Am. J. Pot Res (2011) 88:387–397
DOI 10.1007/s12230-011-9204-5

Intraclonal Selection for Improved Processing of NB 'Russet Burbank' Potato

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Published online: 27 July 2011

Abstract 'Russet Burbank' has limited fertility and has not parented improved cultivars through traditional breeding efforts. This study showed that 'Russet Burbank' (NB clone) could be improved through selection of intraclones (somatic embryos derived from specific tuber tissues) based on field performance and/or processing characteristics. In 2005 and 2006, approx. 800 intraclones were regenerated from field-grown tubers or microtubers. Intraclones were micropropagated, acclimatized, and field-tested to identify the highest yielding lines. Each season, following storage, tubers of selected lines were tested for glucose content and French fry-processing quality. In 2007, the best intraclones from earlier seasons were increased through micropropagation and retested for yield and processing features. Approx. 2–9% of intraclones had similar yield to controls but superior processing features. Neither tuber source nor explant tissue type affected intraclone tuber yield, type, or processing characters. We recommend the incorporation of somatic embryogenesis into potato improvement programs for processing quality traits.

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Resumen "Russet Burbank" tiene fertilidad limitada y no ha sido progenitor que haya mejorado variedades a través de esfuerzos de mejoramiento tradicional. Este estudio demostró que "Ruset Burbank" (clon NB) pudo mejorarse por selección de intraclones (embriones somáticos derivados de tejidos específicos de tubérculo) con base en el comportamiento en el campo y/o en características de procesamiento. En 2005 y 2006, se regeneraron aproximadamente 800 intraclones de tubérculos cultivados en el campo o de microtubérculos. Los intraclones se micropropagaron, se aclimataron, y se probaron en el campo para identificar las líneas de rendimiento más alto. En cada ciclo de cultivo, después del almacenamiento, se probaron tubérculos de líneas selectas para el contenido de glucosa y de calidad de fritura para papas a la francesa. En 2007, los mejores intraclones de siembras previas se incrementaron por micropropagación y se volvieron a probar para rendimiento y características de procesamiento. Aproximadamente el 2–9% de intraclones tuvieron rendimiento similar al de los testigos pero mejores características de procesamiento. Ni la fuente del tubérculo ni el tipo de tejido del explante (inóculo) afectaron el rendimiento de tubérculo de los intraclones, o el tipo o características del procesamiento. Recomendamos la incorporación de embriogénesis somática en los programas de mejoramiento de papa para las características de calidad en el procesamiento.

Keywords Somatic embryogenesis · Potato · Processing · Reducing sugars · Yield · Somaclonal variation

Introduction

'Russet Burbank' potato (*Solanum tuberosum* L.) originated in 1914 as a sport of 'Burbank', quickly supplanted it in

importance (Davis 1992), and currently dominates the North American French fry industry (Salaiz et al. 2005; Rommens et al. 2006; Gagnon et al. 2007). 'Russet Burbank' has limited fertility and has not parented improved cultivars despite numerous breeding trials (Iritani and Weller 1978; Shepard et al. 1980; PAA 2008).

'Russet Burbank' improvement could take place through chance identification of a mutant (uncertain) or screening for high-performing geographic variants. For example, selection for 'Norgold Russet' with greater stem vigour lead to the release of 'Norgold Russet M' which has replaced the original cultivar in many USA states (Lever et al. 1994). Similarly, selection of giant hill mutants followed by recurrent selection for improved geographic variants or ecotypes of 'Russet Norkotah' in Colorado and Texas has resulted in improved strains that out-yield 'Russet Norkotah' by 20–30% (Miller et al. 1999, 2004). While giant hill mutants of 'Russet Burbank' have not been selected, there is evidence that different germplasm accessions of 'Russet Burbank' held at various repositories in North America are essentially geographic variants, grown for decades at different locations. Accessions differed in yield and processing components (Wright and Mellor 1976; Love et al. 1992; Coleman et al. 2003). The New Brunswick accession of 'Russet Burbank' (NB 'Russet Burbank') is paramount in Atlantic Canada.

Plant tissue culture technology offers many permutations and combinations to produce variant genotypes for potential plant improvement. For potato, this has included haploid production including androgenesis (microspore or anther cultures) or gynogenesis (unfertilized ovule or ovary culture), embryo culture, protoplast culture (protoclones), shoot regeneration from callus (calliclones), and direct or indirect regeneration of somatic embryos (somaclones). 'Russet Burbank' mesophyll-derived protoclones varied in resistance to crude extracts of Early Blight (*Alternaria solani*) (Matern et al. 1978) in the laboratory but were not field-tested. Approximately 3.8% of field-tested clones of 'Russet Burbank' varied in 13/22 traits including tuber weight, number, sucrose level at harvest, leaf, flower morphology, and tolerance to Late Blight (*Phytophthora infestans* Mont.) (Secor and Shepard 1981; Ayers and Shepard 1981). Protoclones of other genotypes varied in regeneration potential in vitro (Coleman et al. 1991) and in yield and other agronomic characteristics in the field (Wenzel et al. 1979; Jelenić et al. 2001).

Phenotypic variation (plant morphology, yield) occurred among calliclones regenerated adventitiously on tuber discs of 'Russet Burbank', 'Superior', and 'Kennebec' (Rietveld et al. 1991, 1993). Similarly, changes in plant morphology occurred among calliclones from nodal cuttings (Austin and Cassells 1983). Field evaluation of 'Desiree' clones that produced adventitiously on callused shoot explants varied in tuber characteristics (Evans et al. 1986). One out of 325 calliclones (0.3%) of 'Desiree' showed resistance to *Verticillium dahlia* in a growth chamber evaluation (Sebastiani et al. 1994). Field-evaluated calliclones from nodal and internodal segments of 'Multa' and 'Diamant' showed variation in plant height, number of leaves, and yield components (Nasrin et al. 2003). Thieme and Griess (1996) evaluated approx. 13,000 calliclones from stem internodes and leaves of 14 (1996) and 17 (2005) potato cultivars in both the greenhouse and field. From 2% to 10% of calliclones were superior to control genotypes for haulm growth, earliness, and tuber yield. Only 0.1%–1.4% of calliclones were better than control genotypes for a range of tuber characters (Thieme and Griess 2005). A variant of 'Russet Burbank' with resistance to potato leafroll virus was selected from a population of calliclones and released in Canada in 2002 as 'AC LR Russet Burbank' (Plant Breeders' Rights Office 1997; CFIA 2008). To the best of our knowledge, it is the only cultivar derived from 'Russet Burbank' through tissue culture and among very few with 'Russet Burbank' in their genealogy; 'Butte', for example (Pavek et al. 1978) in almost 100 years of cultivation.

Somatic embryos of potato have been produced from a wide assortment of explants, including tuber discs (Lam 1975; Bragd-Aas 1977), shoot meristem tips (Powell and Uhrig 1987; Fiegert et al. 2000), microspores (Dunwell and Sunderland 1973; Johansson 1986), immature zygotic embryos (Pretova and Dedicova 1992), leaves (JayaSree et al. 2001), single-node stem cuttings (Reynolds 1986; Garcia and Martinez 1995; Sharma and Millam 2004; Sharma et al. 2007), inter-nodal stem cuttings, leaves, microtubers, and roots (Seabrook and Douglass 2001; Seabrook et al. 2001). Of 14 studies of potato somatic embryogenesis, only one group (Seabrook and Douglass 2001) evaluated somaclones in the greenhouse, noting "off-types", and none tested somaclones in the field. The objective of our research was to investigate the possibility of improving NB 'Russet Burbank' for French fry processing through in vitro regeneration of somatic embryos explanted from specific tuber tissues (known as somatic regenerants, SR_1, or intraclones) and field evaluation of intraclones in New Brunswick to select for improved yield and processing-quality characteristics.

Materials and Methods

Source of Plant Materials

In vitro control plantlets of NB 'Russet Burbank' (clone #179) and 'Burbank' were obtained from the Plant Propagation Center, New Brunswick Dept. of Agriculture,

Fisheries & Aquaculture (Fredericton, NB) and the United States Department of Agriculture (USDA) Research Service, Inter-Regional Potato Introduction Station (Sturgeon Bay, WI), respectively. Certified field-grown tubers of NB 'Russet Burbank' were from the Bon Accord Elite Seed Potato Center (Bon Accord, NB, Canada).

Production and Maintenance of Intraclones

The entire procedure used to produce and evaluate intraclones over the 3 years of the study is schematically represented in Fig. 1. In vitro-produced microtubers (Leclerc et al. 1994) and field-grown tubers of NB 'Russet Burbank' were used as a source of periderm, cortex, and pith explants in fall 2005 and 2006. Explants (~0.50× 0.35×0.50 cm) were established in petri dishes and sub-cultured 2-wk later onto medium for somatic embryo regeneration in Magenta boxes (Carolina Biological Supply Co., NC, USA) using a procedure modified from Seabrook and Douglass (2001). Cultures were kept at 23±2 C under 100 μmol m^{-2} s^{-1} cool white fluorescent light (16-h photoperiod). Embryoids approx. 2-cm-long were collected at 1, 2, and 3 mo and assigned an intraclone code. For example, each had a source designation of M or F (microtuber or field tuber) followed by S, C, or P (skin or periderm, cortex, or pith), and a sequential number. Coded intraclones were sub-cultured to micropropagation medium without growth regulators (Murashige and Skoog 1962).

Control cultivars and intraclones were kept in vitro under standard micropropagation conditions and sub-cultured at 4–5 week intervals or, to reduce work-load, were maintained in cold storage at 15 C, 50% RH, with 100 μmol m^{-2} s^{-1} cool white irradiation and a 16-h photoperiod in a growth chamber (CONVIRON CMP 4030 Controlled Env. Ltd., MB, Canada). Following each field season, a decision was made concerning which intraclones to retain in vitro for further evaluation and which to discard.

Field Planting and Design

Plantlets were rinsed of medium and transferred into ProMix-BX (Premier Horticulture Inc., QC, Canada) in Kord trays (6× 12 plastic units; Kord Ltd., ON, Canada). Greenhouse-grown transplants were exposed to 500 μmol m^{-2} s^{-1} light from 400 W HP sodium lamps (P.L. Light Systems, ON, Canada) with a 16-h photoperiod. An automatic retractable curtain (Frank Jonkman & Sons Ltd., ON, Canada) was used to reduce incident sunlight and ambient temperatures (maintained at 29–36 C) in 2006 and 2007. For the first 4 d, transplants were watered twice daily, and after this, as needed. Hardening to the outdoors lasted for 1 wk beginning 1 wk following transplant. Each day, trays were placed outdoors for 3–4 h for the first 5 d, then kept outdoors, and regularly fertilized with 0.5 gl^{-1} of 10-52-10.

Each June (2005–2007), hardened plantlets were transplanted into the field by hand and covered with a floating row cover (Vesey Seed Ltd., PE, Canada) for 2 wk. The moisture level was maintained at 75% field capacity through drip irrigation (Netafim, CA, USA). Fertilization occurred at 1,113 $Kgha^{-1}$ of 18.5-15-15. Plants were hilled twice mechanically with a tractor and mid-mount cultivators. Harvest occurred in late September or early October with a field season duration of 109 d (2005), 119 d (2006), and 110 d (2007).

In Seasons 1 and 2 (2005, 2006) 495 intraclones (tested as single plantlets) and 310 intraclones (tested as duplicate plantlets) respectively, as well as control NB 'Russet Burbank' (both plantlet (40 and 20, respectively)—and

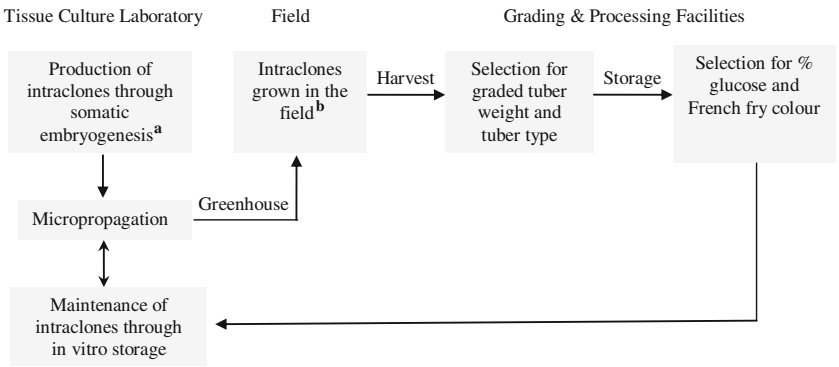

Fig. 1 Intraclone production and evaluation. Step-wise flow chart illustrates the annual cycle of in vitro production of somatic embryos and increase through micropropagation for the purpose of storage in vitro and evaluation in the field. Following harvest, the first selection for improved French fry processing quality was based on graded tuber weight and tuber type. Following storage, the next selection was based on % glucose and French fry colour. Intraclones superior for processing characteristics were transferred from in vitro storage and increased via micropropagation for testing as clonal lines the following year, while intraclones with insufficient yield were discarded from in vitro storage. [a]Explants were derived from specific tuber tissues (periderm, cortex, pith) of microtubers or field-grown tubers. [b]New intraclones were represented by 1 plantlet (season 1), two plantlets (season 2) and put into the field in increased numbers in seasons 2 and 3

seed tuber-derived (0 and 55, respectively)), and 'Burbank' (plantlet-derived; 20 and 45, respectively) were field-planted in a randomized complete design (RCD). The same within-row (45 cm) and between-row (90 cm) spacing was used for the entire study. Also in 2006, two replicates of each of the best 15 intraclones from season 1 and control plantlets were field planted in 17 plots of 15 plants each in a randomized complete block design (RCBD). In season 3 (2007), 26 of the best intraclones selected from seasons 1 and 2, along with controls, were tested in eight rows in a RCBD with three replicates each of 12 plants.

Yield and Processing Quality Evaluation

Field performance data collected for each plant included: total tuber yield (weight (Kg) and number), graded tuber yield (weight (Kg) and number ≥5 cm), average weight per tuber (Kg), and tuber type (appearance including size, shape, and overall quality). Intraclones with graded (marketable) tuber yield ≥ plantlet-derived NB 'Russet Burbank' control plants (RBP) were retained and the others discarded. The selected intraclones were bagged, labelled, and stored in wooden crates at 10 C, 97% RH, in the dark at the NB Department of Agriculture & Rural Development (NBDARD) Wicklow Station (Florenceville, NB, Canada). Tubers were removed from storage for French fry processing tests after 3 mo (season 1 and 2) or 5 mo (season 3). Where tuber numbers were insufficient after seasons 1 or 2, estimates were performed on reduced samples. In season 3, larger samples were evaluated and replication increased. For specific gravity, weight in air and in water was done using a pre-prepared balance (Murphy and Goven 1959).

Glucose (% glucose on a FW basis) and sucrose (mgg^{-1} FW) contents were estimated from 300 g of combined sample from 5 tubers of each genotype using the YSI Biochemical Analyzer (Model 2700 Select, Yellow Springs Instrument Co., OH, USA) as in Sowokinos and Preston (1988). For French fry colour assessment, a new set of 10 tubers of each genotype was tested. Each of these tubers was cut longitudinally to remove 1 or 2 slices (1-cm-thick) and 1 or 2 tuber discs (5-cm-diameter) were cut from each slice with a disc cutter. Eighteen to twenty tuber discs were randomly cut from central, apical and/or stem ends of these slices for each genotype. Tubers discs were fried at 190 C for 2.5 min and colour measured with an Agtron M45 Process Analyzer (Agtron Inc., NV, USA). Spectrophotometer (Agtron) readings were converted to USDA values (1–7, where 1 is the best) according to Iritani and Weller (1974). Agtron values of 88–100, 70–87, 54–69, 36–53, 21–35, 5–20, and 0–4 are equated with USDA rankings of 1, 2, 3, 4, 5, 6, and 7, respectively.

Statistical Analysis

Differences between the main factors (year and source tubers; microtubers and field tubers) and the three sub-factors (source tissues; periderm, cortex, and pith) were determined using the General Linear Model (GLM) of SAS (SAS Institute Inc. NC, USA, 2007). Data were tested for normality using the UNIVARIATE procedure before analyses. Then the differences between field-selected intraclones from each season were statistically analyzed using the GLM. A single or duplicate plant (intraclone) represented an experimental unit. The means were compared using Scheffe's Multiple Comparison Test and Dunnett's Test ($P<0.05$).

Results

Yield Comparison Between Populations of Intraclones

Statistical analysis of the effects of season (year), tuber source (microtubers and field-grown tubers), and tissue source (periderm, cortex, or pith) on yield components; total tuber number (TTN), graded tuber numbers (GTN), graded tuber weight (GTW), total tuber weight (TTW), and average weight per tuber (AWT) are presented in Table 1. The summary of the statistical analysis reveals a significant interaction between year and tuber source, and year and tissue source, on almost all yield components. Average yield data from control cultivars and all intraclones derived from different source tubers and tissues and field-tested in seasons 1 and 2 are summarized in Table 2. In season 1, averaged yield components (graded tuber number, total tuber weight) were greatest in 'Burbank' (B). Average total tuber number was similar in B and MC-regenerated intraclones and greater than the other groups of genotypes. The two control cultivars had greater average graded tuber weight than other genotypes. NB 'Russet Burbank' control plantlet-derived (NBRB) and FC-derived intraclones had similar average graded tuber weight and the later had similar graded tuber weight to the other groups of intraclones. NBRB had the greatest average weight per tuber but was not different from that of B and FC genotypes while MC-derived intraclones had the least. Cortex-derived intraclones had greater average total tuber number and weight than pith-derived intraclones from both source tubers but other averaged yield components were similar. Microtuber- and field-tuber derived intraclones were similar for most averaged yield components although microtuber-derived intraclones had greater average total tuber number.

In season 2, NB 'Russet Burbank', 'Burbank' controls, MC, MS, FP, and FC intraclones had similar averaged total tuber number and graded tuber weight. All intraclonal

Table 1 ANOVA mean square values of yield components; total tuber number (TTN), graded tuber number (GTN), graded tuber weight (GTW), total tuber weight (TTW), and average weight per tuber (AWT) of intraclones that were field-evaluated for 2 years, 2005 and 2006, respectively

Source	Years	df	Yield components				
			TTN	GTN	GTW	TTW	AWT
Year		1	4.73	121.82**	2.45*	0.01	0.03
Tuber sources	2005	1	310.28*	0.26	0.02	0.01	0.02
	2006	1	1039.23*	245.57**	0.81**	16.27**	0.02**
Tissue sources	2005	1	2188.23**	0.49	0.16	1.89*	0.01
	2006	2	128.73	1.44	0.04	0.61	0.002
Clones	2005	369	63.43*	9.99*	0.37	0.45	0.005**
	2006	283	62.53*	15.72**	0.53**	0.62**	0.004**
Year*Tuber sources		1	1055.68**	82.45*	2.33*	5.39*	0.001
Year* Tissue sources		3	758.77**	121.66*	4.46**	5.17**	0.03*

*, **Significant at $P<0.05$ and $P<0.01$, respectively

groups had similar total tuber number and graded tuber weight except the MP intraclones which had less graded tuber weight but not different from that of MC and MS groups. Control cultivars, FP, and FC genotypes had similar graded tuber number and total tuber weight and MP-derived intraclones had less graded tuber number and total tuber weight but were similar to MC and MS intraclones. For average weight per tuber, almost all genotypes had similar weights except MP which had the least but not different from B, MC, and FC genotypes. Cortex- and pith-derived intraclones were similar to each other and to periderm-derived intraclones for all averaged yield parameters. In contrast to the first season, microtuber-derived intraclones had lesser average yield components than field tuber-derived intraclones. Due to the wide range of variation among intraclonal populations, average yield of control cultivars was greater than or similar to average yield of populations of intraclones regenerated from different tuber sources and tissues.

Tuber source tissues were similar in generating useful variation for yield and processing traits. No differences in tuber type were attributed to explant source, just as no differences in intraclone periderm russeting were attributed to explant source (Nassar et al. 2008). Explants from the pith and cortex were relatively easier to isolate and more regenerative compared with periderm explants (data not shown).

Selection of Superior Intraclones for Processing Based on Graded Yield, Type, and Processing Criteria

Selection of promising intraclones for the processing industry was done at two steps. At harvest primary selection was based on average graded tuber weight and acceptable type. After 3 or 5 mo storage, a secondary selection was based on processing characteristics including % glucose and French fry color. In 2005, primary selection identified 28 intraclones with traits better or equal to control ‘Russet Burbank’. Table 3 data is pooled from three seasons and includes a subset of the season 1 selections retained through season 3. For example, FP3405 was among three intraclones from the first season with greater graded tuber weight (2.47 Kg) and greater total tuber number (40) compared with NB ‘Russet Burbank’ control plantlet-derived. Based on processing quality traits, determined after 3 mo storage, a smaller subset of 15 intraclones were retained for field-evaluation the following year. Average intraclone tuber sucrose concentrations (mgg^{-1}) and specific gravities were similar to control values. However, several intraclones, including FP3405 and MP18405, had at least 10% less glucose than mean control values. Control plantlet tubers of NB ‘Russet Burbank’ and ‘Burbank’ fried at Agtron 78 and 84, respectively; USDA 2. Tubers of many intraclones fried similarly or more poorly (one was Agtron 64; USDA 3). However, tubers of several intraclones, including MP18405 and FP3405, had better fry color (Agtron 98 and 96, respectively; USDA 1).

No differences were found in yield and processing characteristics between controls of NB ‘Russet Burbank’ field tuber-derived (NBRBF) and plantlet-derived (NBRBP) and ‘Burbank’ and new intraclones tested in season 2 (Table 3) except FP3405 which had lesser average graded tuber weight compared with control ‘Russet Burbank’. A total of 17 intraclones with graded tuber yield ≥ that of control NB ‘Russet Burbank’ and at least 10% less % glucose were reserved for additional study in season 3, including, FC2806, FP106, FP306, FP906, FP2106, FP2906, MC1606, and MS1406 (Table 3).

In 2006, differences occurred in average yield or processing characteristics between controls of NB ‘Russet Burbank’ and ‘Burbank’ and the 15 intraclones selected from the 2005 field season and re-evaluated in 2006. A confounding difficulty that may have depressed yield in 2006 was end-of-season water-logging in 9 of 15 plots in one replicate. Nevertheless, nine superior intraclones were identified with lesser % glucose than plantlet-derived

Table 2 Yield comparison

Control or intraclonal population	No. of harvested plants[a]		Tuber number				Tuber weight (Kg)				AWT (Kg)	
	2005	2006	Total		Graded		Total		Graded		2005	2006
			2005	2006	2005	2006	2005	2006	2005	2006		
NBRB	39	10	13.17b	20.90a	6.36b	9.50ab	1.74b	2.12a	1.36ab	1.69a	0.21a	0.18a
B	18	10	23.12a	20.20ab	9.65a	9.70a	2.46a	2.07ab	1.78a	1.49ab	0.21ab	0.16ab
MP	172	38	14.88b	13.74b	4.51c	3.53d	1.34b	0.93d	0.80c	0.55c	0.16bc	0.11b
MC	104	106	21.05a	15.46ab	4.49c	5.98cd	1.53b	1.41cd	0.73c	1.01bc	0.15c	0.16ab
MS[b]	–	111	–	15.68ab	–	6.40bcd	–	1.47bcd	–	1.09abc	–	0.17a
FP	67	179	15.30b	18.31ab	4.34c	7.24abc	1.41b	1.74abc	0.77c	1.24ab	0.17bc	0.17a
FC	9	111	12.11b	17.68ab	5.11bc	6.98abc	1.32b	1.67abc	0.94bc	1.22ab	0.21abc	0.16ab
CV[c]			42.83	40.03	58.92	49.71	41.67	41.21	63.67	53.90	42.48	32.52
Pith	239	217	15.00b	17.51a	4.46a	6.59a	1.36b	1.60a	0.79a	1.12a	0.16a	0.16a
Cortex	113	217	20.34a	16.59a	4.54a	6.49a	1.51a	1.54a	0.75a	1.12a	0.15a	0.16a
Periderm	–	111	–	15.68a	–	6.40a	–	1.47a	–	1.09a	–	0.17a
CV			44.84	41.17	65.65	52.61	44.76	43.88	71.97	56.79	44.02	33.67
Microtubers	276	255	17.02a	15.30b	4.50a	5.80b	1.41a	1.37b	0.77a	0.98b	0.16a	0.16b
Field tubers	76	290	14.92b	18.07a	4.43a	7.14a	1.40a	1.71a	0.79a	1.23a	0.17a	0.17a
CV			46.94	40.50	65.65	51.55	45.06	42.51	71.92	55.58	43.94	33.48

Average yield data and average weight per tuber (AWT; Kg) of control plantlet-derived NB 'Russet Burbank' (NBRB) and 'Burbank' (B) and intraclone populations explanted from microtuber pith (MP), cortex (MC), or periderm (MS) tissues or seed tuber pith (FP) or cortex (FC) tissues of NB 'Russet Burbank'

[a] Some control or intraclone plants did not survive; harvested numbers are less than planted numbers

[b] MS; Intraclones from periderm tissues were produced for the 2006 field season only

[c] CV; coefficient of variation. Means were compared using Scheffe's Multiple Comparison Test ($P<0.05$)

Table 3 Yield and processing results

Control Cvs and select intraclones	Average graded tuber weight (Kg)			% Glucose			French fry color (average Agtron value)		
	2005	2006	2007	2005	2006	2007	2005	2006	2007
NBRBP	1.36b	1.69a	0.77b	0.142a	0.139a	0.047b	78b	77.9b	93.6a
NBRBF	NT	2.28a	1.51a	NT	0.097a	0.032b	NT	NT	92.1a
FP106	NT[a]	1.86a	0.66b	NT	0.061a	0.016c	NT	NT	95.1a
FP2106	NT	1.76a	0.82b	NT	0.109a	0.020c	NT	NT	89.0a
FP3405	2.47a	0.71b	0.72b	0.087b	0.040b	0.026c	96a	96.4a	92.9a
MS1406	NT	1.82a	0.70b	NT	0.021b	0.030b	NT	NT	90.8a
MC1606	NT	2.04a	0.60b	NT	0.036b	0.032b	NT	NT	91.5a
FP306	NT	1.83a	0.77b	NT	0.076a	0.032b	NT	NT	89.1a
MC405	1.52b	1.01a	0.91b	0.097b	0.103a	0.035b	84b	94.8a	84.9a
MP18405	2.29a	0.86a	0.97b	0.070b	0.031b	0.037b	98a	94.8a	87.5a
FP2906	NT	2.15a	0.88b	NT	0.063a	0.040b	NT	NT	97.0a
FP906	NT	1.68a	0.66b	NT	0.062a	0.045b	NT	NT	87.5a
FC2806	NT	1.77a	1.04b	NT	0.076a	0.047b	NT	NT	93.5a
MP19805	2.21a	0.92a	0.89b	0.080b	0.030b	0.078a	NT	96.9a	85.7a

Selective results of average graded tuber weight (Kg), glucose (%), and French fry color (average Agtron value) for controls of NB 'Russet Burbank' plantlet-derived (NBRBP) and seed-derived (NBRBF) and select intraclones produced from microtuber pith (MP), cortex (MC), or periderm (MS) tissues or seed tuber pith (FP) or cortex (FC) tissues and tested in the field for 3 years. Results in the table were arranged based on 2007 % glucose values of intraclones

[a] NT; not tested.% glucose and French fry color tests were assayed after 3 mo (in 2005 and 2006) or 5 mo (in 2007) in storage. Means were compared with 'Russet Burbank' plantlet-derived using Dunnett's test ($P<0.05$). Data presented in this Table are results of 1 plantlet in 2005 and averages of 2 plantlets in 2006, while in 2007, intraclones were tested in three replicates of 15 plantlets each

control NB 'Russet Burbank' including FP3405, MS1406, MC1606, MP18405, and MP19805 (Table 3).

Field evaluation in 2007 showed differences in tuber number (total and graded) and tuber weight (total and graded), but no differences in average weight per tuber, or processing quality criteria between controls NBRBF and NBRBP plants (Table 3). Overall yields were generally less in season 3 than in previous seasons. No differences were found between control 'Russet Burbank' plantlet-derived and intraclones, or between intraclones for any averaged tuber yield component. At 5 mo storage, tubers of FP3405, FP2106, FP106, and MS1406 had lesser % glucose compared with tubers of other genotypes and control NBRBP plants (Table 3) based on Dunnett's test. After 9 mo storage, less material was available for the 9 mo evaluation. For this reason, the sugar tests performed at 9 mo were indicative only. MP3405 and FP2106 were not available for testing, while FP106 was in the average range of NBRBP% glucose.

Selection Percentages Based on Tuber Yield and Processing Quality

The relative proportion of superior intraclones for processing quality was calculated each season based on a combination of the two most important criteria; total graded tuber weight and % glucose (Table 4). For example, from 2005, selected intraclones derived from pith and cortex tissues of microtubers were 7.76 and 5.98% (based on graded tuber weight) and 4.11 and 2.56% (based on % glucose), respectively. Overall, the proportion of superior intraclones selected from microtubers and field-grown tubers of NB 'Russet Burbank' were 7.14 and 3.11% (2005) and 5.66 and 13.64% (2006) based on total graded tuber yield, respectively while 3.57 and 1.86% (2005) and 2.52 and 9.09% (2006) were selected based on % glucose, respectively.

Discussion

We used two tuber sources and produced somatic embryos from specific tuber parts (intraclones) from NB 'Russet Burbank' in 2005 and 2006 and field-evaluated them for 3 successive years in New Brunswick, Canada. Selection for graded tuber yield (≥control NB 'Russet Burbank') and tuber type at harvest time and improved processing quality traits (lesser % glucose and better fry colour) after 3 or 5 mo in storage was applied after each field season.

Table 4 Selection efficiency

Tuber source	Year	Tissue sources	Number of intraclones tested in the field	Selected intraclone number and percentage							
				Graded tuber weight				% Glucose			
				Tissue sources[a]	%	Tuber sources[b]	%	Tissue sources	%	Tuber sources	%
Microtubers	2005	Pith	219	17	7.76			9	4.11		
		Cortex	117	7	5.98	24	7.14	3	2.56	12	3.57
	2006	Pith	40	1	2.50			1	2.50		
		Cortex	59	3	5.08			1	1.69		
		Periderm	60	5	8.33	9	5.66	2	3.33	4	2.52
Field tubers	2005	Pith	124	5	4.03			3	2.42		
		Cortex	37	0	0.00	5	3.11	0	0.00	3	1.86
	2006	Pith	92	10	10.87			8	8.70		
		Cortex	62	11	17.74	21	13.64	6	9.68	14	9.09

Number and percentage of selected intraclones of NB 'Russet Burbank' produced from each tuber source (microtubers or field tubers) and specific tissue (periderm, cortex, or pith) each season (2005 or 2006) based on graded tuber weight followed by % glucose

[a] Tissue sources; number of selected intraclones produced from various tuber tissues (periderm, cortex, or pith) separately

[b] Tuber sources; number of selected intraclones produced from microtuber and field-grown tuber tissues collectively

Significant difference occurred among intraclones in % glucose and French fry colour. We noticed poor correlations between % glucose and the French fry color. Also, there were inconsistencies in % glucose and fry color traits from year to year for some intraclones perhaps because we used different tubers for the estimations of % glucose and French fry color. Tuber type and source tissue were clearly unimportant, so somaclones can be randomly produced from any part of the tuber. Intraclonal selection was a useful means of generating better lines that will store longer with better French fry processing quality. Multigenic traits such as yield (Cassells et al. 1983) with high variability (Neele et al. 1988; Jones and Cassells 1995) are difficult to improve as are processing traits (Douches and Freyre 1994), although processing traits have been considerably improved over the last 2 decades (Love et al. 1998).

Intraclones were evaluated in 2005 as single plants and in 2006 as duplicate plants. Field evaluation of lines as duplicate plants is generally better than as single plants since this reduces the error variance (explained by Brown 1987), decreases environmental effects, and slightly enhances selection efficiency (Neele et al. 1988). Statistical analysis revealed clear environmental effects on yield, sugars, and French fry color characteristics from year to year. Several studies reported the environmental effects including Brown (1987), Maris (1988), Neele et al. (1988). However, increasing the number of replicates per clone during early years of selection has a down-side that we experienced. Increased replication reduces the total number of evaluated clones, is generally more labor- and time-consuming, and requires more land area if the same number of lines are to be evaluated.

The relative speed of regeneration and the greater likelihood of stability of these regenerants from single cells, compared with other tissue culture approaches that are more likely to yield chimeric plants, are among the advantages of somaclones. Still, selection for superior variants is a "numbers game"; the greater the somaclone numbers, the greater the chance of identifying promising clones for new cultivar development. This statement is reminiscent of similar findings by potato breeders working with potato seedlings. To obtain 3–5 promising seedlings for new variety development, selection from a population of 1,000–1,000,000 seedlings was necessary with the selection

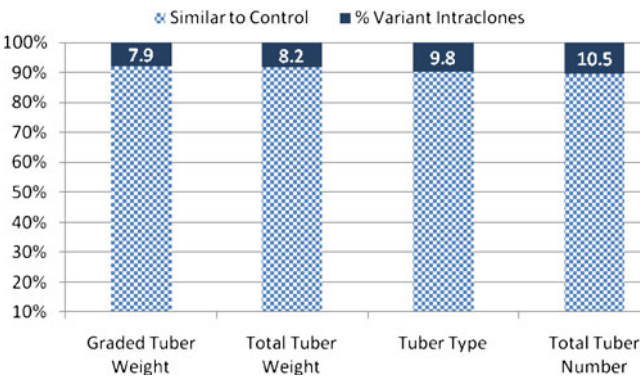

Fig. 2 Percentage somaclonal variation. Percentages of variant intraclones (approx. 800 intraclones) with significantly lesser or greater values compared with control plantlet-derived NB 'Russet Burbank' for various agronomic characteristics studied over a 2 year interval

and field evaluation process lasting from 10 to 15 years (Maris 1988; Neele et al. 1988). Similarly, Shepard et al. (1980) suggested that field evaluation of large populations of 60,000–80,000 seedlings was necessary for the identification of one promising seedling-based clone (0.000012%–0.000016%). In contrast, Thieme and Griess (2005) estimated that 5,000–10,000 somaclones are required to obtain one new variety (0.0001%–0.0002%). Our results from field evaluation of somaclones suggest that the extreme numbers used for seedlings may not be essential to obtain improved clones but the effort and expense remain considerable. For various agronomic characteristics studied over a 2 year interval (approx. 800 intraclones), the % of variant intraclones (with significantly lesser or greater values compared with control plantlet-derived NB 'Russet Burbank') ranged from 7.9% to 10.5% (Fig. 2). Based on these numbers, somaclonal assessment is a useful cultivar improvement strategy, as sufficient positive variation occurs to justify this effort. Comparing our results with previously reviewed studies we can highlight the importance of our somatic embryogenesis technology in the initiation of useful variation in yield and storage quality features that can be incorporated into potato improvement programs.

Somaclonal variation generates clones with improved or worsened agronomic characters (Rietveld et al. 1991) that may appear less arbitrary as our understanding of these phenomena increases. The underlying mechanisms of tissue culture-derived variations are numerous, affecting the nuclear and organellar genomes, and have been extensive described by others. These include changes in chromosome number (aneuploidy, aneusomy, mixploidy, and/or polyploidy) or structure (Evans and Sharp 1986; Gavrilenko et al. 1999), DNA sequence rearrangements (deletion, and/or addition), mitochondrial DNA changes such as sequence alteration or presence of low molecular weight species (Gengenbach et al. 1977; Kemble and Shepard 1984), alteration of a single gene base pair, or deamplification of ribosomal-RNA genes (Landsmann and Uhrig 1985). Also, Kaeppler et al. (2000) suggested an epigenetic role in somaclonal variation. Several mechanisms of epigenetics were reported; DNA methylation, histone modifications, and transposable elements (Springer and Kaeppler 2008). Moreover, an environmental component may also affect clones (Li 2009). In the current study, the aim was to determine if improved somaclones could be generated using current somatic embryogenesis technology. The underlying mechanism of genetic change was not integral to this investigation; this can follow.

Conclusions

We studied the potential of NB 'Russet Burbank' improvement through in vitro production of somatic embryos from specific tuber tissues (periderm, cortex, and pith), of microtubers and field tubers followed by field-evaluations of these intraclones for yield and processing traits. No particular source tuber type or specific tuber tissue contributed more useful variation than any other. This suggests that somaclones could be regenerated randomly from any part of the tuber in the future. Average yield components showed no differences between control NB 'Russet Burbank' plantlet-derived and intraclone-derived plants. Overall, 2–9% of intraclones had improved processing quality. Two intraclones were optioned to local industry and others have been retained for further evaluation. These will all be subject to molecular analysis. The single cell origin of these somatic variants suggests that they are more likely to be stable in comparison to organogenesis-derived regenerants from culture, which was supported by the release of AC LR-Russet Burbank (Plant Breeders' Rights Office 1997; CFIA 2008). Our results suggest that field-based somaclonal selection of NB 'Russet Burbank' has the potential to significantly improve this cultivar. This technology can be recommended for incorporation into potato improvement programs.

Acknowledgements The authors acknowledge Dr. A. Kushalappa and Dr. J. Singh (Plant Science Department, McGill University, QC) for manuscript review. We thank McCain Foods Canada Ltd. for extensive field support and the NB Department of Agriculture & Rural Development (NBDARD) Wicklow Station (Florenceville, NB, Canada) for generous use of their processing and storage facilities. We also thank the Bon Accord Elite Seed Potato Centre (Bon Accord, NB, Canada), the Plant Propagation Center, NB Department of Agriculture, Fisheries & Aquaculture (Fredericton, NB, Canada) and The USDA Research Service, Inter-Regional Potato Introduction Station (Sturgeon Bay, WI, USA) for supplying plant materials. We are grateful for financial support from the NSERC Discovery Grant Program (D.J. Donnelly) and for a graduate scholarship from the Government of Egypt (A.M.K. Nassar). We are most grateful to Dr. J. Bamberg and the editorial staff at AJPR for their supportive review process.

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