed among the trials, but the relative performance of the two checks (Seaside (high) and Unipack 12 (low)) remained consistent; mean values show that the two checks differed in Cd content. Based on results from the first two trials, we identified high and low accessions to retest (Fig. 1a). The final trial, Fall 2017, reevaluated these accessions in a replicated trial to determine the repeatability of the Cd concentrations. The observed Cd values (not BLUP adjusted Cd) showed that these accessions were consistent across the three trials (Fig. 1b). The trial-to-trial variation is shown in this figure. These findings show there is a wide range of Cd uptake among screened accessions. We have identified 10 low Cd selections for further testing and potential use in our breeding program.

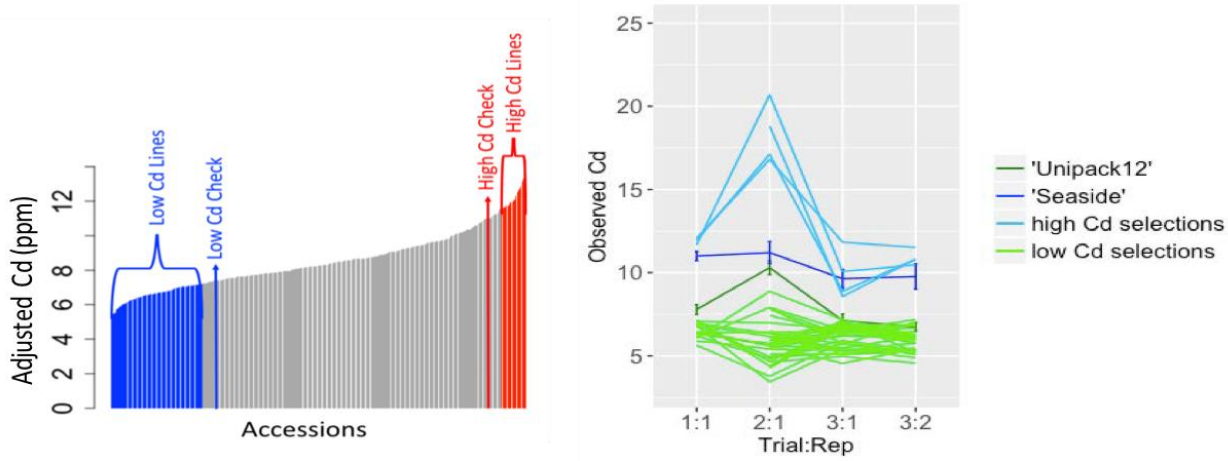

Figure 1. (a) Left panel. The adjusted Cd levels of ~600 spinach accessions, showing the range of variation after adjusting for effects of different evaluation environments. (b) Right panel. Consistency of observed Cd concentrations (not BLUP adjusted values) across three trials and two replications of the third trial. Although variation among testing environments is present, the checks and the high and low selections remain consistent.

Objective 2
We evaluated seven SNP markers present in three candidate genes associated with low cadmium accumulation in leaves using a KASP marker assay. Four of the markers had not association with Cd. The other three markers were associated with Cd but the strength of the association was weak. This can be due to low frequency of positive alleles in the population, population structure or that the markers are not closely linked to Cd accumulation in spinach. Further work scanning the entire genome is needed in order to identify markers associated with Cd uptake and to create structured bi-parental populations for testing. We are scanning additional markers with funding from the spinach industry.

Objective 3.
We identified accessions with low Cd to be used in field trials. However, before we could plant trials, we need to increase the amount of seed we have on hand. We have increased seed of the lowest 10 to date. Field trials will be conducted in 2018.

Objective 4.
We are planning to hybridize low Cd accessions with our breeding populations after confirmation in the field.

A peer-reviewed publication is being submitted to summarize these results. A Masters student, Rachel Frank Greenhut, was trained in plant breeding with this research. She has obtained a position in the seed industry.

Citations

Alia, N., Khan S., Muhammad S., Alam S., Siddique S., Ahmed T., and Schilz M. 2015. Toxicity and bioaccumulation of heavy metals in spinach (*Spinacia oleracea*) grown in a controlled environment. Int. J. Environ. Res. and Public Health 12:7400–7416.

Greenhut, R. 2018. Developing Baby Leaf Spinach with Reduced Cadmium Accumulation. MS thesis. University of California-Davis.

Majmundar, H. 1980. Distribution of heavy elements hazardous to health, Central Coast region, CA. Special Report. Sacramento, CA: California Division of Mines and Geology.