DISSERTATION

RELATIVE TOTAL PHENOLICS IN POTATO (*SOLANUM TUBEROSUM* L.) PROGENY FROM 15 FAMILIES

Submitted by

Mohammed Ibrahim Al-Daej

Department of Horticulture and Landscape Architecture

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2009

COLORADO STATE UNIVERSITY

March 4, 2009

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MOHAMMED IBRAHIM AL-DAEJ ENTITLED RELATIVE TOTAL PHENOLICS IN POTATO (*SOLANUM TUBEROSUM* L.) PROGENY FROM 15 FAMILIES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTORAL OF PHILOSOPHY.

Committee on Graduate Work

Patrick F. Byrne

Advisor

Department Head

ABSTRACT OF DISSERTATION

RELATIVE TOTAL PHENOLICS IN POTATO (*SOLANUM TUBEROSUM* L.) PROGENY FROM 15 FAMILIES

Potatoes are the fourth most important food crop by consumption in the world. Since antioxidants have been identified as having health benefits related to reducing free radicals, there is interest in increasing the levels of antioxidants in new potato cultivars. The objectives of this study were to: 1) Determine the total phenolics content (TPC) of progenies of 15 families derived from crosses of clones grown in the San Luis Valley (SLV), Colorado (CO). 2) Determine if pigment level as measured by MiniScan® XE Plus could be used as a method to identify high TPC tubers.
3) Study the correlation between TPC of all tubers and families' means harvested from the field with corresponding families' means and tubers harvested from the greenhouse (GH).

Progeny from 15 families were evaluated for TPC in the GH (448 progeny) and field (223 progeny) in the SLV, CO in 2007. MiniScan® XE Plus assay was also evaluated as a tool to select superior progeny for TPC in the GH. TPC of seedling tubers grown in the GH were also compared to TPC of the same tubers grown in the field. TPC was measured using Folin-Ciocalteu reagent and tuber flesh color was measured using the MiniScan® XE Plus from Hunter Lab (Reston, VA).

The TPC varied considerably among individual progeny lines of tubers grown in the GH and field and ranged from 1.74 to 17.94 and 1.82 to 19.38 mg Gallic Acid Equivalent (GAE)/g dry weight (DW), respectively. Three families (CO0463, CO04045, and CO97307) were found to have the highest average TPC in the GH

(7.43, 7.1, and 6.1 mg GAE/g DW) and field (14.45, 9.88, and 9.1 mg GAE /g DW) respectively. Most families' means and tubers exhibited a considerable increase in TPC in the field when compared to the same families' means and seedling tubers grown in the GH.

Correlation of TPC means of families grown in the GH with those grown in the field was r=0.83 (P= 0.0002). However, correlation of TPC of all individual lines of tubers harvested from the field with corresponding seedling tubers harvested from the GH was r = 0.70 (P <0.0001). In addition, the correlations between TPC for individual tubers grown in the GH and field within each family varied from r= -0.13 (P = 0.5) to -0.82 (P<0.0001) within CO04045 and CO97306 families, respectively.

Color as determined by MiniScan® XE Plus was not useful as a tool to select superior progeny for TPC. Correlation of flesh color as indicated by L value and total phenolic content of 448 seedling tubers was moderate (r = -0.65, P<0.0001) (L value ranges from 0 to 100, where 0 is black and 100 is white).

In conclusion, TPC of progeny in the greenhouse can be used to select among families but selections of superior individuals within families are better done in the field where greater overall levels of TPC are noted. Progenies of CO0463, CO04045, and CO97307 families can be used to select for high TPC clones. Colored-fleshed potato clones were higher in TPC than white-fleshed clones. The highest TPC contents were observed in red-fleshed clones in the GH and field. Thus, the purple-fleshed potatoes are not necessarily higher in TPC than red-fleshed as previously reported by Reddivari et al. (2007). Many environmental factors such as higher light intensity, UV-B radiation, lower fertilizer rates applied, and large diurnal temperature differences in the field were likely factors associated with the differences in TPC observed between the GH and field.

Mohammed Ibrahim Al-Daej
Department of Horticulture
and Landscape Architecture
Colorado State University
Fort Collins, CO 80523
Summer 2009

ACKNOWLEDGMENTS

Initially, I would like express my grateful gratitude to Almighty Allah for his guidance and help throughout my life. This research would not have been completed without the help and support of many people. First, I would like to express my heartfelt gratitude and appreciation to my advisor, Dr. Harrison Hughes who gave me the honor of working with him and for his patience, guidance, encourage and enthusiasm during graduate school. He will always be remembered as a wonderful advisor and a good friend.

Sincere thanks go my committee members, Dr. Cecil Stushnoff, Dr. David Holm, and Dr. Patrick Byrne for their assistance and support to complete this research. Special thank to Dr. Stushnoff who gave me a full access to his laboratory and offers me all technical and chemical support. He was constantly willing to help and welcome to answer my questions. Heartfelt thanks go to Dr. Holm for his kind support, patience and timely help in the greenhouse and field. Also special thanks due to Dr. Patrick Byrne for his significant impact on my knowledge of quantitative and molecular genetics during graduate school.

With deep respect and esteem regards, I extend my thanks to Dr. Fahrettin Goktepe, Dr. Samuel Essah, Mitzi Sisneros, Savanah Fugok, and Steve Keller for their help during my work in the greenhouse and field.

Moreover, I would like to express my appreciation to my friends, gradate students and the faculty and staff at the department of Horticulture and landscape architecture.

Sincere appreciation and deep gratitude is due to my parents, my brothers and my sisters for their love and support. There is no single word describes my grateful gratitude to my wife and my kids for their patience, understanding, and unlimited encouragement during graduate school.

Finally, I'd like to thank my government represented in King Faisal University in Saudi Arabia for their financial support and encouragement.

DEDICATION

To the soul of my father (God's mercy upon him), who passed away in November 2007 and didn't share with me these moments, to my mother who was always behind all successful endeavors I have ever made, without her incessant prayers this work could not be completed, to my lovely wife and children for their encouragement and support throughout my graduate school, to my brothers, my sisters, and all of my sincere friends who enthused me to achieve this degree, I appreciatively dedicate this work.

Mohammed Ibrahim AL-Daej
Fort Collins, Colorado, USA
Spring 2009

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the major food crops in the world. It provides an important source of vitamins, minerals, and high quality proteins. Potato is considered to be an excellent source of vitamin C, and also contains significant levels of vitamin B and E (Lachman et al., 2000). Potatoes also contain phenolic compounds and antioxidants, which are directly related to human health through protection against oxidative and free radical processes (Al-Saikhan, 1995, 2000; Brown, 2005).

It has been suggested that consumption of fruits and vegetables containing high levels of polyphenols may reduce chronic diseases such as cancer and heart disease (Halliwell et al., 1992). There is evidence that a diet rich in fruits and vegetables has a positive effect on human health, providing protection against age-related diseases, such as heart disease, cardiovascular disease, Alzheimer's disease, cataracts and several forms of cancer (Gandini et al., 2000; Joshipura et al., 2001; Kang., et al, 2005; Liu et al., 2000; Liu et al., 2001; Williamson, 1996). These diseases occur as a result of cellular damage caused by the reaction of free radicals with a range of biological molecules such as lipids, nucleic acids, and protein.

Phenolics in fruits and vegetables have received a great deal of attention because of their antioxidant activity (Chinnici et al., 2004). Phenolic compounds can suppress free radical-induced oxidative stress and thus may reduce the beginning of cancer and heart disease (Halliwell et al., 1992). All of these health-related properties have stimulated the search for new phenolic-rich plant sources as well as the development of strategies to increase the content of phenolic compounds in plant tissue. For example, purple and red-fleshed potatoes offer a valuable novel source of natural colorants and antioxidants, both associated with their phenolic compounds (Reyes et al., 2003).

A group of eight purple- and one red-flesh potatoes were evaluated for their total anthocyanins, total phenolic content (TPC) and antioxidant capacity (Reyes et al., 2005). Positive correlations were observed between antioxidant capacity and total anthocyanins (r= 0.87) and TPC (r= 0.88). Moreover, a positive correlation was observed between total anthocyanins and TPC (r= 0.91) (Reyes et al., 2005). These results are consistent with previous reports indicating that phenolic compounds are important contributors to the antioxidant capacity of many fruits and vegetables, as compared to other well-known antioxidants such as Vitamin C, Vitamin E and carotenoids (Cao et al., 1996; Gil et al., 2002; Wang et al., 1996).

Seven potato cultivars (Russet Burbank, Chipeta, CO94183-R/R, CO94165-3P/P, Yukon Gold, Russet Norkotah, and Russet Nugget) grown in 4 cultivation sites in Colorado were evaluated for their antioxidant properties (Al-Obeidani, 2005). A strong correlation was observed between antioxidant capacity and gallic acid equivalent TPC (r=0.966), again suggesting that phenolics are the major an important source of antioxidant capacity.

Prompt research in the field of horticulture and food science is recommended to improve the antioxidant content of fruit and vegetables through cultivar development, production techniques and post-harvest practices (Kalt et al., 1999). The objectives of this study were to: 1) Determine the TPC of specific families derived from crosses of clones grown in the San Luis Valley (SLV) of Colorado. 2) Study the potential use of MiniScan® XE Plus to assay pigment levels as a method to select high TPC clones.

3) Study the correlation between TPC as determined in potatoes grown under greenhouse conditions with those grown in the field.

REFERENCES

Al-Obeidani, J.S., 2005. Antioxidant Properties of Colorado Grown Potatoes. Ph.D Dissertation, Colorado State University.118p.

Al-Saikhan, M.S., L.R. Howard, and J.C. Miller, Jr., 1995. Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum,* L.). J. Food Sci. 60:341-348.

Al-Saikhan, M. S., 2000. Antioxidants, Proteins, and Carotenoids in Potato (*Solanum tuberosum* L.). Ph.D Dissertation, Texas AM University, College Station, TX.170p

Brown, C.R., 2005. Antioxidants in potato. Am. J. Pot. Res. 82:163-172.

Cao, G., E. Sofic, and R.L. Prior. 1996. Total antioxidants capacity of tea and common vegetables. J. Agric. Food Chem. 44:3426-3431.

Chinnici, F., A. Bendini, A. Gaiani, and C. Riponi. 2004. Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. J. Agric. Food Chem. 52:4684-4689.

Gandini, S., H. Merzenich, C. Robertson, and P. Boyle. 2000. Meta-analysis of studies on breast cancer risk and diet: The role of fruit and vegetable consumption and the intake of associated micronutrients. Eur. J. Cancer. 36:636-646.

Gil, M.I., F.A. Tomas, B. Iless-Pierce, and A.A. Kader. 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin contents of nectarine, peach and plum cultivars from California. J. Agric. Food Chem. 50:4976-4982.

Halliwell, B., J.M.C. Gutteridge, and C.E Cross. 1992. Free radicals, antioxidants, and human disease: Where are we now? J. Lab. Clin. Med. 119:598–620.

Joshipura, K.J., F.B. Hu, , J.E. Manson, M.J. Stampfer, E.B. Rimm, F.E. Speizer, G. Colditz, A. Ascherio, B. Rosner, D. Spiegelman, and W.C. Willett. 2001. The effect of fruit and vegetable intake on risk for coronary heart disease. Ann. Intern. Med. 134: 1106-1114.

Kalt ,W., Forney, C.F., Martin, A., and R.L. Prior. 1999. Antioxidant capacity, vitamin C, phenolics and anthocyanins after fresh storage of small fruits. J. Agric. Food Chem. 47:4638-4644.

Kang, J.H., A. Ascherio, and F. Grodstein. 2005. Fruit and vegetable consumption and cognitive decline in aging women. Ann. Neurol. 57:713-720.

Lachman, J., K. Hamouz, M. Orsak, and V. Pivec. 2000. Potato tubers as a significant source of antioxidants in human nutrition. Rost. Vyroba. 46: 231-236.

Liu, S., J.E. Manson, I.M. Lee, S.R. Cole, C.H. Hennekens, W.C. Willett, and J.E. Buring. 2000. Fruit and vegetable intake and risk of cardiovascular disease: The women's health study. Am. J. Clin. Nutr.72: 922-928.

Liu, S., I.M. Lee, U. Ajani, S.R. Cole, J.E. Buring and J.E. Manson. 2001. Intake of vegetables rich in carotenoids and risk of coronary heart disease in men: The physicians' health study. Int. J. Epidemiol. 30: 130-135.

Reyes, L.F. and L. Cisneros-Zevallos. 2003. Wounding stress increases the phenolics content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum* L.). J. Agric. Food Chem. 51:5296-5300.

Reyes, L.F., J.C. Jr. Miller and L. Cisneros-Zevallos. 2005. Antioxidant capacity, anthocyanins and total phenolics in purple-and red-fleshed potato (*Solanum tuberosum* L.) genotypes. Am. J. Pot. Res. 82:271-277.

Wang, H., G. Cao and R.L. Prior. 1996. Total antioxidant capacity of fruits. J. Agric. Food Chem. 44:701-705.

Williamson, G., 1996. Protective effects of fruit and vegetables in the diet. Nutr. Food Sci. 96:6-10.

CHAPTER 2

LITERATURE REVIEW

INTRODUCTION

Potato (*Solanum tuberosom* L.) is a member of the *Solanaceae* family which also includes pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), and tomato (*Solanum lycopersicum* L.). It is one of the major world food crops and has been ranked as the fourth most important crop for human consumption after wheat, rice and maize (Ross, 1986).

The Andes Mountains of South America, particularly in Peru and Bolivia, represent the center of origin of the potato. Potatoes have been grown and eaten for more than 2,000 years there. Wild potato species still grow all over South America and are consumed by the locals who have given them the name *papas criollas*.

The potato was taken from South America to Europe in 1570 by the Spanish conquistadors who were searching for gold in Peru. The potato entered Spain first then spread elsewhere in Europe (Burton, 1989; Hawkes, 1992). When potatoes entered Europe for the first time, they were cultivated on a small scale as food for livestock and did not become a staple food crop until the late 1700s.

European immigrants introduced potatoes to North America several times throughout the 1600's, but they were not widely grown until the early 1700's. The first major planting of potatoes in North America was in New Hampshire in 1719. Eighty years later, the french fry was served at the White House during the presidency of Thomas Jefferson (FAOSTAT, 2007).

The potato tuber is an enlarged portion of an underground stem that is used by the plant for storage. The "eyes" are buds that can sprout under certain conditions to regenerate new plants. The potato is considered to have five growth stages: sprout development, vegetative growth, tuber initiation, tuber bulking, and maturation. Potatoes can be managed to produce very high yields per acre, generally many times greater than any grain crop on a fresh weight basis (Burton, 1969). They have many uses including table, processed, livestock feed, and industrial (Talburt, 1987).

Potato is a cool-season crop adapted to a wide range of field conditions. Average daytime temperatures of 21°C are optimum for the highest yield. Night temperatures play an important role in accumulation of carbohydrates and dry matter in the tubers. Low night temperatures lead to greater starch storage in the tubers because of low tuber respiration. The most favorable soil temperature for tuber initiation is 16 to 19°C. Development of tubers are reduced when soil temperatures increase above 20°C and almost stops at soil temperatures above 30°C (Stark and Love, 1995).

WORLD POTATO PRODUCTION

The potato is one of the major world food crops. During the last two decades, the world production of potato has increased remarkably especially in Asia, Africa, and Latin America (FAO, 2007). Production in developing countries has increased from 84.86 million tons in 1991 to 165.15 million tons in 2007 while developed countries production has decreased from 183.1 millions tons in 1991 to 155.6 millions tons in 2007 (Table 1). According to the latest FAO data, world potato production stands at 320.7 millions tons and covers more than 19 millions hectares (FAOSTAT, 2007).The major world potato producers in rank order by production are China, Russia, India, United States, and Ukraine (Table 2).

Table 2.1. World potato production, 1991-2007

	1991	1993	1995	1997	1999	2001	2003	2005	2007
Countries	million tones								
Developed	183.13	199.31	177.47	174.63	165.93	166.94	160.97	159.99	155.56
Developing	84.86	101.95	108.50	128.72	135.15	145.92	152.11	160.12	165.15
World	257.25	301.27	285.97	303.36	301.08	312.86	313.09	320.11	320.71

Source: FAOSTAT, 2007

Table 2.2. Top world potato producers (2007)

No.	Countries	Quantity (tons)
1	China	72,040,000
2	Russian Fed	36,784,000
3	India	26,280,000
4	USA	20,373,267
5	Ukraine	19,102,300
6	Poland	11,791,072
7	Germany	11,643,769
8	Belarus	8,743,976
9	Netherland	7,200,000
10	France	6,271,000

Source: FAOSTAT, 2007

POTATO PRODUCTION IN THE U.S.

Commercial potato production occurs in every state in the U.S. The total potato production in 2007 was 17.6 million tons grown on about 456,000 hectares with an average yield of 38.7 t/ha. This is significantly lower than the 2006 production which recorded 20 millions tons (FAOSTAT, 2007). States with the greatest productions are Idaho, Washington, Wisconsin, North Dakota, Colorado, Oregon, Maine, Minnesota, California and Michigan which together produce about half of the U.S. potato crop.

Ninety percent of U.S. potatoes are planted in the spring and harvested in the fall. Potatoes harvested in the winter, spring, and summer account for only 10 percent of U.S. potato production (www.usda.gov). About 60 percent of total potato production in the U.S. is processed into frozen products, dehydrated potato products, chips, and starch. Only around 33% of U.S. potato production is consumed fresh while 6 percent is re-used as seed potato. Potato consumption of each American is more than 54 kg/ year (www.potato2008.org).

COLORADO POTATO PRODUCTION

Colorado is one of the largest potato production areas in the U.S. with a ranking of fifth in 2007. Potato production in 2007 was about 994,546 tons covering 25,025 hectares (ha) (www.usda.gov). Over 87 % of those hectares are in the San Luis Valley (SLV) located in south central Colorado. The SLV is a large flat valley that varies from 20 to 50 miles in width and is about 100 miles north to south. Almost all growers in the SLV are using center pivot sprinkler systems to apply irrigation. Irrigation water comes from the abundant snow in the surrounding mountains and recharged ground water wells (www.coloradopotato.org).

Colorado grows over 100 potato cultivars. The vast majority of these fall into three major groups that include russet, red, and yellow. Russet cultivars make up most of the Colorado crop. They are characterized by their even oblong to long shape, russet brown color, net-textured skin and few shallow eyes. Red cultivars, mostly Durango Red and Sangre, are round potatoes with red colored skin and white flesh. Yukon Gold is the fastest-growing yellow variety in SLV with a golden flesh. The SLV grows many other specialty potato varieties such as All Blue, Purple Majesty, and fingerling types (www.coloradopotato.org).

BREEDING POTATO CULTIVARS

The potato has many ploidy levels with a basic haploid number of X=12 chromosomes. These levels include diploid (2n=24), triploids, tetraploids, pentaploids, and hexaploid (Dodds, 1962). The cultivated potatoes are autotetraploid (4n=48), so there are four interchangeable genes at each locus (Ross, 1986). Many of the wild species are diploid, but range up to hexaploid.

Conventional potato breeding methods involve hybridization of parental clones. The large numbers of offspring are then screened to select superior individuals with the desired combination of traits. Single plant selections are then propagated vegetatively and evaluated as clones for relevant agronomic and quality attributes. This breeding approach has developed many elite clones which have become highly successful potato cultivars.

There are some difficulties associated with traditional potato breeding that are related to the autotetraploid nature of the cultivated potato. Genes of interest thus have multiple copies. Cultivars are also highly heterozygous. A true-breeding line produced by self-pollination leads to inbreeding depression. In addition, some potatoes are sterile or partially sterile which limits their use as parents.

Some nontraditional methods have been used in potato breeding. For example, diploids of *S. tuberosum* have been generated through several techniques. The diploid level can then be used in breeding programs to integrate the desired traits into the parents, and then, tetraploids can be reconstituted via protoplast fusion (Poehlman and Sleper, 1995).

Biotechnology also has been used to integrate new genes into potato. Bt potatoes have a modified gene from *Bacillus thuringiensis* which produces a protein that kills the Colorado potato beetle (Reed et al., 2001). Moreover, potatoes with modified starch have also been developed that have only amylopectin rather than the usual mix of amylose and amylopectin starch for nonfood purposes (Wandelt, 2006). A genetically modified potato variety of Ranger Russet has been developed with improved potato storage, french fry aroma, and reduced amount of acrylamide, a carcinogen that is created when starchy foods are baked, roasted, fried, or toasted (Rommens et al., 2006). However, these genetically modified potatoes were rejected by the fry industry as well as foreign and domestic consumers.

POTATO PROPAGATION

The potato "seed" of commerce is not true botanical seed. The potato is usually propagated vegetatively from tuber seedpieces (Everett, 1981).The resulting crop will be genetically identical to the plants that are produced from the tubers. This type of cloning assures genetic uniformity and genetic stability in perpetuity, except for mutation (Shepard et al., 1980). Plants grown from tuber seedpieces will generally be higher yielding, more vigorous, and mature more quickly than plants grown from true seed. The disadvantage of this method of production is that diseases can also be more readily transmitted from one generation to the next via vegetative propagules. Thus it's important that farmers plant only certified tubers which are screened for specific diseases.

True potato seed (TPS) are botanical seed of the potato, and currently are limited in use for commercial and garden culture (Page, 1982, 1985; Park, 1989). TPS has many advantages that include reduced disease transmission, easy storage and shipment, and a decrease of acreage used for seed production (Ross, 1986). However, sterility of some potatoes limits the use of TPS. Furthermore, the time from planting to harvest is much longer with true seed. True seed generally have a high degree of variation.

Northern vigor in potato is very significant in terms of yield and production. Seed tubers produced from plants grown in northern latitudes are more vigorous and higher in yield than those produced from southern latitudes.This dominance was presumed to be due to low levels of seed borne disease and/or some inherent physiological merits of the seed tuber itself. Studies were conducted from 1986 to 1992 at the Department of Horticulture Science, University of Saskatchewan, Canada to determine the basis for "Northern Vigor". The results of these studies indicated that the yield differences were not caused by seed-borne disease but rather some physiological changes in the seed-tuber. Northern potato seed produce vigorous plants with delayed senescence, larger tubers with less variation between hills and thus more even crop development, and higher yield. Temperature during the seed production season is thought to influence the vigor and yield potential. Locations with low diurnal temperature differences (cool days, cool nights) produce reduced vigor seed compared to those produced at locations with strong diurnal temperature (warm days and cool nights) (Wahab, 1992). However, 'Northern

Vigor" may be a misleading term since vigorous seed could also be produced from plants grown in a cooler climate and at high latitude such as the SLV (Holm, 2009).

PHENOLIC COMPOUNDS

Phytochemicals are plant chemicals that may be defined as bioactive non-nutrient compounds in fruits, vegetables, and grains and other plant foods that have been linked to reducing the risk of major chronic diseases (Liu, 2004). Phytochemicals can be categorized into five major groups including phenolics, carotenoid, alkaloids, nitrogen-containing compounds, and organo-sulfur compounds. Phenolic compounds are secondary plant metabolites (Friedman, 1997) that make up one of the most broadly distributed groups of substances in plants. More than 8,000 phenolic structures have been identified (Bravo, 1998; Dewick, 1994). There are two main pathways in which plants synthesize phenolic compounds: the shikimate pathway and the acetate pathway (Bravo, 1998). Natural phenolic compounds vary from simple molecules such as phenolic acids to highly polymerized compounds (tannins). Phenolic compounds are mostly found as conjugates with one or more sugars linked to the hydroxyl group or to an aromatic carbon atom. The attached sugar can be present as a monosaccharide, disaccharide or oligosaccharide with glucose as the most familiar type. However, galactose, rhamnose, xylose, and arabinose are also present in addition to glucuronic and galactaronic acids (Bravo, 1998). Depending on their chemical structures, phenolic compounds can be classified into at least 10 different classes (Table 2.3). Flavonoids, with a basic structure of C_6-C_3-C_6, contain the most important phenolic groups and can be further divided into 13 subclasses (Table. 2.4).

Table 2.3. Main classes of the phenolic compounds (Bravo, 1998).

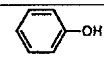
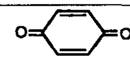
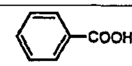
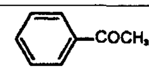
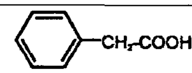
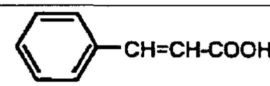
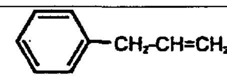
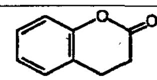
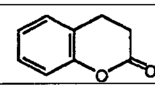
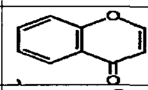
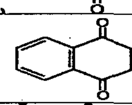
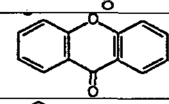
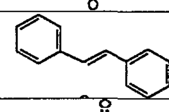
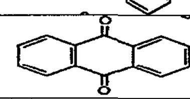
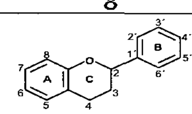
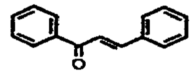
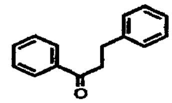
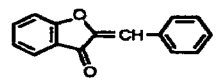
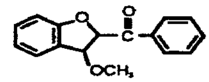
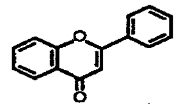
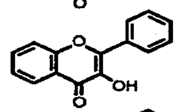
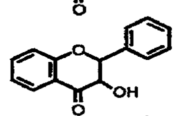
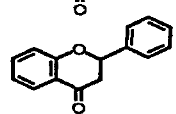
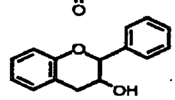
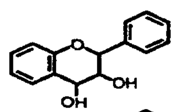
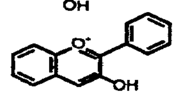
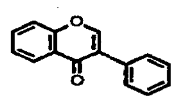
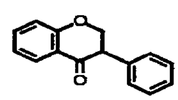
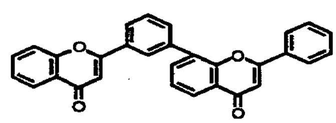
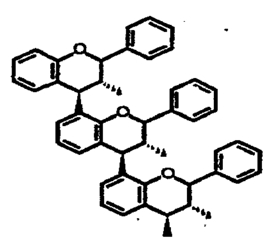
Class	Basic Skeleton	Basic structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acid	C_6-C_3	
Phenylpropenes	C_6-C_3	
Coumarins, isocoumarins	C_6-C_3	 
Chromones	C_6-C_3	
Naftaquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoid	C_6-C_3-C_6	
Lignans, neolignans	$(C_6$-$C_3)_2$	
Lignins	$(C_6$-$C_3)_n$	

Table 2.4. Flavonoids subclasses and their basic structures (Bravo, 1998).

Flavonoid	Basic Structure
Chalcones	
Dihydrochalcones	
Aurones	 
Flavones	
Flavonols	
Dihydroflavonol	
Flavanones	
Flavanol	
Flavandiol or leucoanthocyanidin	
Anthocyanidin	
Isoflavonoids	 
Biflavonoids	
Proanthocyanidins or condensed tannins	

Anthocyanins are types of flavonoids and are the strongest antioxidants among over 150 flavonoids tested (Elliott et al., 1992). They are among the many flavonoids that may be found in potato tubers.

Anthocynins are water-soluble pigments that impart to flowers and other plant parts colors ranging from violet and blue to most shades of red (Harborne and Grayer, 1988). They also act as valuable antioxidants in the human diet offering protection against certain cancers, cardiovascular disease and age-related degenerative diseases (Joseph et al., 1999; Hou et al., 2004; Renaud and De Lorgeril, 1992; Seeram et al., 2004).

Anthocynins occur only in the cytoplasm of land plants and they are derived via the shikimic acid and flavonoid biosynthetic pathways (Chalker-Scott 1999). Phenylalanine is the common precursor of anthocyanins. Figure 2.1 shows the biosynthetic pathway of anthocyanins. These pigments act as powerful antioxidants. They protect the plant from radicals formed by UV light and during metabolic processes. They protect plants from strong UV light by absorbing certain wavelengths, thus limiting DNA damage in the cell. They also protect plants from excess light during periods of high light stress by absorbing blue-green light (Chalker-Scott, 1999; Steyn, et al., 2002).

In plants, anthocyanins occur in glycosylated and acylated forms, usually linked with glucose, galactose, arabinose, rhamnose, xylose, or fructose and various simple phenolic acids. The sugar group is most often found on the 3 or 5 position. The most widespread anthocyanidins in nature are cyanidin, delphinidin, pelargonidin, peonidin, petunidin and malvidin with cyanidin glycosides reported to be present in about 90 percent of all fruits (Awad and Bradford, 2005; Mazza and Miniati., 1993).

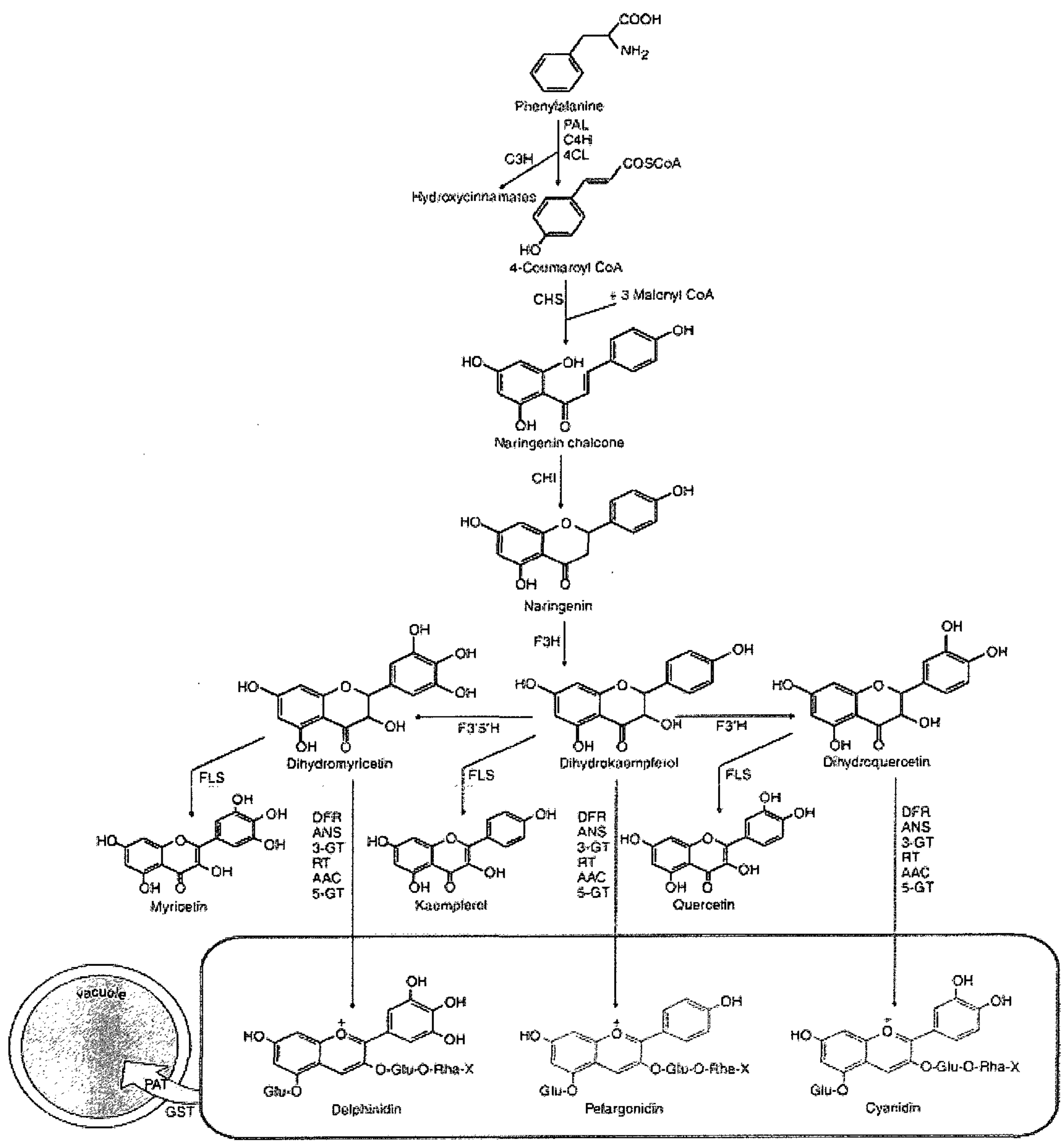


Figure 2.1. Biosynthetic pathway of anthocyanins (Butelli et al., 2008).PAL, phenylalanine ammonia lyase; 4CL, 4-coumarate:coenzyme A ligase; C4H, cinnamate 4-hydroxylase; C3H, 4-coumarate 3-hydroxylase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone-3-hydroxylase; F3′H, flavonoid-3′-hydroxylase; F3′5′H, flavonoid-3′5′-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; ANS, anthocyanidin synthase; 3-GT, flavonoid 3-O glucosyltransferase; RT, flavonoid 3-O-glucoside-rhamnosyltransferase; AAC, anthocyanin acyltransferase; 5-GT, flavonoid-5-glucosyltransferase; GST, glutathione S-transferase; PAT, putative anthocyanin transporter.

POTATO PIGMENTS

Tuber flesh color is determined by the presence of two classes of compounds. Anthocyanins pigments impart red, blue, and purple flesh color, whereas carotenoids, predominantly xanthopylls, produce yellow flesh color (Tevini and Schonecker, 1986).

Brown et al. (2003) have studied the breeding behavior of clones containing high levels of anthocyanins. Generally, red and purple-fleshed clones were accompanied by red and purple skin, respectively. Red flesh has been found to vary from partial pigmentation to complete pigmentation (pigment present in all tuber tissues). The proportion of completely red-fleshed progenies in red x red parents was three times greater (14.5%) than the red x white and reciprocal (4.1%) (Brown et al., 2003). Red-fleshed progeny from two red parents was about two times greater than red-fleshed progeny produced by crossing red x white parents and reciprocals (Brown et al., 2003). The complete distribution of anthocyanins in pigmented flesh may be genetically complex. However, presence and absence of anthocyanins is under monogenic control (Brown et al., 2003; De Jong, 1991).

A series of single genes controls presence and absence of red and blue pigment (Brown et al., 2003). Different genetic systems have been identified to control pigment expression for cultivated diploid vs cultivated tetraploid potatoes (Dodds and Long 1955, 1956; Lunden 1960). However, De Jong, (1991) and Van Eck et al. (1994) have disputed that the genes appear to be syntenic and should be considered as belonging to the same genome (De Jong, 1991; Van Eck et al. 1994). The symbol D denotes a single gene controlling synthesis of red pigment, located on chromosome 2; the symbol P stands for a single gene on chromosome 11 controlling blue pigment synthesis; while *I*, of undetermined location, epistatically controls presence or absence of tuber skin and flesh pigmentation even when P and D are present. Gebhardt et al. (1989) reported a locus controlling purple skin color, Psc, on chromosome 4. The single gene P*f*, linked to *I*, determines whether pigment is present beyond the periderm in the interior tissues of the tuber (De Jong, 1987, 1991; Van Eck et al., 1994).

Diverse kinds of acylated anthocyanidin glycosides determine the pigments (Harborne, 1960; Rodriguez-Saona et al., 1998). HPLC analysis of anthocyanin extracts indicated that red-fleshed clones contained mostly acylated glycosides of pelargonidin while the acylated glycosides of petunidin and peonidin were mostly found in purple-fleshed clones (Brown et al., 2003). Acylation of anthocyanins is controlled by the Ac gene. Acylated anthocyanins are present in tetraploid potato cultivars while both acylated and non-acylated anthocyanins are present in diploid cultivars (Swaminathan and Howard 1953).

White vs yellow flesh is thought to be under single gene control, (Bonierbale et al., 1988; Gebhardt et al., 1989). White and yellow-fleshed potatoes have similar composition of carotenoids; however, the yellow color of the latter group is due to a higher concentration of certain xanthophylls (Brown et al. 1993; Gross 1991). Potatoes have mostly xanthophyll type carotenoids, including predominantly lutein, violaxanthin and zeaxanthin (Brown et al., 2003; Fossen and Andersen, 2000; Fossen et al., 2003; Mazza and Miniati., 1993; Rodriguez-Saona et al., 1998).

FREE RADICALS

Free radicals are highly reactive and unstable due to an unpaired electron in the outer (valence) shell of the molecule. Radicals are constantly generated by normal metabolism, as well as from environmental stress such as exercise, light, excess heat or cold, radiation, smoke, pollutants, and even some food sources (Lister, 2003).

There are many types of radicals, but those of most concern in biological systems are derived from oxygen. These radicals which are known as reactive oxygen species (ROS) include the superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\cdot}$), peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl) (Halliwell and Gutteridge, 1999). The most important reactive oxygen species in the human body are the superoxide ion, hydroxyl and hydrogen peroxide (Smythies, 1998). Even though oxidation reactions (which are the chemical transfers of electrons from one substance to an oxidizing agent) are vital for life, they can cause damage to living cells.

Overproduction of radicals may increase the level of free radicals or reactive oxygen species beyond the level that the antioxidant defense system can handle causing oxidative stress (Finkel and Holbrook, 2000). These radicals may react with cellular components such as lipids, proteins and nucleic acids. Lipids may be especially prone to damage due to a process known as lipid peroxidation. When radicals react with polyunsaturated fatty acids in cellular membrane phospholipids, the fatty acids themselves become radicals and can react with other fatty acids, starting a chain reaction that may cause severe damage to the living organism. Free radicals have been found to be involved in aging, several forms of cancer, heart disease, cardiovascular disease, Alzheimer's disease, inflammatory conditions, rheumatism, insulin resistance and cataracts (Halliwell and Gutteridge, 1999; Houstis et al., 2006). Increasing antioxidant intake through consumption of fruits and vegetables containing high level of phytochemicals is believed to decrease the risk of these diseases (Berger, 2005).

However, free radicals have beneficial effects that cannot be ignored. It has become obvious that ROS play an important role as signals in plants controlling important biological processes like growth, development, response to biotic and abiotic environmental stimuli, and programmed cell death (Bailey-Serres and Mittler, 2006). In recent years, new sources of ROS have been identified in plants, including NADPH oxidases, amine oxidases, and cell wall-bound peroxidases. This has led to the demonstration that plant cells can start and most likely increase ROS production for the purpose of signaling. The short-lived nature of $O_2^{\cdot-}$ and other ROS makes them ideal signaling molecules or for carrying out other localized biochemical activities (Halliwell and Gutteridge, 1999; Levine et al., 1994).This demonstrates that ROS mediated signaling is managed by a delicate balance between production and scavenging. For example, there is evidence that the change in ROS in the particular cases of hypoxia and anoxia in plants are mediated by a group of compounds called the RHO-like GTPases (ROP). Activation of ROP after treatment with a low concentration of O_2 induces H_2O_2 production and this seems necessary for low O_2 signaling (Baxter-Burrell et al., 2002, 2003; Bailey-Serres and Chang, 2005).

ANTIOXIDANTS

Antioxidants are groups of compounds that quench free radicals and delay oxidation of an oxidizable substrate. They protect living cells against the harmful effects of free radicals and other reactive oxygen species (ROS). Free radicals are molecules with one or more unpaired electrons (and therefore reactive) that have a strong tendency for extra electrons. In order to get extra electrons they can react with vital biological molecules such as nucleic acid, protein, and fat causing cell damage (Lister, 1999; Lister, 2003). The antioxidants interfere with oxidation processes through chain-braking reaction processes (primary antioxidant) or through scavenging of free radicals (secondary antioxidants). Depending on the structure, they have the ability to act as antioxidants in a broad range of chemical oxidation systems due to the ease of donation of a hydrogen atom from an aromatic hydroxyl group to a free radical. Also, the aromatic compound is able to support an unpaired electron due to delocalization around the π - electron system (Duthie et al., 2000). Consequently, free radicals prefer to combine with these antioxidants instead of lipids, DNA, RNA, and proteins.

There are several kinds of antioxidants and antioxidant defense mechanisms, some of which are produced in the body while others come from the food that we eat.

The human body is able to produce some antioxidant components such as albumin and enzymes like superoxide dismutase (SOD) and glutathione peroxidase to reduce the harmful effect of the free radicals (Lister, 2003). However, there are times when the formation of free radicals exceeds the capacity of the body's natural defenses leading to oxidative stress. The consumption of antioxidants in our food thus offers the potential to reduce oxidative stress. The phytochemicals include carotenoids, flavonoids and other phenolics besides vitamins C (ascorbic acid) and E (tocopherols and tocotrienols) which are also part of the antioxidant defense system (Lister, 2003; Lurie, 2003). These phytochemical compounds may also be antibacterial or have a hormonal action which may stimulate the immune system or modulate enzyme activity (Lampe, 1999).

Antioxidant compounds are thought to play an important role in plant disease resistance. For example, resistance to soft rot caused by *Pectobacterium carotovorum* (Pc) were compared between 10 purple-fleshed potato cultivars and 10 white/yellow-fleshed potato cultivars (Wegener and Jansen, 2007). The results indicated that average resistance to soft rot in colored potato cultivars was better than white and yellow fleshed potato cultivars. The presence of anthocyanins, higher concentrations of soluble phenols and elevated polyphenol oxidase activity in tuber tissue were associated with the greater resistance of colored potatoes. Significant correlation has been found among these three components and the extent of rotting caused by Pc. On the other hand, there was no big difference between colored and white/yellow-fleshed potatoes in phenylalanine ammonia-lyase and peroxidase activity. In addition, no significant differences were found in the concentration of dry matter, starch, crude protein and glycoalkaloids. The authors concluded that the total soluble phenols and anthocyanins play a crucial role in resistance of colored potato cultivars.

ANALYSIS OF ANTIOXIDANT CAPACITY

Antioxidant activity is the radical absorbance capacity of an antioxidant. There are many assays that have been reported to measure antioxidant activity. Based on the chemical reactions involved, these assays can be classified into two major types:

1) Assays based on hydrogen atom transfer (HAT) reaction. These assays include oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), and crocin bleaching assays. 2) Assays based on single electron transfer (ET) reaction which include Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), Cu (II) complex antioxidant potential, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid (ABTS). ET-based assays determine the capacity of an antioxidant to reduce an oxidant, which then results in the reductant showing a color change. In addition, other assays such as superoxide, hydrogen peroxide, the hydroxyl radical, singlet oxygen and peroxynitrite scavenging capacity are utilized to measure the ability of antioxidants to scavenge respective radicals (Huang et al., 2005; Prior et al., 2005).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) are among the most popular methods that have been developed to estimate the radical-scavenging activity. In addition, Folin-Ciocateu reagent adapted from Spanos and Wrolstad (1990) is used to estimate total phenolic compounds. This assay is based on the color reaction of phenolics with phosphomolybdic-phosphotungstic acid reagent such as Folin-Ciocateu reagent. Total phenolics quantification by the Folin-Ciocateu method is based on the number of phenolic groups or on other potential oxidizable groups present in the sample (Singleton et al., 1999).

POTATO ANTIOXIDANTS

Consumption of fruits and vegetables containing high levels of polyphenols can offer protection against chronic diseases such as cancer and heart diseases (Halliwell et al., 1992). There is increasing evidence suggesting that many human diseases such as heart disease, cancer, inflammation, arthritis, immune system decline, brain dysfunction and cataracts are a result of cellular damage by free radicals. Antioxidants in our diet could play an important role in prevention of these diseases (Buring and Hennekens., 1997; Cao et al., 1998). Most epidemiological studies suggest that diets rich in fruits and vegetables reduce cancer risk, while others disagree. Approximately 200 epidemiological studies have been reviewed by Block et al. (1992). These studies examined the relationship between intake of fruit and vegetables and cancer of the lung, breast, pancreas, stomach, esophagus, ovary, cervix, oral cavity, and bladder. A total of 122 of the 156 dietary studies concluded that the risk of cancer was 2 fold higher in people who had low intake of fruits and vegetables compared to those with high intake (Block et al., 1992).

Phenolics, in fruits and vegetables, have received a great deal of attention because of their antioxidant activity (Chinnici et al., 2004). Al-Saikhan et al. (1995) studied antioxidant properties in different potato genotypes. They compared the antioxidant activity between potato and other vegetables such as broccoli, onion, carrot and bell peppers. Antioxidant activity in potato was reported to be higher than all except broccoli. Different antioxidant components were found in potato cultivars including chlorogenic acids, glutathione, quercetin, and patain. Liu et al. (2003) indicated that the patatin family of glycoproteins can be found only in potato. Purple- and red-flesh potatoes have been evaluated for their total anthocyanins, total phenolic and antioxidant capacity (Reyes et al., 2005). The concentration at the stem-end of the tuber was higher. The total anthocyanins and total phenolic concentrations in potato peel (1-2 mm thick) were 0.9 to 1.6 fold higher than potato flesh. The overall contribution of the peel to total phenolic and total anthocyanins of a potato slice was approximately 20%.

Antioxidant properties of cultivars and selections from the Colorado potato breeding program have been investigated for the major phenolic compounds, anthocyanins, and glyco-alkaloid content (Stushnoff et al., 2008). They reported that the major compounds in the potato extracts were the glyco-alkaloids, α-solanine and α-chaconine, three chlorogenic acid isomers, and a range of anthocyanins that were only found in the pigmented cultivars. Concentration of chlorogenic acid isomers of pigmented cultivars 'Purple Majesty' and 'Mountain Rose' was significantly higher than non- pigmented cultivars. Among non-pigmented cultivars, 'Rio Grande Russet' had the highest chlorogenic acid concentration.

Recent biochemical studies have revealed several anti-carcinogenic properties of chlorogenic acid such as the inhibition of the proliferation of A549 human cancer cells *in vitro* (Feng et al., 2005). They reported that pretreatment of JB6 mouse epidermal cells with chlorogenic acid blocked UV-B or TPA-induced transactivation of AP-1 and NF-KB, all known to be carcinogenesis inducers. Chlorogenic acid is one of the most plentiful antioxidant phenolic compounds in human diets and potato is considered to be a great source of it.

Biosynthetically, chlorogenic acid is derived from phenylalanine (Friedman, 1997) which is also a precursor for flavonol and anthocyanins biosynthesis in potatoes (Dixon and Paiva, 1995; Van Eldik et al., 1997). Phenylalanine is formed via the shikimate pathway starting from phosphoenol pyruvate and erythrose 4-phosphate. The biosynthetic alteration of phenylalanine to chlorogenic acid is illustrated in Figure 2.2.

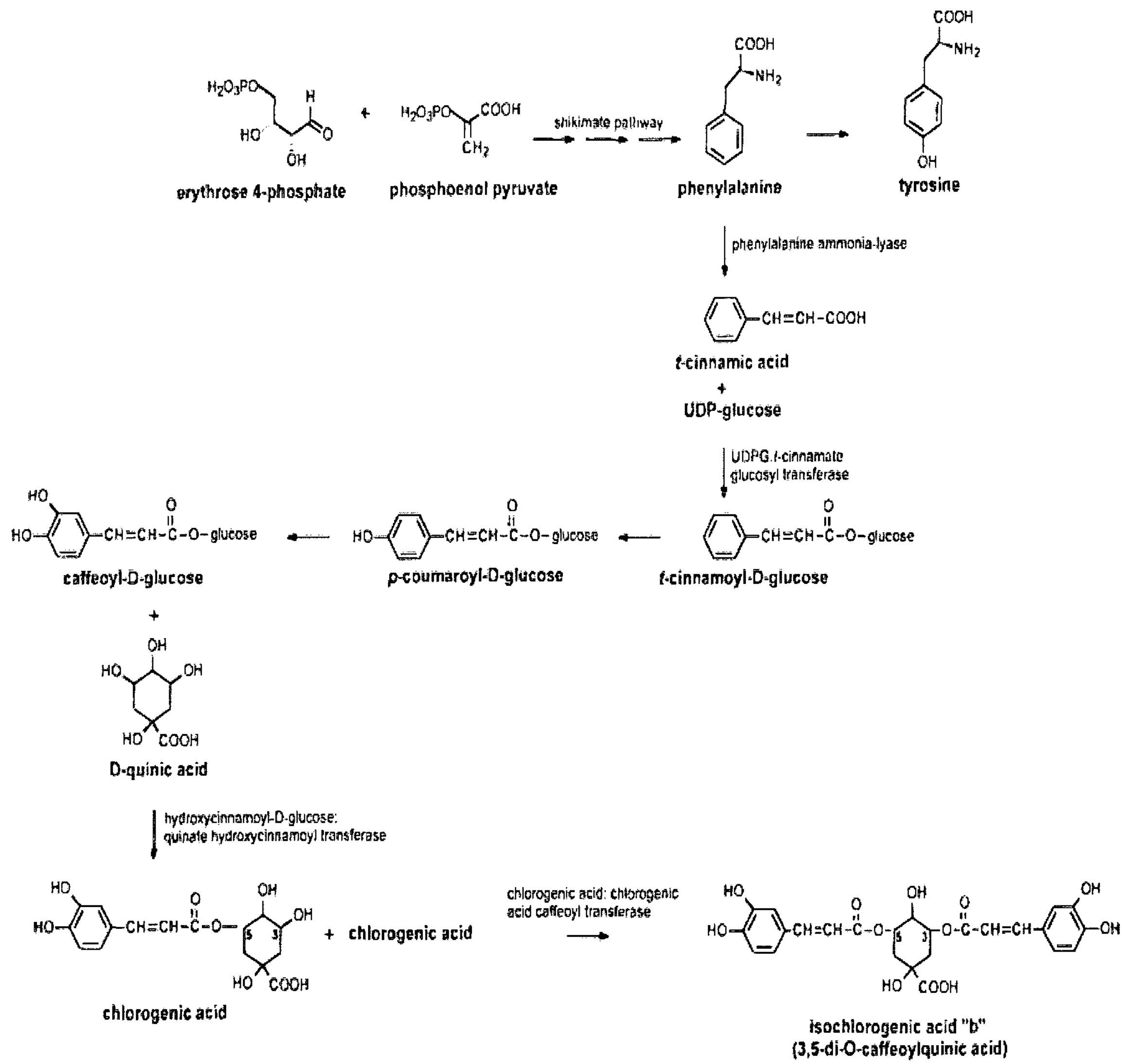


Figure 2.2. Biosynthetic transformations of phenylalanine to chlorogenic acid (Friedman, 1997).

Tubers with variegated flesh color from two Colorado selections (CO97216-3P/PW and CO97277-2P/PW) have been used to compare the content of total phenolics in the pigmented sector with the non-pigmented sector (Stushnoff et al., 2008). Total phenolic compounds in pigmented sectors were 3 to 4 times higher. In addition, vitamin C in the pigmented sector was slightly higher. These results suggest that the major antioxidant properties of these variegated genotypes are derived from phenolics, anthocyanins, and associated compounds.

The amount and type of anthocyanins have been investigated in the red cultivar 'Montain Rose' and the purple cultivar 'Purple Majesty' and found to be different. In 'Mountain Rose, five pelargonidin glycosides and peonidin were identified while 'Purple Majesty' had five petunidin glycosides plus a single glycoside of each of the delphinidin, petunidin and malvidin aglycone (Stushnoff et al., 2008).

EFFECT OF ENVIRONMENT ON CHEMICAL COMPOSITION OF THE POTATO

The concentrations of chemical compounds in potato are certainly not constant and are affected by many environmental factors.

Several environmental factors may influence the biosynthesis of anthocyanins (ACY), total phenolics content (TPC), and potato yields (Vayda, 1994; Dixon and Paiva, 1995; Chalker-Scott, 1999). For example, location, light, and temperatures have been associated with red radish anthocyanin biosynthesis (Giusti et al., 1998). Cool temperatures and long days have been associated with increased growth and yields of potatoes (Vayda, 1994). Variations in content and yield of ACY and TPC were studied during development of purple- and red-flesh potato cultivars grown in Texas and Colorado (Reyes et al., 2004). In both locations, the ACY and TPC decreased with tuber growth and maturity, while tuber weight and total yield increased. The ACY and TPC of potato tubers is enhanced when tubers are grown in a location with higher light intensities followed by lower temperatures. Longer days and cooler temperatures in Colorado favored a 2.5- and 1.4-times higher ACY and TPC, respectively, than in Texas-grown tubers. In addition, harvesting potatoes at later maturity stages maximizes total yield, ACY and TPC yield, and reduced glycoalkaloid content.

A study in the Czech Republic evaluated the effect of location, years, and fertilization on total phenolics in potato (Hamouz et al., 2005, 2006, and 2007). Different potato cultivars including yellow and purple-fleshed varieties were grown in four different locations (Přerov Nad Labem, Praha-Suchdol, Lípa, and Stachy) in the Czech Republic. These locations are different in altitude, average annual temperatures and total annual precipitation. Different site conditions significantly affected TPC in tubers. In both experimental years, the highest TPC was determined to be at the Stachy location. The Stachy area is characterized by the highest altitude, the lowest average year temperature and the highest total precipitation in comparison to the other locations.

The impact of environment on antioxidant status has been investigated with seven potato cultivars grown at 4 location sites in Colorado (Al-Obeidani, 2005). He concluded that the variation of total phenolic content was due more to cultivar than locations, even though locations resulted in highly significant differences. He also evaluated the effect of different potato cooking methods (microwave, boil, and bake) on antioxidant stability of sixteen cultivars. He found that all of these cooking methods caused a significant decrease in total phenolics and ascorbic acid, with baking being the most destructive method.

Induction of anthocyanins by cold temperatures has received less attention than light induction. Low temperature has been shown to induce anthocyanin synthesis in seedlings of arabidopsis (Graham, T. L., 1998; Leyva et al., 1995), *Zea mays* (Christie et al., 1994), and sorghum (Shichijo et al., 1993). Christie et al. (1994) suggests that the anthocyanin biosynthetic pathway involves cold-regulated genes except that very cold temperatures may destroy the biosynthetic capability. McKown et al. (1996) have the same opinion that there is some association between anthocyanin biosynthesis and freezing tolerance, in either the synthetic or regulatory pathways leading to both. However, low temperatures in the absence of either visible light or UV-B prevent anthocyanin biosynthesis (Janda et al., 1996; Oren-Shamir and Levi-Nissim, 1997). The mechanism for cold induction of anthocyanins and the role of light suggest separate or perhaps overlapping pathways (Mol et al., 1996).

Expression regulation of anthocyanin biosynthetic genes, responsible for red coloration of apple (Malus x domestica), are believed to be controlled by MYB transcription factors (Ban et al., 2007). The MYB transcription factor gene (MdMYBA) was isolated from apple skin and characterized. The expression of the gene was dependent on tissue and cultivar/species. It was also found to be induced by UV-B irradiation, low temperature treatment and cauliflower mosaic virus.

Lillo et al. (2008) observed that the content of flavonoids increases in response to nitrogen and phosphorus depletion in plants which induce the gene expression of the falvonoid pathway. On the other hand, flavonol synthase is enhanced by high light intensity and sucrose, not mineral depletion.

STORAGE INFLUENCE ON TOTAL PHENOLIC CONTENT

The potato tuber is a living organism that continues to undergo progressive changes in storage in much the same way as in the field. However, these changes occur more slowly in storage than in the field. Many physical, biochemical and physiological changes have been reported during tuber storage (Knowles and Knowles, 1990; Pang and Scanlon, 1996). These changes are influenced by many factors such as the duration, temperature, humidity, and atmospheric conditions of storage (Butchbaker et al., 1967; Spychalla and Desborough, 1990). In addition, the environmental and cultural conditions experienced by the tubers during the growing season affect the degree of these changes (Boucaron et al., 1990).

Duration of storage and temperature are two of the important factors that affect anthocyanin level in vegetables and fruits throughout storage. Lewis et al. (1999) have reported that anthocyanin concentrations in periderm of 'Desiree' and 'Arran Victory' tubers increased when stored at 4 °C, but decreased in storage at 10 °C or higher temperature. They hypothesized that there was an increase in anthocyanin synthesis caused by an increase in sugar concentration during cold storage. Alenazi, (2005) conducted a study to estimate the influence of different storage temperatures and storage time on anthocyanin concentration in different potato genotypes. Tubers of seven genotypes were stored at 4 °C and 10 °C for 0, 4, 6, 8, 10, 12, 16, and 20 weeks. The anthocyanins present were estimated in freeze-dried and fresh samples. He reported an increase in anthocyanins concentration with time at 4 °C and 10 °C in both types of samples (freeze-dried and fresh samples). In addition, he reported that anthocynin levels of tubers stored at 4 °C were higher than tubers stored at 10 °C.

Rivero et al. (2003) evaluated the effect of storage (at 12 °C and 90% humidity) on moisture, starch, amylose, ash, fructose, sucrose, glucose and vitamin C in five cultivars of Tenerife potatoes. 'Negra' had a greater reduction in levels of compounds in storage while 'Botnia' and 'Colorada' maintained the highest levels. There was a significant decrease of moisture observed after 6 weeks of storage. Starch (dry basis) progressively decreased over time of storage while reducing sugars increased. A significant decrease of Vitamin C was also observed during storage. After 20 weeks, tubers lost more than 50% of there original vitamin C content. During the first 6 weeks of storage there was an increase in the amylose/amylopectin ratio.

STATEMENT OF PURPOSE

Potatoes contain significant levels of antioxidant compounds. The health properties of these antioxidants are encouraging to researchers, especially breeders to increase the phenoilc compounds in plant tissue. The potential to increase antioxidants in potato, particularly phenolics, through breeding efforts has not been extensively investigated. However, conventional plant breeding holds significant promise to improve the antioxidant and nutrient levels in various fruits and vegetables (Kalt and Kushad, 2000). The objectives of this study were to: 1) Determine the TPC of specific families derived from crosses of clones grown in the San Luis Valley (SLV), CO. 2) Study the potential use of MiniScan® XE Plus to assay pigment levels as a method to select high TPC clones. 3) Study the correlation between TPC as determined in potatoes grown under greenhouse conditions with those grown in the field.

REFERENCES

Al-Obeidani, J.S., 2005. Antioxidant Properties of Colorado Grown Ppotatoes. PhD Dissertation. Colorado State University, Fort Collins. 118p.

Al-Saikhan, M.S., L.R. Howard, and J.C. Miller, Jr., 1995. Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum* L.). J. Food Sci. 60:341-348.

Alenazi, M. M., H.G. Hughes, C. Stushnoff, and D.G. Holm. 2006. Influence of storage temperature and time in storage on pigment content of potato (*Solanum tuberosum* L.) (Abstract). Hort. Sci. 41:1014.

Awad, A.B. and P.G. Bradford. 2005. Nutrition and Cancer Prevention. Boca Raton, Fl. CRC, Taylor Francis. 617p.

Bailey-Serres, J. and R. Chang, 2005. Sensing and signalling in response to oxygen deprivation in plants and other organisms. Ann. Bot. 96:507-518.

Bailey-Serres, J. and R. Mittler. 2006. The roles of reactive oxygen species in plant cells. Plant Physiol. 141:311-311.

Ban, Y., C. Honda, Y. Hatsuyama, M. Igarashi, H. Bessho, and T. Moriguchi. 2007. Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red coloration in apple skin. Plant Cell Physiol. 48:958-970.

Baxter-Burrell, A., Z. Yang, P.S. Springer, and J. Bailey-Serres. 2002. RopGAP4-dependent Rop GTPase rheostat control of Arabidopsis oxygen deprivation tolerance. Sci. 296:2026-2028.

Baxter-Burrell, A., R. Chang, P. Springer, and J. Bailey-Serres. 2003. Gene and enhancer trap transposable elements reveal oxygen deprivation-regulated genes and their complex patterns of expression in Arabidopsis. Ann. Bot. 91:129-141.

Berger, M.M. 2005. Can oxidative damage be treated nutritionally? Clin. Nutr. 24: 172-183.

Block, G., B. Patterson, and A. Subar. 1992. Fruit, vegetables and cancer prevention: A review of the epidemiological evidence. Nutr. Cancer. 18:1-29.

Bonierbale, M.W., R.L. Plaisted and S.D. Tanksley. 1988. RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095-1103.

Boucaron, C., L. Collett, and P.V. Zaag. 1990. Influence of growing and storage conditions on the subsequent performance of *Solanum* seed potatoes. Trop. Agric. 67:85-89.

Bravo, L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 1998, 56:317-333.

Brown, C.R., C.G. Edwards, C.P. Yang, and B.B. Dean. 1993. Orange flesh trait in potato: Inheritance and carotenoid content. J. Am. Soc. Hort. Sci. 118:145-150.

Brown C.R., R. Wrolstad, R. Durst, C. P. Yang, and B. Clevidence. 2003. Breeding studies in potatoes containing high concentrations of anthocyanins. Am. J. Potato Res. 80:241-249.

Buring, J.E., and C.H. Hennekens. 1997. Antioxidant vitamins and cardiovascular disease. Nutr. Rev. 55:s53-s60.

Butchbaker, A.F., D.C. Nelson, and R. Shaw. 1967. Controlled-atmosphere storage of potatoes.Trans. Am. Soc. Agric. Eng. 10:534-538.

Burton, W.G. 1969. Potato. In: Encyclopaedia Britannica, Vol. 18, pp. 95-134. Benton, Chicago et alibi. 1197p.

Burton, W.G., 1989. The Potato. 3rd ed. Longman Scientific Technical. New York, USA. 742p.

Butelli, E., L. Titta, M. Giorgio, H.P. Mock, A. Martos, S. Peterek, E.G. Sachijlen, R.D. Hall, A.G. Bovy, J. Luo, and C. Martin. 2008. Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nature Biol. 26:1301-1308.http://www.nature.com/nbt/journal/v26/n11/pdf/nbt.1506.pdf

Cao, G., Booth, S.L, and R.L. Prior. 1998. Increase in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. Am. J. Clin. Nutr. 68:1081-1087

Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70:1-9.

Chinnici, F., A. Bendini, A. Gaiani, and C. Riponi. 2004. Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. J. Agric. Food Chem. 52:4684-4689.

Christie, P.J., M.R. Alfenito, and V. Walbot. 1994. Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: Enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. Planta 194:541-549.

De Jong, H. 1987. Inheritance of pigmented tuber flesh in cultivated diploid potatoes. Am. Pot. J. 64:337-343.

De Jong, H. 1991. Inheritance of anthocyanin pigmentation in the cultivated potato: A critical review. Am. Pot. J. 68:585-593.

Dewick, P.M. 1994. Isoflavonoids. In J.B. Harborne, ed., The Flavonoids: Advances in research since 1986. Chapman and Hall, London, pp. 117-238

Dixon, R.A., and N.L. Paiva. 1995. Stress-induced phenylpropanoid metabolism. Plant cell 7:1085-1097.

Dodds, K.S., and D.H. Long. 1955.The inheritance of colour in diploid potatoes. I. Types of anthocyanidins and their genetic loci. J. Genet. 53:136–149

Dodds, K.S., and D.H. Long. 1956.The inheritance of colour in diploid potatoes. II. Three-factor linkage group. J. Genet. 54:27–41

Dodds, K.S. 1962. Classification of cultivated potatoes. In: D.S. Correll (ed.), The Potato and Its Wild Relatives. Contributions from Texas Res. Found., Bot. Studies 4:517-539.

Duthie, G.G., S.J. Duthie, and J. A.M. Kyle. 2000. Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. Nutr Res Rev 13:79-106.

Elliott, A.J., S.A. Scheiber, C. Thomas and R.S. Pardini. 1992. Inhibition of glutathione reductase by flavonoids. A structure-activity study. Biochem Pharmacol 44: 1603-1608.

Everett, T.H. 1981. The New York Botanical Garden Illustrated Encyclopedia of Horticulture, 10 vols. Garland Publishing, Inc., New York.

FAO, 2007. Food and Agriculture Organization of the United Nations (FAO). http://faostat.fao.org/.

Feng, R.T., Y. Lu, L.L. Bowman, Y. Qian, V. Castranova, and M. Ding. 2005. Inhibition of ap-1, Nf-Kb and MAPKS and induction of phase 2 detoxifying enzyme activity by chlorogenic acid. 21June. http://www.jbc.org/cgi/reprint/M503347200v1.pdf

Finkel, T., and N.J. Holbrook. 2000. Oxidants, oxidative stress and the biology of ageing. Nature. 408:239-247.

Fossen, T., and O.M. Andersen. 2000. Anthocyanins from tubers and shoots of the purple potato, *Solanum tuberosum*. J. Hort. Sci. Biotech. 75:360-363.

Fossen, T., D.O. Ovstedal, R. Slimestad, and O. Andersen. 2003. Anthocyanins from a

Norwegian potato cultivar. Food Chem. 81:433–437.

Friedman, M. 1997. Chemistry, biochemistry, and dietary role of potato polyphenols. A review. J. Agric. Food Chem. 45:1523-1540.

Gebhardt, C., E. Ritter, T. Debener, U. Schachtschabel, B. Walkemeier, H. Uhrig, and F. Salamini. 1989. RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet. 78:65-75.

Giusti, M.M., L.E. Rodriguez-Saona, J.R. Baggett, G.L. Reed, R.W. Durst, and R.E. Wrolstad. 1998. Anthocyanin pigment composition of red radish cultivars as potential food colorants. J. Food Sci. 63:219-224.

Graham, T. L. 1998. Flavonoid and flavonol glycoside metabolism in Arabidopsis. Plant Physiol Bioch. 36:135-144.

Gross, J. 1991. Pigments in Vegetables: Chlorophylls and Carotenoid.Van Nostrand Reinhold, New York. 351p.

Halliwell, B. and J.M.C. Gutteridge. 1999. Free radicals in biology and medicine. 3rd . ed. Oxford University Press. New York. 936p.

Halliwell, B., J.M.C. Gutteridge, and C.E Cross. 1992. Free radicals, antioxidants, and human disease: Where are we now? J. Lab. Clin. Med. 119:598–620.

Hamouz, K., J. Lachman, P. Dvořák, and V. Pivec. 2005. The effect of ecological growing on the potatoes yield and quality. Plant Soil Environ. 51:397–402.

Hamouz, K., J. Lachman, P. Dvořak , and V. Pivec. 2006. The effect of site conditions, variety and fertilizationon the content of polyphenols in potato tubers. Plant Soil Environ. 52:407–412.

Hamouz, K., J. Lachman, J. Čepl, P. Dvořák, V. Pivec, and M. Prášilová. 2007. Site conditions and genotype influence polyphenol content in potatoes. Hort. Sci. 34:132–137.

Harborne, J.B. 1960. Plant polyphenols: I. Anthocyanin production in the cultivated potato. Biochem. J. 74:262-269.

Harborne, J.B. and R.J. Grayer. 1988. The anthocyanins. In Harborne, J. B., ed. The Flavonoids: Advances in Research Since 1980. pp. 1-20. Chapman and Hall, London.

Hawkes, K.J. 1992. History of the potato. In: The Potato Crop. P.M. Harris (ed). Chapman and Hall, London, pp 1-13.

Holm, D. 2009. Personal communication with Dr. David Holm on January 8, 2009. http://www.colostate.edu/depts/SLVRC/pers_dholm.htm.

Hou, D.X., M. Fujii, N. Terahara, and M. Yoshimoto. 2004. Molecular mechanisms behind the chemopreventive effects of anthocyanidins. Biomed. Biotechnol. 5:321–325.

Houstis, N., E.D. Rosen, and E.S. Lander. 2006. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 440:944-948.

Huang, D., B. Ou, and R.L. Prior. 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53:1841-1856.

Janda, T., G. Szalai, and E. Paldi. 1996. Chlorophyll fluorescence and anthocyanin content in chilled maize plants after return to a non-chilling temperature under various irradiances. Biol. Plant. 38:625-627

Joseph, J.A., B. Shukitt-Hale, N.A. Denisova, D. Bielinski , A. Martin, J.J. McEwen, and P.C. Bickford. 1999. Reversals of age-related declines in neuronal signal transduction, cognitive, motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J. Neurosci. 19:8114–8121.

Kalt, W. and M.M. Kushad. 2000. The role of oxidative stress and antioxidants in plant and human health? Introduction to the colloquium. Hort. Sci. 35:572-574.

Knowles, N.R. and L.O. Knowles. 1990. Changes in fatty acid composition of phospholipids from different ages of seed-tubers during sprouting. Ann. Bot. 65:217-223.

Lampe, J.W. 1999. Health effects of vegetables and fruit: Assessing mechanisms of action in human experimental studies. Am. J. Clin. Nutr. 70: 475S-490S.

Levine, A., R. Tenhaken, R. Dixon, and C. Lamb. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79:583–593.

Lewis, C.E., J.R.L. Walker, and J.E. Lancaster. 1999. Changes in anthocyanin, flavonoid and phenolic acid concentrations during development and storage of coloured potato (*Solanum tuberosum* L.) tubers. J. Sci. Food Agric. 79:311-316.

Leyva, A., J.A. Jarillo, J. Salinas, and J.M. Martinez-Zapater. 1995. Low temperature induces the accumulation of phenylalanine ammonia-lyase and chalcone synthase mRNAs of Arabidopsis thaliana in a light-dependent manner. Plant Physiol. 108:39-46.

Lillo, C., U.S. Lea, and P. Ruoff. 2008. Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway. Plant Cell Environ. 31:587-601.

Lister, C.E. 1999. The medicine of life. Vitamins and other bioactive food components. Food Technol. 34:18-20, 35.

Lister, C.E. 2003. Antioxidants a Health Revolution. NewZeland Institute for Crop Food Research Limited, Private Bag 4704, Christchurch, New Zeland. 98p.

Liu, R.H. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J. Nutrition. 134:3479S-3485S.

Liu, Y., C. Han, M. Lee, F. Hsu, and E. How. 2003. Patatin, the tuber storage protein of potato (*Solanum tuberosum* L.), exhibits antioxidant activity in vitro. J. Agric. Food Chem. 51:4389-4393

Lunden, A.P. 1960. Some more evidence of autotetraploid inheritance in the potato (*Solanum tuberosum* L.). Euphytica 9:225-234.

Lurie, S. 2003. Antioxidants. In: Hodges, D.M. (Ed) Postharvest Oxidative Stress in Horticultural crops, pp. 131-150. New York: Food Products Press.

Mazza, G. and E. Miniati. 1993. Anthocyanins in Fruits, Vegetables, and Grains. CRC Press: Boca Raton, FL. 362p.

McKown, R., G. Kuroki, and G. Warren. 1996. Cold responses of Arabidopsis mutants impaired in freezing tolerance. J. Exp. Bot. 47:1919-1925.

Mol, J., G. Jenkins, E. Schafer, and D. Weiss. 1996. Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. Crit. Rev. Plant Sci. 15:525-557.

Oren-Shamir, M. and A. Levi-Nissim. 1997. UV-light effect on the leaf pigmentation of *Cotinus coggygria* `Royal Purple.' Sci. Hort. 71:59-66.

Page, O.T. 1982. Utilization of the Genetic Resources of the Potato III. International Potato Center, Lima, Peru. 235p

Page, O.T. 1985. Innovative Methods for Propagating Potatoes. International Potato Center, Lima, Peru. 342p.

Pang, C.H., and M.G. Scanlon. 1996. Mechanical properties of parenchyma of potato (*Solanum tuberosum* cv. Russet Burbank). Can. J. Botany 74:859-869.

Park, W.J. 1989. Park Seed: Flower and vegetable seed catalogue. Park Seed Company, Greenwood, South Carolina.132p.

Poehlman, J.M. and D.A. Sleper. 1995. Breeding Field Crops. 4th.ed. Iowa State University Press. 494p.

Prior, R.L., X. Wu, and K. Schaich. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food

Chem. 53:4290-4302.

Reed, G.L., A.S. Jensen, J. Riebe, G. Head, and J.J. Duan. 2001. Transgenic Bt potato and conventional insecticides for Colorado potato beetle management: comparative efficacy and non-target impacts. Entomol. Exp. Appl 2001. 100:89–100.

Renaud, S. and M. De Lorgeril. 1992. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339:1523-1526.

Reyes, L. F., J.C. Miller, and L. Cisneros-Zevallos. 2004. Environmental conditions influence the content and yield of anthocyanins and total phenolics in purple- and red-flesh potatoes during tuber development. Am. J. Pot. Res. 81:187-193.

Reyes, L.F., J.C. Miller, Jr., and L. Cisneros-Zevallos. 2005.Antioxidant capacity, anthocyanins and total phenolics in purple-and red-fleshed potato (*Solanum tuberosum* L.) genotypes. Am. J. Pot. Res. 82:271-277.

Rivero R., E. Rodeiguez, and C. Romero. 2003. Effect of current storage conditions on nutrient in several varieties of potatoes from Tenerife. Food Chem. 80: 445-450.

Rodriguez-Saona, L.E., M.M. Giusti, and R.E. Wrolstad.1998. Anthocyanin pigment composition of red-fleshed potatoes. J. Food Sci. 63:458-465.

Rommens, C.M., J. Ye, C. Craig and K. Swords. 2006. Improving potato storage and processing characteristics through all-native DNA transformation. J. Agric. Food Chem. 54:9882-9887.

Ross, H. 1986. Potato Breeding: Problems and Perspectives: Advances in Plant Breeding, Supplement 13 to Journal of Plant Breeding. Paul Parey, Berlin and Hamburg. 132p.

Seeram, N.P., L.S. Adams, M.L. Hardy, and D. Herber. 2004. Total cranberry extract versus its phytochemical constituents: Antiproliferative and synergistic effects against human tumor cell lines. J. Agric. Food Chem. 52:2512–2517.

Shepard, J.F., D. Bidney, and E. Shahin. 1980. Potato protoplasts in crop improvement. Science 208:17-24.

Shichijo, C., T. Hamada, M. Hiraoka, C.B. Johnson, and T. Hashimoto. 1993. Enhancement of red-light-induced anthocyanin synthesis in sorghum first internodes by moderate low temperature given in the pre-irradiation culture period. Planta 191:238-245.

Smythies, J.R. 1998. Every Person's Guide to Antioxidants. Rutgers University press. New Brunswick, New Jersey, and London.140p.

Singleton, V.L., R. Orthofer, R.M. Lamuela-Raventós. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In Packer L (ed) Methods in enzymology, Oxidants and Antioxidants. Academic Press pp: 152-178.

Spanos, G.A. and R.E. Wrolstad. 1990. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. J. Agric. Food Chem. 38:1565-1571.

Spychalla, J.P. and S.L. Desborough. 1990. Fatty acids, membrane permeability, and sugars of stored potato tubers. Plant Physiol. 94:1207-1213.

Stark, J.C. and S.L. Love. 1995. Potato Production Systems. University of Idaho Agricultural Communications, 1st. edition.426p.

Steyn, W.J., S.J.E. Wand, D.M. Holcroft, G. Jacobs. 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol. 155:349–61.

Stushnoff, C., D. Holm, M.D. Thompson, W. Jiang, H.J. Thompson, N.I. Joyce, and P. Wilson. 2008. Antioxidant properties of cultivars and selections from the Colorado Potato Breeding Program. Am. J. Pot. Res. 85:267-276.

Swaminathan, M.S. and H.W. Howard. 1953. The cytology and genetics of the potato (*Solanum tuberosum*) and related species. Bibliogr. Genet. 16:1-192

Talburt, W. F. 1987. History of potato processing. p 1-10. In Talburt, W. F., and O. Smith (eds.) Potato Processing, 4th ed., Van Nostrand, New York. 796p.

Tevini, M. and G. Schonecker. 1986. Occurrence, properties, and characterization of potato carotenoids . Potato Res. 29 (2):265.

United States Department of Agriculture (USDA). National Agricultural Statistics Service (NASS). http://www.nass.usda.gov/Statistics_by_Subject/index.asp

Van Eck, H.J., J.M.E. Jacobs, P.M. Van Den Berg, W.J. Stiekema, and E. Jacobsen. 1994. The inheritance of anthocyanin pigmentation in potato (*Solanum tuberosum* L.) and mapping of tuber skin colour loci using RFLPs. Heredity 73:410–421.

Van Eldik, G. J., W.H. Rejnen, R.K. Ruiter, M.M.A Van Herpen, J.A.M. Schrauwen, and G.J. Wullems. 1997. Regulation of flavonol biosynthesis during anther and pistil development, and during pollen tube growth in *Solanum tuberosum*. Plant. J. 105-113.

Vayda, M.E. 1994. Environmental stress and its impact on potato yield. In: JE Bradshaw, GR Mackay (eds), Potato Genetics, CAB International, Wallingford, UK. pp. 239-261.

Wahab, J. 1992. Physiological Basis for Northern Vigor in Potato Seed Tubers. PhD Dissertation, University of Saskatchewan, Saskatoon, Sask., Canada. 330p.

Wandelt, C. 2006. General surveillance – Cultivation of genetically modified starch potatoes in a closed-loop system. J. Verbr. Lebensm. 1:58–59.

Wegener, C.B. and G. Jansen. 2007. Soft-rot resistance of coloured potato cultivars (*Solanum tuberosum* L.): The role of anthocyanins. Potato Res. 50:31-44.

CHAPTER 3

EVALUATION OF 15 POTATO FAMILIES GROWN IN THE GREENHOUSE AND FIELD FOR HIGH TOTAL PHENOLIC CONTENT (TPC) AND USE OF MINISCAN® XE PLUS DATA TO IDENTIFY HIGH TPC TUBERS

INTRODUCTION

Potato (*Solanum tuberosum* L) is the leading vegetable crop in the U.S. and considered the fourth most important food crop worldwide.The annual consumption of potato for each American has been estimated to be more than 54 kg (www.potato2008.org). Freshly harvested potatoes contain about 80 percent water and 20 percent dry matter. The starch represents about 60 to 80 percent of the dry matter. The potato has high vitamin C content that promotes absorption of the moderate amount of iron found in potatoes. It is also considered a good source of vitamins B_1, B_3 and B_6 and minerals such as potassium, phosphorus and magnesium (www.potato2008.org). In addition, potato contains significant levels of important antioxidants (Al-Saikhan et al., 1995; 2000), including phenolic acids, carotenoids, and flavonoids (Arai et al., 2000; Brown et al., 2005).

In recent years, many studies have focused on the functional properties of a range of fruits and vegetables and their role in human health. A variety of phytochemicals, such as phenolics, carotenoids and flavonoids, have been shown to exhibit functional properties such as antimicrobial, antimutagenic, and free radical scavenging (Friedman, 1997). Free radicals are molecules with one or more unpaired electrons that can react with vital biological molecules such as nucleic acids, proteins, and fats that may lead to cell damage (Lister, 1999; 2003). This damage is thought to lead to the occurrence of chronic illnesses including cancer, inflammation, and cardiovascular diseases (Gomes et al., 2003). Phenolic compounds have the ability to suppress free radical-induced oxidative stress and thus may reduce the initiation of these chronic diseases (Ho et al., 2004).

Phenolics in fruits and vegetables have received a great deal of attention because of their antioxidant activity (Chinnici et al., 2004). Reyes et al. (2003) reported that purple- and red-fleshed potatoes offer a valuable novel source of natural colorants and antioxidants, associated with their phenolic compounds.

Many studies have reported high positive correlation between total phenolics content (TPC) and/or anthocyanins and antioxidant activity in potatoes (Brown, 2005; Lachman et al., 2000; Reyes et al., 2005). Compared to white-fleshed cultivars, red and purple-fleshed potatoes demonstrated 2.5 to 3 times higher antioxidant activity (Brown et al., 2005). This large difference is related to the presence of acylated anthocyanins along with phenolic acids in red- and purple-fleshed cultivars.

Both environmental conditions and genetics have been reported to have an impact on the level of polyphenols contained in potatoes. The impact of environment on antioxidants has been investigated with seven potato cultivars grown at 4 locations in Colorado. The variation of total phenolic content was greater for cultivars as compared to locations, even though locations had highly significant differences (Al-Obaidani , 2005). A study in the Czech Republic evaluated the effect of locations, years, and fertilization on total polyphenols in potato (Hamouz et al., 2005; 2006; 2007). Different potato cultivars including yellow- and purple-fleshed types were grown in four locations in the Czech Republic. These locations were different in altitude, average annual temperature and total sum of precipitation. Different site conditions were found to have a significant effect on TPC in tubers. Hale et al. (2003) also examined the effect of location on antioxidant activity in different potato varieties and advanced selections. Significant differences were reported among cultivars ($P<0.0001$) and locations ($P<0.0001$). Moreover, the interaction between cultivars and locations was also significant ($P<0.0001$). Haynes et al. (1996) also reported significant differences among environments and clones for yellow-flesh color intensity.

Hypothesis Statements

1- Evaluation of progeny sub-sample from different families will aid potato breeders in the identification of families that have high potential in generating tubers with high TPC.

2- Potatoes with darker flesh color may have high TPC related to the high concentration of anthocyanins. Thus, measuring potato flesh color level using the MiniScan® XE Plus might be helpful to identify and select the superior tubers for TPC.

3- High correlation between TPC of individual tubers and families grown under greenhouse conditions with those grown in the field will aid potato breeders in the selection for high TPC in the greenhouse.

The objectives of this study were to:

(1) Determine sub-sample progeny with greatest TPC of 15 families grown in the San Luis Valley (SLV) in Colorado (CO).

(2) Determine if pigment level as measured by MiniScan® XE Plus could be used as a method to identify high TPC tubers.

(3) Study the correlation between TPC of all tubers and families' means harvested from the field with corresponding families' means and tubers harvested from the greenhouse.

MATERIAL AND METHODS

Plant Materials

Fifteen families derived from specific crosses of clones grown in the San Luis Valley (SLV), Colorado (CO) were selected for this study (Table. 3.1). These crosses were made over several years by Dr. David Holm.

Table 3.1. Fifteen families derived from specific crosses of clones grown in SLV, Colorado were selected for this study. W= white, R= red, P= purple, and DR= dark red.

Family	**Female**(skin/flesh color)	**Male** (skin/flesh color)
CO94163	ND 1995- 1(W/W)	All Blue (P/P)
CO94166	ND2109-7 (W/W)	All Blue (P/P)
CO94178	All Blue (P/P)	ND 1995-1(W/W)
CO94179	All Blue (P/P)	ND2109-7 (W/W)
CO94181	All Blue (P/P)	Chipeta (W/W)
CO94198	Chipeta (W/W)	All Blue (P/P)
CO97211	Durango (DR/W)	Mountain Rose (R/R)
CO97219	Purple Majesty (P/P)	Mountain Rose (R/R)
CO97225	Mountain Rose (R/R)	Mountain Rose (R/R)
CO97254	Cherry Red (R/W)	Mountain Rose (R/R)
CO97306	Purple Peruvian (P/P)	Mountain Rose (R/R)
CO97307	Purple Peruvian (P/P)	CO94214-1 (P/P)
CO04045	CO97215-2 (P/P)	CO97216-1 (P/P)
CO04059	CO97219-1(R/R)	CO97306-1(R/R)
CO04063	CO97226-2 (R/R)	CO97222-1(R/R)

Greenhouse experiment

Progeny from these crosses were planted in the greenhouse on November 6, 2006 in the SLV, CO. The temperature of the greenhouse was about 21.5 to 22.5 ± 2°C night and day, respectively. The greenhouse is covered by 8 mm thick clear Lexan, a polycarbonate sheet used for covering greenhouses. According to the Lexan technical manual, the solar transmission of 8 mm clear Lexan is about 85% whereas UV-B transmission is 0%. Approximately 300 true seed from each family were planted in plastic trays (25 cm W x 25 cm L x 10 cm D) filled with a mix of peat moss and vermiculite (1:1) and subsequently transplanted on December 4, 2006 into 7.62 cm x 7.62 cm pots filled with a growing mix (1:1:1, sand: peat moss: vermiculite). Each pot contained a single seedling. The greenhouse was lighted with Philips Son Agro 430 2/3 light bulbs. During the day, the lights were only on during cloudy days, as well as at night from 5:00 pm to 11:00 pm.

All plants were harvested at the end of February, 2007. Thirty plants were selected from each family based on number and size of seedling tubers. One seedling tuber (largest one) from each individual plant was saved for a subsequent experiment. Another seedling tuber from each single plant within each family was selected to measure the level of pigment using MiniScan® XE Plus. The rest of the seedling tubers were used to extract and analyze for total phenolic content (TPC) in the lab.

Procedure of breaking dormancy

Because of the short time between harvesting and planting (about 2.5 months), the seedling tubers were not likely to break dormancy in time for field planting. Thus the selected seedling tubers were treated with a solution to reduce length of dormancy period. The treatment was a soak of seedling tubers in a Florel and ProGibb solution (Florel 250 ppm + 2.28 10^{-6} ProGibb/L water) for 24 hours at 15°C in a dark cooler. They were subjected to constant aeration during soaking with an aquarium air pump (Aquaculture MK-1504). This treatment has been found to effectively reduce the dormancy period

(Külen, 2005). The seedling tubers were then moved from the solution into a chamber at 15°C in the dark. They were screened weekly for bud growth. Seedling tubers that broke dormancy were moved and held in another cool chamber (15°C) under light until time of field planting. The cool chamber had 4 fluorescent lights (32 w/each) at the ceiling and one incandescent light bulb (75 w) at the top of the door.

Field experiment

The field experiment was conducted in the SLV, CO in 2007. The SLV is 2347 m above sea level with sandy soil and warm days in summer but cool nights. According to Colorado Climate Center data, the averages of day/night temperatures in Center, CO for May, June, July, August, and September of 2007 were 19.6/2.2°C, 25.9/6.3 °C, 28.2/10.1, 27.4/9.7, and 23.2/5.1, respectively. On May 16, 2007, the 15 families were randomly planted in 15 rows (one row for each family). Each family had 30 seedling tubers which had been previously selected in the greenhouse and had been treated with dormancy breaking solution. However, some treated seedling tubers had not sprouted by the time of planting in the field. The seedling tubers were spaced 61 cm apart within each row and about 86 cm between rows. The vines were killed on September 10, 2007 and tubers were harvested on September 26, 2007. Fifteen plants from each family were randomly selected. However, CO04045 family had only 13 tubers available, so all of them were selected.

Measurement of seedling tuber flesh color

Flesh color was measured using the MiniScan® XE Plus spectrally based color measurement instrument from HunterLab (Reston, VA). This device represented the color of the potato flesh in three axel values under "**L**", "**a**", and "**b**" symbol. "**L**", "**a**", and "**b**" indicate the relative hue and value. "**L**" values range from 0 to100 where 0 is black and 100 is white. Positive "**a**" is red and negative "**a**" is green. Positive "**b**" is yellow and negative "**b**" is blue. The center of the axels is achromatic. When the values of "**a**" and "**b**" increased, the point moves out from the center and the saturation (chroma) of the color increases. The flesh colors of the potatoes ranged from white to blue including yellow, pink, red and purple. The "L" value decreases from white flesh to blue flesh. This mean that the "L" value could likely be used to represent the value of "**a**" and "**b**". Thus the MiniScan® XE Plus study was discussed based on the relationship between total phenolic content and "L" values. However, the color relationship between total phenolic content and "**a**" and "**b**" values are included.

Reciprocal crosses

CO94163 is a reciprocal of CO94178; CO94166 is a reciprocal of CO94179; and CO94181 is a reciprocal of CO94198 (Table 3.1). These reciprocal crosses were used to study the maternal effect on TPC.

Sample preparation for extraction

The remaining seedling tubers from greenhouse from each single plant within each family were used in the lab to quantify TPC. The whole seedling tubers were cut into slices, weighed, and then held together by tooth picks. (For field samples, three tubers from each harvested plant within each family were used to quantify TPC. Approximately 5mm-thick single slice from the middle of each tuber was collected, weighed, and then held together by toothpicks). The samples were then placed in a Virtis Genesis 25 LL freeze dryer (Gardiner, N.Y. 12525) for 4 days (at -40 °C for 1 day, then -10 °C for 1 day, then 18 °C for 1 day, and finally at 28 °C for 1 day). Dried samples were weighed, ground into powder using mortar and pestle and sieved with a 100 mesh sieve to ensure uniform particle size prior to extraction. Ground samples were held in 15 ml plastic centrifuge tubes sealed with a screw cap to prevent uptake of moisture and then stored at -20 °C.

Extraction procedure

A total of 300 mg was used from each ground sample. This amount was placed into 15 ml plastic centrifuge tubes with 5 ml of 80% acetone, vortexed, and rotated in the dark for one hour at 4°C. They were then placed in a centrifuge at 6000 rpm at 4°C for 15 min. A total of 4 ml of supernatant from each centrifuged tube was distributed into 4 eppendorf tubes (1 ml per eppendorf tube) before drying at 45°C by Vacufuge™ "Eppendorf AG" (Westbury, N.Y.) for 2.5 h. Dried samples were then immediately processed or placed into a freezer (-20°C) for later analysis.

Total phenolic content protocol

The Folin-Ciocalteu assay, a well-known assay developed by Rossi and Singleton (1965), was used to quantify the TPC of potato tuber flesh. The assay was modified for use with a 96-well plate and microplate reader as adapted from Spanos and Wrolstad, (1990).

The principle of this method is based on a color reaction. The phenolic groups in the sample are converted to phenolate ions in the presence of an oxidizing agent such as Folin-Ciocalteu and alkali such as sodium carbonate to give a blue color complex. This color can be read spectrophotometrically at 765 nm and compared with gallic acid standards. A standard curve was prepared using a gallic acid solution to quantify TPC as gallic acid equivalents (GAE). The gallic acid solution was freshly made by dissolving 25 mg gallic acid into 25 ml 80% acetone in a volumetric flask for best accuracy. The standard curve was prepared from 3.5 ml of stock gallic acid solution with 6.5 ml 80% acetone. For best accuracy, gallic acid standard curve dilutions were prepared using volumes as noted in Table 3.2.

Table 3.2. Gallic acid proportions used for total phenolics standard curve dilutions

μg/ ml in assay	μl Stock Standard (GA)	μl de-ionized water
0	0	3500
10	100	3400
20	200	3300
40	400	3100
60	600	2900
80	800	2700
100	1000	2500

Vacufuged stored samples were reconstituted with 1 ml of 80% acetone and sonicated and vortexed for 10-20 min. 100 μl from each reconstituted sample was pipetted into a new Eppendorf tube and then diluted by adding 900 μl dH_2O. 35 μl of each diluted sample as well as a gallic acid standard curve sample were pipetted into triplicate microplate wells (3 reps/sample). 150 μl of 0.2M Folin-Ciocateu reagent (freshly diluted from the full strength Sigma reagent 1/10 with dH_2O) was added to all wells using a multichannel pipetter. The microplate was then covered with adhesive film and mixed on a platform shaker (Thermlyne Maxi-Mix III ™, Type 65800) at 400 rpm for 30 seconds, then held for 5 minutes at room temperature. After that, 115 μl of 7.5% (w/v) Na_2CO_3 (7.5g/ 100ml dH_2O) was added to all wells using a multichannel pipetter. The microplate was then covered with adhesive film and mixed on the platform shaker at 400 rpm for 30 seconds, then held for 5 minutes at room temperature. The microplate was then incubated at 45 °C for 30 min and then cooled to room temperature for 1 hour. Absorbance of each sample in the microplate wells was measured at 765 nm using a spectrophotometer/microplate reader (SPECTRA *max* Plus^{384} UV-vis spectrophotometer, Molecular Devices, Sunnyvale, CA) and SOFT *max* Pro Version 3.1.2 software. A regression spreadsheet from Microsoft Excel® was used to calculate the concentration of gallic acid equivalents (GAE) of each liquid sample in mg GAE/g of dry weight (DW).

Statistical analysis

The average TPC of families were analyzed by analysis of variance (ANOVA). The ANOVA was performed with the general linear model (GLM) of the SAS 9.1 software package (Cary, NC). Tukey's Minimum Significant Difference procedure was used to compare the differences of mean values among families ($P < 0.05$).

The normality of the frequency distribution of TPC in families was evaluated using *Kolmogorov-Smirnov* normality test of the SAS 9.1 software package (Cary, NC).

Correlation between flesh color as determined by MiniScan® XE Plus and TPC of tubers grown in the greenhouse was made using the correlation coefficient (r) obtained from GraphPad Prime 5 software (San Diego, CA). The same software was also use to study the correlation between TPC of tubers and families grown under greenhouse conditions with those grown in the field.

RESULTS

Greenhouse study

Among 448 individual seedling tubers harvested from 15 families in the greenhouse, the highest TPC observed was 17.94 mg GAE/g DW followed by 14.69 mg GAE/g DW and 11.05 mg GAE/g DW (Table 3.3). These individuals were derived from CO04045, CO04063, and CO97307 families, respectively. The flesh colors of these seedling tubers were red, red, and purple respectively. In contrast, the lowest TPC reported were 1.74 mg GAE/g DW, 1.89 mg GAE/g DW, and 1.89 mg GAE/g DW from CO97225, CO94178, and CO97307 families, respectively (Table 3.3). The flesh color of these seedling tubers was white. The top 30 seedling tubers for high TPC (Table 3.3) revealed that 60% of these seedling tubers were red-fleshed followed by purple-fleshed tubers (40%). The majority of these top tubers (86.67%) were derived from CO04063, CO97307, and CO04045 families, respectively. The 30 tubers with the least TPC were white-fleshed and the majority of these tubers (80%) were derived from CO97225, CO94178, and CO97211 families, respectively. The highest and lowest TPC as well as the average of each family is noted in Table 3.5. Additionally, figures from 3.1 to 3.15 illustrate frequency distributions of TPC for each family under greenhouse (A) and field (B) conditions.

The analysis of variance (ANOVA) was carried out with the GLM of SAS to compare TPC means of the families (Table 3.7). CO04063 and CO04045 had the highest mean for TPC at 7.43 and 7.1 mg GAE/g DW, respectively with no significant difference between them followed by CO97307 at 6.07 mg GAE/g DW which was significantly less than the CO04063 family. The lowest TPC means were identified in CO97225, CO94178, and CO97211 families, respectively, with no significant difference among them (Table 3.7).

The reciprocal effect was estimated by comparing the means of total phenolic content among reciprocal crosses. CO94163 is a reciprocal of CO94178; CO94166 is a reciprocal of CO94179; and CO94181 is a reciprocal of CO94198. The analysis of variance (Table 3.7) revealed no significant effect of reciprocal cross on TPC between CO94166 and CO94179 and between CO94181 and CO94198. However, a significant difference in the mean of TPC was found between CO94163 and CO94178 indicating a possible maternal effect on TPC in at least some crosses.

Field study

Among 223 individual tubers harvested from 15 families in the field, the highest TPC observed was 19.38 mg GAE/g DW followed by 16.83 mg GAE/g DW and 16.48 mg GAE/g DW (Table 3.4). These individuals had red flesh color and were derived from CO04063 family. In contrast, the lowest TPC observed were 1.82 mg GAE/g DW, 2.32 mg GAE/g DW, and 2.52 mg GAE/g DW which were tubers derived from CO94163, CO97307, and CO94163 families, respectively (Table 3.4). The flesh color of these tubers was white. The 30 tubers with the highest TPC (Table 3.4) were red-fleshed (about 63%) followed by purple-fleshed tubers (37%). In addition, 50% of these superior tubers were derived from the CO04063 family followed by 16%, 13%, and 10% derived from CO97307, CO97219 and CO04045 families, respectively. However, those 30 tubers with the lowest TPC were white-fleshed tubers and 23% of these tubers were derived from CO97225 family (Table 3.4). Figures from 3.1 to 3.15 illustrate frequency distributions of

TPC for each family grown in the greenhouse (A) and field (B) conditions. The highest and lowest TPC as well as the average of each family is noted in Table 3.6.

The analysis of variance (ANOVA) was carried out with the general linear model (GLM) of SAS to compare TPC means of the families (Table 3.8). CO04063 had the highest mean for TPC (14.45 mg GAE/g DW) followed by CO04045 and CO97307 at 9.88 and 9.01 mg GAE/g DW, respectively. The lowest TPC means were found in CO94166, CO97225, CO94178 and CO94179 families (4.41, 4.83, 4.92, and 4.95 mg GAE/g DW), respectively with no significant difference among them (Table 3.8).

Analysis of variance (ANOVA) among the means of TPC for reciprocal crosses (Table 3.8) revealed no significant effect of reciprocal crosses on TPC.

Correlation between the field and greenhouse regarding total phenolic content

In general, most of the families' means and individual tubers had a greater level of TPC in the field when directly compared with the corresponding families' means and tubers grown in the greenhouse. A total of 178 of 233 tubers (about 80%) grown in the field were from 10 to as much as 300% greater in TPC. Approximately 9 % of the field grown tubers were 10 to 59% lower in TPC when directly compared with corresponding tubers grown in the greenhouse. Families' means for TPC of field lines were generally greater than those grown in the greenhouse (Fig.3.16).

The TPC of 223 individual tubers, harvested from 15 families in the field, were correlated with the TPC of the corresponding seedling tubers grown in the greenhouse ($r = 0.70$, $P < 0.0001$). In addition, the correlations between TPC of individual tubers grown in the greenhouse and field within each family were made and varied between r = -0.13 (P = 0.6387) to r = 0.86 (P< 0.0001) in CO04063 and CO94179, respectively (Table 3.9). Furthermore, TPC means of the families grown in the greenhouse were compared with the means of the same families grown in the field. High correlation was noted between the means of families in the greenhouse and field (r=0.83, P= 0.0002). Moreover, the highest three families for TPC in the greenhouse were the same as in the field. Similarity, the lowest three families for TPC were similar except that CO97211 family was replaced by CO94166 in the field (Table 3.7 and 3.8). However, there was no significant difference between the TPC mean of these two families when grown in the field.

Correlation between flesh color as determined by MiniScan® XE Plus and total phenolic content

Flesh color was measured using the MiniScan® XE Plus spectrally based color measurement instrument from HunterLab. Flesh color of 448 seedling tubers derived from 15 families were determined by MiniScan® XE Plus and correlated with their TPC as determined in the lab (Table 3.10). The correlation between L value and TPC was moderate (r = -0.65 P<0.0001) which means that the MiniScan® XE Plus would likely have a low reliability to select or identify high TPC tubers.

Moreover, the correlation of flesh color and TPC was studied in different relationships in order to get more information about the nature of this correlation. For example, the correlation of flesh color and TPC was conducted within white, red, and purple flesh tubers only and within purple and white; red and white; and purple and red flesh (Table 3.10). In addition, the correlation between flesh color and TPC were made within each family alone (Table 3.11). The correlation between L value and TPC varied from $r = -0.13$ ($P = 0.5$) to -0.82 ($P<0.0001$) within CO04045 and CO97306 families, respectively.

Table 3.3. The highest and lowest 30 seedling tubers in total phenolics content (TPC) (mg/g DW) among greenhouse harvested tubers. P=Purple flesh, R=Red flesh, W=White flesh.

Highest 30 tubers in TPC (mg/g DW)				Lowest 30 tubers in TPC (mg/g DW)		
Family	TPC	Flesh color		family	TPC	Flesh color
CO04045-21	17.94	R		CO97225-13	1.74	W
CO04063-30	14.69	R		CO94178-8	1.89	W
CO97307-21	11.05	P		CO97307-9	1.89	W
CO04063-3	10.77	R		CO97306-29	1.94	W
CO97307-12	10.59	R		CO94178-3	1.95	W
CO04063-16	10.54	R		CO97225-15	1.97	W
CO94163-16	10.24	P		CO97225-7	2.04	W
CO04059-18	9.99	R		CO97225-2	2.06	W
CO04045-9	9.54	P		CO97225-1	2.08	W
CO04063-5	9.50	R		CO97225-20	2.12	W
CO97307-29	9.35	P		CO97225-18	2.16	W
CO04045-5	9.27	R		CO94178-1	2.19	W
CO04063-24	8.99	R		CO94178-17	2.20	W
CO97307-4	8.63	R		CO97225-17	2.23	W
CO04063-21	8.53	R		CO97211-17	2.25	W
CO97307-6	8.51	P		CO04059-26	2.25	W
CO94163-25	8.42	P		CO97225-22	2.28	W
CO04045-13	8.41	P		CO94178-23	2.29	W
CO04063-14	8.37	R		CO94179-17	2.30	W
CO04063-25	8.36	R		CO97225-14	2.30	W
CO97225-17	8.29	R		CO97225-16	2.30	W
CO04063-29	8.28	R		CO97211-14	2.33	W
CO97307-1	8.25	P		CO94178-5	2.35	W
CO04045-22	8.09	P		CO97211-30	2.35	W
CO04063-15	8.04	R		CO97219-27	2.39	W
CO04045-11	8.00	P		CO97307-14	2.44	W
CO97307-26	7.97	P		CO97225-9	2.45	W
CO04063-26	7.86	R		CO97225-29	2.46	W
CO04045-20	7.83	R		CO97211-3	2.46	W
CO97307-25	7.82	P		CO97225-11	2.47	W

Table 3.4. The highest and lowest 30 tubers in total phenolics content (TPC) (mg/g DW) among tubers grown in the field. P=Purple flesh, R=Red flesh, W=White flesh.

Top 30 tubers in TPC (mg/g DW)				Lowest 30 tubers in TPC (mg/g DW)		
Family	TPC	Flesh Color		Family	TPC	Flesh Color
CO04063-18	19.38	R		CO94163-17	1.82	W
CO04063-29	16.83	R		CO97307-14	2.32	W
CO04063-21	16.48	R		CO94163-21	2.52	W
CO04063-24	15.7	R		CO97225-22	2.67	W
CO04063-20	15.63	R		CO94179-17	2.73	W
CO04063-14	14.62	R		CO94166-9	2.76	W
CO04063-25	14.24	R		CO94198-25	2.78	W
CO04063-30	14.16	R		CO97225-13	2.84	W
CO97307-19	14.11	P		CO97225-7	2.88	W
CO04063-1	14.02	R		CO97225-19	3.02	W
CO04063-15	13.98	R		CO94198-9	3.12	W
CO04063-23	13.69	R		CO94163-8	3.13	W
CO97307-26	13.52	P		CO94178-12	3.16	W
CO04045-14	13.44	P		CO04059-26	3.19	W
CO97306-12	13.18	P		CO97225-20	3.22	W
CO04063-16	12.98	R		CO94179-3	3.24	W
CO94163-16	12.97	P		CO97254-20	3.28	W
CO04063-22	12.39	R		CO94179-1	3.29	W
CO04045-26	12.32	P		CO94166-10	3.33	W
CO04045-16	12.12	P		CO94163-28	3.36	W
CO97307-6	11.92	P		CO94181-10	3.36	W
CO94181-21	11.85	R		CO97254-7	3.46	W
CO97307-13	11.62	P		CO94166-17	3.47	W
CO97219-13	11.58	P		CO97225-5	3.48	W
CO04063-26	11.44	R		CO94166-20	3.55	W
CO04063-27	11.18	R		CO94178-8	3.56	W
CO97219-19	11.11	R		CO97225-14	3.57	W
CO97219-5	10.95	P		CO94178-15	3.61	W
CO97307-4	10.94	R		CO94181-24	3.63	W
CO97219-17	10.9	R		CO94179-13	3.65	W

Table 3.5. Minimum, maximum and average total phenolics content (TPC) (mg GAE/g DW) within each family harvested from the greenhouse in the SLV, CO in 2007. SD= Standard deviation.

Family	Minimum TPC mg GAE/g DW	Maximum TPC mg GAE/g DW	Family average	SD	Family range
CO94163	3.81	10.24	5.21	1.53	6.43
CO94166	2.81	7.40	4.52	1.12	4.59
CO94178	1.89	5.24	3.09	0.77	3.35
CO94179	2.30	5.26	3.70	0.80	2.96
CO94181	2.60	7.61	4.81	1.35	5.01
CO94198	2.52	7.04	4.48	1.23	4.52
CO97211	2.25	4.99	3.32	0.78	2.74
CO97219	2.39	8.29	4.64	1.44	5.9
CO97225	1.74	7.31	2.85	1.14	5.57
CO97254	2.74	7.16	4.32	0.98	4.42
CO97306	1.94	6.17	4.64	1.13	4.23
CO97307	1.89	11.05	6.07	2.39	9.16
CO04045	3.53	17.94	7.10	2.43	14.41
CO04059	2.25	9.99	4.04	1.61	7.74
CO04063	4.56	14.69	7.43	2.07	10.13

Table 4.6. Minimum, maximum and average of total phenolic content (mg GAE/g DW) within each family harvested from the field in the SLV, CO in 2007. SD= Standard deviation.

Family	Minimum TPC mg GAE/g DW	Maximum TPC mg GAE/g DW	Family average	SD	Family range
CO94163	1.82	12.97	5.56	2.83	11.15
CO94166	2.76	6.56	4.41	1.05	3.81
CO94178	3.16	8.47	4.92	1.55	5.31
CO94179	2.73	8.26	4.95	1.49	5.52
CO94181	3.36	11.85	6.30	2.32	8.49
CO94198	2.78	10.25	5.52	1.94	7.47
CO97211	3.77	9.26	6.11	1.64	5.49
CO97219	4.79	11.58	8.38	2.36	6.79
CO97225	2.67	10.02	4.83	2.52	7.35
CO97254	3.28	8.88	6.13	1.74	5.60
CO97306	4.26	13.18	8.20	2.08	8.91
CO97307	2.32	14.11	9.01	3.40	11.80
CO04045	5.63	13.44	9.87	2.16	7.81
CO04059	3.19	10.52	6.96	2.73	7.33
CO04063	11.18	19.38	14.45	2.14	8.19

Table 3.7. Analysis of variance (ANOVA) among the means of total phenolic content for 15 families grown in the greenhouse at SLV, CO in 2007. Means with the same letter are not significantly different according to Turkey's Minimum Significant Difference.

The GLM Procedure	
Alpha	0.05
Minimum Significant Difference	1.3058

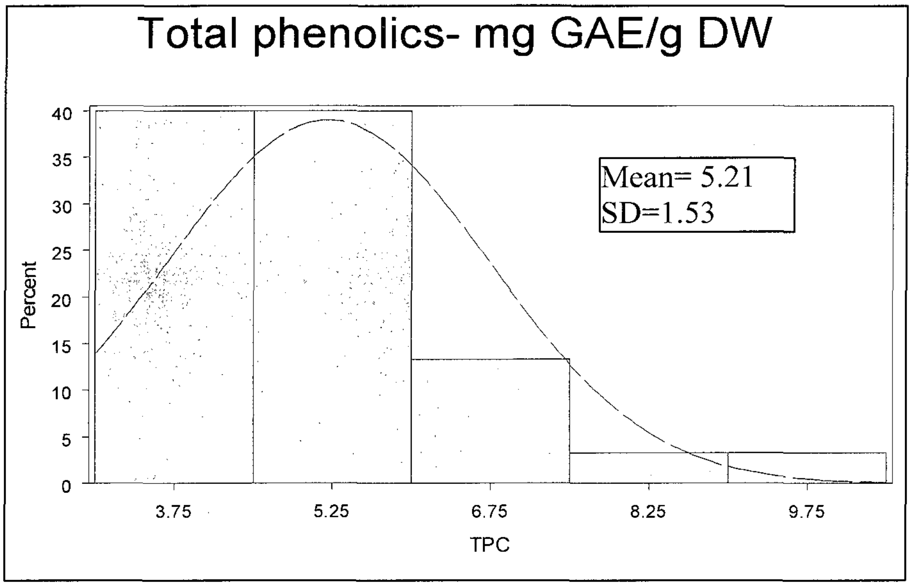
Tukey Grouping								*Mean*	*N*	*Families*
A								7.4272	30	CO04063
A	B							7.0993	30	CO04045
	B	C						6.0720	30	CO97307
		C	D					5.2063	30	CO94163
		C	D	E				4.8084	29	CO94181
			D	E	F			4.6402	30	CO97306
			D	E	F			4.6357	30	CO97219
			D	E	F			4.5238	30	CO94166
			D	E	F			4.4781	30	CO94198
			D	E	F	G		4.3167	29	CO97254
			D	E	F	G	H	4.0409	30	CO04059
				E	F	G	H	3.7014	30	CO94179
					F	G	H	3.3807	30	CO97211
						G	H	3.0927	30	CO94178
							H	2.8534	30	CO97225

Table 3.8. Analysis of variance (ANOVA) among the means for total phenolic content for 15 families grown in the field. Means with the same letter are not significantly different according to Turkey's minimum significant difference.

The GLM Procedure	
Alpha	0.05
Minimum Significant Difference	2.7824

Tukey Grouping						*Mean*	*N*	*Families*
A						14.4478	15	CO04063
	B					9.8750	13	CO04045
	B	C				9.0100	15	CO97307
	B	C	D			8.3844	15	CO97219
	B	C	D	E		8.1959	15	CO97306
		C	D	E	F	6.9581	15	CO04059
		C	D	E	F	6.2968	15	CO94181
			D	E	F	6.1340	15	CO97254
			D	E	F	6.1079	15	CO97211
				E	F	5.5554	15	CO94163
				E	F	5.5173	15	CO94198
					F	4.9542	15	CO94179
					F	4.9245	15	CO94178
					F	4.8324	15	CO97225
					F	4.4145	15	CO94166

A



B

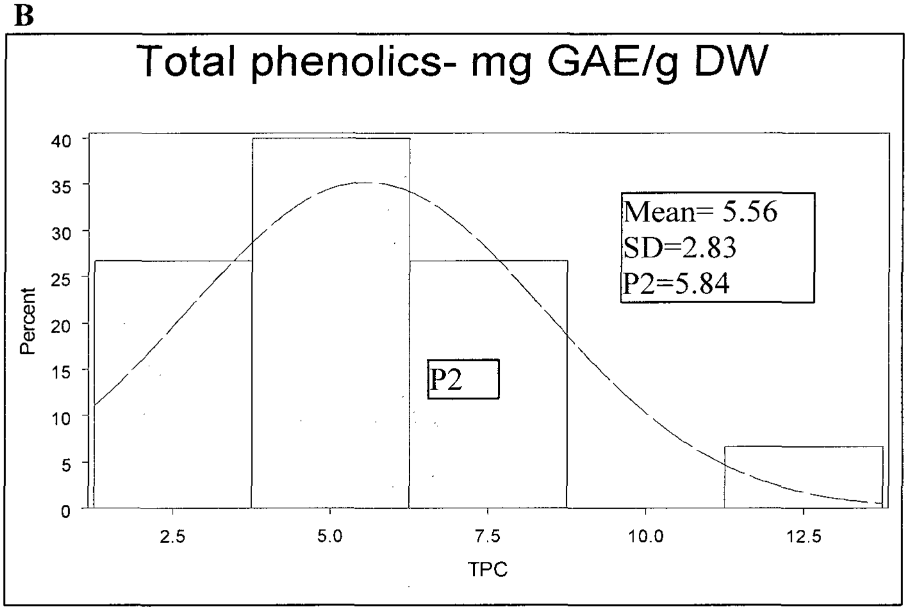
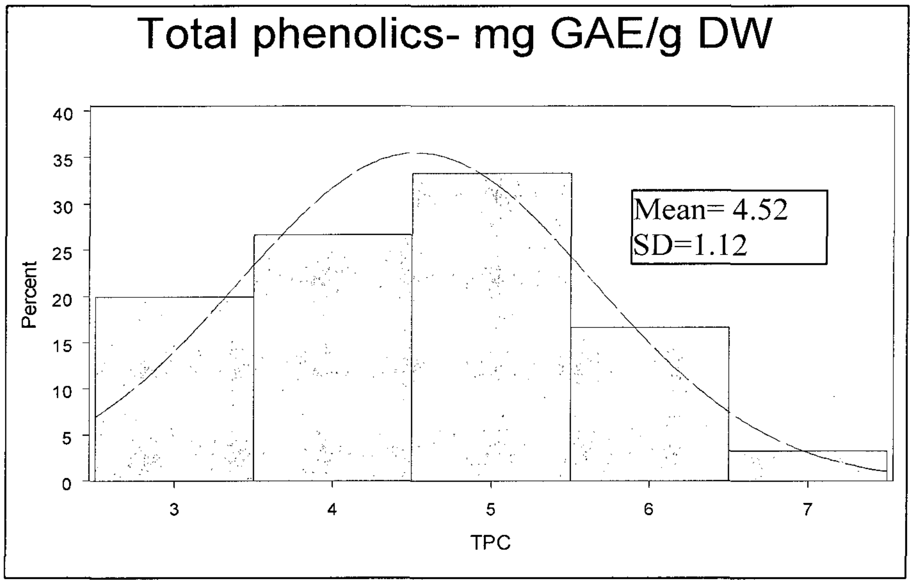


Figure 3.1. Frequency distribution of total phenolic content (TPC) in CO94163 family [ND 1995-1(W/W) (♀) x All Blue (P/P) (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. P/P=purple skin/purple flesh, W/W=white skin/white flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were <0.01 and >0.150, respectively.

A



B

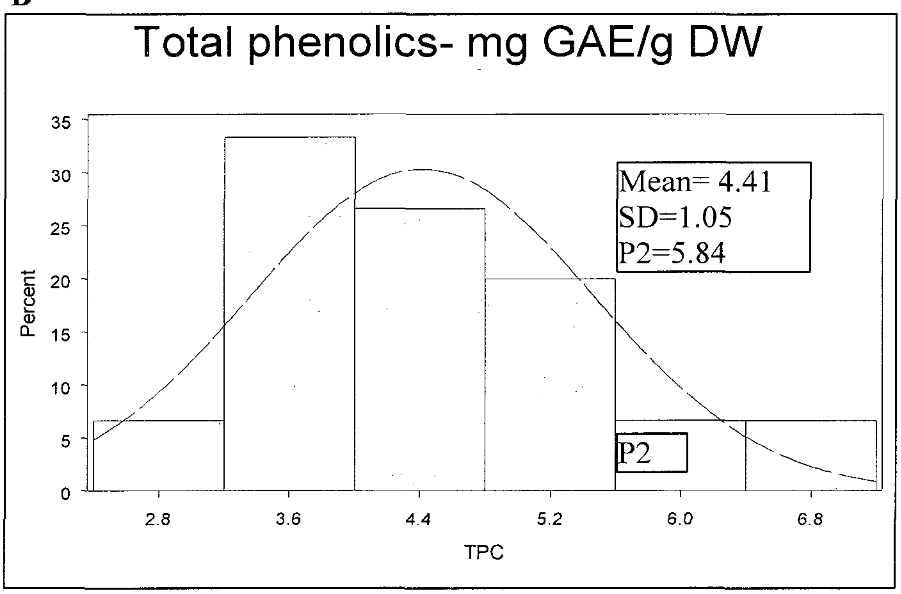
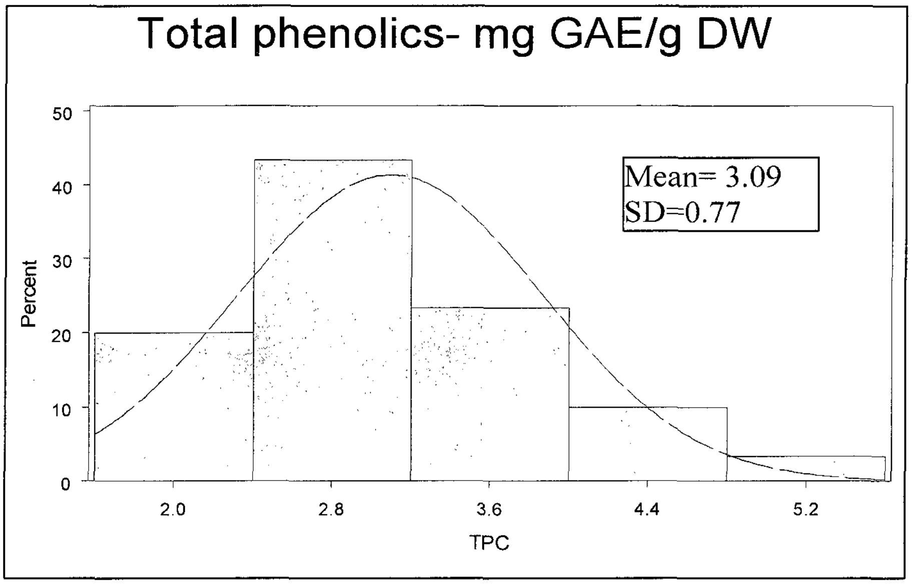


Figure 3.2. Frequency distribution of total phenolic content (TPC) in CO94166 family [ND2109-7(W/W) (♀) x All Blue (P/P) (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. P/P=purple skin/purple flesh, W/W=white skin/white flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were >0.150 and >0.150, respectively.

A



B

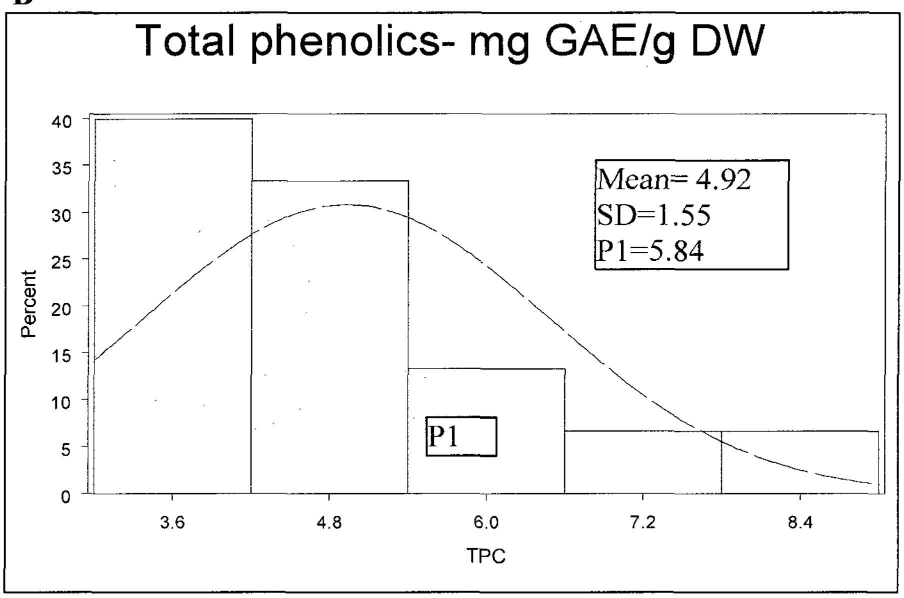
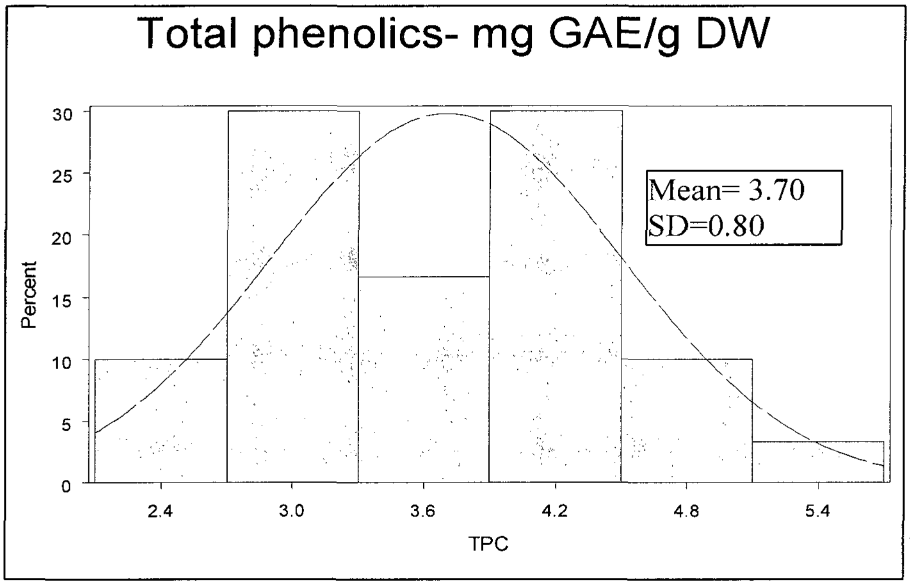


Figure 3.3. The distribution of total phenolic content (TPC) in CO94178 family [All Blue (P/P) (♀) x ND 1995-1(W/W) (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. P/P=purple skin/purple flesh, W/W=white skin/white flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were 0.136 and 0.071, respectively.

A



B

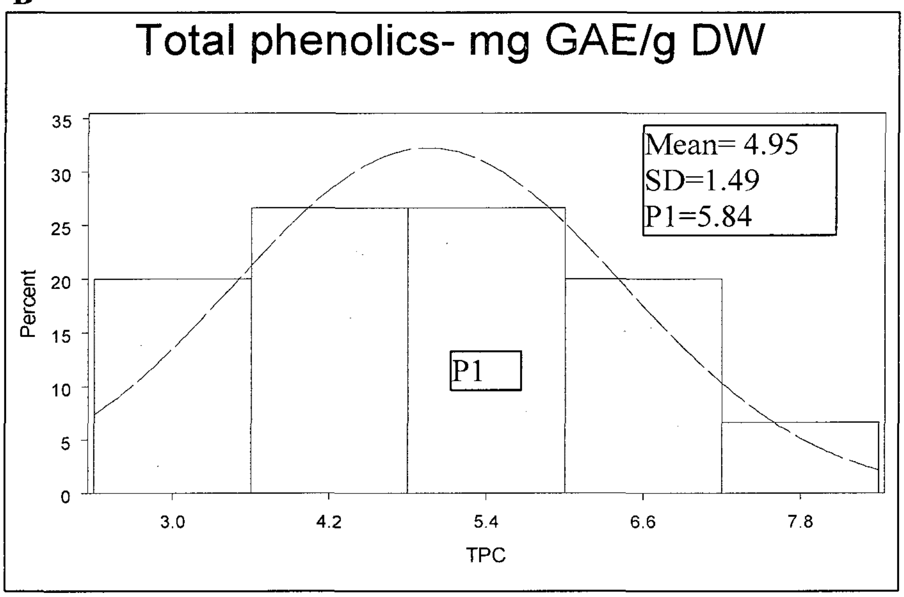
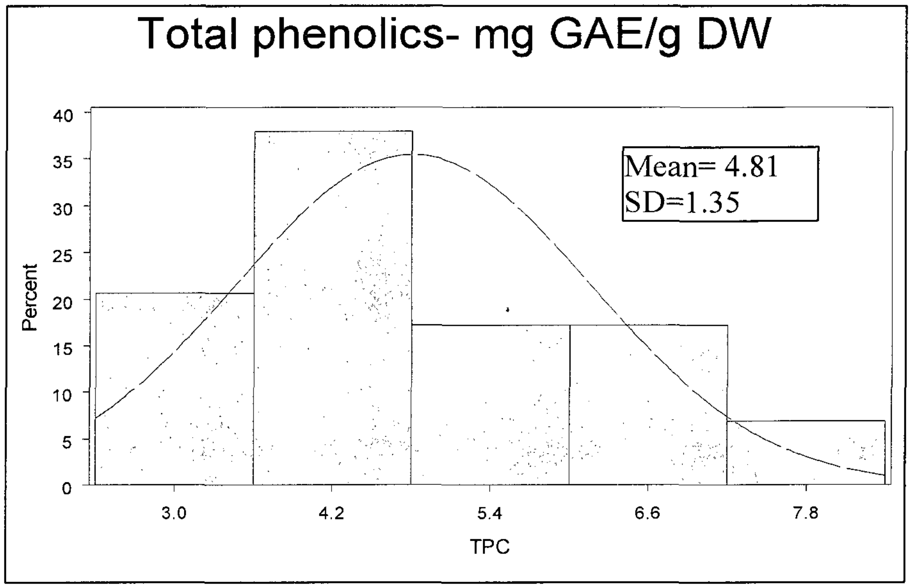


Figure 3.4. Frequency distribution of total phenolic content (TPC) in CO94179 family [All Blue P/P (♀) x ND2109-7W/W (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. P/P=purple skin/purple flesh, W/W=white skin/white flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were >0.150 and >0.150, respectively.

A



B

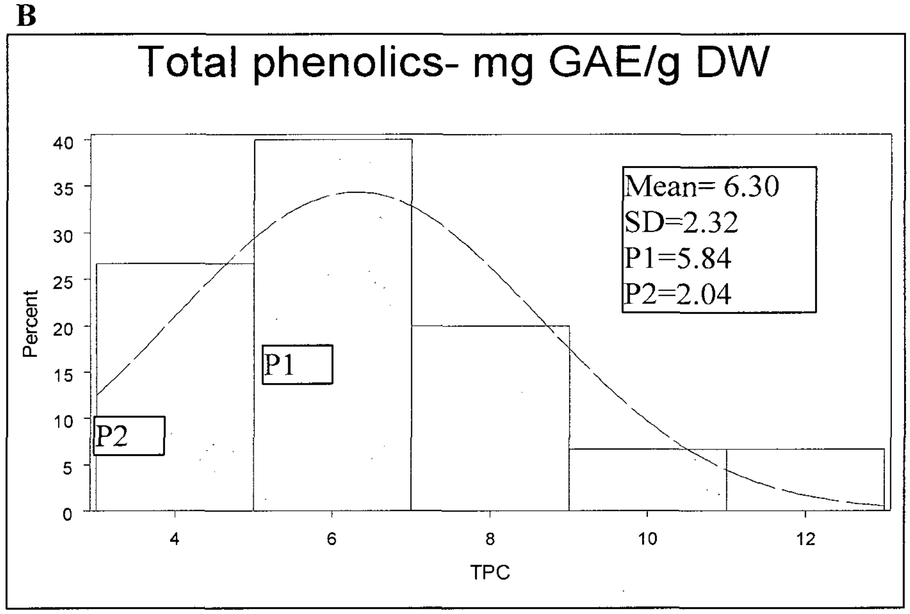
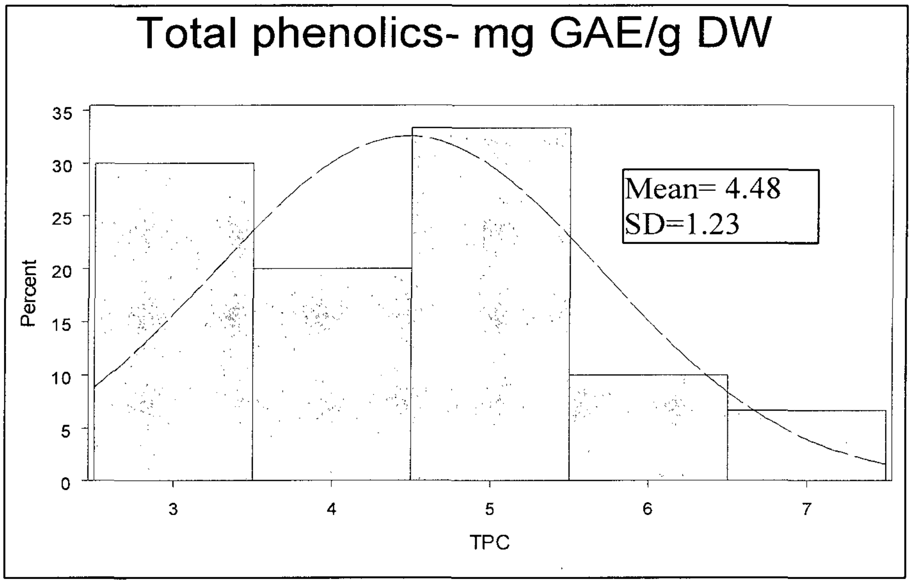


Figure 3.5. Frequency distribution of total phenolic content (TPC) in CO94181 family [All Blue P/P(♀) x Chipeta W/W (♂)]grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. P/P=purple skin/purple flesh, W/W=white skin/white flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were >0.150 and >0.150, respectively.

A



B

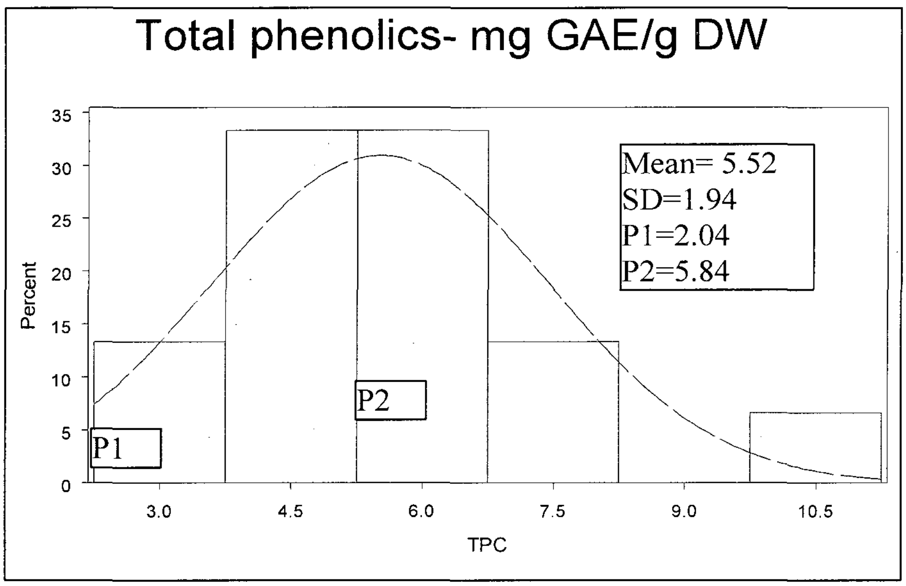
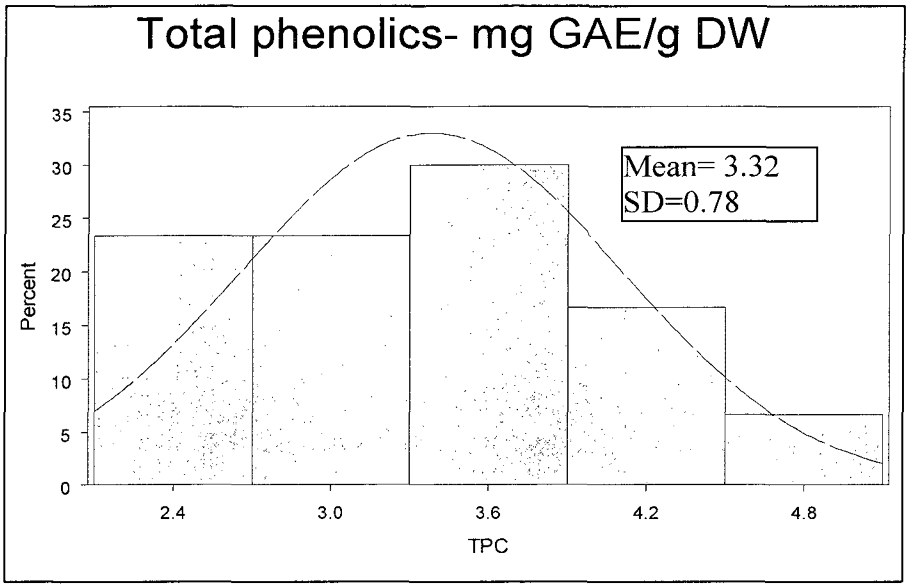


Figure 3.6. Frequency distribution of total phenolic content (TPC) in CO94198 family [Chipeta W/W (♀) x All Blue (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. P/P=purple skin/purple flesh, W/W=white skin/white flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were >0.150 and >0.150, respectively.

A



B

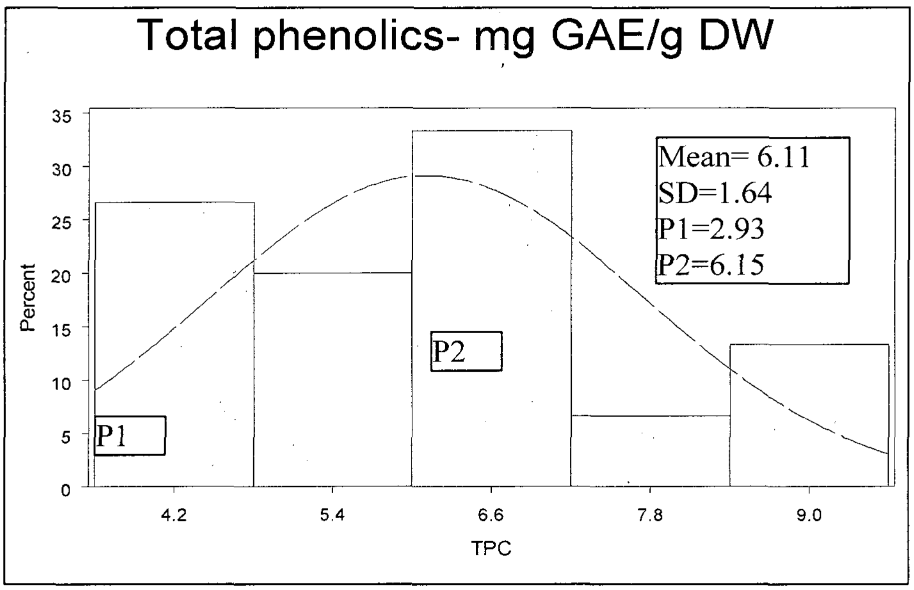
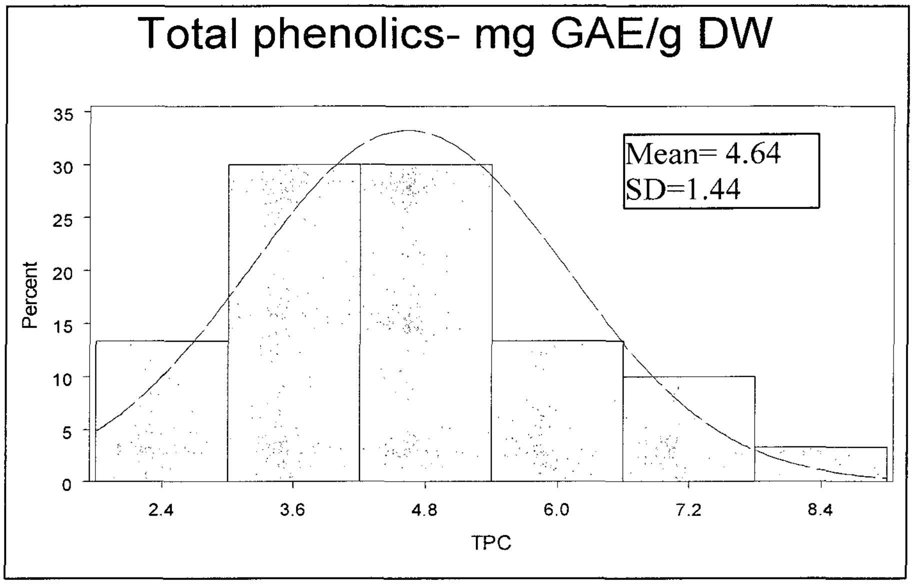


Figure 3.7. Frequency distribution of total phenolic content (TPC) in CO97211 family [Durango DR/W (♀) x Mountain Rose 1R/R (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. DR/W=dark red skin/white flesh, W/W=white skin/white flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were >0.150 and >0.150, respectively.

A



B

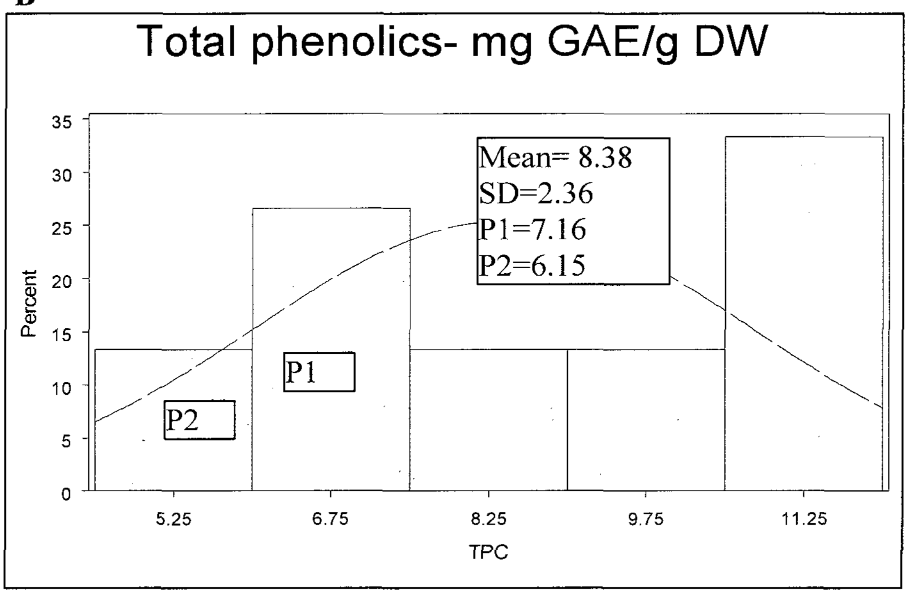


Figure 3.8. Frequency distribution of total phenolic content (TPC) in CO97219 family [Purple Majesty (♀) x Mountain Rose (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. P/P=purple skin/purple flesh, R/R=red skin/red flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were >0.150 and >0.150, respectively.

A

B

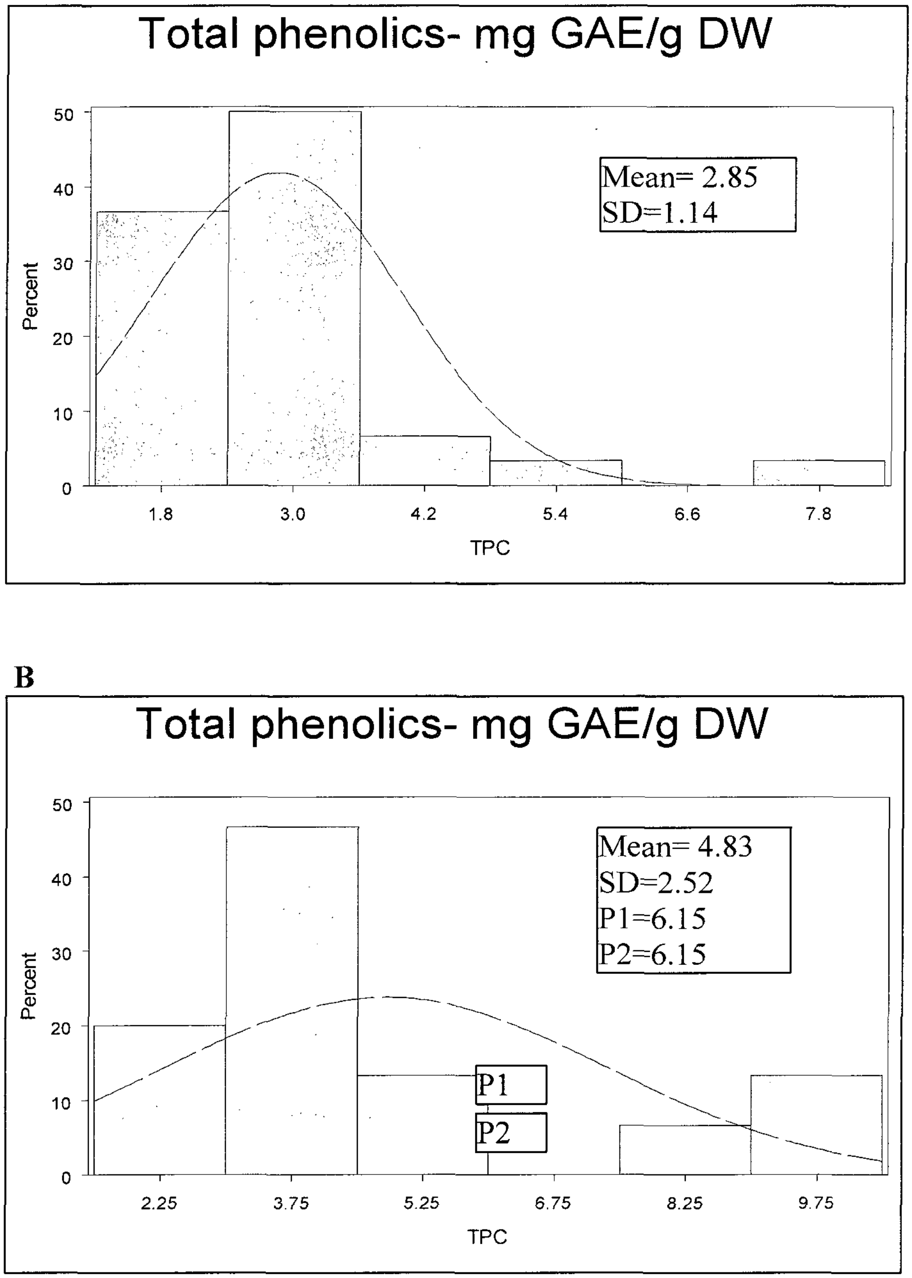
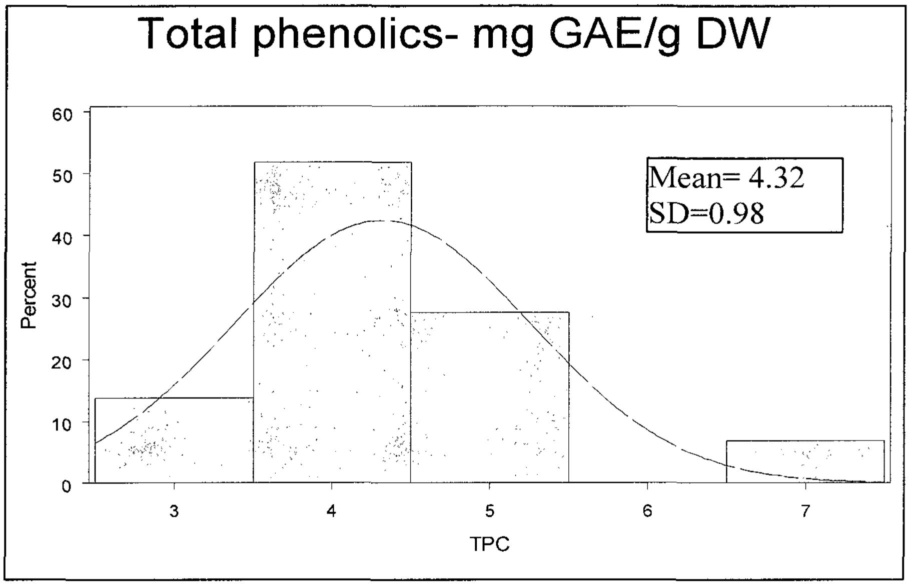


Figure 3.9. Frequency distribution of total phenolic content (TPC) in CO97225 family [Mountain Rose R/R (♀) x Mountain Rose R/R (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. R/R=red skin/red flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were <0.01 and <0.015, respectively.

A



B

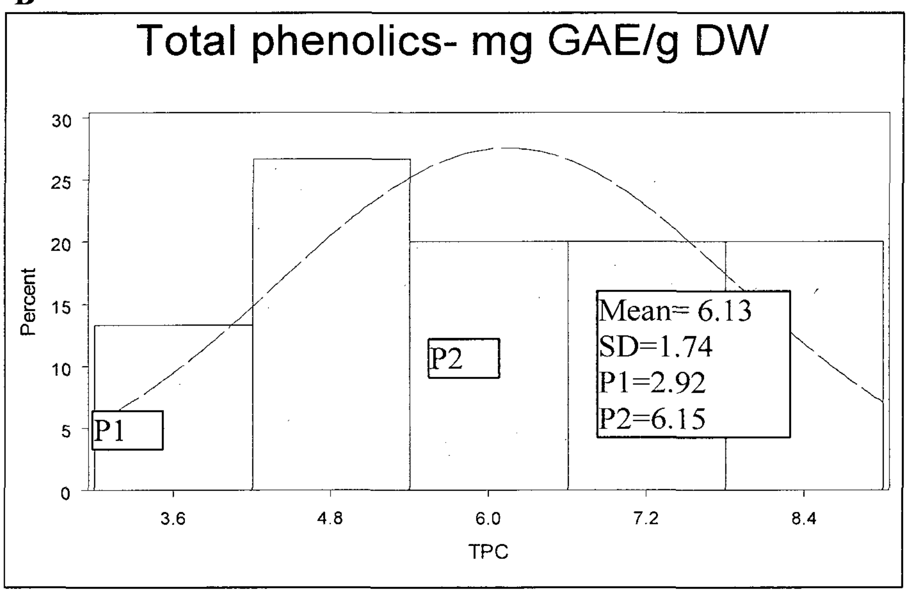
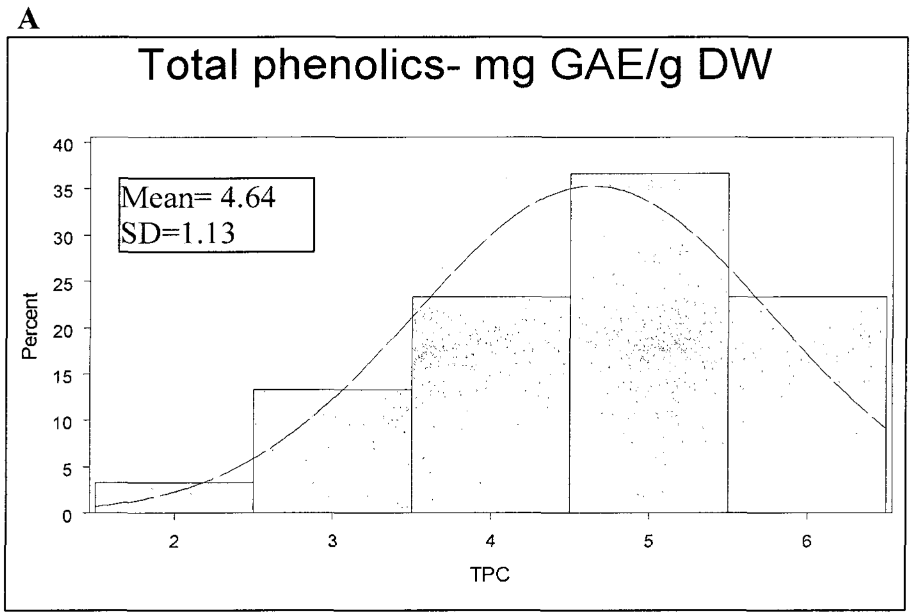


Figure 3.10. Frequency distribution of total phenolic content (TPC) in CO97254 family [Cherry Red R/W (♀) x Mountain Rose R/R (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. R/W=red skin/white flesh, R/R=red skin/red flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were >0.150 and >0.150, respectively.



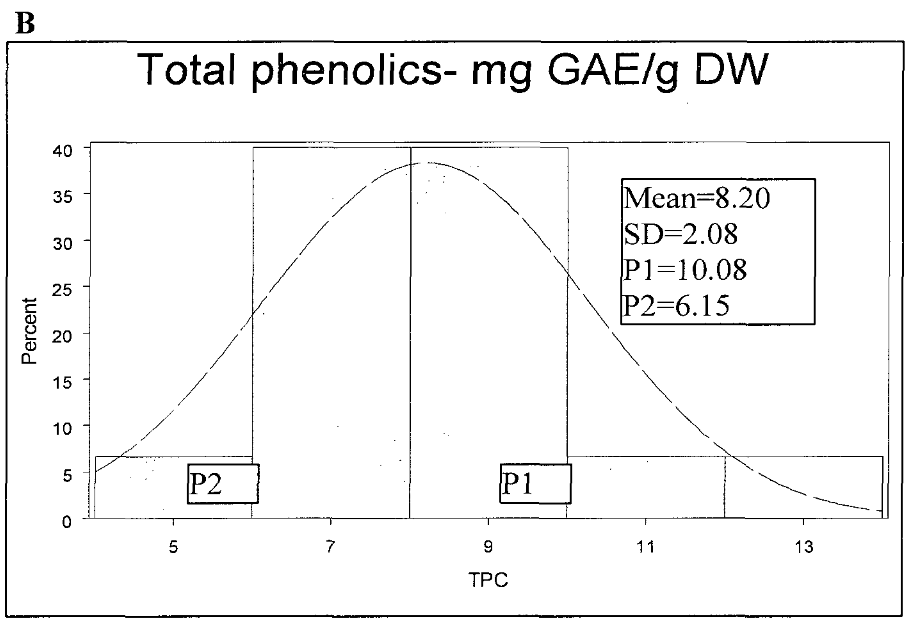
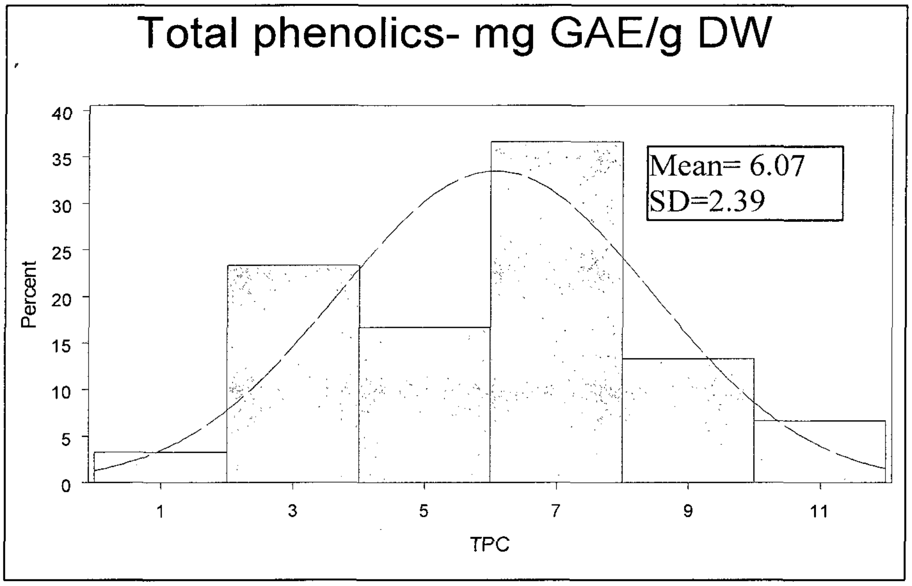


Figure 3.11. Frequency distribution of total phenolic content (TPC) in CO97306 family [Purple Peruvian P/P (♀) x Mountain Rose R/R (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. P/P=purple skin/purple flesh, R/R=red skin/red flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were 0.084 and >0.150, respectively.

A



B

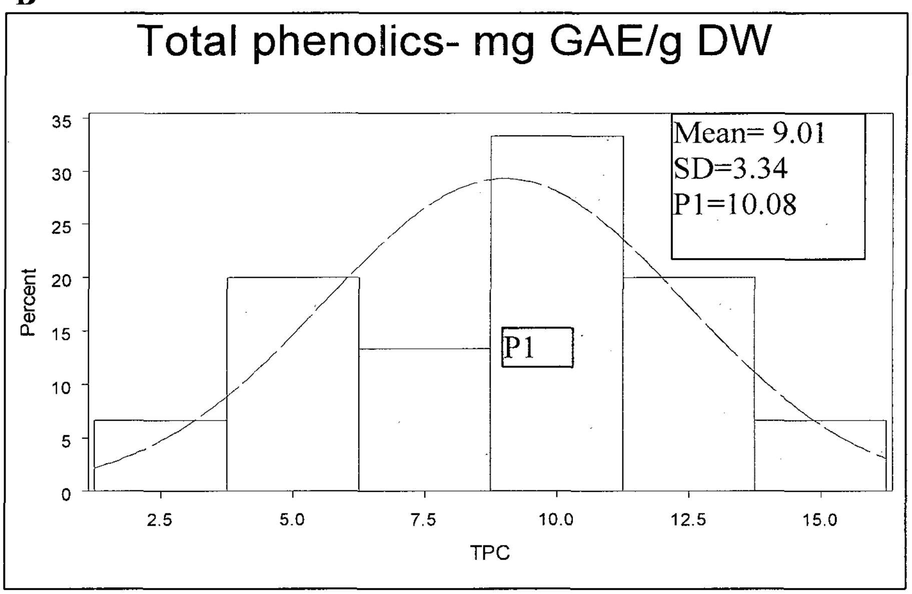
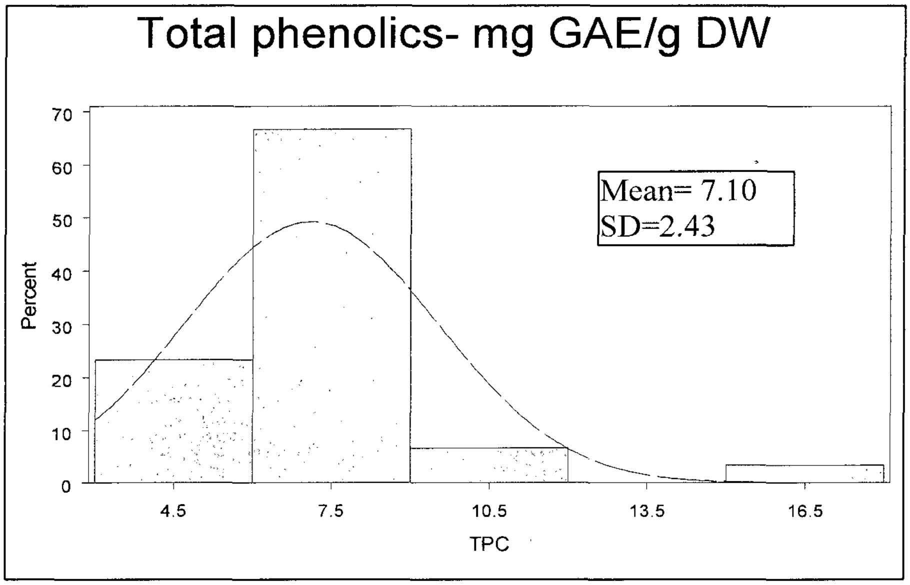


Figure 3.12. Frequency distribution of total phenolic content (TPC) in CO97307 family [Purple Peruvian P/P(♀) x CO94214-1P/P (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. P/P=purple skin/purple flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were >0.150 and >0.150, respectively.

A



B

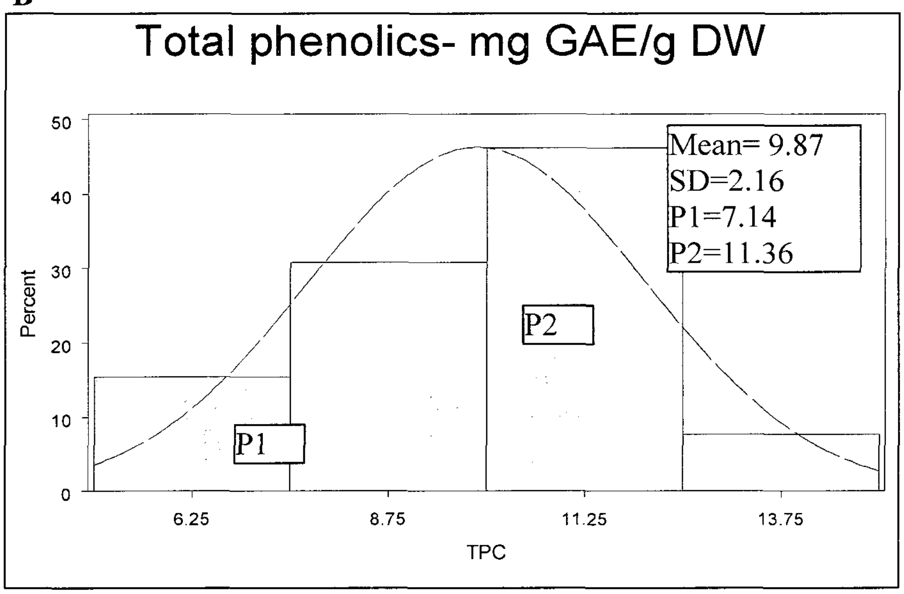
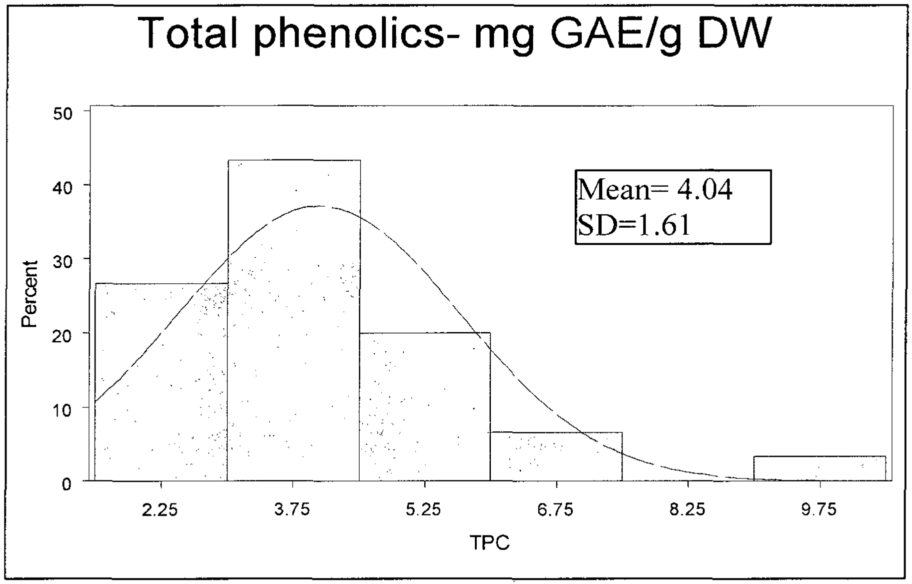


Figure 3.13. Frequency distribution of total phenolic content (TPC) in CO04045 family [CO97215-2P/P (♀) x CO97216-1P/P (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 13 tubers. P/P=purple skin/purple flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were <0.01 and >0.150, respectively.

A



B

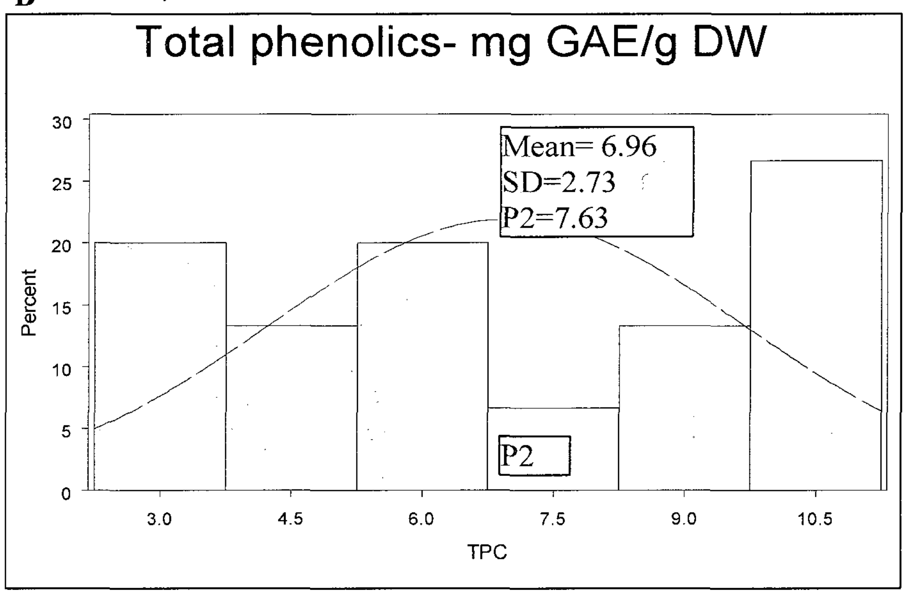
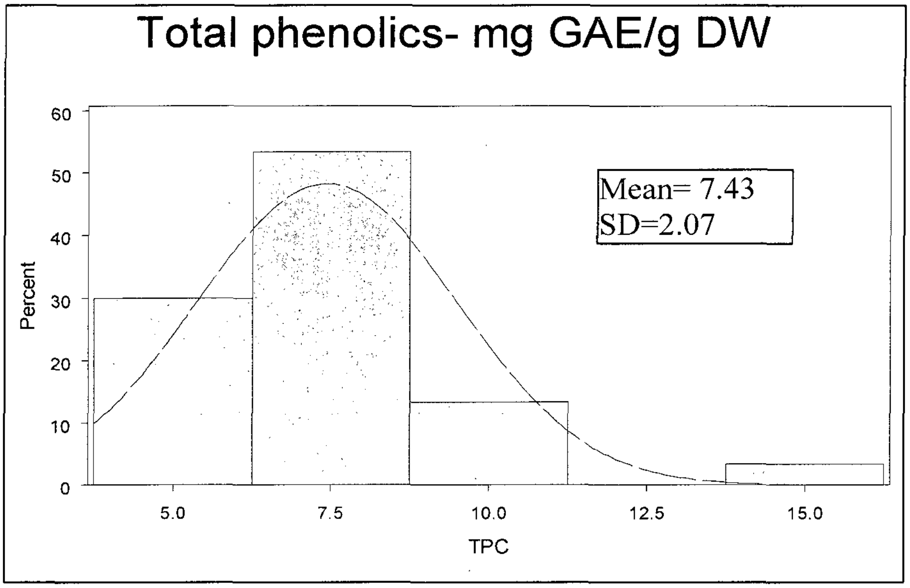


Figure 3.14. Frequency distribution of total phenolic content (TPC) in CO04059 family [CO97219-1R/R (♀) x CO97306-1R/R (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. R/R=red skin/red flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were <0.015 and >0.150, respectively.

A



B

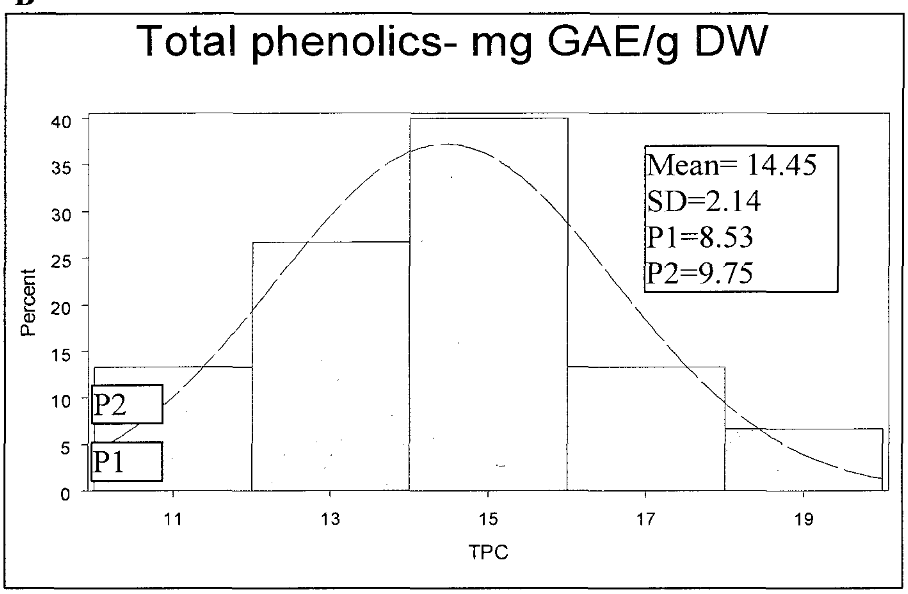


Figure 3.15. Frequency distribution of total phenolic content (TPC) in CO04063 family [CO97226-2R/R (♀) x CO97222-1R/R (♂)] grown in the greenhouse (A) n=30 tubers, and field (B) n= 15 tubers. R/R=red skin/red flesh, percent= percent of tubers, P1 (♀) and P2 (♂) = parent value for TPC if available. SD=Standard deviation. P-value for *Kolmogorov-Smirnov* normality test in GH and field were 0.035 and >0.150, respectively.

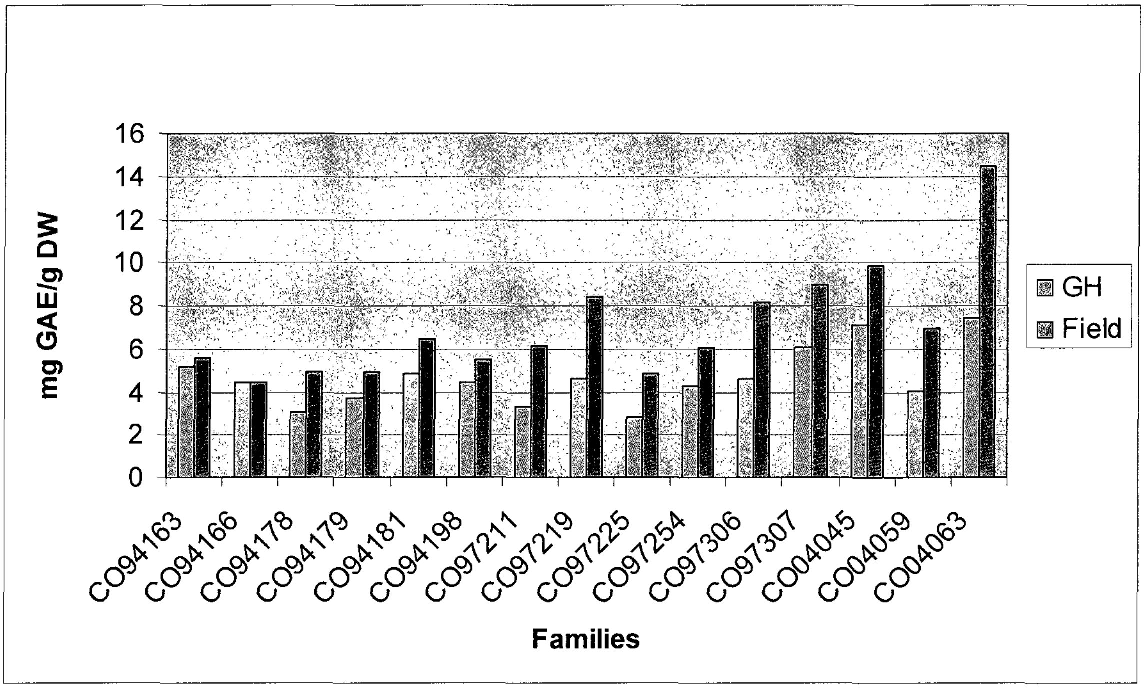


Figure 3.16. Average of total phenolic content (TPC) of tubers for 15 potato families harvested from the greenhouse (GH) and field in SLV, CO in 2007.

Table 3.9. Correlations between total phenolic content (TPC) of tubers harvested from the greenhouse (GH) and field for each potato family.

Families	N	r	P-value
CO94163	15	0.77	0.0008
CO94166	15	0.60	0.0176
CO94178	15	0.76	0.0010
CO94179	15	0.86	< 0.0001
CO94181	14	0.64	0.0142
CO94198	15	0.64	0.0098
CO97211	15	0.71	0.0033
CO97219	15	0.62	0.0130
CO97225	15	0.76	0.0009
CO97254	14	0.49	0.0752
CO97306	15	0.74	0.0016
CO97307	15	0.50	0.0593
CO04045	15	0.58	0.0395
CO04059	13	0.72	0.0024
CO04063	15	-0.13	0.6387

Table 3.10. Correlation between color as measured by MiniScan® XE Plus from HunterLab and total phenolics content (TPC). **L**, **a**, **b** indicate relative hue and value. **"L"** values range from 0 to100 where 0 is black and 100 is white. Positive **"a"** is red and negative **"a"** is green. Positive **"b"** is yellow and negative **"b"** is blue. P= purple flesh, R=Red flesh, W= White flesh, N= number of seedling tubers. Spearman correlation from GraphPad Prism 5 was used to calculate Spearman correlation for all groups except "only purple flesh" calculated by Pearson.

Groups	**Flesh color**			N	**L**	**a**	**b**
					r	r	r
Correlation using all flesh color types from all families.	P	R	W	448	-0.65 P<0.0001	0.58 P<0.0001	-0.56 P<0.0001
Correlation using only purple and white flesh types.		P	W	277	-0.67 P<0.0001	0.55 P<0.0001	-0.55 P<0.0001
Correlation using only red and white flesh types.		R	W	312	-0.66 P<0.0001	0.73 P<0.0001	-0.68 P<0.0001
Correlation using only purple and red flesh types.		P	R	307	-0.48 P<0.0001	0.22 P<0.0001	-0.21 P<0.0003
Correlation using only white flesh types.		W	W	141	-0.06 P>0.05 ns	0.49 P<0.0001	-0.35 P<0.0001
Correlation using only red flesh types.		R	R	171	-0.68 P<0.0001	0.66 P<0.0001	-0.56 P<0.0001
Correlation using only purple flesh types.		P	P	136	-0.38 P<0.0001	-0.54 P<0.0001	0.38 P<0.0001

Table 3.11. Correlations between total phenolic content (TPC) & color as measured by MiniScan® XE Plus within each family alone.

		L	a	b
Families	**N**	**r**	**r**	**r**
CO94163	30	-0.60 P 0.0004	0.58 P 0.0009	-0.55 P 0.0015
CO94166	30	-0.45 P 0.0127	0.54 P 0.0019	-0.29 P 0.122
CO94178	30	-0.50 P 0.0052	0.68 P <0.0001	-0.49 P 0.0061
CO94179	30	-0.63 P 0.0002	0.73 P <0.0001	-0.63 P 0.0002
CO94181	29	-0.42 P 0.0224	0.67 P <0.0001	-0.41 P 0.026
CO94198	30	-0.64 P 0.0001	0.72 P <0.0001	-0.59 P 0.0006
CO97211	30	-0.71 P<0.0001	0.65 P 0.0001	-0.67 P<0.0001
CO97219	30	-0.31 P 0.1002	-0.15 P 0.444	0.09 P 0.61
CO97225	30	-0.18 P 0.344	0.13 P 0.49	-0.34 P 0.063
CO97254	29	-0.73 P<0.0001	0.58 P 0.001	-0.66 P 0.0001
CO97306	30	-0.82 P<0.0001	0.14 P 0.47	-0.30 P 0.1115
CO97307	30	-0.51 P 0.0038	0.12 P 0.54	-0.07 P 0.713
CO04045	30	-0.13 P 0.5005	0.18 P 0.334	0.26 P 0.1674
CO04059	30	-0.79 P<0.0001	0.80 P<0.0001	-0.83 P<0.0001
CO04063	30	-0.47 P 0.0087	0.31 P 0.091	-0.20 P 0.30

DISCUSSION

Cultivated potatoes are tetraploids and are cross-pollinated (Poehlman and Sleper, 1995). A cross of two potato clones will produce a highly heterozygous plant and a highly heterogeneous population. Thus the 448 seedling tubers in this study that were derived from fifteen crosses of specific potato lines and cultivars varied in TPC and flesh color.

Significant variation in TPC among seedling tubers and among families grown in the GH was directly related to genetic difference as all 448 seedling tubers were grown, harvested and analyzed similarly. Similarly, the variation in TPC among tubers and among families grown in the field was related to true genetic variance. The TPC varied among the individual tubers harvested from the GH and field and ranged from 1.74 to 17.94 and 1.82 to 19.38 mg GAE/g DW, respectively. Range of TPC content within each family grown in the GH and field was reported (Table 3.5 and Table 3.6). Analysis of average TPC among the 15 families grown in the GH and among those grown in the field showed significant differences (Table 3.7 and Table 3.8) according to Tukey's Minimum Significant Difference (MSD) test, at $\alpha = 0.05$.

Recent studies on colored potatoes have compared the antioxidant activity among different cultivars at different locations and times. Significant differences in TPC were observed among cultivars in several studies (Lachman et al., 2000; Lewis et al., 1998; Reyes et al., 2005). The genotype was the most important factor that influenced both total phenolics and total anthocyanin content in potato.

A recent study in Texas used specialty (colored) potatoes from the Texas Potato Variety Development Program to evaluate the influence of genotype, location and year on antioxidant activity, total phenolics, total carotenoids, phenolic and carotenoid composition (Reddivari et al., 2007). The study involved 25 potato selections with different skin and flesh colors (red, purple, yellow, and white). Genotype, location and year were all found to have significant effects on antioxidant activity, total phenolics, total carotenoids, and phenolic composition. However, the effect of genotype was greater than location and year effects. Al-Saikhan et al. (1995) did a similar study using several potato cultivars. They reported that variation of total phenolics among cultivars was genotype dependent. Al-Obeidani (2005) also concluded that the variation in total phenolic content among potato cultivars was due more to cultivar than locations, even though locations resulted in highly significant differences.

In this study, the families that had the highest means for TPC in the GH and field (CO04063, CO04045, and CO97307) were derived from crosses of (red-fleshed x red-fleshed), (purple-fleshed x purple-fleshed) and (purple-fleshed x purple-fleshed) parents, respectively. The lowest families in TPC means were derived from crosses of (red-fleshed x white-fleshed) or (purple-fleshed x white-fleshed) parents (Table 3.1). It appeared that greater TPC was found in progeny of crosses between parents with red and/or purple flesh color. This can be helpful for the potato breeding program in the SLV to predict progeny TPC for crosses. However, the CO97225 family, a self of 'Mountain Rose' (red-fleshed tuber), produced a majority of offspring (about 85 %) that had white flesh. This was reasonable since "Mountain Rose" is derived from a cross between red and a white-fleshed parent (All Red, R/R x ND2109-7 W/W).

Many studies have demonstrated that colored cultivars have greater TPC than white or yellow-fleshed cultivars. For example, colored potato cultivars contained three to four times more phenolic acids and twice the concentration of flavonoids than white cultivars (Lewis et al., 1998). The white-fleshed tubers with colored skins had higher concentrations of phenolic acids than those with white flesh and white skin. However, no significant differences were reported in flavonoid concentration between purple and red-fleshed, or between white-fleshed tubers with or without colored skin. The phenolic composition of potato selections with different skin and flesh colors (red, purple, yellow, and white) was also investigated by Reddivari et al. (2007). The purple fleshed color selections were highest in anthocyanins and TPC followed by red- fleshed and yellow-fleshed selections.

In this research, the colored-fleshed potato tubers (purple and red) were higher in TPC as compared to white-fleshed tubers. However, the purple-fleshed tubers were not always higher than the red-fleshed tubers. The results found that the majority of the top 30 tubers in the GH and field for high TPC were red-fleshed (Table.3.3). Furthermore, the highest TPC was noted in the red-fleshed tubers. This is in contrast to Raddivari et al. (2007) who found that the purple-fleshed potatoes were higher in TPC.

Correlation between field and greenhouse regarding total phenolic content

Notable variations were reported between the greenhouse and field regarding production for TPC (Fig. 3.16). In general, most of the families' means and individual tubers had a greater level of TPC in the field when directly compared with the corresponding families' means and individual seedling tubers grown in the greenhouse.

This was expected as the accumulation of phenolic compounds in plant tissues may be induced by different abiotic stresses (Vayda, 1994; Dixon and Paiva, 1995; Chalker-Scott, 1999). For example, location, light, and temperatures have been associated with red radish anthocyanin biosynthesis (Giusti et al., 1998). Variations in content and yield of anthocyanins and TPC were studied during development of purple- and red-flesh potato (*Solanum tuberosum* L.) cultivars grown in Texas and Colorado (Reyes et al., 2004). The anthocyanins and TPC of potato tubers was enhanced when tubers were grown in locations with higher light intensities and lower temperatures. Longer days (higher solar radiation) and cooler temperatures in Colorado favored 2.5 and 1.4-fold increases in anthocyanins and TPC, respectively, when compared to Texas-grown tubers.

More recent studies in Czech Republic by Hamouz et al. (2005; 2006; 2007) on the effect of location, years, and fertilizations on TPC in potato found that site conditions significantly affected TPC in tubers. In all experimental years, the highest TPC was found at the Stachy location which had the highest altitude, the lowest average year temperature and the highest year sum of precipitation in comparison to the other locations.

Expression regulation of anthocyanin biosynthetic genes, responsible for red coloration of apple (Malus x domestica), is believed to be controlled by MYB transcription factors (Ban, et al., 2007). The MYB transcription factor gene (MdMYBA) was isolated from apple skin and characterized. The expression of the gene was dependent on tissue and cultivar/species. It was also found to be induced by UV-B irradiation, low temperature treatment and cauliflower mosaic virus. Lillo et al. (2008) observed that the content of flavonoids increases in response to nitrogen and phosphorus depletion in plants which induce the gene expression of the flavonoid pathway. On the other hand, flavonol synthase is mainly enhanced by high light intensity and sucrose, not mineral depletion.

In this study, GH and field experiments were conducted in the SLV, CO (2347 m above sea level) in 2007. Many environmental factors may have caused the differences in TPC between tubers grown under greenhouse conditions and the corresponding tubers grown in the field. For example, the solar intensity in the greenhouse is approximately 15% less than the field besides the elimination of UV-B by Lexan in the greenhouse (Lexan is a polycarbonate sheet used for covering the greenhouse in the SLV research center, CO). In addition, the day/night temperature differential in the greenhouse (22/21 2± °C, respectively) is lower than the field. According to the Colorado Climate Center, the average of day/night temperatures in Center, CO for May, June, July, August, and September of 2007 were 19.6/2.2°C, 25.9/6.3 °C, 28.2/10.1, 27.4/9.7, and 23.2/5.1, respectively. Moreover, more fertilizer was applied to plants in the greenhouse to mange the growth stages as compared to the field. In addition to these environmental factors, some physiological factors might also be involved. For instance, the seedling tubers harvested from the greenhouse were produced from true potato seeds whereas tubers harvested from the field were produced from seedling tubers previously grown from true seed. So, higher light intensity, UV-B radiation, lower fertilizer rates applied, and large diurnal temperatures in the field were likely factors associated with the differences in TPC observed between the GH and field. The observation that some tubers in the field were lower in TPC might be explained by differential genetic response to the environment as the parents had highly diverse backgrounds.

Correlation between flesh color and total phenolic content

Color of potato tubers is determined by two main classes of pigments, the carotenoids and the anthocyanins. Anthocyanin pigments are responsible for red, purple, and blue flesh and skin color of the tuber, while carotenoid imparts yellow flesh color (Howard, 1970). White and yellow potatoes contain the same composition of carotenoids. However, the yellow potatoes are higher in carotenoid concentration than white potatoes (Brown et al., 1993; Gross, 1991). Other flavonoids, such as flavonols and flavones which have a yellow color, could be found in tiny amounts, but are not likely to have any significant effect on flesh or skin color (Burton, 1989). Therefore, the major contribution to potato tuber color is imparted by anthocyanins.

The correlation of flesh color and TPC was also investigated in this study. The flesh color of 448 seedling tubers derived from 15 families was determined using MiniScan® XE Plus and correlated to their TPC as determined by Folin-Ciocalteu assay (Table 3.6). A moderate correlation was reported between L value and TPC ($r= -0.65$, $P<0.0001$). This implies that the MiniScan® XE Plus may not be a reliable tool for selection or identification of high TPC tubers.

Moreover, the correlation of flesh color and TPC was studied in different relationships in order to get more information about the nature of this correlation. For example, the correlation of flesh color and TPC was conducted using the "L" value within white ($r=-0.06$, $P>0.05$), red (-0.68, $P<0.0001$), and purple fleshed (-0.38, $P<0.0001$) tubers only and within purple and white ($r=-0.67$, $P<0.0001$); red and white ($r=-0.66$, $P<0.0001$); and purple and red ($r=-0.48$, $P<0.0001$) fleshed tubers (Table 3.6). In addition, the correlation between flesh color and TPC was conducted within each family alone (Table 3.7). The correlation varied from r = -0.13 (P = 0.5005) to r = -0.82 (P<0.0001) for "L" value within CO04045 and CO97306 families, respectively.

This low to moderate correlation between TPC and flesh color would seem to indicate the existence of other important phenolic compounds that have a major contribution to TPC of potato tubers and which significantly varied among tubers. Reddivari et al. (2007) investigated the phenolic composition of 25 potato selections with different skin and flesh colors (red, purple, yellow, and white) grown in Texas. Chlorogenic acid, gallic acid, catechin, caffeic acid, and malvidin-3-(p-coumaryl rutinoside)–5-galactoside were the major phenolic compounds, while the major carotenoids identified were lutein and violaxanthin. Chlorogenic acid was found to be the major phenolic acid in potatoes, and contributed 50 – 70% of total phenolics depending on the selection, followed by gallic acid (3 to 18%) and caffeic acid (5 - 10%). Furthermore, Brown (2005) has reported that chlorogenic acid is one of the predominant phenolics compounds in potato and comprises 80% of the total phenolic acids.

Antioxidant properties of cultivars and selections from the Colorado potato breeding program have been investigated for the major phenolic compounds, anthocyanins, and glycolalkaloid content (Stushnoff et al., 2008). The major compounds in the potato extracts were the glycolalkaloids α-solanine and α-chaconine, three chlorogenic acid isomers, and a range of anthocyanins that were only found in the pigmented cultivars. Chlorogenic acid isomers were recognized as the major phenolic compounds present. In addition, chlorogenic acid isomers of pigmented cultivars 'Purple Majesty' and 'Mountain Rose' were about 10-fold higher when compared to 'Yukon Gold'.

In another study that looked at the phenolic acids of potato peels of different cultivars, Onyeneho and Hettiarachchy (1993) found that the phenolic acid composition of different cultivars was not linked to their color (anthocyanin content).The major phenolic acids in all peel samples investigated were chlorogenic, protocatechuic and caffeic acids.

CONCLUSION

Most families' means and individual tubers were considerably higher in TPC when grown in the field as compared to the greenhouse (GH). Progenies of CO0463, CO04045, and CO97307 crosses exhibited high TPC tubers in both GH and field. Since the ranking of families grown in the GH was similar to the field, it is likely that selection among families grown in the GH offers a potential benefit. However, field selection for individual tubers is critical for the identification of truly superior individuals.

Many environmental factors such as high light intensity, UV-B, applied fertilizers, and large diurnal temperatures in SLV, CO may have induced greater TPC production of field harvested tubers.

The maternal effect on TPC was evaluated in the GH and field. Significant maternal effect was only found in the GH with one of the three reciprocal crosses.

Colored-fleshed potato tubers were higher in TPC than white-fleshed tubers. The highest TPC was reported in the red-fleshed tubers. Thus, the purple-fleshed potatoes are not necessarly higher in TPC than red-fleshed as previously indicated by Reddivari et al. (2007).

The correlation of flesh color and TPC was examined. Moderate correlation was observed between flesh color as determined by MiniScan® XE Plus and TPC. This would indicate that the use of MiniScan® XE Plus to select or identify high TPC tubers is not practical.

REFERENCES

Al-Obeidani, J.S., 2005. Antioxidant Properties of Colorado Grown Ppotatoes. PhD Dissertation. Colorado State University, Fort Collins. 118p.

Al-Saikhan, M.S., L.R. Howard, and J.C. Miller, Jr., 1995. Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum* L.). J. Food Sci. 60:341-348.

Al-Saikhan, M. S., 2000. Antioxidants, Proteins, and Carotenoids in Potato (*Solanum tuberosum* L.). Ph.D Dissertation, Texas A&M University, College Station, TX.170p

Arai, Y., S. Watanabe, M. Kimira, K. Shimoi, R. Mochizuki, and N. Kinae. 2000. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J. Nutr. 130:2243-2250.

Ban, Y., C. Honda, Y. Hatsuyama, M. Igarashi, H. Bessho, and T. Moriguchi. 2007. Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red coloration in apple skin. Plant Cell Physiol. 48:958-970.

Brown, C.R., 2005. Antioxidants in potato. Am. J. Pot. Res. 82:163-172.

Brown, C.R., D. Culley, C.P.Yang, R. Durst, and R. Wrolstad. 2005. Variation of anthocyanin and carotenoid contents and associated antioxidant values in potato breeding lines. J. Amer. Soc. Hort. Sci. 130:174-180.

Brown, C.R., C.G. Edwards, C.P. Yang, and B.B. Dean. 1993. Orange flesh trait in potato: Inheritance and carotenoid content. J. Am. Soc. Hort. Sci. 118:145-150.

Burton, W.G., 1989. The Potato. 3rd ed. Longman Scientific Technical. New York, USA. 742p.

Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70:1-9.

Chinnici, F., A. Bendini, A. Gaiani, and C. Riponi. 2004. Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. J. Agric. Food Chem. 52:4684-4689.

Dixon, R.A., and N.L. Paiva. 1995. Stress-induced phenylpropanoid metabolism. Plant cell 7:1085-1097.

Friedman, M. 1997. Chemistry, biochemistry, and dietary role of potato polyphenols. A review. J. Agric. Food Chem. 45:1523-1540.

Giusti, M.M., L.E. Rodriguez-Saona, J.R. Baggett, G.L. Reed, R.W. Durst, and R.E. Wrolstad. 1998. Anthocyanin pigment composition of red radish cultivars as potential food colorants. J. Food Sci. 63:219-224.

Gomes, C.A., T.G. da Cruz, J.L. Andrade, N. Milhazes, F. Borges, and M.P. Marques. 2003. Anticancer activity of phenolic acids of natural or synthetic origin: A structure-activity study. J. Med. Chem. 46:5395-5401.

Gross, J. 1991. Pigments in Vegetables: Chlorophylls and Carotenoid.Van Nostrand Reinhold, New York. 351p.

Hale, A., 2003. Screening Potato Genotypes for Antioxidant Activity, Identification of the Responsible Compounds, and Differentiating Russet Norkotah Strains Using AFLP and microsatellite marker analysis. Ph.D Dissertation, Texas A&M University, College Station, TX. P245.

Hamouz, K., J. Lachman, P. Dvořák, and V. Pivec. 2005. The effect of ecological growing on the potatoes yield and quality. Plant Soil Environ. 51:397–402.

Hamouz, K., J. Lachman, P. Dvořak , and V. Pivec. 2006. The effect of site conditions, variety and fertilizationon the content of polyphenols in potato tubers. Plant Soil Environ. 52:407–412.

Hamouz, K., J. Lachman, J. Čepl, P. Dvořák, V. Pivec, and M. Prášilová. 2007. Site conditions and genotype influence polyphenol content in potatoes. Hort. Sci. 34:132–137.

Haynes, K.G., J.B. Sieczka, M.R. Henninger, D.L. Fleck. 1996. Clone x environment interactions for yellow-flesh intensity in tetraploid potatoes. J. Amer. Soc. Hort. Sci. 121: 175-177.

Ho, E., T.W. Boileau, and T.M. Bray. 2004. Dietary influences on endocrine-inflammatory interactions in prostate cancer development. Arch. Biochem. Biophys. 428: 109-117.

Howard, H.W. 1970. Genetics of potato: Fertility and sterility problems. Logo Press, London.

Külen, O. 2005. Potato minituber bud dormancy release. Ph.D Dissertation, Colorado State University, Fort Collins. 130p.

Lachman, J., K. Hamouz, M. Orsak, and V. Pivec. 2000. Potato tubers as a significant source of antioxidants in human nutrition. Rost. Vyroba. 46: 231-236.

Lewis, C.E., J.R.L. Walker, J.E. Lancaster, K.H. Sutton, 1998. Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of Solanum tuberosum L. J. Sci. Food Agric. 77:45-57.

Lillo, C., U.S. Lea, and P. Ruoff. 2008. Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway. Plant Cell Environ. 31:587-601.

Lister, C.E. 1999. The medicine of life. Vitamins and other bioactive food components. Food Technol. 34:18-20, 35.

Lister, C.E. 2003. Antioxidants a Health Revolution. NewZeland Institute for Crop Food Research Limited, Private Bag 4704, Christchurch, New Zeland. 98p.

Onyeneho, S.N. and N.S. Hettiarachchy. 1993. Antioxidant activity fatty acids and phenolic acids compositions of potato peels. J. Sci. Food Agric. 62:345-350.

Poehlman, J.M. and D.A. Sleper. 1995. Breeding field crops. 4th.ed. Iowa State University Press. 494p.

Reddivari, L., A.L. Hale, and J.C. Miller, Jr., 2007. Genotype, location, and year influence antioxidant activity, carotenoid content, phenolic content, and composition in specialty potatoes. J. Agric. Food Chem. 55:8073-8079.

Reyes, L.F. and L. Cisneros-Zevallos. 2003. Wounding stress increases the phenolics content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum* L.). J. Agric. Food Chem. 51:5296-5300.

Reyes, L. F., J.C. Miller, and L. Cisneros-Zevallos. 2004. Environmental conditions influence the content and yield of anthocyanins and total phenolics in purple- and red-flesh potatoes during tuber development. Am. J. Pot. Res. 81:187-193.

Reyes, L.F., J.C. Miller, Jr., and L. Cisneros-Zevallos. 2005.Antioxidant capacity, anthocyanins and total phenolics in purple-and red-fleshed potato (*Solanum tuberosum* L.) genotypes. Am. J. Pot. Res. 82:271-277.

Singleton, V.L. and J.A. Rossi, Jr. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 16:144-158.

Spanos, G.A. and R.E. Wrolstad. 1990. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. J. Agric. Food Chem. 38:1565-1571.

Stushnoff, C., D. Holm, M.D. Thompson, W. Jiang, H.J. Thompson, N.I. Joyce, and P. Wilson. 2008. Antioxidant properties of cultivars and selections from the Colorado Potato Breeding Program. Am. J. Pot. Res. 85:267-276.

Vayda, M.E. 1994. Environmental stress and its impact on potato yield. In: JE Bradshaw, GR Mackay (eds), Potato Genetics, CAB International, Wallingford, UK. pp. 239-261.

CHAPTER 4

SUMMARY

Greenhouse study

In this study, 448 individual seedling tubers derived from 15 families were harvested from the greenhouse and TPC was quantified. The TPC varied among individual tubers and ranged from 1.74 to 17.94 mg GAE/g DW. In addition, means for TPC of 15 potato families were significantly different among families (Table 3.7). CO04063, CO04045 and CO97307 had the highest mean for TPC (7.43, 7.1, and 6.07 mg GAE/g DW), respectively. The lowest TPC means were observed in CO97225, CO97211, and CO94178 families, respectively, with no significant difference among them.

The maternal effect on TPC has been evaluated in the GH and field. Significant maternal effect was found in the GH with only one of the reciprocal crosses.

Correlation of flesh color as determined by MiniScan® XE Plus and total phenolic content of 448 seedling tubers was examined. Moderate correlation between flesh color and TPC was observed ($r = -0.65$ $P<0.0001$ for "L" value). This means that the MiniScan® XE Plus cannot be a reliable for selection of tubers with high TPC.

Field study

A total of 223 individual tubers derived from the 15 families were grown and harvested in the field in the San Luis Valley in Colorado. The TPC varied among these individual tubers and ranged from 1.82 to 19.38 mg GAE/g DW. CO04063, CO04045 and CO97307 had the highest mean for TPC (14.45, 9.88, and 9.01 mg GAE/g DW), respectively. In contrast , the lowest TPC means were observed in CO94166, CO97225, and CO94178 families, respectively, with no significant difference among them (Table 3.8). Most of the families' means and individual tubers exhibited a remarkable increase in TPC when compared with corresponding families' means and seedling tubers grown in the greenhouse. A total of 178 of the 233 tubers (about 80%) grown in the field were from 10 to as much as 300% greater in TPC. Approximately 9 % of the field tubers were 10 to 59% lower in TPC when compared to the corresponding seedling tubers grown in the greenhouse. Some environmental factors such as high light intensity, UV-B radiation, lower fertilizer rates applied, and large diurnal temperatures in SLV, CO were likely the factors associated with the differences in TPC observed between the GH and field.

A high correlation was determined between the means of families in the greenhouse and field ($r= 0.83$, $P= 0.0002$). Moreover, the highest three families for TPC in the greenhouse were identical to those grown in the field. Families with the lowest TPC were similar in field and greenhouse except that CO97211 (C7) was switched with CO94166 (C2) in the field. However, there was no significant difference in TPC means of these two families in the field. Correlation of TPC of all tubers harvested from the field with corresponding seedling tubers grown in the greenhouse was $r= 0.71$, $P <0.0001$. In addition, the correlations between TPC of individual tubers grown in the greenhouse and field within each family varied between $r = -0.13$ ($P = 0.6387$) to 0.86 ($P< 0.0001$) in CO04063 and CO94179, respectively (Table 3.9). This would imply that selection of individual tubers for high TPC within families is better done in the field. However, TPC of progeny in the greenhouse can be used to select among families.

Colored-fleshed potato tubers were higher in TPC than white-fleshed tubers. The highest TPC were reported in red-fleshed tubers in the greenhouse and field. Thus, the purple-fleshed potatoes are not necessarily higher in TPC than red-fleshed as previously indicated by Raddivari et al. (2007).

APPENDIX

Table 1. Color of potato flesh as measured by MiniScan® XE Plus from HunterLab and total phenolics (TPC) determined by Folin Ciocalteu for all seedling harvested from the greenhouse. **L**, **a**, **b** indicate relative hue and value. **L** values range from 0 to100 where 0 is black and 100 is white. Positive **a** is red and negative **a** is green. Positive **b** is yellow and negative **b** is blue. Aver= four readings average of flesh color. SD=standard deviation of the 4 readings of flesh color. TPC= total phenolic.

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO94163-1	L	28.73	3.12	6.94		CO94166-1	L	47.82	2.94	3.59
	a	10.18	1.06				a	4.73	1.68	
	b	-4.30	1.45				b	1.21	2.03	
CO94163-2	L	52.07	3.52	4.70		CO94166-2	L	59.81	1.65	3.74
	a	6.48	2.72				a	1.86	0.84	
	b	5.37	2.14				b	6.72	0.41	
CO94163-3	L	42.44	0.96	5.77		CO94166-3	L	39.26	1.69	4.79
	a	12.42	0.90				a	12.65	0.62	
	b	0.27	0.29				b	-5.62	0.89	
CO94163-4	L	60.58	0.45	4.70		CO94166-4	L	56.64	0.44	3.29
	a	0.14	0.63				a	-0.56	0.10	
	b	8.64	0.29				b	7.30	0.27	
CO94163-5	L	56.05	1.51	3.91		CO94166-5	L	54.87	1.20	3.73
	a	-0.26	0.11				a	0.90	0.32	
	b	9.06	0.38				b	6.22	0.30	
CO94163-6	L	42.38	2.09	6.26		CO94166-6	L	32.61	3.26	4.98
	a	12.34	0.92				a	10.54	0.36	
	b	0.41	0.36				b	-5.11	0.70	
CO94163-7	L	24.98	1.64	5.64		CO94166-7	L	33.91	1.53	5.90
	a	10.84	0.78				a	11.22	0.57	
	b	-5.93	0.61				b	-6.21	0.70	
CO94163-8	L	58.51	0.44	4.94		CO94166-8	L	56.62	1.76	4.69
	a	-0.42	0.18				a	0.11	0.45	
	b	7.86	0.56				b	7.22	0.29	
CO94163-9	L	16.51	1.13	7.41		CO94166-9	L	58.89	0.66	4.68
	a	7.08	0.61				a	-0.67	0.03	
	b	-4.50	0.41				b	7.94	0.33	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO94163-10	L	58.82	2.33	3.81		CO94166-10	L	61.49	0.52	4.00
	a	0.37	0.65				a	-0.52	0.01	
	b	7.61	1.07				b	8.61	0.10	
CO94163-11	L	41.16	3.10	4.12		CO94166-11	L	46.20	1.28	7.40
	a	11.28	1.13				a	10.05	1.44	
	b	-4.41	1.72				b	2.44	0.66	
CO94163-12	L	31.62	2.87	4.51		CO94166-12	L	63.94	0.19	4.69
	a	11.83	0.23				a	-0.23	0.18	
	b	-6.67	0.42				b	8.28	0.48	
CO94163-13	L	57.10	0.38	4.81		CO94166-13	L	40.22	0.21	5.91
	a	1.81	0.58				a	14.29	0.90	
	b	6.16	0.07				b	-0.88	0.44	
CO94163-14	L	57.01	0.61	3.90		CO94166-14	L	56.58	0.58	3.01
	a	0.27	0.27				a	0.14	0.12	
	b	6.04	0.48				b	5.90	0.25	
CO94163-15	L	54.45	1.71	3.90		CO94166-15	L	56.35	1.37	5.44
	a	5.36	1.01				a	-0.16	0.18	
	b	5.50	0.32				b	8.12	0.35	
CO94163-16	L	42.02	5.95	10.24		CO94166-16	L	45.90	0.43	6.05
	a	8.47	0.57				a	11.54	0.83	
	b	-2.83	1.51				b	2.30	0.92	
CO94163-17	L	58.92	0.43	4.39		CO94166-17	L	58.88	2.55	3.38
	a	0.87	0.38				a	-0.27	0.14	
	b	7.29	0.47				b	7.76	0.12	
CO94163-18	L	53.03	2.94	4.20		CO94166-18	L	59.15	1.75	4.95
	a	3.47	2.56				a	-0.20	0.05	
	b	4.66	1.98				b	8.17	0.43	
CO94163-19	L	62.38	1.46	4.86		CO94166-19	L	60.57	1.18	3.29
	a	-0.02	0.23				a	-0.53	0.05	
	b	7.96	0.43				b	6.43	0.42	
CO94163-20	L	44.18	0.42	7.36		CO94166-20	L	60.65	1.01	4.65
	a	11.53	0.43				a	-0.57	0.12	
	b	0.57	0.33				b	8.33	0.13	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO94163-21	L	61.17	0.92	4.73		CO94166-21	L	53.07	1.46	4.30
	a	-0.59	0.08				a	0.20	0.73	
	b	8.85	0.47				b	6.70	0.46	
CO94163-22	L	57.30	1.33	5.42		CO94166-22	L	49.09	0.98	5.35
	a	0.44	0.19				a	9.50	0.51	
	b	7.99	0.57				b	2.52	0.44	
CO94163-23	L	48.48	0.69	5.62		CO94166-23	L	41.87	1.31	5.93
	a	5.14	0.85				a	12.73	0.13	
	b	3.82	0.37				b	-0.22	0.67	
CO94163-24	L	59.05	1.49	4.06		CO94166-24	L	57.88	2.29	3.86
	a	-0.49	0.10				a	-0.42	0.13	
	b	7.68	0.41				b	6.43	0.34	
CO94163-25	L	20.42	0.54	8.42		CO94166-25	L	59.26	1.57	3.85
	a	9.08	0.99				a	-0.25	0.14	
	b	-5.19	0.73				b	8.09	0.35	
CO94163-26	L	58.37	1.44	4.11		CO94166-26	L	28.54	0.91	3.71
	a	1.51	0.69				a	10.99	0.46	
	b	6.97	1.37				b	-7.43	0.31	
CO94163-27	L	38.36	12.97	5.40		CO94166-27	L	61.32	1.84	3.05
	a	11.09	0.41				a	-0.18	0.18	
	b	-5.08	0.72				b	7.75	0.54	
CO94163-28	L	61.69	2.07	3.81		CO94166-28	L	41.76	2.37	4.67
	a	-0.47	0.17				a	5.15	0.99	
	b	8.04	0.14				b	0.20	1.56	
CO94163-29	L	57.26	1.63	4.23		CO94166-29	L	36.57	1.82	6.05
	a	-0.23	0.12				a	15.61	0.32	
	b	7.51	0.13				b	-0.19	0.90	
CO94163-30	L	62.64	0.89	4.03		CO94166-30	L	52.95	1.89	2.81
	a	-0.61	0.11				a	1.75	0.44	
	b	10.15	0.18				b	5.39	0.31	
CO94178-1	L=	46.76	1.55	2.19		CO94179-1	L=	57.39	1.00	2.83
	a=	6.11	1.29				a=	-0.43	0.07	
	b=	-0.50	1.21				b=	6.82	0.08	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO94178-2	L=	37.83	3.18	2.87		CO94179-2	L=	27.25	0.77	3.95
	a=	10.56	1.05				a=	10.63	0.31	
	b=	-4.48	1.56				b=	-6.23	0.18	
CO94178-3	L=	57.15	2.90	1.95		CO94179-3	L=	59.68	0.64	2.49
	a=	-0.65	0.22				a=	-0.49	0.16	
	b=	8.33	0.97				b=	7.48	0.36	
CO94178-4	L=	63.40	1.32	2.68		CO94179-4	L=	25.74	1.11	4.89
	a=	-0.60	0.21				a=	11.08	1.20	
	b=	12.37	0.40				b=	-6.80	1.31	
CO94178-5	L=	54.49	0.79	2.35		CO94179-5	L=	55.17	0.52	4.24
	a=	-0.65	0.06				a=	0.93	0.39	
	b=	8.27	0.31				b=	5.74	0.23	
CO94178-6	L=	33.91	2.34	3.68		CO94179-6	L=	26.01	0.88	3.81
	a=	11.43	0.73				a=	10.39	0.51	
	b=	-5.78	1.54				b=	-6.02	0.59	
CO94178-7	L=	49.60	1.26	3.80		CO94179-7	L=	52.14	0.57	3.40
	a=	8.67	1.06				a=	-0.43	0.28	
	b=	4.01	1.63				b=	6.35	0.15	
CO94178-8	L=	59.26	0.98	1.89		CO94179-8	L=	61.07	0.33	3.20
	a=	-0.78	0.06				a=	-0.29	0.06	
	b=	8.60	0.21				b=	7.83	0.13	
CO94178-9	L=	54.96	2.52	2.75		CO94179-9	L=	52.12	0.91	4.78
	a=	0.58	0.53				a=	6.87	0.72	
	b=	7.91	0.43				b=	4.05	0.27	
CO94178-10	L=	23.14	1.15	2.87		CO94179-10	L=	55.10	1.23	3.11
	a=	10.37	0.54				a=	2.69	0.48	
	b=	-5.80	0.44				b=	5.90	0.29	
CO94178-11	L=	46.44	1.24	3.08		CO94179-11	L=	34.08	1.06	4.45
	a=	11.96	0.46				a=	11.62	0.40	
	b=	0.05	0.84				b=	-8.23	0.28	
CO94178-12	L=	58.11	0.43	3.07		CO94179-12	L=	53.39	2.43	2.90
	a=	0.79	0.32				a=	6.02	1.88	
	b=	7.91	0.09				b=	3.53	3.68	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO94178-13	L=	35.65	1.02	3.09		CO94179-13	L=	61.96	0.75	3.66
	a=	11.18	0.49				a=	-0.34	0.14	
	b=	-5.95	0.82				b=	7.86	0.41	
CO94178-14	L=	57.08	1.06	3.23		CO94179-14	L=	25.60	0.72	4.18
	a=	0.01	0.16				a=	11.67	0.45	
	b=	9.20	0.20				b=	-6.32	0.21	
CO94178-15	L=	53.80	1.23	2.66		CO94179-15	L=	52.85	1.28	3.48
	a=	3.32	0.87				a=	2.97	0.70	
	b=	6.02	0.58				b=	5.20	0.52	
CO94178-16	L=	30.28	2.46	2.88		CO94179-16	L=	42.96	2.03	3.67
	a=	10.39	0.36				a=	6.20	1.20	
	b=	-5.03	0.59				b=	-1.32	1.24	
CO94178-17	L=	58.15	0.88	2.20		CO94179-17	L=	55.56	1.49	2.30
	a=	-0.27	0.16				a=	-0.47	0.08	
	b=	8.03	0.13				b=	6.78	0.19	
CO94178-18	L=	59.66	1.20	2.52		CO94179-18	L=	60.36	1.16	2.95
	a=	-0.51	0.08				a=	-0.40	0.22	
	b=	7.91	0.13				b=	7.86	0.35	
CO94178-19	L=	53.28	0.32	3.81		CO94179-19	L=	58.24	1.05	2.83
	a=	9.42	1.20				a=	2.29	1.10	
	b=	3.13	0.72				b=	4.22	1.03	
CO94178-20	L=	24.90	0.99	4.22		CO94179-20	L=	35.60	0.69	4.50
	a=	12.15	1.11				a=	9.26	0.38	
	b=	-6.67	1.56				b=	-6.32	0.31	
CO94178-21	L=	46.99	2.30	2.88		CO94179-21	L=	26.60	1.83	4.43
	a=	6.90	0.91				a=	10.63	0.43	
	b=	0.13	1.18				b=	-7.75	0.56	
CO94178-22	L=	35.95	1.13	3.96		CO94179-22	L=	56.30	1.01	4.37
	a=	10.92	0.68				a=	-0.11	0.17	
	b=	-5.26	0.73				b=	7.31	0.28	
CO94178-23	L=	60.21	0.80	2.29		CO94179-23	L=	40.95	0.83	4.84
	a=	-0.52	0.09				a=	12.92	0.61	
	b=	8.21	0.21				b=	1.43	0.87	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO94178-24	L=	59.40	1.09	3.35		CO94179-24	L=	39.90	1.08	4.28
	a=	-0.51	0.14				a=	10.63	0.64	
	b=	9.50	0.24				b=	-6.04	0.94	
CO94178-25	L=	62.38	0.78	2.88		CO94179-25	L=	51.02	0.65	2.56
	a=	-0.35	0.14				a=	5.06	0.20	
	b=	7.38	0.07				b=	0.07	0.35	
CO94178-26	L=	60.85	0.34	2.62		CO94179-26	L=	51.12	0.72	3.85
	a=	-0.65	0.10				a=	7.31	0.29	
	b=	9.25	0.37				b=	3.49	0.33	
CO94178-27	L=	57.85	0.50	3.51		CO94179-27	L=	34.60	2.17	3.26
	a=	-0.53	0.07				a=	10.14	0.72	
	b=	8.67	0.39				b=	-5.56	0.39	
CO94178-28	L=	30.54	1.64	4.05		CO94179-28	L=	25.08	0.91	3.94
	a=	12.88	0.66				a=	12.42	0.42	
	b=	-6.05	0.44				b=	-7.91	0.57	
CO94178-29	L=	27.24	0.61	5.24		CO94179-29	L=	25.76	1.28	5.26
	a=	12.19	0.75				a=	11.91	0.46	
	b=	-7.52	0.46				b=	-7.98	0.41	
CO94178-30	L=	54.85	1.60	4.23		CO94179-30	L=	58.60	1.44	2.90
	a=	3.53	0.61				a=	-0.01	0.05	
	b=	7.63	1.08				b=	7.93	0.21	
CO94181-1	L	44.84	1.32	5.53		CO94198-1	L	64.13	1.39	3.58
	a	9.20	0.37				a	0.11	0.41	
	b	-1.53	2.36				b	7.70	0.60	
CO94181-2	L	47.24	0.80	4.07		CO94198-2	L	30.00	3.14	4.75
	a	8.63	1.03				a	12.72	1.32	
	b	1.88	0.65				b	-6.96	1.42	
CO94181-3	L	54.28	0.44	3.35		CO94198-3	L	27.20	0.81	5.27
	a	0.47	0.14				a	11.05	0.85	
	b	8.67	0.33				b	-6.39	0.90	
CO94181-4	L	41.29	1.26	6.70		CO94198-4	L	54.15	0.74	2.95
	a	17.08	0.41				a	3.79	1.09	
	b	-0.68	0.85				b	4.42	0.43	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO94181-5	L	38.03	1.98	6.51		CO94198-5	L	60.48	1.28	3.21
	a	9.97	0.99				a	0.15	0.21	
	b	-4.45	0.80				b	8.02	0.23	
CO94181-6	L	39.96	0.30	3.77		CO94198-6	L	60.74	1.01	3.31
	a	11.75	0.33				a	0.37	0.21	
	b	-4.86	0.31				b	8.26	0.51	
CO94181-7	L	x	x	x		CO94198-7	L	51.45	1.09	4.45
	a	x	x				a	8.24	1.86	
	b	x	x				b	3.36	0.95	
CO94181-8	L	60.55	1.15	4.11		CO94198-8	L	28.04	2.61	6.01
	a	-0.53	0.13				a	10.89	0.37	
	b	10.15	0.51				b	-5.95	0.56	
CO94181-9	L	58.10	0.59	2.60		CO94198-9	L	54.16	1.17	2.52
	a	-0.18	0.28				a	-0.59	0.12	
	b	8.06	0.77				b	8.67	0.26	
CO94181-10	L	62.63	0.82	3.52		CO94198-10	L	43.88	0.56	7.04
	a	-0.91	0.04				a	12.34	0.72	
	b	10.22	0.18				b	-0.08	0.43	
CO94181-11	L	31.14	0.77	4.17		CO94198-11	L	26.74	0.56	5.67
	a	11.72	0.35				a	12.86	0.09	
	b	-6.79	0.62				b	-8.62	0.12	
CO94181-12	L	40.97	1.42	7.41		CO94198-12	L	25.07	1.06	4.80
	a	13.45	2.76				a	12.45	0.55	
	b	1.14	1.23				b	-7.50	0.26	
CO94181-13	L	60.06	0.26	5.21		CO94198-13	L	63.78	0.83	3.18
	a	-0.08	0.06				a	-0.72	0.04	
	b	8.05	0.44				b	9.48	0.15	
CO94181-14	L	39.30	0.68	5.48		CO94198-14	L	25.57	1.40	4.92
	a	14.89	1.27				a	11.35	0.46	
	b	-1.44	0.94				b	-7.07	0.22	
CO94181-15	L	63.29	0.57	4.43		CO94198-15	L	30.01	1.85	5.27
	a	0.38	0.34				a	10.36	1.36	
	b	8.77	0.54				b	-5.48	1.26	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO94181-16	L	56.89	0.89	3.81		CO94198-16	L	32.99	1.48	5.15
	a	2.72	0.88				a	12.71	0.37	
	b	4.00	0.70				b	-7.18	0.40	
CO94181-17	L	52.01	0.73	5.82		CO94198-17	L	63.94	0.27	3.02
	a	8.72	0.57				a	-0.71	0.03	
	b	4.31	0.51				b	8.17	0.08	
CO94181-18	L	57.47	0.68	3.60		CO94198-18	L	57.30	0.83	4.98
	a	-0.47	0.14				a	1.66	0.92	
	b	7.80	0.27				b	6.43	0.45	
CO94181-19	L	17.99	1.28	4.83		CO94198-19	L	26.45	2.07	6.17
	a	8.57	0.95				a	10.85	0.43	
	b	-5.45	0.49				b	-6.31	0.11	
CO94181-20	L	38.70	1.64	4.17		CO94198-20	L	38.57	0.39	6.87
	a	7.61	0.54				a	17.80	0.25	
	b	-0.88	0.56				b	-1.89	0.09	
CO94181-21	L	38.00	0.69	6.59		CO94198-21	L	48.78	0.50	5.49
	a	16.13	1.01				a	10.60	0.35	
	b	-0.58	0.95				b	2.65	0.47	
CO94181-22	L	60.60	0.70	6.10		CO94198-22	L	42.79	0.41	4.42
	a	0.44	0.36				a	11.76	0.85	
	b	7.64	0.34				b	0.11	0.24	
CO94181-23	L	54.68	1.09	3.85		CO94198-23	L	64.28	0.18	3.18
	a	7.24	0.86				a	-0.45	0.09	
	b	4.93	0.31				b	8.28	0.51	
CO94181-24	L	55.79	1.11	3.15		CO94198-24	L	37.35	2.36	3.23
	a	-0.57	0.14				a	10.19	0.86	
	b	7.98	0.22				b	-6.24	1.17	
CO94181-25	L	51.14	0.53	6.08		CO94198-25	L	63.06	0.50	3.05
	a	4.49	1.14				a	-0.46	0.20	
	b	5.77	0.90				b	8.79	0.19	
CO94181-26	L	54.48	2.82	4.65		CO94198-26	L	41.59	1.68	3.51
	a	5.48	1.28				a	9.18	1.09	
	b	4.53	1.44				b	-3.27	0.76	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO94181-27	L	51.49	1.46	4.64		CO94198-27	L	51.33	0.33	4.08
	a	6.39	0.19				a	9.75	0.34	
	b	5.83	0.39				b	2.19	0.19	
CO94181-28	L	62.53	0.82	2.93		CO94198-28	L	28.99	0.62	5.08
	a	-0.69	0.08				a	12.06	0.20	
	b	8.34	0.40				b	-6.48	0.16	
CO94181-29	L	40.14	0.59	7.61		CO94198-29	L	48.02	3.09	3.91
	a	15.16	1.05				a	7.64	1.01	
	b	-0.23	0.54				b	0.16	1.32	
CO94181-30	L	50.00	0.71	4.75		CO94198-30	L	54.61	1.56	5.25
	a	6.52	0.48				a	-0.58	0.09	
	b	4.02	0.34				b	7.88	0.49	
CO97211-1	L	48.79	0.65	3.49		CO97219-1	L	24.06	0.53	4.00
	a	7.56	1.37				a	12.02	0.11	
	b	2.68	0.42				b	-7.53	0.11	
CO97211-2	L	42.22	0.68	3.80		CO97219-2	L	47.49	0.77	2.52
	a	14.94	0.25				a	10.45	0.61	
	b	0.56	0.30				b	2.47	0.57	
CO97211-3	L	56.99	1.25	2.46		CO97219-3	L	19.74	0.96	3.42
	a	0.31	0.38				a	9.80	0.25	
	b	6.48	0.05				b	-5.67	0.21	
CO97211-4	L	54.17	1.01	3.16		CO97219-4	L	22.55	3.60	6.38
	a	1.15	0.26				a	9.30	0.54	
	b	6.18	0.22				b	-6.17	0.39	
CO97211-5	L	49.04	1.47	3.45		CO97219-5	L	20.81	0.11	4.43
	a	5.68	0.88				a	8.96	0.19	
	b	4.23	0.52				b	-5.61	0.15	
CO97211-6	L	44.20	0.50	4.33		CO97219-6	L	52.04	5.48	5.79
	a	16.06	0.41				a	9.63	3.52	
	b	0.06	0.32				b	2.66	1.88	
CO97211-7	L	49.81	0.59	3.40		CO97219-7	L	18.41	0.43	4.15
	a	9.39	0.76				a	9.24	0.16	
	b	2.46	0.29				b	-5.84	0.21	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO97211-8	L	61.12	0.84	2.60		CO97219-8	L	51.73	0.56	3.27
	a	-0.59	0.05				a	9.99	0.51	
	b	7.45	0.27				b	2.10	0.59	
CO97211-9	L	47.16	0.50	3.99		CO97219-9	L	45.05	0.68	3.78
	a	5.86	0.35				a	9.53	0.66	
	b	4.12	0.12				b	1.98	0.25	
CO97211-10	L	55.08	0.71	3.10		CO97219-10	L	40.95	3.58	3.60
	a	4.98	0.15				a	10.75	0.45	
	b	5.39	0.22				b	-4.19	0.87	
CO97211-11	L	45.71	1.13	3.74		CO97219-11	L	21.06	4.42	5.14
	a	11.42	0.48				a	9.69	0.87	
	b	2.57	0.59				b	-5.89	0.29	
CO97211-12	L	53.88	0.50	3.16		CO97219-12	L	30.80	0.87	2.84
	a	4.97	0.39				a	12.99	0.31	
	b	4.54	0.05				b	-7.12	0.27	
CO97211-13	L	57.43	0.75	2.66		CO97219-13	L	20.38	0.64	5.03
	a	-0.28	0.19				a	10.56	0.37	
	b	7.75	0.41				b	-6.88	0.30	
CO97211-14	L	61.91	0.24	2.33		CO97219-14	L	39.57	1.22	4.95
	a	-0.13	0.20				a	17.00	0.24	
	b	8.35	0.27				b	-1.50	0.49	
CO97211-15	L	47.63	2.33	3.44		CO97219-15	L	29.77	1.19	2.74
	a	12.67	2.16				a	13.91	0.67	
	b	0.98	1.42				b	-7.32	0.42	
CO97211-16	L	44.07	0.69	4.77		CO97219-16	L	14.46	0.21	6.68
	a	11.45	0.58				a	4.88	0.17	
	b	1.65	0.43				b	-3.65	0.18	
CO97211-17	L	58.97	0.45	2.25		CO97219-17	L	27.58	0.72	8.29
	a	-0.46	0.03				a	19.83	0.99	
	b	7.05	0.10				b	-2.13	0.74	
CO97211-18	L	57.24	0.23	2.73		CO97219-18	L	33.30	0.66	3.04
	a	-11.10	21.47				a	14.46	0.73	
	b	6.50	0.09				b	-7.64	0.61	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO97211-19	L	55.28	0.99	3.12		CO97219-19	L	41.79	1.04	4.57
	a	0.24	0.24				a	16.18	0.64	
	b	7.09	0.09				b	-0.47	0.31	
CO97211-20	L	48.12	0.75	3.96		CO97219-20	L	49.68	0.66	5.42
	a	12.12	1.07				a	12.85	0.25	
	b	2.40	0.75				b	0.37	0.27	
CO97211-21	L	45.13	0.53	4.99		CO97219-21	L	21.01	1.37	4.33
	a	9.05	1.15				a	12.13	0.89	
	b	2.74	0.28				b	-7.71	0.45	
CO97211-22	L	53.81	1.31	3.50		CO97219-22	L	22.83	1.28	3.97
	a	4.28	0.38				a	12.75	0.58	
	b	5.28	0.12				b	-7.40	0.35	
CO97211-23	L	47.40	0.82	4.40		CO97219-23	L	27.49	0.47	5.09
	a	5.47	0.60				a	13.61	0.54	
	b	3.46	0.20				b	-7.92	0.43	
CO97211-24	L	45.65	0.98	4.18		CO97219-24	L	60.08	1.78	5.85
	a	11.54	0.82				a	1.75	1.51	
	b	3.01	0.13				b	7.56	0.63	
CO97211-25	L	46.75	1.45	2.99		CO97219-25	L	31.61	0.37	4.03
	a	5.74	0.59				a	17.37	0.68	
	b	3.77	0.11				b	-2.02	0.06	
CO97211-26	L	41.65	0.32	2.61		CO97219-26	L	33.63	0.49	5.15
	a	11.82	0.36				a	19.52	0.49	
	b	1.12	0.11				b	-2.51	0.37	
CO97211-27	L	54.67	0.46	3.70		CO97219-27	L	56.47	0.60	2.39
	a	7.44	0.38				a	2.34	0.51	
	b	4.62	0.21				b	6.72	0.18	
CO97211-28	L	51.47	1.55	3.03		CO97219-28	L	15.57	1.14	6.67
	a	9.58	0.83				a	6.74	0.23	
	b	3.85	0.62				b	-4.62	0.15	
CO97211-29	L	43.68	1.94	3.77		CO97219-29	L	21.22	0.88	4.52
	a	9.95	2.04				a	12.59	0.40	
	b	2.54	0.77				b	-7.49	0.17	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO97211-30	L	55.46	1.17	2.35		CO97219-30	L	19.65	1.28	7.02
	a	-0.44	0.23				a	7.96	0.35	
	b	7.58	0.24				b	-5.25	0.21	
CO97225-1	L	58.00	0.24	2.08		CO97254-1	L	37.42	1.02	6.57
	a	-0.76	0.11				a	9.46	1.54	
	b	18.26	0.17				b	1.02	0.55	
CO97225-2	L	55.64	1.91	2.06		CO97254-2	L	52.98	0.17	3.32
	a	-1.16	0.19				a	7.00	0.89	
	b	12.07	1.95				b	5.54	0.12	
CO97225-3	L	36.24	0.59	2.52		CO97254-3	L	48.38	0.28	4.21
	a	11.74	0.27				a	10.35	0.35	
	b	-6.40	0.22				b	2.03	0.50	
CO97225-4	L	59.08	0.56	2.64		CO97254-4	L	44.65	0.71	4.77
	a	-1.31	0.07				a	13.19	1.19	
	b	15.90	0.56				b	0.39	0.63	
CO97225-5	L	54.21	2.08	2.86		CO97254-5	L	49.39	1.52	3.81
	a	-1.42	0.16				a	8.58	0.76	
	b	16.29	0.93				b	2.51	0.52	
CO97225-6	L	29.76	0.29	7.31		CO97254-6	L	43.13	0.86	4.23
	a	17.08	0.53				a	14.68	0.52	
	b	-1.49	0.29				b	-0.52	0.35	
CO97225-7	L	57.36	1.01	2.04		CO97254-7	L	61.46	1.46	2.74
	a	-1.04	0.07				a	-0.80	0.08	
	b	16.52	0.44				b	9.19	0.50	
CO97225-8	L	52.93	0.74	2.58		CO97254-8	L	47.07	0.57	4.49
	a	4.93	0.36				a	9.97	0.53	
	b	4.79	0.33				b	1.49	0.36	
CO97225-9	L	58.44	1.11	2.45		CO97254-9	L	41.97	1.12	4.62
	a	-1.20	0.10				a	13.33	1.65	
	b	14.60	0.43				b	-0.11	0.90	
CO97225-10	L	41.40	0.72	4.74		CO97254-10	L	42.25	0.60	5.23
	a	11.01	0.40				a	13.57	0.27	
	b	1.53	0.21				b	-0.65	0.14	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO97225-11	L	59.26	2.37	2.47		CO97254-11	L	x	x	x
	a	-0.98	0.13				a	x	x	
	b	15.01	0.46				b	x	x	
CO97225-12	L	39.70	0.36	5.42		CO97254-12	L	43.42	1.51	4.92
	a	10.97	0.52				a	11.41	2.32	
	b	1.39	0.29				b	1.07	0.97	
CO97225-13	L	54.43	0.36	1.74		CO97254-13	L	55.38	0.23	4.00
	a	-1.10	0.08				a	6.22	0.37	
	b	15.37	0.40				b	4.61	0.26	
CO97225-14	L	58.26	1.23	2.30		CO97254-14	L	54.83	0.46	4.25
	a	-1.04	0.06				a	5.85	0.66	
	b	14.78	0.93				b	4.91	0.44	
CO97225-15	L	60.41	0.23	1.97		CO97254-15	L	55.54	0.35	3.45
	a	-0.67	0.04				a	3.90	0.80	
	b	18.71	0.28				b	6.52	0.42	
CO97225-16	L	57.35	1.19	2.30		CO97254-16	L	43.12	1.40	5.20
	a	-1.36	0.13				a	10.38	1.23	
	b	19.51	0.66				b	1.66	0.88	
CO97225-17	L	57.42	2.19	2.23		CO97254-17	L	57.32	1.24	3.60
	a	-1.35	0.34				a	-0.43	0.05	
	b	19.52	0.53				b	8.95	0.26	
CO97225-18	L	58.25	1.41	2.16		CO97254-18	L	43.78	0.43	4.81
	a	-1.51	0.18				a	12.14	1.50	
	b	19.92	1.02				b	0.90	0.66	
CO97225-19	L	60.31	2.25	2.83		CO97254-19	L	52.20	0.46	5.18
	a	-1.21	0.19				a	6.60	0.91	
	b	15.59	1.26				b	3.37	0.40	
CO97225-20	L	57.99	0.78	2.12		CO97254-20	L	48.41	0.70	3.89
	a	-1.35	0.10				a	9.32	1.78	
	b	17.34	0.89				b	2.81	0.34	
CO97225-21	L	61.94	1.72	2.77		CO97254-21	L	47.64	1.28	3.84
	a	-1.12	0.09				a	11.34	0.91	
	b	13.55	0.32				b	2.22	0.38	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO97225-22	L	57.03	0.67	2.28		CO97254-22	L	38.08	1.15	4.40
	a	-0.78	0.04				a	14.21	0.70	
	b	10.27	0.03				b	-1.34	0.27	
CO97225-23	L	58.56	0.76	2.84		CO97254-23	L	54.31	1.56	3.60
	a	-1.33	0.11				a	5.91	1.12	
	b	18.31	0.44				b	5.91	0.62	
CO97225-24	L	58.40	1.48	3.04		CO97254-24	L	38.37	0.54	7.16
	a	-1.26	0.07				a	16.89	1.00	
	b	13.38	0.37				b	-1.24	0.44	
CO97225-25	L	59.22	1.62	2.94		CO97254-25	L	53.23	0.68	3.80
	a	-0.92	0.15				a	6.47	0.23	
	b	19.44	0.49				b	4.51	0.12	
CO97225-26	L	57.63	0.88	2.78		CO97254-26	L	51.45	1.16	3.58
	a	-1.02	0.08				a	5.48	0.70	
	b	12.30	0.42				b	4.74	0.32	
CO97225-27	L	57.06	2.59	3.79		CO97254-27	L	45.43	1.07	3.31
	a	-1.41	0.12				a	13.59	1.24	
	b	21.28	1.21				b	1.04	0.57	
CO97225-28	L	58.98	1.06	2.78		CO97254-28	L	42.74	0.56	4.54
	a	-0.55	0.43				a	11.15	0.43	
	b	9.78	0.35				b	2.53	0.29	
CO97225-29	L	63.44	0.31	2.46		CO97254-29	L	55.77	2.21	3.71
	a	-1.37	0.11				a	0.88	0.20	
	b	19.69	0.56				b	7.08	0.24	
CO97225-30	L	56.78	1.30	3.10		CO97254-30	L	56.11	0.56	3.93
	a	-1.23	0.46				a	8.28	0.43	
	b	12.10	0.26				b	4.45	0.30	
CO97306-1	L	18.20	1.36	6.15		CO97307-1	L=	55.51	3.90	8.25
	a	9.43	0.48				a=	4.13	1.95	
	b	-5.69	0.23				b=	6.03	2.42	
CO97306-2	L	40.90	0.51	4.00		CO97307-2	L=	20.85	1.09	5.13
	a	15.39	0.91				a=	12.64	4.65	
	b	-0.56	0.33				b=	-6.56	0.28	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO97306-3	L	39.92	1.49	5.24		CO97307-3	L=	20.87	1.15	5.36
	a	15.16	1.11				a=	9.70	1.36	
	b	0.19	0.37				b=	-6.39	0.84	
CO97306-4	L	24.17	0.89	4.46		CO97307-4	L=	32.23	1.75	8.63
	a	9.04	0.25				a=	17.65	1.21	
	b	-5.88	0.58				b=	-1.19	0.35	
CO97306-5	L	40.55	0.43	5.26		CO97307-5	L=	17.52	1.12	7.53
	a	14.77	0.57				a=	8.05	0.96	
	b	-0.47	0.23				b=	-5.27	0.49	
CO97306-6	L	51.60	0.82	3.87		CO97307-6	L=	16.59	0.87	8.51
	a	6.16	0.48				a=	8.62	0.72	
	b	4.70	0.35				b=	-5.54	0.54	
CO97306-7	L	65.33	1.25	2.60		CO97307-7	L=	31.18	3.31	6.71
	a	-0.39	0.06				a=	9.35	0.60	
	b	9.81	0.11				b=	-4.43	0.80	
CO97306-8	L	18.00	0.57	5.82		CO97307-8	L=	33.59	0.81	3.73
	a	7.41	0.26				a=	12.26	0.91	
	b	-5.38	0.34				b=	-6.63	0.42	
CO97306-9	L	19.14	1.19	5.65		CO97307-9	L=	65.66	0.34	1.89
	a	9.67	0.92				a=	-0.62	0.17	
	b	-6.37	0.58				b=	10.09	0.27	
CO97306-10	L	22.04	0.60	5.32		CO97307-10	L=	48.86	3.48	3.99
	a	11.28	0.21				a=	5.92	0.77	
	b	-7.21	0.13				b=	1.21	1.34	
CO97306-11	L	20.93	0.60	6.17		CO97307-11	L=	40.98	0.78	6.26
	a	10.64	0.73				a=	15.48	1.54	
	b	-6.08	0.46				b=	-0.36	0.21	
CO97306-12	L	19.42	0.72	5.86		CO97307-12	L=	52.94	2.10	10.59
	a	10.19	0.40				a=	6.00	1.15	
	b	-6.22	0.36				b=	1.61	0.64	
CO97306-13	L	42.48	0.75	4.41		CO97307-13	L=	17.88	0.67	6.02
	a	12.51	0.28				a=	7.04	0.64	
	b	0.42	0.35				b=	-4.75	0.33	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO97306-14	L	40.90	0.68	4.55		CO97307-14	L=	62.58	0.13	2.44
	a	14.58	0.45				a=	-0.91	0.08	
	b	0.00	0.38				b=	11.09	0.61	
CO97306-15	L	45.52	0.90	4.02		CO97307-15	L=	32.95	0.95	3.72
	a	9.98	0.70				a=	14.20	0.44	
	b	1.41	0.20				b=	-10.09	0.61	
CO97306-16	L	20.36	0.30	5.40		CO97307-16	L=	20.10	0.90	6.42
	a	9.70	0.37				a=	7.95	0.51	
	b	-5.22	0.16				b=	-5.65	0.76	
CO97306-17	L	24.72	0.59	5.18		CO97307-17	L=	57.35	0.77	5.18
	a	12.35	0.66				a=	1.90	0.14	
	b	-6.42	0.41				b=	9.87	0.78	
CO97306-18	L	21.24	0.53	5.54		CO97307-18	L=	57.99	0.66	4.26
	a	9.04	0.22				a=	0.48	0.13	
	b	-6.23	0.12				b=	10.48	0.12	
CO97306-19	L	37.93	0.64	4.86		CO97307-19	L=	25.75	0.79	4.54
	a	20.19	0.88				a=	11.48	1.10	
	b	-3.06	0.66				b=	-8.31	0.56	
CO97306-20	L	21.49	1.13	5.37		CO97307-20	L=	18.87	0.72	6.88
	a	11.94	0.83				a=	9.51	0.34	
	b	-6.85	0.44				b=	-7.17	0.50	
CO97306-21	L	27.49	0.61	2.75		CO97307-21	L=	17.14	0.93	11.05
	a	12.40	0.34				a=	6.30	0.21	
	b	-7.35	0.36				b=	-4.58	0.29	
CO97306-22	L	35.91	1.46	3.48		CO97307-22	L=	16.36	0.72	6.54
	a	9.82	0.76				a=	7.91	0.68	
	b	-3.45	0.36				b=	-6.18	0.45	
CO97306-23	L	19.56	1.11	4.93		CO97307-23	L=	37.33	5.71	3.27
	a	8.62	0.44				a=	8.20	2.20	
	b	-5.00	0.18				b=	-3.22	2.35	
CO97306-24	L	22.40	1.49	4.72		CO97307-24	L=	28.54	1.55	3.71
	a	11.07	0.38				a=	14.04	0.55	
	b	-6.10	0.16				b=	-8.52	0.72	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO97306-25	L	29.54	0.97	2.62		CO97307-25	L=	33.10	2.06	7.82
	a	15.05	0.32				a=	13.22	0.26	
	b	-8.25	0.21				b=	-6.88	1.75	
CO97306-26	L	21.50	0.77	5.31		CO97307-26	L=	19.96	1.19	7.97
	a	10.50	0.25				a=	7.59	0.45	
	b	-6.24	0.11				b=	-5.29	0.24	
CO97306-27	L	19.28	0.96	5.87		CO97307-27	L=	66.26	0.53	2.57
	a	7.33	0.42				a=	-0.69	0.16	
	b	-4.64	0.19				b=	12.73	0.57	
CO97306-28	L	24.40	0.60	4.12		CO97307-28	L=	28.94	4.06	7.51
	a	11.96	0.26				a=	12.13	1.44	
	b	-8.33	0.20				b=	-6.04	1.41	
CO97306-29	L	66.56	1.11	1.94		CO97307-29	L=	20.70	0.31	9.35
	a	-0.85	0.12				a=	7.19	0.23	
	b	11.05	0.12				b=	-4.39	0.32	
CO97306-30	L	40.26	1.46	3.72		CO97307-30	L=	20.66	0.70	6.31
	a	13.17	1.15				a=	9.74	0.23	
	b	-5.47	1.06				b=	-7.22	0.11	
CO04045-1	L	20.37	0.37	7.00		CO04059-1	L	41.29	0.08	3.72
	a	7.10	0.39				a	15.30	0.48	
	b	-4.21	0.26				b	-1.55	0.22	
CO04045-2	L	20.65	0.66	7.01		CO04059-2	L	60.31	1.13	3.08
	a	10.35	0.24				a	-0.46	0.11	
	b	-6.32	0.20				b	7.50	0.16	
CO04045-3	L	22.19	1.72	5.79		CO04059-3	L	45.82	0.86	3.96
	a	8.32	0.09				a	12.17	1.22	
	b	-4.52	0.22				b	0.23	0.47	
CO04045-4	L	17.76	1.03	6.84		CO04059-4	L	54.42	1.35	3.08
	a	9.31	0.42				a	6.45	1.23	
	b	-5.42	0.29				b	3.14	0.51	
CO04045-5	L	27.71	0.37	9.27		CO04059-5	L	32.79	0.23	5.54
	a	19.07	0.34				a	19.94	0.42	
	b	-1.25	0.15				b	-2.56	0.54	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO04045-6	L	21.38	1.25	6.68		CO04059-6	L	58.24	0.32	2.70
	a	9.40	0.30				a	-0.62	0.07	
	b	-5.76	0.23				b	7.83	0.09	
CO04045-7	L	19.39	0.46	7.58		CO04059-7	L	51.14	0.91	3.71
	a	7.18	0.38				a	9.93	0.45	
	b	-4.80	0.30				b	1.66	0.42	
CO04045-8	L	22.43	0.96	5.08		CO04059-8	L	48.18	0.78	2.98
	a	10.56	0.97				a	10.18	0.68	
	b	-5.82	0.61				b	1.58	0.40	
CO04045-9	L	18.37	2.07	9.54		CO04059-9	L	34.35	1.11	3.43
	a	7.77	1.14				a	15.26	0.76	
	b	-4.99	0.72				b	-0.71	0.40	
CO04045-10	L	18.06	0.36	5.52		CO04059-10	L	50.30	0.94	3.59
	a	7.36	1.16				a	9.29	0.59	
	b	-4.28	0.78				b	2.73	0.19	
CO04045-11	L	18.00	0.86	8.00		CO04059-11	L	53.79	2.21	3.68
	a	6.09	0.57				a	8.47	1.29	
	b	-3.69	0.29				b	3.45	0.79	
CO04045-12	L	19.56	0.72	5.78		CO04059-12	L	37.13	2.46	6.07
	a	9.30	0.55				a	14.51	3.01	
	b	-5.76	0.58				b	-0.75	1.02	
CO04045-13	L	21.67	5.80	8.41		CO04059-13	L	36.49	0.64	5.58
	a	8.99	2.27				a	17.97	3.64	
	b	-5.50	0.90				b	-1.77	0.54	
CO04045-14	L	19.97	1.64	6.14		CO04059-14	L	35.40	0.99	4.90
	a	9.94	0.19				a	17.21	0.99	
	b	-6.12	0.16				b	-1.51	0.46	
CO04045-15	L	17.13	0.91	6.50		CO04059-15	L	43.64	2.18	5.11
	a	4.76	0.16				a	15.20	0.31	
	b	-3.09	0.32				b	-0.39	0.24	
CO04045-16	L	17.72	0.20	7.09		CO04059-16	L	51.73	1.17	3.15
	a	7.16	0.28				a	4.61	0.26	
	b	-4.89	0.12				b	3.65	0.36	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO04045-17	L	23.25	0.78	7.80		CO04059-17	L	49.47	0.97	4.27
	a	7.77	0.31				a	10.21	0.52	
	b	-4.74	0.28				b	1.97	0.50	
CO04045-18	L	23.29	0.38	4.53		CO04059-18	L	35.82	0.90	9.99
	a	10.49	0.16				a	18.36	1.52	
	b	-6.33	0.06				b	-0.85	0.71	
CO04045-19	L	26.53	1.36	6.42		CO04059-19	L	57.00	1.32	2.54
	a	12.29	0.63				a	4.02	0.75	
	b	-6.57	0.72				b	6.73	0.67	
CO04045-20	L	30.77	0.38	7.83		CO04059-20	L	48.81	0.35	3.28
	a	19.02	0.44				a	11.91	0.85	
	b	-1.76	0.10				b	0.87	0.27	
CO04045-21	L	28.35	2.03	17.94		CO04059-21	L	59.38	1.00	2.70
	a	18.86	1.08				a	-0.59	0.05	
	b	-1.46	0.05				b	8.39	0.39	
CO04045-22	L	16.47	0.65	8.09		CO04059-22	L	63.99	0.28	2.71
	a	6.00	0.79				a	-0.77	0.02	
	b	-3.79	0.46				b	7.24	0.12	
CO04045-23	L	39.14	0.57	3.53		CO04059-23	L	63.10	0.40	2.67
	a	14.97	0.23				a	-0.68	0.05	
	b	-1.42	0.27				b	8.68	0.37	
CO04045-24	L	20.94	0.51	6.78		CO04059-24	L	61.79	1.87	4.11
	a	8.68	0.33				a	1.04	0.16	
	b	-5.28	0.33				b	6.73	0.36	
CO04045-25	L	16.44	0.37	6.12		CO04059-25	L	31.22	0.53	6.65
	a	5.54	0.58				a	19.15	0.36	
	b	-3.62	0.32				b	-2.35	0.22	
CO04045-26	L	21.32	0.47	6.39		CO04059-26	L	62.49	0.78	2.25
	a	9.34	0.27				a	0.11	0.53	
	b	-5.52	0.18				b	8.32	0.13	
CO04045-27	L	40.72	0.80	5.04		CO04059-27	L	49.03	3.09	5.06
	a	13.97	0.89				a	10.11	2.06	
	b	0.61	0.22				b	2.12	0.91	

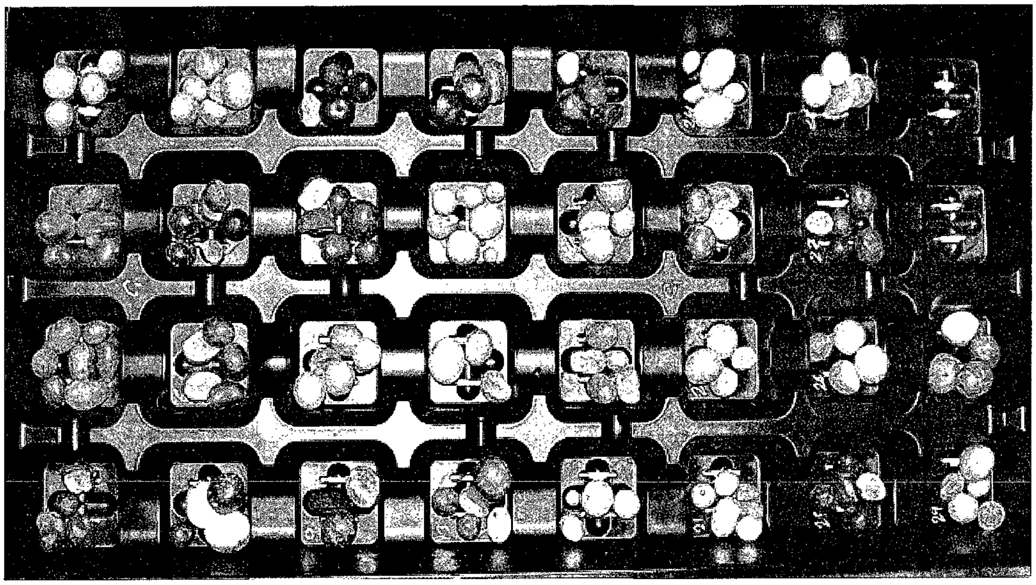
Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO04045-28	L	20.36	1.43	6.26		CO04059-28	L	47.75	0.20	3.35
	a	9.28	0.92				a	10.35	0.19	
	b	-6.02	0.44				b	1.25	0.13	
CO04045-29	L	17.89	0.69	6.45		CO04059-29	L	61.41	0.48	2.48
	a	9.02	0.41				a	0.88	0.48	
	b	-5.41	0.25				b	8.42	0.36	
CO04045-30	L	18.09	0.64	7.58		CO04059-30	L	44.90	0.61	4.88
	a	6.42	0.44				a	9.10	0.32	
	b	-4.10	0.18				b	0.58	0.12	
CO04063-1	L	27.25	0.38	6.76		CO04063-16	L	32.99	0.60	10.54
	a	18.36	0.19				a	16.92	0.65	
	b	-1.73	0.10				b	-1.42	0.38	
CO04063-2	L	32.91	0.96	4.56		CO04063-17	L	39.62	1.60	5.90
	a	17.78	0.66				a	15.09	1.33	
	b	-1.49	0.25				b	-0.68	0.70	
CO04063-3	L	25.97	0.69	10.77		CO04063-18	L	45.93	2.86	6.60
	a	18.09	0.45				a	8.84	2.68	
	b	-0.71	0.11				b	2.88	1.45	
CO04063-4	L	35.70	0.63	6.09		CO04063-19	L	33.47	0.73	6.24
	a	19.74	0.53				a	17.28	2.19	
	b	-1.41	0.48				b	-1.64	0.39	
CO04063-5	L	30.94	1.05	9.50		CO04063-20	L	29.39	0.49	6.55
	a	18.45	0.44				a	15.65	0.42	
	b	-3.64	0.33				b	-0.33	0.67	
CO04063-6	L	33.55	1.16	7.80		CO04063-21	L	34.13	1.46	8.53
	a	19.58	0.32				a	18.71	0.90	
	b	-0.59	0.66				b	-1.71	0.63	
CO04063-7	L	37.15	1.08	6.68		CO04063-22	L	41.67	0.20	6.89
	a	16.08	0.43				a	13.86	0.60	
	b	-0.26	0.82				b	0.51	0.20	
CO04063-8	L	31.97	0.13	6.01		CO04063-23	L	39.54	0.75	6.44
	a	17.55	0.23				a	16.81	1.88	
	b	-1.39	0.26				b	0.14	0.52	

Continue

clone ID		Aver.	SD	TPC		clone ID		Aver.	SD	TPC
CO04063-9	L	35.32	1.58	5.37		CO04063-24	L	32.43	0.75	8.99
	a	16.74	1.29				a	19.46	0.60	
	b	-1.10	0.52				b	-1.39	0.26	
CO04063-10	L	34.69	0.69	5.35		CO04063-25	L	27.26	1.16	8.36
	a	19.13	0.55				a	18.45	0.86	
	b	-1.61	0.23				b	-1.94	0.06	
CO04063-11	L	40.00	10.18	6.12		CO04063-26	L	31.74	0.33	7.86
	a	16.54	0.66				a	19.43	0.42	
	b	-0.77	0.47				b	-1.07	0.23	
CO04063-12	L	35.71	1.19	4.60		CO04063-27	L	32.20	0.33	6.91
	a	16.59	0.44				a	20.07	0.45	
	b	-0.39	0.18				b	-2.37	0.05	
CO04063-13	L	35.18	1.05	7.71		CO04063-28	L	29.65	0.56	6.30
	a	18.11	0.32				a	20.92	0.59	
	b	-1.16	0.43				b	-1.54	0.22	
CO04063-14	L	31.92	0.13	8.37		CO04063-29	L	35.12	0.89	8.28
	a	19.52	0.17				a	17.04	0.46	
	b	-0.99	0.24				b	-1.45	0.40	
CO04063-15	L	33.20	0.27	8.04		CO04063-30	L	29.13	1.42	14.69
	a	21.26	0.23				a	17.96	0.90	
	b	-2.21	0.28				b	-1.39	0.34	

A



B

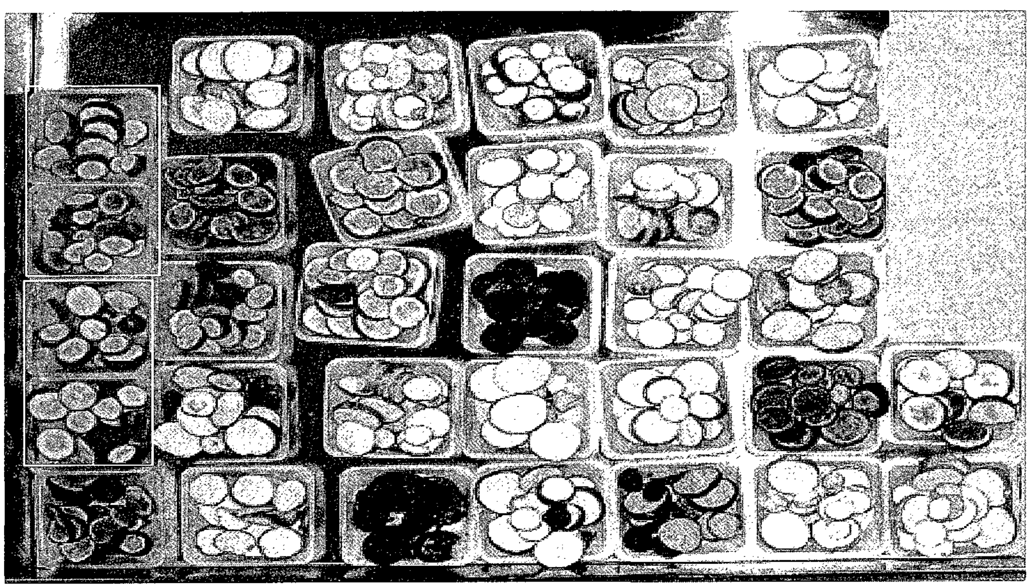
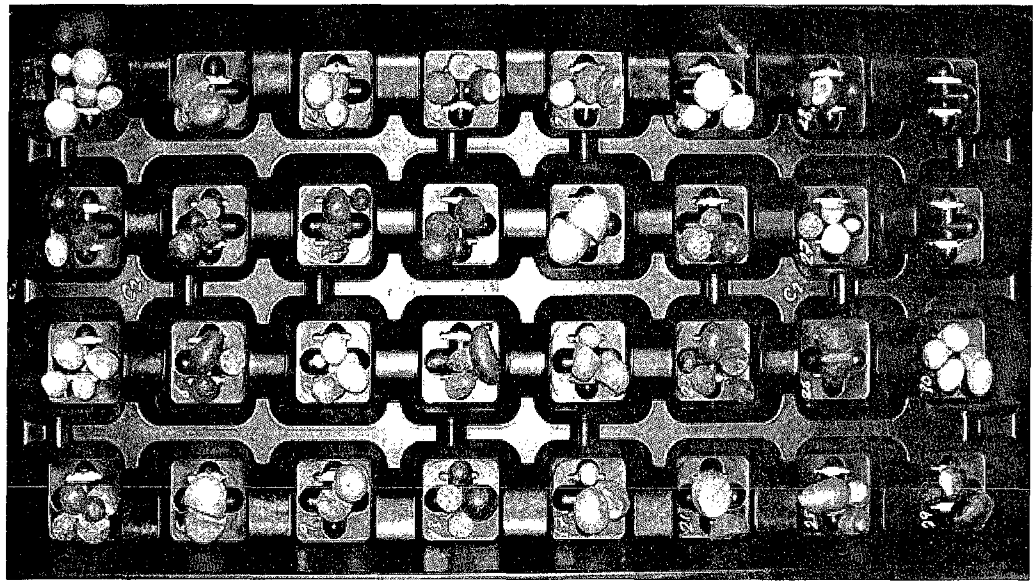


Figure 1. Photograph illustrating the range of skin (A) and flesh (B) color of CO94163 family [ND 1995- 1W/W (♀) x All Blue (P/P) (♂)]. W=white, P= purple, R= red (skin color/flesh color). Trays in the same highlighted yellow box contain the same sample.

A



B

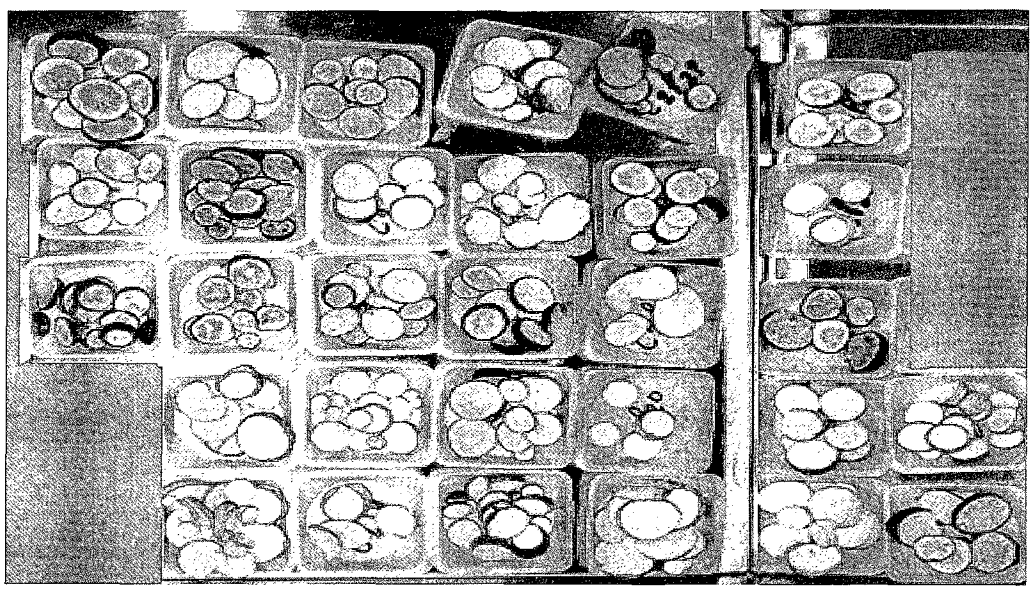
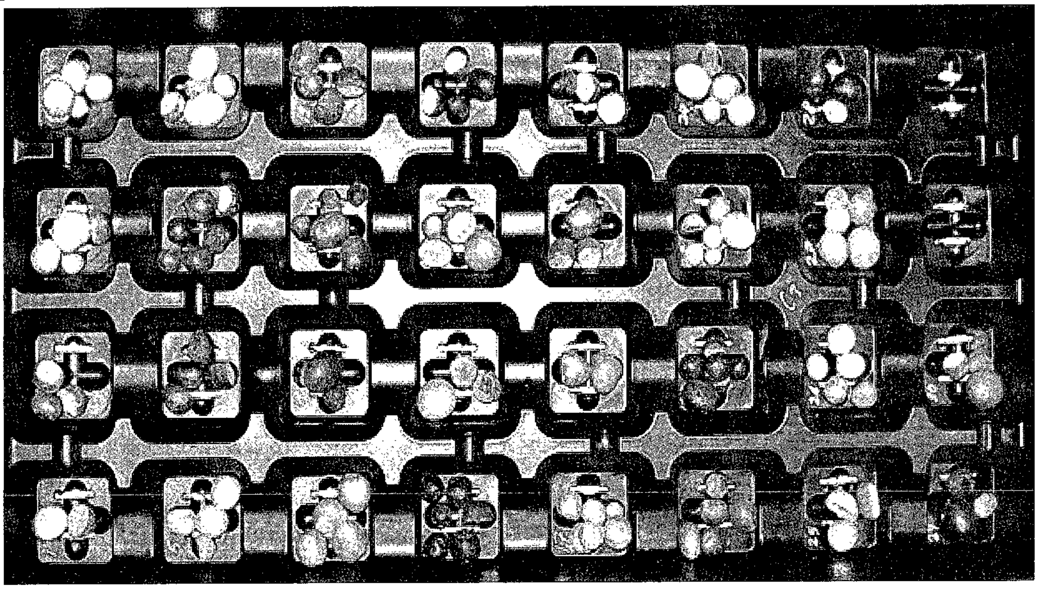


Figure 2. Photograph illustrating the range of skin (A) and flesh (B) color of CO94166 family [ND2109-7W /W (♀) x All Blue P/P (♂)].W=white, P= purple, R= red (skin color/flesh color).

A



B

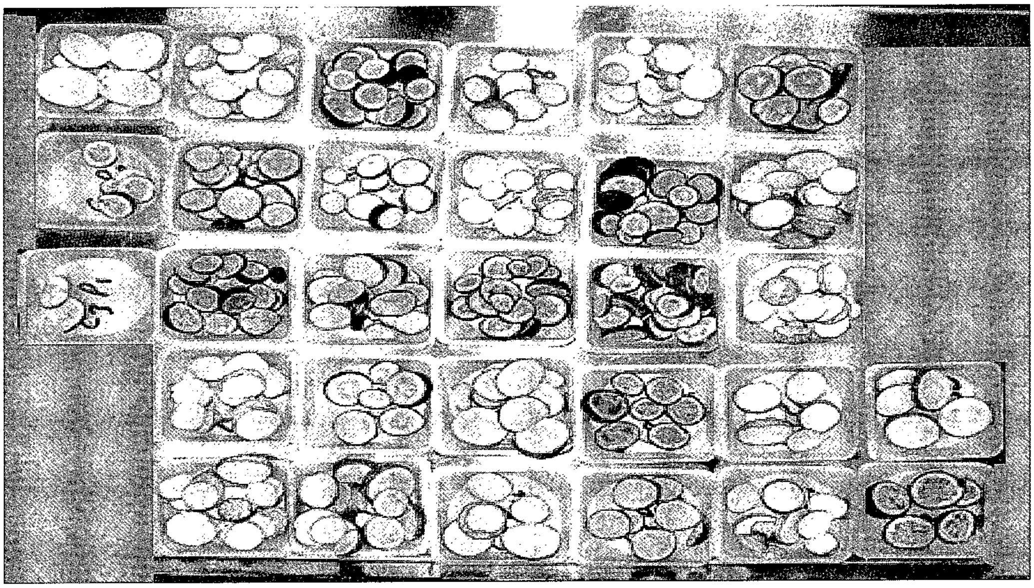


Figure 3. Photograph illustrating the range of skin (A) and flesh (B) color of CO94178 family [All Blue P/P (♀) x ND 1995-1W/W (♂)].W=white, P= purple, R= red (skin color/flesh color).

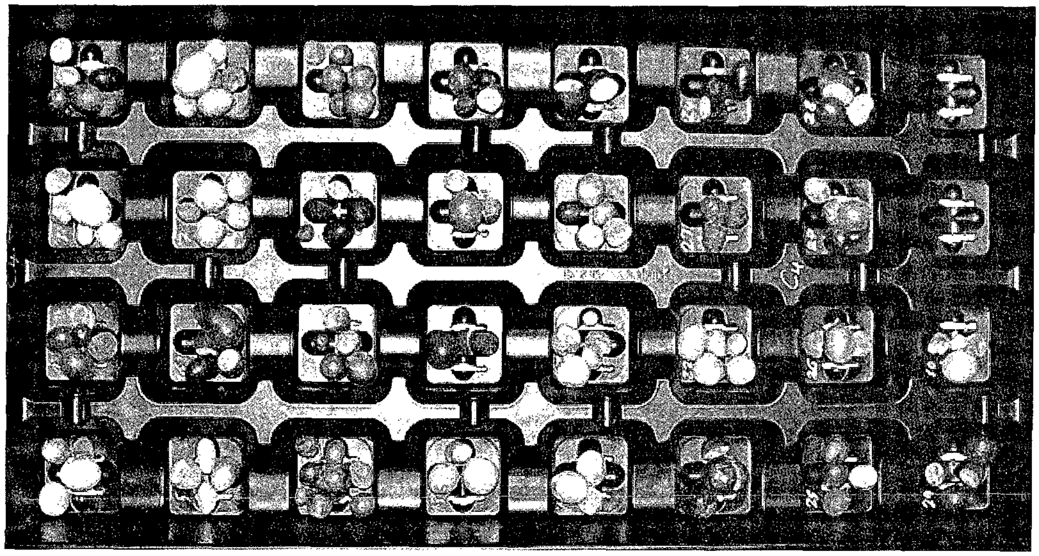
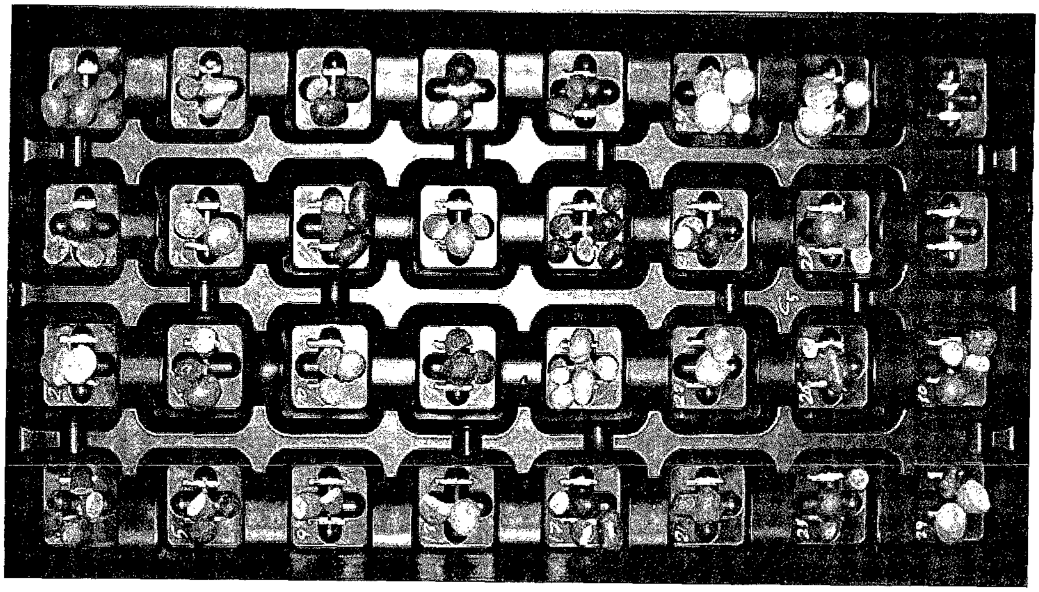


Figure 4. Photograph illustrating the range of skin color of CO94179 family [All Blue P/P (♀) x ND2109-7W/W (♂)].W=white, P= purple, R= red (skin color/flesh color).

A



B

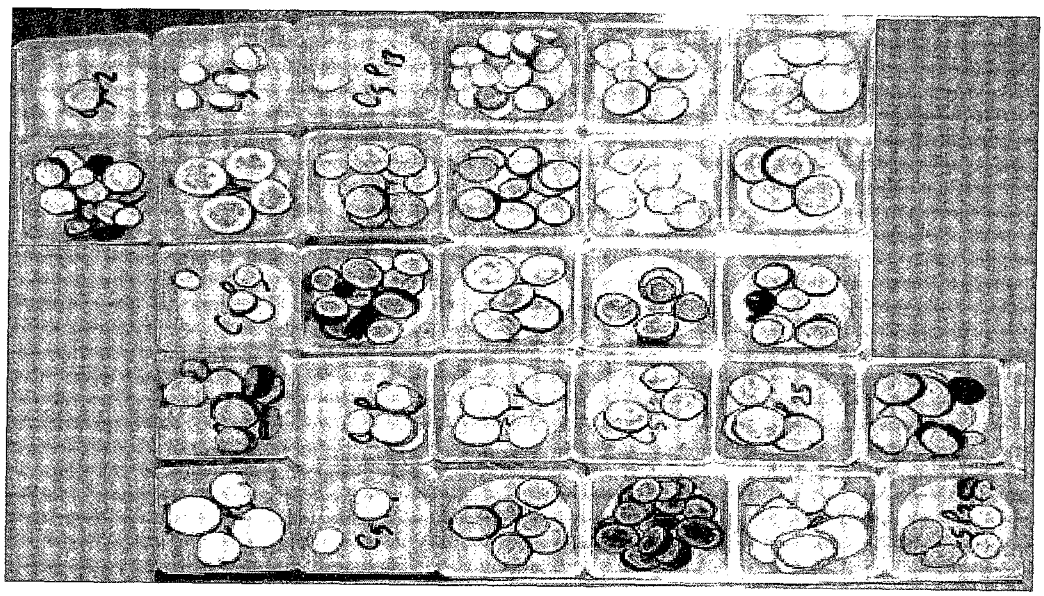
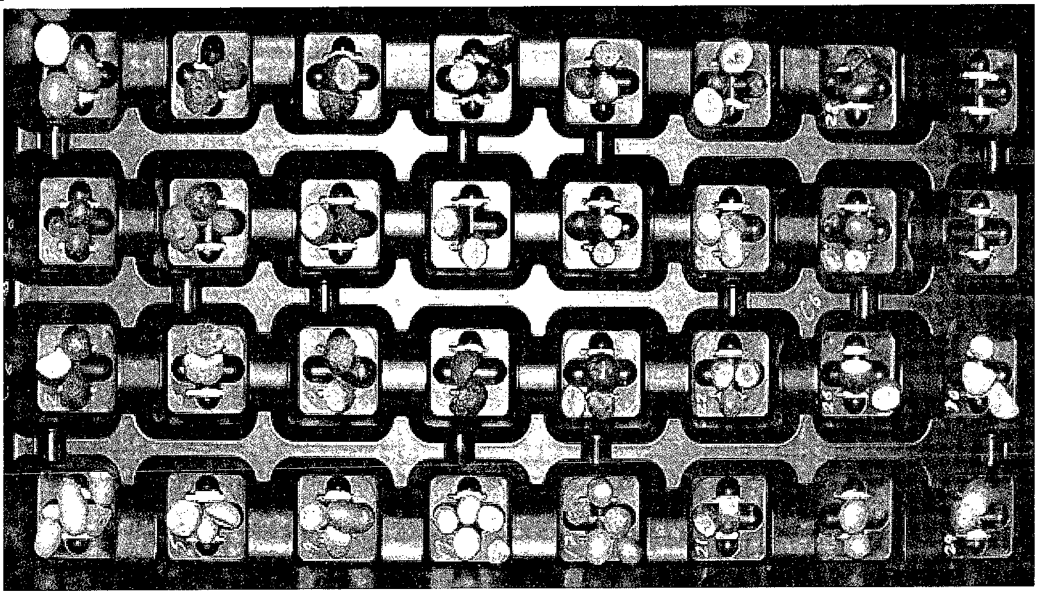


Figure 5. Photograph illustrating the range of skin (A) and flesh (B) color of CO94181 family [All Blue P/P(♀) x Chipeta W/W (♂)].W=white, P= purple, R= red (skin color/flesh color).

A



B

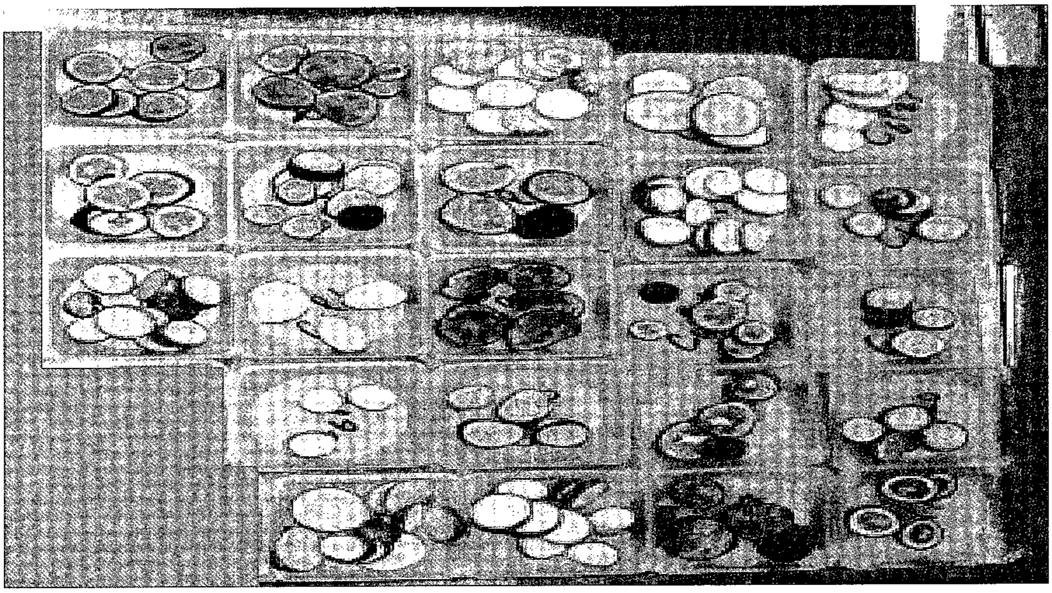


Figure 6. Photograph illustrating the range of skin (A) and flesh (B) color of CO94198 family [Chipeta W/W (♀) x All Blue (♂)]. W=white, P= purple, R= red (skin color/flesh color).

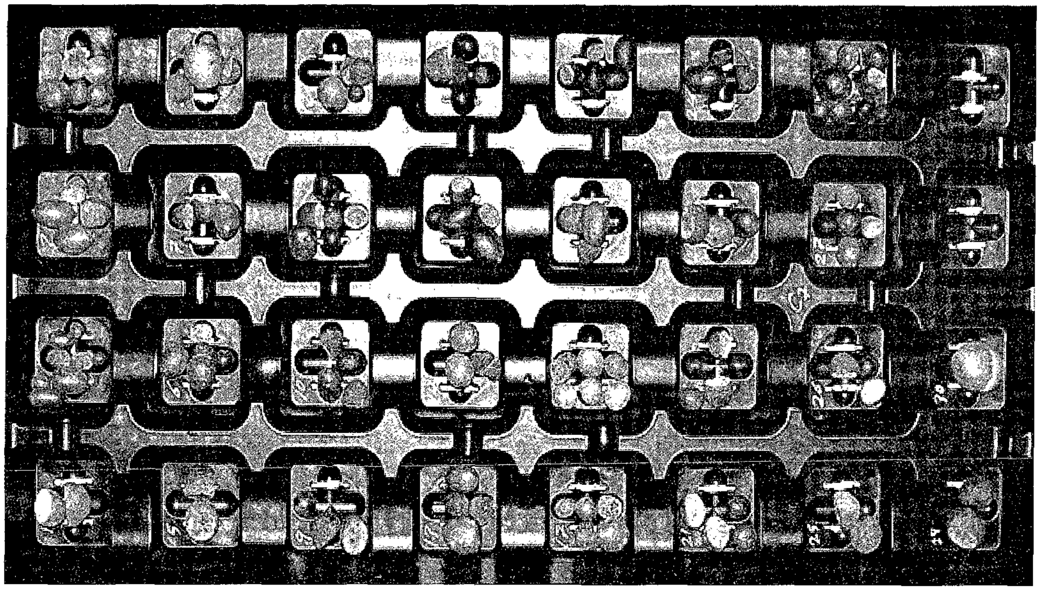
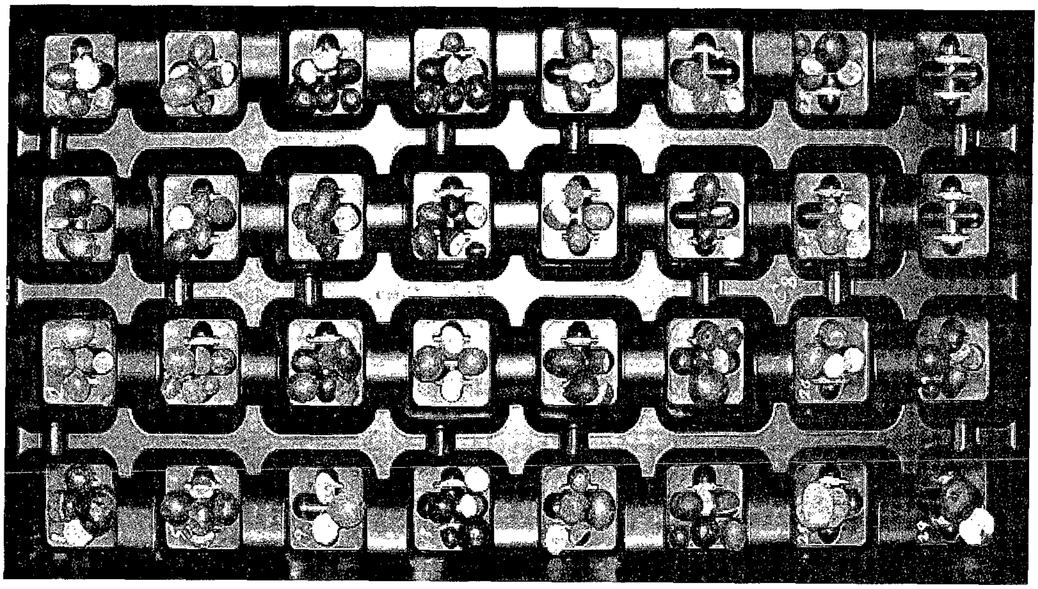


Figure 7. Photograph illustrating the range of skin color of CO97211 family [Durango DR/W (♀) x Mountain Rose 1R/R (♂)].W=white, DR= dark red, R= red (skin color/flesh color).

A



B

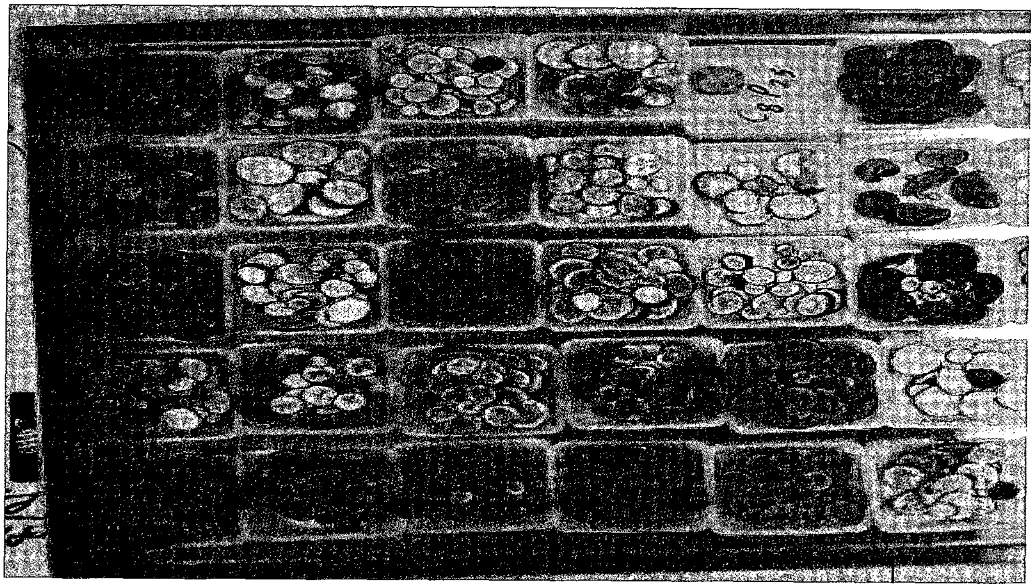
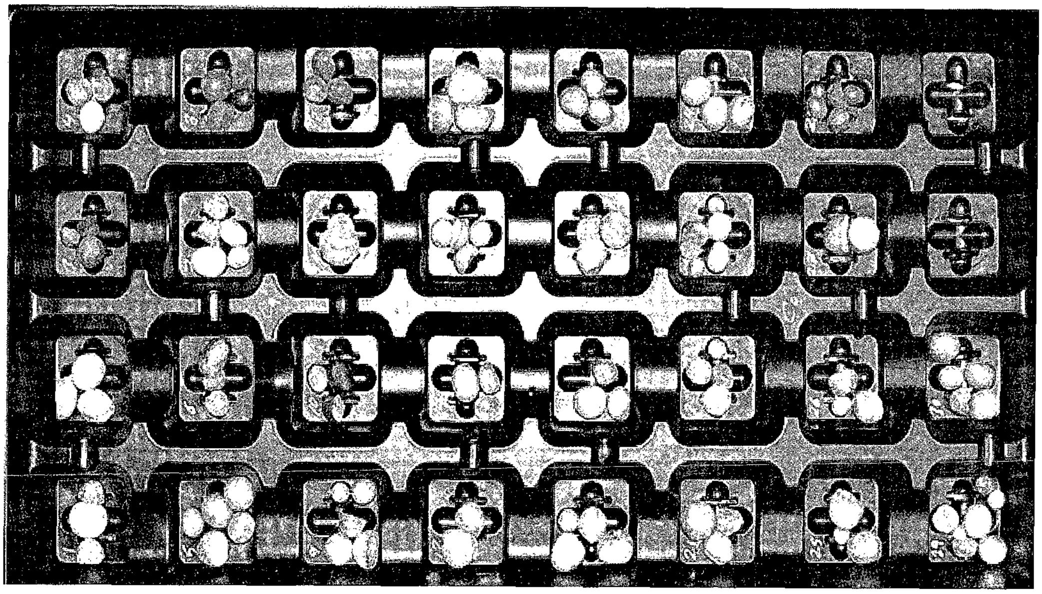


Figure 8. Photograph illustrating the range of skin (A) and flesh (B) color of CO97219 family [Purple Majesty (♀) x Mountain Rose (♂)].W=white, P= purple, R= red (skin color/flesh color).

A



B

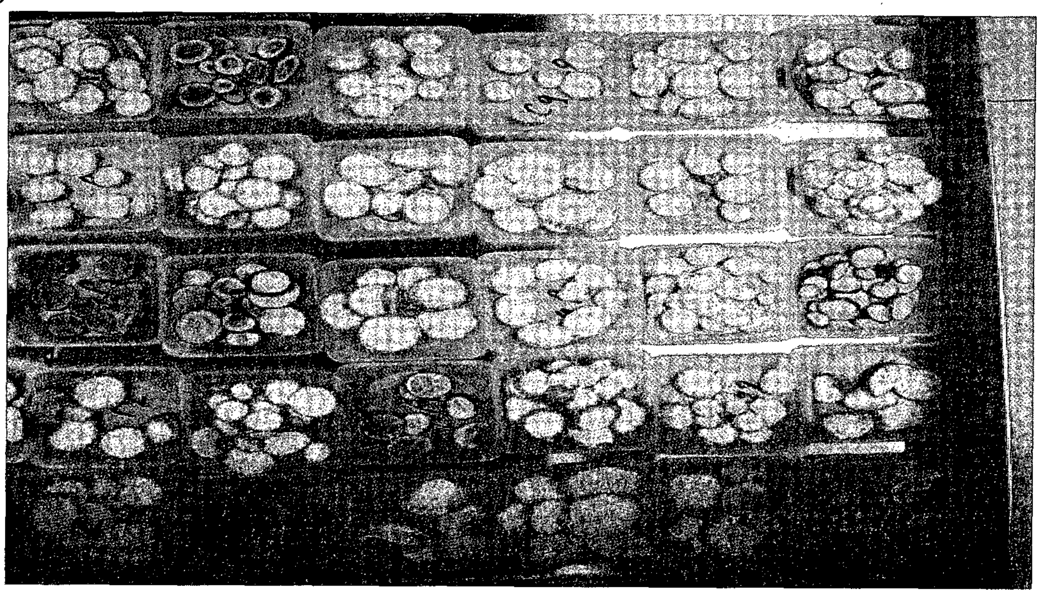
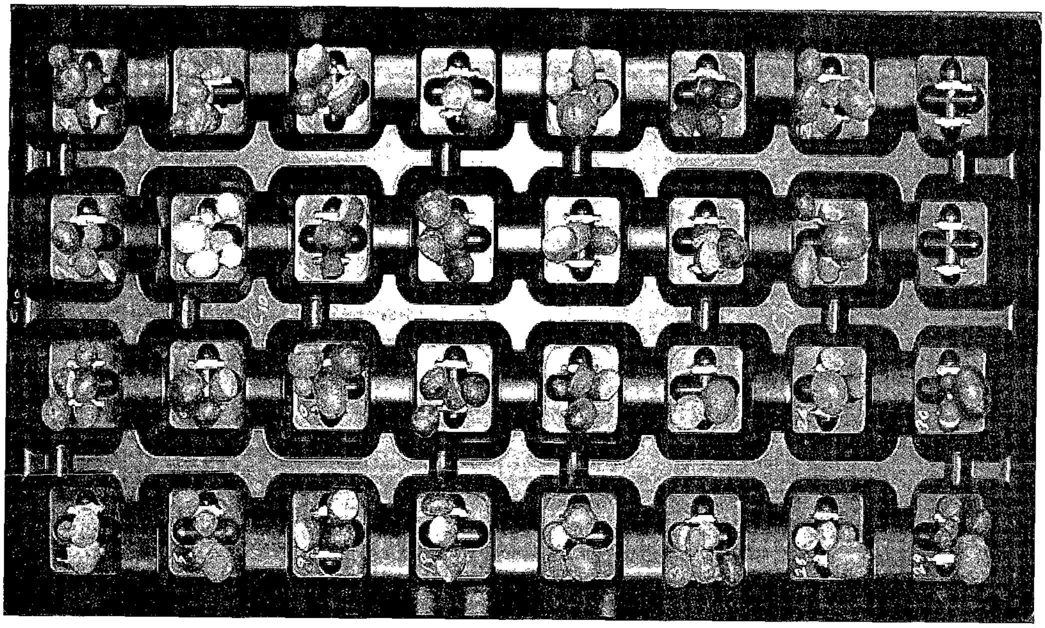


Figure 9. Photograph illustrating the range of skin (A) and flesh (B) color of CO97225 family [Mountain Rose R/R (♀) x Mountain Rose (♂)].W=white, P= purple, R= red (skin color/flesh color).

A



B

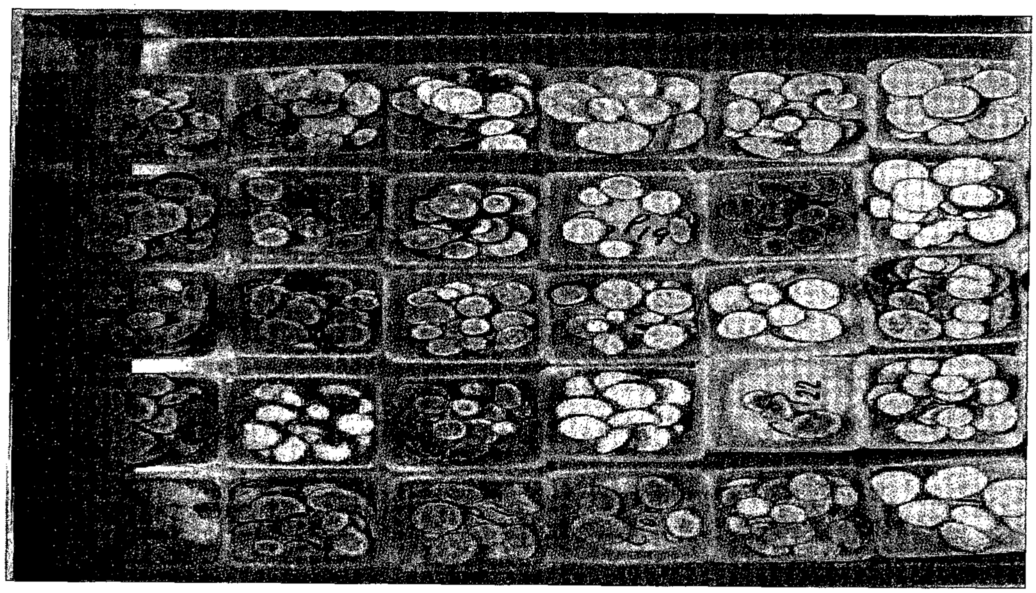
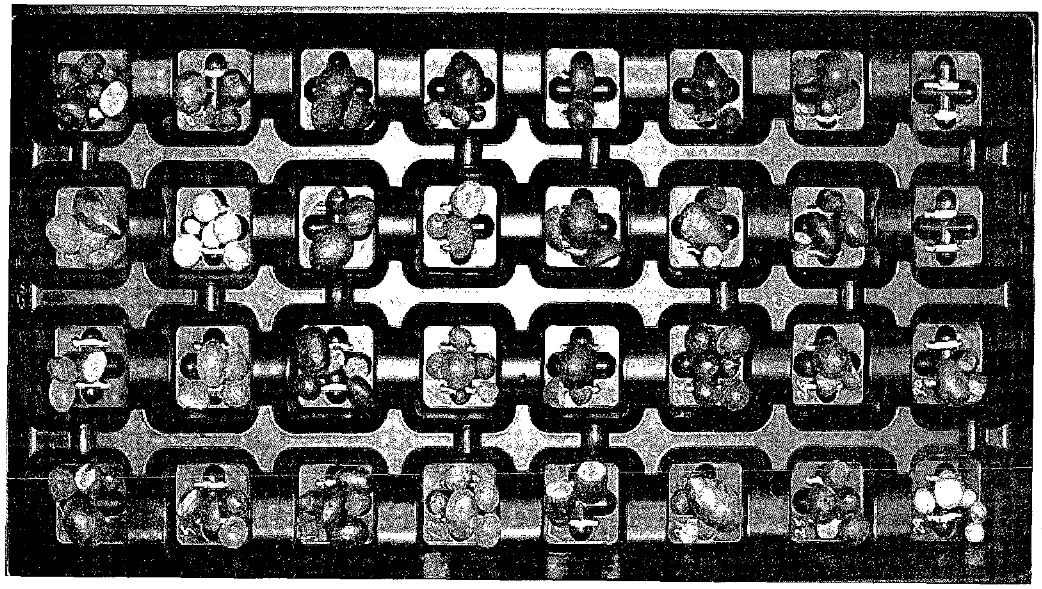


Figure 10. Photograph illustrating the range of skin (A) and flesh (B) color of CO97254 family [Cherry Red R/W (♀) x Mountain Rose R/R (♂)].W=white, P= purple, R= red (skin color/flesh color).

A



B

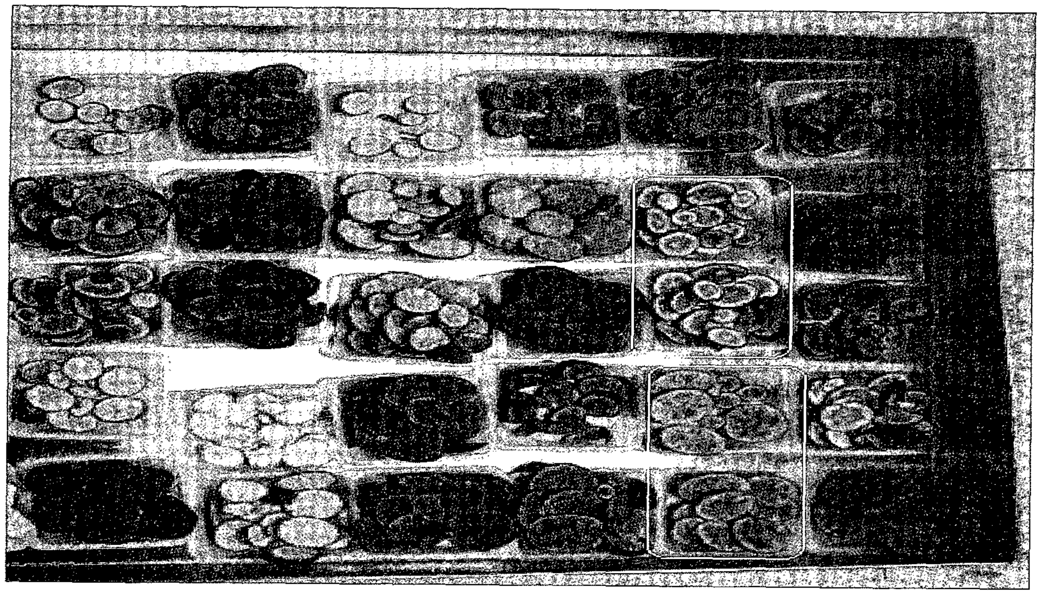
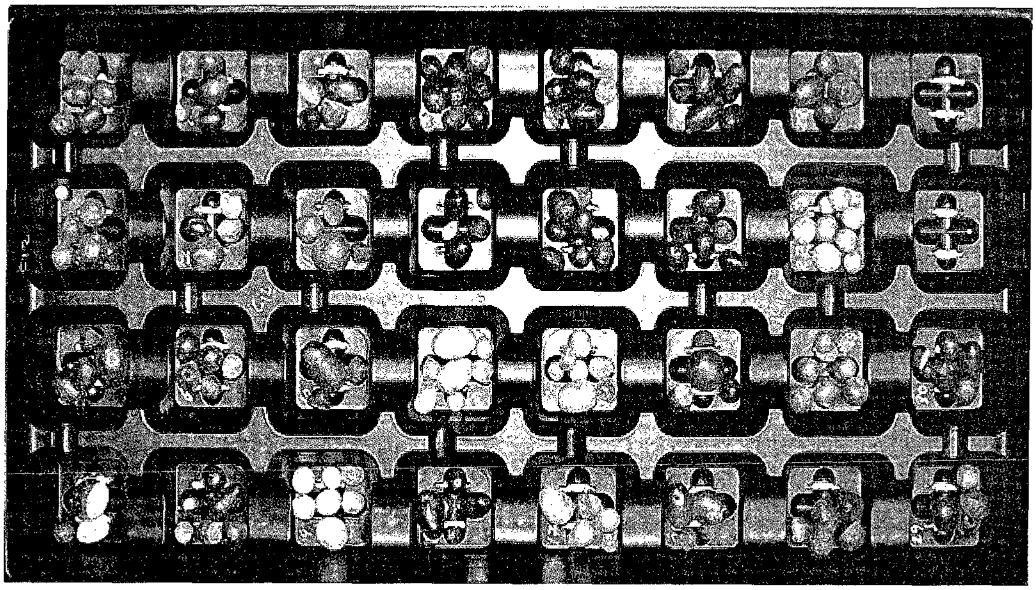


Figure 11. Photograph illustrating the range of skin (A) and flesh (B) color of CO97306 family [Purple Peruvian P/P (♀) x Mountain Rose R/R (♂)].W=white, P= purple, R= red (skin color/flesh color). Trays in the same highlighted yellow box contain the same sample.

A



B

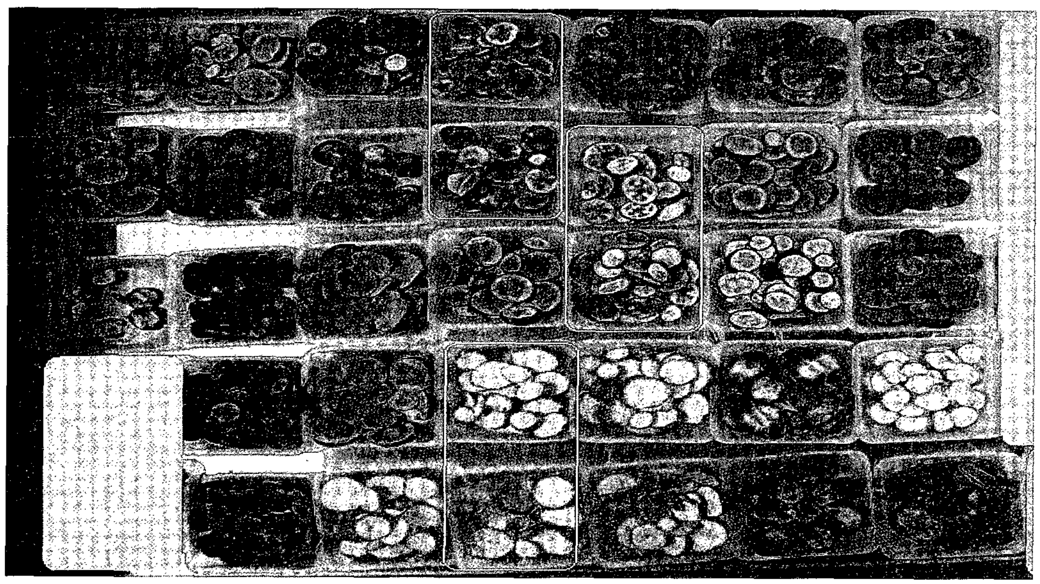
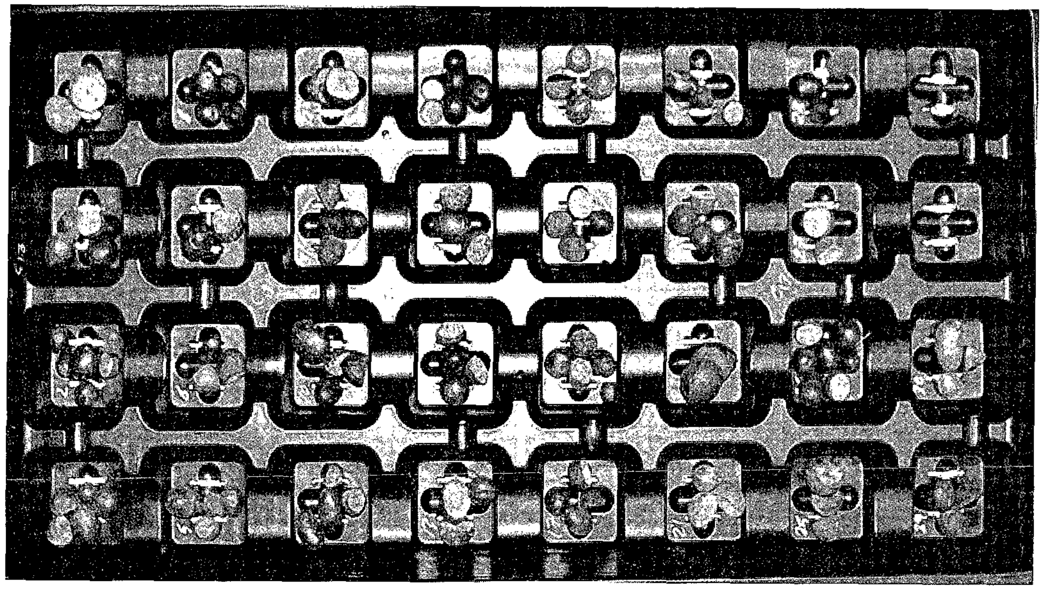


Figure 12. Photograph illustrating the range of skin (A) and flesh (B) color of CO97307 family [Purple Peruvian P/P (♀) x CO94214-1P/P (♂)].W=white, P= purple, R= red (skin color/flesh color). Trays in the same highlighted yellow box contain the same sample.

A



B

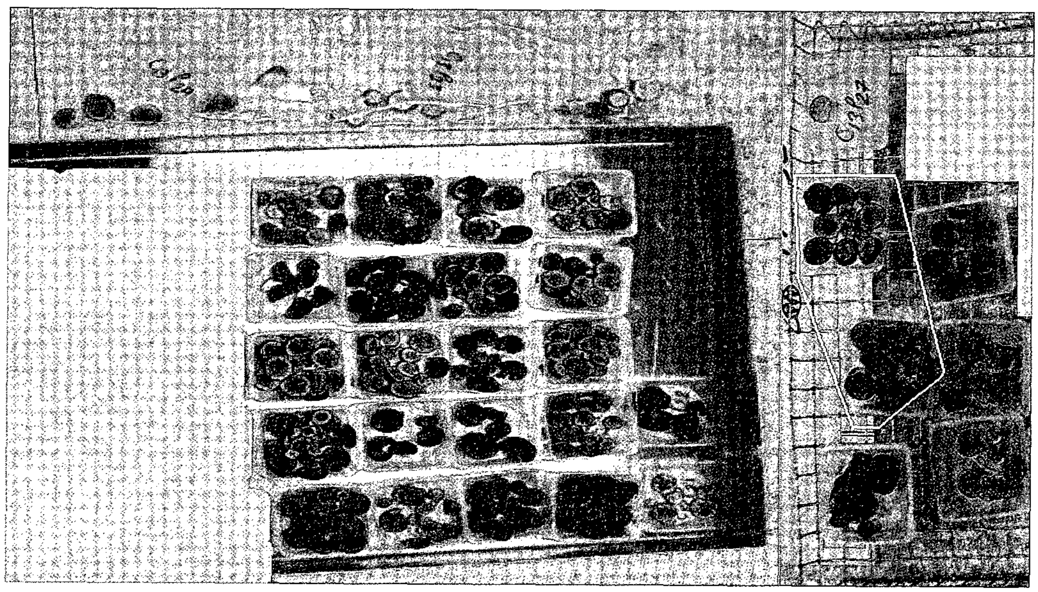
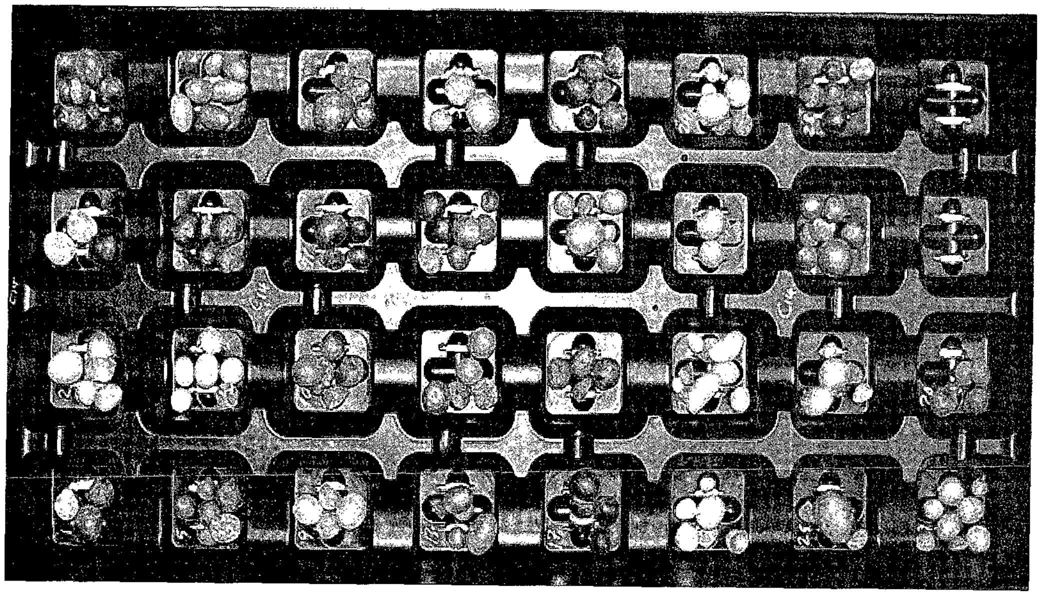


Figure 13. Photograph illustrating the range of skin (A) and flesh (B) color of CO04045 family [CO97215-2P/P (♀) x CO97216-1P/P (♂)].W=white, P= purple, R= red (skin color/flesh color). Trays in the same highlighted yellow box contain the same sample.

A



B

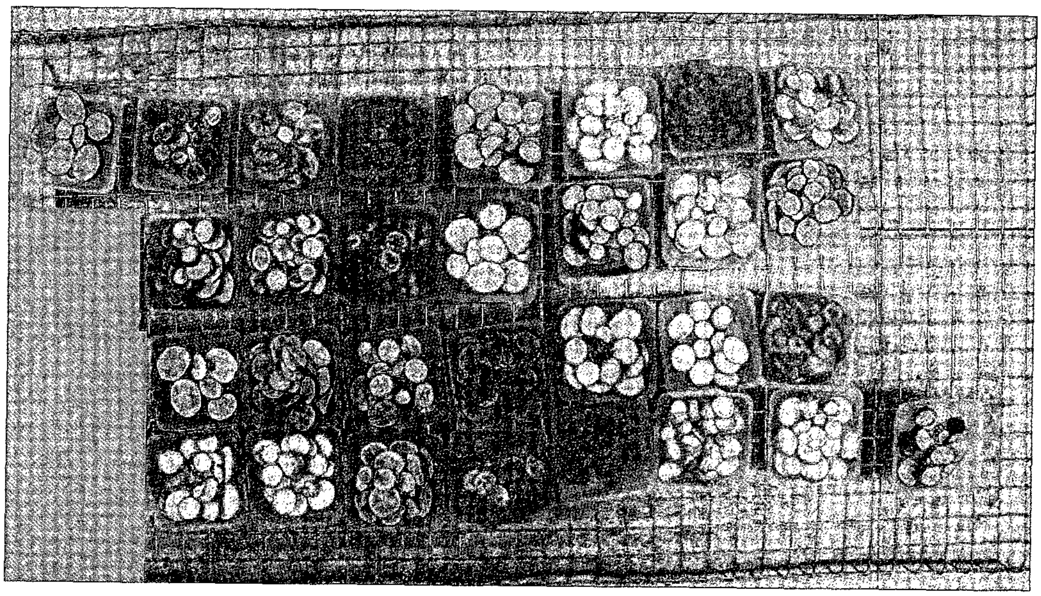
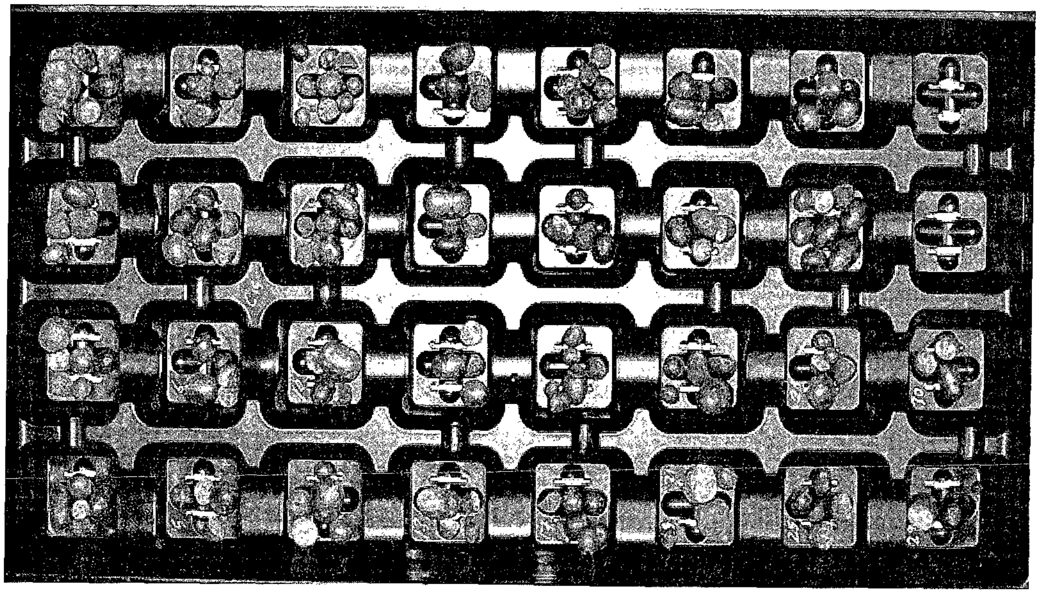


Figure 14. Photograph illustrating the range of skin (A) and flesh (B) color of CO04059 family [CO97219-1R/R (♀) x CO97306-1R/R (♂)].W=white, P= purple, R= red (skin color/flesh color).

A



B

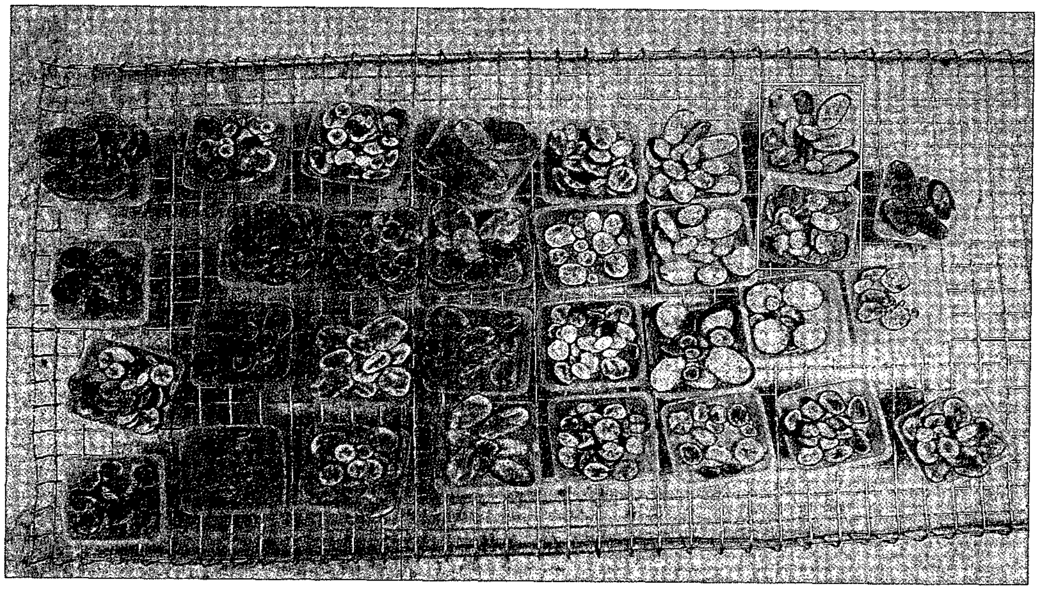


Figure 15. Photograph illustrating the range of skin (A) and flesh (B) color of CO04063 family [CO97226-2R/R (♀) x CO97222-1R/R (♂)].W=white, P= purple, R= red (skin color/flesh color). Trays in the same highlighted yellow box contain the same sample.