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Somatic Mining for Phytonutrient Improvement of 'Russet Burbank' Potato

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Abstract Screening of >800 somaclones of 'Russet Burbank', North America's leading French fry cultivar, for improved yield and processing quality, led to the selection of 25 advanced lines. Three replicates of 3–5 tubers each from advanced lines were assessed after 5 months storage for antioxidant and polyphenol attributes; a subject receiving increased attention among plant breeders, nutritionists, and consumers. Phytonutrients affecting antioxidant components and total antioxidant capacity per serving (150 g fresh matter; one serving size) varied significantly among tubers of the 25 somaclones as well as between these somaclones and 'Russet Burbank' control plants (field tuber-derived and plantlet-derived). Several phenolics, including chlorogenic acid, caffeic acid derivatives, ferulic acid derivatives as well as the flavonoid rutin, ranged in concentration from 10- to 100-fold with some lines exceeding control tuber concentrations by >7-fold. Similarly, ascorbic acid ranged >3-fold (47.21 to 208.63 mg) on a per serving basis with some lines showing significantly greater concentrations than the control plantlet derived tubers (93.82 mg) by >2-fold. Antioxidant capacity, estimated using 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), ranged up to 5-fold for the advanced somaclones (2,121.34 to 11,163.07 μM trolox equivalent/serving). Less variation occurred with other antioxidant capacity tests (DPPH, Folin-Ciocalteu). Overall 17/25 lines had increased antioxidant components. HPLC assays were necessary to confirm lines with better phytonutrient profiles. Somaclonal selection offers clear benefits for phytonutrient improvement in potato and can follow selection for yield and processing attributes.

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Resumen Las evaluaciones de más de 800 somaclones de "Russet Burbank", variedad líder en Norteamérica para elaborar papas a la francesa, para mejorar el rendimiento y la calidad del procesamiento, condujo a la selección de 25 líneas avanzadas. Se analizaron tres repeticiones de 3–5 tubérculos de cada una de las líneas avanzadas después de cinco meses en almacenamiento, para atributos de antioxidantes y polifenoles; que son aspectos de la mayor atención entre fitomejoradores, nutriólogos y consumidores. Los fitonutrientes que afectan a los componentes antioxidantes y la capacidad total antioxidante por ración (150 g de materia fresca; tamaño de una ración) variaron significativamente entre tubérculos de los 25 somaclones, así como entre estos somaclones y las plantas testigo "Russet Burbank" (derivadas de tubérculos del campo y derivadas de plántulas). Varios fenoles, incluyendo el ácido clorogénico, ácido caféico, ácido ferúlico y el flavonoide rutina, variaron en concentración de 10 a 100 veces con algunas líneas, excediendo a las concentraciones de los tubérculos testigo por más de siete veces. Similarmente, el ácido ascórbico varió por más de tres veces (47.21 a 208.63 mg) con base a una ración, con algunas líneas mostrando significativamente mayores concentraciones que la plántula testigo derivada de tubérculos (93.82 mg) por el doble. La capacidad antioxidante, estimada usado 2,2′-azino-bis (3-ethylbenzthiazoline-6-ácido sulfónico) (ABTS), varió hasta por cinco veces más para los somaclones avanzados (2,121.34 a 11,163.07 μM trolox equivalentes/ración). Se presentó menor variación con otras pruebas de capacidad antioxidante (DPPH, Folin-Ciocalteu). En general, 17/25 líneas aumentaron sus componentes antioxidantes. Fueron

necesarios ensayos con HPLC para confirmar las líneas con mejores perfiles de fitonutrientes. La selección somaclonal ofrece beneficios claros para el mejoramiento de fitonutrientes en papa y se puede seguir la selección para rendimiento y atributos de procesado.

Keywords Breeding · Nutrition · *Solanum tuberosum* L · Somatic embryogenesis

Introduction

In populations where potatoes are a dietary staple, they are typically the most consumed vegetable and an underestimated source of antioxidant phytonutrients including phenolic compounds, anthocyanins, and ascorbic acid that can play a major role in maintaining human health. Antioxidants may protect against degenerative diseases such as diabetes, cardiovascular diseases, and cancer that can be initiated by cellular damage resulting from excessive reactive oxygen species (ROS) that disrupt nucleic acids, proteins, and lipid molecules (Halliwell et al. 1992; Kang et al. 2005; Liu et al. 2000, 2001). Potato has been ranked as the third most important source of phenolics in the American diet after apples and oranges (Chun et al. 2005). As a source of total phenolics and antioxidants, potato is moderate among fruits and vegetables (Cao et al. 1996; Wu et al. 2004), greater than onion and carrot (Al-Saikhan et al. 1995), and greater and lesser (Al-Saikhan et al. 1995) or similar (Navarre et al. 2011) to bell peppers and broccoli, respectively.

Phenolics are a large group of small molecular weight compounds that determine organoleptic properties and contribute antioxidant activity (Bravo 1998; Chinnici et al. 2004). Caffeic acid derivatives, particularly chlorogenic acid, are the main phenolic constituents in potatoes and can contribute approximately 90 % of the total phenolic content (Malenberg and Theander 1985; Mattila and Hellstrom 2007). Intensive efforts have been made to identify the factors affecting phytonutrient composition of potato cultivars and wild potato relatives with potential as breeding parents for phytonutrient improvement. Tuber content of antioxidant compounds is affected by genotype, agronomic factors, post-harvest storage, cooking method, processing conditions, and environmental factors including light level, UV-B radiation, and diurnal temperature differences (Al-Daej 2009; Ezekiel et al. 2011). Stushnoff et al. (2008) and Navarre et al. (2011) screened 90 and 50 genotypes, respectively, in the search for genotypes with elevated phenolic levels and high antioxidant capacity, but neither group has reported on hybridization activities. Others have screened for promising parental lines (Hale 2003; Hale et al. 2008; Al-Daej 2009; Wegener and Jansen 2011) and described advanced breeding lines with 5-fold (Hale 2003) or 10-fold (Al-Daej 2009) greater total phenolic concentrations over cultivated potato. Red- and purple-fleshed genotypes generally have greater antioxidant capacity (> 4-fold), total anthocyanins and phenolics, and chlorogenic acid content compared to white- or yellow-fleshed genotypes (Stushnoff et al. 2008; Wegener et al. 2008; Al-Daej 2009). Metabolomics profiling confirmed greater antioxidant activity and phenolic components in pigmented genotypes (Ji et al. 2011).

Potato generally has greater amounts of vitamin C on a fresh mass (FM) basis than apple, carrot, cucumber, grape, lettuce, squash, sweet corn, and tomato (Love and Pavek 2008). Ascorbic acid can be improved through potato breeding programs, better crop management, and choice of suitable cooking methods (Love and Pavek 2008). Vitamin C content in 75 North American potato genotypes ranged from 11.5–29.8 mg/100 g FM with approximately 3-fold difference between the greatest and least (Love et al. 2003). Six parental genotypes had consistent concentrations of vitamin C across multiple years and growing sites; inter-seasonal differences were greater than effects of growing site (Love et al. 2003; 2004). Several wild accessions were identified with greater antioxidant potential assessed as ascorbic acid and trolox-equivalent antioxidant capacity (Davies et al. 2002; Love et al. 2003; Love and Pavek 2008; Wegener and Jansen 2011).

Improvement of potato for phytonutrients, particularly for components affecting antioxidant scavenging capacity such as tuber concentrations of total phenolics, anthocyanins, carotenoids, and ascorbic acid, is important where potato is grown as a staple crop. Production of new cultivars with greater nutritional value can eventually result from conventional methods such as sexual hybridization, utilizing wild potato relatives for parents with greater concentrations of specific or collective antioxidants than cultivated potatoes. Field screening of large numbers (tens of thousands) of seedlings is followed by selection of superior lines, which are then propagated vegetatively and evaluated for agronomic and quality characteristics. All told, this is extremely costly, laborious, and time consuming. For example, the one release to date ('AmaRosa') with relatively high anthocyanins, occupied a large collaborative team for more than a decade (Brown et al. 2012). To expedite this process, non-traditional breeding methods are required. Somatic embryogenesis, a tissue culture-based technique, has been effectively used for 'Russet Burbank', the most important processing cultivar in North America, to select promising somatic lines with good yields and less reducing sugars after long term (5 months) storage (Nassar et al. 2011). The objective of the current study was to evaluate the potential for nutritional selection among 25 advanced somaclones based on their content of total and specific phenolics, rutin, and ascorbic acid that contribute largely to the antioxidant capacity of potatoes.

Materials and Methods

Source of Plant Materials and Sample Preparation

Potato somaclones (approximately 800) were produced through somatic embryogenesis technology and field-evaluated during 2005–2007 (Nassar et al. 2011). Greenhouse-hardened ex vitro somatic plantlets were planted by hand into the field (Florenceville, NB, Canada) in a randomized complete design. The spacing within-row was 45 cm and between-rows was 90 cm. Plantlets were covered with floating row cover (Vesey Seed Ltd., PE, Canada) for 2 weeks. Soil moisture was kept at 75 % field capacity using drip irrigation (Netafilm, Ca, USA). Plants were fertilized with 18.5-15-15 (1,113 $Kgha^{-1}$). At harvest time (109 day season), tuber yield and type were recorded and somaclones with good tuber type and ≥control plantlet-derived yield were retained and stored for 5 months in dark, 10° C, and >95 % RH. After storage, tubers were tested for reducing sugars and French fry processing. Twenty-five advanced somaclones were selected on the basis of yield, tuber type, low reducing sugar levels and good French fry color during late storage (≥ 5 months). In the current study, tubers from field-grown micropropagated plantlets (ex vitro plantlets) were chosen based on their weight confidence interval. From each somatic line, periderm, cortex, and pith tissues were separated. Three replicates each of a homogenized tissue sample of 3–5 tubers were cut and fast-frozen using liquid nitrogen, then freeze-dried at −60 to −70 °C (Christ Freeze-Dryer, Gamma 1–16 LSC, Osterode, Germany). Freeze-dried samples were ground to a fine powder under liquid nitrogen and stored in a −80 °C freezer (Thermo Electron Corporation, OH, USA) until analysis.

Freeze-dried powder (100 mg) was extracted with 3 ml of 90 % methanol (MeOH). Samples were vortexed at maximum speed for 60 s, sonicated (Branson 2200, Branson Ultrasonics Corporation, CT, USA) for 30 min, and centrifuged at 2,630×g for 15 min at 4 °C (AccuSpin 3R, Fisher Scientific, USA). Supernatants were collected and the remaining pellet was re-extracted with 2 ml of 90 % MeOH and the supernatants combined. This crude extract was used for total soluble phenolics (Folin Ciocalteu (FC) assay) and the antioxidant scavenging activity determined using 2,2-diphenyl-1-picrylhydrazyle (DPPH) and 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (hydrophilic phase) assays. The remaining pellet was re-extracted twice, each time with 1 ml hexane, and supernatants combined. This extract was dried in a Speed-Vac (Thermo Savant, Waltham, MA, USA), and the residues re-solubilized in 2 ml of 95 % ethanol and used for the evaluation of antioxidant scavenging capacity using ABTS (lipophilic phase).

Total Soluble Phenolic Contents

Total extractable phenolic contents of samples were evaluated with Folin-Ciocalteu reagent using gallic acid (Chirinos et al. 2007) or chlorogenic acid as standard materials. A 100 µl aliquot of sample extract or a standard dilution was mixed with 2 ml water followed by 200 µl of FC reagent (2 N). Tubes were vortexed and incubated at room temperature (RT) for 5 min, and then 1 ml of aqueous sodium carbonate solution (10 %) was added. Samples were vortexed and kept at RT for 1 h. Absorbance was measured at 765 nm in a Beckman DU 640 spectrophotometer (Beckman Instruments, Fullerton, CA, USA) using 1-cm disposable cells. All measurements were replicated two times. Total phenolic content was expressed as milligrams of gallic acid equivalent (mg GAE) or chlorogenic acid equivalent (mg CAE) per 150 g FM. Standard curves using chlorogenic and gallic acids as standard materials are presented in Fig. 1. Percentage of intra-CV (coefficient of variation) and inter-CV for total phenolic assays were 5.22 and 5.07, respectively.

Antioxidant Capacity (ABTS Hydrophilic and Lipophilic Phases)

2, 2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (7 mM) stock solution was prepared in 18 Ωcm^{-1} water. Radical cation of ABTS ($ABTS^{\bullet+}$) was produced by reacting ABTS solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) in the dark at RT for 12–16 h before use for complete oxidation of ABTS (Re et al. 1999). Oxidation of ABTS starts immediately after adding the $K_2S_2O_8$ but absorbance is not maximal or stable until after >6 h. The radical is stable in this form for more than 2 days when stored at RT in the dark. The $ABTS^{\bullet+}$ solution was diluted with 95 % ethanol to an absorbance of 0.70 (±0.02) at 734 nm. About 1.2 ml of diluted $ABTS^{\bullet+}$ solution (A734 nm=0.700±0.020) was added to 100 µl of sample extract (hydrophilic or lipophilic) or trolox

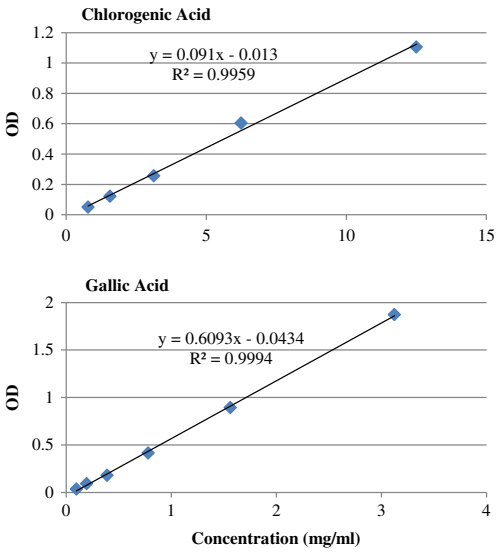

Fig. 1 Calibration curves that were used for total phenolic content estimation using chlorogenic acid or gallic acid as standard materials

standards (0–15 μM) in ethanol and absorbance recorded 1 min after initial mixing and for up to 5 min. Antioxidant scavenging activity was expressed as μM trolox equivalent (TE)/150 g FM. Percentage of intra- and inter-CV for ABTS assays were 3.89 and 3.69, respectively.

Antioxidant Capacity (DPPH Radical Scavenging Capacity)

Radical scavenging capacity assay was performed as described by Martinez-Valverde et al. (2002). Methanolic crude extract (100 μl) was added to 1.5 ml of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (0.5 mM in MeOH) and shaken vigorously. After incubation at RT for 30 min, the absorbance of the remaining DPPH molecules was determined at 517 nm. The mean values were obtained from triplicate determinations. Antioxidant activity was expressed as mg GAE/150 g FM. Percentage of intra- and inter-CV for DPPH assays were 2.36 and 4.57, respectively.

Quantification of Phytonutrients Using High Performance Liquid Chromatography (HPLC)

Polyphenolic acids (chlorogenic, caffeic, and ferulic), the flavonoid rutin, and ascorbic acid were quantified using HPLC (Varian 9012, Varian Chromatography Systems, Walnut Creek, CA) (Shakya and Navarre 2006; Vipin et al. 2007) equipped with a tertiary pump, refrigerated auto-sampler, and a single variable wavelength detector. Samples were detected using a reverse-phase HPLC Gemini-NX column (C18; 5 μm, 100×4.6 mm) (Phenomenex) and a 4.6×2.0 mm guard column was used when needed. Samples were prepared for HPLC analysis as follows: 100 mg of freeze-dried sample was extracted with 0.9 ml of 90 % MeOH (with 0.5 mM meta-phosphoric acid and 0.02 mM EDTA) in 1.5 ml Eppendorf tubes. The tubes were vortexed for 60 s, sonicated for 30 min in cold water (4 C), and centrifuged for 15 min at 4 °C at 2,630×g. The supernatant was collected into a 1.5 ml glass vial. The samples were re-extracted by adding 0.6 ml of extraction buffer as mentioned before and the supernatants were combined into the same 1.5 ml glass vial. Supernatants in glass vials were vacuum-dried for 6–8 h in a speed-vac. Dried-samples were re-solubilized in 500 μl of extraction buffer. Extracts were filtered into a 1 ml HPLC glass vial using a 1 ml syringe and a 0.2 μm nylon filter (Fisher Scientific, Ottawa, ON). About 20 μl of each sample was injected twice into the HPLC and the compounds of interest were identified and quantified based on the retention time and area of the peaks in the chromatographs compared to pure standards.

HPLC running condition was as follows: mobile phase consisted of buffer A and buffer B. Buffer A consisted of 10 mM formic acid. Buffer B had 5 mM ammonium formate in 1 L 100 % MeOH with agitation on a magnetic stirrer. Running conditions were flow rate of 1 ml min^{-1}, a gradient elution of 0–1 min 100 % buffer A, 1–5 min 0–30 % buffer B, 5–6.5 min 40–70 % buffer B, 6.5–8.5 min 70–100 % buffer B. UV detection was done at 280 nm. The column was re-equilibrated with 100 % buffer A for 1 min after each sample injection. The external standards were used for calibration of each compound; each standard curve was prepared from 6 to 8 different concentrations of standard solutions (Figs. 2, 3 and 4).

Calculation of Mean Content of Phytonutrients and Antioxidant Capacity

Mean tissue concentration values were used to estimate the amount of total phenolics, antioxidant capacity (ABTS and DPPH), ascorbic acid, polyphenolics (chlorogenic, caffeic, and ferulic acids), and rutin in a typical tuber of 100 g FM using conversion factors established for average tuber tissue volumes of control NB 'Russet Burbank' (Ortiz-Medina et al. 2009). For periderm, cortex, and pith, phytonutrient concentrations were multiplied by 0.403, 9.336, and 11.791, respectively. For example, total phenolic content in a whole tuber of 100 g FW was estimated by summation of values for pith, cortex, and periderm and then results were converted into 150 g FM. These estimates enabled logical comparison of phytonutrient content among tested potato genotypes.

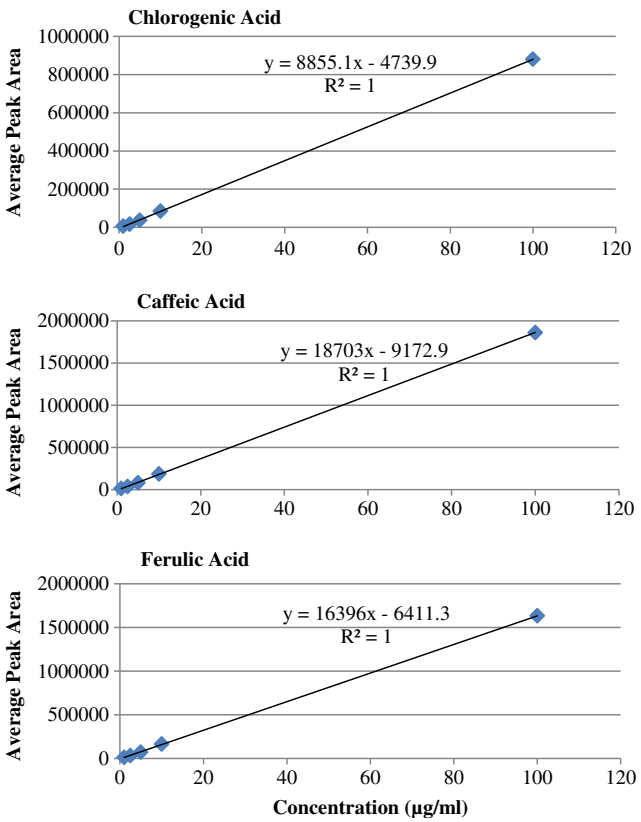

Fig. 2 Standard curves for HPLC measurement of polyphenolics using chlorogenic acid, caffeic acid, and ferulic acid standard materials

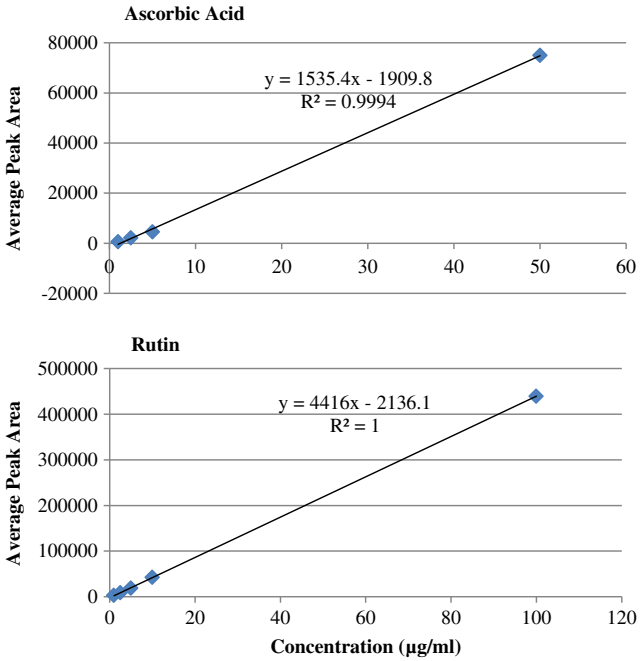

Fig. 3 Standard curves for HPLC measurement of ascorbic acid and rutin using standard materials of each compound

Quality Control of Ascorbic Acid

Method reliability, quantification limits, and detection limits were assessed for ascorbic acid using certified reference material (Brussels sprouts; Sigma CRM® 431) (modified from Tarrago-Trani et al. 2012), because vitamin C was measured using a single wavelength HPLC detector (A 280), which was a limitation of the method. Certified reference materials were not available for other compounds estimated. Extraction and analysis of the certified freeze-dried Brussels sprouts powder with known concentration of ascorbic acid (459–507 mg/100 g DM) was done using the same technique described for ascorbic acid analysis for HPLC. About 20 µl of the methanolic extract was injected into the HPLC using the same run conditions described above. Ascorbic acid content in the certified material was 488.77±20.03 mg/100 g DM.

Limits of Detection and Quantification of Ascorbic Acid

Limits of detection (LD) and quantification (LQ) were estimated statistically using a standard curve of ascorbic acid (Ermer 2005). Dilution series of ascorbic acid from 0.76 to 24.41 (µg/ml) were repeated 3 times each and standard error (SE) was calculated. A calibration curve was plotted and the slope (S) was calculated from the regression equation ($Y=3{,}000{,}000*X-1713.2$; $R^2=0.9997$) and then $LD=3.3*SE/S$ and $LQ=10*SE/S$ were estimated on a dry matter basis. Results of detection and quantitation limits of ascorbic acid were 2.08 and 6.31 µg, respectively.

Percentage Variation

Percentage variation among somaclones and between highest and least somaclone values and control values were calculated. Percentage variation among somaclones=(highest $value_{somaclone}$−least $value_{somaclone}$)/highest $value_{somaclone}$×100. While percentage variation among somaclones and control plantlets of 'Russet Burbank'=(highest or least $value_{somaclone}$−value of the control)/value of control×100.

Experimental Design and Statistical Analysis

The experiment was designed as a Completely Randomized Design (CRD) with one main factor, genotype, with three replicates, where each replicate was represented by a mixture of

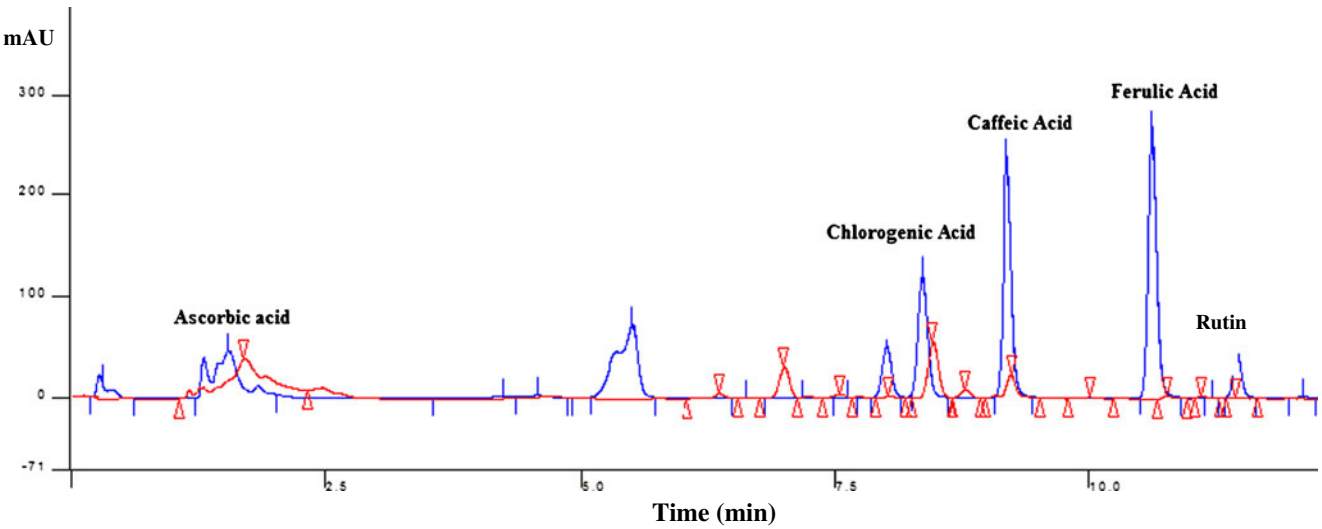

Fig. 4 Chromatogram (*labelled, in blue*) is for standard materials of ascorbic acid, the polyphenolic acids: chlorogenic, caffeic derivatives, and ferulic derivatives, and the flavonoid rutin while chromatogram (*unlabelled, in red*) is for sample separation

tissues from 3 to 5 tubers. The tubers that were used for analysis in each cultivar were selected within the confidence intervals of individual tuber weights. Results were analyzed using the General Linear Model (GLM) of Statistical Analysis System (SAS) (SAS 2011). Means of the results were compared using Tukey's honestly significant difference *post-hoc* test ($P \leq 0.05$).

Results and Discussion

Antioxidant Scavenging Activity

ABTS

The ABTS measure of antioxidant activity is applicable for both hydrophilic and lipophilic antioxidants, including flavonoids, hydroxyl cinnamates, and carotenoids (Re et al. 1999). Sixteen of 25 advanced somaclones had greater ABTS activity than control genotypes ('Russet Burbank' plantlet-derived and field-derived tubers) (Tables 1 and 2). Total ABTS values (sum of the ABTS hydrophilic and lipophilic extracts) ranged from 1,655.74 (tubers from control plantlet of 'Russet Burbank') to 11,163.07 (MS1406) μM TE/150 g FM. All but two somaclones (FC2006 and FP2906) had greater ABTS content than control plantlet-derived 'Russet Burbank'. Tubers from 'Russet Burbank' field tuber-derived controls showed greater ABTS activity compared to 'Russet Burbank' plantlet-derived tubers.

Wide variation occurred for ABTS values among tubers of somaclones (81.00 %) and between tubers of somaclones and control 'Russet Burbank' plantlet-derived (28.12 (FP2906) to 574.20 % (MS1406)) (Table 1 and Fig. 5). These results were

Table 1 LS Mean values[a] of average graded tuber weight (Kg) and antioxidant scavenging activity using ABTS (μM trolox equivalent (TE)/150 g FM) and DPPH (mg gallic acid equivalent (GAE)/150 g FM) and total phenolic contents (mg GAE/150 g FM and mg chlorogenic acid equivalent (CAE)/150 g FM) using FC reagent of tubers from advanced somatic lines and control cultivars including Russet Burbank (ex vitro plantlets (P) and field tubers (F))

Genotype	Average Graded Tuber Weight (Kg)	Antioxidant Scavenging Capacity		Total Phenolic Content (FC)	
		ABTS (TE)	DPPH (GAE)	GAE	CAE
'Russet Burbank' (P)	0.77^{cde}	$1{,}655.74^{l}$	149.29^{a}	103.43^{abc}	572.62^{abc}
'Russet Burbank' (F)	1.51^{a}	$5{,}113.90^{fgh}$	132.51^{bc}	116.95^{ab}	663.15^{ab}
MS1406	0.70^{cde}	$11{,}163.07^{a}$	118.25^{ij}	68.17^{de}	336.58^{de}
FP806	0.56^{e}	$10{,}733.45^{a}$	$122.34^{e–j}$	64.18^{de}	309.83^{de}
FP106	0.66^{de}	$1{,}0280.8^{ab}$	135.25^{b}	71.27^{de}	357.33^{de}
FP306	0.77^{cde}	$9{,}740.89^{abc}$	$120.93^{g–j}$	61.12^{e}	289.35^{e}
MP19805	0.89^{bcde}	$9{,}616.50^{abc}$	$121.18^{f–j}$	63.80^{de}	307.33^{de}
MP405	0.79^{cde}	$9{,}573.76^{abc}$	$124.64^{d–i}$	82.58^{cd}	433.05^{cd}
FC1106	0.75^{cde}	$9{,}520.80^{a–d}$	130.63^{bcd}	69.78^{de}	347.33^{de}
MP706	0.76^{cde}	$9{,}375.91^{a–d}$	$128.12^{b–g}$	70.35^{de}	351.14^{de}
FC606	0.82^{cde}	$9{,}287.95^{a–d}$	$126.59^{d–h}$	70.38^{de}	351.35^{de}
MP11505	0.66^{de}	$8{,}298.48^{bcd}$	118.04^{ij}	72.74^{de}	367.16^{de}
FC2806	1.04^{bc}	$8{,}298.40^{bcd}$	$129.57^{b–e}$	65.76^{de}	320.40^{de}
MP18405	0.97^{bcd}	$8{,}278.17^{bcd}$	$128.43^{b–f}$	58.90^{e}	274.47^{e}
FP2106	0.82^{cde}	$7{,}961.56^{cde}$	116.16^{j}	69.89^{de}	348.07^{de}
MP7405	0.73^{cde}	$7{,}949.79^{cde}$	119.42^{hij}	64.77^{de}	313.78^{de}
MC10605	0.70^{cde}	$7{,}820.73^{cde}$	$122.50^{e–j}$	63.96^{de}	308.40^{de}
MC1606	0.60^{de}	$7{,}513.71^{cde}$	147.49^{a}	63.27^{de}	303.78^{de}
FP8106	0.67^{de}	$7{,}294.18^{def}$	132.79^{bc}	65.47^{de}	318.53^{de}
MS906	0.64^{de}	$5{,}975.32^{efg}$	117.53^{ij}	64.80^{de}	314.00^{de}
MC405	0.91^{bcde}	$4{,}785.15^{ghi}$	131.55^{bcd}	102.82^{bc}	568.56^{bc}
MP9605	0.86^{bcde}	$4{,}358.59^{g–j}$	118.30^{ij}	62.29^{e}	297.24^{e}
FP3405	0.72^{cde}	$4{,}006.43^{g–k}$	116.64^{j}	65.84^{de}	320.98^{de}
FC406	0.83^{cde}	$3{,}689.30^{h–k}$	119.95^{hij}	72.77^{de}	367.37^{de}
FP906	0.66^{de}	$3{,}314.19^{h–k}$	118.86^{ij}	68.92^{de}	341.58^{de}
FC2006	0.68^{cde}	$2{,}451.18^{jkl}$	130.86^{bcd}	122.59^{a}	700.96^{a}
FP2906	0.88^{bcde}	$2{,}121.34^{kl}$	132.74^{bc}	110.15^{ab}	617.64^{ab}

[a] LS Means were compared using Tukey's Studentized Range (HSD) Test ($P \leq 0.05$). Means with the same superscript letter are not significantly different

Table 2 LS Mean values[a] of polyphenolic acids (chlorogenic, caffeic derivatives, and ferulic derivatives), rutin, total phenolics, and ascorbic acid (mg/150 g FM) of potato advanced somatic lines and control cultivars including Russet Burbank (ex vitro plantlets (P) and field tubers (F)), evaluated using high pressure liquid chromatography (HPLC)

Genotype	Polyphenolic Acids			Rutin	[b]Total Phenolics (HPLC)	Ascorbic Acid
	Chlorogenic	Caffeic	Ferulic			
Russet Burbank (P)	46.97^{ij}	4.73^{hi}	4.12^{ghi}	4.10^{efg}	59.92^{d}	93.82^{bcd}
Russet Burbank (F)	119.41$^{d–j}$	36.51^{efg}	10.70$^{d–h}$	19.52^{bc}	186.14$^{a–d}$	76.24^{cd}
MS1406	272.57^{abc}	78.73^{b}	22.28^{b}	18.17^{bc}	391.75^{a}	169.64^{ab}
FP806	244.53$^{a–d}$	4.22^{i}	16.18^{bcd}	13.11^{cd}	278.04$^{a–d}$	93.91^{bcd}
FP106	252.50^{abc}	55.95$^{c–f}$	9.55$^{d–i}$	1.37^{g}	319.37^{abc}	60.63^{cd}
FP306	205.37$^{b–f}$	34.43^{fg}	4.13^{ghi}	30.93^{a}	274.86$^{a–d}$	84.98^{bcd}
MP19805	203.39$^{b–g}$	50.48$^{c–g}$	3.65^{ghi}	17.64^{bc}	275.16$^{a–d}$	149.38^{abc}
MP405	46.07^{j}	1.60^{i}	1.20^{i}	1.77^{g}	50.64^{d}	80.09^{bcd}
FC1106	173.83$^{c–i}$	37.52^{efg}	4.50^{ghi}	6.04^{efg}	221.89$^{a–d}$	70.25^{cd}
MP706	313.73^{ab}	65.38^{bcd}	17.09^{bed}	1.55^{g}	397.75^{a}	56.22^{cd}
FC606	209.66$^{b–e}$	59.78$^{b–e}$	2.26^{hi}	7.09$^{d–g}$	278.79$^{a–d}$	99.95^{bcd}
MP11505	280.45^{abc}	57.34$^{b–f}$	34.11^{a}	3.99^{efg}	375.89^{a}	129.04$^{a–d}$
FC2806	45.48^{j}	1.18^{i}	2.79^{hi}	3.18^{efg}	52.63^{d}	95.77^{bcd}
MP18405	344.20^{a}	114.97^{a}	15.63$^{b–e}$	32.24^{a}	507.04^{ab}	120.28$^{a–d}$
FP2106	46.92^{ij}	8.98^{hi}	5.15^{ghi}	7.82$^{d–g}$	68.87^{cd}	112.41$^{a–d}$
MP7405	69.88^{hij}	27.73^{gh}	6.51$^{e–i}$	5.24^{efg}	109.36^{bcd}	92.48^{bcd}
MC10605	114.85$^{e–j}$	3.52^{i}	3.02^{ghi}	4.82^{efg}	126.21^{bcd}	208.63^{a}
MC1606	77.70$^{g–j}$	7.30^{hi}	2.53^{hi}	3.99^{efg}	91.52^{cd}	131.46$^{a–d}$
FP8106	229.14$^{a–e}$	70.92^{bc}	22.52^{b}	21.66^{b}	344.24^{ab}	77.82^{bcd}
MS906	185.63$^{c–h}$	46.89$^{d–g}$	6.03$^{f–i}$	2.75^{fg}	241.3$^{a–d}$	47.21^{d}
MC405	77.74$^{f–j}$	8.95^{hi}	15.22$^{b–f}$	4.11^{efg}	106.02^{bcd}	100.07^{bcd}
MP9605	58.07^{hij}	9.24^{hi}	1.13^{i}	3.32^{efg}	71.76^{cd}	60.82^{cd}
FP3405	38.02^{j}	4.90^{hi}	6.53$^{e–i}$	7.37$^{d–g}$	56.82^{d}	83.52^{bcd}
FC406	71.89^{hij}	47.87$^{c–g}$	16.69^{bcd}	34.00^{a}	170.45$^{a–d}$	115.07$^{a–d}$
FP906	36.14^{j}	2.24^{i}	6.34$^{e–i}$	13.92^{cd}	58.64^{d}	80.90^{bcd}
FC2006	58.40^{hij}	6.97^{hi}	3.13^{ghi}	6.14^{efg}	74.64^{cd}	125.60$^{a–d}$
FP2906	202.04$^{b–g}$	47.72$^{c–g}$	20.47^{bc}	9.90^{de}	280.13$^{a–d}$	172.69^{ab}

[a] LS Means were compared using Tukey's Studentized Range (HSD) *post-hoc* Test ($P \leq 0.05$). Means with the same superscript letter are not significantly different

[b] Total phenolics were the sum of the four phenolics chlorogenic acid, caffeic acid, ferulic acid, and rutin

low compared to the antioxidant capacity (2,700–21,900 μM TE/100 g DM) reported for white- and purple-fleshed potato cultivars and breeding lines (determined by the ORAC method; Navarre et al. 2011).

DPPH

'Russet Burbank' plantlet-derived control and the somaclone MC1606 had similar tuber DPPH values (mg GAE/150 g FM) and were greater in DPPH than the other somaclones and the field-grown cv. Russet Burbank (Table 1). Less variation occurred among DPPH values compared to that found in the ABTS results. The DPPH assay is best correlated with the alcoholic (methanolic) soluble antioxidants while ABTS is best correlated with polar- (water and alcohols) and non-polar-soluble antioxidants (Arnao 2000). Variation in DPPH among somaclones was 21.24 % and between somaclones and 'Russet Burbank' plantlet-derived control it ranged from −22.19 (FP2106) to −1.21 % (MC1606). Antioxidant activity determined by the DPPH assay varied widely among tuber-bearing potato species (7,200–123,600 μg TE/150 g FM) (Hale et al. 2008).

Total Phenolic Content

Somatic lines generally had reduced tuber phenolic contents (TP) based on the Folin-Ciocalteu (FC) assay gallic acid equivalent (GAE) (Table 1). Only three lines FC2006,

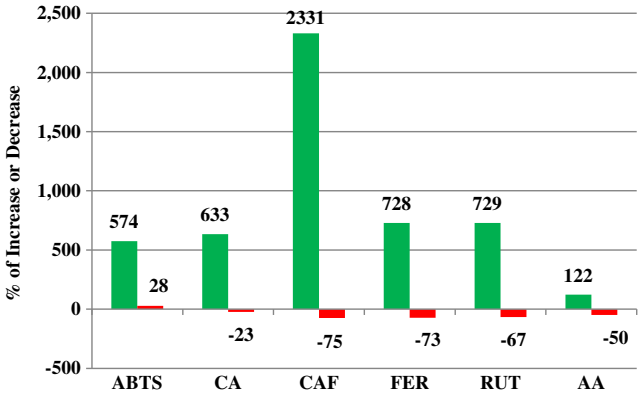

Fig. 5 Variation (percent of increase or decrease from control plantlet-derived 'Russet Burbank') in ABTS scavenging activity and phytonutrient content: CA; chlorogenic acid, CAF; caffeic acid derivatives, FER; ferulic acid derivatives, RUT; rutin, and AA; ascorbic acid compared with control tubers from plantlet-derived 'Russet Burbank'

FP2906, and MC405 had similar phenolic content to tubers of plantlets or field-grown tuber controls of 'Russet Burbank', which were similar. Variation occurred in TP content among somaclones (51.95 %) and between somaclones and 'Russet Burbank' plantlet- and field tuber-derived controls (−43.05 (MP18405) to 18.52 % (FC2006)). Red- and purple-fleshed potato cultivars and breeding lines had 114 to 271 mg chlorogenic acid equivalent (CAE)/150 g FM (Reyes et al. 2005), The total phenolic content of 'Russet Burbank' controls based on GAE were similar to previously reported values (105–120 mg/150 g FM; utilizing an average moisture content of 80 %) (Külen et al. 2013). The utilization of GAE to determine TP resulted in lesser polyphenolic amounts compared to use of chlorogenic acid equivalent (CAE). Results in Table 1 showed that TP contents ranged from 274.47–700.96 mg CAE compared to 58.90–122.59 mg GAE/150 g FM. The latter values are comparable to TP values that ranged from 54.6–581.4 mg GAE/150 g FM (considering DM content as 20 %) among tubers of advanced lines (Al-Daej 2009).

The TP values estimated using the GAE standard generally corresponded to the lower range of total phenolic content estimated by HPLC. Conversely, the TP content using the CAE standard tended to match closer to the higher range of values estimated by HPLC. Clearly, CAE is more credible since most of the total phenolic content of potato tubers is composed of chlorogenic acid. The HPLC estimates of total phenolics may be on the low side relative to the CAE estimates of TP content partly due to several unidentified peaks (Fig. 4). The current study clearly shows that measurement of total phenolic content as mg of GAE consistently underestimated the content of total phenolics of "chlorogenic-rich samples" like potato (by 4.7–5.7-fold). This latter finding strongly supports the work of Chun and Kim (2004) who recommended replacement of GAE with CAE to measure total phenolics in chlorogenic acid-rich plant samples. It is quite possible that many published values where the FC test using GAE for potato can have substantially underestimated the total phenolic content. Major differences in total phenolic content based on the use of different standards have been previously noted by others. For example, Ah-Hen et al. (2012) noted that 'Violette' showed relatively high total phenolic content (1,283 mg/150 g FM) based on ferulic acid equivalents.

Values of total phenolics estimated by HPLC were less compared to that measured by Folin Ciocalteu CAE (Tables 1 and 2) since the FC assay may overestimate plant phenolic content due to the presence of non-phenolic components such as proteins, amino acids, thiol compounds, and vitamins (Escarpa and González 2001; Everette et al. 2010). The use of FC assay was recommended as an antioxidant scavenging measure and not for total phenolic content (Everette et al. 2010). Total phenolic contents of individual compounds estimated by HPLC ranged from 50.64–507.04 mg/150 g FM (Table 2). When total phenolic content was based on the addition of the individual compounds as quantified via HPLC, variation occurred from 9–59.4 mg/150 g FM among *S. tuberosum* genotypes (Hale 2003) whereas greater variation was observed among wild *Solanum* species from 5.3 to 237.6 mg/150 g FM (Hale et al. 2008). These values were much greater for purple-fleshed breeding lines that showed 234- to 266-fold greater total phenolic content than white- and yellow-fleshed genotypes (Wegener et al. 2008). In the current study, tubers of six clones showed greater content of total phenolics compared to plantlet-derived but not field-tuber-derived controls of 'Russet Burbank' which showed similar content to all somaclones and the plantlet-derived control. The sum of the individual identified phenolics showed that chlorogenic acid ranged from 42 to 91 % of total phenolics (Table 2).

Polyphenolic Acids

Chlorogenic Acid

Wide variation was found among somaclones and between somaclones and controls in tuber chlorogenic acid content (mg/150 g FM) (Table 2). Five somaclones including MP18405 (344.20), MP706 (313.73), MP11505 (280.45), MS1406 (272.57), and FP106 (252.50) had greater chlorogenic acid content than both plantlet-derived (46.97) and field tuber-derived (119.41) controls of 'Russet Burbank'. The remaining somaclones had similar tuber content of chlorogenic acid to the two controls, which were similar to each other.

Wide variation in chlorogenic acid (533 %) was reported among purple-, white-, and yellow-fleshed breeding lines (Wegener et al. 2008) and 95.35 % among a population of 55 potato genotypes including cultivars, breeding lines, and primitive germplasm and wild species (6.6 to 141.9 mg/150 g

FM; considering dry matter content as 20 % on average) (Navarre et al. 2011). Variation among somaclones and plantlet-derived control 'Russet Burbank' (> 7 fold) ranged from −23.06 to 632.81 % (Fig. 5) and was clearly great enough to select somatic lines with lesser or greater chlorogenic acid content. However, values of chlorogenic acid reported herein are higher than any previously reported especially for white potatoes.

Caffeic Acid Derivatives

Four somaclones (MP18405, MS1406, FP8106, and MP706) showed greater tuber caffeic acid derivatives content (mg/150 g FM) than control 'Russet Burbank' both plantlet-derived and field grown tuber-derived (Table 2). All remaining somaclones had tuber caffeic acid derivatives levels similar to that of 'Russet Burbank' control plantlet-derived. 'Russet Burbank' control field tuber-derived tubers had greater caffeic acid derivatives content than the plantlet-derived tubers.

Wide variation occurred in caffeic acid derivatives content among somaclones and the plantlet-derived control plants (from −75.05 (FC2806) to 2,330.66 % (MP18405); Fig. 5). This range, while substantive, is less than that reported by Navarre et al. (2011) for white- and purple-fleshed potato cultivars and breeding lines (up to 9,520 %). Nevertheless, selection of somatic lines with improved tuber caffeic acid content is clearly possible.

Ferulic Acid Derivatives

Four somatic lines (MP11505, FP8106, MS1406, and FP2906) had the greatest concentration of tuber ferulic acid derivatives (Table 2). The remaining lines had similar contents to both 'Russet Burbank' controls, which were similar in content of ferulic acid derivatives. Wide variation occurred in ferulic acid content among somaclones and control plantlet-derived tubers (−72.69 to 727.91 %) (Fig. 5). For this reason, selection for somatic lines with improved tuber ferulic acid content is possible.

The Flavonoid Rutin and its Derivatives

Three advanced somaclones (FC406, MP18405, and FP306) had greater tuber rutin content than both plantlet- and field tuber-derived controls (Table 2). The control 'Russet Burbank' field tubers had five times the rutin content than the plantlet-derived tubers. Most lines (17/25) had similar content of rutin to the control plantlet-derived tubers. Variation in rutin concentration ranged from −66.69 to 729.27 % (Fig. 5). Variation in tuber rutin was less than that reported by Navarre et al. (2011) who found variation of about 4,862 % (DM basis) between the greatest and least rutin concentrations among parental genotypes and breeding lines.

Ascorbic Acid Content

Ascorbic acid levels ranged from 47.21–208.63 mg/150 g FM (Table 2). The somaclone MC10605 had the greatest tuber ascorbic acid content (208.63 mg/150 g FM) compared with control cultivars and all tested somaclones (Table 2). The somaclones FP2906 and MS1406 had greater ascorbic acid content compared to control 'Russet Burbank' (field tuber-derived). All other somaclones had similar ascorbic acid content to the plantlet- and field tuber-derived 'Russet Burbank' controls. The range of ascorbic acid levels among somaclones (47.21–208.63 mg/150 g FM) was greater than those reported in potato (17.25–44.70 mg/150 g FM) among parental genotypes examined by the microflourometric method by Love et al. (2003, 2004) while ascorbic acid content ranged from 24–69 mg/150 g FM in four Korean potato cultivars quantified using HPLC (Han et al. 2004). Dale et al. (2003) reported vitamin C content of 12–45 mg/150 g FM (~ 4- fold differences) among 33 genotypes grown in Europe while Kwon et al. (2006) showed more variability in vitamin C levels (0.74–45.3 mg/150 g FM) in 'Superior', 'Atlantic', 'Shepody', 'Jopung', and 'Nomsuh' grown in Korea. Ascorbic acid levels in potato advanced breeding lines ranged from 48.45–58.35 mg/150 g FM (Cho et al. 2013). Previous work showed ascorbic acid content of 'Russet Burbank' to be substantially lower—about 2.5-fold lower also via the microflourometric method (29.25 mg/150 g FM; Novy et al. 2006). 'Russet Burbank' had lesser ascorbic acid when the season was extended (i.e. 36 mg/150 g FM when harvested after 150 days but 54 mg/150 g FM after 120 days) measured using the indophenol titration method (Augustin et al. 1975).

As ascorbic acid methodology reported herein was confirmed to be valid using the certified plant reference material, the higher values reported in the present study are likely attributable to genotypic differences known to occur among somaclones and perhaps superimposed upon other factors shown to cause wide variation in potatoes such as agricultural practices, tuber maturity, temperature, season, and storage (Zhang et al. 1997; Hamouz et al. 2009; Cho et al. 2013). For example, differences in heavy metal content of soil caused significant variation in ascorbic acid content in potatoes (Musilova et al. 2009). In that regard, they reported that 'Asterix' grown in soil with greater cadmium level had ascorbic acid content of 84.84 mg/150 g FM measured by HPLC, which is similar to the ascorbic acid content in 'Russet Burbank' plantlet- or field tuber-derived controls in the current study. HPLC determination of ascorbic acid is more sensitive compared to spectrophotometric methods which may underestimate its content due to interference of analytes (ferrous or cuprous), sugars, or glucuronic acid (Nováková et al. 2008). Developmental stage had a significant effect on ascorbic acid content (Cho et al. 2013) and younger tubers had greater content than mature tubers (Augustin et al. 1975; Cho et al.

2013). It is possible that our ex vitro plantlets produced tubers that were physiologically less mature; similar to the shorter-season tubers with greater vitamin C content. Vitamin C content was almost double when 'Russet Burbank' was harvested at 120 d versus 150 d (Augustin et al. 1975) whereas the potatoes in Canada including our clones were harvested at even earlier time point of 109 days and this could strongly account for the higher vitamin C content in the current report. Warmer seasonal temperatures tended to increase vitamin C content. For example, high temperatures at planting time resulted in tubers with elevated vitamin C content (Hamouz et al. 2009). Also, spring cultivation showed increased ascorbic acid levels compared with potato planted during the fall season (Cho et al. 2013).

Variation in ascorbic acid content among somaclones was 77.37 %; from −49.68 (MS906) to 122.37 % (MC10605) compared with 'Russet Burbank' (plantlet-derived) (Table 2 and Fig. 5). This wide variation range strongly suggests that ascorbic acid levels could be improved by selection among somaclones. A wide variation in ascorbic acid content was observed by Davies et al. (2002). Wild tuber-bearing species showed greater ascorbic acid content compared with *S. tuberosum* cultivars and hybrids. By comparison, a three-fold difference was found among 75 genotypes in the North American potato germplasm repository (Love et al. 2003). The stability of potato genotypes should be tested over years (Love et al. 2004). Phytonutrient content will be retested at several sites over the next few growing seasons to determine relative stability in somaclone tubers.

Screening for Phytonutrient Improvements

In the current study, the increase in antioxidant capacity via ABTS was more strongly related to observed values of the antioxidant components of interest than was DPPH or total phenolics (Tables 1 and 2). The discrepancies might be related to different mechanisms of antioxidant action measured among the tested assays. Results of TP, polyphenolics, rutin, and ascorbic acid reported in the current study were greater than previously reported in the literature maybe due to environmental factors, agricultural practices, genotypes, and/or the method that was used to calculate summative content of different tissue layers (periderm, cortex, and pith) which might have affected the phytonutrient content. Similar to vitamin C content, the relative earlier stage of maturity due to harvesting at 109 days could be an important factor that could explain the substantially greater phenolic values reported in the current study. Thus, the impact of an earlier harvest time on potato phytonutrient content requires further study. Clearly, HPLC determinations of polyphenolics, rutin, and ascorbic acid were required along with these indicators of antioxidant capacity for screening somaclones for improved phytonutrient components. Approximately, 6/16 lines with increased ABTS values related to increased phenolic components; among these were the two lines with the greatest ABTS values, where two or more components were increased. On the other hand, three lines with one or two increased phenolic components showed low ABTS values. Overall, 17/25 lines had increased antioxidant component(s) (5 with chlorogenic acid, 4 with caffeic acid derivatives, 4 with ferulic acid derivatives, 3 with rutin, and 1 with ascorbic acid (Table 3)).

Success of Using Somatic Embryogenesis Technology in Phytonutrient Improvement

Considering the high per capita consumption of potatoes compared to other vegetables, widespread adoption of high-phytonutrient cultivars could significantly increase dietary intake of phytonutrients. Antioxidant levels have varied widely among tested genotypes suggesting genetic control and the possibility of improving this trait (Hale 2003). Advanced somatic lines displayed enough variation to enable selection for greater antioxidant capacity (17/25 advanced lines) despite the very small sample size tested and long storage interval (5 months) prior to evaluation (Table 3 and Fig. 5). It is important to put these numbers into context based on the original population of 800 somatic regenerants from tuber tissues, selected in a realistic, prioritized manner for yield, type, and processing attributes, and subsequently for antioxidant capacity using HPLC. Overall, percentages of selected lines that were improved for chlorogenic acid, caffeic acid derivatives, ferulic acid derivatives, rutin, and ascorbic acid were 0.63, 0.50, 0.50, 0.38 and 0.13 %, respectively. Comparing these percentages with what has been reported in the literature and taking into consideration the challenges of conventional potato breeding, somaclonal selection appears to be a useful strategy to combine both processing and phytonutrient improvements. In a typical breeding program, tens of thousands of seedlings are grown from crosses between dozens of parents

Table 3 Selection efficiency. Number and percentage of selected lines with possibly improved phytonutrient content in terms of ABTS and HPLC content of phenolics (caffeic, chlorogenic, and ferulic acids, and rutin), and ascorbic acid

Traits	No. of Improved Lines	% of 25 Advanced Lines	% of 800 Lines Evaluated
ABTS	16	64	2.00
Chlorogenic acid	5	20	0.63
Caffeic acid derivatives	4	16	0.50
Ferulic acid derivatives	4	16	0.50
Rutin	3	12	0.38
Ascorbic acid	1	4	0.13
Overall using HPLC	17	68	2.13

with less than 1 % of hybrids retained for successive selection over several years to evaluate stability and performance. So, it might take up to 10 years or more for a clone to be considered for new cultivar status (Jansky 2009). Selection among advanced somatic lines, previously selected for yield and type, lower reducing sugars and associated better processing, highlights the possibility of multiple-trait selection for successful use in phytonutrient improvement programs.

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