Exploring Vitamin B9 Diversity for the Nutritional Improvement of Potato

Bruce Reid Robinson II
Crop Science
Hermiston Agricultural Research and Extension Center
Oregon State University

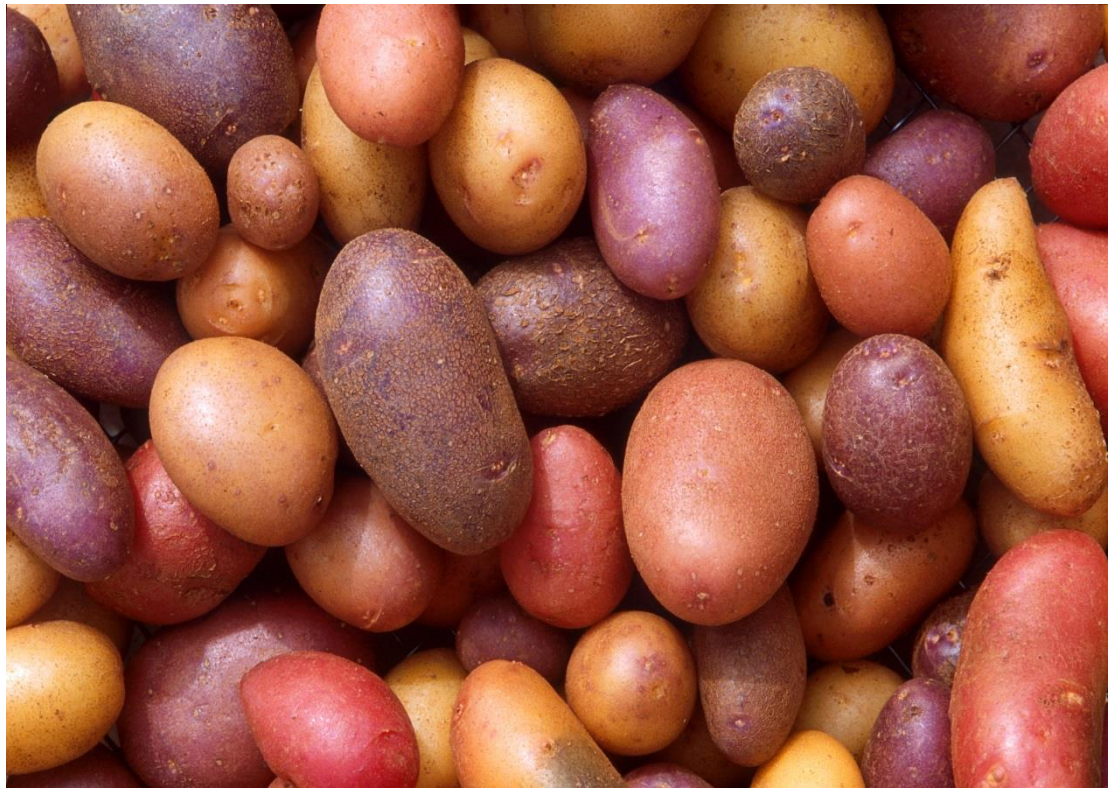
Outline

I. Introduction/Background

II. Objectives

III. Conclusions/Perspectives

IV. Acknowledgements



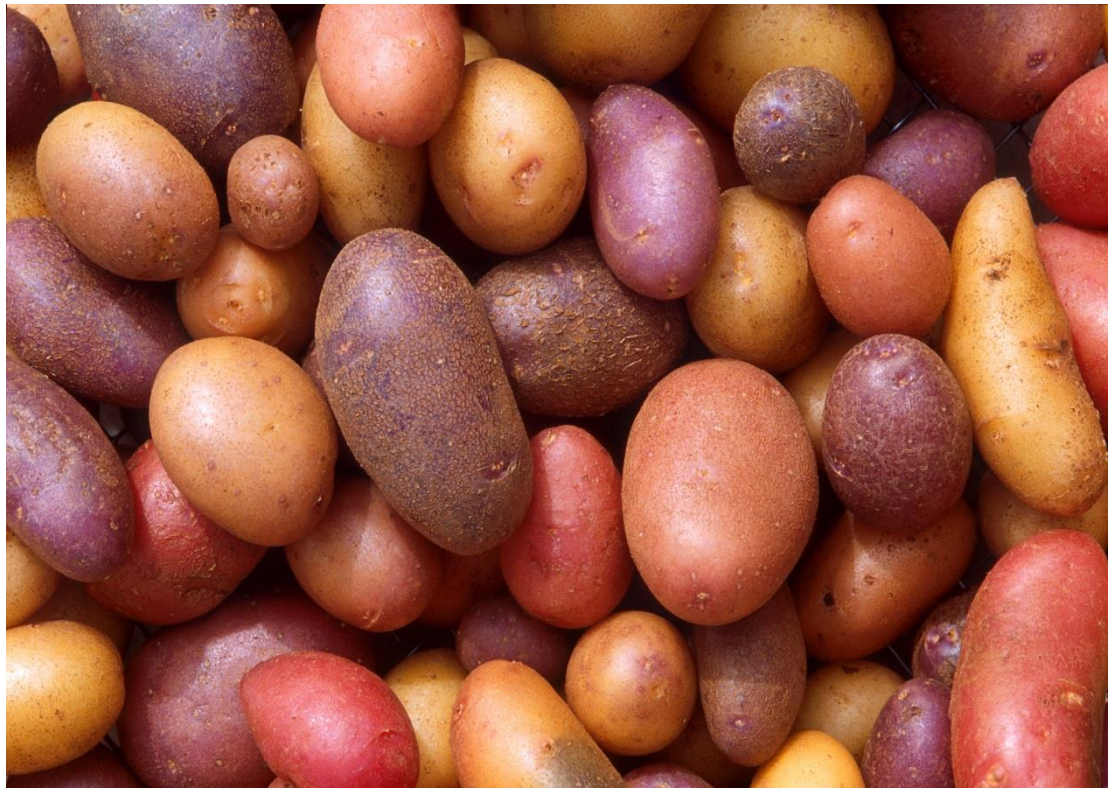
Outline

I. Introduction/Background
- -Micronutrient Malnutrition
- -Folate
- -Sources and Deficiency
- -Biofortification
- -Potatoes

II. Objectives

III. Conclusions/Perspectives

IV. Acknowledgements



Oregon State University

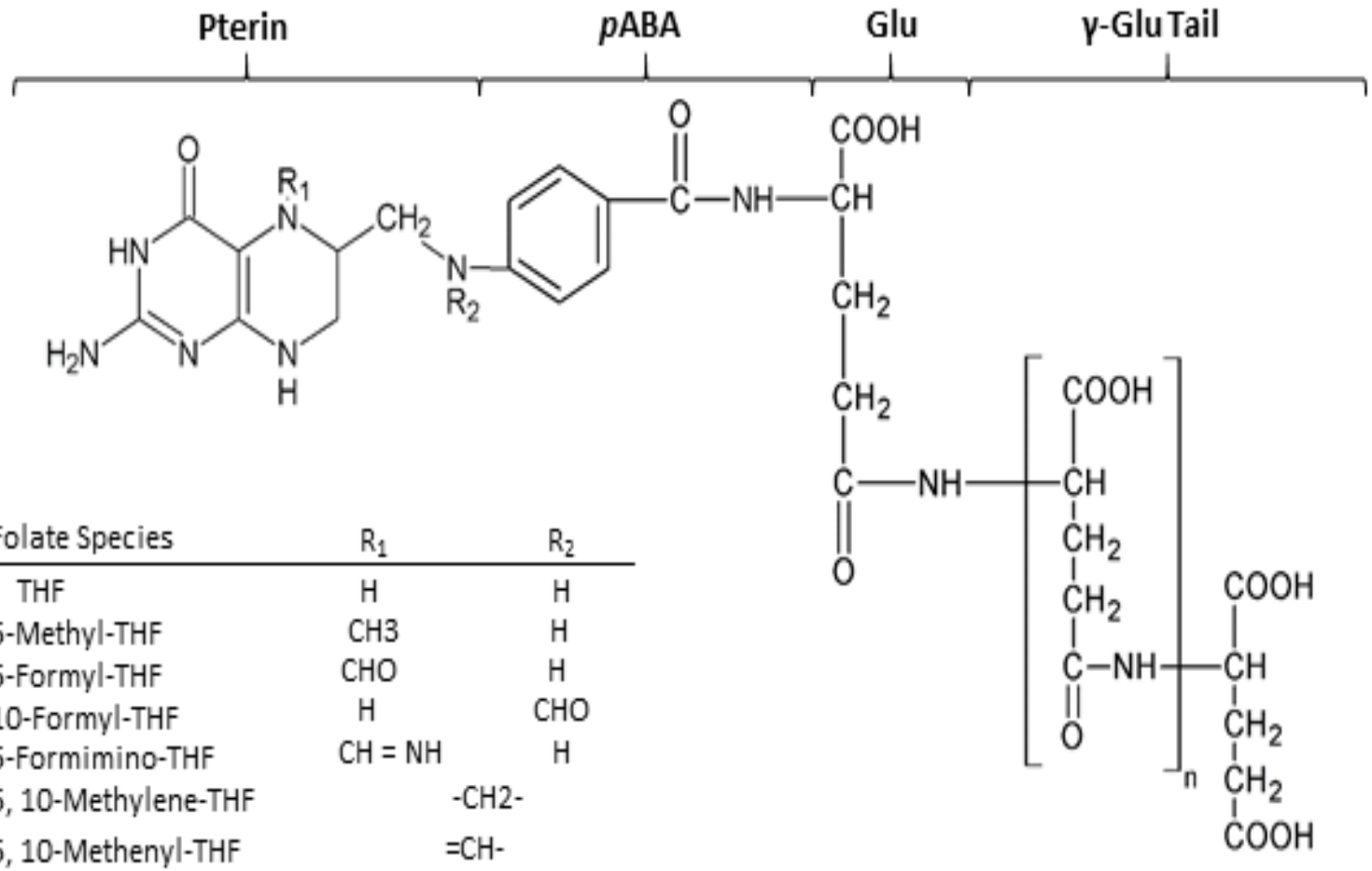
Micronutrient Malnutrition

- Negatively affects as many as 2 billion people worldwide
- Most commonly a deficiency in dietary intake of:
- Minerals: Ca, I, Fe, P, K, Na, Zn
- Vitamins: A, B1, B2, B3, B5, B6, B9, B12, C, D, E, K
- Phytochemicals: Carotenoids, Flavonoids…
- Main sources in human diets are plants

Bailey et al. 2015

Folate – Water Soluble Vitamin B9

- Without adequate folate levels, cells are not able to biosynthesize nucleotides, metabolize amino acids, or utilize the methylation cycle properly



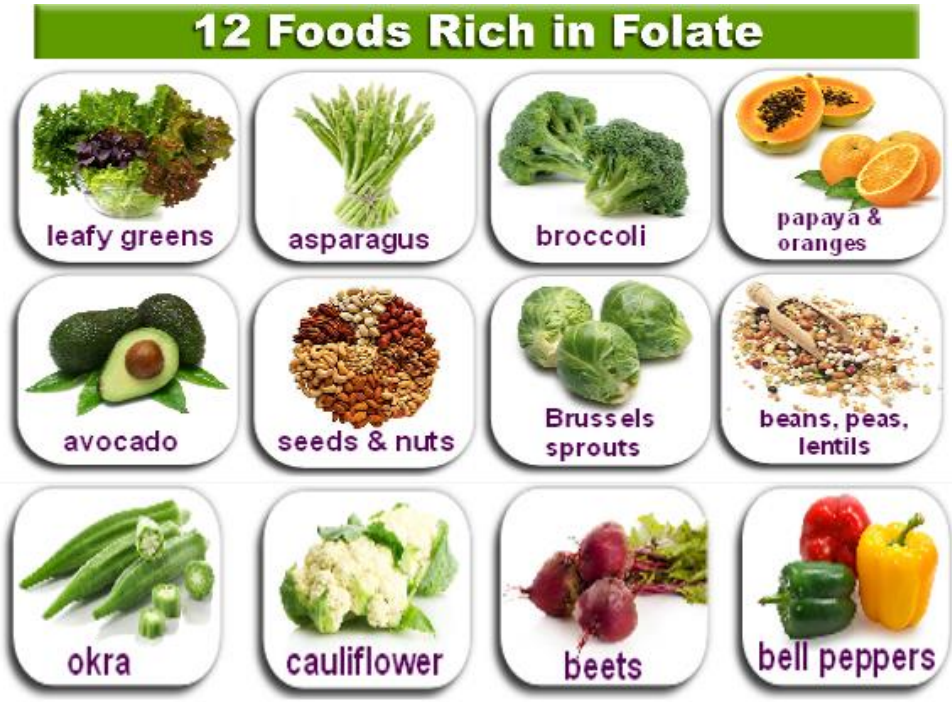
Folate Species	R_1	R_2
THF	H	H
5-Methyl-THF	CH3	H
5-Formyl-THF	CHO	H
10-Formyl-THF	H	CHO
5-Formimino-THF	CH = NH	H
5, 10-Methylene-THF	-CH2-	
5, 10-Methenyl-THF	=CH-	

Folate Sources and Deficiency

- Plants are the major source of dietary folate
- Folate deficiency has been linked to:

a. Neural Tube Defects (NTDs) such as spina bifida and anencephaly
b. Cardiovascular diseases
c. Stroke
d. Anemia
e. Development of certain types of cancers
f. Impaired cognitive performance

- More that 75 countries have instituted folic acid fortification programs



Biofortification

- The process by which the nutritional quality of food crops is improved through conventional plant breeding or modern biotechnology (W.H.O.)

- Has additional advantages compared to industrial fortification alone:
    a. More cost-effective and sustainable over time
    b. Can impact areas that lack the political will, infrastructure, and money to utilize current fortification practices

Importance of Potato (*Solanum tuberosum* L.)

- Third most important food crop behind rice and wheat

- Considered as significant source of folate in their diets

Oregon State UNIVERSITY

Additional Potato Information

- 150g serving of potato (one medium sized russet) provides 6-10% of the 400µg RDA of folate
- Folate retention is high in potato tubers even after storage, processing, and cooking
- ~200 tuber bearing *Solanum* species representing enormous genetic diversity

Oregon State UNIVERSITY

Outline

I. Introduction/Background

II. Objectives

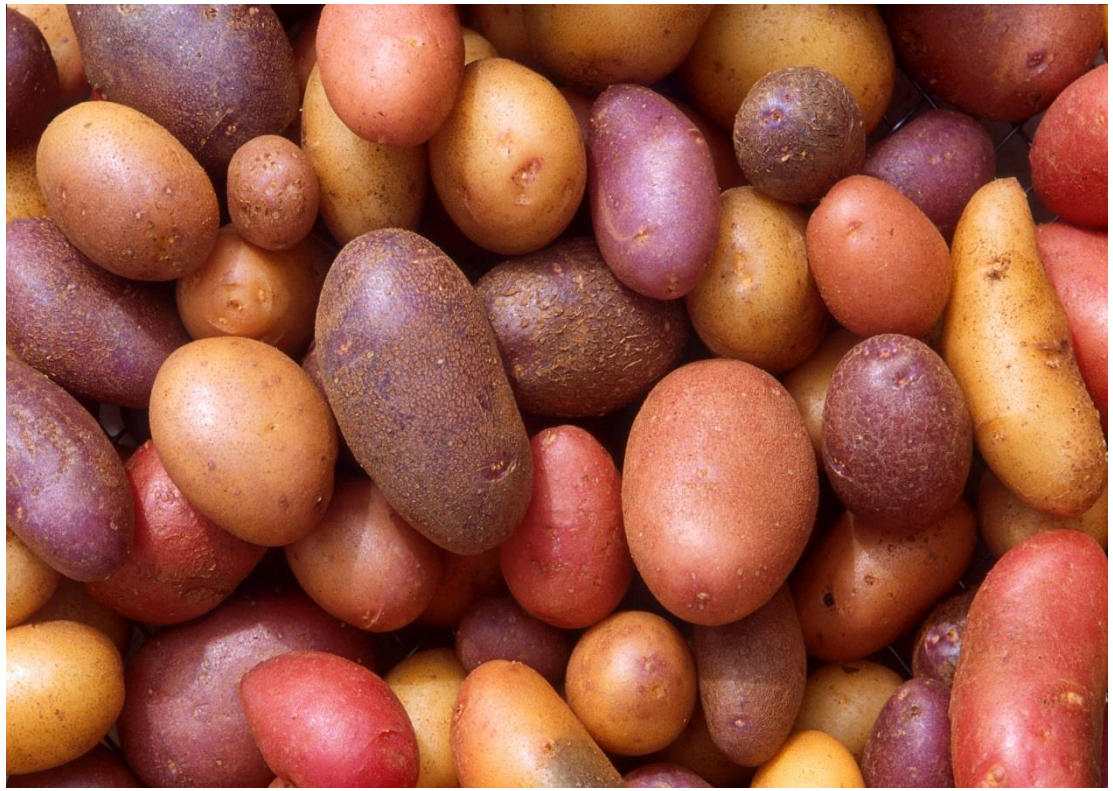
-Germplasm diversity with respect to folate levels

-Expression of folate related genes

-SNP Genotyping

III. Conclusions

IV. Acknowledgements



Oregon State University

Exploring Folate Diversity in Wild and Primitive Potatoes for Modern Crop Improvement

Oregon State University

Previous Work in Folate Variability in Potatoes

- Wild and primitive cultivated species show the greatest range of folate content

- Further evaluating this wild and primitive germplasm is useful in identifying sources of high folate germplasm

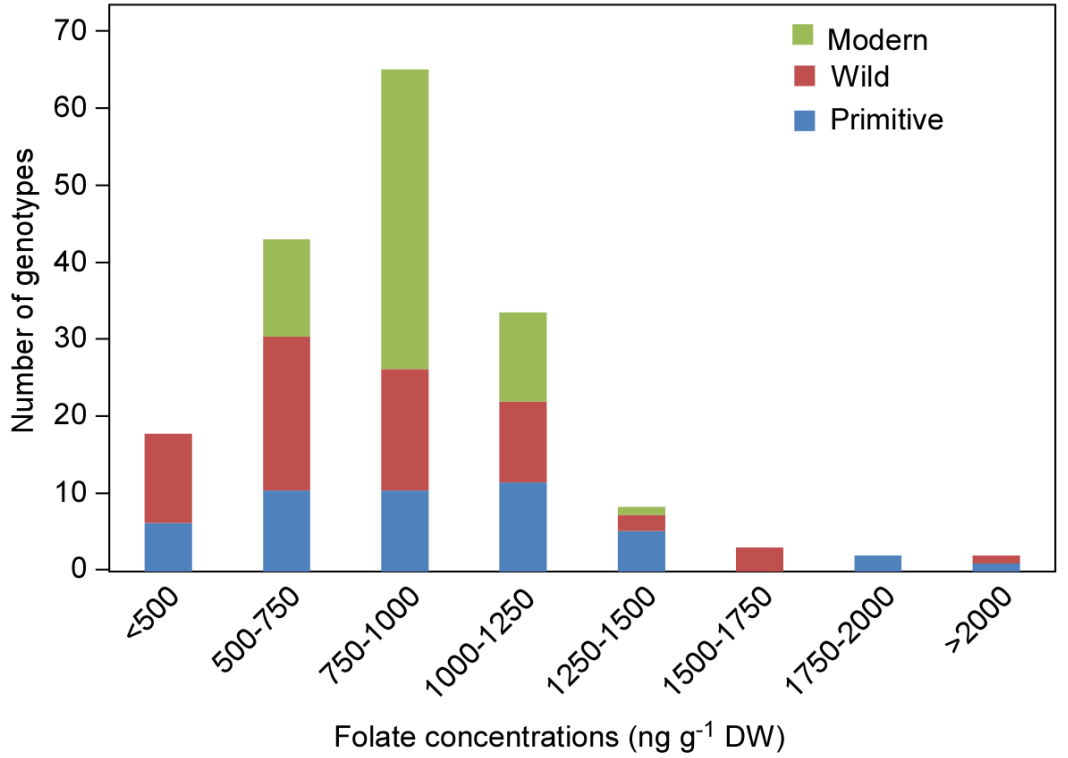


Figure 1. Frequency of modern, primitive and wild potatoes within folates concentration brackets.

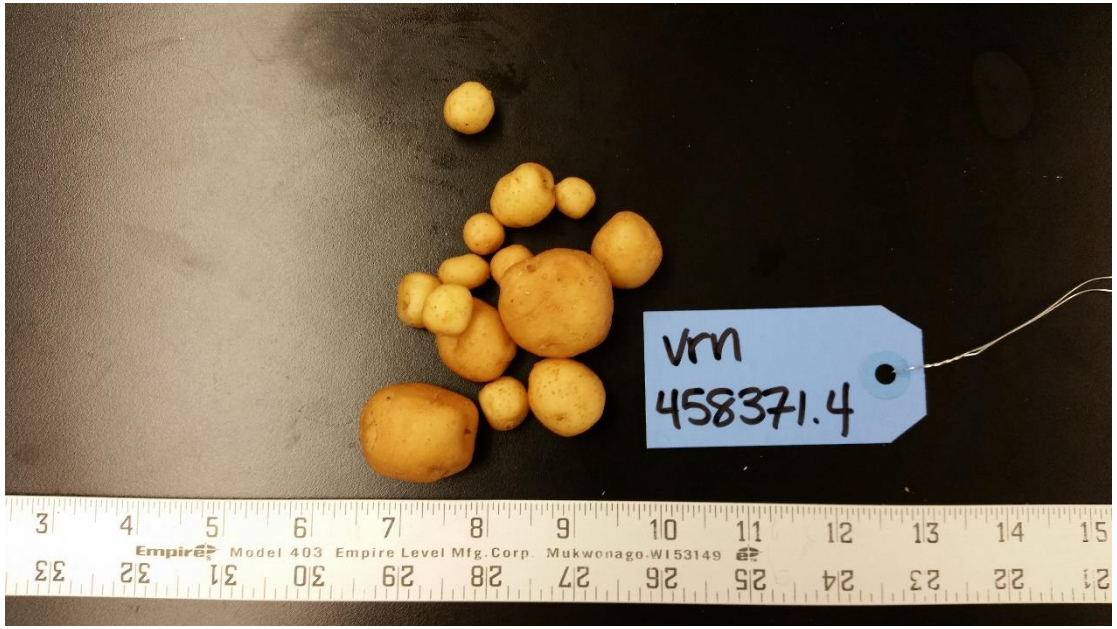
Goyer and Sweek (2011)

Oregon State University

Objectives

- Quantify folate content via tri-enzyme extraction and *Lactobacillus rhamnosus* microbiological assay

- Identify wild and primitive accessions that have high folate content



Oregon State University

Potato Materials – Wild and Primitive Species

- 257 individual plants from 77 accessions representing 10 species evaluated with Russet Burbank as control

- Accessions were obtained from the U.S. Potato Genebank

Oregon State UNIVERSITY

Potato Materials – Wild and Primitive Species

Harvested Selections:

1. *S. acuale* (3 accessions, 4X)
2. *S. boliviense* (25 accessions, 2X)
3. *S. candolleanum* (3 accessions, 2X)
4. *S. chacoense* (2 accessions, 2X)
5. *S. stipuloideum* (3 accessions, 2X)
6. *S. demissum* (3 accessions, 6X)
7. *S. microdontum* (3 accessions, 2X)
8. *S. okadae* (3 accessions, 2X)
9. *S. tuberosum* subsp. *andigenum* (9 accessions, 2X & 4X)
10. *S. vernei* (23 accessions, 2X)

Oregon State University

Tri-Enzyme Extraction Method

- General Principle: Folate species must be released from food matrices and processed without degrading the sample so determination can be performed
- HEPES/CHES buffer, protease, α-amylase, and conjugase allow for this with reasonable throughput

Tuber Sample

Homogenize in HEPES/CHES Buffer

Heat (10min at 100° C)

Ice Bath

Incubate with Protease (2hrs at 37° C)

Heat (5 min at 100° C)

Ice Bath

Incubate with α-amylase and conjugase (2-3hrs at 37° C)

Heat (10min at 100° C)

Ice Bath

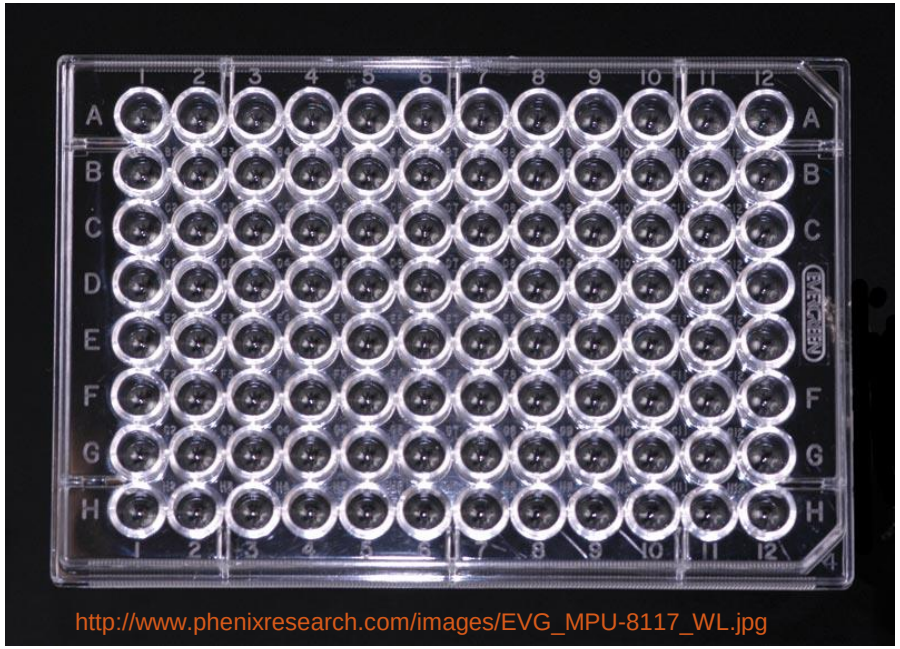
Centrifuge

Storage at -80° C

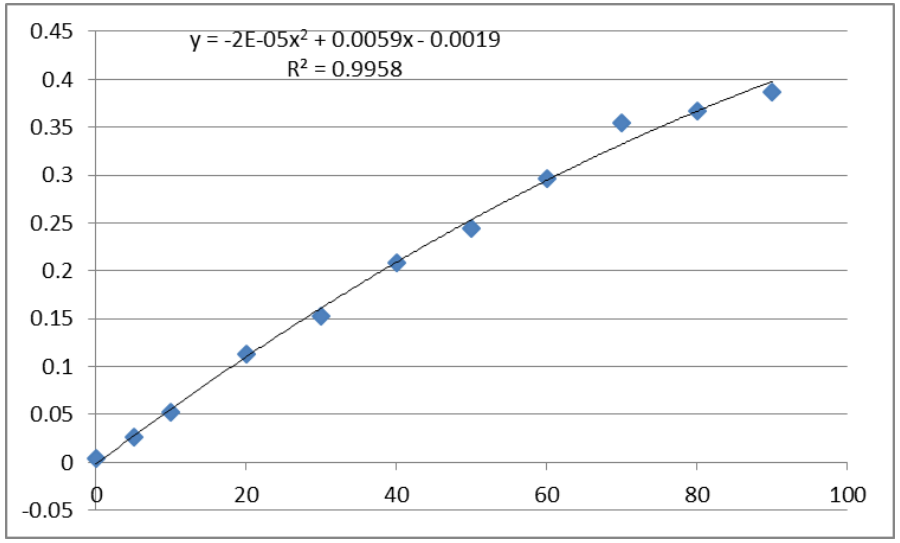
Oregon State University

Folate Determination

- Microbiological Assay using *L. rhamnosus*
- Wells loaded with Folic Acid Medium, standards, or samples
- Incubated for 18-24 hours
- Read with microplate reader
- Folate values calculated from standard curve

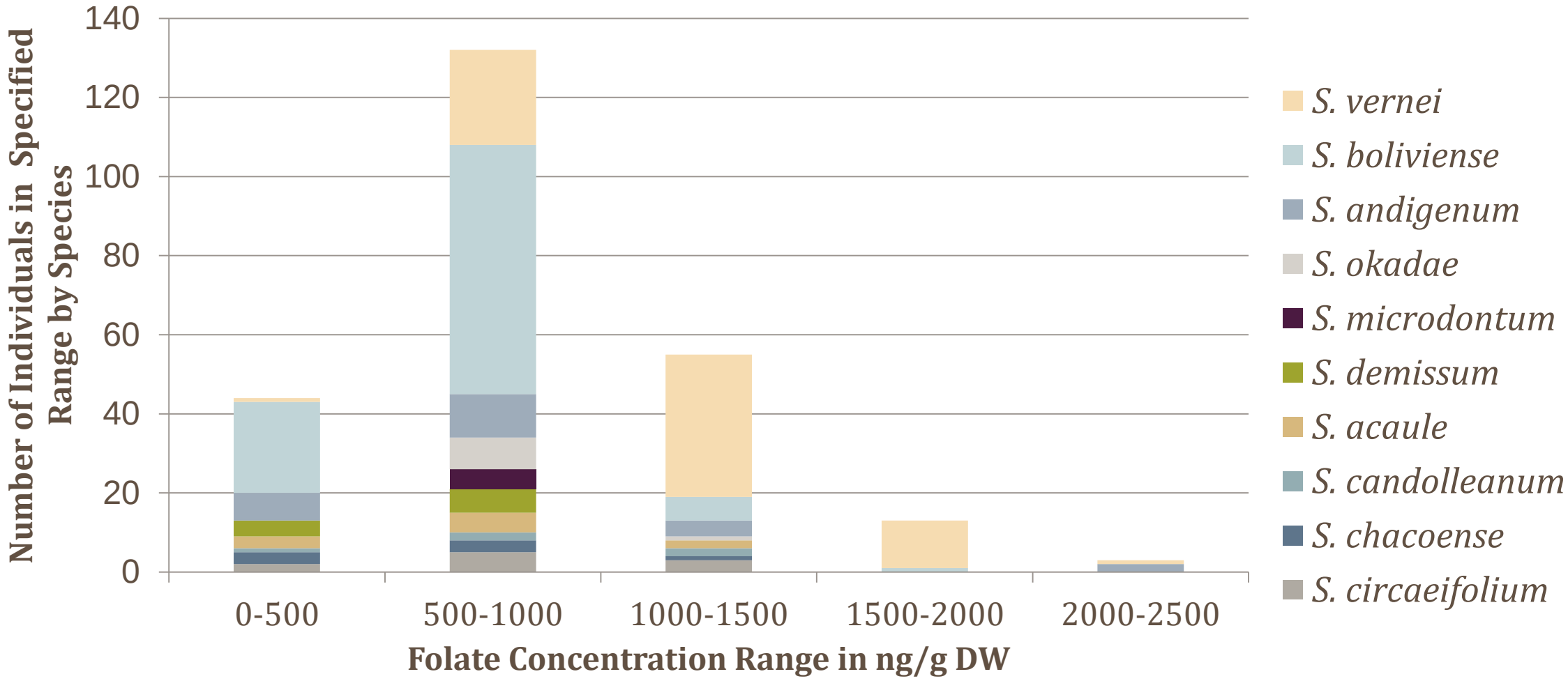


http://www.phenixresearch.com/images/EVG_MPU-8117_WL.jpg



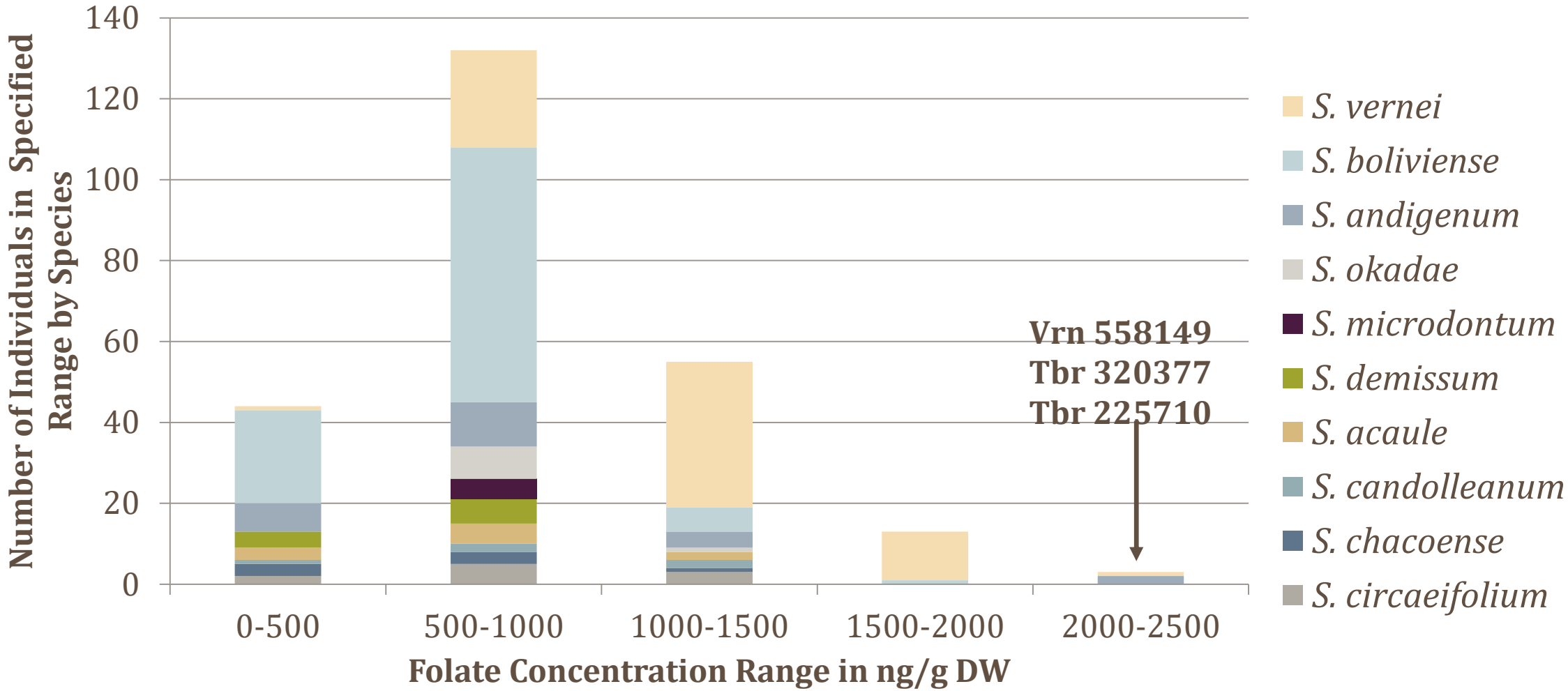
Oregon State University

Wild and Primitive Species Folate Distribution



Oregon State University

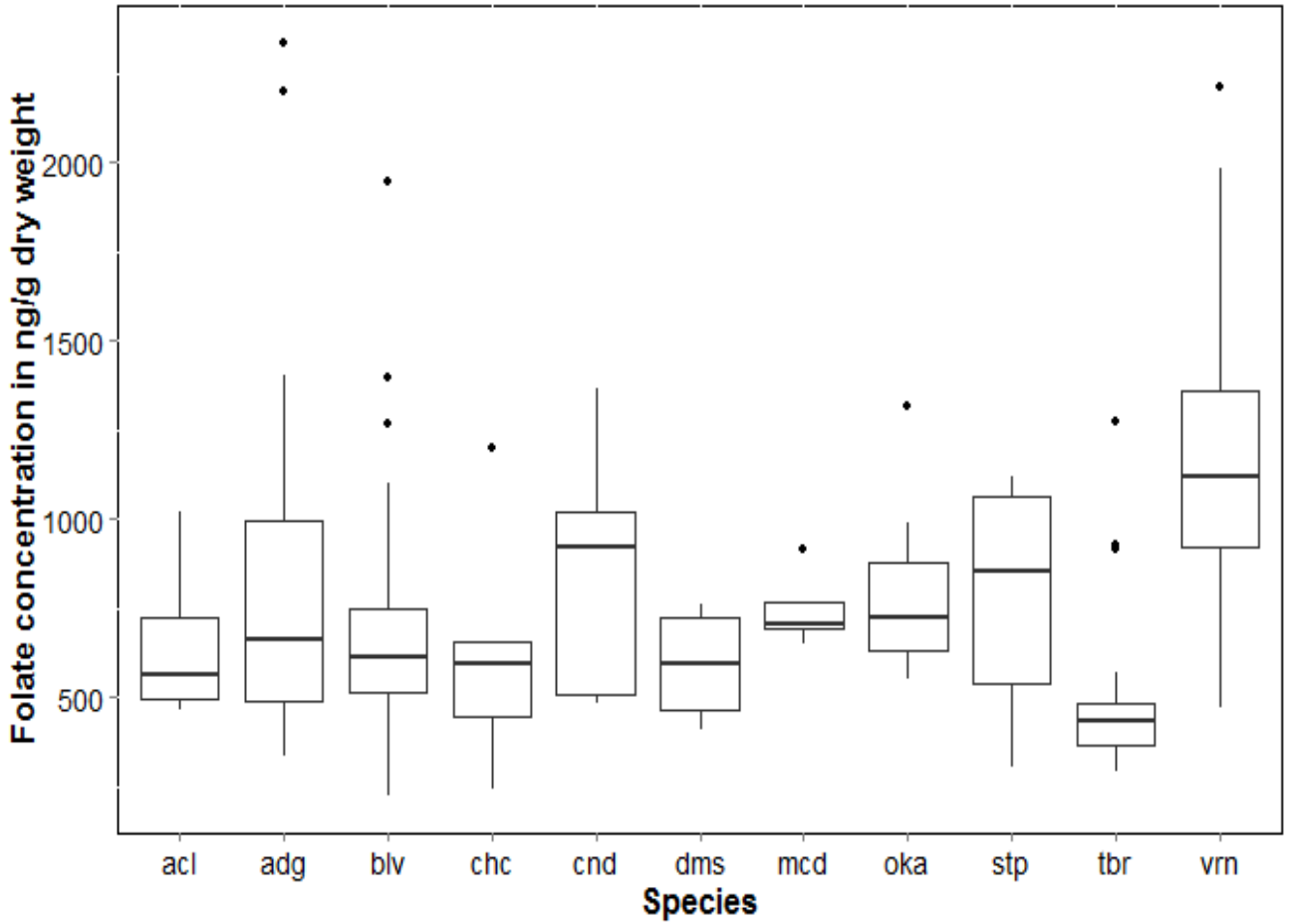
Wild and Primitive Species Folate Distribution



Oregon State University

Summary

- Wild and primitive cultivated species showed a range of 220 – 2200 ng/g folate DW
- *S. Vernei* and *S. tuberosum* subsp. *andigenum* showed highest folate levels
- Increasing commercial cultivar's folate content to more than 2000 ng/g dry weight or more represents a 4X increase



Oregon State UNIVERSITY

Genes **2015**, *6*, 1300-1314; doi:10.3390/genes6041300

OPEN ACCESS

genes

ISSN 2073-4425

www.mdpi.com/journal/genes

Article

Exploring Folate Diversity in Wild and Primitive Potatoes for Modern Crop Improvement

Bruce R. Robinson [1,2], Vidyasagar Sathuvalli [1,2], John Bamberg [3] and Aymeric Goyer [1,4,*]

[1] Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston, OR 97838, USA; E-Mails: Bruce.Robinson@oregonstate.edu (B.R.R.); Vidyasagar@oregonstate.edu (V.S.)

[2] Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97330, USA

[3] USDA/Agricultural Research Service, Sturgeon Bay, WI 54235, USA; E-Mail: john.bamberg@ars.usda.gov

[4] Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97330, USA

* Author to whom correspondence should be addressed; E-Mail: aymeric.goyer@oregonstate.edu; Tel.: +1-541-567-8321; Fax: +1-541-567-2240.

Academic Editors: Sean Mayes, Festo Massawe, Prakit Somta and Wai Kuan Ho

Received: 27 October 2015 / Accepted: 25 November 2015 / Published: 8 December 2015

Abstract: Malnutrition is one of the world's largest health concerns. Folate (also known as vitamin B_9) is essential in the human diet, and without adequate folate intake, several serious health concerns, such as congenital birth defects and an increased risk of stroke and heart disease, can occur. Most people's folate intake remains sub-optimal, even in countries that have a folic acid food fortification program in place. Staple crops, such as potatoes, represent

Oregon State UNIVERSITY

Outline

I. Introduction/Background

II. Objectives

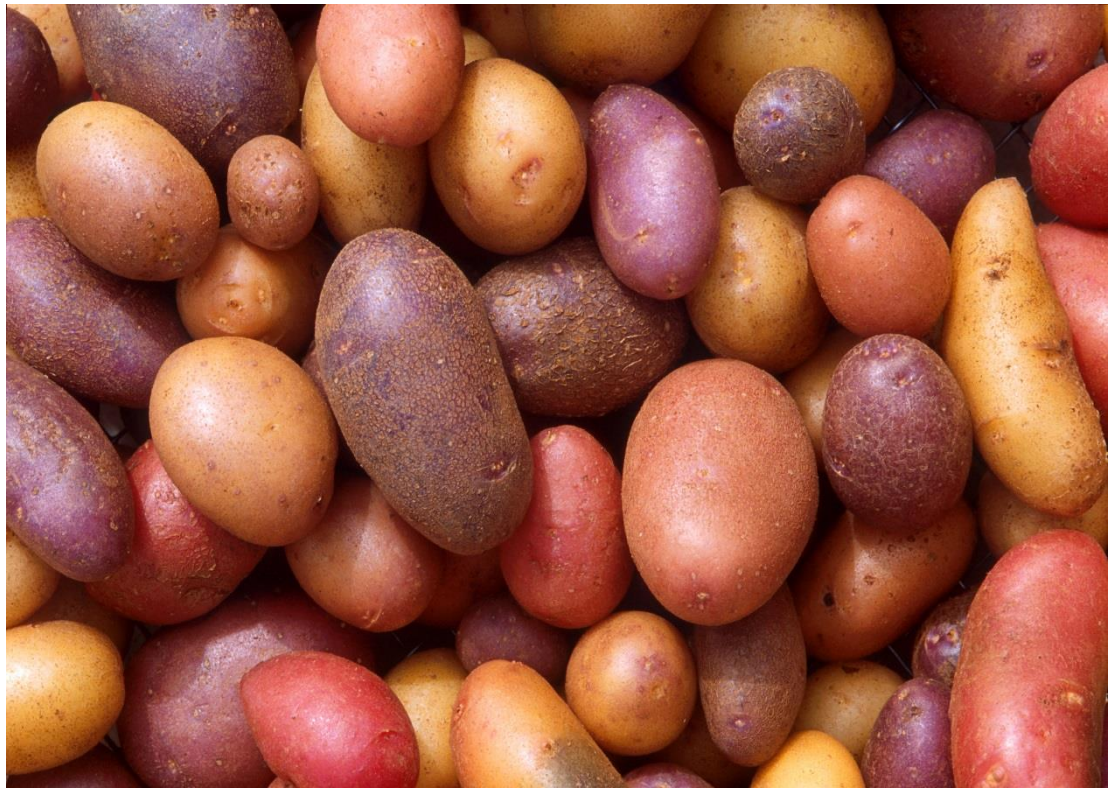
-Germplasm Diversity with respect to folate levels

-Expression of folate related genes

-SNP Genotyping

III. Conclusions

IV. Acknowledgements



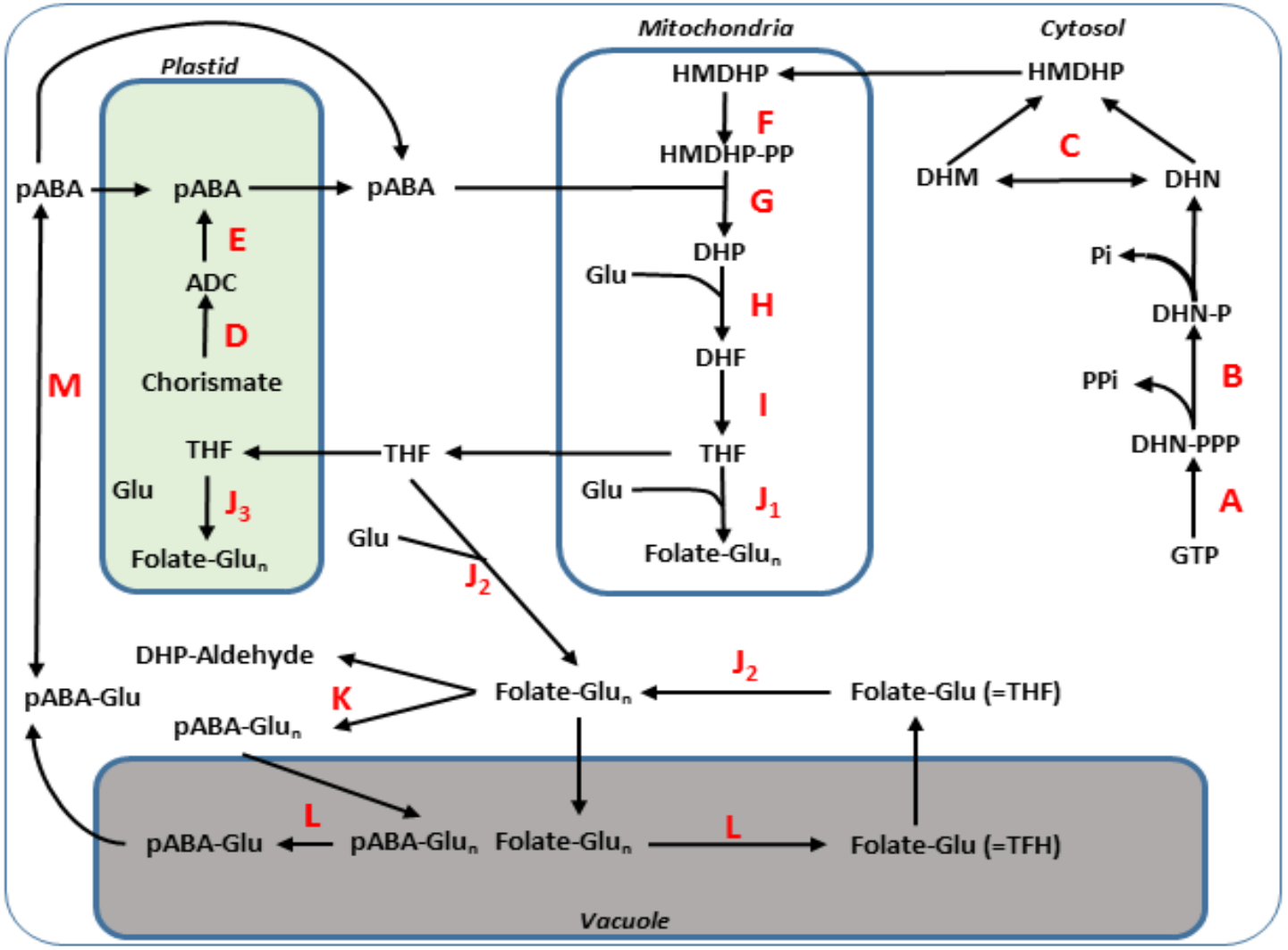
Oregon State University

Expression Levels of The γ-Glutamyl Hydroplase I Gene Correlate With Vitamin B9 Content in Potato Tubers

Oregon State University

Objectives

- Identify how expression of folate-related genes contributes to folate accumulation
- Analyze RNA-Seq data to identify genes with differential expression in high/low folate genotypes
- Perform quantitative PCR (qPCR) to confirm the results of RNA-seq results in diverse germplasm



Oregon State University

Materials

- High: fol 1.3, fol 1.6
- Low: fol 1.5, fol 1.11

Solanum boliviense PI 597736

RNA-Sequence analysis (2 technical reps, each rep made of tubers pooled from 3-4 plants)

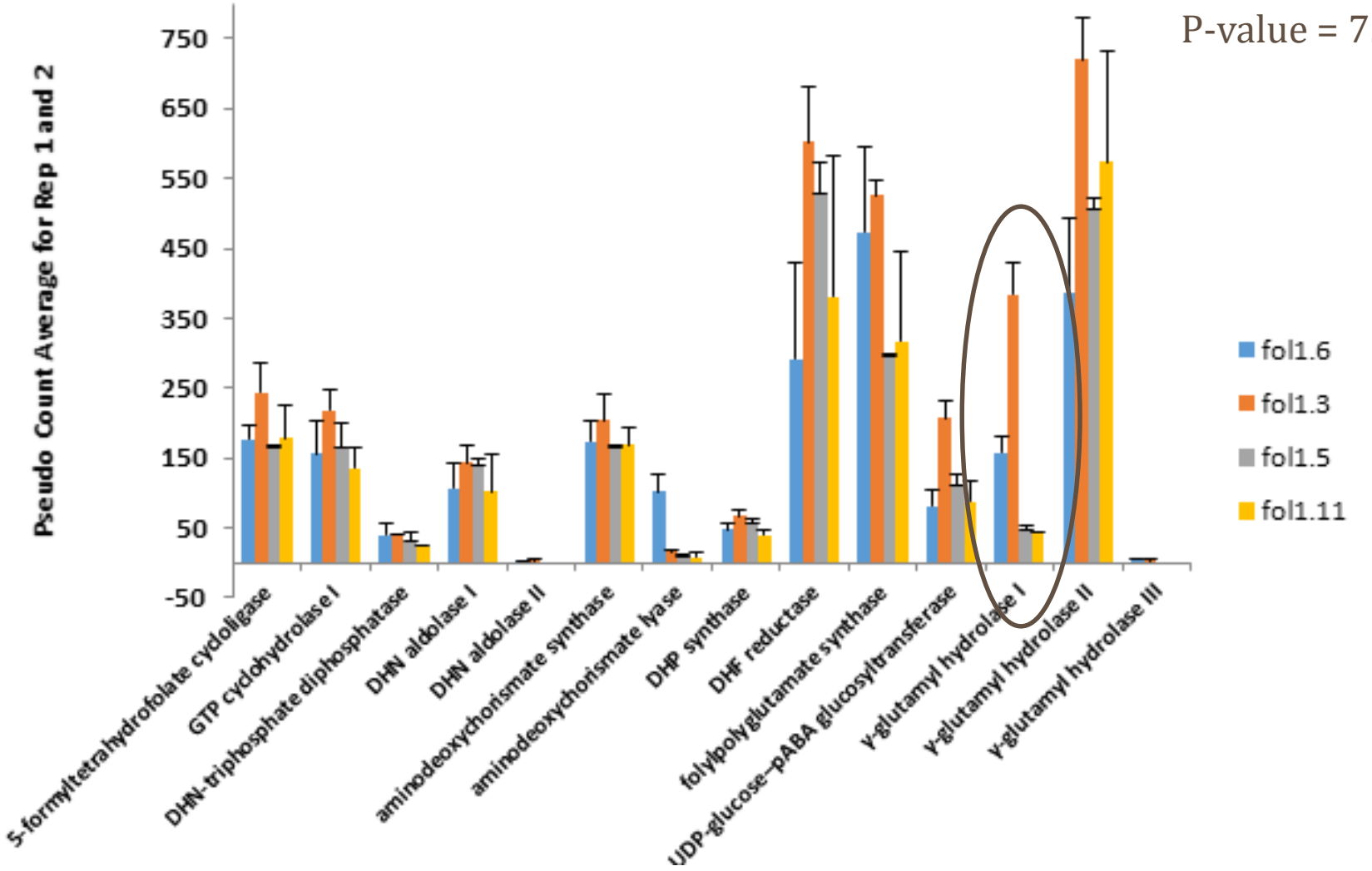
Oregon State University

Methods

- 1 Illumina HiSeq2000 lane (51 cycle V3 single end)
- TruSeq RNA Libraries quantified by qPCR
- Normalized to β–tubulin pseudocounts
- Mapping, assembly, and differences in expression determined by JEANS

Oregon State UNIVERSITY

Methods – RNA-sequence analysis

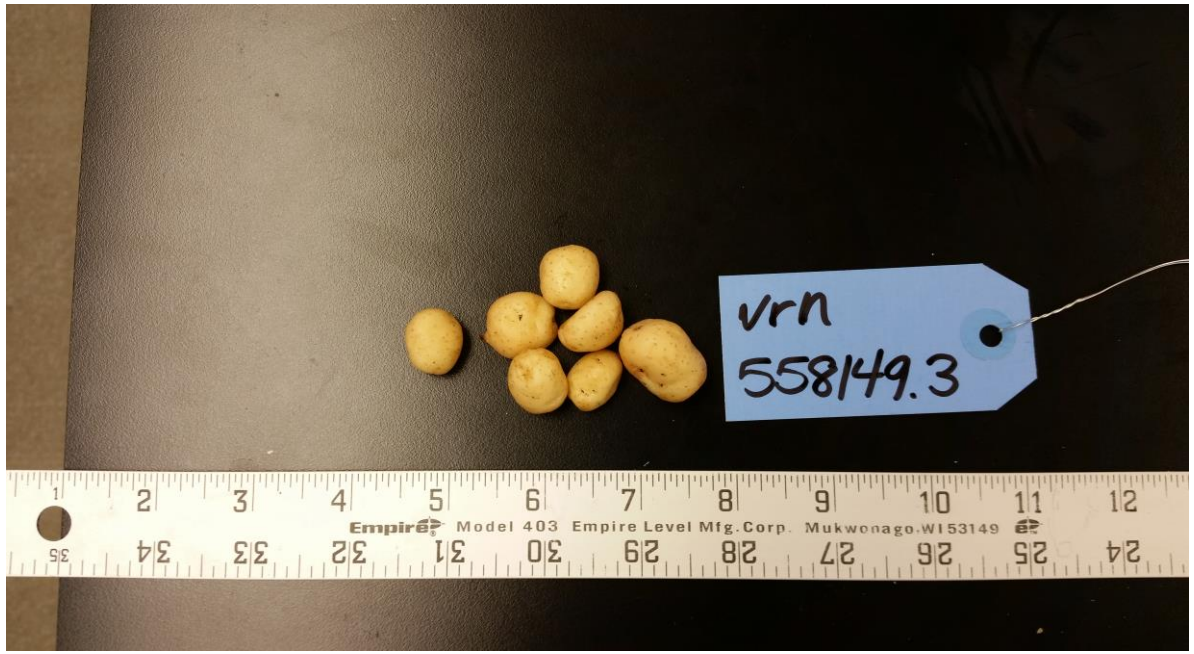


P-value = 7.52624E-14

Oregon State University

Materials

Sample	Folate concentration (ng/g DW)
BRR1 12	2373 ± 29
BRR1 27	471 ± 20
BRR3 90	2952 ± 277
BRR3 56	326 ± 21
Tbr 225710.3	2336 ± n.d.
Tbr 546023.4	626 ± 21
Vrn 558149.3	1688 ± 18
Vrn 500063.1	469 ± 16
Fol 1-3	1667 ± 113
Fol 1-5	810 ± 269
Fol 1-6	2137 ± 473
Fol 1-11	911 ± 67



Oregon State University

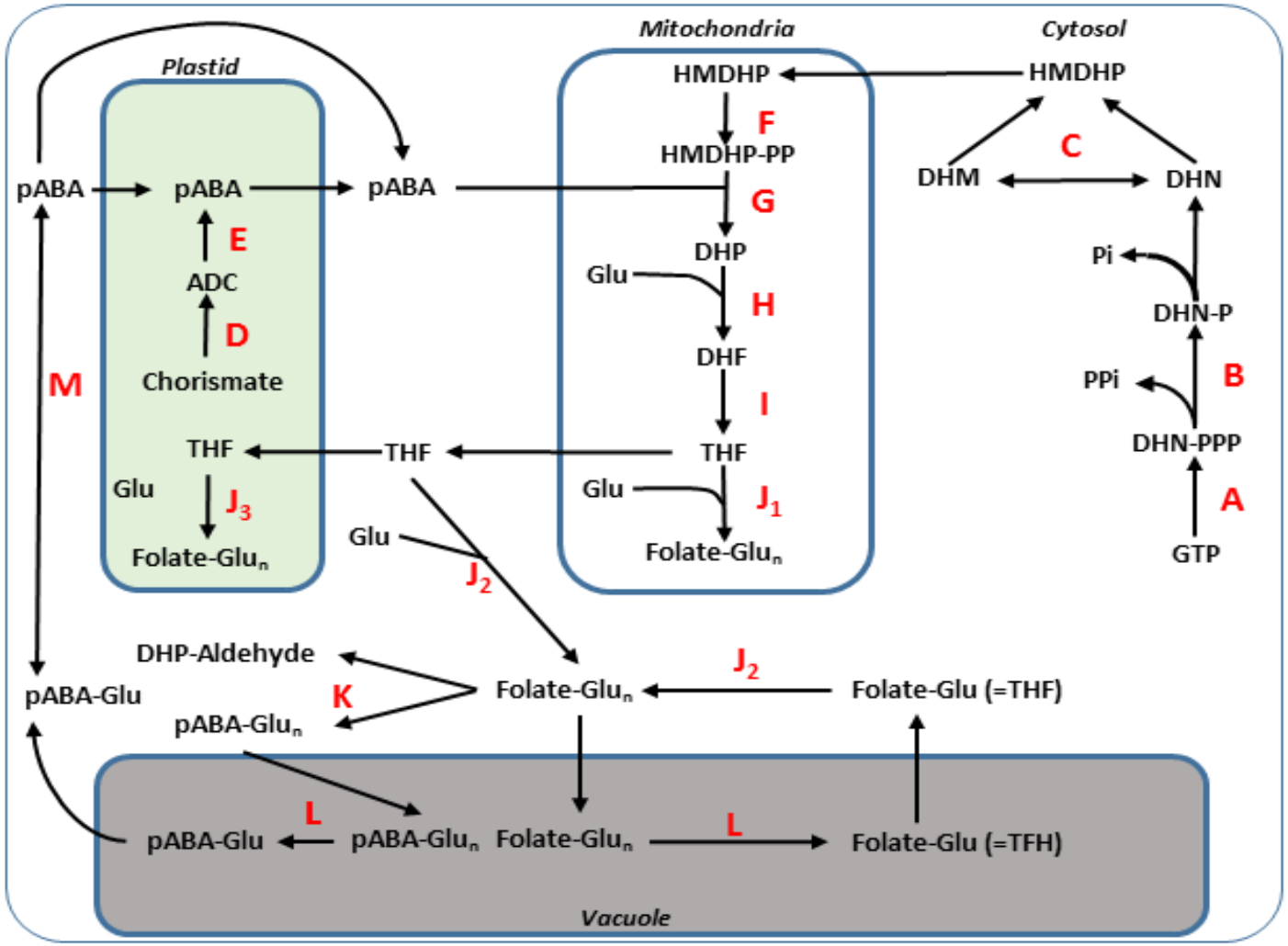
Results

High Folate Genotype	C_t Value	Low Folate Genotype	C_t Value	High/Low $2^{-\Delta Ct}$	Fold Change in GGH1 Expression
BRR1 12	34.18	BRR1 27	31.74	0.189/0.018	10
BRR3 90	40.44	BRR3 56	36.71	3.33E -05/4.53E -04	0.1
Tbr PI 225710	29.66	Tbr PI 546023	38.84	3.00E -02/1.55E -02	2
Vrn PI 558149	35.33	Vrn PI 500063	40.78	6.25E -02/1.29E -04	481
Fol 1-6	32.01	Fol 1-11	35.41	7.10E -03/4.76E -04	15
Fol 1-6	32.01	Fol 1-5	39.82	7.10E -03/8.07E -05	88
Fol 1-3	30.90	Fol 1-11	35.41	1.13E -02/4.76E -04	24
Fol 1-3	30.90	Fol 1-5	39.82	1.13E -02/8.07E -05	140

Oregon State UNIVERSITY

Summary

- RNA-Seq data identified GGH1 with differential expression in high/low folate genotypes
- qPCR results confirmed this trend



Oregon State University

Outline

I. Introduction/Background

II. Objectives

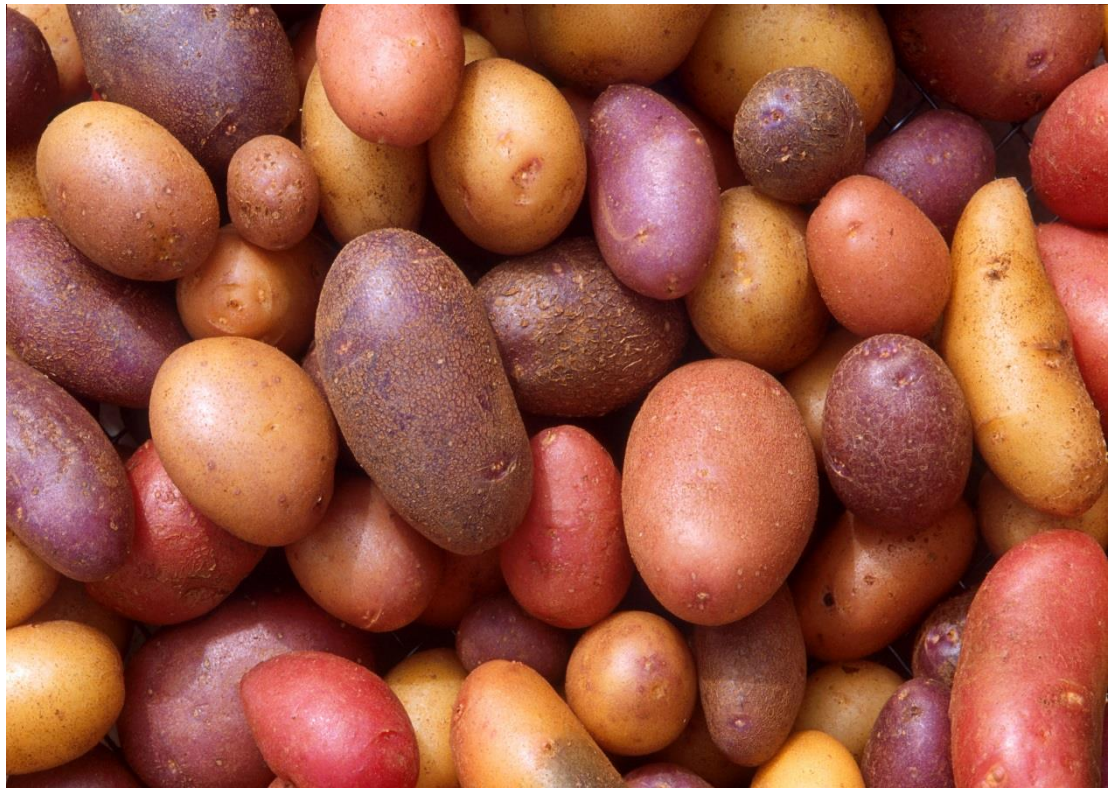
-Germplasm Diversity with respect to folate levels

-Expression of folate related genes

-SNP Genotyping

III. Conclusions

IV. Acknowledgements



Oregon State University

Single Nucleotide Polymorphism Markers Associated With High Folate Content from Wild Potato Species

Oregon State University

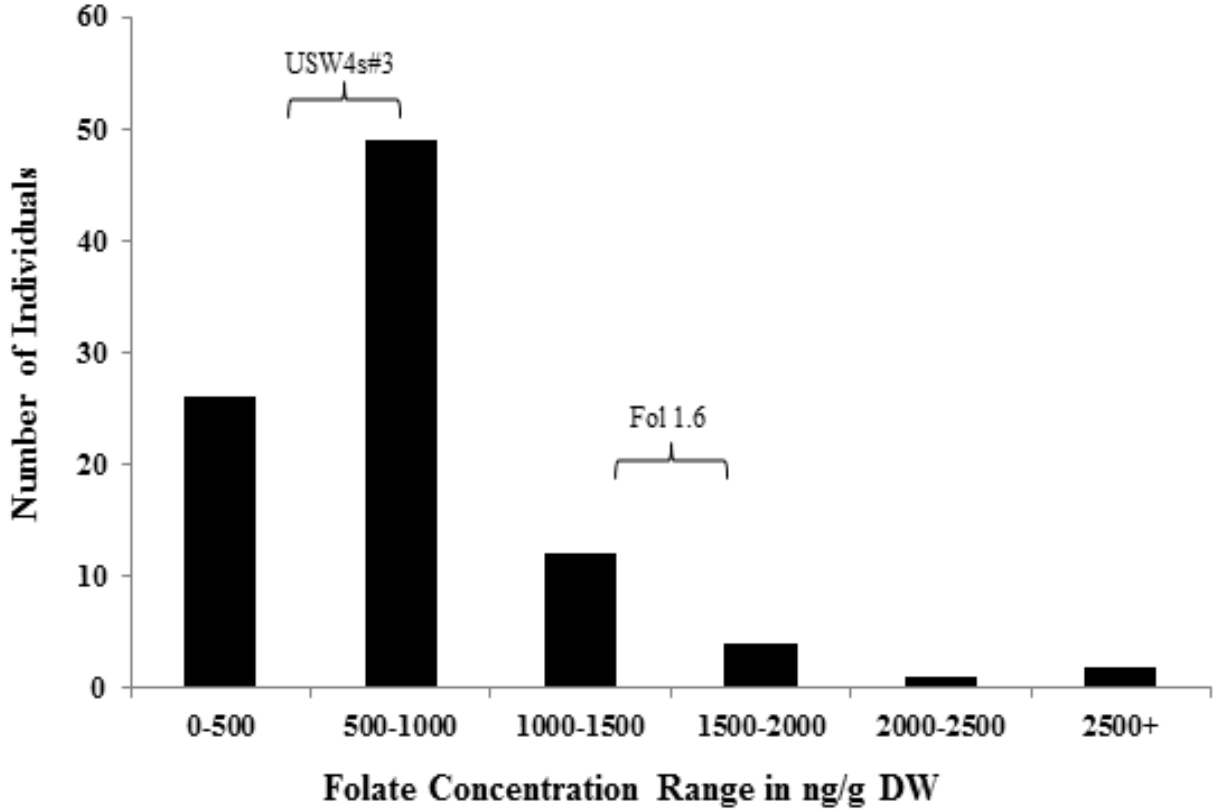
Objectives

- Use SNP genotyping platform to develop linkage maps
- Perform SNP-trait association
- Perform QTL single marker analysis
- Identify potential SNPs associated with high folate

Oregon State University

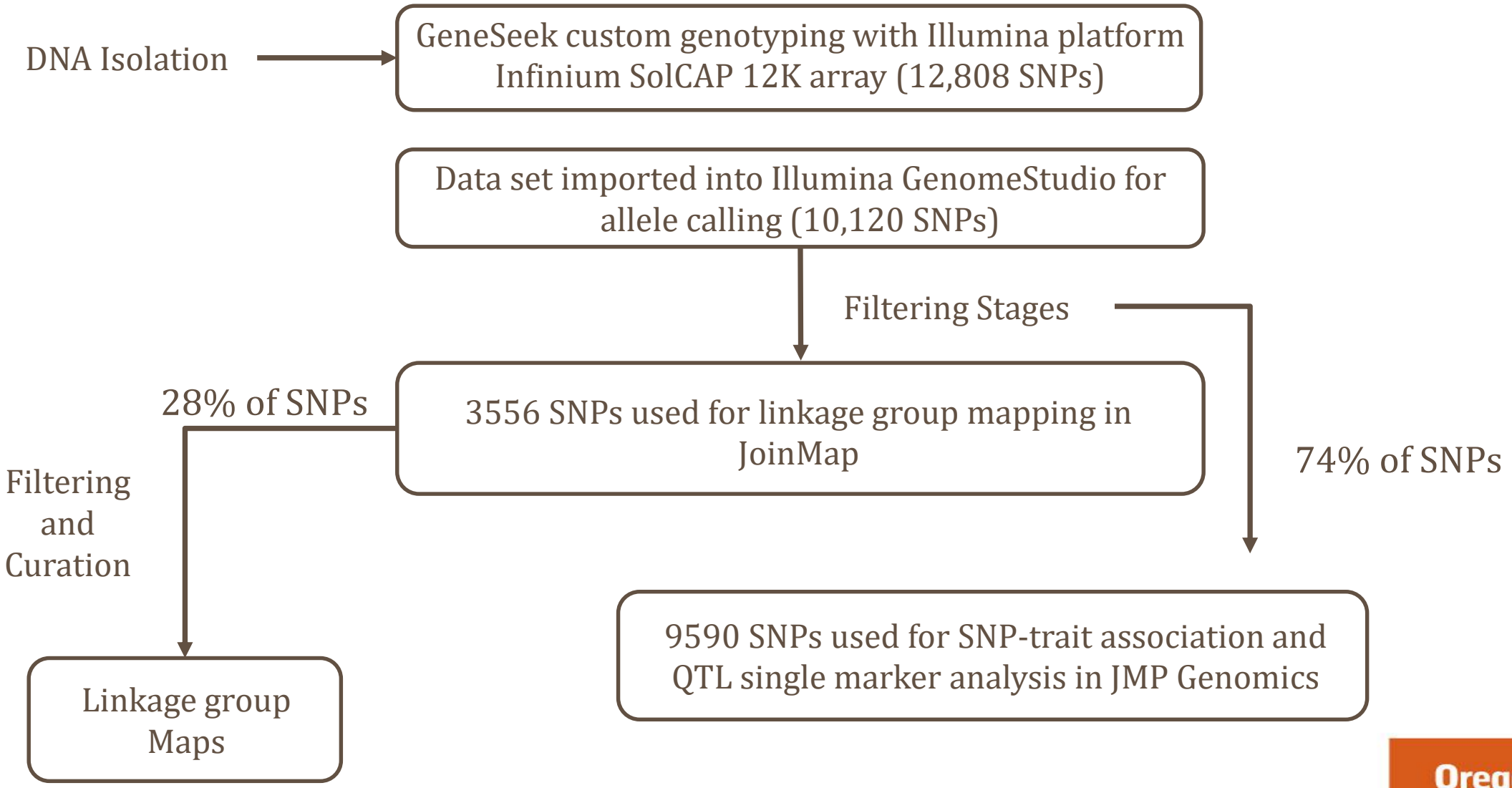
Materials

- BRR3 – F2 Diploid mapping population
- 94 individuals
- [$USW4_{self}$#3 x $fol1.06_{blv597736}$]F2



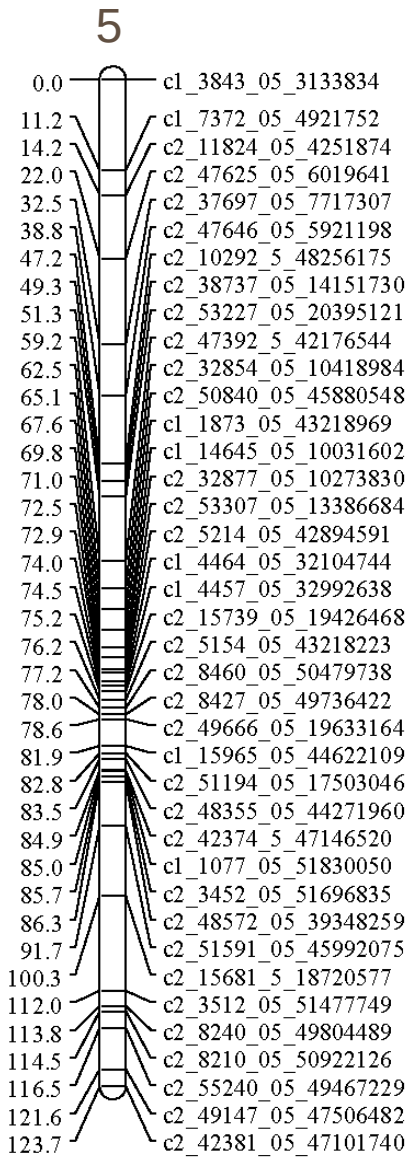
Oregon State UNIVERSITY

Workflow for SNP genotyping, mapping, and QTL Analysis



Oregon State University

Results

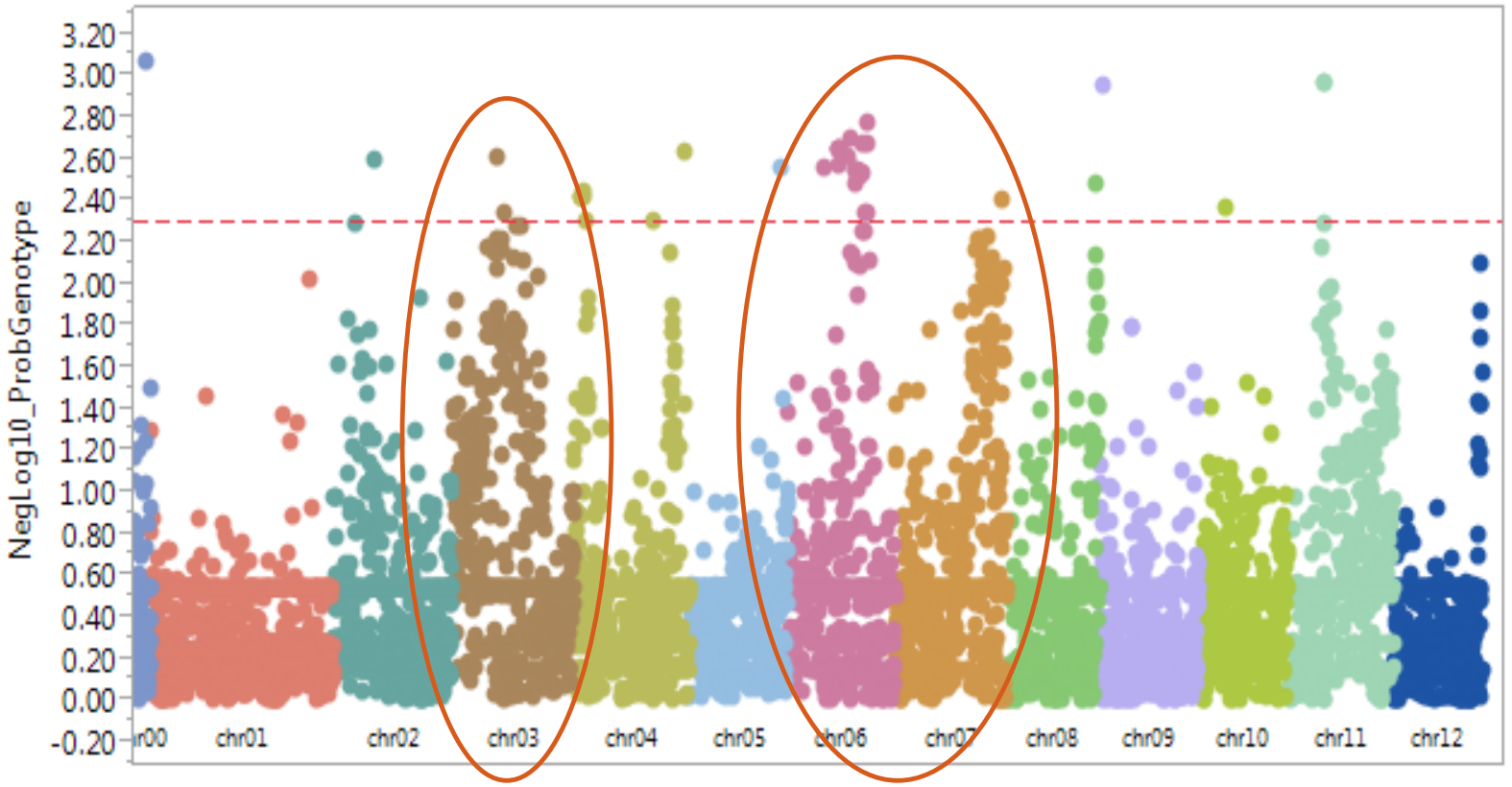


	SNPs from Parents					
Linkage Group	USW4s#3	Fol 1.6	Number of Codominant markers	Group Length (cM)	Total SNPs per linkage group	Marker Coverage (markers/cM)
1	59	7	2	98.177	68	1.44
2	22	15	9	124.182	46	2.69
3	51	2	3	165.488	56	2.95
4	49	4	4	140.512	57	2.40
5	29	6	4	123.679	39	1.65
6	36	6	4	113.482	46	2.67
7	24	5	10	59.681	39	2.58
8	52	0	0	126.636	52	2.43
9	48	4	2	157.314	54	3.41
10	51	1	3	113.187	55	2.05
11	58	4	6	101.767	68	1.41
12	51	8	5	107.182	64	1.67
Total	530	62	52	1431.227	644	2.22

Oregon State University

Results

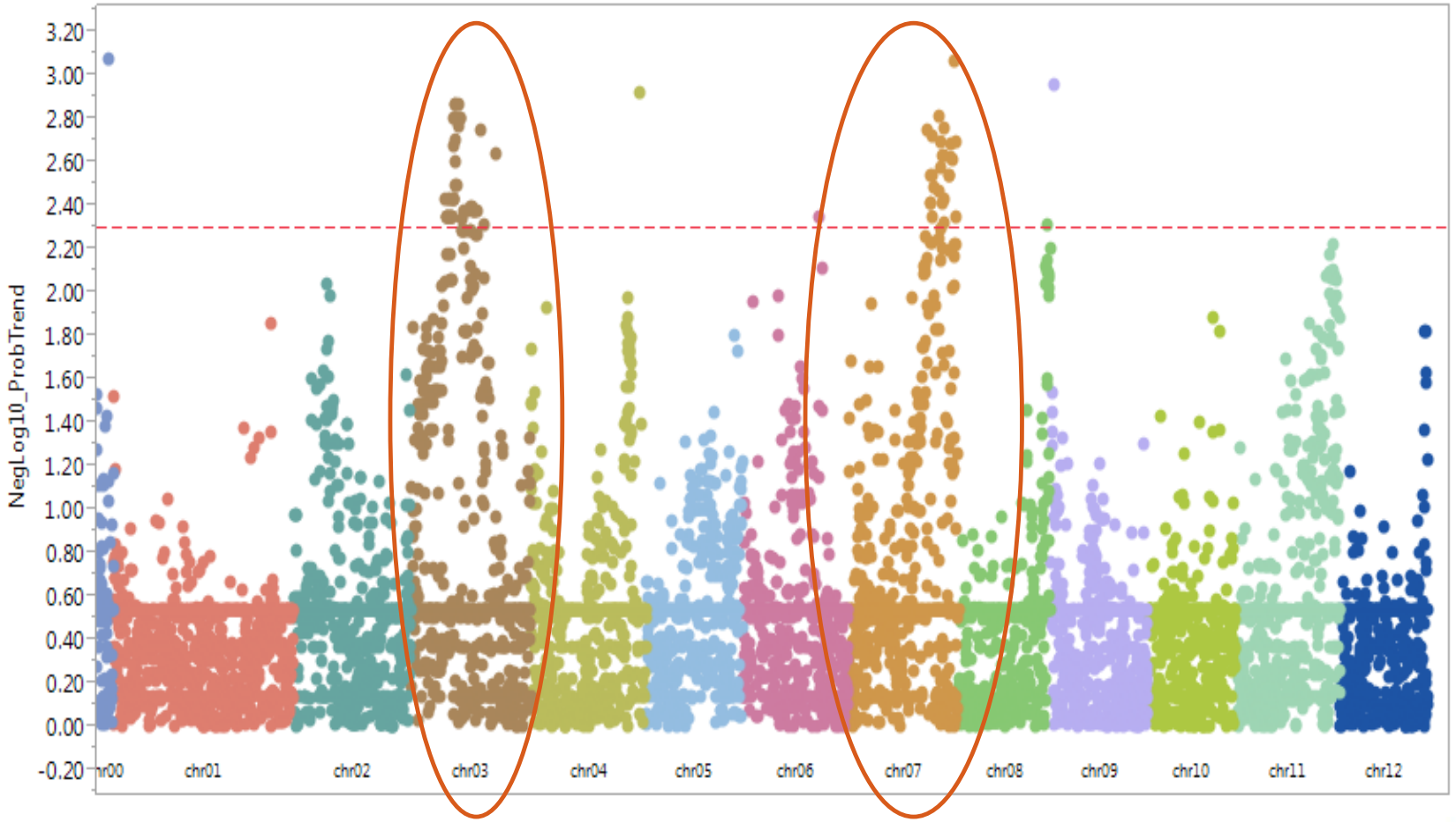
- SNP-trait association identified 109 SNPs
- 86% or 94 SNPs were associated with chromosomes 3, 6, and 7
- 5-Formyltetrahydrofolate cycloligase (chromosome 3)
- Dihydrofolate (DHF) synthase (chromosome 6)
- γ-glutamyl hydrolase 1 (chromosome 7)



Oregon State University

Results

- QTL single marker analysis identified 80 SNPs
- 94% or 75 SNPs were associated with chromosomes 3 and 7
- Potential QTLs are located in areas previously identified by SNP-trait association



Oregon State University

Summary

- 73 common SNPs were identified from both analysis, 66 are located on chromosome 3 and 7

SNP_ID	CHR	POS	R-squared trend
solcap_snp_c2_53198	chr00	29279410	0.114572486
solcap_snp_c2_48372	chr03	39255217	0.105971323
solcap_snp_c2_48371	chr03	39255236	0.105971323
solcap_snp_c2_48369	chr03	39257162	0.105971323
solcap_snp_c2_35234	chr03	40992986	0.105971323
solcap_snp_c1_6875	chr03	41994529	0.103532909
solcap_snp_c2_10688	chr04	71592216	0.108221677
solcap_snp_c2_28223	chr07	51604961	0.10388895
solcap_snp_c2_18680	chr07	55283766	0.114219648
solcap_snp_c2_48597	chr09	778420	0.109861634

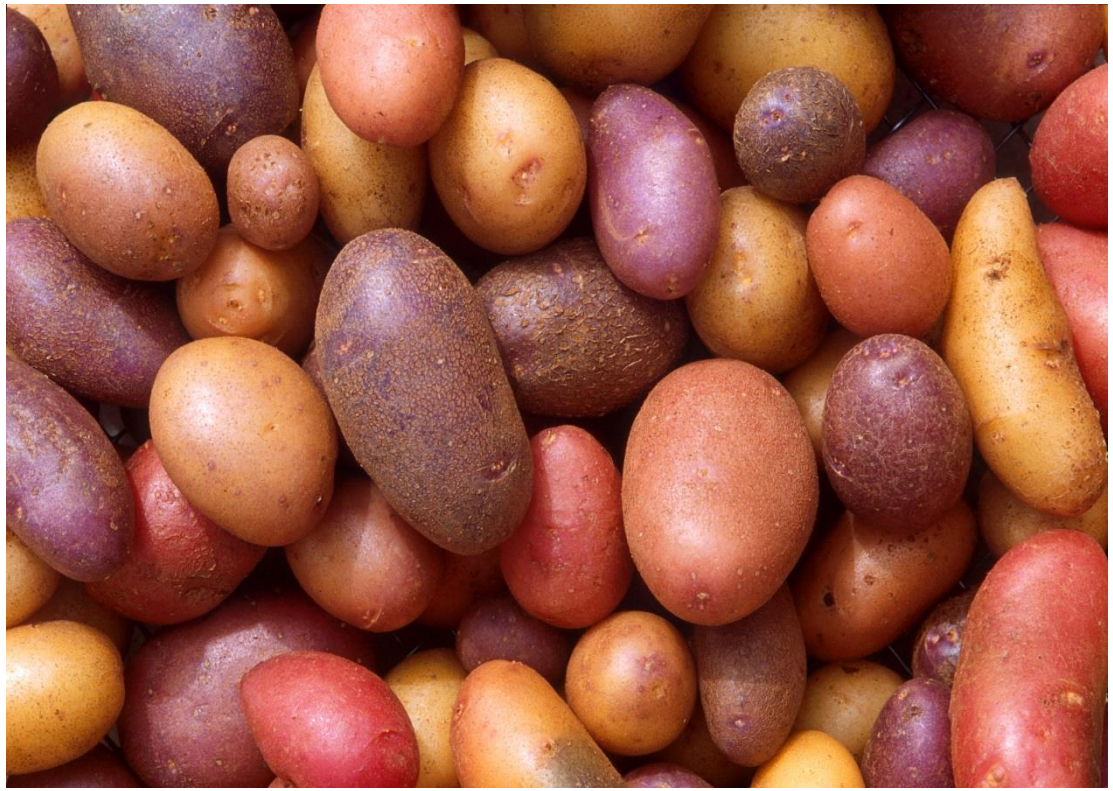
Outline

I. Background/Justification

II. Objectives

III. Conclusions

IV. Acknowledgements



Conclusions

- There is genetic material with significantly higher folate concentrations available for breeding purposes
- GGH1 expression correlates with high folate in tubers
- SNP genotyping and subsequent studies identified areas of the genome that are associated with high folate content and folate related genes

Oregon State UNIVERSITY

Future Research

- Continue folate studies in *S. vernei* and *S. tuberosum* subsp. *andigenum*
- Evaluate heritability of high folate traits
- Study gene expression of FPGS in conjunction with GGH1 to better understand folate accumulation in tubers
- Validation of identified SNPs for their potential to use in marker assisted breeding of high folate genotypes

Oregon State UNIVERSITY

Acknowledgements

- Dr. Aymeric Goyer

  Matt Warman, Wei Dong, Taryn Goodwin, Mark Barnett

- Dr. Vidyasagar Sathuvalli

  Solomon Yilma, Moises Aguilar, Stan Li, Sapinder Bali

- Dr. Laurent Deluc
- Dr. Ramesh Sagili
- Dr. John Bamberg, US Potato Gene Bank
- OSU HAREC Station

Funding Sources

WESTERN SARE
Sustainable Agriculture Research & Education

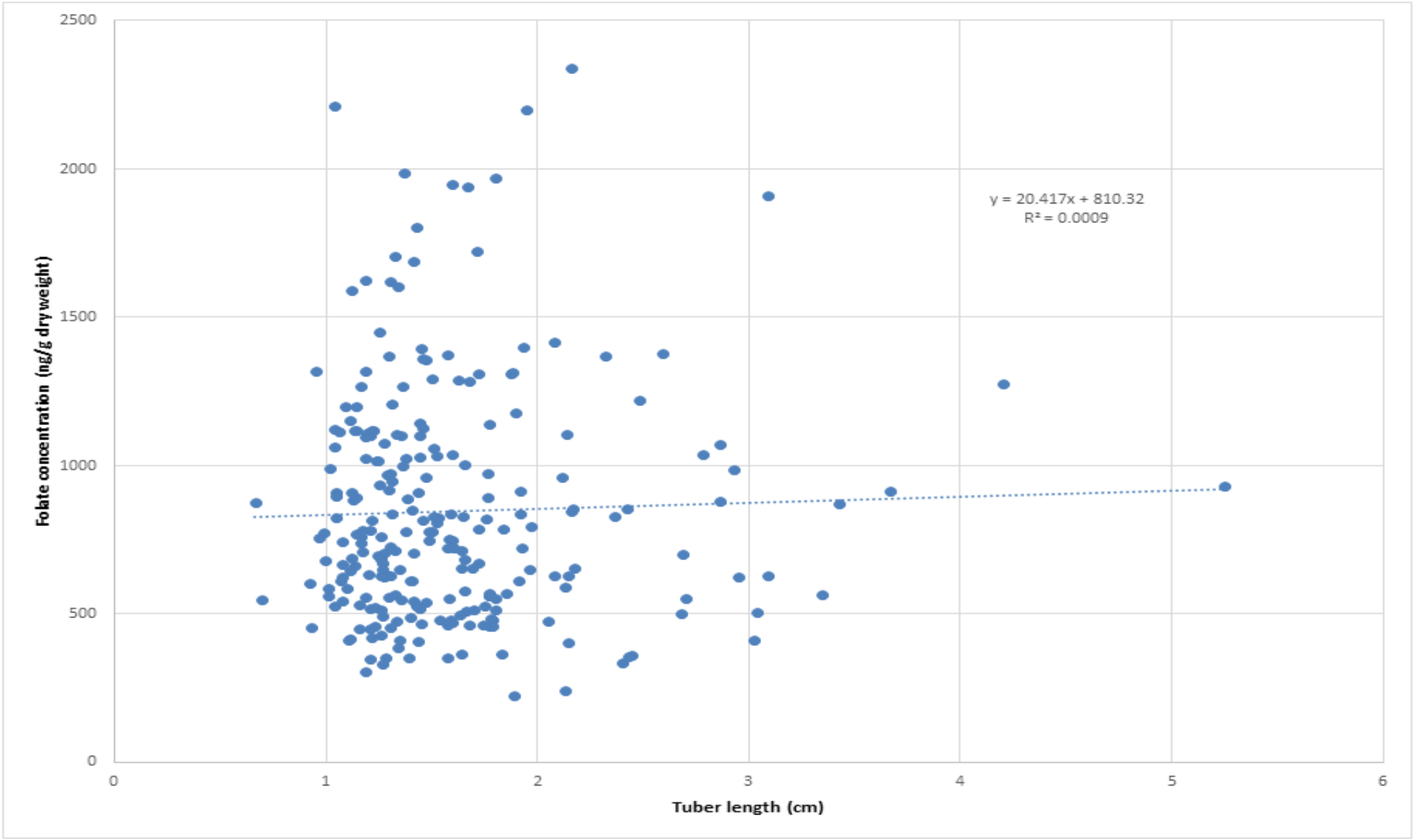
USDA NIFA

United States Department of Agriculture
National Institute of Food and Agriculture

Oregon State University

THANK YOU

Oregon State UNIVERSITY



Oregon State UNIVERSITY