Use of Somatic Embryogenesis in Potato (*Solanum tuberosum* L.) cv. Russet Burbank Improvement

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To Somaia, Mohamed, and parents

ABSTRACT

‘Russet Burbank’, the most important potato in North America, was described as a putative periclinal mutation from ‘Burbank’ and has not parented outstanding cultivars. The current study aimed to: (1) investigate the present chimeral status of NB ‘Russet Burbank’ based on tuber periderm phenotype, (2) determine if yield and/or processing characteristics of NB ‘Russet Burbank’ could be improved through selection of intraclones, (3) determine whether pre-selected somaclones had sufficient variation in protein content (crude (CP), total soluble (TSP)), or resistance to *Phytophthora infestans* (US-8) to select for, and (4) examine long-term stability in protein content. Somatic embryogenesis technology was used to regenerate intraclones of the NB clone of ‘Russet Burbank’ potato. Approx. 800 intraclones were regenerated and field-tested from 2005 to 2007. At harvest, we selected somaclones with the greatest yield compared with NB ’Russet Burbank’ control. Following storage, tubers of selected clones were tested for processing quality characteristics and protein content. Late blight resistance was examined in the greenhouse for 2 years.

Results showed that most intraclones had tubers with russet periderm. About 4 % of intraclones had non-russet tubers and 21 % had patchy periderm. Presently, NB ‘Russet Burbank’ is not organized as periclinal chimera (Nassar et al. 2008). From 2-9 % of intraclones had superior reducing sugar characteristics. Two promising somaclones with good yield and reducing sugars were selected by industry for further evaluation. Molecular characterization is now needed for advanced somaclones with improved processing features. Somatic embryogenesis technology generated sufficient somaclonal variation for potential improvement of NB ‘Russet Burbank’ (Nassar et al. 2009a). One advanced somaclone had greater CP content than control in 2008. One somaclone had lesser TSP content for 3 consecutive years and 7 somaclones exhibited reduced TSP content for 2 consecutive years compared with control (Nassar et al. 2009b). This is the first study to report inter-seasonal variation in protein content in potato. A full characterization of protein content in advanced somaclones is

needed. Most intraclones showed resistance to *P. infestans* (US-8) similar to controls; field study is required. In conclusion, these studies underline the advantages of using somaclonal technology and its suitability in potato improvement programs.

RÉSUMÉ

La Russet Burbank, le cultivar de pommes de terre le plus important en Amérique du Nord, a longtemps été considérée comme dérivé d'une mutation périclinale de la 'Burbank'. Cette étude avait pour but: (1) d'évaluer le statut chimérique de la NB ' Russet Burbank' en se basant sur le phénotype péridermique des tubercules; (2) de déterminer si les caractéristiques de performance au champ et/ou de transformation peuvent être améliorées à l'aide d'une sélection d'intraclones; (3) de déterminer si les somaclones sélectionnés montrent suffisamment de variation dans leur contenu protéique (protéine brute (PB) et protéines solubles totales (PST)) ou leur résistance à *Phytophthora infestans*, pour permettre le triage de ces traits phénotypiques et 4) d'examiner la stabilité a long terme du contenu protéique. Environ 800 intraclones du clone NB générés utilisant la technologie d'embryogenèse somatique ont été testés au champ (2005 à 2007). Les somaclones ayant les meilleurs rendements, comparés aux témoins, ont été sélectionnés. Après l'entreposage, leurs qualités à la transformation et leur contenu protéique furent évalué.

Presque tous les intraclones générés possédaient des tubercules avec des péridermes 'russet', 4% avaient des tubercules non-'russet' et 21% montraient des péridermes tachetés. La NB 'Russet Burbank' n'est pas actuellement organisé dans un arrangement périclinal chimérique (Nassar *et al.* 2008). Deux somaclones prometteurs ayant des rendements et des qualités de transformation améliorées fut fournis à l'industrie locale pour des évaluations futures. Suffisamment de variation entre les somaclones fut générée pour potentiellement permettre d'améliorer ce cultivar (Nassar *et. al* 2009a). Un somaclone possédait des teneurs en PST significativement plus faibles, comparés aux témoins, pour 3 années consécutives et 7 avaient des teneurs plus basses pour 2 années consécutives (Nassar *et al.* 2009b). Cette etude est la premiere à démontrer une variation inter-saisonnière du contenu protéique chez la pomme de terre. Une caractérisation complète du contenu protéique est nécessaire. La majorité des intraclones démontrent une résistance au *P.*

infestans similaire aux témoins; des études aux champs sont requises. En conclusion, cette étude démontre l'applicabilité de la technologie d'embryogénèse somatique dans les programmes d'amélioration des pommes de terre.

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LIST OF ABBREVIATIONS

AAFC	Agriculture and Agri-Food Canada
AFLP	amplified fragment length polymorphism
AGTW	average graded tuber weight
Bt	*Bacillus thuringiensis*
C	Celsius
CFIA	Canadian Food Inspection Agency
CRBD	completely randomized block design
CRD	completely randomized design
cv(s)	cultivar(s)
DNA	deoxyribo nucleic acid
DW	dry weight
FAO	Food and Agricultural Organization of the United Nations
FW	fresh weight
GA3	gibberellic acid
GI	glycemic index
GISH	genomic in situ hybridization
GLM	general linear model
Ha	hectare
IAA	indole-3-acetic acid
IgE	immunoglobulin E
ISSR	inter simple sequence repeat
IYP	International Year of Potato
L_1	outer tunic layer (differentiates into the periderm)
L_2	inner tunic layer (differentiates into the cortex)
L_3	corpus (differentiates into the pith)
LRR	leucine rich repeat
MAS	marker assisted selection
MHa	million hectares
MSAP	methylation-sensitive amplification polymorphism

Mt	million metric tonnes
NB	New Brunswick
NBDARD	New Brunswick Department of Agriculture & Rural Development
NBS	nucleotide binding site
NR	non-russet tubers
OECD	Organisation for Economic Co-operation and Development
P	patchy (the tuber surface shows patches of russet)
PAA	The Potato Association of America
PCR	polymerase chain reaction
PLRV	potato leafroll virus
PVY	potato virus Y
QTL	quantitative trait loci
R	russet tubers
RAPD	random amplified polymorphic DNA
RH	relative humidity
SR_1	somatic regenerants first generation
SAS	Statistical Analysis System
SE	Standard error
SAM	shoot apical meristem
SSR	Single sequence repeats
TDZ	thidiazuron
TP	total or crude protein
TSP	total soluble protein
USDA	United States Department of Agriculture
Z	Zeatin

CONTRIBUTIONS OF AUTHORS

This thesis has been written in the form of manuscripts. This format has been approved by the Faculty of Graduate Studies as described in “Guidelines for Thesis Preparation and Submission”. This research was designed in cooperation with Dr. D.J. Donnelly, thesis supervisor and a co-author of publications. I conducted the field and laboratory work, analyzed the data, and wrote the manuscripts and thesis under the supervision of Dr. Danielle J. Donnelly. The current thesis is composed of 7 chapters. The first and second chapters are Introduction and Literature Review, respectively. Chapters III, IV, and V represent the field and laboratory work and were written in the form of manuscripts either published or submitted for publication to refeered journals. Chapters VI and VII are Summary & Conclusions and Contributions to Knowledge, respectively.

The first manuscript (chapter III) was co-authored by Dr. Danielle J. Donnely helped planning, supervised, reviwed written manuscripts and thesis, and provided valuable suggestions at all stages of this work. Dr. Estela Ortiz-Medina did the preliminary greenhouse work. Dr. Yves Leclerc provided the field land and care of plants, storage of tubers of select somaclones, laboratory for grading, and processing trais tests.

The second manuscript (chapter VI) was co-authored by Dr. Jihad Abdulnour, Dr. Yves Leclerc, Dr. Xiu-Qing Li, and Dr. Danielle J. Donnelly. The contributions of Dr. Danielle J. Donnelly and Dr. Yves Leclerc were as described in chapter III. Dr. Jihad Abdulnour contributed with field harvest, grading, and processing quality traits tests. Dr. Xiu-Qing Li provided the molecular biology work for ‘Russet Burbank’ and ‘Burbank’.

The third manuscript was co-authored by Dr. Danielle J. Donnelly, Dr. Ajjamada C. Kushalappa, and Dr. Yves Leclerc. The contributions of Dr. Danielle J. Donnelly and Dr. Yves Leclerc were as described for Chapter III. Dr. Ajjamada C. Kushalappa helped with the planning of the disease work and reviewed manuscript.

CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the world's most important vegetable crop. In 2007, more than 321 million tons (Mt) of tubers were harvested from over 19 million hectares (MHa) of potato grown around the world (FAO 2008). Potato is an indispensable part of the diet of a large proportion of the world's population. Global consumption of potatoes is increasing mostly in developing countries, like China and India (FAO 2008). The annual consumption in Africa, Asia/Oceania, Europe, Latin America, North America, and the world average is 14.18, 25.83, 96.15, 23.65, 57.94, and 33.69 Kg/capita/year, respectively (Gagnon et al. 2007; FAO 2008).

Potato is a rich source of nutrients including carbohydrate, protein, vitamins (e.g. vitamin C, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B6 (pyridoxine), folic acid (the anionic form of vitamin B9)), minerals (e.g. calcium, potassium, and phosphorus), and phytonutrients (e.g. anthocyanins, carotenoids (xanthophylls; zeaxanthin and lutein), flavonoids, and phenolic acids) (Davies 2002; Brown 2008). There is an interest among potato breeders and researchers in improving potato qualities including yield, storability and processing, nutritional features including protein concentration, and disease resistance (especially to *Phytophthora infestans* (Mont.) de Bary), (Beukema and Zaag 1990; Mullins et al. 2006).

The need for potato cultivars with greater tuber yield is important to cope with increasing world population and food scarcity, especially in developing countries (Cotes et al. 2002; IYP 2008). Little progress was made in improving potato yield during the 20th century. Nevertheless, potato yield has been increased about six-fold per unit growing area in the USA since the 1920s due to improved cultural practices, fertilization, irrigation, pest management, a shift in production to the western USA, and through the introduction of improved cultivars

through public breeding efforts (Lucier et al. 1990; Douches et al. 1996). However, improving yield, a multi-gene controlled-trait (Cassells et al. 1983) with high variability (Neele et al. 1988; Jones and Cassells 1995), is not an easy task. Some cultivars have been released for high yield and other important characteristics (e.g. Alta Russet (Lynch et al. 2004), Premier GemStar Russet (Love et al. 2006), and Premier Russet (Novy et al. 2008)).

Processing is an important sector within the potato industry. Potato are processed into French fries and chips, frozen or dried, etc. in developing (~ 10 % of the crop) and in developed countries (4–5 times > developing countries) (Morrow and Jecha-Beard 2003). In USA, 82 % of consumed potatoes are in processed form (National Potato Council, 2009). Processing quality traits are multigenic (Douches and Freyre 1994) but have been considerably improved over the last 2 decades in North America (Love et al. 1998). About 44 potato cultivars have been released for the processing industry (Love et al. 1998). Improvements to cultivars include increased tuber solids, lesser reducing sugar content and lighter chip color, and the ability to produce defect-free chips (Dale and Mackay, 1994; Douches et al. 1996; Love et al. 1998). Potatoes must be stored at low temperature (4 or 10 C) for months, for later use for the fresh market, seed, or processing industries (Kerby et al. 2007). During long-term storage of potato tubers, an accumulation of sucrose occurs and later converts to reducing sugars (Burton 1989). Accumulation of reducing sugars causes low-temperature sweetening and bitter taste in fried potato products. Also, a strong positive correlation was found between reducing sugars and acrylamide formation (Amerin et al. 2003 and 2004; Kumar et al. 2004; Ohara-Takada et al 2005; Silva and Simon 2005; Mestdagh et al. 2008).

Improving potato nutritional components, such as proteins, is equally import to improving yield and/or processing quality traits. Potatoes contain a lower concentration of protein than cereal grains, but potato protein yield per unit growing area is far greater than that of grains, including wheat or rice (Markakis 1975). Potato protein has a greater nutritional quality and biological value for

humans and it provides the amino acid requirements of more people from a unit area of land than wheat, maize, or legumes such as beans or peas (Kaldy et al. 1972). Protein content of potato tubers varies between cultivars and tissues (periderm, cortex, pith) within the same cultivar (Ortiz-Medina and Donnelly 2003). 'Russet Burbank' had a protein distribution map with greater protein concentration in the periderm (derived from histogenic tissue L_1) and relatively low concentration in the cortex and pith tissues (derived from histogenic tissues L_2 and L_3, respectively Fig. 1.1).

Improving potato cultivars for pathogen resistance, especially against late blight (*Phytophthora infestans* (Mont.) de Bary) is also important. *P. infestans* caused and still causes the most destructive disease of potato and tomato in the world, late blight (Fry and Goodwin 1997a, b; Sogin and Silberman 1998; Schiermeier 2001; and Ristaino 2002). Late blight is responsible for losses of billions of dollars in potato and tomato production (Fry and Goodwin 1997a, b). It is important to explore and to utilize various sources and techniques to introduce *P. infestans* resistance including somatic hybridization between wild species and cultivated potato (Douches et al. 2001). Mesophyll protoplasts were used as a source of more resistant 'Russet Burbank' (Ayers and Shepard 1981; Secor and Shepard 1981). A small incidence of these protoclones (3.8 %) was selected for several agronomic traits and late blight resistance (Ayers and Shepard 1981).

The first report of a potato improvement program was in the early nineteenth century, in the United Kingdom (UK), by Knight (1807). He cross-pollinated different varieties (Bradshaw and Mackay 1994). During the 20th century, many programs for potato improvement were developed. For example crossing of tetraploids x diploids which lead to the release of 'Yukon Gold' (Johnston and Rowberry 1981) and identification of superior clones with lighter chip colour and generally good tuber appearance (Lauer et al. 1988; Darmo and Peloquin 1991). Tetraploid cultivars were successfully produced by hybridizing diploids and dihaploid species of *S. tuberosum* followed by recurrent phenotypic mass selection of the tetraploid cultivars (discussed by Bradshaw and Mackay

1994). Haploid breeding through ploidy manipulation lead to enhancement of genetic diversity of germplasm holding through introduction of genes of desirable traits from wild species into cultivated potato (Jansky et al. 1990; Carputo et al. 1997).

Potato improvement programs include *neo*tuberosum, somatic hybridization, in-strain selection, marker-assisted selection, and tissue culture techniques. Production of *neo*tuberosum, a new breeding technology, was achieved by crossing the wild *S. andigena* with cultivated *S. tuberosum* followed by recurrent mass selection of promising clones. *S. andigena*-selected clones were then backcrossed with *S. tuberosum* and recurrent selection was performed (reviewed by Bradshaw and Mackay 1994). Somatic hybridization was used to identify some promising clones with greater disease resistance (Helgeson et al. 1998). In-strain selection from giant hills lead to the identification of 'Norgold Russet M' (Leever et al 1994) and 'Russet Norkotah' (Miller et al. 1995, 2004). Marker-assisted selections lead to identification of QTL for freezing tolerance from *S. commersonii* and *S. cardiophyllum* (reviewed by Mullins et al. 2006). However, their results were considered preliminary (Vega et al. 2003). Tissue culture techniques e.g. protoplast methods and shoot regeneration from callus were used and generated somaclonal variation among somatic-derived clones (Shepard et al. 1980; Cassells et al. 1983).

Tissue culture technologies played and will continue to play a key role in potato improvement because of the highly heterozygous nature of the tetraploid cultivated potatoes and associated tetrasomic inheritance (Wenzel 1994; Bhojwani and Razdan 1996). Protoplast extraction from leaf mesophyll tissues was used by Shepard et al. (1980) in the production of ~ 2,500 protoclones from 'Russet Burbank'. They identified ~ 2.4 % of protoclones with improved agronomic as well as Early (*Alternaria solani* Sorauer) and late blight resistance traits. Thompson et al. (1986) selected potato protoclones with increased resistance to potato virus Y and leafroll virus. Cassells et al. (1983) in their comparative study between calliclones, potato clones produced through a callus

phase indirectly or directly on explant tissue, including stem pieces (lateral buds and stem segments), recommended the involvement of calliclones in potato improvement programs. The big success was the selection of 'AC LR Russet Burbank' among a population of calliclones resistant to potato leafroll virus; it was released in Canada in 2002 (CFIA 2009).

Tissue culture-based methods of potato improvement depend on the identification of useful genetic variation among plants using screening following tissue culture-based regeneration techniques. These variant plants were first called "somaclonal variants" by Larkin and Scowcroft (1981). Somaclonal variants may reflect a combination of processes that lead to differences among regenerants from culture; variation between cells present in the explant (endogenous variation), or variation resulting from the culture process (exogenously caused variation). Endogenous and exogenous variation could come about through mutations in the shoot growing points in tissue culture.

Plants in which mutations have occurred are referred to as chimeras; plants composed of more than one genotype (Norris et al. 1983; Tilney-Basset 1986). Chimeral arrangements in which mutations occur at an early stage in the shoot growing point, so that mutated cell lines occupy a significant portion of an histogenic layer(s), include periclinal, sectorial, or mericlinal. The periclinal chimera is the most stable chimeral type. In the periclinal chimera one or more complete cell layers in the shoot meristem, the outer histogenic layer known as the outer tunic or L_1 layer, is genetically different from the internal (wild-type) layer(s) (Stewart et al. 1972). The internal layers include the inner tunic layer (L_2) and the corpus (L_3). In the most common periclinal chimeras, layer L_1 carries the mutation and layers L_2 and L_3 have the wild-type unchanged genetic composition (Howard, 1971). Apparently, in some periclinal chimeras L_2 could be mutant and L_1 and L_3 wild type (Simmonds 1965).

'Russet Burbank' is one of four cultivars that account for 75 % of the potato acreage in the United States (Acquaah 2007). It was believed to have

originated as a mutation from ‘Burbank’ (Davis 1992). The tubers of ‘Russet Burbank’ have a periderm (skin) that is russet (reddish brown) with a heavily netted (raised “fish net”) pattern, in contrast to the white skin of ‘Burbank’, while the inner flesh (cortex and pith) was apparently unaffected by the mutation and remained white (Davis 1992). ‘Russet Burbank’ was described as a periclinal chimera (Asseyeva 1931; Hardenburg 1949; Krantz 1951; Idaho Potatoes 2008). Miller (1954), Brown (1993), and Coleman et al. (2003) referred to ‘Russet Burbank’ as a somatic mutant of ‘Burbank’. It was listed by Klopfer (1965) and Tilney-Basset (1986; adapted from Klopfer 1965) among many russet sports of potato that are periclinal for mutated (russet) L_1 and wild type for L_2 and L_3.

A central question of this thesis was the investigation of the periclinal chimeral status of the New Brunswick (NB) clone of ‘Russet Burbank’ (Fig. 1.1) (Chapter III; Nassar et al. 2008a). An important inference that was investigated in this thesis was could a potato cultivar be improved through separation of putative periclinal chimeral genotypes and reconstitution of non-chimeral plants? Subsequently, we hypothesized that the isolation and propagation of non-chimeral genotypes of ‘Russet Burbank’ potato (intraclones or somaclones) using somatic embryogenesis would have utility for agronomic (Chapter IV; Nassar et al. 2009), and nutritional, or antipathogenic qualities (Chapter V).

1.1. Hypotheses

1.1.1. Intraclonal somatic regenerants exhibit topophysis. R_0 plantlets derived from histogenically different tissues will be non-chimeral and contain anatomical and physiological features that are the same as that present in the explanted tissue from the source tuber.

1.1.2. Intraclonal/somaclonal potato lines produced from R_0 plantlets are useful for improvement of ‘Russet Burbank’ (NB Clone). These show altered agronomic characteristics in the field (yield or type) or in storage, affecting processing (specific gravity, reducing sugars, fry quality), or other

biochemical or physiological traits (protein characteristics or disease resistance), that could be selected for in a cultivar improvement program.

1.2. Objectives

The objectives of the current study were to:

1.2.1. Test periclinal chimeral theory using a novel tissue culture application for somatic embryogenesis. Tissue-specific explantation from periderm, cortex, and pith tissues enabled separation of each putative genotype and regeneration of non-chimeral R_0 plants from tissue derived from the histogenic layers (L_1, L_2, and L_3) within the putative periclinal chimeral cv. Russet Burbank (NB Clone). Micropropagation of intraclones was followed by evaluation of tuber periderm characteristics from field-grown intraclonal plants (Chapter III).

1.2.2. Determine whether somaclonal variation can be exploited in the improvement of yield, type, or processing characteristics of cv. Russet Burbank (NB Clone) through intraclone production followed by field (yield, type) and lab (specific gravity, reducing sugars, fry quality) selection (Chapter IV).

1.2.3. Determine whether somaclonal variation can be exploited in the improvement of nutrient characteristics of cv. Russet Burbank (NB Clone) through assessment of select intraclones for distribution and concentration of proteins (Chapter V).

1.2.4. Determine whether somaclonal variation can be exploited in the improvement of disease resistance characteristics of cv. Russet Burbank (NB Clone) through assessment of select intraclones for resistance to the late blight organism, *P. infestans* (strain US-8) (Chapter V).

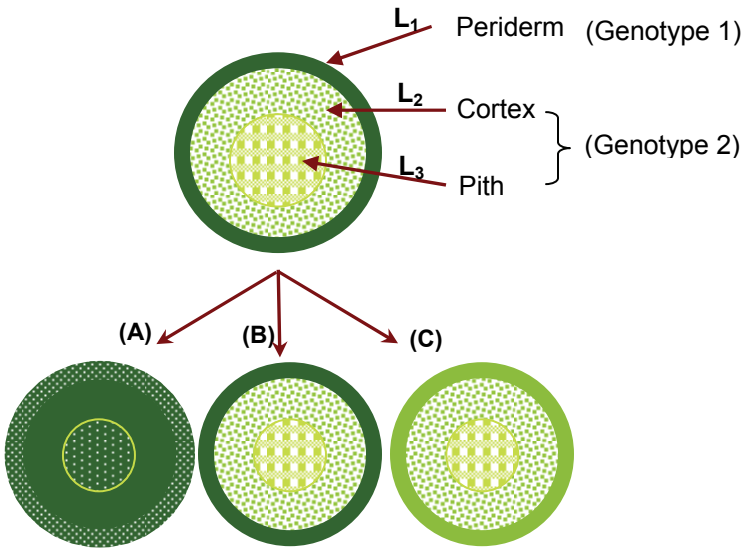

Fig. 1.1. Diagram shows the periclinal chimera concept and possible genotypes that could emerge from isolating chimeral components. (A) A non-chimeral tuber derived from the mutated (L_1) genotype. (B) Control, chimeral tuber, such as one derived from eye tissue. (C) A non-chimeral tuber derived from the wild-type internal genotype (L_2, L_3).

CHAPTER II

GENERAL REVIEW OF THE LITERATURE

2.1. Potato (*Solanum tuberosum* ssp. *tuberosum* L.)

2.1.1. Anatomy

The potato is an annual field vegetable crop. It is characterized by large and swollen stem tubers that range from round to elongate in shape with various skin (periderm) and flesh colours based on the cultivar (Burton 1989; Harris 1992; Acquaah 2007; Elzebroek and Wind 2008). Potato has branched, angular, erect, semi-erect, and weak stems of about 30-80 cm in length at the flowering stage. Its root system is fibrous. Potato leaves are pinnately compound with three or four pairs of oval leaflets with smaller ones in between. The flower if produced, depending on the cultivar, consists of a greenish calyx composed of five sepals, a corolla with five petals (these give an open flower a star-shape), and five stamens. The potato fruit is a globular, green or yellowish berry, about 2 cm across, with many seeds (~ 200 seeds per berry), similar to a tiny unripe tomato fruit. Potato is mainly self-pollinated but is less commonly propagated by seed. There is a “true potato seed” industry. However, most cultivated potatoes are clonally multiplied, and most commercial potato are cultivated from small (golf ball sized) “seed tubers” or “seed pieces” cut from larger stem tubers.

2.1.2. Taxonomy

Family Solanaceae contains many important crop plants; tomato (*Lycopersicon esculentum*), eggplant (*Solanum melogena*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*), potato (*Solanum tuberosum*), and many others. The genus Solanum has > 1,000 species (OECD 1997); Burton (1989) estimated > 2,000 species. It is subdivided into several subsections; the subsection potatoe contains all tuber-bearing potatoes and is divided into several series including the tuberosa series, which contains about 54 species, wild and

cultivated. One of these species is *S. tuberosum* (Hawkes 1990) which is divided into two subspecies: *tuberosum* and *andigena*. *Tuberosum* subspecies is the cultivated potato widely grown almost all over the world while the subspecies *andigena* is cultivated primarily in Central and South America (Hawkes 1990; OECD 1997) (Table 2.1).

2.1.3. History

Potato was first found in South America in the Andean mountains from Colombia and Venezuela through Ecuador, Peru, and Bolivia to northern Argentina, more than 7,000 years ago (Glendinning 1983; OECD 1997; Bradshaw, 2007). The oldest confirmation of potato use was in the area between Bolivia and Peru around 11,000 BC (Elzebroek and Wind 2008). There is no agreement between scientists on the specific date of potato introduction into Europe and later to the rest of the world. The Spanish Conquistador Pedro Cieza de Leon mentioned in his Journal of 1553 that potato was introduced into Europe between 1565 and 1580 and from England it was brought to USA in 1621 (Acquaah 2007).

Four main introductions established the foundation of modern potato germplasm in Europe and North America. The first two were the introduction of *Solanum tuberosum* subsp *andigena* from South America into Spain and England around 1570 and 1590, respectively. The third one was around 1830 when the South American cultivar Daber was imported into Germany. Finally, the fourth introduction was in 1861 when Goodrich brought 'Rough Purple Chili' into the US (Hawkes 1979; Glendinning 1983; Burton 1989). For many years, potato was used as food only at monasteries, hospitals, and palaces. It was not before the 18th century that potato was available to ordinary people (Elzebroek and Wind 2008). Potatoes spread from Europe to the world during the seventeenth century (Pandey and Kaushik 2003). Nowadays, potato is the number one non-grain food commodity and the fourth most important field crop after wheat, maize, and rice;

grown in > 160 countries (AAFC 2007; Bradshaw 2007; FAO 2008) and represented by > 4,000 cultivars (Hils and Pieterse 2007).

2.2. Potato Statistics

Potato can be grown under a wide range of altitudes, latitudes, and climatic environments. It can be grown from sea level to > 4,000 m in altitude and from the equator to > 40º north and south (Harris 1992). World production of potato was 321 million metric tonnes (Mt) in 2007 (Table 2.2). Potato consumption (Kg capita^{-1}yr^{-1}) was highest in the Russian Federation (125), Canada (89), and USA (63). China and India are the first and third potato producers in the world, but consumption is still relatively low compared with other countries; 35 and 17 (Kg capita^{-1}yr^{-1}), respectively, in 2003. Table 2.2 shows FAO statistics for potato consumption and amount of total energy and protein provided by potato (FAO 2008).

2.3. Plant Chimeras

Winkler (1907) who obtained a “graft-hybrid” plant from callus formed at the juncture between grafted tomato and nightshade plants first mentioned the term chimera. The new plant shoot was divided longitudinally into tomato and nightshade. Winkler called this new plant a chimera to underline its two genetically discrete tissues (Tilney-Bassett 1986).

Quite apart from grafting events, plant chimeras usually occur through mutation. A plant chimera is defined as a plant that contains two or more genetically dissimilar tissues because of mutation (Norris et al. 1983; Tilney-Bassett 1986). Chimeras usually arise from spontaneous or induced early-stage somatic mutations of the shoot meristem. If a mutant cell lineage stabilizes in any of the histogenic layers, this mutant condition is perpetuated in all lateral shoot outgrowths from the chimeral meristem (Stewart et al. 1972; Tilney-Bassett 1986; Poethig 1989; Marcotrigiano 1990; Marcotrigiano and Gradziel 1997; Burge et al. 2002; Hartmann et al. 2002). The chimeral components may vary according to

their nuclear chromosome or organellar (plastid or mitochondrial) DNA structure (Norris et al. 1983).

The division of the shoot apical meristematic (SAM) cells govern plant growth. SAM develops in two stages: initial formation and subsequent growth (Schmitz and Theres, 1999; Chatfield et al. 2000). SAM are found at the shoot tip (apex, or apical growing point) or laterally (axillary growing points) on the stem, and consist of cells that are arranged in three layers in most flowering dicot plants (Fig. 2.1 A). The outer cell layers cover a centre of inner cells in the arrangement known as the tunica-corpus meristem (Tilney-Bassett 1986; Poethig 1989). During shoot and tuber differentiation from the shoot meristem, the epidermis (and subsequent periderm) are derived from the outer tunica layer (L_1), the cortex and germ cells from the inner tunica layer (L_2), and the vascular ring and pith from the corpus (L_3) (Dermen 1960).

Variations or natural mutations take place in plants continuously during tissue interactions with micro-environmental conditions. The shoot apices (apical or lateral) are a common site of mutagenesis (Norris et al. 1983). Accordingly, mutations may develop with many chimeral arrangements according to any change in chromosomes of the cell genome or organelles (plastids, mitochondria). The chimeras that involve the nuclear genome have been classified according to their structure as sectorial, mericlinal, or periclinal (Tilney-Bassett 1986; Hartmann et al. 2002).

In a sectorial chimera, the mutated cells are present in a section of the meristem that transects several histogenic layers. As the organ grows, the mutated cells extend into a pie-shaped area from the epidermis into the center of the stem or root (Fig. 2.1 B). This kind of chimera is rare and unstable. The affected organ (shoot, or more commonly root) reverts either to a periclinal chimera or to the wild type. In the mericlinal chimera, cells containing the mutant gene occur only in a part of the outer histogenic layer (L_1) of the plant (Fig. 2.1

C). Mericlinal chimeras are also unstable and the shoot usually reverts to a periclinal chimera or to the wild type.

Plants with mutated outer and/or inner tunica layer (s) (L_1 and/or L_2), with the corpus wild-type, are called periclinal or “hand-in-glove” chimeras (Asseyeva 1931; Dermen 1960; Howard 1961; Marcotrigiano and Gradziel 1997; Hartmann et al. 2002) (Fig. 2.1 D). Periclinal chimeras usually arise from spontaneous or induced early-stage somatic mutations of the meristem (sectorial, mericlinal, or more extensive) that become incorporated into a lateral shoot. The concept of periclinal chimerism is relevant to current horticultural research, where separation of chimeral plants into pure (non-chimeral) types is a common and recurring theme in the development of improved plants, including thornless blackberry and other *Rubus* spp. (reviewed by Skirvin et al. 1994), thornless roses (Canli 2003), and wine grapes (Franks et al. 2002; Hocquigny et al. 2004; Bertsch et al. 2005). Periclinal chimerism was recently confirmed in two *Vitis vinifera* ‘Pinot gris’ clones through analysis of 50 microsatellite loci (Hocquigny et al. 2004).

2.3.1. Potato Chimeras

Satina et al. (1940) was first to suggest that periclinal chimeras are important phenomena for scientific study. From these we can learn more about the initiation and development of tissues and organs and the contribution of epidermal cells in the formation of inner tissues. They induced periclinal chimeras in Datura using solutions of 0.2-0.4 % colchicine for 3 to 8 days. Histological examination, showed three independent germ layers in the shoot apex.

In general, mutations happen in single cells and the stability of the somatic mutant depends on the cell growth rate and its location in the meristem (Simmonds 1965). Several investigators have studied somatic mutation in relation to periclinal chimeras. For example, Simmonds propagated a number of somatic mutants of potato tubers of several cultivars using adventitious shoots and deep shoots (from sub-epidermal tissues). He mentioned that ‘Gladstone Red’ is an L_1 mutant and ‘Majestic Holly’, ‘Majestic Dock’, ‘Eclipse Raspberry’,

‘Doon Star’, ‘Dunbar Rover’, and ‘Great Scott’ produced several “mutant-type shoots” suggesting that in some of them L_2 was mutant and L_1 and L_3 were not.

Cultivars Red Craige and Red King are periclinal chimeras with L_1 red, and L_2 and L_3 layers white-splashed-pink in colour (Howard 1971). Howard noted a reversion of L_1 layer red to white-splashed-pink in a tuber or part of a tuber in 1 of 100 plants. He suggested that the reversion from red to white-splashed-pink could result from a back-mutation or replacement at the growing point of an L_1 layer cell by a cell from L_2 (this proposed phenomenon was termed “perforation” by Bergann and Bergann (1959)).

In a trial to find the most appropriate technique to study the plants derived from L_1 tissues in assumed (putative) periclinal chimeras, Van Hatren (1972) tried to induce growth in adventitious buds and shoots removed from epidermal tissues. It was not possible for him to induce adventitious buds and shoots for potato. Van Harten suggested that the production of sufficient numbers of adventitious shoots from L_1 tissues of potato plants could be used to show the L_1 origin of these shoots in a periclinal chimera. In this thesis, this strategy was elaborated to separate derivatives from all three histogenic layers and employed to test the periclinal status of the NB clone of ‘Russet Burbank’. ‘Russet Burbank’ tubers have a periderm (skin) that is russet (reddish brown) with a heavily netted (raised “fish net”) pattern, while the inner flesh (cortex and pith) is white (Davis 1992).

Periclinal chimerism is characteristic of mutations of potato (Asseyeva 1931; Howard 1961), and was believed to be true of ‘Russet Burbank’. Asseyeva (1931), Hardenburg (1949), Krantz (1951) Idaho Potatoes (2008) assumed that ‘Russet Burbank’ is a periclinal chimera. Miller (1954) and Brown (1993) referred to ‘Russet Burbank’ as a somatic mutant of ‘Burbank’. It was listed by Klopfer (1965) and Tilney-Basset (1986; adapted from Klopfer 1965) among many russet sports of potato that are periclinal for mutated (russet) L_1 and wild type for L_2 and L_3. The only experimental evaluation of ‘Russet Burbank’ chimeral status found

by the authors was conducted by Clark (1930, 1933) using an “eye excision” method developed by Asseyeva (1927) and a crossing trial with the white-skinned cv. Katahdin. Thirty cut tubers of ‘Russet Burbank’ yielded plants with tubers that were all russet (Clark 1930).

This work was repeated with 100 cut tubers in 1931 (Clark 1933). At the end of the season small tubers, from which periderm features were not distinct, were collected from each hill and replanted in 1932, yielding tubers that were 85 % russet, and 15 % patchy russet. Results of crossing experiments were limited in number but not as expected for a periclinal chimeral arrangement; some seedlings had russet tubers and some had white tubers. Clark concluded that ‘Russet Burbank’ was probably a seedling and not a periclinal chimera of ‘Burbank’. Clark’s experimental work generated confusing results that were disparaged (Howard 1959) or overlooked. A sequence of repeated “hearsay” has contributed to the present day assumption that ‘Russet Burbank’ is a periclinal chimera. We investigated the periclinal chimeral status of ‘Russet Burbank’ (complete work presented in Chapter III; Nassar et al. 2008a).

2.3. Methods Used in Potato Improvement

Potato improvement programs in North America started mid 19th century by both private and public breeders and have been successful in releasing more than 250 cultivars (Sieczka and Thornton 1993). There is no easy method to achieve potato improvement, partly because many potato cultivars are closely related (Hawkes 1979). The main potato cultivar to be introduced to North America was ‘Rough Purple Chili’ (Goodrich 1863). ‘Garnet Chili’, and then ‘Early Rose’ were selected, followed by the seedling selection of ‘Burbank’ from ‘Early Rose’ by Luther Burbank 1910 (Davis 1992). ‘Russet Burbank’, the most famous potato cultivar in North America and the focus of this thesis, was found in 1914 by a farmer in Denver, Colorado, USA and described as a mutation of ‘Burbank’ (Plaisted and Hoopes 1989; Davis 1992; Douches et al. 1996; Ortiz 2001).

To widen the genetic base of the European and North American potato cultivars, researchers have suggested the incorporation of wild species genes into the cultivated potato gene pool using crossing or hybridization (Simmonds 1962; Plaisted and Hoopes 1989). Field screening and selection among germplasm repository materials is conducted to identify desirable alleles that could result in promising parents (Birhman and Kang 1993; Ortiz 1998).

Potato plant breeding methods have included traditional crossing and supplementary methods including: use of haploids, somatic hybridization, use of wild species, neotuberosum, molecular marker assisted selection, and genetic engineering. Each method is briefly reviewed in the following sections.

2.3.1. Haploids

Haploids are plants with half the chromosome number (equal to the gametophytic chromosome number) of their parents (Tai 2005). They are used in gene mapping, identification of major and quantitative trait loci (QTL), genetic transformation, somatic fusion, and marker-assisted selection in breeding for new cultivars. Haploids are produced by androgenesis (paternal haploids) and/or gynogenesis (maternal haploids) (Tai 2005). Maternal haploids can be produced parthenogenetically by interspecific hybridization of cultivated tetraploid potato cultivars (4 x) (maternal; pistillate) using *S. phureja* (Juz. and Bukasov) pollen (2 x) (pollinator; parent) (Peloquin et al. 1996; reviewed by Ortiz 1998). Parthenogenesis can occur when both paternal nuclei fertilize the polar nuclei leaving none to fertilize the egg. Parental haploids can be obtained using male gametes via anther or microspore culture (Uhrig and Salamini 1987; Calleberg and Johansson 1993; Rokka et al. 1998). De Maine (1995) examined effects of a second haploid generation on inbreeding of somatically chromosome-doubled (homozygous dihaploid) potato from crosses with *S. phureja* clones. The dihaploids had greater seed yield than the original haploid generation, but no other apparent differences including tuber yield. While haploids have use in plant

improvement programs, including potato, no reports were found describing potato cultivar release based on this method.

2.3.2. Somatic Hybridization

Somatic hybridization of plants can be done via protoplast fusion. Somatic cells from leaves are digested enzymatically to remove the cell walls then fused using either chemical compounds or an electric current, termed electrofusion (Austin et al. 1986). Calliclones are produced from hybrid cells (cybrids) through a callus phase. Calliclone shoots are removed and rooted on a rooting medium, and then transferred to the greenhouse and/or later to the field for screening for desired trait(s) (Novy and Helgeson 1994a and b; Novy 2007). Somatic hybridization can overcome sexual incompatibility and male or female sterility encountered in conventional sexual crossing (Oryczyk et al. 2003; Guo et al. 2004). Somatic hybrids have been reported for many crops including potato (Trabelsi et al. 2005).

Several methods have been used for the characterization of regenerated somatic hybrid lines in plants including: morphological evaluation, isozyme analysis, cytological evaluation by chromosome counting and flow cytometry analysis, and molecular characterization using DNA markers. For potato somatic hybrid clones, inter simple sequence repeat (ISSR)-PCR is used for detecting the insertion of foreign DNA into cultivars through somatic hybridization (Matthews et al. 1999). Semi-random PCR primers designed to detect intron-exon splice junctions were used by Przetakiewicz et al. (2002) to identify tetraploid somatic hybrids derived from diploid species. A genomic in situ hybridization (GISH) technique was used to detect the genome fragments in potato *S. tuberosum* and *S. brevidens* hybrid cells (Gavrilenko et al. 2002).

Somatic hybridization has been used for a long time in potato improvement programs. For example, it was used in the production of F1 hybrid seeds to convert fertile potato cultivars into cytoplasmic male sterile seed-parents (Perl et al. 1990). Cytoplasmic male sterility is a maternally inherited phenotype,

which is identified by inability of a plant to produce functional pollen (Perl et al. 1990). This is a mitochondria-controlled trait. Cytoplasmic male sterility was transferred from an alloplasmic male sterile into two male fertile potato cultivars that were previously used as seed-parents in F1 hybrid seed-production.

Somatic hybridization and protoplast technologies were used for developing virus- and insect-resistant cultivars (Novy 2007). Hybrids were produced from the *S. etuberosum* clone PI 245939 (potato virus Y (PVY)-resistant) and a *S. tuberosum* dihaploid x *S. berthaultii* cross using somatic hybridization (Novy and Helgeson 1994b; Novy 2007). These tri-species hybrids gave poor tuber yield but vigorous leaf growth so Novy and Helgeson backcrossed the somatic hybrids with *S. tuberosum* to improve yield. They tested the fusion parents, the somatic hybrids, and the sexual progeny of the somatic hybrids for resistance to PVY and potato leafroll virus (PLRV). Novy and Helgeson identified three somatic hybrids with significant resistance to PVY compared with 'Russet Burbank' and 2 clones that were resistant to PLRV. This work may lead to the production of important clones for release as new specific virus resistant or tolerant cultivars.

2.3.3. Exploitation of Wild Species

About 235 species of the genus *Solanum* are tuber-bearing. Only the *tuberosum* specie is cultivated (Hawkes 1990). This means a narrow genetic base is present in most cultivated potato which is problematic for potato improvement. Incorporation of genes from wild species into the *tuberosum* specie has been necessary to increase disease resistance (Bradshaw and Mackay 1994; reviewed by Bradshaw et al. 2006).

Several wild diploid species are tuber-bearing and resemble the dihaploids of *S. tuberosum* in their ability to be crossed (Bradshaw and Mackay 1994). These wild populations are potential sources of resistance to disease, pest, abiotic stress, and other useful genetic diversity. Hybrids of diploids and dihaploids were adapted for long-days and able to set tubers under long day

conditions. Backcrossing of these hybrids with the tetraploid was proposed to incorporate about 25 % of the wild hybrid into the new clone (Bradshaw and Mackay 1994). It was anticipated that this new breeding strategy could widen the genetic base of cultivated varieties and introduce new characteristics (Jansky et al. 1990).

A scheme was started to produce a population of *Phureja*/*Stenotomum* adapted to long-days (Carroll 1982). Natural seeds were used and both seedling and tuber populations were produced over a 2-year cycle. After several such cycles, the population became adapted to long-day conditions and yield improved; mainly an increase in tuber size and reduction in tuber numbers. Direct hybridization of members of this improved diploid *S. phureja* x *S. stenotomum* population with tetraploid *S. tuberosum* cultivars resulted in superior tetraploid hybrids in both marketable and total yield, and more tubers per plant, with slightly low mean tuber weights (Carroll and Maine 1989). Use of multiple bridge crosses permitted the transfer of the R-genes from *S. bulbocastanum* to *S. tuberosum* (Hermsen and Ramanna 1973). Multiple bridge crossing of *S. bulbocastanum* with *S. acaule* and *S. phureja* and then backcross of hybrids with *S. tuberosum* resulted in the release of 'Toluca' (Jacobsen and Schouten 2008) and 'Biogold' (Visser 2009). These cultivars were released around 2005, described as resistant to *P. Infestans*, and are apparently in use for organic farming in the Netherlands (Visser 2009).

2.3.4. *Neo*tuberosum

Since *S. tuberosum* was originally selected from *S. andigena*, breeding programs were established in many countries to identify new *Tuberosum* from *Andigena*-based populations: in 1959 in the UK (Simmonds 1969), in 1963 in the USA on materials supplied by Simmonds (Plaisted 1987), in 1968 in Canada (Glendinning 1987), and in 1976 in the Netherlands (Maris 1989). Large numbers of seedlings from non-adapted *Andigena* accessions were grown in the field and selection was applied for greatest yield with acceptable tuber size, shape, and

colour. Moreover, berries were collected to produce sexual generations. Selection of resistance to late blight was improved by imposing severe late blight infection in the field (Bradshaw and Mackay 1994).

After four generations, Simmonds reported potential success in selecting better *S. andigena* clones comparable in yield and maturity to *S. tuberosum* and with increased late blight resistance (Bradshaw and Mackay 1994). Field results of clones selected after crossing clones of *S. andigena* with *S. tuberosum* showed tuber uniformity and yield heterosis (Tarn and Tai 1983; Plaisted 1987). These programs showed that clones of *S. andigena* were adapted and produced parents suitable for incorporation into modern potato. *Neo*tuberosum material has been employed in cultivar development. For example, both cultivars Shelagh, highly resistant to late blight (The European Cultivated Potato Database 2009) and Rosa, resistant to Early Blight (Pelletier and Fry 1989), resulted from crosses between *S. tuberosum* and *neo*tuberosum (Bradshaw and Mackay 1994).

2.3.5. Molecular Marker Assisted Selection (MAS)

Marker assisted selection (MAS) technology is based on genetic markers related to genes expressing beneficial phenotypes (Mullins et al. 2006). This can enable rapid and efficient selection for the traits of interest, allow the identification of the genetic components of the same trait, and potentially shorten the breeding process by several years. Despite its potential, there have been few instances of MAS use in potato breeding. This is largely because of the out-breeding, tetraploid genetics of cultivated potato, and the fact that many traits of interest to breeders are polygenic in nature and greatly affected by environment.

MAS is in use for improvement of traits controlled by single dominant genes (Mullins et al. 2006). Molecular marker-based maps of potato have identified at least 19 major disease resistance genes and numerous quantitative trait loci (QTL) for disease resistance (Gebhardt et al. 2001), morphological, developmental, and quality traits that are single gene-controlled (Mullins et al. 2006). For example, Gebhardt and Valkonen (2001) mentioned that inherited

disease resistance and large-effect QTL for disease resistance were mediated by a single class of genes that share conserved nucleotide-binding-site (NBS) and leucine-rich repeat (LRR) motifs. Based on map location, several NBS-LRR-type disease resistance genes have been cloned and characterized for use in MAS. Caromel et al. (2005) identified two separate QTL for potato cyst nematode resistance in the wild potato *S. sparsipilum* and inserted them into the potato genotype *S. tuberosum* Casper H3. This work apparently resulted in highly resistant phenotypes but has not yet lead to new cultivar release.

2.3.6. Genetic Transformation

The idea of inserting genes into potato cultivars to improve various traits is an interesting proposition. Scientists have found it relatively easy to insert foreign genes into potato (Destefano-Beltran et al. 1991; Ghislain et al. 1998). Direct introgression of resistance genes into potato material using a transgenic approach was first reported by An (1986) and Shahin and Simpson (1986). The success of nuclear incorporation of heterologous gene(s) into the potato genome was described for the *Agrobacterium tumefaciens*-mediated transformation of leaf tissue (De Block 1988), tuber tissue (Ishida et al. 1989), and internodal stem segments (Newell et al. 1991), and for direct gene transfer through particle bombardment into a similar range of tissues (Romano et al. 2001).

Genetic engineering of potato is an area of active current research, much of which is in the pharmaceutical arena. This review is limited to articles related to improvement of agronomic, disease resistance and nutritional characteristics. The most widely used genetic modification technology involved transformation using *A. tumefaciens*. Most of these studies focused on resistance to different pest, virus, and fungal diseases (Davies 2002; Mullins et al. 2006). Some examples include resistance to black scurf (*Rhizoctonia solani*) (Broglie et al. 1991), Colorado potato beetle (*Leptinotarsa decemlineata*) through expression of the *Bacillus thuringiensis* (Bt) toxin gene (Adang et al. 1993; Perlak et al. 1993), late blight (*P. infestans*) (Cornelissen and Melchers 1993; Song et al. 2003; van

der Vossen et al. 2003 and 2005; Osusky et al. 2004), potato leafroll luteovirus (PLRV), potato tuber moth (*Phthorimaea operculella*) (Davidson et al. 2002), PVY (Hassairi et al. 1998), and soft rot (*Erwinia carotovora*) (During et al. 1993). Examples of transgenic potato for increased nutritional characteristics include increase in total amino acid composition especially lysine, tyrosine, and sulphur amino acids (Chakrakorty et al. 2000), selective increase in the essential amino acid methionine (Zeh et al. 2001), increased inulin (Hellwege et al. 2000), increased β-carotene and lutein (Ducreux et al. 2005), and decreased solanin (glycoalkaloid) (Lukaszewicz et al. 2004).

Commercial release of genetically modified potato cultivars was reported in North America by Monsanto (Davies 2002). They used *A. tumefaciens*, in some cases with a sequence of repeated transformation events, to improve a small set of popular North American cultivars. Monsanto's NewLeaf transgenic potatoes were released for commercial use in 1998 (Davies 2002; Kaniewski and Thomas 2004). NewLeaf™ was the trade name for transformed 'Atlantic', 'Superior', and 'Russet Burbank' containing the Bt cry3A and cry3C genes (encoding Bt endotoxin proteins) conferred resistance to Colorado potato beetle. NewLeaf™ Plus was a trade name for 'Russet Burbank' that in addition to resistance to *L. decemlineata* and other insects also had resistance to potato leafroll virus. NewLeaf™ Y was a trade name for 'Russet Burbank' and 'Shepody' with resistance to *L. decemlineata* and other insects as well as resistance to potato virus Y (PVY). NewLeaf™ 6 'Russet Burbank' was released for high tuber yield and improved (longer) storage ability. NewLeaf™ Ultra 'Russet Burbank' and 'Atlantic' were high-yielding, with good processing traits, and resistance to PVY, PLRV, and *L. decemlineata* and other insects. Genetically modified potatoes were increased within the certification industry in Canada but public opposition and concerns resulted in withdrawal of all of the NewLeaf™ varieties from the Canadian certification program and from the Canadian market in 2000 (Kaniewski and Thomas 2004).

2.3.7. Tissue Culture Methods

2.3.7.1. Origin, Definition, and Mechanism of Somaclonal Variation

In vitro tissue culture techniques (callus (calliclones), anther, microspore, and ovule (gametoclones), embryo, protoplast (protoclones), and somatic cell (somaclones)) may exploit or induce natural variation that can be used in crop improvement (Wenzel et al. 1979; Evans and Sharp 1986; Brown and Thorpe 1995; Brar and Jain 1998; Gavrilenko et al. 1999). Those variations were coined “somaclonal variation” (Larkin and Scowcroft 1981). Somaclonal variation could result from inherent variation and/or induced variation from prolonged growth of callus or cell suspension cultures, regeneration of plants from long-term cultures, exposure to specific components of the medium, etc. (reviewed by Evans 1989; Karp 1995; reviewed by Duncan 1997; Brar and Jain 1998).

Several factors were reported to affect the overall proportion of somaclonal variants such as genotype, explant source, medium composition, and the duration of plant culture (Evans and Sharp 1986; Brown and Thorpe 1995; Brar and Jain 1998). The exact mechanism of somaclonal variation is not yet understood either genetics or epigenetics.

The possible genetic mechanisms affecting DNA changes in potato somaclones could vary extensively. These could include changes in chromosome number (aneuploidy, aneusomy, mixploidy, and polypoidy) or structure (Evans and Sharp 1986; Gavrilenko et al. 1999). DNA changes could include nuclear DNA amplification or deletion, DNA sequence rearrangements (deletion, and/or addition), non-active transposable elements e.g. reterotransposable (Flavell et al. 1992), DNA methylation (Brar and Jain 1998), mitochondrial DNA changes e.g., 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).

High degree of natural DNA sequence variation was reported in potato varieties or clones (Gebhardt et al. 1989). Epigenetics, heritable phenotypic variation without changes in DNA sequence, has a role in somaclonal variation (Kaeppler et al. 2000). Several mechanisms of epigenetics were reported; DNA methylation, histone modifications, and transposable elements (Springer and Kaeppler 2008).

DNA methylation refers to 5-methylcytosine and some methylated adenines (Springer and Kaeppler 2008). The methyl moiety is added to cytosine residues found in DNA by DNA methyltransferases. The majority of DNA methylation in plants was found in chloroplast genome dinucleotide and trinucleotide sequences. DNA methylation is a normal system by which plant genes are regulated, especially those involved in development or response to stress (Finnegan et al. 2000). For example, in dormant potatoes, large-scale, transient demethylation (50–70 %) of 5'-CCGG-3' sequences occurs before transcription of genes involved in cell division and meristem growth (Law and Suttle 2002).

Histone modifications take place on the histone tails including acetylation, methylation, and ubiquitination (Kouzarides 2007; Pfluger and Wagner 2007). These histone modifications reveal transcriptional activation, transcriptional repression, efficient assembly into chromatin, and DNA replication. The epigenetic information of histone modifications is less stable than that of DNA methylation. Most histone modifications are reversible and the equilibrium for a particular locus is controlled by modifiers and demodifiers (Kouzarides 2007; Pfluger and Wagner 2007). Cytogenetic studies showed chromosomal distribution for several histone modifications (Jackson et al. 2004; Baroux et al. 2007). Histone modifications are studied by chromatin immunoprecipitation with microarray hybridization (Zhang et al. 2007; Bernatavichute et al. 2008).

Epigenetic mechanisms include silencing of expression and/or mobility of transposable elements (Girard and Freeling 1999). Active transposable elements are mobile DNA segments in the genome and are highly mutagenic (Slotkin and Martienssen 2007). They usually affect the protein-coding genes by insertion,

chromosome breakage, useless recombination, and genome rearrangement. Also, transposable elements change neighbouring genes by altering splicing and polyadenylation patterns, or by functioning as enhancers or promoters (Girard and Freeling 1999).

DNA-based genetic markers were used to detect epigenetic variation including point mutations, insertions, deletions or inversions in allelic DNA fragments, which can be used to differentiate between individuals of the same species (Gebhardt et al. 2005). For example single-nucleotide polmorphisms (SNPs) are markers that were used to study the comprehensive population genetics and linkage disequilibrium (Niewöhner et al. 1995; Sattarzadeh et al. 2006). SNPs-based mapping technique was used to assess the frequency of SNPs and insertion/deletion (Indels) in the potato genome (Rickert et al. 2003). They performed comparative sequencing of 78 amplicons in 17 tetraploid and 11 diploid genotypes. They reported one SNP every 21 base pairs and one Indel every 243 base pairs (Rickert et al. 2003). After identification of the presence of SNPs, a variety of techniques, e.g. pyrosequencing or single-nucleotide primer extension (SNuPE) were used (Rickert et al. 2002) to measure SNP allele frequencies in DNA pools which accurately detect variation between genotypes (Gruber et al. 2002; Neve et al. 2002; Wasson et al. 2002).

Also, somaclonal variation can be detected based on morphological, biochemical (such as isoenzyme), or DNA markers. Isozyme variations were found in some regenerated somaclones from stem internodes of three potato cultivars (Binsfield et al. 1996). Several molecular techniques have been employed to detect somaclonal variation including: random amplified polymorphic DNA (RAPD) (Evans and Sharp 1986; Brar and Jain 1998; Ehsanpour et al. 2007), ISSR (Albani and Wilkinson 1998), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) (Jain 2001), and methylation sensitive amplified polymorphism (MSAP) (Xiong et al. 1999; Joyce and Cassells 2002).

2.3.7.2. Application of Tissue Culture Methods to Plant Improvement

Tissue culture methods are useful in supplementing conventional plant breeding methods and are applicable to almost all plant species (Compton and Veilleux 1991). Selection and release of new somatically-derived cultivars has not always been successful. In some cases, the majority of observed variation was negative or lacked persistence with time (Karp 1995).

Somaclonal variation has been reported in potato for various agronomic traits such as tuber yield, plant morphology (abnormal leaflets or stems, leaf discoloration, or dwarfs), maturity, and physiological and biochemical traits (Brar and Jain 1998). 'Russet Burbank' mesophyll-derived protoclones were tested in the field; 65/1,700 (3.8 %) varied in 13/22 traits including tuber weight, number, sucrose level at harvest, leaf and flower morphology (Secor and Shepard 1981; Ayers and Shepard 1981). Variation occurred among potato leaf mesophyll-derived protoclones of 'Russet Burbank' (Shepard et al. 1980). Changes in growth habit, tuber shape and size, skin color, photoperiod requirements, and maturation date were documented. Similar variation was observed in protoplast-derived regenerants in later studies with other potato cultivars (Sree-Ramulu et al. 1983; Creissen and Karp 1985). Protoclones of other genotypes varied in yield and other characters in the field (Wenzel et al. 1979; Jelenić et al. 2001).

Phenotypic variation (plant morphology, yield) also occurred among calliclones regenerated adventitiously on tuber discs of 'Russet Burbank', 'Superior', and 'Kennebec' (Reitveld et al. 1991, 1993). Similarly, potentially useful changes in plant morphology, leaf anatomy, and anthocyanin pigmentation occurred among calliclones from nodal cutting explants (Austin and Cassells 1983). Field evaluation of 'Desirée' clones that produced adventitiously on callused leaf, rachis, and stem pieces revealed variation in tuber characteristics (Evans et al. 1986). 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). Although many of these

calliclones were described as having some agronomic trait exceeding the source cultivar, most displayed too many accompanying undesirable changes to merit continued breeding efforts.

Several calliclones of ‘Superior’ were selected for desirable improvements in yield, vigour, tuber number, and shape (Rietveld et al. 1991, 1993). Most of them showed phenotypic stability over more than two consecutive tuber generations and maintained their horticulturally desirable characteristics. About 13,000 calliclones from stem internodes and leaves of 14 (Thieme and Griess 1996) and 17 (Thieme and Griess 2005) potato cultivars were evaluated in both the greenhouse and field. Thieme and Griess (1996) observed 2-10 % positive variation among calliclones and 0.5-2.1 % of these had superior haulm growth, earliness, and tuber yield. These variations were stable; they persisted over three field generations. They reported a low incidence of yield improvement (0.1-1 %). Thieme and Griess (2005), noted 0.7-18 % of calliclones that varied positively, 0.7-22 % that varied negatively at $P \leq 0.05$, 0-9 % that varied positively at $P \leq 0.01$ and 78-100 % that were similar compared with source cultivar controls in phenotype, for a range of tuber characters.

The variation rate for regenerated plants using tissue culture methods was estimated at 1-3 % per culture cycle (Skirvin et al. 2000) while others believe it can be greater than 10 % per cycle (Evans and Sharp 1986; Larkin et al., 1989). A statistical approach to evaluate variation rate in micropropagated plants was described by Cote et al. (2001). They concluded that: 1) increase of variation rate can be expected as an exponential function of the number of culture cycles, and 2) after a given number of culture cycles, a percentage of variable off-types can be expected. To be of practical value, the expression of variation among new plants derived in vitro should be frequent enough to enable selection of desirable traits, and the selected lines should perform well under a range of environments (Karp 1995; Duncan 1997). Increasing the number of parameters under evaluation during in vitro or ex vitro screening will increase the opportunity to select material with improved characteristic(s).

Once in vitro selection has been performed (if practical) field selection can follow (Duncan 1997). Many desirable traits of potato should be screened directly in the field, including yield and tuber type. The number of new potato cultivar releases resulting from somaclonal variation has been modest (total of one). Cultivar White Baron, a somatic variant of cv. Danshakuimo (Irish Cobbler) which does not turn brown after peeling, is the sole registered somatic variant (Arihara et al. 1995). This cultivar was field-selected from among 31 protoclones for low tyrosine content. It contains 25 % of the tyrosine concentration of the source cultivar. We compared the yield and processing features of somaclones of 'Russet Burbank' NB Clone that were produced in vitro and grown in the field (Chapter IV; Nassar et al. 2009).

2.3.8. Potato Somatic Embryogenesis

In somatic embryogenesis, bipolar structures with root and shoot are formed from any sporophytic plant tissue (Steward et al. 1958). Somatic embryos develop through the same phases of development as zygotic embryos (globular, heart, torpedo, and cotyledonary stages). This phenomenon demonstrates the totipotency of plant cells.

Potato somatic embryogenesis has been extensively studied. Early scientists tested the possibility of enhanced embryoid production from different tissue explants through media component alterations. Explants included tuber discs (Lam 1975; Bragd-Aas 1977, shoot meristem tips (Fiegert et al. 2000; Powell and Uhrig 1987), microspores (Dunwell and Sunderland 1973; Johansson 1986), immature zygotic embryos (Pretova and Dedicova 1992), leaves (JayaSree 2001), single-node stem cuttings (Reynolds 1986; Garcia and Martinez 1995), stem internodal cuttings, leaves, microtubers, and roots (Seabrook and Douglass 2001; Seabrook et al. 2001; Sharma and Millam 2004; Sharma et al. 2007). Only Sharma et al. (2008 a, b) studied the genetic mechanisms of potato somatic embryogenesis. Of 14 studies of potato somatic embryogenesis, only one evaluated somaclones in the greenhouse and noted

"off-types" (Seabrook and Douglas 2001) and somatic embryoids of potato have apparently not been tested in the field. This technique was used in the regeneration of intraclones of 'Russet Burbank' NB clone from specific source tissues of tubers and microtubers (Chapters III and IV).

2.3.9. Field Evaluation of In Vitro-Produced Potato Plantlets and Microtubers

Field performance of in vitro plantlets and tubers (microtubers) of potato has been studied extensively. In vitro plants and microtubers offer possible alternative propagules to field-grown seed-tubers for plant breeders, as they are specific disease-free, and convenient for handling, storage, and transport (Ranalli et al. 1994). Moreover, microtubers do not require greenhouse hardening, and could be mechanically field-planted on a large scale (Ranalli et al. 1994). Another alternative propagule to field-grown seed tubers are minitubers; tubers produced under protected cultivation in a greenhouse/screenhouse from in vitro plantlets or microtubers. It was recommended to use large-size microtubers or minitubers as seed tubers for good field performance (Struik and Lommen 1999). Using microtubers and minitubers imported from France as alternative seed tuber sources was cheaper than importing seed tubers in Mali (Vanderhofstadt 1999).

Yield of tubers is the determining factor in the successful use of in vitro plantlets, microtubers, or minitubers as field propagules. No significant differences were found between ex vitro plantlet- or microtuber-derived plants of 'Russet Burbank' in plant performance and tuber yield in Quebec, Canada (Leclerc and Donnelly 1990). Total tuber biomass and average tuber weight were less for ex vitro plantlets, microtubers, and minitubers compared with conventional seed tubers. Ex vitro propagules produced more tubers than conventional seed tubers (Leclerc and Donnelly 1990). Ranalli et al. (1994) compared the field performance of conventional seed-tubers, minitubers, and microtubers of 'Monalisa'. Tuber yield data showed (in descending order) that certified seed-tubers > minitubers > microtubers. Also, seed-tubers were superior

in yield compared with minitubers of ‘Bintje’, ‘Ostara’, and ‘Elkana’ (Lommen and Struik 1994 and 1995).

The effects of jasmonic acid, light, and dormancy-breaking treatments on the field performance of potato microtubers were compared with ex vitro plantlets in the field (Pruski et al. 2003). Microtubers gave only 30-40 % of the pre-elite tuber yield of ex vitro plantlets. Microtubers of ‘Amisk’ and ‘Russet Burbank’ out-yielded ‘Shepody’ and ‘Atlantic’. Cultivar maturity affected the performance of microtubers in the field. In a study conducted in Japan, the field performance of microtubers and conventional seed tubers of the early ‘Kitaakari’ and late ‘Konafubuki’ and ‘Norin1’ were compared (Kawakami et al. 2004). They noticed low vigour and yield of microtubers of the early maturity cultivar compared with the later maturity cultivars and conventional seed tubers. Irrespective of their maturity status, microtuber-derived plants yielded 86 % of the tuber dry weight of seed tubers. Microtubers have use as an alternative propagule for potato (Kawakami et al. 2004).

2.3.10. Potato Processing Quality Traits

2.3.10.1. Importance of Potato Processing

Processed potato products have been popular for 150 years (Garayo and Moreira 2002; Clark 2003). Processed products are favoured for home and industry because of the good appearance, taste, texture, and reduced food preparation time (Pedreschi et al. 2007). Industrial production of French fries started around 1945 (Kirkman 2007). Jack Simplot, the founder of Simplot Company, was the inventor of the industrial process for French fries. McCain, Simplot, and Lamb Weston with McCain own 55 plants in 13 countries and market French fries to 110 countries (Kirkman 2007). About 50 to 60 % of the crop is processed in North America and some European countries (Li et al. 2006; Acquaah 2007; Kerby et al. 2007; Kirkman 2007). In Canada, in 2004, about 2.5 Mt were processed into French fries (Keijbets 2005). USA, Canada, and the Netherlands are the world leaders in potato processing. Canada (0.89 Mt) is the

second largest exporter of frozen potato after the Netherlands (1.18 Mt) (USDA 2004).

Potato varieties processed as French fries should have: suitable tuber morphology, high content of solids (20-22 %), less than 2 mgg^{-1} (FW) glucose content (Burton 1989; Kirkman 2007). Potatoes with higher glucose values than these will usually show color problems after cooking. Varieties with less glucose result in better texture, higher yields, and lower oil absorption (Lisinska and Leszczynski 1989). The French fry process involves tuber washing, peeling, slicing, or cutting, defect removal, blanching, and frying at 180 C (Burton 1989; Li et al. 2006; Kirkman 2007).

2.3.10.2. Storage of Potato and Processing Quality

Most potato are stored at low temperature for months for later use in the fresh market, for seed potatoes, or within the processing industry, so safe storage is very important (Kerby et al. 2007). During long-term storage of potato tubers, the accumulation of sucrose is initiated which eventually converts to reducing sugars (Burton 1989). Concentrations of reducing sugars in potato tubers are affected by genotype, mechanical stress factors, and storage conditions (Kumar et al. 2004).

Storage temperatures affect the sugar content of potato cultivars. Tubers of potato cultivars AO82283.1, Augsberg Gold, Norchip, Russet Burbank, Red Gold, Saginaw Gold, and Yukon Gold that were stored at 10 C had lower content of sucrose, glucose, and fructose than tubers stored at 3.3 or 8.3 C, respectively (Edwards et al. 2002). At the same storage temperature (8.3 C), cvs. Saginaw Gold, Augsberg Gold, and AO82283.1 had lower concentration of reducing sugars than cvs. Norchip, Red Gold, Russet Burbank, and Yukon Gold (Edwards et al. 2002). Storing potato tubers at 20 C did not alter the level of reducing sugars while at 4 C the concentration of reducing sugars was increased (Matsuura-Endo et al. 2004).

Storage of potato at low temperature (10 C or lower) delayed sprouting and reduced storage losses from various microorganisms (Burton and Wilson 1978). Conversely, storage at low temperature induced rapid conversion of starch to reducing sugars (glucose and fructose) (Isherwood 1973; Kerby et al. 2007). Accumulation of reducing sugars caused sweetening (low-temperature sweetening) and an unpleasant bitter taste in fried potato products. A strong positive correlation between reducing sugars and acrylamide formation was reported (Amerin et al. 2003 and 2004; Kumar et al. 2004; Ohara-Takada et al 2005; Silva and Simon 2005; Mestdagh et al. 2008). Glucose, fructose, and sucrose with amino acids of nitrogenous compounds (maybe asparagine) play a major role in acrylamide formation but asparagine and glycoalkaloids produced more acrylamide during high temperature processing (Mottram et al. 2002; Becalski et al. 2003; Yaylayan et al. 2003; Kumar 2004; Stadler et al. 2004).

2.3.10.3. Breeding for Low Reducing Sugars

For a cultivar to be used for processing, it must have a high specific gravity, low dry matter levels, large tuber size, white colour after cooking, and low reducing sugar concentration (Gould 1999; Kerby et al. 2007). From 1876 to 1998, about 48 potato cultivars were released for improved processing quality traits (Love et al. 1998). Several potato cultivars are used as French fry processing cultivars in Europe and North America, including Agria, Astrix, Atlantic, Bintje, Hermes, Lady Olympia, Maris Piper, Ranger Russet, Russet Burbank, Shepody, Umatilla Russet, and Victoria (Kirkman 2007). Examples of potato cultivars that were used as chipping cultivars are Atlantic, Hermes, Innovator, Lady Claire, Lady Rosetta, Monona, Norchip, Record, Russet Burbank, Saturna, Snowden, and Umatilla Russet.

Significant differences in reducing sugar content were found between 33 potato genotypes in a 2-year study at 5 locations in Europe after storage at 4 and 10 C (Kerby et al. 2007). One genotype was indentified with superior reducing sugar concentration after 12 weeks at 4 C. Pronounced variation in glucose

content was found in advanced clones of diploid species (*S. phureja* and *S. stenotomum*) evaluated for important processing traits including glucose and chip color (Amoros et al. 2000). They identified 6 clones with lighter chip color.

2.4. Nutritive Value of Potato

2.4.1. Nutritive Components of Potato

Potato belongs on any food menu (Haase 2008). Potato tubers are consumed either as table stock (fresh, stored) or as processed products. Fresh consumption includes all cooking methods (boiled, steamed, baked, fried, roasted, or microwaved) and processed products include French fries, canned, frozen, chilled, and chips (Tarn et al. 2006; McGregor 2007). Consumption of fresh potato is more nutritious compared with processed. Fresh potatoes are relatively low in calories, almost free of fat and cholesterol, high in vitamin C, have considerable amounts of potassium and magnesium, and are high in fibre, especially if the skins are eaten. The major nutritive components of potatoes are carbohydrates, proteins, vitamins, minerals, dietary fibres, and some other phytonutrients including antioxidants (Table 2.3). Only the protein content was reviewed here; improvement to protein content was one objective addressed in this research (Chapter V).

2.4.2. Proteins

2.4.2.1. Importance of Potato Proteins

Potatoes contribute to the protein requirements of more people per hectare than any other major crop (Kaldy et al. 1972) except soybean (Jadhav et al. 1981). Potato provides a complete source of protein for human nutrition (Woodward and Tally 1953; Haase 2008). Potatoes constitute a considerable proportion of total protein consumption in many areas (Millard 1986). The biological value of potato protein, based on the essential amino acid index and nitrogen balance, is greater than beef, tuna fish, wheat flour, soybean, rice, corn,

beans, or seaweed; it is exceeded only by egg protein (Hughes 1958; Schupan 1959; Kofranyi and Jekat 1965, 1967; Markakis 1975). Nutritional studies on humans and animals have confirmed these findings. For example, a woman and a man were kept in good health and in N equilibrium for 167 days on a diet where the entire N was supplied by potatoes (Kon and Klein 1928). Potato protein has a satisfactory ratio of total essential amino acids/total amino acids (Lopez de Romana et al. 1981).

Tuber nitrogen is composed of soluble and insoluble protein and non-protein nitrogen (NPN) (Woolfe and Poats 1987; Dale and Mackay 1994). Eighty-five % of total protein content is soluble (Racusen and Foote 1981) while 8–10 % is insoluble (Desborough 1985). Non-protein nitrogen is formed of organic and inorganic compounds. The organic portion includes the free amino acids (~ 75 %) and amides. Glutamine, asparagine, and valine were the three major free amino acids and constituted more than 50 % of the total free amino acids (Kapoor et al. 1975).

Protein content varies among potato cultivars and ranged on a DW basis from 3.5–23 % (Schwimmer and Burr 1976), from 8.1–12.3 % among six cultivars (Kaldy and Markakis 1972), 4.8–10.1 % among 34 cultivars (Miedema et al. 1976), and 6-8 % among 6 potato hybrids of *S. phureja* X *S. tuberosum*, 5 diploids and 1 tetraploid (Desborough and Weiser 1974). Proteins from potato were classified into three categories; patatin (40-60 %), proteinase inhibitors (20-30 %) and other proteins with high molecular weight (20-30 %) (Pots et al. 1999).

Patatin, 43 Kb, is the main tuber storage protein, contributing 40 to 45 % of the total soluble protein (TSP) in tubers (Pavia et al. 1983; Rajapakse et al. 1991; Shewry 2003). Patatin physiologically functions as lipid acylesterase which is involved in plant defense (Dennis and Galliard 1974). It is heat labile, digested in 15 sec, and binds specifically to the immunoglobulin E (IgE) in adults and children. This immune-reaction identifies patatin as the major allergen in potato (Pearson 1966; Seppälä et al 1999; Majamaa et al. 2001; Koppelman et al. 2002;

De Swert et al. 2007). There are other proteins, named cathepsin D, cysteine, and aspartic protease inhibitors, which belong to the soybean trypsin inhibitor group (Kunitz type) and have been identified as allergens to children (Seppälä et al 2001). Allergenic response to potatoes could be initiated by eating cooked or raw potatoes. Several allergenic symptoms were noticed in adults including sneezing, wheezing, urticaria, and anaphylaxis (Pearson 1966; Seppala et al 1999; Majamaa et al. 2001; Koppelman et al. 2002). In children, the allergenic symtopms were asthma, atopic dermatitis (infantile eczema), rihinitis (runny nose), and urticaria (Seppala et al 1999; Majamaa et al. 2001).

Total nitrogen measurement was used for rapid estimation of potato tuber crude protein. Nitrogen-to-protein was determined by multiplying total nitrogen content from Kjeldahl tests by 6.24 (Van Gelder 1981). This conversion factor is very close to the empirical factor (6.25), the nitrogen content in protein of 16 g per 100 g DW. Wu and Lakin (1993) employed the elemental nitrogen analyzer in the determination of protein in powdered potato and confirmed accurate estimation of potato proteins. Also, total nitrogen was measured by Narvaez-Vasquez and Ryan (2002) using LECO® combustion analysis. That enabled them to estimate the total protein in potato transformed with the tomato prosystemin gene which regulates the defensive and developmental genes. LECO®, nitrogen/protein analyzer, burns the samples in a pure oxygen environment. Combusted gases are collected after loss of water vapour. The gasses are scrubbed and all nitrogen oxides are reduced to nitrogen gas, which is then read as % nitrogen. A LECO® combustion apparatus was employed in protein studies in this thesis and enabled estimation of crude protein content (Chapter V).

2.4.2.2. Improvement of Potato for Protein

Only a few breeding studies have attempted to improve potato tuber protein quantity or quality. Total protein content was found to be significantly different among 40 clones of *S. andigena* (Li and Sayre 1975). These authors concluded that tuber protein content is controlled by genetic, environmental, and

cultural factors. They recommended that clones be evaluated at different field locations to obtain stable higher-protein clones.

Hybrids high in protein were selected from a 4-year study of clones produced by crossing *S. phureja* and *S. tuberosum* (Desborough and Lauer 1977). These hybrids had about 10 % protein – a level more than double that of the potato cultivar Red Pontiac. Selection for high protein from the *andigena* hybrids which has higher protein content than *tuberosum* specie was recommended (Desborough and Lauer 1977). Snyder and Desborouh (1978) selected one hybrid with high protein content from four hybrids of *Phureia-Tuberosum-Andigena* that were compared to the relatively low protein- containing 'Norland' and 'Kennebec'.

Improving the amino acid methionine levels in 'Russet Burbank' potato was attempted through the production of protoclones on a medium that contained ethionine, a methionine analogue (Langille et al. 1998). In six of the 48 protoclones selected, tubers produced significantly increased free methionine content, up to 2.66 times the control level (Langille et al. 1998). Unfortunately, this work did not lead to a new cultivar; it led only to a recommendation for the incorporation of the strategy into future programs for improved nutritional components. Patatin, the main storage protein in potato, can be highly allergenic for some people (Seppälä et al. 1999). Therefore, selection for reduced protein content somaclones is as important as selection for high protein content. We investigated the possibility of selecting improved clones for altered (high and/or low) protein content (Chapter V).

2.5. Potato Late Blight Disease

2.5.1. Late Blight (*Phytophthora infestans* (Mont.) de Bary)

Phytophthora infestans (Mont.) de Bary causes the most devastating disease of potato in the world, late blight (Fry et al. 1993; Vleeshouwers et al. 2000; Agrios 2005; Möller and Reents 2007). This disease results in yield losses

of 40 to 70 % and reduces crop quality depending on variety susceptibility and environmental conditions (Hausladen 2006). *P. infestans* is very destructive in cool and moist environments in areas such as the northern United States and the east coast of Canada (Agrios 2005). This fungus attacks both tuber and foliage of potato plants and can destroy the complete plant in 1-2 weeks depending on the weather.

Prior to 1990, it was believed that North America had only one clonal population of *P. infestans* (A1 mating type, strain US-1) known to be sensitive to phenylamide fungicides including metalaxyl, oxadixyl, benalaxyl, and ofurace (Fry et al. 1993; Goodwin et al. 1994; Miller et al. 1998). *P. infestans* resistant to phenylamide fungicides (A2 mating type, e.g. strain US-8) was first detected in Mexico and subsequently migrated to European countries and then around the world. By 1991, A2 (including the three strains US-6, US-7, and US-8) were reported in the United States and Canada on potato and tomato plants.

Disease starts with the spread of mycelium, germinating oospores, or zoospores from infected plants (Agrios 2005). When mycelium reaches the leaves it produces spore-bearing sporangia that can be transferred from one plant to another by wind or rain. Once the environmental conditions are suitable for infection (15-25 C and ~ 100 % RH), the spores germinate and these germ tubes penetrate leaves through the stomata. Mycelia grow between cells and send absorptive haustoria into cells. Mycelia continue to grow from infected cells to fresh cells. After a few days of infection, sporangiophores, consisting of stalk with apical sporangia, emerge from the stomata and spores are spread by wind or rain. Tuber infection starts in the field with spores spread from leaves to the soil by rain. Zoospores germinate and penetrate the tuber tissues through lenticels or wounds. Also, within the tuber, the mycelia grow between cells and penetrate the cells by haustoria. Oospores can stay alive in the soil for 3-4 years and may produce more virulent strains through genetic recombination of the mating strains A1 and A2 through sexual reproduction (Hardham 1992; Colon et al. 1993; Kramer et al. 1997; Agrios 2005).

Susceptibility of potato to *P. infestans* was proposed to result from inhibition and/or delay of the defensive response by a soluble glucan synthesized by the fungus (Andreu et al. 1998). The pathogen secrets several cell wall degrading enzymes to penetrate the plant. Glucanases, polygalacturonases, pectin esterases, pectin lyases and xylanases enzymes were identified (Hardham and Shan 2009). Resistance to *P. infestans* not only depends on the production of defensive molecules, but also depends on their time and location of production. Eleven major genes (R1-R11) were identified in resistant hybrids of *S. demissum* that were responsible for resistance to *P. infestans* (Swiezynski and Zimnoch-Guzowska 2001; Rauscher et al. 2006).

2.5.1. Improvement of Potato for Late Blight (*Phytophthora infestans*) Resistance

Numerous studies were conducted to improve resistance of potato cultivars to *P. infestans*, particularly to the very destructive US-8 strain (Kato et al. 1997; Goodwin et al. 1998; Douches et al. 2001a and b, 2002, and 2004). Potato history provides dark memories of late blight fungus that destroyed the 1845-1846 potato crops and caused widespread famine in Europe. An estimated one million people starved to death and more than a million were forced to migrate to escape famine conditions (IYP 2008). Since then, breeding for resistance to *P. infestans* became crucial for potato.

Several methods have been applied in breeding for *P. infestans* resistance including genetic modification, cisgenesis, crossing cultivated varieties and wild species (Jacobsen and Schouten 2008), marker assisted selection (reviewed by Mullins et al. 2006; Regan et al. 2006), and various tissue culture techniques (Shepard et al. 1980). Selection for *P. infestans* resistance is combined with selection for agronomically important traits (Umaerus et al. 1983).

Genetically modified potatoes with resistance to *P. infestans* have been reported. Among these are potato plants that produce hydrogen peroxide (Wu et al. 1995) and a transgenic line expressing the antimicrobial protein temporin A (Osusky et

al. 2004). However, at the current time, genetically engineered potatoes are not acceptable for cultivation in North America due to perceived environmental and health issues (Jacobsen and Schouten 2008). Cisgenesis is a transgenic approach which uses natural genes and regulatory elements from the same plant species or from crossable species (Schouten et al. 2006; Schubert and Williams 2006). This technology needs refinement for use in in plant breeding. It has been applied in the development of *P. infestans* resistant selections (Haverkort et al. 2008; Jacobsen and Schouten 2008) but no resistant cultivar has been released yet.

Combining selection for tuber quality, yield, and resistance to late blight has been done. For example, unadapted potato cultivars known to have elevated resistance levels to *P. infestans* were crossed with susceptible cultivars (Bisognin and Douches 2002). Eighty clones with moderate to strong late blight (US-8 strain) resistance were identified in the greenhouse and field. Identification of clones resistant to the US-8 strain among 22 genotypes (10 cultivars and 12 selections) was performed at different field locations (Haynes et al. 2002). Environment x genotype had great effect on stability of resistance and 4 genotypes were identified for strong resistance levels. Relative resistance levels of 147 cultivars including the European resistant 'Libertas' and breeding lines, to *P. infestans* US-8, was estimated in the greenhouse (Douches et al. 1997). Hybrids of *S. tuberosum* and *S. bulbocastanum* and their backcrosses (seven advanced lines) had relatively strong resistance to late blight and were similar to the resistance level of 'Zarevo', a highly resistant European potato cultivar.

Selection of seedling hybrids or clones produced by crossing wild and cultivated species conferring increased resistance to late blight has been demonstrated. For example, introgression of R-genes from *S. bulbocastanum* using multiple bridge crosses of *S. tuberosum* with *S. acaule* and *S. phureja* resulted in the release of 'Toluca' and 'Biogold' after 30 years of evaluation (Jacobsen and Schouten 2008; Visser 2009). Helgeson et al. (1998) produced somatic hybrids of *S. bulbocastanum* and *S. tuberosum* then crossed the cybrids

with economically important potato cultivars for relative resistance to the strain US-8. They reported hybrids with an effective resistance to *P. infestans* and high yield compared with 'Russet Burbank' in an infested field. Evaluation of 281 clones from 72 families of a diploid hybrid population of *S. phureja* x *S. stenotomum* showed 75 % of clones with significantly more resistance to *P. infestans* than the marker cultivar 'Atlantic' (Haynes and Christ 1999). Similar work was done by Costanzo et al. (2004), a student of Haynes. They field-evaluated 230 *S. phureja* x *S. stenotomum* hybrid populations and reported greater field resistance within 26 clones than control 'Atlantic', 'Kennebec', and 'Katahdin'. After all that work, a few cultivars were released as resistant cultivars including 'Jacqueline Lee' (Douches et al. 2001b) and 'Defender' (Novy et al. 2006). However, 'Russet Burbank' has not parented any cultivar with resistance to the US-8 strain (Staples 2004).

Tissue culture approaches have been used in the "mining process" for finding resistance to *P. infestans* with no reported success. For example, Behnke (1979 and 1980) regenerated plants from resistant callus that was initially treated with P. infestans filtrate. Behnke noticed that plants regenerated from resistant callus had greater resistance than control plants. 'Russet Burbank' mesophyll-derived protoclones were tested in the field; 3.8 % varied in disease resistance to *P. infestans* and other agronomically important traits (Secor and Shepard 1981; Ayers and Shepard 1981).

Screening of potato clones for resistance to *P. infestans* in the field is a reliable technique. However, the risk of spreading of the inoculum restricts large-scale field screening (Douches et al. 2004). Using the greenhouse for *P. infestans* evaluation minimizes the risk of inoculum spread and is a suitable approach for screening clones for late blight resistance (Colon et al. 1995; Dorrance and Inglis 1997; Helgeson et al. 1998; Douches et al. 2002 and 2004). We used the greenhouse for screening advanced selections of 'Russet Burbank' to strain US-8 compared with 'Libertas' which has a foliar resistance to *P. infestans* (Douches et al. 2004) (Chapter V).

Table 2.1. Taxonomy of *S. tuberosum* spp. *tuberosum* (OECD 1997; Acquaah 2007)

Rank	Latin Name
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Genus	*Solanum* L.
Species	*Solanum tuberosum* L.
Subspecies	*Tuberosum*, *Andigena*

Table 2.2. Statistics for potato production and harvested area in 2007 and consumption, energy, and protein in 2003; Mt, million metric tonnes; MHa, million hectares (from FAO 2008).

Area	Production (Mt)	Harvested area (MHa)	Consumption (Kg capita^{-1}yr^{-1})	Energy (Kcal capita^{-1}d^{-1})	Protein (g capita^{-1}d^{-1})
World	321.74	19.33	32	60	1
Asia	135.61	8.70	23	45	1
Europe	129.40	7.49	93	170	4
China	72.04	5.00	35	68	1
Americas	38.75	1.59	63	136	3
Russia	36.78	2.86	125	230	5
India	26.28	1.60	17	31	0
USA	17.65	0.46	63	100	2
Africa	16.32	1.50	13	25	0
Canada	4.97	0.16	89	144	3
Egypt	2.60	0.11	18	38	0

Table 2.3. Potato tuber contents of major (g) and minor nutrients (mg) per 100 g DW (compiled from Woolfe and Poats 1987; Li et al. 2006; and Buckenhüskes 2005).

Component	Per 100 g DW	
Energy	70.00	Kcal
Dry matter	20.00	g
Carbohydrate	14.80	g
Nitrogen total	3.00	g
Dietary fiber	2.10	g
Protein	2.00	g
Sugars		
Sucrose	0.68	g
Glucose	0.58	g
Lipids	126.00	mg
Fat	100.00	mg
Minerals		
Potassium	418.00	mg
Manganese	21.00	mg
Phosphorous	50.00	mg
Calcium	6.40	mg
Sodium	2.70	mg
Iron	0.43	mg
Zinc	0.34	mg
Magnesium	0.15	mg
Amino Acids		
Asparagine	529.00	mg
Glutamine	409.00	mg
Proline	209.00	mg
Other amino acids	117.00	mg
Vitamins		
C	20.00	mg
B6	0.31	mg
B1	0.11	mg
E	0.10	mg
B2	0.04	mg
B9	0.02	mg

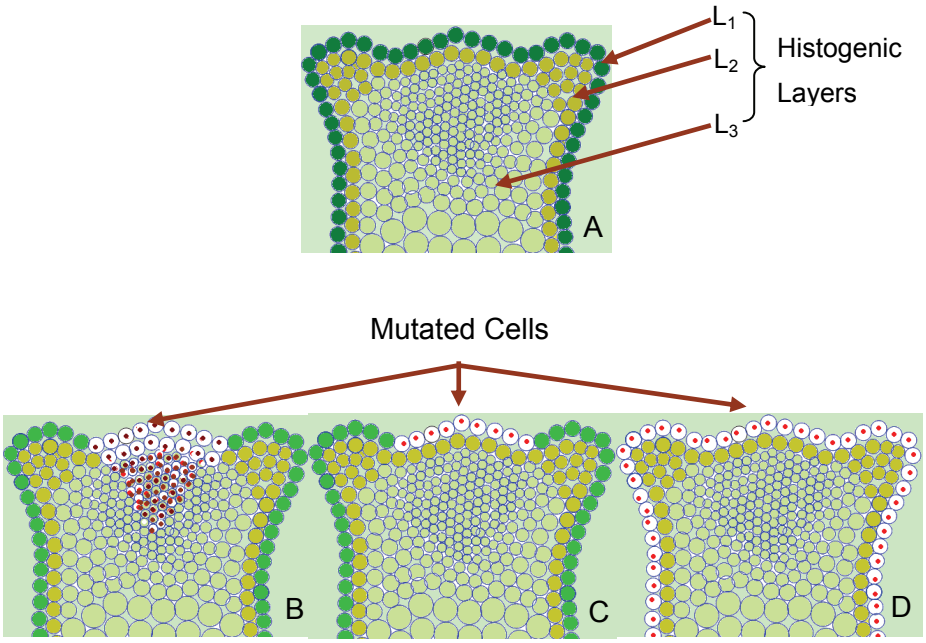

Fig. 2.1. Diagram illustrates (A) the theoretical situation in the apical meristem of dicots; there are two outer (periclinal) layers comprising the tunica (L_1, L_2) and the inner, a mass-meristem or corpus is designated L_3; (B) the sectorial chimera; (C) the mericlinal chimera; and (D) the periclinal chimera.

CONNECTING STATEMENT FOR CHAPTER III

Chapter III consists of a manuscript prepared by myself, Dr. D.J. Donnelly, Dr. Estela Ortiz-Medina, and Dr. Yves Leclerc under the title “Periclinal Chimeral Status of New Brunswick ‘Russet Burbank’ Potato”. This study was presented orally at the First Montreal Plant Meeting held at McGill University, November 17, 2007. It was published in the American Journal of Potato Research (2008) 85: 432 - 437.

Russet Burbank is an important and famous North American potato cultivar. It is described as a periclinal chimera of cv. Burbank, with a mutated periderm (skin) and wild-type inner tissues (cortex and pith). We investigated the chimeral status of cv. Russet Burbank with a novel application of tissue culture technology; through regeneration of somatic embryos from specific tuber tissues (intraclones) derived from the three histogenic layers (periderm, cortex, and pith) of the shoot meristem from microtubers and field tubers. Intraclones were evaluated for tuber periderm characteristics in two field seasons. We predicted that if cv. Russet Burbank was a periclinal chimera, periderm-derived intraclones would have russet tubers like cv. Russet Burbank while cortex- and pith-derived intraclones would have non-russet tubers like cv. Burbank. Results were definitive; NB ‘Russet Burbank’ is not presently organized in a periclinal chimeral arrangement.

CHAPTER III

PERICLINAL CHIMERAL STATUS OF NEW BRUNSWICK 'RUSSET BURBANK' POTATO

•Atef M. K. Nassar[1], Estela Ortiz-Medina[1], Yves Leclerc[2], and Danielle J. Donnelly[1,3]

3.1. Abstract

The widely grown and important 'Russet Burbank' potato has now been almost 100 years under cultivation. 'Russet Burbank', derived from 'Burbank', is described as the classic example of a periclinal chimeral cultivar that is (by definition) a stable entity. This research investigated the chimeral status of the New Brunswick (NB) clone of 'Russet Burbank'. This was done through regeneration of somatic embryos from specific tuber tissues representing the three histogenic layers of the shoot meristem from microtubers and field tubers. Intraclones were evaluated for tuber periderm characteristics in two field seasons. Most intraclones had tubers with russet periderm regardless of tuber source tissues. The frequency of up to 4 % for non-russet (wild-type) and up to 21 % for patchy periderm suggests that one or more types of gene expression modification should be investigated for these phenotypic changes. Clearly, NB 'Russet Burbank' is not presently organized in a periclinal chimeral arrangement.

3.2. Resumen

La ampliamente cultivada e importante papa 'Russet Burbank' tiene ahora casi 100 años bajo cultivo. 'Russet Burbank', derivada de 'Burbank', es descrita como el clásico ejemplo de cultivar de quimera periclinal que es (por definición)

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una entidad estable. Se investigó la condición quimérica del clon de Nueva Brunswick (NB) de 'Russet Burbank'. Esto se hizo a través de la regeneración de embriones somáticos provenientes de tejidos específicos del tubérculo que representan las tres capas histogénicas del meristemo de brotes de microtubérculos y de tubérculos provenientes del campo. Los intraclones fueron evaluados para características del peridermo del tubérculo en dos temporadas de campo. La mayoría de los intraclones tuvieron tubérculos con peridermo rugoso independientemente de la procedencia del tejido del tubérculo. La frecuencia de hasta el 4 % para peridermo no-rugoso (tipo silvestre) y hasta 21 % para peridermo rugoso sugiere el estudio de uno o más tipos de modificaciones en la expresión génica para determinar estos cambios fenotípicos. Claramente, el NB 'Russet Burbank' no está organizado en un arreglo de quimera periclinal.

3.3. Introduction

Solanum tuberosum L. 'Russet Burbank' has many synonyms, including: 'California Russet', 'Golden Russet', 'Idaho Baker', 'Idaho Russet', and 'Netted Gem' (Clark and Lombard 1946; Hardenburg 1949; Stevenson 1949; Darling 1968). It is the most important cultivar in North America, grown extensively in the USA (primarily in Idaho, Montana, Oregon, and Washington, but also other north-central and mid-western states) and right across Canada (Darling 1968; Atkinson et al. 2003; PAA 2008). Its enduring popularity is a tribute to its excellent storage and cooking qualities. It is favored for table use as a baking potato and for commercial processing, primarily for French fries (Clark and Lombard 1946; Darling 1968; CFIA 2008; PAA 2008).

'Burbank', was a seedling selection made by Luther Burbank in the 1870s from a rare chance fruit of 'Early Rose' (Davis 1992). Potato lore suggests that 'Russet Burbank' originated as a mutation from 'Burbank' and was discovered in 1914 by Lou D. Sweet, a farmer in Denver, Colorado, USA. It was assumed (but not rigidly tested) to have considerable resistance to potato scab (*Streptomyces*

scabies) and late blight (*Phytophthora infestans*) compared with other cultivars of that time (Davis 1992). The popularity of 'Russet Burbank' supplanted that of 'Burbank' immediately and has continued to this day; almost 100 years under cultivation. The release date for 'Russet Burbank' is often confused with that of 'Burbank' and generalized ("around 1880", Canadian registration 1923, CFIA 2008) or listed for both cultivars as 1876 (Thornton and Sieczka 1980).

The tubers of 'Russet Burbank' have a periderm (skin) that is russet (reddish brown) with a heavily netted (raised "fish net") pattern, in contrast to the white skin of 'Burbank', while the inner flesh (cortex and pith) was apparently unaffected by the mutation and remained white (Davis 1992). A plant chimera is defined as a plant that contains two or more genetically dissimilar tissues as a result of mutation (Norris et al. 1983; Tilney-Bassett 1986). Chimeras usually arise from spontaneous or induced early stage somatic mutations of the shoot meristem. If a mutant cell lineage stabilizes in any of the histogenic layers, this mutant condition is perpetuated in all lateral shoot outgrowths from the chimeral meristem (Stewart et al. 1972; Tilney-Bassett 1986; Poethig 1989; Marcotrigiano 1990; Marcotrigiano and Gradziel 1997; Burge et al. 2002; Hartmann et al. 2002). During shoot and tuber differentiation from the shoot meristem, the epidermis (and periderm) are derived from the outer tunica layer (L_1), the cortex and germ cells from the inner tunica layer (L_2), and the vascular ring and pith from the corpus (L_3; Dermen 1960). Plants with mutated outer and/or inner tunica layer (s) (L_1 and/or L_2), with the corpus wild-type, are called periclinal or "hand-in-glove" chimeras (Asseyeva 1931; Dermen 1960; Howard 1961; Marcotrigiano and Gradziel 1997; Hartmann et al. 2002).

The concept of periclinal chimerism is relevant to current horticultural research, where separation of chimeral plants into pure types is a common and recurring theme in the development of improved plants, including thornless blackberry and other *Rubus* spp. (reviewed by Skirvin et al. 1994), thornless roses (Canli 2003), and wine grapes (Franks et al. 2002; Hocquigny et al. 2004; Bertsch et al. 2005). Periclinal chimerism was recently confirmed in two *Vitis*

vinifera 'Pinot gris' clones through analysis of 50 microsatellite loci (Hocquigny et al. 2004).

Periclinal chimerism is characteristic of mutations of potato (Asseyeva 1931; Howard 1961), and was believed to be true of 'Russet Burbank'. Asseyeva (1931), Hardenburg (1949), Krantz (1951) Idaho Potatoes (2008) assumed (but did not prove) that 'Russet Burbank' is a periclinal chimera Miller (1954) and Brown (1993) referred to 'Russet Burbank' as a somatic mutant of 'Burbank'. It was listed by Klopfer (1965) and Tilney-Basset (1986; adapted from Klopfer 1965) among many russet sports of potato that are periclinal for mutated (russet) L_1 and wild type for L_2 and L_3. The only experimental evaluation of 'Russet Burbank' chimeral status found by the authors was conducted by Clark (1930, 1933) using an "eye excision" method developed by Asseyeva (1927) and a crossing trial with the white-skinned cv. Katahdin. Thirty cut tubers of 'Russet Burbank' yielded plants with tubers that were all russet (Clark 1930). This work was repeated with 100 cut tubers in 1931. At the end of the season small tubers, from which periderm features were not distinct, were collected from each hill and replanted in 1932, yielding tubers that were 85 % russet, and 15 % patchy russet (Clark 1933). Results of crossing experiments were limited in number but not as expected for a periclinal chimeral arrangement; some seedlings had russet tubers and some had white tubers. Clark concluded that 'Russet Burbank' was probably a seedling and not a periclinal chimera of 'Burbank'. Clark's experimental work generated confusing results that were disparaged (Howard 1959) or overlooked. A sequence of repeated "hearsay" has contributed to the present day assumption that 'Russet Burbank' is a periclinal chimera.

Somatic cell technology provides a more precise tool for investigation of chimeral structure than the early-mid twentieth century technique of eye-excision as suggested, but not realized, by Van Harten (1972). We hypothesized that 'Russet Burbank' intraclones produced from tissues derived from L_1, L_2, and L_3 (periderm, cortex, and pith, respectively) would produce non-chimeral plants and tubers. We predicted that if 'Russet Burbank' was an L_1 periclinal chimera,

periderm explants would produce intraclones with russet tubers like ‘Russet Burbank’ while cortex- and pith-derived explants would produce intraclones with non-russet tubers like ‘Burbank’ (Fig. 3.1 schematically represents this hypothesis).

The objective of this study was to investigate the current periclinal chimeral status of an important eastern North American accession, the New Brunswick (NB) ‘Russet Burbank’ through: (a) regeneration of plants called somatic regenerants first generation (SR_1 or intraclones) dissected from specific tuber tissues (periderm, cortex, and pith) derived from the L_1, L_2, and L_3, respectively, of the shoot meristem, and (b) examination of tuber periderm from these non-chimeral field-grown plants to determine whether the source tissue phenotype was mutated (russet) or ‘Burbank’-type (smooth and white).

3.4. Materials and Methods

3.4.1. Source of Cultivars Russet Burbank and Burbank

Seed tubers of NB ‘Russet Burbank’ were obtained from the Bon Accord Elite Seed Potato Centre (Bon Accord, NB). In vitro plantlets of this clone (#179) were obtained from the Plant Propagation Center, New Brunswick Department of Agriculture, Fisheries and Aquaculture (Fredericton, NB). In vitro plantlets of ‘Burbank’ and reference photographs of ‘Russet Burbank’ and ‘Burbank’ minituber periderm were obtained from the United States Department of Agriculture (USDA) Research Service, Inter-Regional Potato Introduction Station (Sturgeon Bay, WI).

3.4.2. Intraclone Production

NB ‘Russet Burbank’ intraclones were produced through somatic embryogenesis, using a two-step procedure modified from Seabrook and Douglass (2001) in McGill University’s Micropropagation Facility. This is a method that regenerates new plants from individual cells (Pedroso and Pais

1995; Sharma and Millam 2004). Tissue-specific (periderm, cortex, and pith) explants were aseptically removed from fresh field-grown NB 'Russet Burbank' tubers obtained from Bon Accord and from microtubers produced in our laboratory (Leclerc et al. 1994). After 2 weeks, callused explants were transferred onto somatic embryo regeneration medium. The first somatic plantlets were collected after 4–5 weeks and assigned an intraclone number, then micropropagated using single-node cuttings on MS basal salt medium (Murashige and Skoog 1962).

In preliminary experiments, a relatively small number of intraclones were regenerated from a few source tubers and transferred to pots for minituber production in the greenhouse (Ortiz-Medina 2006). This preliminary greenhouse trial was followed by a large field trial replicated over 2 years. Intraclones were regenerated from tissue-specific explants of two sources, field tubers and microtubers, during the winters of 2004 and 2005. Micropropagated control plantlets and intraclones were hardened-off in plug trays over a 3-week interval then trucked to McCain Foods Canada Ltd. research farm (Greenfield, NB). Plants of each intraclone, represented as a single plant in 2005 and as two plants in 2006, were transferred into irrigated plots in a completely randomized design (CRD) and harvested after 111 days (2005) or 119 days (2006). The number of intraclones that were grown in the field are shown in Table 3.1.

3.4.3. Classification of Tubers

At maturity, plants were individually dug, the tubers bagged, tagged, and graded. Tubers were then washed and digitally photographed. These photographs were used to classify tuber periderm into: R (russet) = the entire surface was russet; P (patchy) = the surface showed patches of russet, russet and non-russet tubers were mixed, or (2006 only) two plants representing one intraclone showed inconsistent periderm characters; NR (non-russet) = the entire surface was smooth and white. Each designation was based on a picture that

included all graded tubers (at least four tubers) and consensus by three of the authors.

3.4.4. Exprimental Design and Statistical Analysis

The experiment was designed as a factorial completely randomized design with three factors (tissue sources): periderm, cortex, and pith. The experimental unit was the population of intraclones regenerated from each tissue source. Data were tested for normality using the UNIVARIATE procedure of the Statistical Analysis System (SAS 2007) and statistically analyzed using the general linear model (GLM) procedure to compare freshly harvested tuber periderm phenotypes (R, P, and NR) from different factors ($P \leq 0.05$) for each year of field evaluations.

3.5. Results

3.5.1. Preliminary Greenhouse Trial

Periderm features on minitubers were recorded (data not shown). NB 'Russet Burbank' control minitubers all had russet periderm. All cortex-derived and some pith-derived intraclones produced minitubers with smooth white periderm similar to our USDA photographic reference for 'Burbank' periderm (Fig. 3.1). However, some pith-derived intraclones produced minitubers with periderm that was clearly russet like NB ‘Russet Burbank’, in direct contrast to expected periderm characteristics.

3.5.2. Field Trials

Control plant tubers of NB ‘Russet Burbank’ usually had periderm that was russet (Table 3.1) but occasionally russet with some patchy areas. Control plant tubers of ‘Burbank’ usually had periderm that was non-russet; smooth, shiny, and white. Only one tuber, from one ‘Burbank’ control plant was non-russet with patches of russet. The incidence of NB ‘Russet Burbank’ intraclones with russet or patchy periderm was 84-88 % and 9-14 % (2005) and 79-91 % and 8-21 % (2006), respectively, with no significant difference related to explant source

tissues. Only 0-4 % of all intraclones showed non-russet periderm. Among periderm-derived intraclones, where 100 % of the intraclones were expected to have tubers with russet periderm, 91 % of intraclones had tubers that were russet while 9 % were patchy and 0 % were non-russet. Supplemntary field data are presented in Appendix 1 (Table A.1.1 and Fig. A.1.1.; pages 145-146).

3.6. Discussion

A periclinal chimera is believed to be a stable arrangement consistent with evolutionary advantage to retention of mutations in stratified apices of angiosperms (Kletkowski et al. 1985). Results from a preliminary greenhouse trial with minitubers and 2 years of field trials consistently showed that NB 'Russet Burbank' does not fit the classic definition of a periclinal chimera. The field data show that most intraclones produced tubers with russet periderm, regardless of source tissue origin (Table 3.1). We interpret this to mean that the genes for russet are currently present with similar incidence in all tissues of this clone regardless of the histogenic layer from which they are derived. From our data we cannot conclude that 'Russet Burbank' was never a periclinal chimera. However, our results support experimental observations of Clark (1930, 1933) who did not find evidence of periclinal chimeral structure in 'Russet Burbank' only 15 years or so following its discovery.

Russet periderm is proposed to be governed by three complementary genes, and the loss of any of the three genes can result in non-russet periderm (De Jong 1981). Somatic recombination can occur and cause somatic instability (Hu et al. 1998). Tissue culture is known to enhance genetic variation at both phenotypic and chromosomal levels (Larkin and Scowcroft 1981; Lee and Phillips 1988). Tissue culture can also cause DNA methylation changes and cause phenotypic modifications (Kaeppler and Phillips 1993). Potential sources of intraclones with non-russet periderm can be cell mix-up between histogenic layers, DNA somatic recombination, or gene expression changes of russet-involved genes. The occurrence of intraclones with non-russet and patchy

periderm suggests that one or more types of gene expression modification are the most suspected cause to be investigated for these phenotypic changes.

3.7. Conclusions

We tested the current periclinal chimeral status of the NB ‘Russet Burbank’ over two field seasons with populations of intraclones produced in culture through somatic embryogenesis. The tubers of intraclones derived from all histogenic layers were almost always russet. Clearly, NB ‘Russet Burbank’ is not organized in a periclinal chimeral arrangement for an L_1 and/or L_2, mutation of 'Burbank'.

Table 3.1. Field plants were established from micropropagated control plantlets of NB 'Russet Burbank' and USDA 'Burbank', or intraclones of NB 'Russet Burbank' derived from specific tissue (periderm, cortex, or pith) from two sources (microtubers or field tubers). Intraclones were represented by one plant in 2005 and two plants in 2006. Tuber periderm phenotype (R (russet) = the entire surface was russet; P (patchy) = the surface showed patches of russet, russet and non-russet tubers were mixed, or two plants representing one intraclone (2006) showed inconsistent periderm characters; NR (non-russet) = the entire surface was smooth and white).

Field Season	Source		Explant	Total No. of Plantlets or Intraclones	Number of Intraclones		
	Cultivar	Plantlets / Tubers			R	P	NR
2005[a]	NB 'R. Burbank'	Plantlets		10	8	2	0
	USDA 'Burbank'	Plantlets		6	0	1	5
	NB 'R. Burbank'	Microtubers	Cortex	101	85	15	1
			Pith	171	151	15	5
		Field Tubers	Pith	96	83	13	0
2006	NB 'R. Burbank'	Plantlets		10	10	0	0
		Field Tubers		6	6	0	0
	USDA 'Burbank'	Plantlets		9	0	0	9
	NB 'R. Burbank'	Microtubers	Periderm	54	49	5	0
			Cortex	51	42	8	1
			Pith	14	11	3	0
	NB 'R. Burbank'	Field Tubers	Cortex	53	47	4	2
			Pith	87	79	8	0

[a] Within each year, data were normally distributed. No significant differences occurred in periderm phenotype between intraclones regenerated from the different source tissues ($P \leq 0.05$).

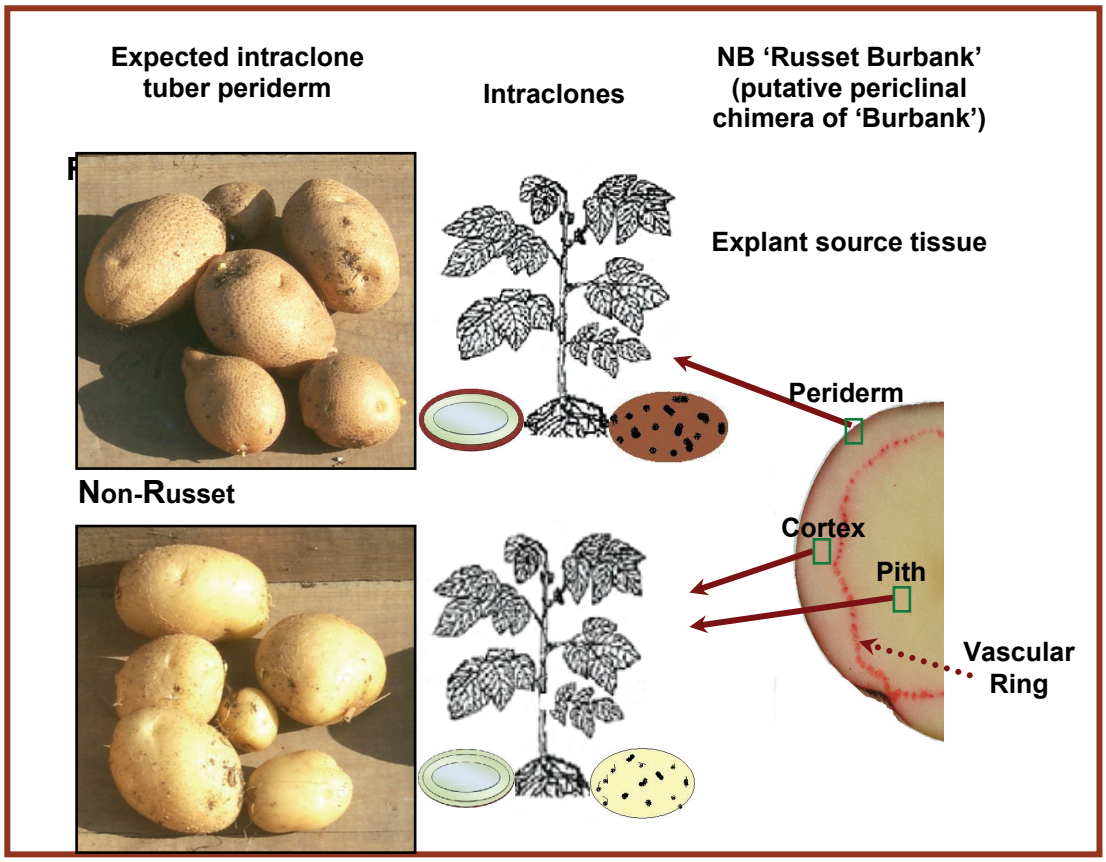

Fig. 3.1. Schematic representation of the hypothesis of this study. If NB `Russet Burbank` is an L_1 periclinal chimera, periderm-derived intraclones will have tubers with russet periderm like 'Russet Burbank', while cortex- and pith-derived intraclones will have tubers with non-russet periderm like 'Burbank'. Reference pictures of USDA 'Russet Burbank' and USDA 'Burbank' minitubers are from Bamberg & Martin, 2004. US Potato Genebank, Sturgeon Bay, WI, USA.

CONNECTING STATEMENT FOR CHAPTER IV

Chapter IV consists of a manuscript prepared by myself, Dr. D.J. Donnelly, Dr. J. Abdulnour, Dr. Y. Leclerc, and Dr. Xiu-Qing Li under the title “Intraclonal Selection for Improved Processing of NB ‘Russet Burbank’ Potato”. It was submitted to the European Journal of Potato Research (March 2009). This study was presented both orally (Nassar et al. 2008b) and in poster form (Nassar et al. 2008c) at the American Society for Horticultural Science Annual Conference held in Orlando, FL, USA (July 21-25, 2008).

Russet Burbank is the most important French fry processing cultivar in North America. In Chapter III, it was shown that NB ‘Russet Burbank’ is not organized in a periclinal chimeral structure. We studied the potential for using somatic embryogenesis to improve the New Brunswick clone (NB clone) of cv. Russet Burbank. Somatic embryos were produced from specific tuber tissues, including periderm, cortex, and pith of microtubers and field tubers. These specific-tissue-derived somatic embryos (intraclones) were tested over three consecutive field seasons for tuber yield and French fry-processing quality traits. Somatic embryogenesis generated useful variation that was not correlated with tuber source or tissue type. Several superior intraclones were identified. Among these were three with lower concentrations of reducing sugars (% glucose) and better fry colour following extended storage (5 or 9 mo). Field and processing evaluation of these advanced lines will continue over the next few years.

CHAPTER IV

INTRACLONAL SELECTION FOR IMPROVED PROCESSING OF NB 'RUSSET BURBANK' POTATO

•Atef M. K. Nassar[1] • Jihad Abdulnour[1] • Yves Leclerc[2] • Xiu-Qing Li[3] • Danielle J. Donnelly[1,4]

4.1. Abstract

'Russet Burbank', the most important potato (*Solanum tuberosum* L.) cultivar in North America, has limited fertility and has not parented improved cultivars through traditional breeding efforts. The goal of this study was to determine if '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 seasons 1 and 2 (2005, 2006) approx. 800 intraclones were regenerated from tubers that were field-grown or produced in vitro. Intraclones were micropropagated, acclimatized, and field-tested to identify the highest yielding lines. Each season, following storage, tubers of selected lines were tested for French fry-processing quality. In season 3 (2007), the best intraclones from seasons 1 and 2 were increased through micropropagation and retested for yield and processing features. Results showed that neither tuber source nor explant tissue type affected intraclone tuber yield, type, or processing characters. About 2-9 % of intraclones had similar yield to controls but superior processing features. We recommend the incorporation of somatic embryogenesis into potato improvement programs for processing quality traits.

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Keywords somatic embryogenesis · potato · processing · reducing sugars · yield · somaclonal variation

4.2. 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 (Leever 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; Miller et al. 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) and minor DNA differences were reported (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 production from callus (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 lab 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 resistance 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 2-10 % 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' (AAFC 1997; CFIA 2008). To the

best of our knowledge, it is the only cultivar derived from ‘Russet Burbank’ in almost 100 years of cultivation.

Recurrent selection is an important component of breeding for new cultivars because it confirms results of previous selections and applies another season of stringent field selection pressure. This is the norm for conventional breeding programs (Miller et al. 2004) and has lead to the identification of many new cultivars including; for example, ‘Alta Russet’ (Lynch et al. 2004) and ‘GemStar Russet’ (Love et al. 2006). Recurrent selection has been used in all field evaluation of clones produced from tissue culture (Shepard et al. 1980; Cassells et al. 1983; Maris 1988; Neele et al. 1988; Jones and Cassells 1995; Thieme and Griess 2005).

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), inter-nodal stem cuttings, leaves, microtubers, and roots (Seabrook and Douglass 2001; Seabrook et al. 2001; Sharma and Millam 2004; Sharma et al. 2007). Of 14 studies of potato somatic embryogenesis, only one group (Seabrook and Douglas 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.

4.3. Materials and Methods

4.3.1. Source of Plant Materials

In vitro control plantlets of NB 'Russet Burbank' 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).

4.3.2. 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. 4.1. 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.

4.3.3. 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 to 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 to 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- and seed-derived), and 'Burbank' (plantlet-derived) 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, 2 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 8 rows in a RCBD with 3 replicates each of 12 plants.

4.3.4. Yield and Processing Quality Evaluation

Field performance data 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 selected for type. Following selection, retained 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 and 9 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 content (% glucose on a FW basis) and sucrose (mgg^{-1} FW) were estimated using the YSI biochemical analyzer (Model 2700 Select, Yellow Springs Instrument Co., OH, USA) as in Sowokinos and Preston (1988). For each somaclonal sample, 4 to 5 potato tubers were peeled and the apical and basal ends removed (0.6 to 0.8 cm-thick slices). Tuber slices were mixed, then a 200 gm sample collected. This was homogenized in 300 ml distilled water using an Omega Fruit and Vegetable Juicer (Omega Products Inc., PA, USA). Approximately 400 ml of potato homogenate was collected in a 600 ml beaker and left to settle for 20 min at room temperature. A 5 ml sample was transferred into each of two test tubes. To the first test tube, 4 ml of 3 % sodium phosphate dibasic (Na_2HPO_4) solution was added for glucose measurement, and to the

second test tube, 4 ml of 0.08 % invertase solution was added for sucrose measurement. The concentrations of glucose and sucrose were recorded (as dextrose) for both test tubes using an YSI Biochemistry Analyzer (Model 2700, YSI Incorporated, Ohio, USA). Dextrose reading from the first test tube was reading “A” and from the second test tube was reading “B”. At least 2 readings were recorded for each test tube to check machine precision. If the first 2 readings were inconsistent, a third reading was taken.

% of glucose = Reading A X 0.43 (dilution factor divided by total sample wt)
Sucrose (mgg^{-1}) = (Reading B – Reading A) X 8.17

For French fry colour assessment, 10 tubers of each genotype were sliced longitudinally (1-cm-thick) and a tuber disc (5-cm-diameter) was cut from each tuber slice with a disc cutter. 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 readings were converted to USDA values (1 to 7, where 1 is the best) according to Iritani and Weller (1974).

4.3.5. Experimental Design and Statistical Analysis

The experiment was designed as a 2x2x3 factorial completely randomized design with two main factors; years: 2005 and 2006, two levels; source tubers: microtubers and field tubers and three sub-levels; tissue sources: periderm, cortex, and pith. Experimental unit was intraclones regenerated from different tissue sources. Yield components, specific gravity, % glucose, French fries and sucrose results were subjected to ANOVA, SAS (SAS Institute Inc. NC, USA, 2007). Data were tested for normality using the UNIVARIATE procedure before analyses. 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 Duncan’s New Multiple Range test ($P \leq 0.05$). Pearson correlation coefficient for tuber yield

components: total tuber weight, total tuber number, graded tuber weight, graded tuber number, and average weight per tuber were estimated. Also, the Pearson correlation coefficients were calculated for specific gravity, % glucose, sucrose, and French fry colour traits (Tables A.2.7 and A.2.8).

4.4. Results

4.4.1. Yield Comparison between Populations of Intraclones

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 4.1. In season 1, most averaged yield components (graded tuber number, total and graded tuber weight) were greatest in 'Burbank'. Average total tuber number was similar in 'Burbank' and MC intraclones and greater than the other genotypes. The two control cultivars and FC intraclones had greater average graded tuber weight (AGTW) than other genotypes. 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, both NB 'Russet Burbank' and 'Burbank' controls had greater averaged yield components (except graded tuber weight) compared with intraclones. All genotypes had similar averaged graded tuber weight except MP intraclones which had lesser graded tuber weights. Cortex- and pith-derived intraclones were similar to each other and to periderm-derived intraclones for most averaged yield parameters but both had greater total tuber number than periderm-derived intraclones. In contrast to the first season, microtuber-derived intraclones had lesser average yield components than field tuber-derived intraclones.

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. 2008a). Explants from the pith and cortex were relatively easier to isolate and more regenerative compared with periderm explants (data not shown). The complete field data are tabulated in Appendix 2 (Table A.2.1 to A.2.18; pages 147-166).

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

Primary selection of promising intraclones for the processing industry used average graded tuber weight and acceptable type and resulted in identification of 28 superior intraclones. Table 4.2 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 compared with other genotypes. 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 USDA classification 2. Tubers of many intraclones, including MC405, fried similarly or more poorly (one was USDA 3). However, tubers of several intraclones, including MP18405, and FP3405, had better fry colour properties (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 4.2). Only one intraclone had significantly greater total tuber weight than control NB '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 4.2).

Also, in 2006 no 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. Confounding difficulties 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 graded tuber yield ≥ control NB 'Russet Burbank', at least 10 % less % glucose, including FP3405, MP18405, and MP19805 (Table 4.2).

Field evaluation in 2007 showed differences in tuber number (total and graded) but no differences in tuber weight (total, graded, and average weight per tuber) and processing quality criteria between controls NBRBF and NBRBP plants (Table 4.2). 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, and FP106 had lesser % glucose compared with tubers of other genotypes and control NBRBP plants (Table 4.2). 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.

4.4.3. Tuber Yield and Processing Quality Selection Efficiency

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 4.3). For example, from 2005, the proportion of 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.

4.5. Discussion

We used two tuber sources and produced somatic embryos (intraclones) from specific tuber parts 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 mo or 5 mo in storage was applied after each field season. Significant difference occurred among intraclones in % glucose and French fry colour. 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). The latter have been considerably improved over the last two 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). 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.

Somatic embryogenesis has the potential to initiate useful variation in yield and storage quality features in potato improvement programs. 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. Sill, 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 to 1,000,000 seedlings was necessary with the selection and field evaluation process lasting from 10-15 years (Maris 1988; Neele et al. 1988). Similarly, Shepard et al. (1980) suggested that field evaluation of large populations of 60,000 to 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 to 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 7.9-10.5 % (Fig. 4.2). Based on these numbers, somaclonal assessment is a useful cultivar improvement strategy, as sufficient positive variation occurs to justify this effort.

Somaclonal variation arbitrarily generates clones with improved or worsened agronomic characters (Rietveld et al. 1991). The underlying mechanisms of tissue culture-derived variations are numerous, affecting the

nuclear and organellar genomes, and have been extensive described by others. An environmental component may also affect clones (Li 2009).

4.6. 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. 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. 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 for fingerprinting to discriminate between them.

Table 4.1. Average yield data (total and graded tuber number, total and graded tuber weight (Kg), and average weight per tuber (ATW; Kg) of control plantlet-derived NB 'Russet Burbank' (NBRB) and 'Burbank' (B) and intraclone plantlet-derived populations of NB 'Russet Burbank' explanted from microtuber pith (MP), cortex (MC), or periderm (MS) tissues or field tuber pith (FP) or cortex (FC) tissues.

Control or Intraclonal Population	No. of Harvested Plants[1]		Tuber Number				Tuber Weight (Kg)				ATW (Kg)	
	2005	2006	Total		Graded		Total		Graded		2005	2006
			2005	2006	2005	2006	2005	2006	2005	2006		
NBRB	39	10	13.17^{b}	20.90^{a}	6.36^{b}	9.50^{a}	1.74^{b}	2.12^{a}	1.36^{b}	1.69^{a}	0.21^{a}	0.18^{a}
B	18	10	23.12^{a}	20.20^{a}	9.65^{a}	9.70^{a}	2.46^{a}	2.07^{a}	1.78^{a}	1.49^{ab}	0.21^{ab}	0.16^{a}
MP	172	38	14.88^{b}	13.74^{c}	4.51^{c}	3.53^{c}	1.34^{c}	0.93^{d}	0.80^{c}	0.55^{d}	0.16^{c}	0.11^{b}
MC	104	106	21.05^{a}	15.46^{bc}	4.49^{c}	5.98^{b}	1.53^{bc}	1.41^{c}	0.73^{c}	1.01^{c}	0.15^{c}	0.16^{a}
MS[2]	-	111	-	15.68^{bc}	-	6.40^{b}	-	1.47^{c}	-	1.09^{c}	-	0.17^{a}
FP	67	179	15.30^{b}	18.31^{ab}	4.34^{c}	7.24^{b}	1.41^{bc}	1.74^{bc}	0.77^{c}	1.24^{bc}	0.17^{bc}	0.17^{a}
FC	9	111	12.11^{b}	17.68^{ab}	5.11^{bc}	6.98^{b}	1.32^{c}	1.67^{c}	0.93^{c}	1.22^{bc}	0.21^{ab}	0.16^{a}
CV[3]			42.83	40.03	58.92	49.71	41.67	41.21	63.67	53.90	42.48	32.52
Pith	239	217	15.00^{b}	17.51^{a}	4.46^{a}	6.59^{a}	1.36^{b}	1.60^{a}	0.79^{a}	1.12^{a}	0.16^{a}	0.16^{a}
Cortex	113	217	20.34^{a}	16.59^{ab}	4.54^{a}	6.49^{a}	1.51^{a}	1.54^{a}	0.75^{a}	1.12^{a}	0.15^{a}	0.16^{a}
Periderm	-	111	-	15.68^{b}	-	6.40^{a}	-	1.47^{a}	-	1.09^{a}	-	0.17^{a}
CV			44.84	41.17	65.65	52.61	44.76	43.88	71.97	56.79	44.02	33.67
Microtubers	276	255	17.02^{a}	15.30^{b}	4.50^{a}	5.80^{b}	1.41^{a}	1.37^{b}	0.77^{a}	0.98^{b}	0.16^{a}	0.16^{b}
Field Tubers	76	290	14.92^{b}	18.07^{a}	4.43^{a}	7.14^{a}	1.40^{a}	1.71^{a}	0.79^{a}	1.23^{a}	0.17^{a}	0.17^{a}
CV			46.94	40.50	65.65	51.55	45.06	42.51	71.92	55.58	43.94	33.48

[1]Some control or intraclone plants did not survive; harvested numbers are less than planted numbers.
[2]MS; Intraclones from periderm tissues were produced for the 2006 field season only.
[3]CV; % coefficient of variation. Means were compared using Duncan's New Multiple Range test ($P \leq 0.05$).

Table 4.2. 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 field tuber-derived (NBRBF) and select intraclones produced from microtuber pith (MP), cortex (MC), or periderm (MS) tissues or field-grown 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 (Standard deviation values where available are in brackets beside each number) of intraclones.

Control Cultivars 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	1.36 (0.38)	1.69 (0.53)	0.77 (0.18)	0.142 (0.013)	0.139 (0.100)	0.047 (0.004)	78	77.9 (11.5)	93.6 (2.4)
NBRBF	NT	2.28 (1.13)	1.51 (0.28)	NT	0.097 (0.027)	0.032 (0.001)	NT	NT	92.1 (5.0)
FP106	NT*	1.86 (0.19)	0.66 (0.19)	NT	0.061 (0.041)	0.016 (0.001)	NT	NT	95.1 (2.9)
FP2106	NT	1.85 (0.62)	0.82 (0.18)	NT	0.109 (0.034)	0.020 (0.006)	NT	NT	89.0 (3.5)
FP3405	2.47	0.71 (0.13)	0.72 (0.16)	0.087	0.040 (0.031)	0.026 (0.012)	96	96.4 (1.6)	92.9 (0.3)
MS1406	NT	1.82 (0.40)	0.70 (0.07)	NT	0.021 (0.001)	0.030 (0.013)	NT	NT	90.8 (8.0)
MC1606	NT	2.04 (0.30)	0.60 (0.04)	NT	0.036 (0.002)	0.032 (0.002)	NT	NT	91.5 (2.8)
FP306	NT	1.83 (0.92)	0.77 (0.11)	NT	0.076 (0.000)	0.032 (0.011)	NT	NT	89.1 (4.0)
MC405	1.52	1.01 (0.55)	0.91 (0.25)	0.097	0.103 (0.002)	0.035 (0.009)	84	94.8 (14.7)	84.9 (4.3)
MP18405	2.29	0.86 (0.59)	0.97 (0.07)	0.07	0.031 (0.019)	0.037 (0.007)	98	94.8 (3.7)	87.5 (4.2)
FP2906	NT	2.15 (0.09)	0.88 (0.21)	NT	0.063 (0.024)	0.040 (0.000)	NT	NT	97.0 (2.4)
FP906	NT	1.68 (0.60)	0.66 (0.02)	NT	0.062 (0.037)	0.045 (0.002)	NT	NT	87.5 (1.6)
FC2806	NT	1.77 (1.22)	1.04 (0.05)	NT	0.076 (0.020)	0.047 (0.007)	NT	NT	93.5 (9.2)
MP19805	2.21	0.92 (0.33)	0.89 (0.11)	0.08	0.030 (0.003)	0.078 (0.005)	NT	96.9 (11.7)	85.7 (1.4)

*NT; not tested.

Table 4.3. Number and percentage of selected intraclones of NB 'Russet Burbank' produced from each tuber source (microtubers or field tubers) and specific tissue (skin, cortex, or pith) each season (2005 or 2006) based on graded tuber weight followed by % glucose.

Tuber Source	Year	Tissue Sources	Number of Intraclones tested in the field	Selected Intraclone Number and Percentage							
				Graded Tuber Weight				% Glucose			
				Tissue Sources[1]	%	Tuber Sources[2]	%	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	2-1	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

[1] Tissue sources; number of selected intraclones produced from various tuber tissues (periderm, cortex, or pith) separately.
[2] Tuber sources; number of selected intraclones produced from microtuber and field-grown tuber tissues collectively.

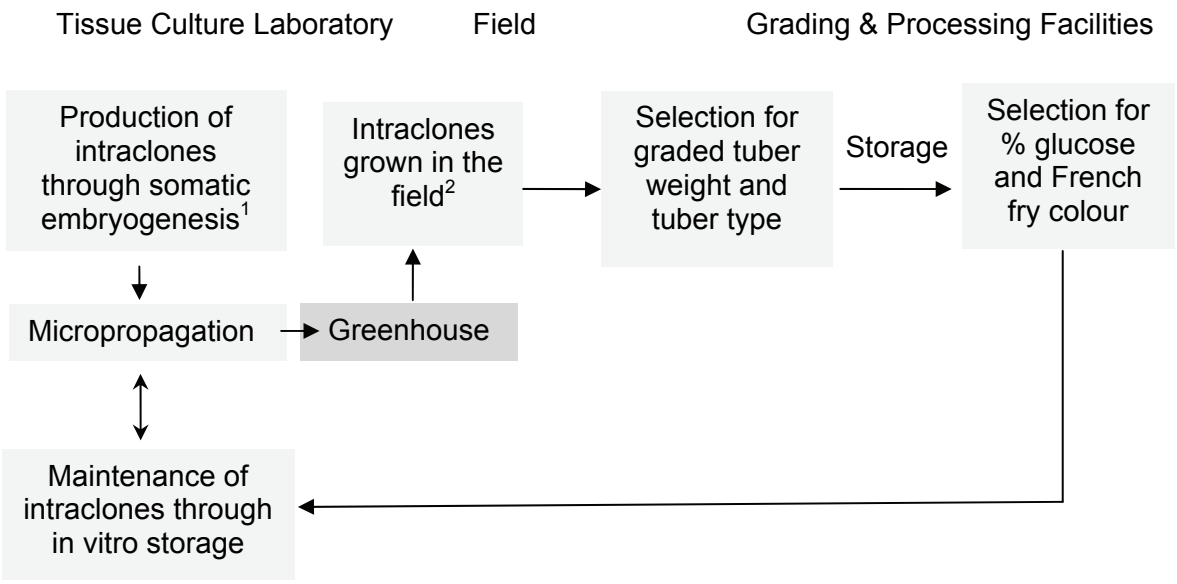

Fig. 4.1. Step-wise flow chart illustrates the annual cycle of in vitro production of somatic embryos and increase through micropropagation for the purpose of storage in vitro and evaluation in the field. Following harvest, the first selection for improved French fry processing quality was based on graded tuber weight. 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.

[1]Explants were derived from specific tuber tissues (periderm, cortex, pith) of microtubers or field-grown tubers.

[2] 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.

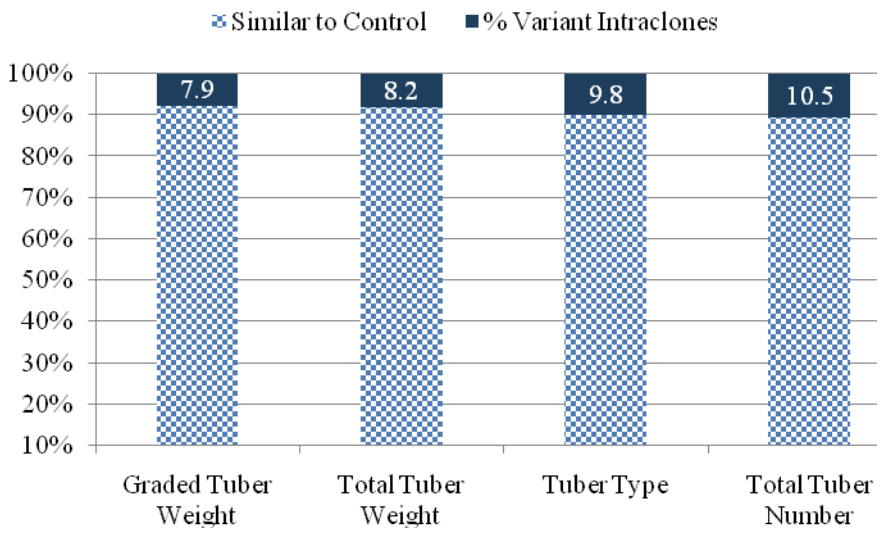

Fig. 4.2. For various agronomic characteristics studied over a 2 year interval (approx. 800 intraclones), the % variant intraclones (with significantly lesser or greater values compared with control plantlet-derived NB 'Russet Burbank') ranged from 7.9-10.5 %.

CONNECTING STATEMENT FOR CHAPTER V

Chapter 5 consists of a manuscript prepared by myself, Dr. D.J. Donnelly, Dr. A.C. Kushalappa, and Dr. Y. Leclerc under the title “Somaclonal selection of NB ‘Russet Burbank’ potato for altered protein content and resistance to *Phytophthora infestans*”. This manuscript was submitted to the American Journal of Potato Research.

Russet Burbank is among the most important potato cultivars in North America and the most studied. We investigated the potential for improvement of ‘Russet Burbank’ (NB Clone) through regeneration of somatic embryos from specific tuber tissues (intraclones) derived from microtubers and field tubers. The best intraclones determined from yield and processing quality evaluations (Chapter IV) were selected for this trial. Tubers from these advanced selections were evaluated for protein content over a 3–year interval. Also, plantlets of advanced selections were tested for late blight (*Phytophthora infestans* (Mont.) de Bary strain US-8) resistance over a 2- year interval in the greenhouse. Several promising intraclones were identified with variant (significantly lesser) total soluble protein concentrations; FC2006, FP306, FP906, MP405, MP9605, MP11505, MP18405, and MS1406 for 2 consecutive years and one (MC405) with significantly greater TSP content compared with control NB ‘Russet Burbank’. Most somaclones expressed similar disease resisatnce to NB ‘Russet Burbank’ and the rest were lesser. Further study need to be done to evaluate the tuber and foliar field resistance of these advanced selections to *P. infestans*. Somatic embryogenesis technology has clear utility in the generation of beneficial variation that can be exploited in potato improvement programs.

CHAPTER V

SOMACLONAL SELECTION OF NB 'RUSSET BURBANK' POTATO FOR ALTERED PROTEIN CONTENT AND RESISTANCE TO *PHYTOPHTHORA INFESTANS*

•Atef M.K. Nassar[1], A.C. Kushalappa[1], Y. Leclerc[2], and D.J. Donnelly[1,3]

5.1. Abstract

Russet Burbank, the most important potato cultivar in North America, has rarely parented outstanding cultivars with improved protein content or resistance to late blight (*Phytophthora infestans* (Mont.) de Bary). The current study aimed to determine whether somaclones of 'Russet Burbank' (NB clone) pre-selected for better field performance and processing quality (advanced somaclones) a) had sufficient variation in protein content or late blight resistance to make screening for these traits possible and b) exhibited long-term (3-4 years) protein stability. Tubers of advanced somaclones were tested for crude protein (CP) and total soluble protein (TSP) content, after 5 months storage. Resistance to *P. infestans* (US-8 strain, A2 mating type) was measured based on ex vitro plantlet foliar assessment in the greenhouse for 2 years. There was substantial inter-seasonal differences in protein content among control plants. This is the first study to show sufficient variation among somaclones to permit screening for altered tuber protein content. Curiously, some of these selections had less inter-seasonal variation in protein level. Advanced somaclones had less variation (approx. 0-5 %) for CP and and greater variation (12-24 %) for TSP content.One advanced somaclone had significantly greater CP content than control NB

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‘Russet Burbank’ for 2 consecutive years. One advanced somaclone had significantly lesser TSP content for 3 consecutive years and 5 somaclones showed reduced TSP content for 2 consecutive years compared with controls. Greenhouse studies showed similar *P. infestans* disease resistance in somaclones compared with control NB ‘Russet Burbank’. Further study is required to compare foliar and tuber resistance of somaclones to *P. infestans* in the field. Advanced somaclones are sufficiently variable to permit screening for improved protein, and perhaps other traits of interest.

Keywords *Solanum tuberosum* · Phytonutrient · Somatic Embryogenesis · Total Soluble Protein · Late Blight

5.2. Introduction

Russet Burbank, released in 1914, is the most commonly grown potato (*Solanum tuberosum* L.) cultivar in North America (Davis 1992). Despite its popularity for processing, particularly for French fries, only two improved cultivars were found that have been parented or derived from ‘Russet Burbank; ‘AC LR Russet Burbank’ with resistance to potato leaf roll virus (PLRV) (AAFC 1997) and ‘Butte’ with high yield and greater protein content (Pavek et al. 1978). Investigation of somaclones of ‘Russet Burbank’ (NB clone) determined the utility of this approach for improved processing characteristics (Nassar et al. 2009). Other potential characters for screening include nutritional parameters such as protein content and resistance to various diseases.

Selection for either high or low protein content of potato is important for human nutrition. Potato is a source of quality protein and a good menu candidate for low income families and/or people in developing countries, who cannot afford meat (Haase 2008). In contrast, potato with low total soluble protein (TSP) content may also be desirable. For example, low TSP may lead to reduced acrylamide formation in processing (Fitzpatrick and Porter 1966; Vattem and Shetty 2003). Acrylamide forms during potato frying in the presence of free amino

acids (mainly asparagine and glutamine) and reducing sugars (glucose and fructose) as part of the Maillard reaction (Maillard 1986; Mottram et al. 2002; Amerin et al. 2003, 2004; De Wilde et al. 2005).

Apart from genetic transformation, few studies have attempted to alter potato protein quality/quantity. Selection for high protein content was achieved by crossing cultivated *S. tuberosum* and wild species known to have high protein contents (Li and Sayre 1975; Desborough and Lauer 1977; Snyder and Desborough 1978; Veilleux et al. 1981). For example, 4 years of field evaluation of hybrids produced by crossing *S. phureja* and *S. tuberosum* resulted in 4 hybrids with 6 % and one hybrid with 9 % greater protein content than 'Red Pontiac' (Desborough and Lauer 1977). In another study, Snyder and Desborough (1978) evaluated the protein content of four hybrids produced by crossing *S. phureja* x *S. tuberosum* x *S. andigena* and selected for high protein and yield. They found 2 lines with greater protein content than 'Kennebec' and 'Norland'. About 250 lines were produced from each of 4 hybrid populations of *S. phureja* and *S. tuberosum* (Veilleux et al. 1981). The hybrid population derived from the tetraploid high protein selections was greatest in protein content among tested populations. One cultivar, 'Butte', with 25 % 'Russet Burbank' in its pedigree, was released in US in 1977 for high yield and about 20 % greater crude protein content than 'Russet Burbank' (Pavek et al. 1978).

Tissue culture techniques have rarely been employed for potato protein improvement. The methionine (met) levels were increased in 'Russet Burbank' through regeneration of protoclones from protoplast-derived calli grown in the presence of ethionine, an analogue of met (Langille et al. 1998). In 6/48 protoclones, tubers produced significantly increased free met content, up to 2.66 times the control level. This work did not lead to new cultivar release but the incorporation of this useful strategy into potato improvement programs appears to have been neglected.

A plethora of studies have been conducted to improve resistance of potato cultivars to *P. infestans* in recent years with focus on the US-8 strain, which is particularly destructive (Kato et al. 1997; Goodwin et al. 1998; Douches et al. 2001a, b, 2002, and 2004). These involved several approaches to breeding for resistance to *P. infestans*, including genetic modification, cisgenesis, crossing cultivated varieties with wild species (Jacobsen and Schouten 2008), marker-assisted selection (reviewed by Mullins et al. 2006; Regan et al. 2006), and tissue culture techniques (Shepard et al. 1980). Genetically modified potato with resistance to *P. infestans* has been reported. Potato plants with resistance to *P infestans* (race 0) that produce hydrogen peroxide (Wu et al. 1995). Potato plants with resistance to *P infestans* (US-8 A2) that express the antimicrobial protein temporin A (Osusky et al. 2004). Genetically engineered potatoes have not reached the market in North America primarily due to public opposition (Jacobsen and Schouten 2008). Cisgenesis is a transgenic approach which uses natural genes and regulatory elements from the plant species or from crossable species (Schouten et al. 2006; Schubert and Williams 2006). However, this technology still needs refinement for use in plant breeding; it was used to develop *P. infestans* resistance (non-specified strain) in potato (Haverkort et al. 2008; Jacobsen and Schouten 2008) but no new cultivar release has yet ensued.

Somatic hybridization was used in the production of hybrids of *S. bulbocastanum* and *S. tuberosum* that were backcrossed with economically important potato cultivars and field-screened for relative resistance to *P. infestans* (US-8 A2) (Helgeson et al. 1998). Two hybrids were identified with effective resistance to *P. infestans* (US-8 A2) and high yield compared with control ‘Russet Burbank’. Field evaluation of 281 clones from 72 families of a diploid hybrid population of *S. phureja* x *S. stenotomum* resulted in 75 % of clones with significantly greater resistance to *P. infestans* than control ‘Atlantic’ (Haynes and Christ 1999). Similarly, Costanzo et al. (2004), field-evaluated 230 clones of a *S. phureja* x *S. stenotomum* hybrid population and found greater field resistance within 26 clones compared with control ‘Atlantic’, ‘Kennebec’, and ‘Katahdin’. A few cultivars were released with resistance to *P. infestans* (US-8 A2), including

‘Jacqueline Lee’ (Douches et al. 2001b) and ‘Defender’ (Novy et al. 2006). However, no release with resistance to the US-8 strain has been reported from ‘Russet Burbank’ (Staples 2004).

Tissue culture approaches have been used to develop clones with resistance to *P. infestans*. For example, plants were regenerated from callus that showed resistance to filtrate from a *P. infestans* strain, but this did not lead to new cultivar release (Behnke 1979, 1980). Calliclones from resistant callus showed greater resistance than control plants. Among mesophyll-derived protoclones of ‘Russet Burbank’ tested in the field, 3.8 % varied in agronomically important traits including resistance to *P. infestans* (non-specified strain) (Ayers and Shepard 1981). These efforts did not translate to release of a cultivar resistant to *P. infestans.*

Ideally, selection for improved protein content and/or disease resistance should be done on somaclones that were pre-selected for better field performance; mainly yield (Desborough and Lauer 1977). The somatic lines evaluated in this study were “advanced lines” of NB ‘Russet Burbank’ pre-selected for better yield and processing traits as described in Nassar et al. (2008, 2009). From 479 (2005 field season) and 313 (2006 field season) total lines, 26 and 21 advanced lines respectively, were pre-selected. These were tested for protein content each year and again on a subset of 25 lines following the 2007 and 2008 field seasons. The objectives of this study were to determine if advanced somaclonal lines of NB ‘Russet Burbank’ had a) sufficient variation to enable further selection for improvement of protein content or increased relative foliar resistance to late blight (US-8) fungus and b) long-term (3-4 years) protein stability.

5.3. Materials and Methods

5.3.1. Plant Materials

Control plantlets of 'Russet Burbank' (NB clone # 179) were obtained from the Plant Propagation Center, New Brunswick Dept. of Agriculture, Fisheries and Aquaculture (Fredericton, NB, Canada). Potato genotypes tested in this study included NB 'Russet Burbank' and advanced somaclonal lines of NB 'Russet Burbank' pre-selected for better yield components under field conditions and better processing quality traits including reducing sugars and French fry color (Nassar et al. 2008, 2009). A total of 26 and 36 genotypes were evaluated for protein and assessed for disease resistance in 2005 and 2006, respectively. Of these, 25 lines were retested for protein content in 2007 and 2008.

5.3.2. Crude Protein and Total Soluble Protein Estimation

Testing occurred after tuber storage for 5 mo in the dark at 10 C and > 95 % RH. Tubers were separated into skin (periderm), cortex, and pith and then immediately frozen in liquid nitrogen. Tissue samples were collected into 20 ml plastic vials, and freeze-dried at -60 to -70 C for up to 36 h in a freeze-dryer (SNL216V, Savant Instruments Inc. NY, USA). Dried samples were ground to a fine powder in liquid nitrogen and stored in a - 86 C Freezer (Thermo Electron Corporation, OH, USA). Samples were weighed and analyzed for percent nitrogen using a LECO® combustion analyzer (FP-428, LECO Co., MI, USA). Crude protein (CP) was estimated by multiplying percent nitrogen by 6.25 and expressed as mgg^{-1} DW (Narvaez-Vasquez and Ryan 2002).

Total soluble protein (TSP) was measured following the method of Jones et al. (1989). Ten mg of freeze-dried powdered sample was stirred into 1.5 ml of 0.1 M sodium phosphate buffer (pH 7.5) and vortexed for 5 s (Vortex-2 Gene, Scientific Industries Inc., NY, USA). The samples were extracted at 4 C for 30 min, vortexed for 5 s, centrifuged (HN-S Centrifuge, International Equipment Co.,

MA, USA) at 5,000 g for 10 min, and the supernatant was collected for protein analysis. TSP was measured by the Bradford (1976) method using bovine serum albumin (BSA) (BIO-RAD Laboratories, ON, Canada) as a standard. TSP concentration in each separated tuber tissue was calculated as mgg^{-1} DW.

Mean tissue concentration values were used to estimate the amount (g) of CP or TSP in a typical tuber of 100 g FW (g/100 g FW) using conversion factors established for average tuber tissue volumes of control NB 'Russet Burbank' (Ortiz-Medina et al. 2009). For periderm, cortex, and pith, CP or TSP concentrations were multiplied by 0.403, 9.336, and 11.791, respectively. The CP and TSP in a whole tuber of 100 g FW was then estimated by summation of values for pith, cortex, and periderm. These estimates enabled logical comparison of total protein content among tested potato genotypes (Ortiz-Medina et al. 2009).

Inter-seasonal variation in protein content among genotypes was estimated by comparison of % variation between individual somaclones compared with control. Stability of protein content (CP and TSP) was measured by calculating % variation (increase or decrease) from year-to-year, over 3-4 successive years, for each genotype.

5.3.3. Pathogen Production and Inoculation

A culture of *P. infestans* strain US-8 (A2 mating type, isolate No. 1661) was obtained from Dr. H. Platt (AAFC Charlottetown, PEI, Canada) and kept on V8 agar medium in Petri dishes in the fridge (4 C) (Miller 1955). Fungal mycelial plugs (1-cm-diameter) from the source culture were transferred into Petri dishes and incubated at 15 C in the dark. Fifteen d prior to inoculation, a fungal suspension was prepared in autoclaved double distilled water then filtered through a double layer of sterile Miracloth of pore size 22–25 µm (Calbiochem Co., CA, USA). The concentration of spores in the inoculum suspension was adjusted to 25-30 × 10^4 sporangia ml^{-1} using a haemocytometer.

In vitro plantlets of NB 'Russet Burbank' and advanced selections were grown individually in 25-cm- diameter pots contain PRO-Mix BX$^{(R)}$ (Premier Horticulture Ltd., QC, Canada). Plants were irrigated, as needed, and fertilized weekly with 1.5 gl^{-1} of Plant-Prod$^{(R)}$ 20-20-20 (Plant Products Co. ON, Canada). After 5 wk, five leaflets were inoculated per plant. Twin spots (10 µl of spore suspension containing 0.02 % Tween 80 or distilled water with 0.02 % Tween 80) were placed in the middle of the leaflet on each side of the mid-vein on the abaxial surface. Plants were misted with distilled water and covered with plastic bags (50 × 80 cm) for 24 h. Lesion length and width were measured 2, 4, and 6 d post inoculation to calculate lesion area (A = 0.25 × length × width × π) (Vleeshouwers et al. 1999). The lesion area over time was used to calculate the area under the disease progress curve (AUDPC) (Shaner and Finney 1977):

$$AUDPC = \sum_{i}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} + t_i)$$

where

i is the number of the individual observation (i = 1 – n)

n is the total number of observations.

t is time after inoculation (days),

y is the disease severity,

5.3.4. Experimental Design and Statistical Analysis

The experiment was designed as a factorial completely randomized design with genotypes as main factors. Experimental unit was an individual somalcone regenerated from different tissue sources.Four separate experiments were carried out over a 4-year period. Each experiment was conducted as a randomized complete design, consisting of genotypes as main treatments (26,

36, 25, and 25 genotypes in 2005, 2006, 2007, and 2008 field seasons, respectively) with three replications. For each genotype, CP and TSP were estimated for 3-4 years while AUDPC was determined for 2006 and 2007. For protein analyses, each experimental unit consisted of three tubers. The data on CP and TSP for different number of genotypes for each year were separately analyzed based on ANOVA, using the Statistical Analysis System (SAS 2007). Means were compared using Duncan's New Multiple Comparison Test ($P \leq 0.05$) to compare results of somaclones individually with results of control plants. Pearson correlation coefficients were calculated for CP and TSP using the CORR procedure of SAS. Inter-seasonal variation in CP and TSP contents of select clones for 3-4 years were tested using the ANOVA procedure and compared with control 'Russet Burbank' using Duncan's New Multiple Comparison Test ($P \leq 0.05$).

The experimental unit for disease severity assessment consisted of 30 inoculation sites, from 3 plants. The disease severity was assessed as lesion area and AUDPC. The data on AUDPC for different genotypes, separately for 2 years, was analyzed using the GLM procedure of SAS. Means of AUDPC were compared using Duncan's New Multiple Comparison Test ($P \leq 0.05$) in SAS (2007).

5.4. Results & Discussion

5.4.1. Crude and Total Soluble Protein Concentrations

Control plantlets (RBP) and seed tubers (RBS) showed essentially the same results each year, and varied inter-seasonally in the same way (Figs. 5.1, 5.2). For selection purposes, protein content of tubers from somaclonal lines were compared with that of tubers from control plantlets.

Mean estimated values for tuber CP and TSP content (g/100 g FW) over four growing seasons are shown in Fig. 5.1. There were no differences between

advanced somaclones and controls in estimated CP content in the first 2 years (Fig. 5.1, upper). However, FC2006 had significantly greater CP content than plantlet controls in both year-3 (24.6 %) and -4 (20.2 %). One of 25 lines showed increased CP content over control in year-3 only (FC1106) while 10/25 lines had increased CP content over controls in year-4 only.

Generally, CP values varied inter-seasonally for each tested genotype; a 25 % increase was apparent in year-3 compared with year-2 and a 50 % decrease occurred in year-4 compared with year-3 (Fig. 5.2, upper). CP values of tubers of somaclonal lines varied inter-seasonally compared with the control RBP. Percentages ranged from 53 to -13 %, 71 to 13 %, and -26 to -64 % in year-2 compared with year-1, year-3 compared with year-2, and year-4 compared with year-3, respectively.

In the first year, no differences were noted in TSP between genotypes and control plantlets (Fig. 5.1, lower). In the second year, most somaclones (22/36) had significantly lesser TSP compared with controls. In the third year, only 1/25 somaclones (MC405) had significantly greater TSP (39.6 % greater than control) while 8 somaclones had significantly lesser tuber TSP contents compared with control plantlets (1.82 ± 0.15 g/100g FW) and the rest were similar to controls. In year-4, 8/25 lines had significantly greater TSP than controls. None of these led in protein content in the previous years and 3/8 of these had significantly less protein in the previous year. FC2006, the most outstanding line for CP was high in TSP in year-4, and was significantly less than control in years 2 and 3. FP1106, also of interest for CP, was similar in year-4, significantly greater in year-3, and lesser than control in year-2. TSP values for tubers of FP3405, MP11505, and MP18405 varied inter-seasonally compared with TSP values of plantlet controls (Fig. 5.2, lower).

As with CP results, TSP content of tested genotypes generally varied inter-seasonally (Fig. 5.2). Inter-seasonal variation between control plantlets ranged greatly from + 25 to – 50 % and +34 to -41 % in CP (Fig. 5.2, upper) and TSP

(Fig. 5.2, lower) content, respectively, over the 4 years of this study. Similarly, somaclones varied inter-seasonally in CP (12/25) and TSP (5/25) contents. Inter-seasonal variation in protein content could result from field location (Li and Sayre 1975; Hunnius et al. 1976), climatic and micro-climatic effects, cultivation practices (Rexen 1976; Hoff et al. 1978), and varietal differences (Hunnius et al. 1976; Woolfe 1987).

Despite inter-seasonal variation in CP content, in year-3 and -4, FC2006 exceeded the protein content of 'Butte', a registered cultivar with 20 % more crude protein than 'Russet Burbank' (Pavek et al. 1978). It is unknown to what extent 'Butte" might vary in protein content inter-seasonally. This underlines the potential utility of somaclonal selection for phytonutrient improvement but also illustrates the difficulty of identifying superior lines in the context of inter-seasonal variation. Variation in somatic lines could reflect genetic or epigenetic variation (Kaeppler et al. 2000; Joyce and Cassells 2002; Li et al. 2009) but this has not been investigated for protein content. Characterization of these proteins is important in relation to potential processing features. Techniques such as SDS-PAGE (2-D) or proteomic analysis could be used (Delaplace et al. 2006). Characterization of these lines could be done using chromosomal cytometry, single nucleotide polymorphism, DNA methylation, or chromatin changes (Springer and Kaeppler 2008). Results support previous conclusions that regeneration of somaclones can be done randomly from any tuber tissue (Nassar et al. 2009). Based on tissue origin, lines with desirable protein content (significantly low or high) ranged from 1.1-1.7 % of all tested somaclones (approx. 800); 1/60, 3/275, and 5/475 of periderm-, cortex-, and pith-derived somaclones, respectively.

5.4.2. Relative Resistance to *P. infestans*

The disease severity assessed as AUDPC for somaclones tested here is presented in Table 5.1. Most advanced somaclones expressed similar disease resistance to control plantlets. In 2006, 2/26 somaclones were less resistant than

control plantlets. In 2007, most somaclones (14/36) had similar disease resistance to the control cultivar. High variation in disease resistance was noted among tested somaclones. This was also reported by Stewart et al. (1983) and Dorrance and Inglis (1997) who evaluated *P. infestans* resistance of somaclones in the greenhouse. Statistical analyses for both trials of *P. infestans* revealed a high coefficient of variation and low R-square values. This could be attributed to greenhouse conditions and/or other causes but it was clear that the greenhouse was not ideal for this work, as noted by others (Vleeshouwers et al. 1999; Kirk et al. 2001; Douches et al. 2002). However, intraclones MP19805, FP3405, and MC10605 had consistently higher AUDPC values (Table 5.1). These may be of interest to researchers studying late blight resistance using potato mutants. Further work is required to reassess advanced somaclones with similar disease responses to control NB 'Russet Burbank' for relative tuber and foliar resistance to *P. infestans*, both A1 and A2 mating types, in the field.

5.5. Conclusions

This is the first study to show sufficient variation among somaclones to permit screening for lines with altered tuber protein content and less inter-seasonal variation than control plants. Most somaclones showed similar *P. infestans* foliar resistance to the control cultivar NB Russet Burbank in the greenhouse but plants and tubers should be further evaluated in the field. Somatic embryogenesis technology appears to have the potential to significantly improve NB 'Russet Burbank' and could be integrated into potato improvement programs. This selection technique has the potential to reduce the time for release of new cultivars since it is based on selection following identification of lines with improved yield and processing traits.

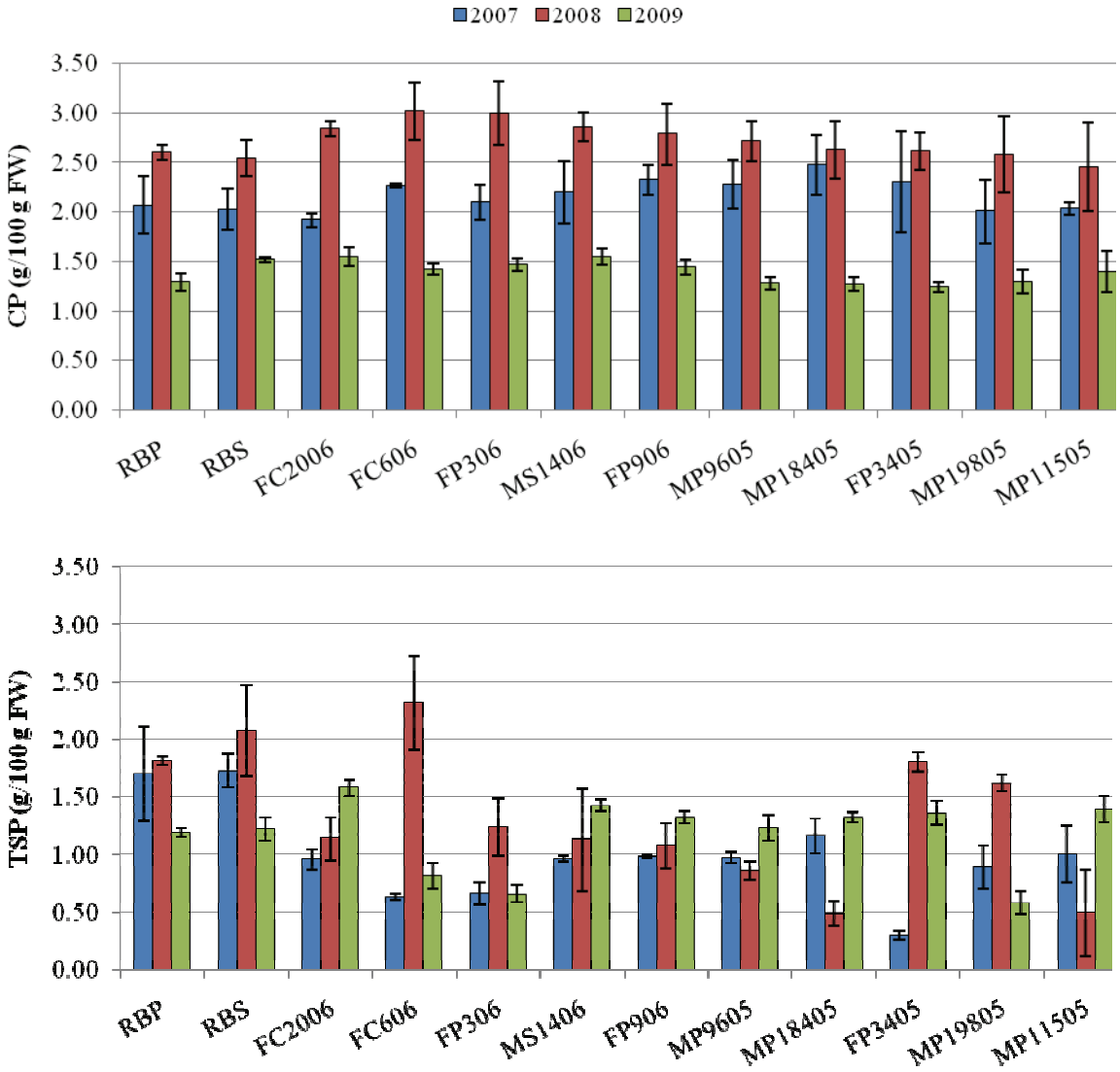

Fig. 5.1. Crude protein (CP; upper) and total soluble protein (TSP; lower) content of a sub-set of advanced somaclones. Mean estimates for CP and TSP content based on a tuber of 100 g FW (g/100g FW) for control cultivar NB 'Russet Burbank', both plantlet-derived (RBP) and seed tuber-derived (RBS), and advanced somaclones. Protein testing was done in 2006-2009 on stored tubers that had been field-grown during the 2005 (n = 26 selected from 475 produced clonal population), 2006 (n = 21 selected from 313 somaclones plus 15 somaclones selected from previous year), 2007, and 2008 (n = 25) growing seasons, respectively. Means were compared using Duncan's New Multiple Range Test ($P \leq 0.05$).

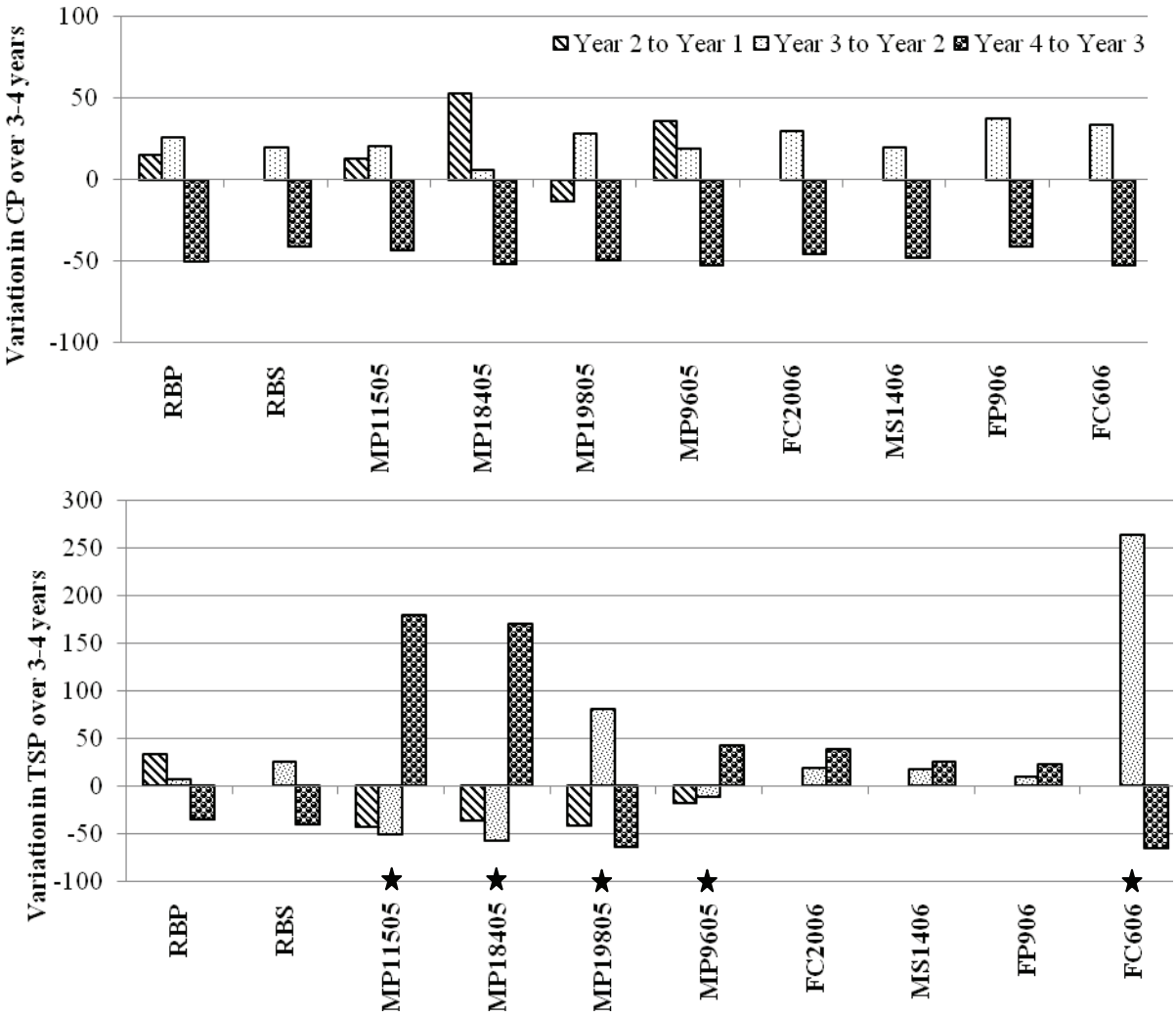

Fig. 5.2. Positive and negative seasonal somaclonal variation estimated for 100 g FW tubers of a sub-set of selected somaclones based on yield and processing quality traits. Starred-somaclones (★) had significantly greater or lesser seasonal variation for crude protein (CP; upper) and total soluble protein (TSP; lower) content compared with control plantlet-derived NB 'Russet Burbank' (RBP) for one or more years. Testing was done in 2006 - 2009 on stored tubers of advanced somaclones that had been selected for yield and processing traits during the 2005 (n = 26 selected from a population of 497 somaclones), 2006 (n = 36; 21 selected from a population of 313 somaclones plus 15 lines tested in 2005), 2007 (n = 25), and 2008 (n = 25) growing seasons, respectively. Means were compared using Duncan's New Multiple Range Test ($P \leq 0.05$).

Table 5.1. Relative disease resistance. Disease severity assessed as lesion area on leaves of potato genotypes; control NB 'Russet Burbank' and advanced somaclones, selected from the 2005 and 2006 field seasons, tested in the greenhouse in 2006 and 2007, respectively. Genotypes were inoculated with *Phytophthora infestans* (US-8) and areas under the disease progress curve (AUDPC) were calculated using the lesion areas (cm^2) 2, 4, and 6 days after inoculation. Genotypes were ranked according to disease severity results in 2007.

Genotype	AUDPC	
	2006	2007
'Russet Burbank'	6.40^{l-p}	6.66^{f}
MS906	NT	6.58^{f}
FP2106	NT	6.79^{f}
MS1406	NT	7.38^{e-f}
FP106	NT	8.38^{d-f}
MP19805	9.77^{h-m}	8.46^{d-f}
FC2806	NT	9.00^{c-f}
FP806	NT	9.85^{b-f}
MP405	5.47^{m-p}	9.93^{d-f}
FP2906	NT	10.19^{b-f}
MP11505	8.00^{i-o}	10.38^{b-f}
FC606	NT	10.81^{b-f}
MP9605	17.43^{b}	10.96^{bf}
MP7405	2.40^{p}	11.02^{a-f}
MC405	7.93^{j-o}	11.47^{a-f}
FP306	NT	11.86^{a-e}
FP3405	10.66^{g-l}	11.90^{a-e}
MC10605	12.10^{c-k}	12.05^{a-e}
FP8106	NT	12.08^{a-e}
FC2006	NT	12.51^{a-d}
MP18405	3.94^{o-p}	12.81^{a-d}
MP706	NT	13.89^{a-c}
FC1106	NT	13.90^{a-c}
MC1606	NT	14.17^{a-b}
FP906	NT	14.37^{a-b}

NT: not tested. Means were compared using Duncan's New Multiple Range Test ($P \leq 0.05$).

CHAPTER VI

SUMMARY, CONCLUSION, AND SUGGESTIONS FOR FUTURE RESEARCH

6.1. General Summary and Conclusion

'Russet Burbank' is the most important potato in North America particularly as a French fry cultivar. It is also the most studied potato cultivar worldwide; numbers of hits were 6,480, 5,725, 1,242, 977, 428, and 302 found by Google Scholar, Scirus, CABI Abstracts, BIOSIS Previews, Agricola, and ISI Web of Knowledge search engines, respectively, as of April 2009. 'Russet Burbank' was described as the perfect model for a periclinal chimeral structure since its discovery in a field of 'Burbank' about 100 years ago (PAA 2008). We tested the current periclinal chimeral status of the NB clone of 'Russet Burbank' and the possibility of improving its agronomic, processing quality, nutritional (protein content), and anti-pathogenic (resistance to late blight disease) traits. This study is presented as three manuscripts published or submitted to scientific journals. A schematic representing an overview of the thesis research is presented as Fig. 6.1.

A novel chimeral disassembly approach was used to separate the three histogenic layers of the tuber using derivative tissue (periderm, cortex, and pith). Intraclones were a term we used to describe somaclones regenerated from specific tuber source tissue. Intraclones were produced using a two-step somatic embryogenesis technique, micropropagated, acclimatized in the greenhouse, hardened-off for field-planting, shipped to N.B, and field-evaluated at the research farm of McCain Foods Canada Ltd. (Florenceville, NB). 2004-regenerated intraclones (~ 500) were field-tested for 3 consecutive years and 2005-regenerated intraclones were field-evaluated for 2 consecutive years.

Chapter III describes the current periclinal chimeral status of NB 'Russet Burbank'. Tuber periderm (skin) colour was used as a determinant of chimeral

status of each field-grown intraclone. The tubers of intraclones derived from all histogenic layers were almost all russet, which indicated that the genes for russet periderm were located in all three histogenic layers of the shoot (stolon) meristems. No significant difference related to explant source tissue was found. Frequencies of russet and patchy periderm were 84-88 % and 9-14 %, respectively, (2005) and 79-91 % and 8-21 %, respectively (2006). Only 0-4 % of all intraclones showed non-russet (putative wild-type/'Burbank'-type) periderm. Furthermore, among periderm-derived intraclones, where 100 % of the intraclones were expected to have tubers with russet periderm, 91 % were russet, 9 % were patchy, and 0 % were non-russet. Patchy periderm could result from cell mix-up between histogenic layers or gene expression changes of russet-involved genes. Perhaps 'Russet Burbank' was originally organized in a periclinal chimeral manner. Results show clearly that NB 'Russet Burbank' is not currently organized in a periclinal chimeral arrangement for an L_1 and/or L_2, mutation of 'Burbank'. Dr. X.Q. Li's lab (Potato Research Centre, Fredericton, NB) was unable to distinguish 'Russet Burbank' from 'Burbank' control plantlets using multiplex PCR; state-of-the-art genetic fingerprint technology (Li et al. 2008, personal communication). However, we do believe our somaclones can be distinctly fingerprinted with molecular technology (for more details see p. 25-26).

Currently, it is clear that 'Russet Burbank' is not a periclinal chimera. Does this finding apply to any other potato cultivars with putative periclinal chimera status? Based on a greenhouse experiment, 'Red Gold' was described as a putative "uncovered" L_2 periclinal chimera (Red-Gold-Red) (Ortiz-Medina 2006). To address that question, somatic regenerants from specific source tissues of 'Red Gold' were produced and field-evaluated (in 2008) as described for 'Russet Burbank' in Chapter III. Results are presented in Appendix to Chapter III. Micropropagated control plants and explants derived from specific source tissues produced pinkish-red tubers (83 %), but occasionally produced gold tubers (17 %) (Table A1.1 and Fig. A1.1). These results disagree with the periclinal chimeral structure proposed by Ortiz-Medina (2006). No current periclinal chimeral potato construct is known. Molecular genetics and epigenetic work could be used to

clarify the genetic constitution of both ‘Red Gold’ and ‘Russet Burbank’ and to more rigorously explore chimeral theory.

Following each field season, the best performing intraclones, based on yield (type, tuber weight), and processing (% glucose, French fry colour) quality traits compared with control ‘Russet Burbank’, were selected, and results were presented in Chapter IV. Processing quality characteristics were estimated for tubers of selected intraclones after a period of storage of 5 mo. or longer. Frequencies of useful variants for processing criteria among the selected intraclones regenerated from different tuber sources or specific tuber tissue were the same. This suggested that somaclones could be regenerated randomly from any part of the tuber in the future. Average tuber yield components showed no differences between control NB ‘Russet Burbank’ plantlet-derived and intraclone-derived plants. Overall, 2-9 % of intraclones had superior processing quality traits compared with control ‘Russet Burbank’ NB clone. McGill optioned two intraclones to local industry for further evaluation for yield and processing quality characteristics. Significant positive or negative variations among approx. 800 intraclones compared with control plantlet-derived NB ‘Russet Burbank’ was 7.9-10.5 % for all tested agronomic and processing quality traits during 2 years of field trails.

During early years of selection, field evaluation of somaclones as two or more replicates 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). However, increasing the number of replicates per clone is generally more labor- and time-consuming, and requires more land area if the same number of lines are to be evaluated. Based on our field experience, it is recommended to evaluate intraclones as single plants in the first year followed by increased replicates in subsequent years. This will allow the evaluation of greater numbers of intraclones since selection of improved intraclones is a “number’s game”. Complete field results are presented in Appendix to Chapter IV.

Chapter V describes results of protein content (crude protein (CP) and total soluble protein (TSP)) and the relative foliar resistance to late blight disease (strain US-8) of “advanced” somaclones (pre-selected for yield and processing characteristics). Tubers of advanced somaclones were tested for crude protein (CP) and total soluble protein (TSP) content, after 5 months storage. Resistance to P. infestans (US-8 strain, A2 mating type) was measured based on ex vitro plantlet foliar assessment in the greenhouse for 2 years. There was substantial inter-seasonal differences in protein content among control plants. This is the first study to show sufficient variation among somaclones to permit screening for altered tuber protein content. Curiously, some of these selections had less inter-seasonal variation in protein level. Advanced somaclones had less variation (approx. 0-5 %) for CP and and greater variation (12-24 %) for TSP content.One advanced somaclone had significantly greater CP content than control NB ‘Russet Burbank’ for 2 consecutive years. One advanced somaclone had significantly lesser TSP content for 3 consecutive years and 5 somaclones showed reduced TSP content for 2 consecutive years compared with controls. Greenhouse studies showed similar *P. infestans* disease resistance in somaclones compared with control NB ‘Russet Burbank’. Further study is required to compare foliar and tuber resistance of somaclones to *P. infestans* in the field. Advanced somaclones are sufficiently variable to permit screening for improved protein, and perhaps other traits of interest.

Somatic embryogenesis technology generated variant plants with positive attributes for processing quality, TSP, and relative resistance (greenhouse) to *P. infestans* traits. Field-based somaclonal selection of NB ‘Russet Burbank’ has the potential to significantly improve this cultivar for other possible (hitherto untested) characters. These results underline the importance of somatic embryogenesis in potato improvement programs.

6.2. Suggestions for Future Research

1. Further field evaluation of advanced somaclones should be conducted. A list of advanced somaclones identified with each useful attribute in Table A.2.6 have been kept as in vitro plantlets and are available to others for continued testing or licensing.
    a. Multi-location field trial should be conducted for advanced intraclones with better processing features.
    b. Acrylamide levels should be measured for selected somaclones with superior processing quality traits.
    c. Advanced intraclones with elevated crude protein or reduced total soluble protein should be fully characterized for protein profile using SDS-PAGE (1D or 2D) or proteomic analysis techniques.
    d. Advanced intraclones should be field-tested for *P. infestans* US-8 resistance.
    e. Advanced somaclones that were selected with improved processing, protein, and/or diseases resistance should be tested for other useful traits.
2. The mechanism behind the somaclonal variation is still not fully understood. Epigenetic variation could be one mechanism of somaclonal variation. Advanced somaclones should be discriminated using modern molecular tools, chromosomal cytometry, single nucleotide polymorphism, DNA methylation, or chromatin alteration.
3. As current technology that was used in this thesis has the potential to significantly reduce the time required to release new cultivars, large numbers of somaclones should be produced from any tuber tissue of ‘Russet Burbank’ or ‘Burbank’ and screened in the field for improved yield, processing, nutrients, anti-pathogenic, and other traits in an attempt to produce newly improved ‘Russet Burbank’.
4. NB ‘Russet Burbank’ is not currently arranged in a periclinal chimeral manner. Molecular genetics work is required for identification of the russet gene(s) and their location(s) and expression pattern(s) within the histogenic layers of the potato tuber. Identification of the russet gene(s) of ‘Russet Burbank’ will better

help understand chimeral theory as it applies to this potato cultivar and potato in general.

5. Chimeral structure of the eleven clones of ‘Russet Burbank’ located in North America should be studied to confirm the thesis findings.
6. Chimeral theory examines the origin of mutation incorporation into shoot growing points (meristems) (Howard, 1959; Derman, 1960; Marcotrigiano, 1990). This theory of reported chimeral potato cultivars should be re-examined using molecular technology.

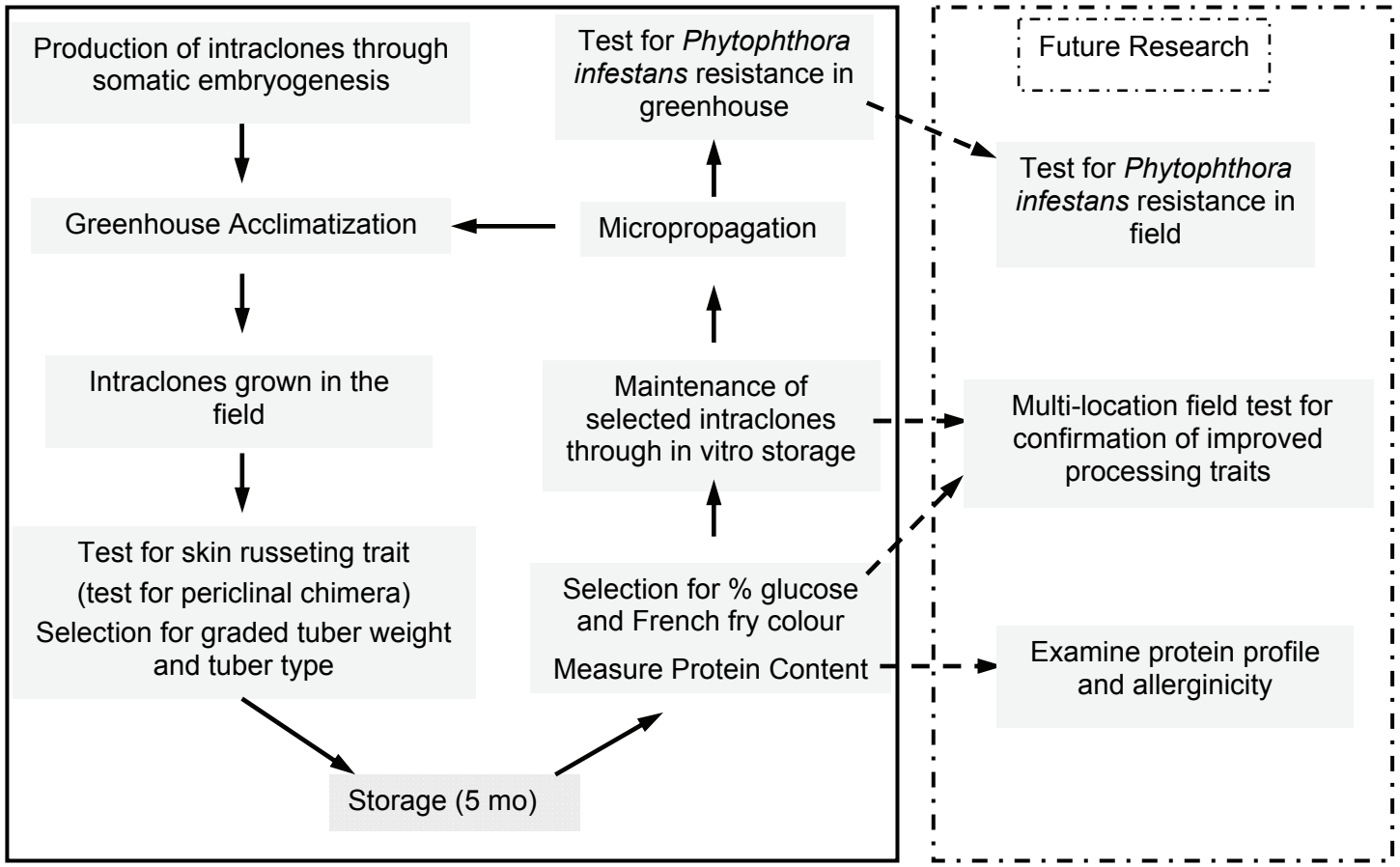

Fig. 6.1. Step-wise flow chart illustrates thesis work. Firstly, somatic embryos (intraclones) were produced in vitro from explants that were derived from specific tuber tissues (periderm, cortex, pith) of microtubers or field-grown tubers. Intraclones were increased through micropropagation for the purpose of storage in vitro and evaluation in the field. Intraclones were field-grown as 1 plantlet (season 1), two plantlets (season 2) and put into the field in increased numbers in seasons 2 and 3. Secondly, harvest and first

selection for improved French fry processing quality was based on graded tuber weight. Following storage of 5 mo or longer, the next selection was based on % glucose and French fry colour. The best intraclones 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 the in vitro storage. Thirdly, intraclones superior in processing characteristics were tested for protein content and fourthly, these intraclones were tested for late blight (strain US-8/A2 mating type) disease resistance in the greenhouse.

CHAPTER VII

CONTRIBUTIONS TO KNOWLEDGE

The following scientific contributions to knowledge can be considered as original in this thesis:

1. Regarding the chimeral structure of potato, an original application of somaclonal technology was used to address the periclinal chimera constitution of 'Russet Burbank', the classical example of an L_1 periclinal chimera, and the putative L_2 periclinal chimera 'Red Gold'. NB 'Russet Burbank' and 'Red Gold' are not currently organized as periclinal chimeras.
2. This was the first study to investigate the use of somatic embryogenesis strategy to produce useful somaclonal variation for potato improvement. It was clear that somatic embryogenesis technology (Figs. 4.1, 6.1) has the potential to generate useful variation for improvement of yield and processing (reducing sugars and French fry) characteristics of NB 'Russet Burbank'.
    a. Two advanced somaclones with improved % glucose and French fry colour after 5 mo storage, have been optioned to potato processing industry (McCain Foods Ltd.).
    b. One somaclone was identified with elevated crude protein content and 8 somaclones were selected with reduced total soluble protein content.
    c. Fourteen somaclones exhibited similar disease resistance to *P. infestans* compared with the control.
3. For agronomic purposes, regeneration of somaclones can be done randomly from any tuber tissue (internal tissues are more regenerative than periderm) or from any tuber source (in vitro-grown tubers (microtubers) or field grown tubers). Fig. 4.1 describes an original schematic for generation and testing of potato somaclones.

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APPENDICES

Appendix 1. Supplement to Chapter III - Investigation of putative periclinal chimeral status of 'Red Gold'

This appendix contains one table and one figure supplementary to Chapter III, Nassar, A.M.K., E. Ortiz-Medina, Y. Leclerc, and D.J. Donnelly. 2008. Periclinal chimeral status of New Brunswick 'Russet Burbank' Potato. American Journal of Potato Research 85: 432-437. Results were not included in Chapter III because field-evaluation of intraclones of 'Red Gold' were done after manuscript submission.

'Red Gold' was the product of a collaborative breeding program between Agriculture and Agri-Food Canada (AAFC, Guelph, ON), the University of Guelph, and the Ontario Ministry of Agriculture and Food, (OMAFRA, Simcoe, ON) (Coffin et al. 1988; CFIA 2009). 'Red Gold' was a seedling selection from the cross (G68211 (periderm and flesh colour not described) x G6521-4RY (red periderm and yellow flesh)) made in 1970. It was registered in Canada in 1987 as a mid-season cultivar primarily for fresh market use. Tubers of 'Red Gold' are round to oval with a pinkish-red skin and a bright yellow flesh. Micropropagated 'Red Gold' plants were obtained from The Potato Gene Resources Repository, Agriculture and Agri-Food Canada, Potato Research Centre, Fredericton, NB, Canada.

When it became clear that 'Russet Burbank' was not a periclinal chimera, it became important to test other known examples of periclinal chimeras. On the basis of greenhouse pot trials, in which cortex-derived intraclones yielded tubers with gold periderm, and pith-derived intraclones yielded tubers with pinkish-red periderm, 'Red Gold' was described as a putative "uncovered" L_2 periclinal chimera (Red-Gold-Red) (Ortiz-Medina 2006). To further investigate this putative chimera, somatic regenerants from specific source tissues of 'Red Gold' were produced and field-evaluated (in 2008) as described for 'Russet Burbank' in

Chapter III. In addition to explants from cortex and pith of microtubers, root explants were taken from control plantlets. Regenerants from root tissue are believed to be derived from L_1 histogenic tissue (Baurle and Laux 2003) and were expected to share periderm characteristics with pith-derived explants. At harvest, intraclone plant tubers were bagged, tagged, and photographed for periderm evaluation.

Field results of control plants and intraclones of 'Red Gold' are described in Table A.1.1 and Fig. A.1.1. Curiously, control plants produced tubers with either all pinkish-red or all gold periderm (Fig. A.1.1. A, B). Most plants regenerated from pith and roots produced tubers with pinkish-red periderm but, as in the control group, a smaller number produced tubers with gold periderm. Plants regenerated from the cortex produced tubers that were all pinkish-red, contrary to what was seen in our preliminary greenhouse pot trials. Based on current field results (Table A.1.1.), it is apparent that micropropagated control plants and explants derived from specific source tissues produce pinkish-red tubers most of the time (83 %), but occasionally produce gold tubers (17 %). These results contradict the periclinal chimeral structure proposed by Ortiz-Medina (2006). No current periclinal chimeral potato construct is known. It is clear that histogenic theory may need revision; this awaits molecular tools that can address this issue. Molecular genetics work is required to clarify the genetic constitution of both 'Red Gold' and 'Russet Burbank'.

Table A.1.1. Field plants were established from micropropagated control plantlets of 'Red Gold' (control) or intraclones of 'Red Gold' derived from specific microtuber tissue (cortex or pith), or root segments of control plantlets. Field tuber periderm phenotype (Pinkish-Red = the entire surface was pinkish red or Gold = the entire surface was gold).

Control/Explant Source Tissue	Number of Plants/Intraclones			
	Planted	Harvested	Periderm	
			Pinkish- red	Gold
control plantlet	10	6	4 (66.7 %)	2 (33.3 %)
control plantlet root	69	27	23 (85.2 %)	4 (14.8 %)
microtuber cortex	8	4	4 (100. %)	0 (00.0 %)
microtuber pith	53	28	23 (82.1 %)	5 (17.9 %)
Total number	140	65	54 (83.0 %)	11 (17.0 %)

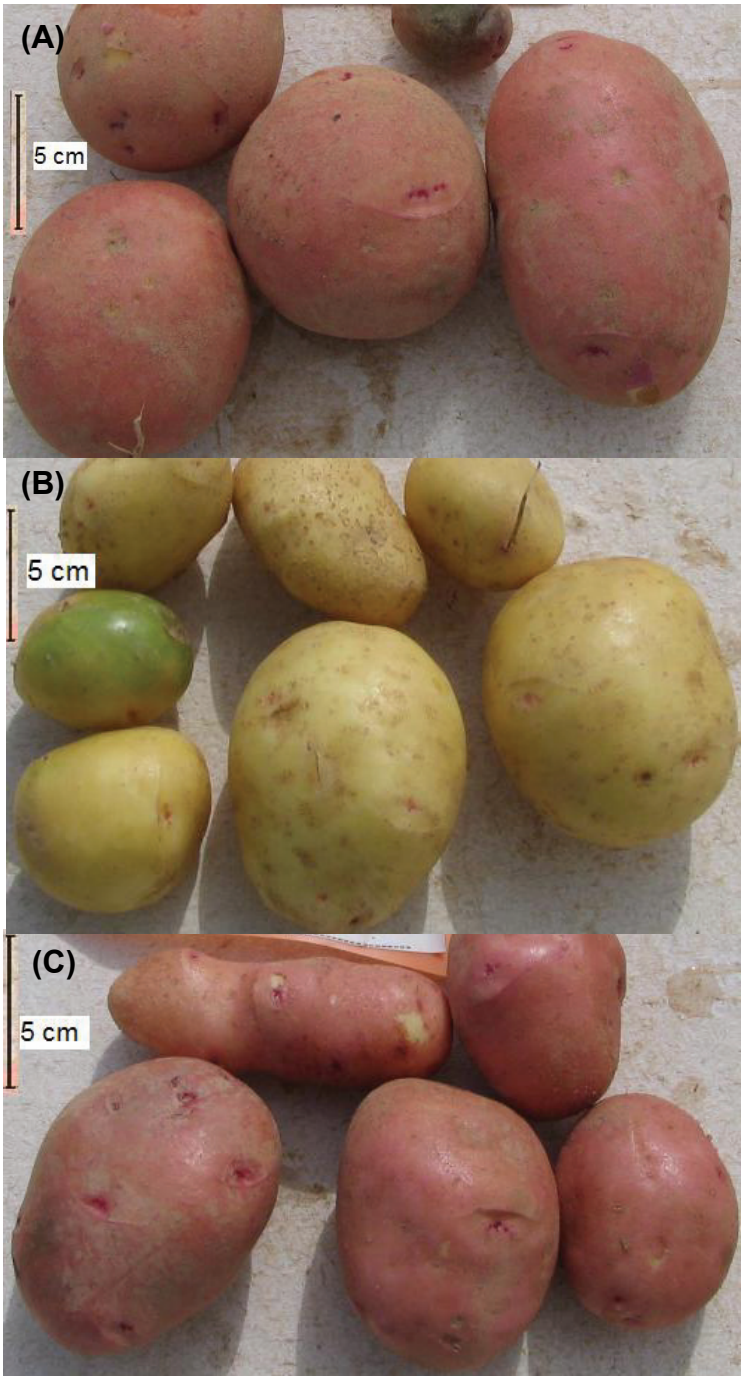

Figure A.1.1. Field-tested control plants and/or intraclones of ‘Red Gold’: (A) Control plant tubers with pinkish-red periderm, (B) Control plant tubers, root- or pith-derived intraclones with gold periderm, and (C) Root, pith- or cortex-derived intraclones with pinkish-red periderm.

Appendix 2. Supplement to Chapter IV – Data Tables and Figures

This appendix contains 18 tables and 3 figures supplementary to Chapter IV that, in the interests of space, were not included in the manuscript "Nassar, A.M.K., J. Abdulnour, Y. Leclerc, X-Q. Li, and D.J. Donnelly. Intraclonal Selection for Improved Processing of NB 'Russet Burbank' Potato. Submitted to the European Journal of Potato Research.

Table A.2.1. Total number of field-grown (2005 and 2006) control NB 'Russet Burbank' (plantlets and seed tubers) and Burbank (plantlets) and intraclone (plantlets) regenerated from microtuber or field-grown tuber tissues (periderm, cortex, or pith).

Tuber Source	Explant Source	Total Number of Field-Grown Plants*	
		2005 Season	2006 Season
Controls			
Russet Burbank	Plantlets	40	10
Russet Burbank	Seed tubers	0	7
Burbank	Plantlets	20	10
Intraclones			
Microtuber	Periderm (skin)	0	60
	Cortex	115	59
	Pith	219	28
Field tuber	Cortex	37	62
	Pith	124	92
Intraclone Total		495	301

*Intraclones were field-tested in the 2005 season as 1 plantlet (single hill) and in the 2006 season as 2 plantlets (duplicate hills).

Table A.2.2. Yield and processing results for selected-intraclones from the 2005 field season. Intraclones derived from 'Russet Burbank' pith (microtuber (MP) or field-grown tuber (FP)) or cortex (microtuber (MC)) explants were compared with control 'Russet Burbank' (RBP) and 'Burbank' (B). Data were arranged based on % glucose from the lowest to the highest value.

Controls / Intraclones	Tuber Number		Tuber Weight (Kg)		ATW[1] (Kg)	% Glucose	Sucrose (mgg^{-1})	Specific Gravity	FF[2]
	Total	Graded	Total	Graded					
RBP	13.2^{d-i}	6.4^{b-e}	1.74^{a-e}	1.36^{abc}	0.21^{b}	0.142^{a-d}	0.731^{abc}	1.085^{ab}	78
B	23.1^{b-g}	9.3^{a-e}	2.46^{a-e}	1.76^{a-d}	0.21^{b}	0.136^{a-e}	0.689^{abc}	1.082^{ab}	84
MP13905	11.0^{e-i}	9.0^{a-e}	1.64^{b-e}	1.49^{abc}	0.17^{b}	0.053^{f}	0.817^{abc}	1.081^{ab}	NA
MP17205	6.0^{ghi}	$4.0d^{e}$	1.26^{e}	1.08^{cd}	0.27^{ab}	0.065^{ef}	0.809^{abc}	1.087^{ab}	NA
MP18405	29.0^{a-d}	13.0^{abc}	3.37^{a-c}	2.29^{abc}	0.18^{b}	0.070^{ef}	0.874^{abc}	1.091^{ab}	98
MP5605	4.0^{i}	4.0^{de}	1.88^{a-e}	1.88^{a-d}	0.47^{a}	0.071^{def}	0.711^{abc}	1.083^{ab}	NA
FP9405	19.0^{c-i}	14.0^{ab}	3.41^{ab}	3.22^{a}	0.23^{b}	0.080^{c-f}	0.931^{abc}	1.087^{ab}	92
MP19805	15.0^{c-i}	8.0^{a-e}	2.59^{a-e}	2.21^{abc}	0.28^{ab}	0.080^{c-f}	0.882^{abc}	1.088^{ab}	NA
FP3405	40.0^{a}	14.0^{ab}	3.53^{a}	2.47^{abc}	0.18^{b}	0.087^{b-f}	0.645^{abc}	1.084^{ab}	96
FP9305	11.0^{e-i}	10.0^{a-e}	1.44^{de}	1.38^{bcd}	0.14^{b}	0.089^{b-f}	0.768^{abc}	1.084^{ab}	81
MC405	13.0^{d-i}	5.0^{cde}	1.95^{a-e}	1.52^{bcd}	0.30^{ab}	0.097^{a-f}	1.217^{a}	1.098^{a}	84
MC8905	22.0^{b-h}	7.0^{b-e}	1.86^{a-e}	1.00^{cd}	0.14^{b}	0.097^{a-f}	1.062^{ab}	1.096^{ab}	77
MP405	28.0^{a-e}	3.0^{e}	1.66^{b-e}	0.50^{d}	0.17^{b}	0.097^{a-f}	0.662^{abc}	1.091^{ab}	105
MP8205	19.0^{c-i}	10.0^{a-e}	2.29^{a-e}	1.76^{a-d}	0.18^{b}	0.098^{a-f}	0.964^{abc}	1.077^{b}	NA
MP14605	14.0^{c-i}	5.0^{cde}	1.61^{b-e}	0.89^{cd}	0.18^{b}	0.100^{a-f}	0.221^{c}	1.082^{ab}	NA
MC11205	19.0^{c-i}	11.0^{a-e}	2.41^{a-e}	2.16^{abc}	0.20^{b}	0.101^{a-f}	0.915^{abc}	1.097^{ab}	96
MP11505	18.0^{c-i}	9.0^{a-e}	2.30^{a-e}	1.74^{a-d}	0.19^{b}	0.107^{a-f}	1.062^{ab}	1.088^{ab}	NA
FP1205	22.0^{b-h}	7.0^{b-e}	2.45^{a-e}	1.12^{cd}	0.16^{b}	0.109^{a-f}	0.621^{abc}	1.082^{ab}	81
MC505	31.0^{a-c}	16.0^{a}	3.21^{a-d}	2.74^{ab}	0.17^{b}	0.111^{a-f}	0.596^{abc}	1.092^{ab}	64
MP11205	14.0^{c-i}	10.0^{a-e}	2.30^{a-e}	2.05^{a-d}	0.21^{b}	0.111^{a-f}	0.441^{bc}	1.082^{ab}	84
MP8805	7.0^{ghi}	6.0^{b-e}	1.45^{de}	1.39^{bcd}	0.23^{b}	0.116^{a-f}	0.882^{abc}	1.093^{ab}	97
MP19405	18.0^{c-i}	9.0^{a-e}	2.31^{a-e}	1.80^{a-d}	0.20^{b}	0.122^{a-f}	0.874^{abc}	1.088^{ab}	NA
FP6405	18.0^{c-i}	9.0^{a-e}	2.73^{a-e}	2.11^{abc}	0.23^{b}	0.127^{a-f}	0.572^{abc}	1.084^{ab}	81
MC4005	18.0^{c-i}	9.0^{a-e}	2.11^{a-e}	1.48^{bcd}	0.16^{b}	0.132^{a-e}	0.850^{abc}	1.092^{ab}	75
MC10605	37.0^{ab}	12.0^{a-d}	3.43^{ab}	2.45^{abc}	0.20^{b}	0.150^{abc}	0.980^{abc}	1.087^{ab}	NA
MP6505	9.0^{f-i}	7.0^{b-e}	1.54^{c-e}	1.45^{bcd}	0.21^{b}	0.150^{abc}	0.801^{abc}	1.087^{ab}	NA
MP7405	26.0^{a-f}	10.0^{a-e}	2.68^{a-e}	1.86^{a-d}	0.19^{b}	0.150^{abc}	0.670^{abc}	1.086^{ab}	NA
MP7205	5.0^{hi}	4.0^{de}	1.08^{e}	1.05^{cd}	0.26^{ab}	0.159^{ab}	0.801^{abc}	1.084^{ab}	NA
MC7305	20.0^{c-i}	10.0^{a-e}	2.49^{a-e}	1.93^{a-d}	0.19^{b}	0.164^{a}	0.686^{abc}	1.088^{ab}	NA
MP6105	15.0^{c-i}	11.0^{a-e}	1.85^{a-e}	1.55^{bcd}	0.14^{b}	0.165^{a}	0.850^{abc}	1.090^{ab}	88
MP9605	15.0^{c-i}	9.0^{a-e}	1.98^{a-e}	1.61^{bcd}	0.18^{b}	NA	NA	NA	78

[1]ATW, average tuber weight; [2]FF, French fry colour; NA, not available; Means were compared using Duncan's New Multiple Range test at probability level of 0.05.

Table A.2.3 Yield and processing data for selected- intraclones from the 2005 season and re-evaluated for the second year in the 2006 season. Intraclones were derived from 'Russet Burbank' pith (microtuber (MP) or field-grown tuber (FP)) or cortex (microtuber (MC)) explants compared with control 'Russet Burbank' (RBP) and 'Burbank' (B) and were re-evaluated (2006). Data were arranged based on % glucose from the lowest to the highest value.

Controls / Intraclones	Tuber Number		Tuber Weight (Kg)		ATW[1] (Kg)	% Glucose	Sucrose (mgg^{-1})	Specific Gravity	French Fry
	Total	graded	Total	Graded					
RBP	14.87^{abc}	6.90^{abcd}	1.57^{abc}	1.23^{abcdef}	0.18^{bcd}	0.066^{bcde}	1.422^{a}	1.102^{a}	77.85^{b}
B	11.40^{bc}	3.60^{cd}	0.95^{c}	0.58^{ef}	0.16^{cd}	0.053^{bcde}	1.610^{a}	1.100^{a}	99.30^{ab}
MP11505	11.13^{bc}	4.10^{bcd}	1.09^{bc}	0.78^{bcdef}	0.18^{abcd}	0.027^{e}	1.626^{a}	1.099^{a}	93.05^{ab}
MP13905	16.55^{abc}	5.53^{bcd}	1.26^{bc}	0.85^{bcdef}	0.15^{cd}	0.029^{e}	1.348^{a}	1.004^{a}	98.13^{ab}
MP19805	14.77^{abc}	5.05^{bcd}	1.33^{bc}	0.92^{bcdef}	0.18^{abcd}	0.030^{e}	1.503^{a}	1.100^{a}	96.95^{ab}
MC11205	13.43^{abc}	5.00^{bcd}	1.29^{bc}	0.82^{bcdef}	0.16^{cd}	0.031^{de}	1.442^{a}	1.099^{a}	90.60^{ab}
MP18405	18.00^{ab}	5.97^{bcd}	1.57^{abc}	0.86^{bcdef}	0.14^{d}	0.031^{de}	1.360^{a}	1.101^{a}	94.80^{ab}
MP8205	16.70^{abc}	4.23^{bcd}	1.41^{bc}	0.87^{bcdef}	0.20^{abc}	0.032^{de}	1.430^{a}	1.098^{a}	96.35^{ab}
FP3405	17.87^{ab}	4.67^{bcd}	1.37^{bc}	0.71^{cdef}	0.15^{cd}	0.040^{cde}	1.311^{a}	1.099^{a}	96.35^{ab}
MP17205	13.67^{abc}	4.30^{bcd}	1.16^{bc}	0.66^{cdef}	0.15^{cd}	0.047^{bcde}	1.585^{a}	1.101^{a}	95.50^{ab}
MP405	11.13^{bc}	6.73^{abcd}	1.47^{abc}	1.22^{abcdef}	0.18^{bcd}	0.053^{bcde}	1.463^{a}	1.103^{a}	92.00^{ab}
FP9305	16.42^{abc}	3.17^{cd}	1.07^{c}	0.49^{f}	0.16^{cd}	0.058^{bcde}	1.091^{a}	1.099^{a}	102.65^{a}
MC8905	10.17^{bc}	4.97^{bcd}	1.23^{bc}	0.82^{bcdef}	0.16^{cd}	0.067^{bcde}	1.614^{a}	1.096^{ab}	88.75^{ab}
FP9405	12.30^{bc}	4.70^{bcd}	1.24^{bc}	0.85^{bcdef}	0.18^{bcd}	0.069^{abcde}	1.430^{a}	1.096^{ab}	90.10^{ab}
MC505	20.67^{ab}	9.00^{ab}	1.89^{abc}	1.63^{abcd}	0.18^{bcd}	0.078^{abcde}	1.385^{a}	1.095^{ab}	NA
MP5605	15.77^{abc}	5.27^{bcd}	1.32^{bc}	0.84^{bcdef}	0.16^{cd}	0.088^{abcde}	1.573^{a}	1.099^{a}	93.85^{ab}
MP9605	23.67^{a}	11.33^{a}	2.46^{a}	2.05^{a}	0.18^{bcd}	0.094^{abcd}	1.413^{a}	1.087^{b}	NA

Table A.2.3. Continued

MC405	15.96^{abc}	6.09^{bcd}	1.48^{abc}	1.01^{bcdef}	0.17^{cd}	0.103^{abc}	1.675^{a}	1.098^{a}	94.80^{ab}
MC10605	15.67^{abc}	7.67^{abcd}	2.14^{ab}	1.75^{ab}	0.23^{ab}	0.105^{ab}	1.473^{a}	1.100^{a}	NA
MP7405	12.00^{bc}	8.00^{abc}	1.68^{abc}	1.56^{abcde}	0.19^{abcd}	0.107^{ab}	1.490^{a}	1.100^{a}	NA
MP8805	11.87^{bc}	5.13^{bcd}	1.35^{bc}	1.00^{bcdef}	0.20^{abc}	0.141^{a}	1.638^{a}	1.096^{ab}	84.25^{ab}
FP1205	10.67^{bc}	6.33^{abcd}	1.18^{bc}	1.01^{bcdef}	0.16^{cd}	NA	NA	NA	NA
MP11205	14.67^{abc}	6.67^{abcd}	1.41^{bc}	1.08^{abcdef}	0.16^{cd}	NA	NA	NA	NA
MP14605	17.67^{ab}	9.33^{ab}	2.00^{abc}	1.64^{abc}	0.18^{bcd}	NA	NA	NA	NA
MP19405	15.00^{abc}	4.67^{bcd}	1.42^{bc}	1.00^{bcdef}	0.22^{ab}	NA	NA	NA	NA
MP6505	6.50^{c}	2.50^{d}	0.94^{c}	0.61^{def}	0.24^{a}	NA	NA	NA	NA
MP7205	15.33^{abc}	7.00^{abcd}	1.69^{abc}	1.33^{abcdef}	0.19^{abcd}	NA	NA	NA	NA

[1]ATW, average tuber weight; NA, not available; Means were compared using Duncan's New Multiple Range test at probability level of 0.05.

Table A.2.4 Yield and processing data for selected- intraclones from the 2006 season. Intraclones derived from NB 'Russet Burbank' pith (microtuber (MP) or field-grown tuber (FP)), cortex (microtuber (MC) or field-grown tubers (FC)) or periderm (microtuber (MS)) explants compared with control 'Russet Burbank' (plantlet-derived (RBP) or seed tubers (RBS)) and plantlet-derived 'Burbank' (B). Data were arranged based on % glucose from the lowest to the highest value.

Controls / Intraclones	Tuber Number		Tuber Weight (Kg)		ATW[1] (Kg)	% Glucose	Sucrose (mgg^{-1})	Specific Gravity
	Total	Graded	Total	Graded				
RBP	20.90^{bc}	9.50^{ab}	2.12^{ab}	1.69^{a}	0.18^{ab}	0.139^{abcde}	1.641^{ab}	1.094^{ab}
RBS	19.25^{abc}	10.50^{ab}	2.57^{ab}	2.28^{a}	0.21^{ab}	0.097^{cde}	1.299^{ab}	1.088^{ab}
B	20.20^{abc}	9.70^{ab}	2.21^{ab}	1.65^{a}	0.16^{ab}	0.089^{cde}	1.419^{ab}	1.093^{ab}
MS1406	15.50^{bc}	8.50^{ab}	2.19^{ab}	1.82^{a}	0.21^{ab}	0.021^{e}	1.377^{ab}	1.092^{ab}
FC206	16.00^{bc}	6.00^{b}	1.57^{b}	1.47^{a}	0.26^{a}	0.030^{e}	1.348^{ab}	1.098^{ab}
MC1606	19.00^{abc}	9.00^{ab}	2.37^{ab}	2.04^{a}	0.23^{ab}	0.036^{e}	1.311^{ab}	1.094^{ab}
MS906	21.00^{abc}	8.00^{ab}	2.36^{ab}	1.87^{a}	0.23^{ab}	0.053^{e}	1.556^{ab}	1.100^{ab}
FP106	24.00^{ab}	10.00^{ab}	2.54^{ab}	1.86^{a}	0.19^{ab}	0.061^{e}	1.499^{ab}	1.093^{ab}
FP906	22.50^{abc}	11.00^{ab}	2.20^{ab}	1.68^{a}	0.15^{ab}	0.062^{de}	1.512^{ab}	1.105^{ab}
FP2906	19.50^{abc}	13.00^{ab}	2.46^{ab}	2.15^{a}	0.17^{ab}	0.063^{de}	1.434^{ab}	1.104^{ab}
FC2806	24.50^{ab}	11.50^{ab}	2.38^{ab}	1.77^{a}	0.15^{b}	0.076^{cde}	1.344^{ab}	1.097^{ab}
FP306	17.00^{bc}	11.50^{ab}	2.12^{ab}	1.83^{a}	0.15^{ab}	0.076^{cde}	1.536^{ab}	1.102^{ab}
FC606	16.50^{bc}	10.00^{ab}	2.14^{ab}	1.88^{a}	0.19^{ab}	0.091^{cde}	1.201^{ab}	1.098^{ab}
FC1106	21.00^{abc}	12.00^{ab}	2.60^{ab}	2.30^{a}	0.19^{ab}	0.102^{cde}	1.634^{ab}	1.096^{ab}
FC2006	16.00^{bc}	8.50^{ab}	2.19^{ab}	1.85^{a}	0.21^{ab}	0.104^{cde}	1.320^{ab}	1.093^{ab}
MP706	21.50^{abc}	11.50^{ab}	2.19^{ab}	1.67^{a}	0.15^{b}	0.104^{cde}	1.712^{a}	1.098^{ab}
FC2106	21.00^{abc}	9.00^{ab}	2.08^{ab}	1.63^{a}	0.19^{ab}	0.106^{cde}	1.352^{ab}	1.108^{a}
FC406	22.50^{abc}	9.50^{ab}	2.14^{ab}	1.79^{a}	0.19^{ab}	0.106^{cde}	1.025^{b}	1.098^{ab}
FP2106	18.50^{abc}	10.00^{ab}	2.23^{ab}	1.85^{a}	0.18^{ab}	0.109^{cde}	1.503^{ab}	1.101^{ab}
FP8106	24.00^{ab}	10.50^{ab}	2.55^{ab}	2.05^{a}	0.20^{ab}	0.117^{bcde}	1.413^{ab}	1.086^{b}
FC106	16.00^{bc}	9.00^{ab}	2.02^{ab}	1.76^{a}	0.19^{ab}	0.122^{bcde}	1.397^{ab}	1.095^{ab}
FP1606	19.00^{abc}	12.00^{ab}	2.20^{ab}	1.95^{a}	0.16^{ab}	0.126^{abcde}	1.164^{ab}	1.096^{ab}
MC3606	17.00^{bc}	9.50^{ab}	1.98^{ab}	1.69^{a}	0.17^{ab}	0.129^{abcde}	1.238^{ab}	1.099^{ab}
MS1006	10.50^{c}	8.00^{ab}	2.19^{ab}	2.02^{a}	0.25^{ab}	0.138^{abcde}	1.324^{ab}	1.085^{b}
FP1806	30.00^{a}	12.00^{ab}	2.82^{ab}	1.76^{a}	0.15^{b}	0.141^{abcde}	1.136^{ab}	1.099^{ab}
MC706	19.00^{abc}	9.00^{ab}	2.20^{ab}	1.97^{a}	0.23^{ab}	0.146^{abcde}	1.458^{ab}	1.094^{ab}
MS1806	19.00^{abc}	11.00^{ab}	2.41^{ab}	2.10^{a}	0.21^{ab}	0.157^{abcde}	1.209^{ab}	1.086^{b}
FP806	27.00^{ab}	14.50^{ab}	3.23^{a}	2.84^{a}	0.20^{ab}	0.162^{abcde}	1.275^{ab}	1.092^{ab}
MS1206	20.50^{abc}	11.00^{ab}	2.14^{ab}	1.79^{a}	0.16^{ab}	0.162^{abcde}	1.577^{ab}	1.093^{ab}
FP2406	20.50^{abc}	7.50^{ab}	2.27^{ab}	1.69^{a}	0.22^{ab}	0.204^{abcd}	1.426^{ab}	1.092^{ab}
FC2406	18.50^{abc}	10.50^{ab}	2.53^{ab}	2.04^{a}	0.21^{ab}	0.213^{abc}	1.279^{ab}	1.100^{ab}
FC1006	19.00^{abc}	10.00^{ab}	2.01^{ab}	1.62^{a}	0.17^{ab}	0.253^{ab}	1.405^{ab}	1.087^{b}
FC3506	26.50^{ab}	10.00^{ab}	2.17^{ab}	1.68^{a}	0.17^{ab}	0.263^{a}	1.172^{ab}	1.088^{ab}

[1]ATW, average tuber weight; NA, not available; Means were compared using Duncan's New Multiple Range test at probability level of 0.05.

Table A.2.5. Yield and processing results of selected intraclones from the 2005 and 2006 seasons re-evaluated during the 2007 field season and controls 'Russet Burbank' (seed tuber-derived) (RBS), 'Russet Burbank' (RBP), 'Burbank' (B), and 'Early Rose' (ER) (plantlet-derived). Intraclones produced from 'Russet Burbank' pith (microtubers (MP) or field tubers (FP)), cortex (microtubers (MC) or field tubers (FC)) or periderm (microtubers (MS)). Data were arranged based on % glucose after 5 mo from the lowest to the highest value.

Controls /		Tuber Number		Tuber Weight (Kg)		ATW[1]	% Glucose			Sucrose (mgg^{-1})		Specific	Fry
Intraclones	N	Total	Graded	Total	Graded	(Kg)	5 mo	N	9 mo	5 mo	9 mo	Gravity	Color
RBP	3	6.76^{abc}	4.14^{abc}	0.90^{cde}	0.77^{cde}	0.19^{bc}	0.047^{ghi}	2	0.025^{bcd}	0.938^{b-f}	1.002^{ab}	1.071^{a}	93.58^{a-d}
RBS	3	9.89^{a}	6.30^{a}	1.67^{a}	1.51^{a}	0.25^{a}	0.032$^{i=l}$	2	0.018^{cd}	0.715^{ef}	0.943^{ab}	1.076^{a}	92.10^{a-d}
B	1	8.71^{abc}	5.71^{ab}	1.41^{ab}	1.20^{b}	0.21^{abc}	0.117^{b}	1	0.035^{a-d}	0.780^{def}	0.626^{b}	1.077^{a}	83.54^{d}
ER	3	5.17^{c}	3.64^{bc}	0.87^{cde}	0.78^{cde}	0.22^{ab}	0.147^{b}	2	0.196^{a}	0.688^{f}	1.804^{ab}	1.075^{a}	66.50^{e}
FP106	3	9.40^{ab}	4.09^{abc}	0.93^{cde}	0.66d^{e}	0.16^{c}	0.016^{l}	2	0.040^{a-d}	1.097^{ab}	1.290^{ab}	1.064^{a}	95.12^{ab}
FP2106	3	6.76^{abc}	4.54^{abc}	0.93^{cde}	0.82^{cde}	0.18^{bc}	0.020^{kl}	0	NA	1.125^{a-d}	NA	1.072^{a}	89.00^{a-d}
FP3405	3	9.33^{ab}	4.53^{abc}	1.03^{cde}	0.72^{cde}	0.16^{c}	0.026^{jkl}	0	NA	1.177^{abc}	NA	1.077^{a}	92.85^{a-d}
MP706	1	9.43^{ab}	4.43^{abc}	0.99^{cde}	0.76^{cde}	0.17^{bc}	0.030^{i-l}	1	0.006^{d}	1.034^{a-f}	0.893^{ab}	1.082^{a}	92.44^{a-d}
MS1406	3	7.00^{abc}	3.98^{abc}	0.86^{cde}	0.70^{cde}	0.18^{bc}	0.030^{i-l}	2	0.012^{d}	1.038^{a-d}	1.379^{a}	1.064^{a}	90.79^{a-d}
FP306	3	8.00^{abc}	4.71^{abc}	0.91^{cde}	0.77^{cde}	0.17^{bc}	0.032^{i-l}	2	0.018^{cd}	1.056^{a-d}	1.261^{ab}	1.074^{a}	89.13^{a-d}
MC1606	3	8.21^{abc}	3.88^{bc}	0.80^{de}	0.60^{de}	0.16^{c}	0.032^{i-l}	2	0.030^{abcd}	0.921^{c-f}	0.901^{ab}	1.072^{a}	91.47^{a-d}
FC1006	3	7.68^{abc}	4.90^{abc}	1.10^{b-e}	0.96^{bcd}	0.19^{bc}	0.033^{i-l}	2	0.023^{bcd}	1.202^{abc}	0.724^{ab}	1.075^{a}	88.09^{a-d}
FP2906	3	8.18^{abc}	5.74^{ab}	0.99^{cde}	0.88^{b-e}	0.15^{c}	0.034^{hij}	1	0.018^{cd}	1.136^{a-d}	0.903^{ab}	1.068^{a}	96.97^{a}

Table A.2.5 Continued

MC405	3	8.32^{abc}	5.13^{abc}	1.05^{bcde}	0.91^{b-e}	0.18^{bc}	0.035^{h-l}	2	0.062^{a}	1.371^{a}	0.963^{ab}	1.069^{a}	84.93^{bcd}
MP18405	3	9.56^{a}	5.28^{abc}	1.18^{bcd}	0.97^{bcd}	0.18^{bc}	0.037^{h-k}	2	0.006^{d}	1.000^{a-f}	0.825^{ab}	1.072^{a}	87.47^{a-d}
FC406	3	8.33^{abc}	4.69^{abc}	0.99^{cde}	0.83^{cde}	0.18^{bc}	0.039^{i-l}	2	0.009^{d}	1.050^{abc}	1.107^{ab}	1.073^{a}	95.33^{ab}
MP405	1	7.75^{abc}	4.75^{abc}	0.96^{cde}	0.79^{cde}	0.17^{bc}	0.040^{hij}	0	NA	0.960^{b-f}	NA	1.081^{a}	96.62^{a}
FP806	3	8.05^{abc}	3.17^{c}	0.73^{e}	0.56^{e}	0.18^{bc}	0.042^{fgh}	0	NA	0.829^{c-f}	NA	1.063^{a}	90.44^{a-d}
FP906	3	8.40^{abc}	4.39^{abc}	0.80^{de}	0.66^{de}	0.15^{c}	0.045^{ghi}	0	NA	0.872^{c-f}	NA	1.071^{a}	87.52^{a-d}
FC2806	3	9.33^{ab}	5.83^{ab}	1.23^{bc}	1.04^{bc}	0.18^{bc}	0.047^{ghi}	0	NA	1.039^{a-f}	NA	1.084^{a}	93.53^{a-d}
MC10605	3	8.33^{abc}	4.11^{abc}	0.89^{cde}	0.70^{cde}	0.17^{bc}	0.048^{ghi}	1	0.024^{bcd}	1.182^{abc}	0.855^{ab}	1.074^{a}	94.16^{abc}
FC2006	3	8.96^{abc}	4.18^{abc}	0.91^{cde}	0.68^{cde}	0.16^{c}	0.054^{ghi}	2	0.050^{abc}	1.021^{abcd}	NA	1.067^{a}	89.19^{a-d}
MP7405	3	9.00^{abc}	4.80^{abc}	0.92^{cde}	0.73^{cde}	0.16^{c}	0.054^{i-l}	0	NA	1.004^{a-f}	1.295^{ab}	1.073^{a}	87.72^{a-d}
FC1106	1	5.40^{bc}	4.00^{abc}	0.75^{e}	0.75^{cde}	0.19^{bc}	0.062^{efg}	0	NA	0.850^{c-f}	NA	1.068^{a}	88.46^{a-d}
MP9605	3	7.33^{abc}	4.64^{abc}	0.99^{cde}	0.86^{b-e}	0.18^{bc}	0.068^{ef}	2	0.016^{d}	1.162^{a-d}	1.062^{ab}	1.072^{a}	94.59^{abc}
MS906	3	6.49^{abc}	3.90^{bc}	0.76^{e}	0.64d^{e}	0.16^{c}	0.071^{e}	2	0.056^{ab}	1.081^{a-e}	1.089^{ab}	1.059^{a}	88.42^{a-d}
MP19805	3	9.71^{a}	5.25^{abc}	1.09^{bcde}	0.89^{b-e}	0.17^{bc}	0.078^{de}	2	0.007^{d}	0.993^{a-e}	0.896^{ab}	1.075^{a}	85.74^{bcd}
FP8106	3	9.33^{ab}	4.25^{abc}	0.89^{cde}	0.67^{de}	0.16^{c}	0.102^{bc}	0	NA	0.956^{b-f}	NA	1.072^{a}	92.57^{a-d}
FC606	3	8.25^{abc}	4.79^{abc}	0.97^{cde}	0.82^{cde}	0.17^{bc}	0.112^{cd}	2	0.012^{d}	0.899^{c-f}	0.871^{ab}	1.071^{a}	85.78^{bcd}
MP11505	3	5.50^{bc}	3.17^{c}	0.71^{e}	0.66^{de}	0.21^{abc}	0.148^{a}	0	NA	1.098^{a-e}	NA	1.062^{a}	84.13^{cd}

[1]ATW, average tuber weight; NA, not available; Means were compared using Duncan's New Multiple Range test ($P \leq 0.05$)

Table A.2.6. Percentages of increased or decreased average total tuber weight, % glucose, or total soluble protein (TSP) of superior select intraclones compared with control NB 'Russet Burbank' for 3 or 2 years of field selection and evaluation.

Intraclones	Average Total Tuber Weight			% Glucose			% TSP (FW)		
	2005	2006	2007	2005	2006	2007	2006	2007	2008
FP3405*	102.9	-12.7	14.4	-38.7	-39.4	-44.7	-5.1	-82.0	-0.6
FP18405	93.7	0.0	31.1	-50.7	-53.0	-21.3	43.6	-31.6	-73.1
MP405	-4.6	-6.4	6.7	-31.7	-19.7	-14.9	4.2	-14.3	-32.8
MP9605	13.8	56.7	10.0	-	42.4	44.7	-6.3	-42.2	-52.6
MP11505	32.2	-30.6	-21.1	-24.6	-59.1	214.9	37.4	-41.0	-72.6
MC405	12.1	-5.7	16.7	-31.7	56.1	-25.5	-4.4	-45.5	39.5
MP7405	54.0	7.0	2.2	5.6	62.1	14.9	-9.9	-30.9	-22.9
FP2906	-	16.0	10.0	-	-54.7	-27.7	-	-22.6	12.9
MS1406	-	3.3	-4.4	-	-84.9	-36.2	-	-43.4	-37.8
FP806	-	52.4	-18.9	-	16.5	-10.6	-	-13.6	-28.9
FP906	-	3.8	-11.1	-	-55.4	-4.3	-	-42.1	-40.7
FC2006	-	3.3	1.1	-	-25.2	14.9	-	-43.8	-37.4

*Intraclone coded names ending with 05 were evaluated for 3 years (2005, 2006, and 2007) while those ending with 06 were evaluated for 2 field seasons (2006 and 2007).

Table A.2.7. ANOVA mean square of yield components; total tuber number (TTN), graded tuber number (GTN), total tuber weight (TTW), graded tuber weight (GTW), and average weight per tuber (ATW) of intraclones that were field-evaluated for 2 years; 2005 and 2006, respectively.

Source	Years	df	Yield Components				
			TTN	GTN	GTW	TTW	ATW
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
Years*Tissue Sources		3	758.77**	121.66*	4.46**	5.17**	0.03**

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

Table A.2.8. Pearson correlation coefficients of yield components; total tuber number (TTN), graded tuber number (GTN), total tuber weight (TTW), graded tuber weight (GTW), and average weight per tuber (ATW) and processing quality traits; specific gravity (SG), % glucose (GLU), sucrose (SUC), and French fry colour (FF), respectively.

	TTN	GTN	GTW	TTW	ATW	SG	GLU	SUC	FF
TTN	1.00	0.57**	0.45**	0.68**	0.08	-	-	-	-
GTN	0.57**	1.00	0.91**	0.89**	0.28**	-	-	-	-
GTW	0.54**	0.91**	1.00	0.93**	0.55**	-	-	-	-
TTW	0.68**	0.89**	0.93**	1.00	0.48**	-	-	-	-
ATW	0.08	0.28**	0.55**	048**	1.00	-	-	-	-
SG	-	-	-	-	-	1.00	-0.10	0.10	0.21
GLU	-	-	-	-	-	-0.10	1.00	-0.29**	-0.50**
SUC	-	-	-	-	-	0.10	-0.29**	0.10	0.30*
FF	-	-	-	-	-	0.21	-0.50**	0.30**	1.00

*, ** significant at $P < 0.05$ and $P < 0.01$, respectively.

Table A.2.9. 2005 field results of total tuber numbers per intraclone of harvested intraclones (single replicate) compared with the average total tuber number of control NB 'Russet Burbank'.

Average tuber number ± SD of control 'Russet Burbank' (15.31± 9.24)									
42	28	22	19	17	15	13	11	8	4
40	27	22	19	17	15	13	11	8	4
39	27	22	19	17	14	13	11	8	4
37	27	22	19	17	14	13	11	8	4
36	27	22	19	17	14	13	11	8	3
36	27	22	19	17	14	13	11	8	3
36	27	22	19	17	14	13	11	8	3
35	26	22	19	16	14	13	11	7	2
35	26	22	19	16	14	12	11	7	2
34	26	21	19	16	14	12	11	7	1
34	26	21	19	16	14	12	11	7	
34	26	21	19	16	14	12	11	7	
34	25	20	19	16	14	12	10	7	
33	25	20	19	16	14	12	10	7	
33	25	20	19	16	14	12	10	7	
32	25	20	19	16	14	12	10	7	
32	25	20	18	16	14	12	10	7	
32	25	20	18	16	14	12	10	7	
31	25	20	18	16	14	12	10	7	
31	25	20	18	16	14	12	10	6	
31	25	20	18	16	14	12	10	6	
31	25	20	18	16	14	12	9	6	
31	24	20	18	16	14	12	9	6	
30	24	20	18	16	14	12	9	6	
30	24	20	18	15	14	12	9	6	
30	24	20	18	15	14	12	9	6	
30	24	20	18	15	14	12	9	6	
30	24	20	18	15	14	12	9	6	
30	23	20	18	15	14	12	9	6	
29	23	20	18	15	14	12	9	6	
29	23	20	18	15	13	11	9	6	
29	23	20	18	15	13	11	9	6	
29	23	20	18	15	13	11	9	6	
29	23	20	17	15	13	11	9	5	
29	23	20	17	15	13	11	9	5	
28	23	20	17	15	13	11	9	5	
28	23	19	17	15	13	11	9	5	
28	23	19	17	15	13	11	9	5	

Table A.2.10. 2005 field results of graded number of tubers per intraclone (≥ 5 cm) of harvested intraclones (single replicate) compared with the average graded tuber number of control NB 'Russet Burbank'.

Average graded tuber number ± SD of control 'Russet Burbank' (6.36 ± 2.11)									
16	8	7	5	5	4	3	2	1	0
14	8	7	5	5	4	3	2	1	0
14	8	7	5	5	4	3	2	1	0
13	8	7	5	5	4	3	2	1	0
13	8	6	5	4	4	3	2	1	0
12	8	6	5	4	4	3	2	1	0
11	8	6	5	4	4	3	2	1	0
11	8	6	5	4	4	3	2	1	0
11	8	6	5	4	4	3	2	1	0
11	8	6	5	4	4	3	2	1	0
11	8	6	5	4	4	3	2	1	
10	8	6	5	4	4	3	2	1	
10	8	6	5	4	4	3	2	1	
10	8	6	5	4	4	3	2	1	
10	8	6	5	4	4	3	2	1	
10	8	6	5	4	4	3	2	1	
10	8	6	5	4	4	3	2	1	
10	7	6	5	4	4	3	2	1	
10	7	6	5	4	4	3	2	1	
10	7	6	5	4	4	3	2	1	
10	7	6	5	4	4	3	2	1	
10	7	6	5	4	4	3	2	1	
9	7	6	5	4	4	3	2	1	
9	7	6	5	4	4	3	2	1	
9	7	6	5	4	4	3	2	0	
9	7	6	5	4	4	2	2	0	
9	7	6	5	4	4	2	2	0	
9	7	6	5	4	3	2	2	0	
9	7	6	5	4	3	2	2	0	
9	7	6	5	4	3	2	2	0	
9	7	6	5	4	3	2	2	0	
9	7	6	5	4	3	2	1	0	
9	7	6	5	4	3	2	1	0	
9	7	5	5	4	3	2	1	0	
9	7	5	5	4	3	2	1	0	
8	7	5	5	4	3	2	1	0	
8	7	5	5	4	3	2	1	0	
8	7	5	5	4	3	2	1	0	

Table A.2.11. 2005 field results of total weight of tubers per intraclone (Kg) of harvested intraclones (single replicate) compared with the average total tuber weight (Kg) of control NB 'Russet Burbank'.

Average total tuber weight ± SD of control 'Russet Burbank' (1.74 ± 0.56)									
3.53	2.18	1.88	1.65	1.46	1.32	1.17	0.97	0.71	0.27
3.43	2.17	1.88	1.65	1.46	1.30	1.17	0.97	0.70	0.22
3.41	2.16	1.87	1.64	1.46	1.30	1.16	0.96	0.70	0.22
3.37	2.16	1.86	1.64	1.45	1.30	1.16	0.95	0.69	0.20
3.21	2.15	1.86	1.64	1.45	1.29	1.15	0.93	0.67	0.17
2.98	2.15	1.85	1.64	1.44	1.29	1.14	0.93	0.67	0.16
2.90	2.14	1.85	1.63	1.44	1.29	1.14	0.92	0.66	0.14
2.74	2.14	1.84	1.63	1.44	1.28	1.13	0.92	0.66	0.13
2.73	2.14	1.84	1.63	1.43	1.27	1.13	0.92	0.66	0.09
2.73	2.11	1.80	1.62	1.43	1.27	1.12	0.90	0.64	0.05
2.69	2.10	1.80	1.62	1.43	1.27	1.11	0.90	0.63	
2.68	2.10	1.80	1.61	1.43	1.27	1.11	0.90	0.63	
2.59	2.08	1.80	1.61	1.41	1.27	1.11	0.89	0.62	
2.53	2.08	1.78	1.61	1.41	1.27	1.10	0.88	0.61	
2.53	2.08	1.78	1.60	1.41	1.26	1.09	0.87	0.58	
2.50	2.07	1.78	1.59	1.41	1.26	1.09	0.86	0.56	
2.49	2.05	1.77	1.59	1.40	1.26	1.08	0.86	0.56	
2.47	2.04	1.77	1.56	1.40	1.26	1.07	0.86	0.54	
2.46	2.01	1.75	1.55	1.40	1.25	1.06	0.85	0.53	
2.45	2.00	1.74	1.55	1.40	1.25	1.04	0.85	0.53	
2.43	2.00	1.74	1.54	1.40	1.25	1.04	0.85	0.52	
2.41	2.00	1.73	1.54	1.39	1.25	1.03	0.84	0.51	
2.40	1.99	1.71	1.54	1.39	1.24	1.03	0.84	0.51	
2.32	1.98	1.71	1.54	1.38	1.23	1.03	0.84	0.51	
2.32	1.96	1.70	1.54	1.38	1.23	1.02	0.83	0.50	
2.31	1.95	1.70	1.53	1.38	1.23	1.02	0.82	0.47	
2.31	1.95	1.70	1.52	1.38	1.22	1.01	0.82	0.44	
2.30	1.93	1.70	1.52	1.37	1.22	1.01	0.81	0.44	
2.30	1.93	1.69	1.51	1.37	1.22	1.01	0.80	0.40	
2.29	1.92	1.69	1.51	1.36	1.22	1.01	0.80	0.40	
2.27	1.92	1.68	1.50	1.36	1.21	1.01	0.80	0.40	
2.27	1.91	1.67	1.49	1.36	1.21	1.00	0.77	0.39	
2.26	1.91	1.67	1.49	1.36	1.20	1.00	0.76	0.33	
2.24	1.90	1.66	1.48	1.35	1.19	1.00	0.75	0.33	
2.22	1.89	1.66	1.48	1.34	1.19	0.99	0.75	0.30	
2.22	1.89	1.66	1.47	1.33	1.18	0.99	0.74	0.30	
2.20	1.88	1.66	1.47	1.32	1.18	0.99	0.74	0.28	
2.19	1.88	1.66	1.47	1.32	1.17	0.98	0.72	0.28	

Table A.2.12. 2005 field results of graded weight of tubers per intraclone (Kg) of harvested intraclones (single replicate) compared with the average graded tuber weight (Kg) (≥ 5cm) of control NB 'Russet Burbank'.

Average graded tuber weight ± SD of control 'Russet Burbank' (1.36 ± 0.55)									
3.22	1.45	1.19	0.98	0.78	0.64	0.50	0.34	0.17	0.00
2.74	1.45	1.19	0.98	0.78	0.64	0.49	0.34	0.16	0.00
2.47	1.41	1.19	0.97	0.77	0.63	0.49	0.32	0.16	0.00
2.45	1.41	1.18	0.97	0.77	0.63	0.49	0.32	0.15	0.00
2.29	1.40	1.17	0.96	0.76	0.63	0.48	0.31	0.15	0.00
2.21	1.39	1.16	0.96	0.76	0.62	0.48	0.31	0.14	0.00
2.16	1.38	1.16	0.95	0.75	0.62	0.48	0.31	0.14	0.00
2.13	1.38	1.16	0.95	0.74	0.62	0.48	0.30	0.13	0.00
2.11	1.37	1.15	0.94	0.74	0.62	0.48	0.30	0.13	0.00
2.05	1.36	1.15	0.93	0.74	0.62	0.46	0.30	0.13	0.00
1.99	1.35	1.14	0.93	0.74	0.61	0.46	0.29	0.12	
1.94	1.34	1.14	0.92	0.73	0.61	0.45	0.29	0.12	
1.93	1.31	1.14	0.91	0.72	0.61	0.45	0.29	0.12	
1.92	1.31	1.12	0.91	0.72	0.61	0.45	0.29	0.11	
1.91	1.31	1.12	0.91	0.71	0.61	0.45	0.29	0.11	
1.89	1.30	1.12	0.91	0.71	0.60	0.44	0.28	0.10	
1.88	1.30	1.12	0.90	0.71	0.59	0.43	0.28	0.10	
1.88	1.29	1.12	0.90	0.71	0.59	0.43	0.28	0.09	
1.87	1.29	1.11	0.90	0.71	0.58	0.43	0.28	0.08	
1.86	1.28	1.11	0.89	0.71	0.58	0.43	0.28	0.08	
1.85	1.27	1.09	0.88	0.71	0.57	0.43	0.27	0.07	
1.80	1.26	1.08	0.87	0.71	0.56	0.42	0.26	0.06	
1.77	1.26	1.08	0.87	0.71	0.55	0.42	0.25	0.06	
1.76	1.26	1.08	0.86	0.70	0.55	0.41	0.25	0.06	
1.74	1.25	1.07	0.85	0.68	0.54	0.40	0.25	0.00	
1.72	1.25	1.06	0.84	0.67	0.54	0.39	0.24	0.00	
1.62	1.25	1.06	0.84	0.67	0.54	0.39	0.22	0.00	
1.61	1.24	1.06	0.82	0.67	0.54	0.39	0.22	0.00	
1.60	1.23	1.05	0.82	0.67	0.53	0.38	0.22	0.00	
1.56	1.23	1.04	0.82	0.66	0.53	0.38	0.22	0.00	
1.55	1.23	1.02	0.82	0.66	0.53	0.38	0.20	0.00	
1.55	1.22	1.02	0.81	0.66	0.51	0.37	0.20	0.00	
1.54	1.21	1.01	0.80	0.66	0.51	0.36	0.20	0.00	
1.54	1.21	1.00	0.80	0.66	0.51	0.36	0.19	0.00	
1.52	1.21	1.00	0.80	0.65	0.51	0.36	0.19	0.00	
1.49	1.20	0.99	0.79	0.65	0.50	0.35	0.19	0.00	
1.48	1.20	0.99	0.79	0.65	0.50	0.35	0.18	0.00	
1.47	1.20	0.98	0.78	0.64	0.50	0.35	0.18	0.00	

Table A.2.13. 2005 field results of average weight per tuber (Kg) of harvested intraclones (single replicate) calculated by dividing graded tuber weight over graded tuber number per intraclone.

Average weight per tuber ± SD of control 'Russet Burbank' (0.21 ± 0.05)									
0.72	0.22	0.19	0.18	0.17	0.16	0.14	0.13	0.12	0.00
0.47	0.22	0.19	0.18	0.16	0.16	0.14	0.13	0.12	0.00
0.39	0.21	0.19	0.18	0.16	0.16	0.14	0.13	0.11	0.00
0.36	0.21	0.19	0.18	0.16	0.16	0.14	0.13	0.11	0.00
0.33	0.21	0.19	0.18	0.16	0.15	0.14	0.13	0.11	0.00
0.32	0.21	0.19	0.18	0.16	0.15	0.14	0.13	0.11	0.00
0.32	0.21	0.19	0.18	0.16	0.15	0.14	0.13	0.11	0.00
0.31	0.21	0.19	0.18	0.16	0.15	0.14	0.13	0.11	0.00
0.31	0.21	0.19	0.18	0.16	0.15	0.14	0.13	0.11	0.00
0.30	0.21	0.19	0.18	0.16	0.15	0.14	0.13	0.10	0.00
0.30	0.21	0.19	0.18	0.16	0.15	0.14	0.13	0.10	
0.28	0.21	0.19	0.17	0.16	0.15	0.14	0.13	0.10	
0.28	0.21	0.19	0.17	0.16	0.15	0.14	0.13	0.10	
0.27	0.21	0.19	0.17	0.16	0.15	0.14	0.13	0.10	
0.27	0.21	0.19	0.17	0.16	0.15	0.14	0.13	0.10	
0.27	0.20	0.19	0.17	0.16	0.15	0.14	0.13	0.10	
0.26	0.20	0.19	0.17	0.16	0.15	0.14	0.13	0.09	
0.26	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.09	
0.25	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.08	
0.25	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.08	
0.25	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.07	
0.24	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.06	
0.24	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.06	
0.23	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.06	
0.23	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.00	
0.23	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.00	
0.23	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.00	
0.23	0.20	0.18	0.17	0.16	0.15	0.14	0.12	0.00	
0.23	0.20	0.18	0.17	0.16	0.15	0.14	0.12	0.00	
0.23	0.20	0.18	0.17	0.16	0.15	0.14	0.12	0.00	
0.23	0.20	0.18	0.17	0.16	0.15	0.14	0.12	0.00	
0.22	0.19	0.18	0.17	0.16	0.15	0.14	0.12	0.00	
0.22	0.19	0.18	0.17	0.16	0.15	0.14	0.12	0.00	
0.22	0.19	0.18	0.17	0.16	0.15	0.14	0.12	0.00	
0.22	0.19	0.18	0.17	0.16	0.15	0.14	0.12	0.00	
0.22	0.19	0.18	0.17	0.16	0.15	0.13	0.12	0.00	
0.22	0.19	0.18	0.17	0.16	0.15	0.13	0.12	0.00	
0.22	0.19	0.18	0.17	0.16	0.15	0.13	0.12	0.00	

Table A.2.14. 2006 field results of total tuber number per intraclone as an average of two replicates of the harvested intraclones compared with the average total tuber number of control NB 'Russet Burbank'.

Total tuber number ± SD of control 'Russet Burbank' (20.9 ±6.17)							
20.90	22.50	20.00	18.50	16.00	14.50	12.50	9.00*
53.00	22.50	20.00	18.50	16.00	14.50	12.50	9.00
30.00	22.50	20.00	18.50	16.00	14.50	12.50	9.00
29.50	22.50	19.50	18.50	16.00	14.50	12.00*	8.50
28.50	22.50	19.50	18.00	16.00	14.50	12.00	8.50
28.00	22.50	19.50	18.00	16.00	14.00*	12.00	8.50
28.00	22.00	19.50	18.00	16.00	14.00	12.00	8.50
28.00	22.00	19.50	18.00	16.00	14.00*	12.00	8.00
27.50	22.00	19.50	18.00	16.00	14.00	12.00	8.00*
27.00	22.00	19.50	18.00	16.00	14.00	12.00	8.00*
27.00	22.00	19.50	18.00	16.00	14.00	12.00	8.00*
26.50	21.50	19.50	18.00	16.00	14.00	12.00	7.50
26.50	21.50	19.50	17.50	16.00	14.00	12.00	7.50
24.50	21.50	19.50	17.50	16.00	14.00	11.50	7.00
24.50	21.50	19.50	17.50	16.00	14.00	11.50	7.00*
24.50	21.50	19.00	17.50	16.00	14.00	11.50	6.50
24.50	21.00	19.00	17.50	15.50	14.00	11.50	6.00*
24.50	21.00	19.00	17.50	15.50	13.50	11.50	5.50
24.00	21.00	19.00	17.50	15.50	13.50	11.50	5.00*
24.00	21.00	19.00	17.50	15.50	13.50	11.50	5.00
24.00	21.00	19.00	17.50	15.50	13.50	11.00*	4.50
24.00	21.00	19.00	17.50	15.50	13.50	11.00	4.50
24.00	21.00	19.00	17.00	15.50	13.50	11.00	3.00*
24.00	21.00	19.00	17.00	15.50	13.00	11.00	3.00*
23.50	21.00	19.00	17.00	15.50	13.00	11.00	2.00*
23.50	21.00	19.00*	17.00	15.50	13.00*	11.00	2.00*
23.50	21.00	19.00	17.00	15.50	13.00	10.50	2.00
23.50	21.00	19.00	17.00*	15.50	13.00	10.50	1.00*
23.50	20.50	19.00	16.50	15.00	13.00	10.50	1.00
23.50	20.50	19.00	16.50	15.00	13.00	10.50	
23.00	20.50	19.00	16.50	15.00	13.00	10.00*	
23.00	20.50	19.00	16.50	15.00	13.00	10.00*	
23.00	20.50	18.50	16.50	15.00	13.00	10.00	
23.00	20.20	18.50	16.50	14.50	13.00	10.00	
23.00	20.00	18.50	16.50	14.50	13.00	10.00	
22.50	20.00*	18.50	16.00*	14.50	12.50	9.50	
22.50	20.00	18.50	16.00	14.50	12.50	9.50	

* Missing second replicate.

Table A.2.15. 2006 field results of graded tuber number per intraclone as an average of two replicates of the harvested intraclones compared with the average graded tuber number of control NB 'Russet Burbank'.

Graded tuber number ± SD of 'Russet Burbank' (9.5±3.44)							
14.50	9.50	8.00	7.50	6.50	5.50	4.00	2.00
14.00	9.50	8.00	7.50*	6.50	5.50	4.00	1.50*
13.00	9.50	8.00	7.00	6.50	5.50	4.00	1.50
12.00	9.50	8.00	7.00*	6.50	5.50	4.00	1.50
12.00	9.50	8.00	7.00	6.50	5.50	4.00	1.50
12.00	9.50	8.00	7.00	6.50	5.50	3.50	1.50
12.00	9.50	8.00	7.00	6.50	5.50	3.50	1.00*
12.00	9.50	8.00	7.00	6.50	5.50	3.50	1.00
12.00	9.00	8.00	7.00*	6.00	5.50	3.50	1.00
12.00	9.00	8.00*	7.00	6.00	5.50	3.50	1.00*
12.00	9.00	8.00	7.00	6.00	5.50	3.50	1.00*
11.50	9.00	8.00	7.00	6.00	5.50	3.50	0.50
11.50	9.00	8.00	7.00	6.00	5.50	3.50	0.00
11.50	9.00	8.00	7.00	6.00	5.00*	3.00*	0.00*
11.50	9.00	8.00	7.00	6.00	5.00	3.00	0.00
11.50	9.00	8.00	7.00	6.00	5.00	3.0*	0.00*
11.00	9.00	7.50	7.00	6.00	5.00	3.00	0.00*
11.00	9.00	7.50	7.00	6.00	5.00	3.00	0.00*
11.00	9.00	7.50	7.00	6.00	5.00	3.00	0.00*
11.00	9.00	7.50	7.00	6.00	5.00	3.00	0.00*
11.00	8.50	7.50	7.00	6.00	5.00	2.50	0.00
10.50	8.50	7.50	7.00	6.00	4.50	2.50	0.00
10.50	8.50	7.50	7.00	6.00	4.50	2.50	
10.50	8.50	7.50	7.00	6.00	4.50	2.50	
10.50	8.50	7.50	7.00	5.50	4.50	2.50	
10.00	8.50	7.50	7.00	5.50	4.50	2.50	
10.00	8.50	7.50	7.00	5.50	4.50	2.50	
10.00	8.00*	7.50	7.00	5.50	4.50	2.50	
10.00*	8.00	7.50	6.50	5.50	4.00	2.50	
10.00	8.00	7.50	6.50	5.50	4.00*	2.50	
10.00	8.00	7.50	6.50	5.50	4.00*	2.00*	
10.00	8.00	7.50	6.50	5.50	4.00	2.00	
10.00	8.00	7.50	6.50	5.50	4.00	2.00*	
10.00	8.00	7.50	6.50	5.50	4.00	2.00*	
9.70	8.00	7.50	6.50	5.50	4.00	2.00	
9.50	8.00	7.50	6.50	5.50	4.00	2.00	
9.50	8.00	7.50	6.50	5.50	4.00	2.00	
9.50	8.00	7.50	6.50	5.50	4.00	2.00	

* Missing second replicate.

Table A.2.16. 2006 field results of total tuber weight (Kg) per intraclone as an average of two replicates of the harvested intraclones compared with the average total tuber weight of control NB 'Russet Burbank'.

Total tuber weight ± SD of 'Russet Burbank' (2.12±0.61)							
3.23	2.17	1.89	1.74	1.53	1.35	1.11	0.52
3.04	2.16	1.88	1.74	1.52	1.35*	1.07	0.51
2.82	2.16	1.87	1.73	1.52	1.35	1.06	0.51
2.61	2.15	1.86	1.71	1.51	1.34	1.05	0.46*
2.60	2.15	1.86	1.71	1.51	1.33	1.05	0.44*
2.58	2.14	1.86	1.70*	1.51	1.33	1.05	0.44
2.55	2.14	1.85	1.68	1.49	1.32	1.03*	0.43*
2.54	2.14	1.85	1.67	1.48	1.32	1.02	0.42
2.53	2.14	1.84	1.67	1.48	1.31	1.00	0.31
2.50*	2.12	1.84	1.66	1.48	1.30	1.00	0.29*
2.46	2.12	1.84	1.66	1.47	1.30	0.99	0.20
2.41	2.10	1.83	1.65	1.47	1.29	0.99	0.19
2.41	2.09	1.83	1.65	1.46	1.28	0.99	0.18*
2.40	2.08	1.83	1.64	1.46	1.28	0.98	0.14*
2.40	2.08	1.81	1.63	1.46	1.27	0.97	0.12*
2.38	2.07	1.81	1.63	1.46	1.26	0.93	0.10*
2.38	2.06	1.81	1.63	1.45	1.25	0.92	0.09
2.37	2.06	1.80	1.62	1.45	1.25	0.92	0.08
2.37	2.04	1.80*	1.62	1.44	1.24*	0.92*	0.07*
2.36	2.03	1.80	1.61	1.44	1.24	0.92	0.04*
2.30	2.02	1.79	1.60	1.43	1.24	0.91	0.02*
2.30	2.01	1.78	1.60	1.43	1.23	0.89	0.00
2.28	1.99	1.78	1.60	1.43	1.23	0.89	
2.27	1.98	1.77	1.60	1.43	1.23	0.86	
2.27	1.97	1.77	1.60	1.42	1.22	0.85	
2.27	1.97	1.76	1.59	1.41	1.21*	0.82	
2.25*	1.95	1.76	1.59	1.41	1.21	0.78	
2.25	1.95	1.76	1.59	1.41	1.20	0.76	
2.23	1.95	1.76	1.59	1.41	1.18*	0.70*	
2.21	1.94	1.76	1.57	1.40	1.17	0.70	
2.20	1.94	1.75	1.57	1.40	1.17	0.69	
2.20	1.92	1.75	1.57	1.38	1.16	0.67	
2.20	1.92	1.75	1.57	1.38	1.15	0.66	
2.19	1.92	1.75	1.57	1.38	1.14	0.65	
2.19	1.91	1.75	1.55	1.38	1.13	0.62	
2.19	1.91	1.74	1.55	1.38	1.13	0.62*	
2.19	1.91	1.74	1.55	1.37	1.13	0.57	
2.18	1.89	1.74	1.54	1.36	1.11	0.55	

* Missing second replicate.

Table A.2.17. 2006 field results of graded tuber weight (Kg) per intraclone as an average of two replicates of the harvested intraclones compared with the average graded tuber weight (Kg) of control NB 'Russet Burbank'.

Graded tuber weight ± SD of 'Russet Burbank' (1.69±0.53)							
2.84	1.69	1.42	1.24	1.06	0.91	0.69	0.24
2.67	1.69	1.42	1.24	1.06	0.90	0.69	0.23
2.30	1.68	1.41	1.24	1.06	0.90	0.67	0.23
2.26	1.68	1.39	1.24	1.05	0.90	0.67	0.22
2.20*	1.68	1.38	1.24*	1.04	0.89	0.66	0.21*
2.15	1.68	1.36	1.23	1.04	0.89	0.65	0.19*
2.10	1.67	1.35	1.23	1.04	0.89	0.63	0.17
2.06	1.67	1.35	1.23	1.03	0.88	0.62	0.12
2.05	1.65	1.35	1.22	1.03	0.88	0.59	0.11*
2.04	1.63	1.35	1.20	1.02	0.87	0.55	0.10*
2.04	1.62	1.35	1.20	1.02	0.86	0.55	0.08*
2.04	1.62	1.34	1.19	1.02	0.85	0.55	0.05
2.04	1.61	1.33	1.18	1.01	0.85	0.54*	0.00
2.02	1.59	1.33	1.18	1.01	0.85	0.49	0.00*
1.99	1.58	1.33	1.18	1.00	0.84	0.47	0.00
1.97	1.56	1.32	1.18	1.00	0.84	0.46	0.00*
1.95	1.56	1.32	1.18	1.00	0.82	0.46	0.00*
1.89*	1.55	1.31	1.16	1.00	0.82	0.45	0.00*
1.88	1.55	1.30	1.16	1.00	0.82*	0.44	0.00*
1.87	1.55	1.30	1.16	1.00	0.81	0.40	0.00*
1.86	1.54	1.30	1.15	0.98	0.80	0.40	0.00
1.86*	1.54	1.29	1.14	0.98*	0.80	0.39	0.00
1.85	1.53	1.28	1.14	0.97	0.79	0.39	
1.85	1.53	1.28*	1.13	0.97	0.79	0.38*	
1.84	1.53	1.28	1.12	0.97	0.79	0.38	
1.83	1.51	1.28	1.12	0.96	0.78	0.36	
1.83	1.50	1.28	1.10	0.94	0.78	0.35	
1.82	1.49	1.27	1.09	0.94	0.77	0.34	
1.80	1.49	1.27	1.09	0.94	0.76	0.34	
1.79	1.49	1.27	1.08	0.93	0.76	0.33	
1.79	1.47	1.26	1.08	0.93*	0.75	0.33	
1.77	1.47	1.26	1.08	0.93	0.75	0.33	
1.76	1.46	1.26	1.08	0.93	0.73	0.32	
1.76	1.46	1.26	1.08*	0.92	0.73	0.31	
1.74	1.46	1.25	1.07	0.92	0.72	0.29	
1.73	1.45	1.25	1.07	0.92	0.72	0.28	
1.70	1.44	1.25*	1.06	0.92	0.72	0.28	
1.69	1.44	1.25	1.06	0.91	0.71	0.26*	

* Missing second replicate.

Table A.2.18. 2006 field results of average weight per tuber (Kg) as an average of two replicates of the harvested intraclones compared with the average weight per tuber of control NB 'Russet Burbank' calculated by dividing graded tuber weight/graded tuber number per intraclone.

Average weight per tuber ± SD of 'Russet Burbank' (0.18±0.04)							
0.41*	0.21	0.19	0.17	0.16	0.15	0.14	0.11*
0.27*	0.21	0.19	0.17	0.16	0.15	0.14	0.11*
0.27*	0.21	0.19	0.17	0.16	0.15	0.14	0.11
0.26	0.21	0.19	0.17	0.16	0.15	0.14	0.11
0.25	0.21	0.19	0.17	0.16	0.15	0.14	0.11
0.25*	0.20	0.19	0.17	0.16	0.15	0.14	0.11
0.25	0.20	0.19	0.17	0.16	0.15	0.14	0.10
0.24	0.20	0.19	0.17	0.16	0.15	0.14	0.10
0.24	0.20	0.19	0.17	0.16	0.15	0.14	0.10*
0.24	0.20	0.19	0.17	0.16	0.15	0.13	0.10
0.24	0.20	0.19	0.17	0.16	0.15	0.13	0.09
0.23	0.20	0.18	0.17*	0.16	0.15	0.13	0.09
0.23*	0.20	0.18	0.17	0.16	0.15	0.13	0.09*
0.23	0.20	0.18	0.17	0.16	0.15	0.13	0.08*
0.23	0.20	0.18	0.17	0.16	0.15	0.13	0.07
0.23	0.20	0.18	0.17	0.16	0.15	0.13	0.07
0.23	0.20	0.18	0.17	0.16	0.15	0.13	0.07
0.23	0.20	0.18	0.17	0.16	0.15	0.13	0.06
0.22	0.20	0.18	0.17	0.16	0.15	0.13*	0.05
0.22	0.20	0.18	0.17	0.16	0.15	0.13*	0.00
0.22	0.20	0.18	0.17	0.16	0.15	0.13	0.00*
0.22	0.20	0.18	0.17	0.16	0.15	0.13	0.00
0.22	0.19	0.18*	0.17	0.16	0.15	0.13	0.00*
0.22	0.19	0.18*	0.17	0.16	0.15	0.13	0.00*
0.22	0.19	0.18	0.17	0.15	0.15	0.13	0.00*
0.22	0.19	0.18	0.16	0.15	0.14	0.13	0.00*
0.22	0.19	0.18	0.16	0.15	0.14	0.13	0.00*
0.21	0.19	0.18	0.16	0.15	0.14	0.12	0.00
0.21	0.19	0.18	0.16	0.15	0.14	0.12	0.00
0.21	0.19	0.18	0.16	0.15	0.14	0.12	
0.21	0.19	0.18	0.16	0.15	0.14	0.12	
0.21	0.19*	0.18	0.16	0.15	0.14	0.12	
0.21	0.19*	0.18	0.16	0.15	0.14	0.12	
0.21	0.19	0.18	0.16	0.15	0.14	0.12	
0.21*	0.19	0.18	0.16	0.15	0.14	0.11	
0.21	0.19	0.18	0.16	0.15	0.14	0.11	
	0.19	0.17	0.16	0.15	0.14	0.11	

* Missing second replicate.

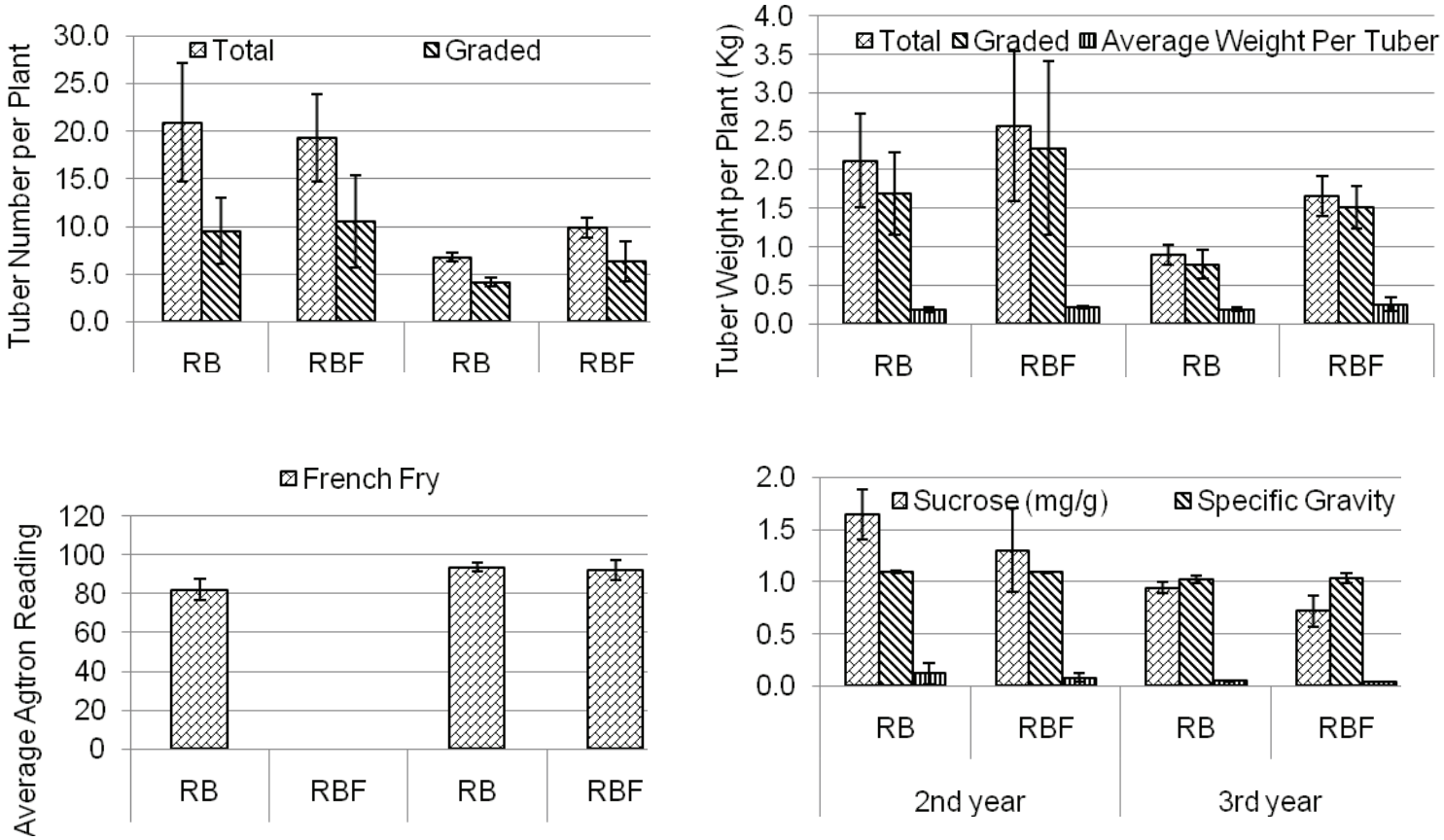

Figure A.2.1. Comparison between (A) sucrose (mgg^{-1}), glucose (%), and specific gravity, (B) French fry colour (average values of the Agtron spectrophotometer), (C) tuber weight (Kg) (total, graded, and average tuber weight), and (D) tuber number (total and graded) of 'Russet Burbank' plantlets and seed tubers after 2 years of field evaluations (2006 and 2007 field seasons).

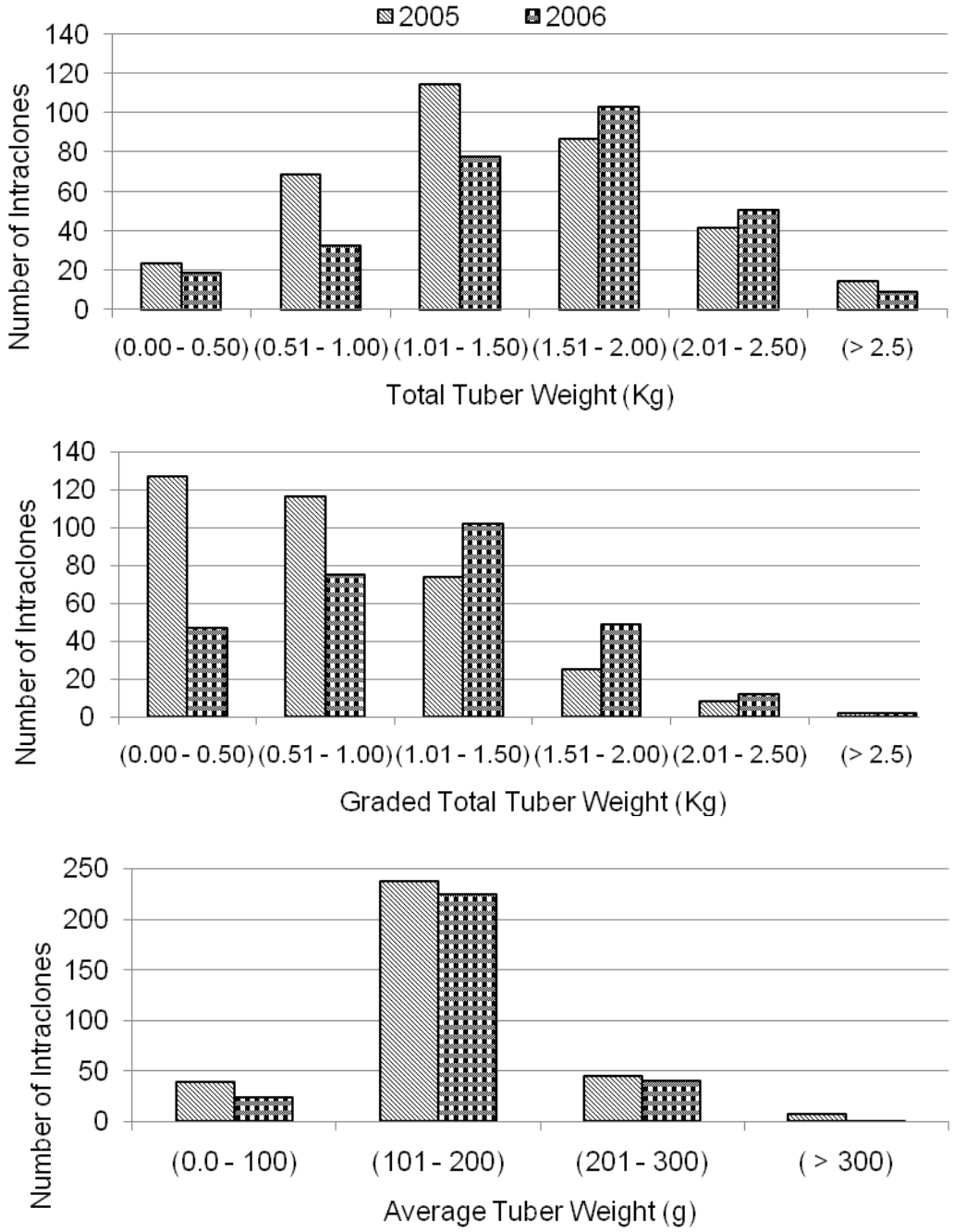

Figure A.2.2. Comparison between field results of grouped total and graded tuber weights (Kg) / intraclones and average tuber weight (g) of field-evaluated intraclones in 2005 (1-plant / plot) and 2006 (2-plants / plot).

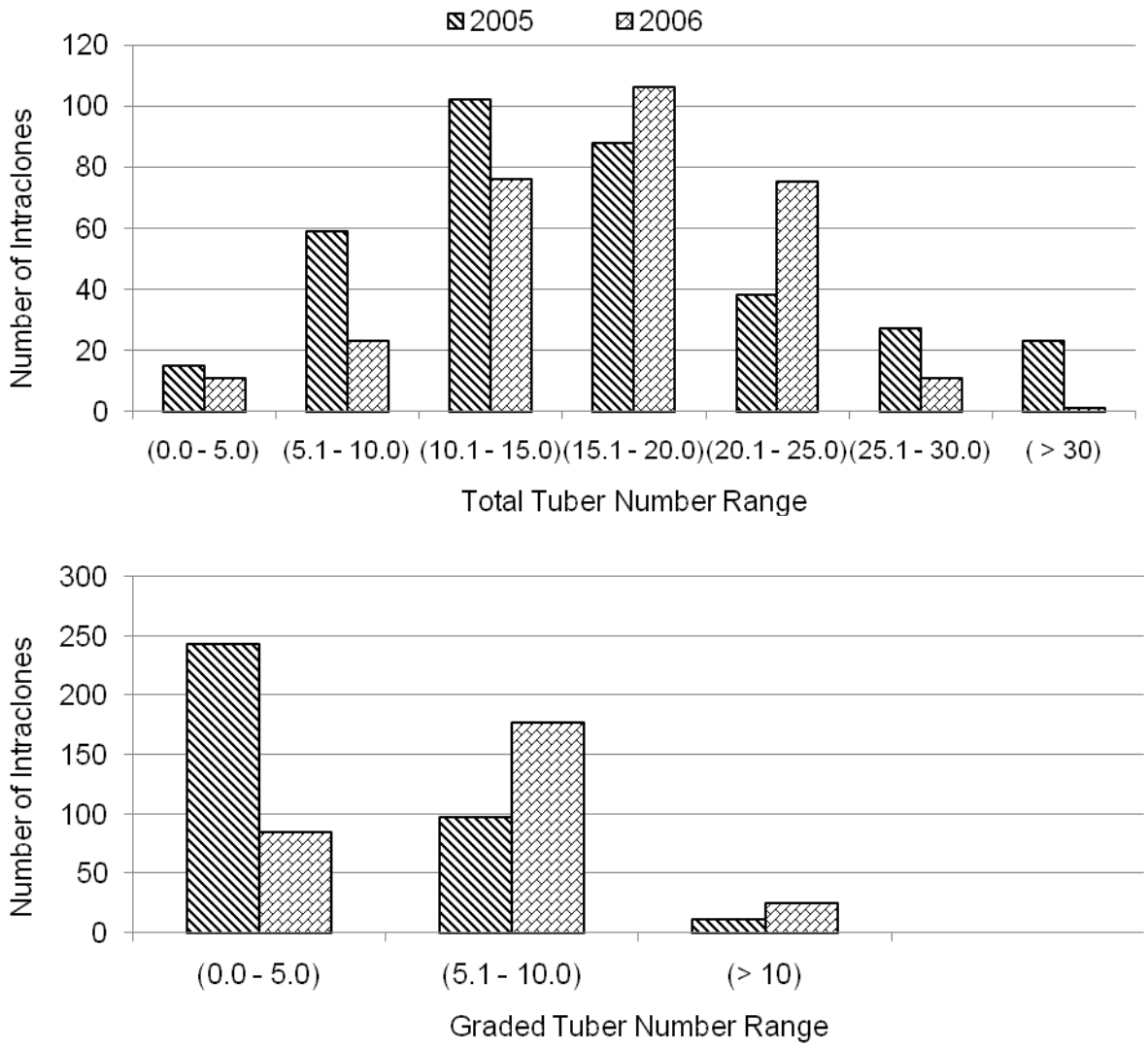

Figure A.2.3. Comparison between grouped total and graded tuber numbers/intraclones of field-evaluated intraclones in 2005 (1-plant / plot) and 2006 (2-plants / plot).