Project Title: Spinach Breeding and Genetics

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Abstract:

Certain soils in the Salinas Valley were derived from marine shale and contain high cadmium (Cd) levels, especially in the southern part of the Valley on the west side. The Cd-mineralized soils extend from the Malibu Canyon through Ventura and Monterey into Santa Cruz Counties. Spinach grown in these soils may have considerably higher than normal Cd content, leading to food safety concerns. We conducted greenhouse experiments to assess the genetic variation in Cd concentrations among 75 spinach cultivars and germplasm accessions. Significant differences in Cd concentrations were detected among the varieties tested. PI 169680, PI 169673, and PI 604782 consistently had low Cd concentrations when grown in soils containing low (0.8 ppm) and high (2.6 ppm) levels of Cd. Among cultivars, Sparrow, Callisto, and LGR 10 had low Cd content consistently. The varieties with low Cd concentrations identified can be used by spinach breeders to develop cultivars with reduced Cd content and by growers for cultivar selection in spinach production. Our results suggest that besides Zn, fertilizers or soil amendments containing Co, Cu, Ni, and Se could also be used to reduce Cd concentrations in spinach crops. We also conducted experiments to breed spinach for resistance to downy mildew disease, leafminer insect, and linuron (Lorox) herbicide.

Objective 1. Assessing the Genetic Variation in Cadmium Concentrations among Spinach Varieties

Certain soils in the Salinas Valley were derived from marine shale and contain high cadmium (Cd) levels, especially in the southern part of the Valley on the west side. The Cd-mineralized soils extend from the Malibu Canyon through Ventura and Monterey into Santa Cruz Counties. Spinach grown in these soils may have considerably higher than normal Cd content.

Besides soil amendments, spinach varieties could play an important role in the integrated Cd management. Cd accumulation and distribution in plants differ with species and among cultivars within a species. Spinach is quite tolerant to Cd and can accumulate a relatively large amount of the mineral. Therefore, spinach is considered to have bioremediation potential for decontamination of cadmium in soil. It was found that lettuce cultivars have a wide range of Cd concentration in the tissue. Varietal variation in Cd content has been found for spinach, and large differences in Ca, Fe, Mg, K, P, Zn, Mn, Cu, Mo, and Ni concentrations have also been detected among the USDA spinach germplasm accessions. The current experiment was conducted to assess the genetic variation in Cd concentrations among spinach cultivars and germplasm accessions.

Procedures

Forty-five current commercial cultivars and 30 USDA spinach accessions were screened in greenhouse experiments. Ten seeds from each variety were planted in a 3-inch pot filled with soils collected from a grower's field in Greenfield containing 0.8 and 2.6 ppm Cd with four replications. Six weeks after planting, spinach leaves were harvested, washed, dried, digested with nitric acid, and analyzed for Cd and other elements by using an Inductively Coupled Plasma (ICP) mineral analyzer.

Data of mineral concentration were analyzed by analysis of variance (ANOVA) using the general linear models (GLM) procedure of JMP Version 5. Genotype is considered a fixed effect, and replication is considered a random effect. For comparisons among genotypes, least significant differences (LSD) were calculated with a Type I (α) error rate of $P = 0.05$.

Results and Discussion

There was significant variation in Cd concentration among 75 spinach varieties grown on 0.8 and 2.6 ppm Cd soil (Figures 1 and 2). We found a wide range of Cd concentrations among the spinach varieties tested. There was about one time (100%) difference in Cd concentrations among varieties grown on the 0.8 ppm Cd soil and three times difference on the 2.6 ppm Cd soil.

Cd concentration was highly correlated with concentrations of Co, Cu, Ni, Se, and Zn (Table 1). Zn has been shown to inhibit Cd uptake by plants, Cd transport to storage organs of many crops, and Cd absorption by animals that consume the crops, due to competition between the two elements for receptors and/or transporters. Fertilizers containing Zn such as $ZnSO_4$ were found to be effective in reducing Cd levels in crops including spinach. Our results suggest that these competitions also exist between Cd and other elements. Besides Zn, fertilizer or soil amendments containing Co, Cu, Ni, and Se could also be effective in reducing Cd concentrations in spinach crops.

We compared the results from the current experiment (Trial 2) with the data from experiments conducted in previous years (Trial 1). PI 169680, PI 169673, and PI 604782 consistently had low Cd concentrations (Table 2). These varieties can be used as source of low

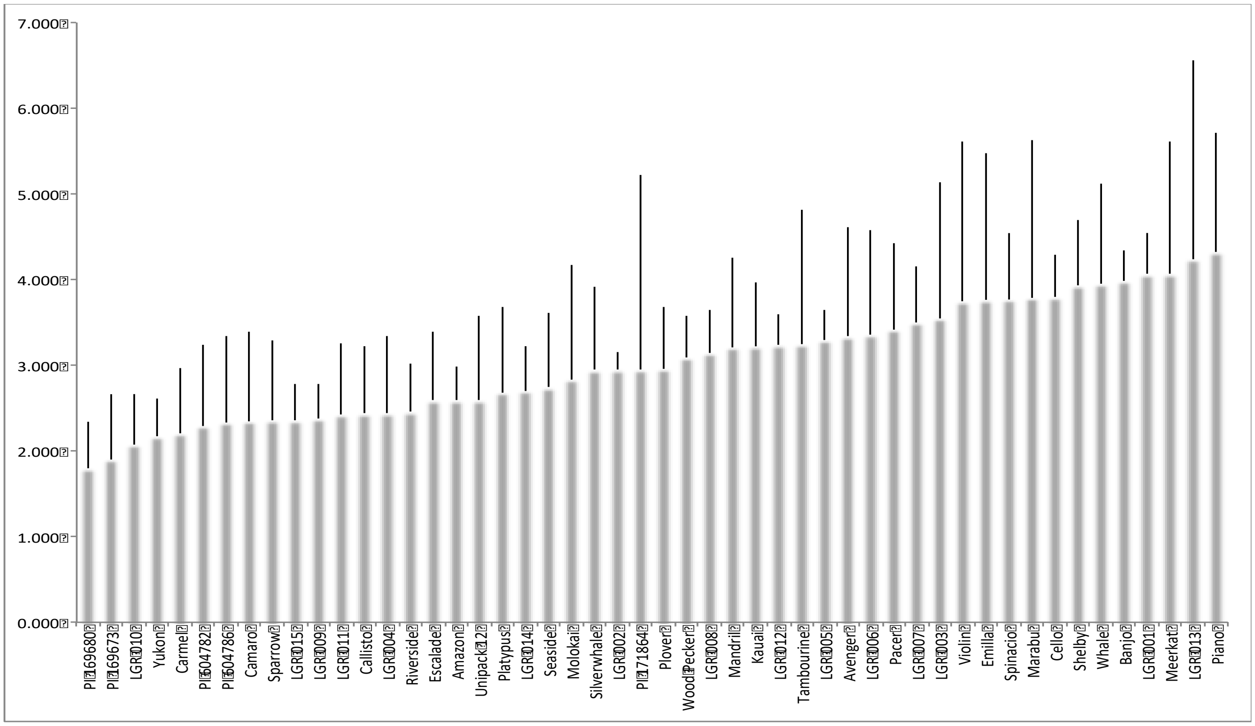

Figure 1. Means and standard deviations of Cd concentrations (ppm on a dry weight basis) for 50 spinach varieties grown in 0.8 ppm Cd soil in a greenhouse test. LGR varieties are commercial cultivars coded by the CA Leafy Greens Research Program.

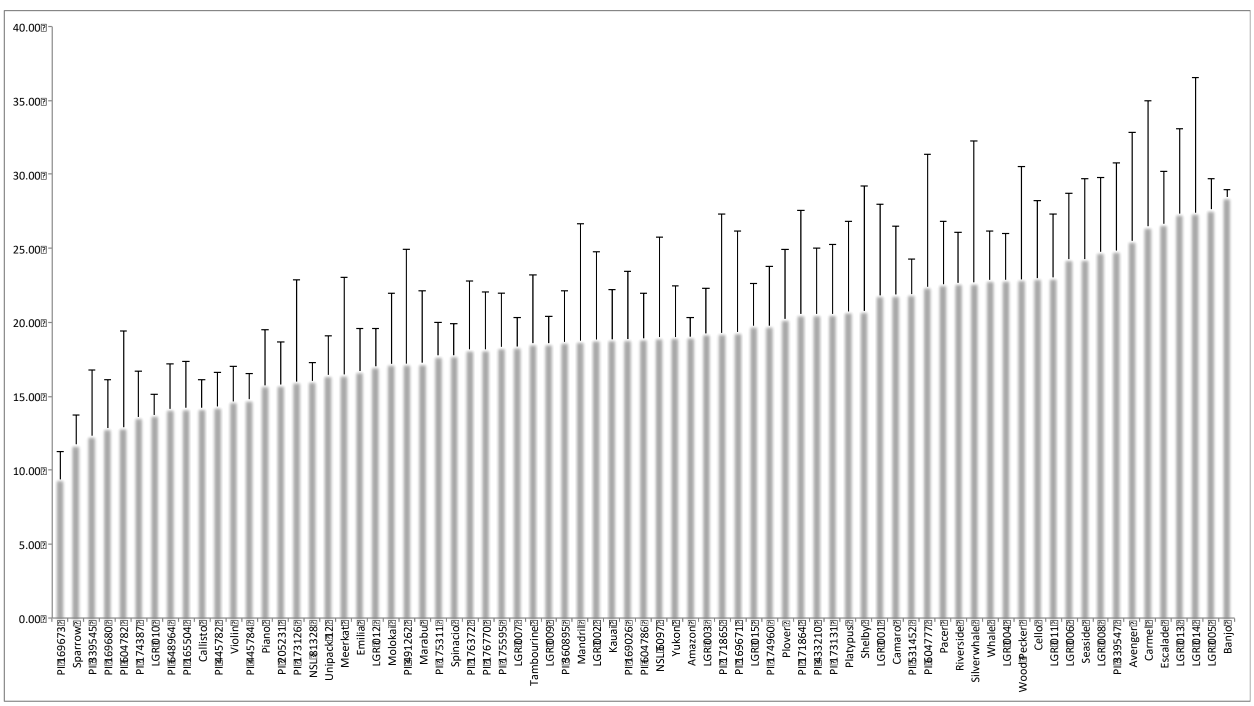

Figure 2. Means and standard deviations of Cd concentrations (ppm on a dry weight basis) for 75 spinach varieties grown in 2.6 ppm Cd soil in a greenhouse test.

Table 1. Correlation coefficients of Cd concentration with concentrations of other elements for 75 spinach varieties grown in 0.8 and 2.6 ppm Cd soils.

	0.8 ppm Cd Soil	2.6 ppm Cd Soil
Al	0.164	---
B	0.455**	0.359*
Ca	-0.127	0.382**
Co	0.763**	0.560**
Cr	-0.137	0.397**
Cu	0.634**	0.415**
Fe	0.261	0.120
K	0.518**	0.253
Mg	-0.122	0.390**
Na	-0.130	-0.020
Ni	0.679**	0.396**
P	0.651**	0.319*
Pb	0.177	---
S	0.777**	0.229
Se	0.756**	0.421**
Sr	-0.148	0.328*
Zn	0.627**	0.707**

*, ** Significant at 0.05 and 0.01 level, respectively.

Table 2. Top ten spinach varieties with lowest Cd concentrations (ppm on a dry weight basis) from two greenhouse trials on 0.8 and 2.6 ppm Cd soils.

0.8 ppm Cd Soil				2.6 ppm Cd Soil			
Trial 1		Trial 2		Trial 1		Trial 2	
PI 604782	3.2	PI 169680	1.8	PI 169673	7.4	PI 169673	9.4
Plover	3.2	PI 169673	1.9	PI 604782	9.2	Sparrow	11.7
PI 169673	3.5	LGR 010	2.1	PI 169680	9.3	PI 339545	12.3
Sparrow	3.6	Yukon	2.2	PI 339545	9.4	PI 169680	12.8
Callisto	3.8	Carmel	2.2	PI 648964	9.5	PI 604782	12.9
PI 169680	3.8	PI 604782	2.3	PI 175595	9.9	PI 174387	13.6
LGR 011	4.2	PI 604786	2.3	PI 173126	10.0	LGR 010	13.7
PI 604786	4.4	Camaro	2.4	PI 445784	10.5	PI 648964	14.1
Yukon	4.4	Sparrow	2.4	LGR 004	10.9	PI 165504	14.2
Whale	4.5	LGR 015	2.4	PI 165504	11.0	Callisto	14.2
Trial Mean	5.5	Trial Mean	3.0	Trial Mean	12.9	Trial Mean	19.3
High	7.8	High	4.3	High	17.8	High	28.4

Cd concentrations in a breeding program to develop commercial spinach cultivars with reduced Cd content. Among cultivars, Sparrow, Callisto, and LGR 10 had low Cd content consistently (Table 3). These cultivars can be used by growers for spinach production on soils with cadmium. The correlation coefficients of Cd concentrations (*r*) between Trial 1 and Trial 2 were 0.294 (significant at 0.05 probability level) for 0.8 ppm soil and 0.665 (significant at 0.01 probability level) for 2.6 ppm soil. For the experiments on the 2.6 ppm Cd soil, leaf samples from both Trial 1 and Trial 2 were analyzed in our lab. For the experiments conducted on the 0.8 ppm Cd soil, Trial 1 samples were sent to the University of California-Davis Analytical Lab for Cd analysis because our ICP mineral analyzer broke down, while Trial 2 samples were analyzed in our lab. The differences in sample preparation, digestion, and analysis between labs might contributed to the low correlation coefficient. Dried leaf samples were ground into powder before digestion in the UC-Davis lab, while samples were digested directly without grinding in our lab.

In summary, significant differences in Cd concentrations were detected among 75 spinach varieties grown on low and high Cd soils. The varieties with low Cd concentrations identified can be used by spinach breeders to develop cultivars with reduced Cd content and by growers for cultivar selection in spinach production. Our results also suggest that besides Zn, fertilizers or soil amendments containing Co, Cu, Ni, and Se could also be used to reduce Cd concentrations in spinach crops.

Other Research Projects:

Downy Mildew Crosses were made among ten cultivars with different downy mildew-resistant genes to combine their resistances. Progenies from 26 crosses, along with resistant and susceptible controls, were planted in a field at the USDA-ARS station in Salinas, CA to test their resistance to downy mildew in conditions of natural infections in September 2017. We also planted downy mildew-susceptible spinach cultivar 'Viroflay' in high density on both sides of the testing plots to attract, multiply, and spread the pathogen. 'Viroflay' was planted twice, with the breeding populations and two weeks before the main experiment. The field was kept wet with sprinkler irrigations two to three times a week to favor downy mildew development. Resistant plants with few downy mildew lesions were selected from each of the 26 populations and transplanted into our isolators for open-pollination. Seeds produced have been harvested and are being cleaned, and will be used for the next round of selection in the field.

We also planted the entire USDA spinach germplasm collection (409 accessions) in the field in two replications and recorded the downy mildew incidence and severity. The data were sent to Dr. Ainong Shi at University of Arkansas to develop molecular markers for downy mildew resistance. We also made crosses between female-only 'Viroflay' and other downy mildew resistance differentials 'Resistoflay', Califlay', 'Clermont', 'Campania', 'Boeing', 'Lion', 'Lazio', 'Whale' in isolators to develop resistance mapping populations.

Leafminer The predominant species of leafminers in central California is *Liriomyza langei.* They have a wide host range including broccoli, cauliflower, celery, lettuce, melons, spinach, tomato, and many weeds. Chemical control is not long-lasting, and it is well documented that leafminers can develop a high degree of resistance to insecticides. Insecticides for adult flies do not provide economic control against a moving target. Chemicals against leafminer larvae may be limited by pre-harvest spray interval (PHI) for a short-season crop like spinach or plantback restrictions for rotational crops.

Table 3. Top ten spinach cultivars with lowest Cd concentrations (ppm on a dry weight basis) from two greenhouse trials on 0.8 and 2.6 ppm Cd soils.

0.8 ppm Cd Soil				2.6 ppm Cd Soil			
Trial 1		Trial 2		Trial 1		Trial 2	
Plover	3.2	LGR 10	2.1	LGR 4	10.9	Sparrow	11.7
Sparrow	3.6	Yukon	2.2	LGR 12	11.4	LGR 10	13.7
Callisto	3.8	Carmel	2.2	LGR 15	11.5	Callisto	14.2
LGR 11	4.2	Camaro	2.4	LGR 7	11.5	Violin	14.6
Yukon	4.4	Sparrow	2.4	LGR 3	12.0	Piano	15.7
Whale	4.5	LGR 15	2.4	LGR 9	13.4	Unipack 12	16.4
LGR 10	4.6	LGR 9	2.4	LGR 2	13.7	Meerkat	16.5
Emilia	4.6	LGR 11	2.4	LGR 5	14.0	Emillia	16.7
Unipack 12	4.6	Callisto	2.4	LGR 8	15.6	LGR 12	17.0
Meerkat	4.6	LGR 4	2.4	LGR 13	15.6	Molokai	17.2
Trial Mean	5.5	Trial Mean	3.0	Trial Mean	12.9	Trial Mean	19.3
High	7.8	High	4.3	High	17.8	High	28.4

Table 4. Seeds planted and survived plants after the linuron herbicide spray in a field for commercial spinach cultivars and breeding populations.

Genotype	Survivors/Seeds planted	% Survived
Polar Bear	0/100	0
Spring Field	0/100	0
Bordeaux	0/100	0
Red Deer	0/100	0
Swan	0/100	0
Melody	0/100	0
Tarpy	0/100	0
Cypress	0/100	0
Whale	0/100	0
Unipack 277	0/100	0
Space	1/100	1.0
Eagle	1/100	1.0
Crocodile	10/600	1.7
Polka	2/100	2.0
Seven R	2/100	2.0
Viroflay	6/150	4.0
16-17-68	11/500	2.2
16-17-11	22/700	3.1
16-17-76	75/600	12.5

A recurrent selection method was used to increase the level of resistance to leafminers in four populations of different leaf types planted in a USDA-ARS research field in Salinas in August 2017. Due to a hot spell after the planting, which might have caused thermo-dormancy in seeds, the seed germination and stand were poor. Plants with fewer leafminer stings or mines were selected and transplanted into isolators to produce seeds for further rounds of evaluation and selection.

Herbicide Tolerance (with Steve Fennimore's Group) Few herbicides are registered for leafy vegetables in California and they are subject to cancellation due to their relatively small acreage, marginal profitability of these products, and regulatory challenges. Linuron (Lorox) is an herbicide that can provide effective control of more than 20 broadleaf weeds and grasses. Differences in tolerance to this herbicide have been observed in spinach varieties. Herbicide-tolerant spinach would allow growers to control weeds with the herbicide without injuring the crop.

We screened spinach germplasm for tolerance to linuron (Lorox) herbicide in the field. Ten breeding populations were planted in the field along with 16 commercial cultivars in August, 2017. There was also a hot spell after planting that could have caused thermo-dormancy and poor seed germination. The stand count of the cultivars and some breeding populations after the linuron herbicide spray (1.0 lb a.i./a) is shown in Table 4. Among commercial cultivars, Viroflay had the highest survival rate at 4% after the linuron spray. A breeding population 16-17-76 had the highest survival rate of 12.5%, showing tolerance to the herbicide. We will repeat the experiment next year to confirm the results. Surviving tolerant plants were transplanted into isolators to produce seeds for testing and selection next year.

Publications relevant to this project in 2017-18:

Xu, C., C. Jiao, H. Sun, X. Cai, X. Wang, C. Ge, Y. Zheng, W. Liu, X. Sun, Y. Xu, J. Deng, Z. Zhang, S. Huang, S. Dai, B. Mou, Q. Wang, Z. Fei, Q. Wang. 2017. Draft genome of spinach and transcriptome diversity of 120 *Spinacia* accessions. Nature Communications 8: 15275. doi: 10.1038/ncomms15275

Qin, J., A. Shi, B. Mou, M.A. Grusak, Y. Weng, W. Ravelombola, G. Bhattarai, L. Dong, and W. Yang. 2017. Genetic diversity and association mapping of mineral element concentrations in spinach leaves. BMC Genomics 18: 941. DOI 10.1186/s12864-017-4297-y

Shi, A., J. Qin, B. Mou, J. Correll, Y. Weng, D. Brenner, C. Feng, D. Motes, W. Yang, L. Dong, G. Bhattarai, and W. Ravelombola. 2017. Genetic diversity and population structure analysis of spinach by single-nucleotide polymorphisms identified through genotyping-by-sequencing. PLoS ONE 12(11): e0188745. https://doi.org/10.1371/journal.pone.0188745