2007

Relative Inheritance of Total Phenolics in Potato and Evaluation of Techniques for the Prediction of Total Phenolics in Production

Investigators: All in Dept of Horticulture & Landscape Architecture
Harrison Hughes, Professor of Horticulture
Mohammed Al-Daej, PhD candidate
David Holm, Professor of Horticulture
Fahrettin Goktepe, Research Associate
Cecil Stushnoff, Professor of Horticulture

B 2,650

Nature of Research: This research is intended to provide information that will assist in the development of highly pigmented potato cultivars.

Objectives:

I. Examine total phenolics of tubers relative to antioxidant activity with time and temperature in storage.

Hypothesis: There will be an increase with length of time in storage as per Mekhled's research with cooler temperatures giving best results.
(We will use different colored potato tubers and freeze dry samples at day 0 storage and at monthly intervals from 1 to 6 months).

II. Compare pigment color and relative phenolics of tubers from plants grown in the greenhouse with filed grown plants.

Hypothesis: There will be a strong correlation between color and phenolics of plants grown in the greenhouse with those grown in the field, ie one can select in the greenhouse for flesh color and phenolics.

(Several pigmented families will be grown in the greenhouse and will then be moved to the field. Tuber pigment will be characterized by visual means, colorimetery and by pigment extraction procedures from potatoes grown in the greenhouse and field and correlations will be run to determine if selection in the greenhouse can be used to identify high, medium and low pigment classes).

III. Evaluate progeny with different parents to determine which parents are best for obtaining highly pigmented tubers with higher phenolic levels.

Hypothesis: Certain parents will give on the average higher pigmented progeny with higher phenolics than other parents. Multiple crosses will be used, ie Purple X Purple, Red X Red and others to evaluate the best parents. Numbers of crosses will be largely associated with availability of seeds from crosses already made.)

Material and Methods:

Fifteen families (Table 1) derived from different crosses of clones and varieties grown in CO, SLV were selected for this study. These crosses have already been made by Dr. David Holm

Table 1: Families that will be used in this study.

	Family	Female	Male
1	CO94163	ND 1995- 1W	All Blue (P/P)
2	CO94166	ND2109-7W	All Blue
3	CO94178	All Blue	ND 1995-1W
4	CO94179	All Blue	ND2109-7W
5	CO94181	All Blue	Chipeta W/W
6	CO94198	Chipeta	All Blue
7	CO97211	Durango	Mountain Rose 1R/R
8	CO97219	Purple Majesty	Mountain Rose
9	CO97225	Mountain Rose	Mountain Rose
10	CO97254	Cherry Red	Mountain Rose
11	CO97306	Purple Peruvian	Mountain Rose
12	CO97307	Purple Peruvian	CO94214-1P
13	CO04045	CO97215-2P/P	CO97216-1P/P
14	CO04059	CO97219-1R/R	CO97306-1R/R
15	CO04063	CO97226-2R/R	CO97222-1R/R

Fifteen families were planted in November of 2006. About 300 true seed from each family was planted in plastic trays (25cm W x 25cm L x 10cm d) and then transplanted in Dec of 2006 into 3″x 3″ pots. Each pot contained a single plant.

Potato clones will be harvested at the end of February and first of March of 2007. One or two tubers from each single plant will be used for extraction for 25-30 plants of each cross. The rest of the mini tubers (1-2) will be kept and planted in May of 2007 for the field experiment.

Flesh color will be measured using MiniScan® XE Plus spectrally based color measurement instrument from HunterLab. After colorimetric measurements, samples from 25-30 clones from each cross will be used to estimate the total phenolic content of each tuber. At the end of September 2007, tubers will be harvested from the field. Flesh color and total phenolics will be evaluated and compared with the previous results from tubers harvested in the greenhouse. The rest of the tubers will be stored for periods up to 6 months with samples taken and evaluated monthly.

Statistical analysis:

GraphPad Prime 4 software (GraphPad software, Inc. San Diego, Calif. 92130) will be used for the analysis of variance (ANOVA). Correlation between color and total phenolics of plants grown in the greenhouse with those grown in the field will be made using Person correlation (r) from GraphPad Prime 4 software.

Sample preparation for extraction:

One or two tubers from each single plant will be used for extraction for 25 randomly selected plants of each cross. The whole mini seed tubers will be cut to three thin slices and held together by tooth picks before placement in the weighing trays. Samples will be then placed in a Virtis Genesis 25 LL freeze dryer (Gadiner, N.Y. 12525) for 5 days (-48 for 1day, -28 for 1day, 18 for 1day, and 28 for 2days). Dried samples will be ground and screened with a 100-mesh sieve to ensure uniform particle size prior to extraction.
Note: for big tubers that will be harvested from the field, three thin slices will be cut from the middle of the tuber and used for extraction.

Extraction procedure:

A total of 600 mgs will be used from each ground sample. This amount will be placed into a tube with 10 ml of 80% acetone and vortexed to ensure that each sample dissolves.

The tubes then will be rotated in the rotator for one hour before putting them in a centrifuge at 6000 rpm at 4°C for 15 min. 4 ml from each centrifuged tube will be distributed into 4 eppendorf tubes (1ml per eppendorf tube) before transfer to vacufuge for at least 2h. Dried samples will then be used immediately or placed into a freezer (-20°C) for later analysis.

Total phenolics protocol:

Total phenolics will be measured using Folin-Ciocalteu reagent as adapted from the method of Spanos and F Wrolasted (1990). 1ml of 80%acetone will be added to each tube of extracted sample followed by sonic for 10-15 min. 100 µl from each tube will be transferred to a new eppendorf tube and then diluted by adding 900 µl dH_2O. 35 µl of each diluted sample and as well as a gallic acid standard curve sample will be transferred into microplate wells (3rep/sample). 150 ul of 0.2M Folin-Ciocateu reagent will be added to each sample using a multi-pipette. Samples in the microplate will be incubated at 45 °C for 30 min and then cooled at room temperature for 1h. Absorbance of each sample in microplate wells will be measured at 765 nm using a spectrophotometer/microplate reader (SPECTRAmax PLUS384 UV-vis spectrophotometer, Molecular Devices, Sunnyvale, Calif.) and Pro Version 3.1.2 software.

Facilities.

Seedlings will be grown in the greenhouse in the SLV. Mini tubers will be sprouted in growth chambers in the SLV and grown at the SLV research center. The colorimeter will be used in the valley as well. Tubers will be brought to Fort Collins and dried and chemically assayed for total phenolics.

Relationship to Potato Growers Needs.

Results from this research will speed the development of potato cultivars with increased nutritional characteristics. This may lead to the development of further proposals on techniques to speed the development of potato cultivars with improved nutrition.

Timeline.

Nov, 06: Germination of the seed—Completed.

Dec, 06: Transplanting—Completed.

End of February and beginning of March, 07: harvesting of the mini tubers, color measurement by the colorimeter, and extraction.

Feb-May, 07: estimate total phenolic and analyze the data.

May, 07: plant the mini tubers in the field.

End of Oct, 07: harvesting, color measurement by the colorimeter, and extraction.

Nov, 07- Jul, 08: estimate total phenolic, analyze data, and compare it with previous data collected from the greenhouse.

Budget.

Chemicals: Acetone, Folin-Ciocateu reagent, Sodium carbonate, pump oil. (chemicals to do the total phenolic assay)	$ 600.
Supplies; tubes, microplates, misc. supplies to run assay.	$ 600.
Travel: Cover expenses in SLV for Al-Daej (2 trips & 3 days each).	
Hughes (1 trip & 3 days).	$ 675.
Potential repairs/maintenance of equipment.	$ 400.
Student hourly to assist during assay.	$ 400.
	$ 2675.

This is the total budget to complete this project.