Breeding for Nutritional Enhancement in Potato: Exploring Vitamin B9 diversity in Wild and Cultivated Potatoes.

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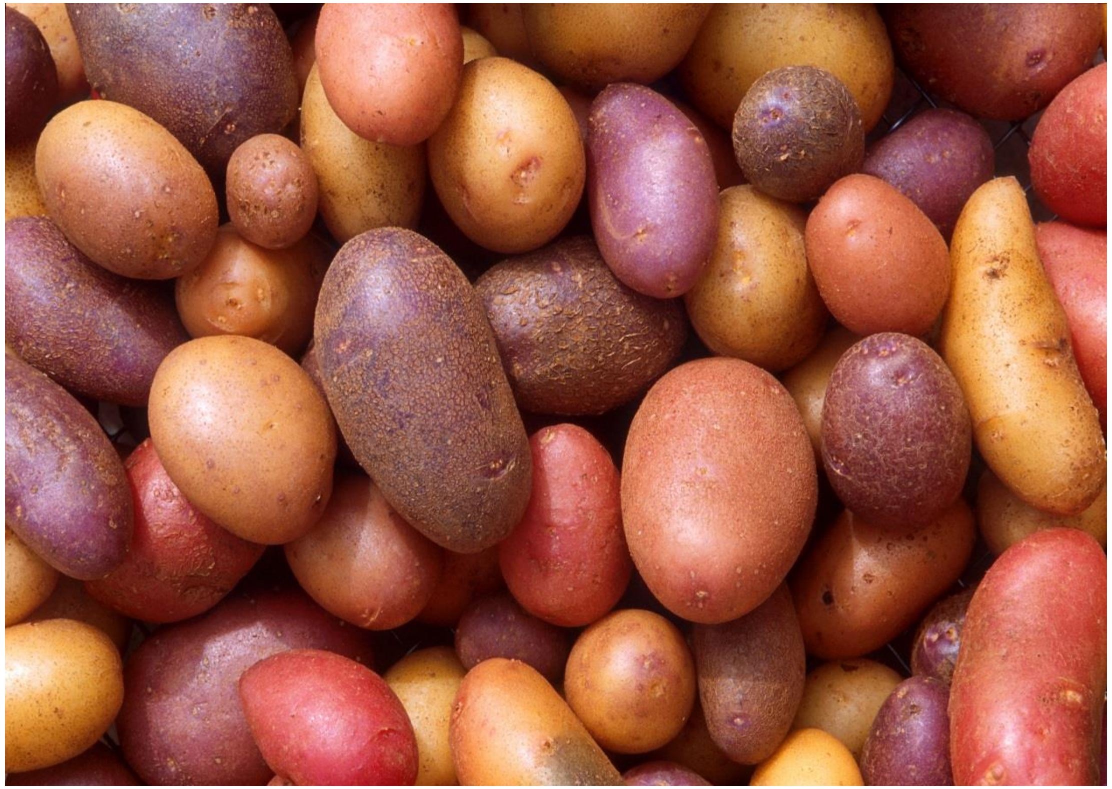
Outline

I. Background/Justification

II. Objectives

III. Conclusions and Perspectives

IV. Acknowledgements



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I. Background/Justification

- -Micronutrient Malnutrition
- -Folate
- -Sources and Deficiency
- -Biofortification
- -Potatoes

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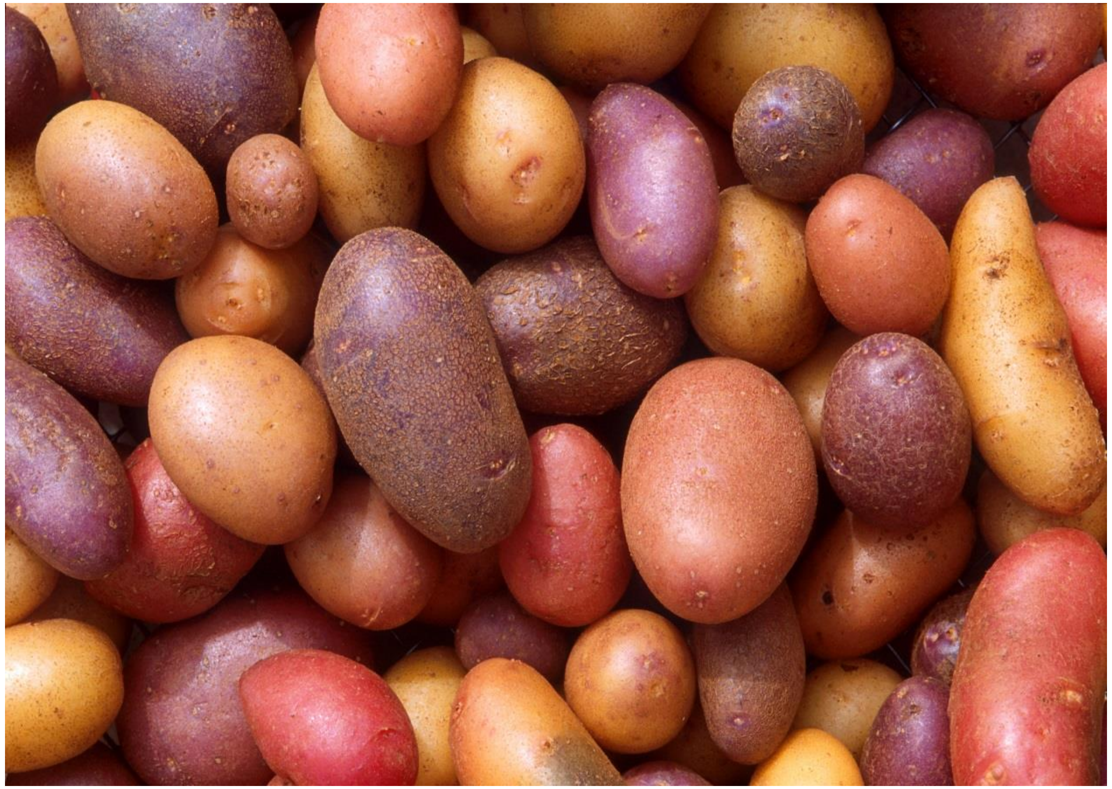
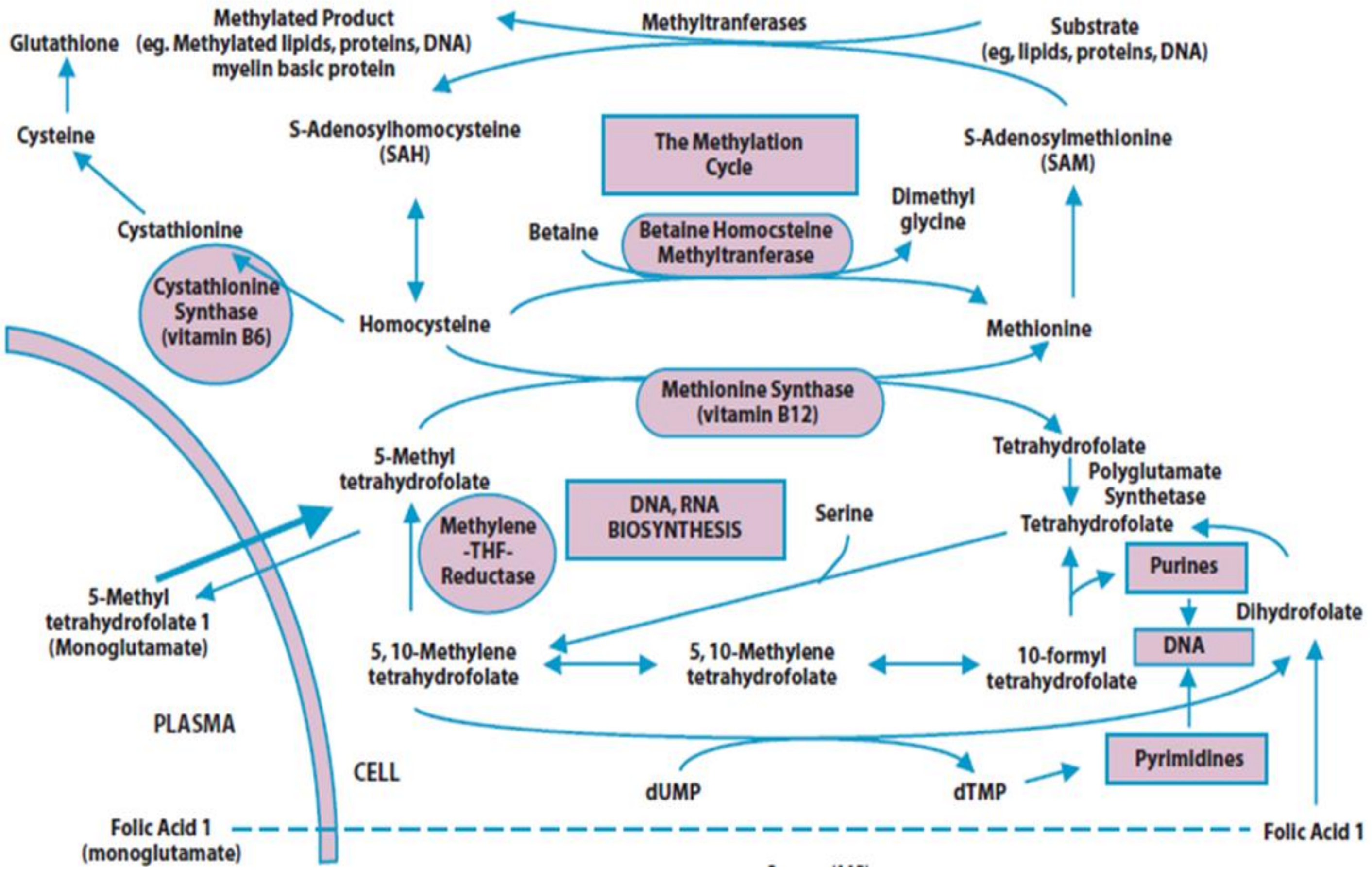


Micronutrient Malnutrition

- Most commonly a deficiency in dietary intake of:
- Minerals: Ca, I, Fe, P, K, Na, Zn
- Vitamins: B1, B2, B3, B5, B6, B9, B12, C, D, E, K
- Phytochemicals: Carotenoids, Flavonoids…
- Main sources in human diets are plants
- Has numerous negative effects on human health
- Global Issue

Folate – Water Soluble Vitamin B9

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

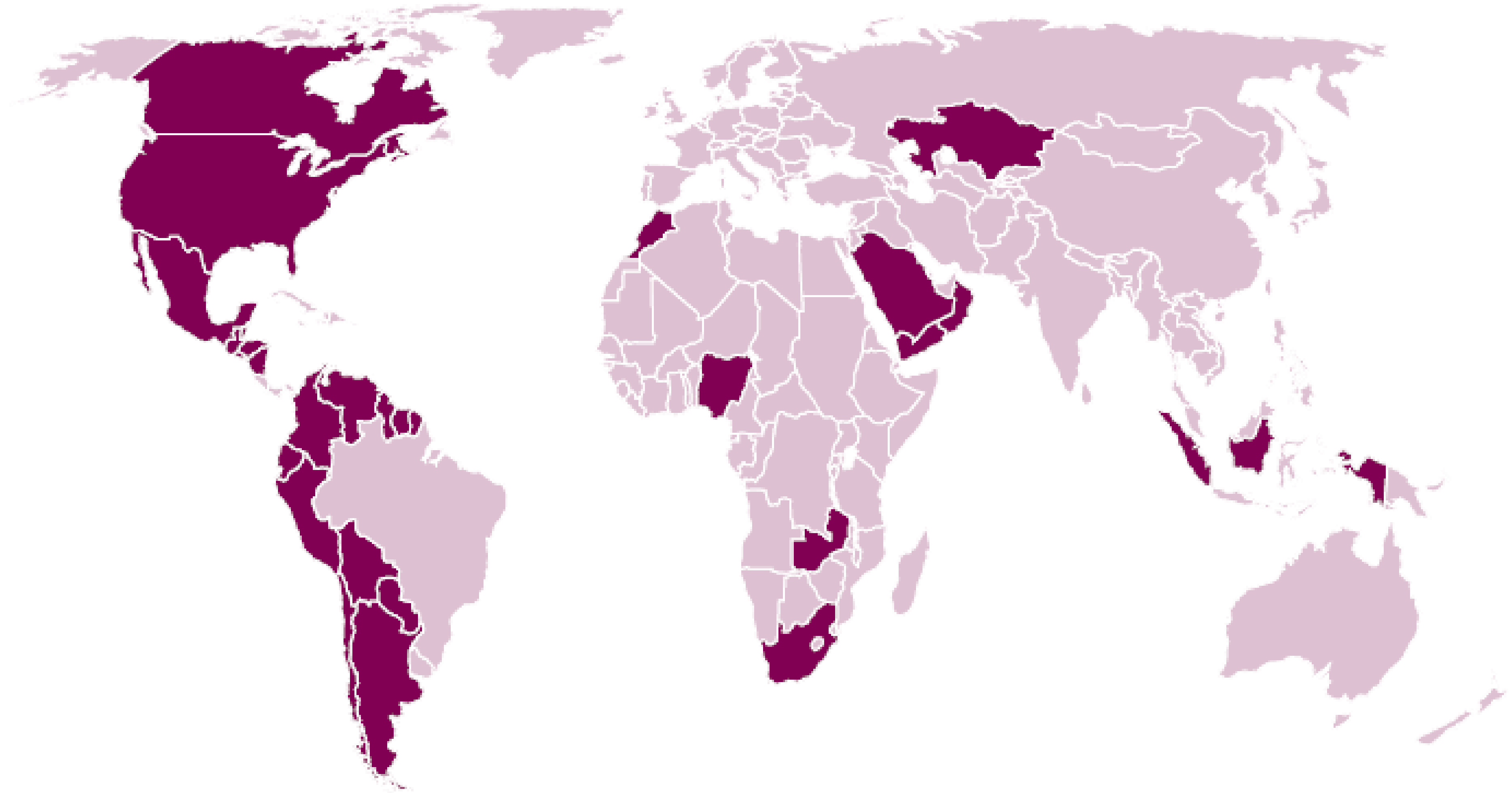


Source: Scott & Weir 1998

Folate Sources and Deficiency

- Plants are the major source of dietary folate : leafy green vegetables, beans, some fruits, tubers
- 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



- 42 countries have mandatory folic acid fortification programs, yet folate intake is still sub-optimal (plum coloring indicates Folic Acid fortification has been implemented)

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Source: Food Safety Authority of Ireland

Biofortification Through Breeding

- Has additional advantages compared to industrial fortification alone:
  - Cost-effective
  - Sustainable
  - Can impact areas that lack the political will, infrastructure, and money to utilize current fortification practices
- Requires that the target of the biofortification is a staple crop
- Requires that this crop demonstrates natural variation, stability, and heritability for the trait you are breeding for

Potatoes are Important

- *Solanum tuberosum* L. is the world's third most important food crop behind rice and wheat.
- Studies from the Netherlands, Norway, Finland, and Spain all report potatoes to be a significant source of folate in their diets, respectively
- A Greek study showed that consumption of potatoes is associated with decreased risk of low serum folate levels

Additional Potato Information

- Currently a 148g serving of potato (a medium sized potato) only provides about 6% of the 400µg RDA of folate
- Folate retention is high in potato tubers even after storage, processing, and cooking
- There are approximately 200 tuber bearing *Solanum* species representing enormous genetic diversity
- Exploiting this variation between species is the paradigm for modern crop improvement, yet potatoes have not been a major focus of biofortification studies until now

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- Germplasm Diversity with respect to folate levels
- RNA-Seq
- Regulation of folate related genes

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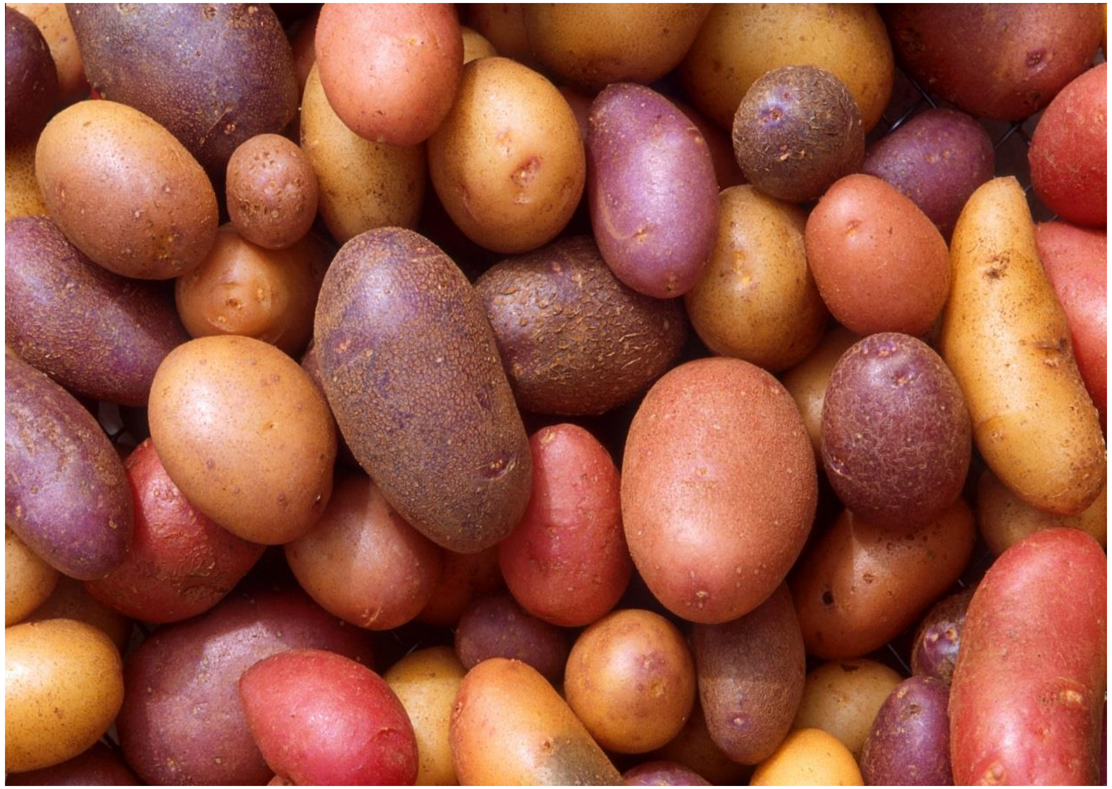

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Folate Content Variability in Potatoes

- Figure one shows genotypes vs. folate concentrations based on dry weight
- Wild type and primitive cultivated species show the greatest range of folate content with some demonstrating significantly higher levels of folate content over modern cultivars
- My focus is on further evaluating this wild and primitive germplasm to identify new sources of high folate germplasm

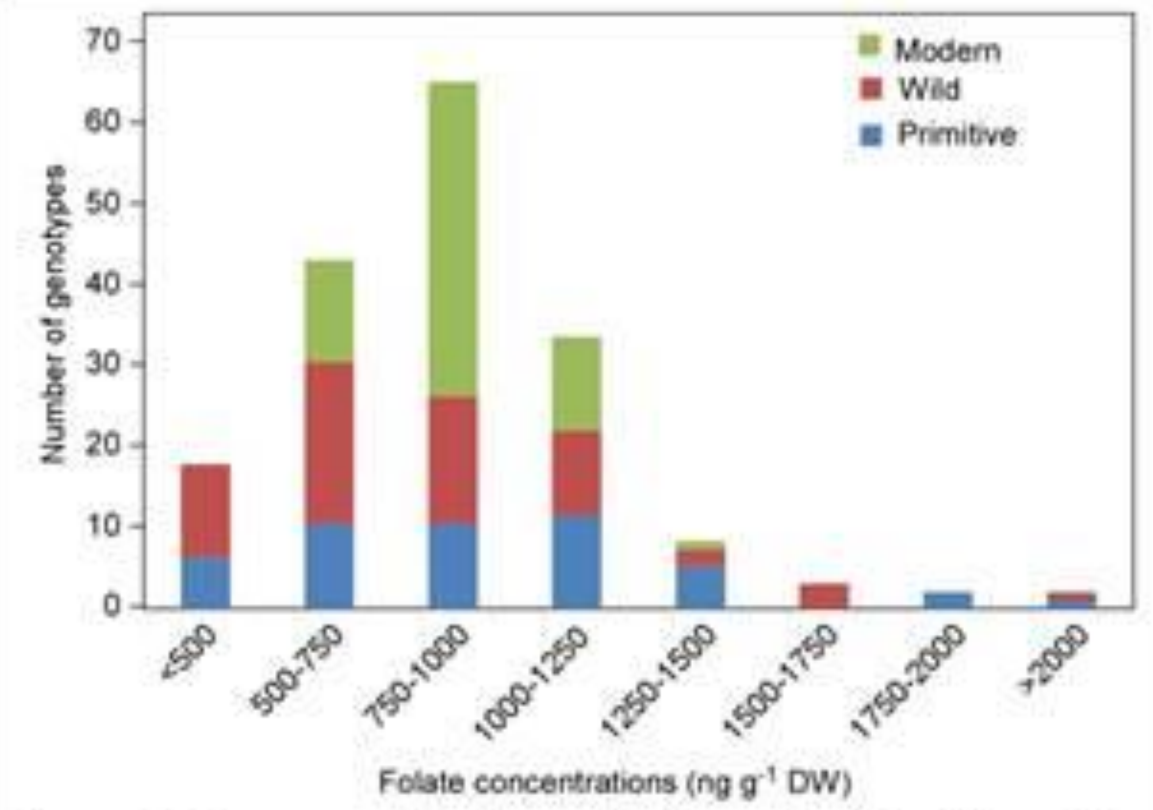

Figure 1. Frequency of modern, primitive and wild potatoes within folates concentration brackets.

Goyer A, Sweek K (2011)

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Previously Evaluated Materials

- **Primitive cultivated** *tuberosum* Group *phureja* (phu): 100 populations, 1,500 genotypes were preselected for folate screening ("RN" series)
- RN_{phu} 18.03 = 225710_{phu} ~ 2000 ng/g DW = 40 µg/100g FW = 16% RDA_{USA}

- **Wild species** (minicore collection): 75 populations made up of 3 populations each of 25 species were screened
- *Solanum boliviense* (blv) PI 597736 was identified as having the highest mean folate content
- Fol 1-6 = 597736_{blv} ~3100ng/g DW = 60 µg/100g FW = 25% RDA_{USA}

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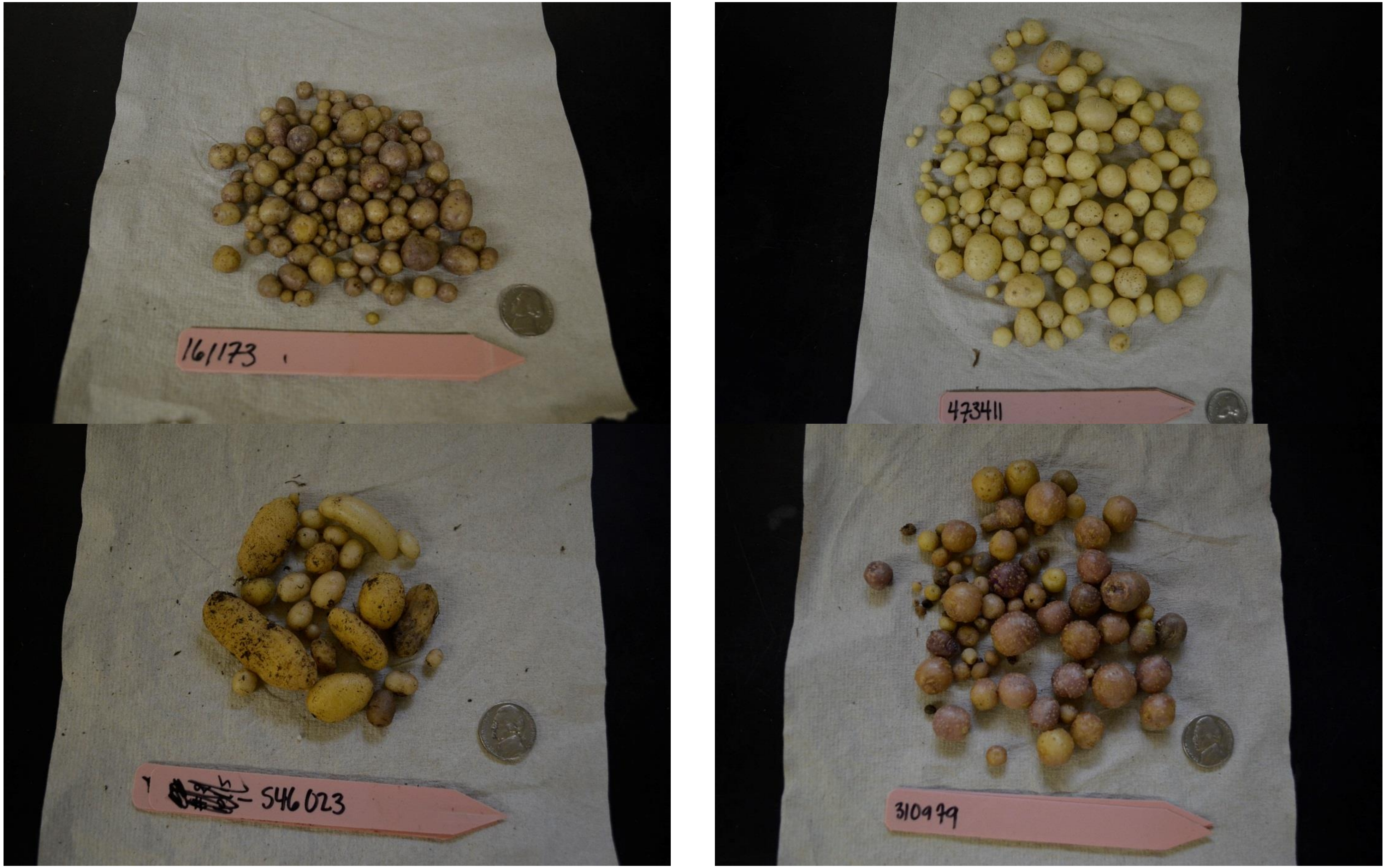

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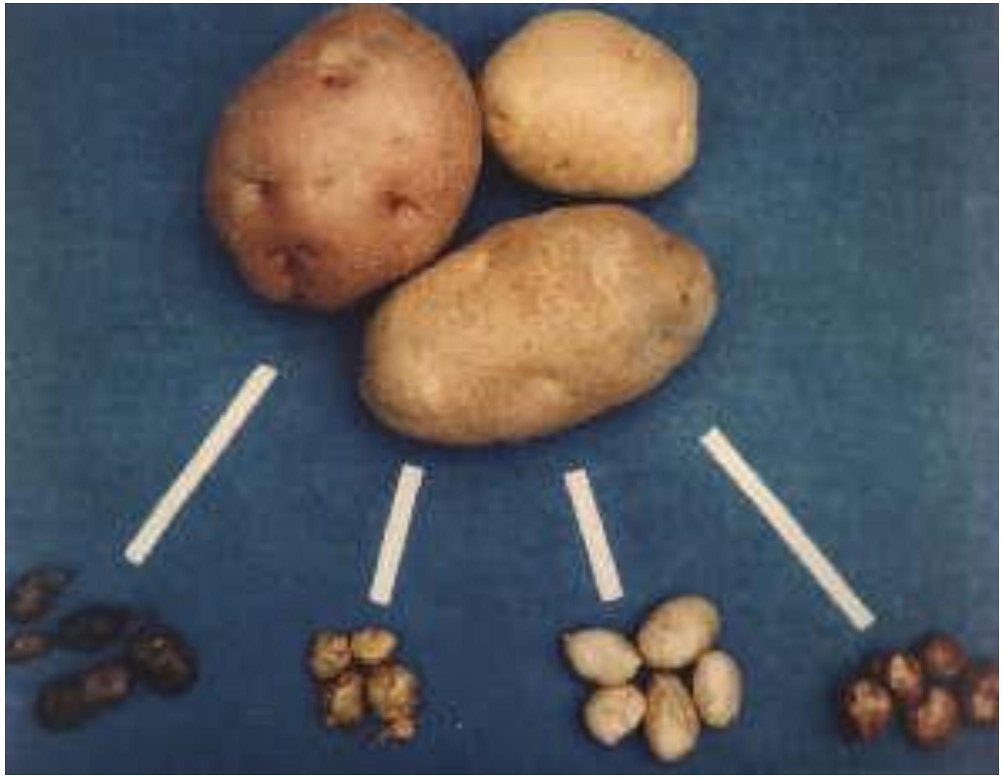
Objectives

- Quantify folate content via tri-enzyme extraction and L. Rhamnosus microbiological assay

- Identify wild and primitive cultivated potato accessions that have relatively high folate content for introgression with “bridge” species USW4$_{self}$#3
  - USW4self#3 x fol1.06
  - USW4self#3 x Phu 18.03

- Use populations from a cross of high folate content with low folate content to characterize folate level segregation.
  - fol1.06 x fol1.07

- If there is adequate segregation in these populations they could be used for eventual identification of QTLs associated with high folate concentration



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Materials for Further Evaluation

- A. Pre-breeding materials: USW4self#3 x fol1.06 and USW4self#3 x Phu 18.03 F1 populations
- B. Segregating populations from high/low folate content crosses: Fol1.06 x Fol1.07
- C. Wild Species Material

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Tri-Enzyme Extraction Method

- General Principle: Folate species must be released from food matrices and processed without degrading the sample so determination by *L. rhamnosus* can be performed

- HEPES/CHES buffer, protease, α-amylase, and conjugase allow for this with reasonable throughput

Food Sample

Homogenize in HEPES/CHES Buffer

Heat (10min at 100C)

Ice Bath

Incubate with Protease (2hrs at 37C)

Heat (5 min at 100C)

Ice Bath

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

Heat (10min at 100C)

Ice Bath

Centrifuge

Storage at -80C

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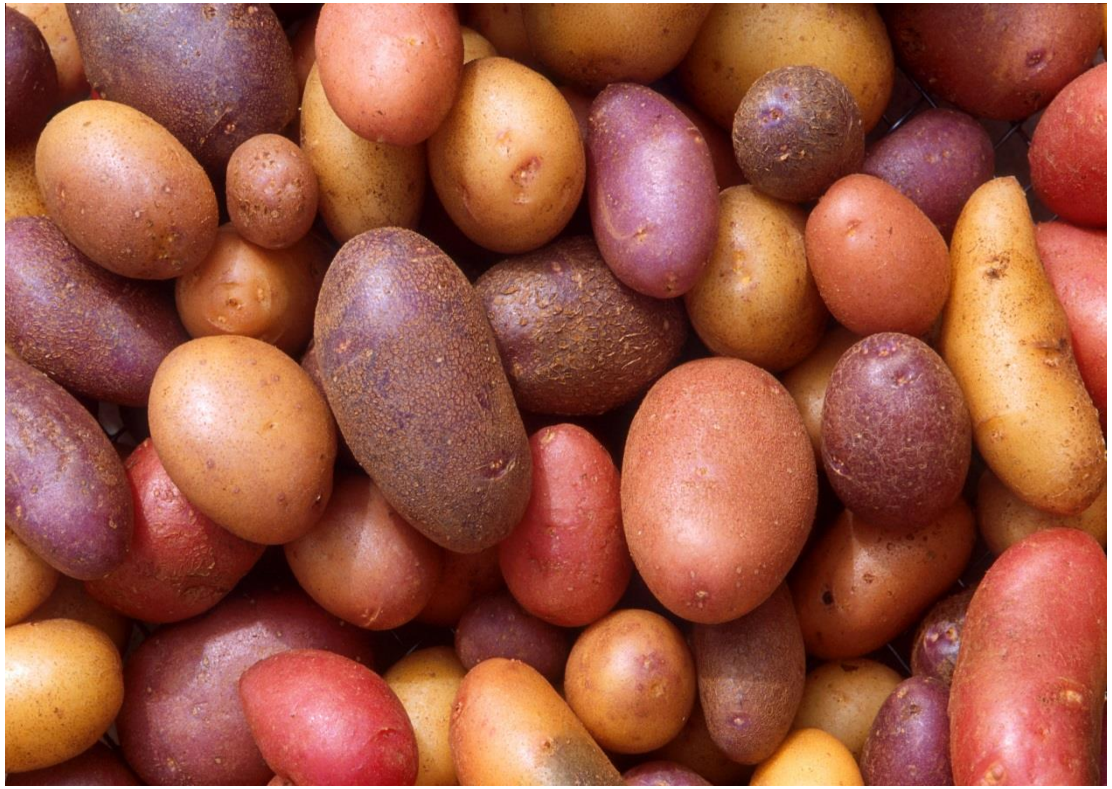

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Objectives

- Perform analysis on RNA-seq data previously generated in order to document genes that are differentially expressed in these samples
- Compare gene expression between varieties that have low folate content vs. high folate content genotypes
- We don’t know if transcriptional regulation is the key determining factor in whether or not a potato tuber has high or low folate content.

TISSUE-SPECIFIC EXPRESSION DETECTED WITH RNA-SEQ

The quantity of individual reads are indicated at each genomic location (y-axis). Expressed exons are clearly seen as peaks, and are consistent with RefSeq annotation (bottom). Sample-specific expression is quantifiable by comparing results from different samples. The brain sample (top) exhibited 3,115 reads, whereas UHR sample (middle) exhibited 31,109 reads, indicating a ten-fold higher level of expression.

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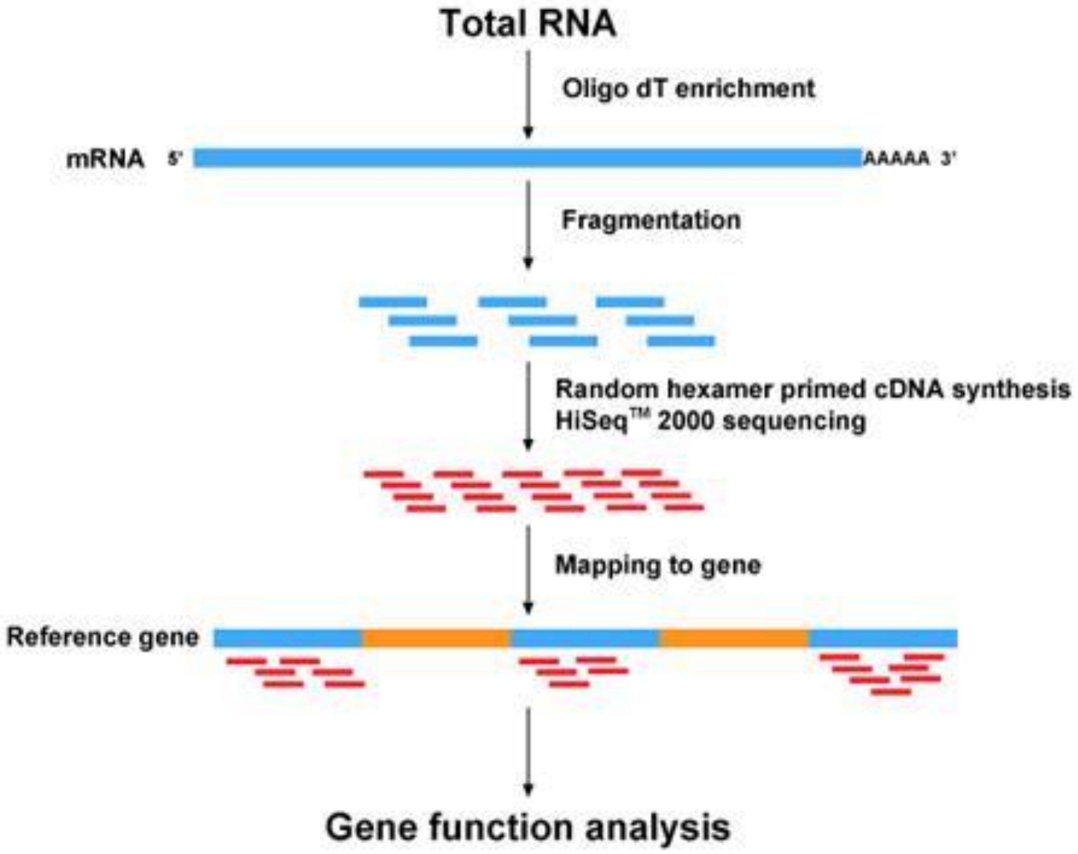
RNA Analysis

- The hope is that this will lead to identification of markers associated with high folate levels
- Why do some accessions produce and retain more folate and what genetic elements are involved?



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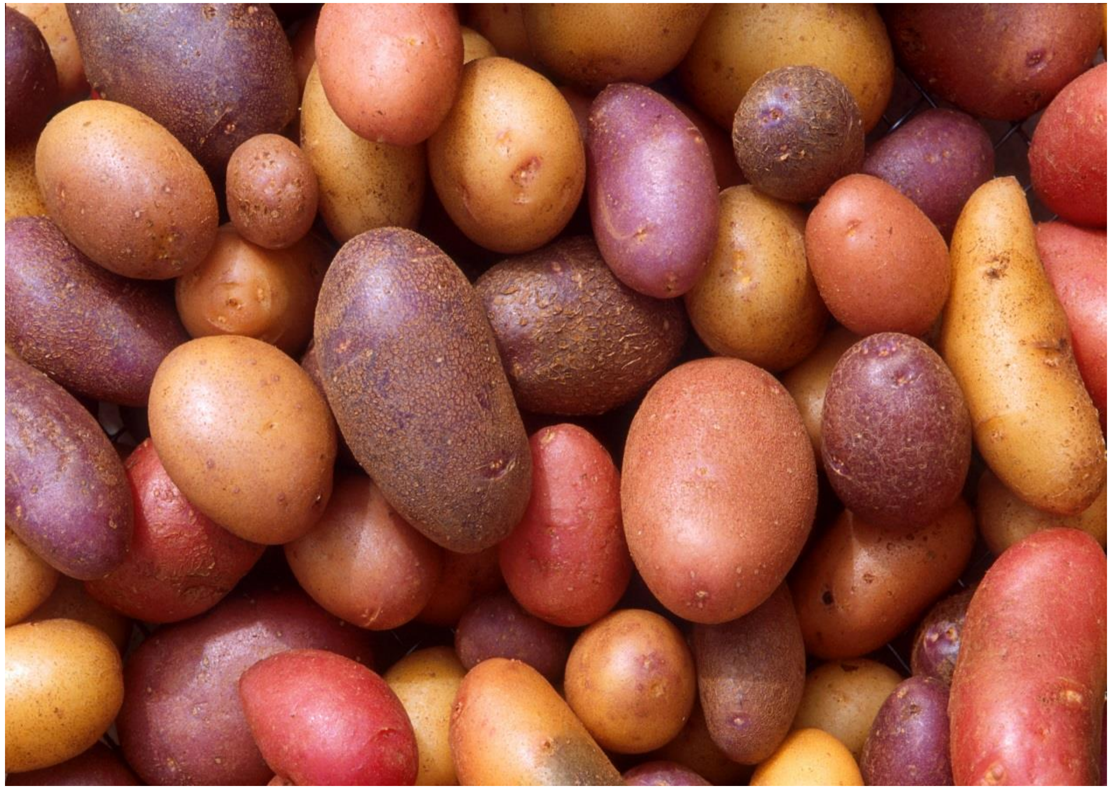

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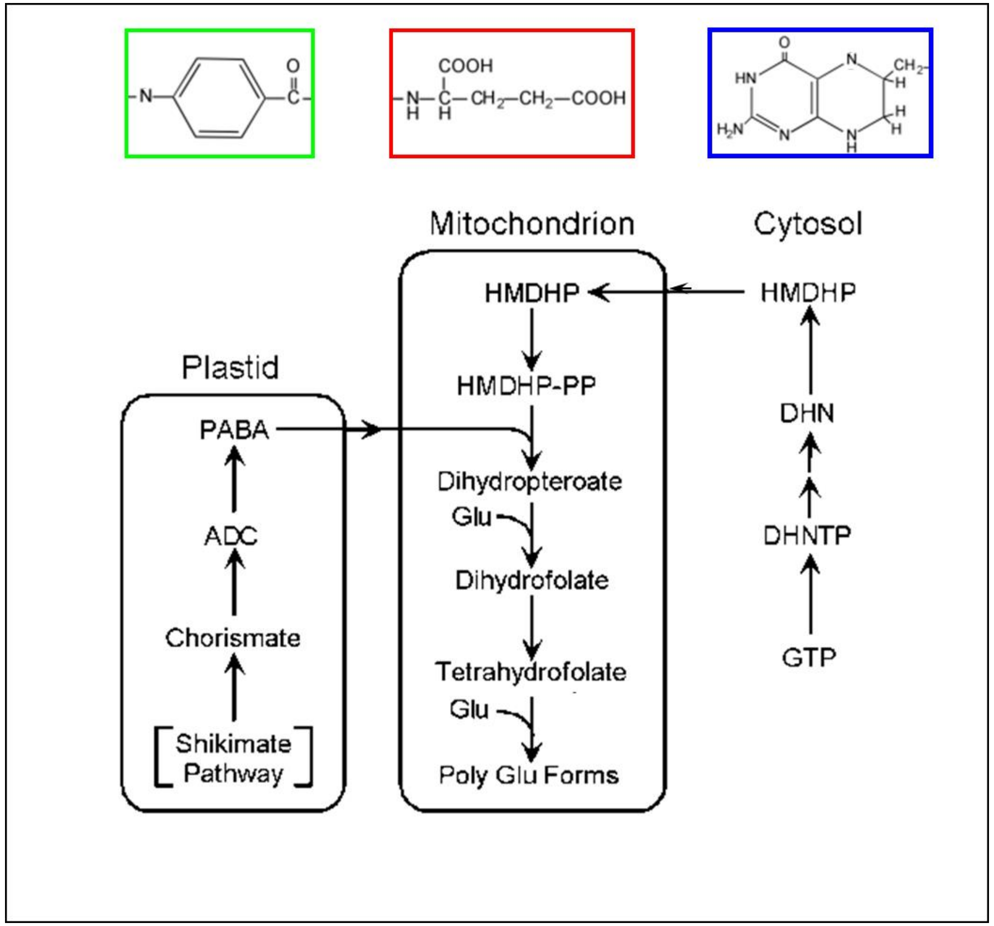
Regulation of Folate Related Genes

- Current data suggests that certain folate pathway genes are developmentally regulated and others are subject to feedforward control by pathway intermediates
- Induction of the folate biosynthesis pathway appears to be relatively specific
- Promoter analysis can help to better understand this mechanism.
- Co-regulation of these processes is a possibility, meaning they may share conserved cis-regulatory elements in their promoter sequences that can be identified



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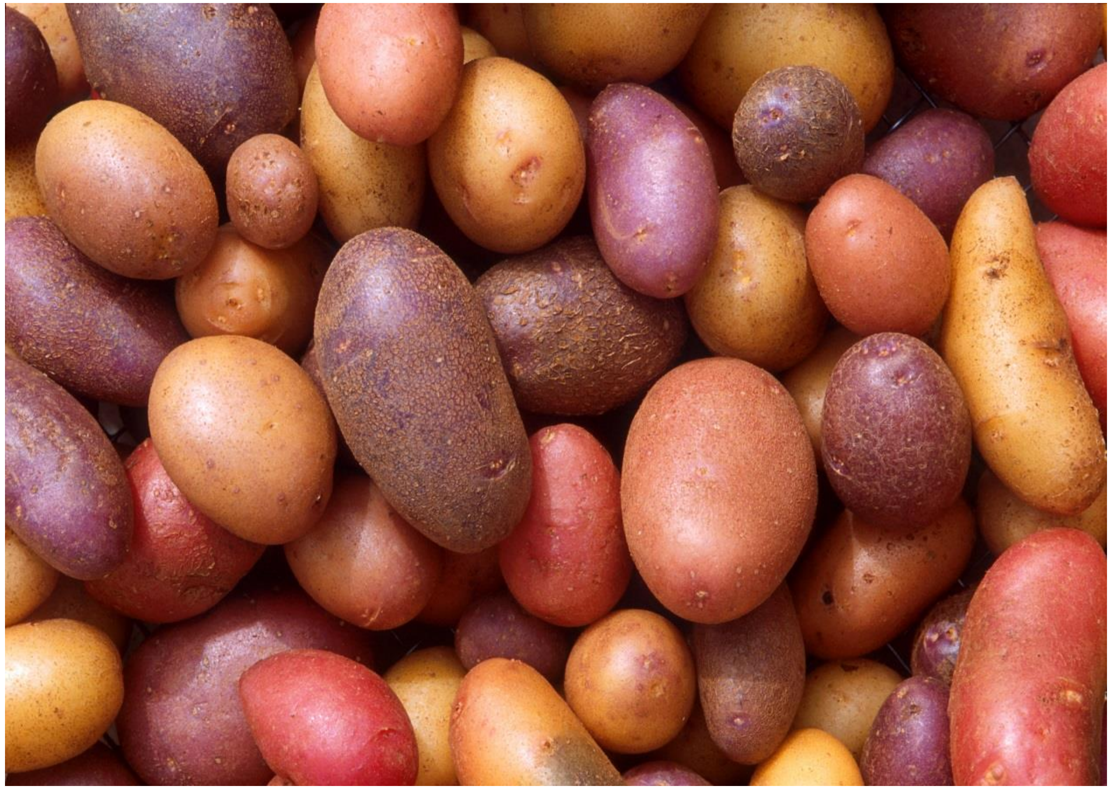

Conclusions

- Continue identification/characterization of potato accessions for introduction to breeding program.
- Evaluate folate levels in segregating populations from crosses in the breeding program
- Perform analysis on RNA-Seq data to determine if transcriptional regulation is responsible for folate levels found in tubers and if so, by which specific genes
- (Exploratory) examine promoter regions of folate biosynthesis genes for conserved cis-regulatory elements

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