Theor Appl Genet (1999) 99: 81–91

J. A. Buso · L. S. Boiteux · S. J. Peloquin

Multitrait selection system using populations with a small number of interploid (4*x*-2*x*) hybrid seedlings in potato: Degree of high-parent heterosis for yield and frequency of clones combining quantitative agronomic traits

Received: 25 November 1998 / Accepted: 19 December 1998

Abstract One of the most attractive features of the 4*x*-2*x* breeding scheme via unilateral sexual polyploidization (USP) is the possibility of obtaining heterotic clones with a combination of desirable traits based upon selection in a small number of hybrid seedlings. A set of experiments was carried out to verify this characteristic of the USP scheme using 42 families (with 20 plants each) derived from 2*x* Phureja-haploid Tuberosum or haploid Tuberosum-*Solanum chacoense* hybrids as male parents. These clones were 2n-pollen-producers by either first-division restitution with crossing over (FDR-CO) or without crossing over (FDR-NCO). The 4*x* parents were eight cultivars of USA and European origin. A total of 168 out 840 clones was initially selected (four clones/family) based on general tuber appearance (GTA). An additional round of selection (not taking into account the parentage of the clone) for total tuber yield (TTY) reduced the sample to 96 clones. These selected clones and the original 4*x* parents were evaluated in two locations at Wisconsin (Hancock – E#1, and Rhinelander – E#2). The average high-parent heterosis values for TTY ranged from 27.8% (E#2) to 48% (E#1). The population of experimental clones also had a TTY range wider than that of the 4*x* parents. The best clone gave a yield of 101.6% and 63.2% over the best 4x parent at E#1 and E#2, respectively. For TTY, a significant genotype × environment (G × E) interaction for the experimental clones was found when a combined analysis of variance for both locations was carried out. However, the G × E interaction was not significant when only the 4*x*-parent group was considered. Clones derived from 4*x* European cultivars had higher GTA scores than clones derived from 4*x* USA cultivars. With two culling levels being set on the TTY ≥ 4*x* parent group mean and the GTA ≥ three, about 56% (E#1) and 48% (E#2) of the clones would be retained for further evaluation. These percentages of selected clones are much higher than those reported using conventional 4*x*-4*x* crosses. Our results indicate that a USP strategy with 4*x*-2*x* (FDR) crosses would be more effective than intra-Tuberosum crosses in generating heterotic clones with a combination of desirable quantitative traits using populations with a small number of hybrid seedlings.

Key words Clonal selection · First-division restitution with crossing over (FDR-CO) · *Solanum chacoense* · *Solanum phureja* · Unilateral sexual polyploidization · 2n-gametes

Communicated by G. Wenzel

J. A. Buso · L. S. Boiteux
Centro Nacional de Pesquisa de Hortaliças (CNPH)/EMBRAPA – C.P. 218, 70359-970 Brasilia-DF, Brazil

S. J. Peloquin (✉)
Department of Horticulture, 1575 Linden Drive, University of Wisconsin-Madison, WI 53706-1590, USA

Introduction

The traditional 4*x*-4*x* potato (*Solanum tuberosum* L.) breeding scheme has been characterized as a method for handling large clonal populations in order to find a few agronomically superior segregant individuals (Simmonds 1969). The often low-frequency of selected clones following 4*x*-4*x* crosses is due, in part, to the utilization of closely related parents (Mendoza and Haynes 1974a; Douches et al. 1996). The use of genetically related cultivars, while facilitating the selection process for some qualitative traits, has imposed a drastic limitation on the rate of improvement for both yield and stability in the cultivated potato (Mendoza and Haynes 1974a; Mendoza and Sawyer 1985). Another possible factor associated with the low frequency of superior clones in the conventional 4*x*-4*x* breeding scheme may be caused by disruption, during normal meiosis, of favorable interlocus interactions present in the high-yield 4*x* parents (Peloquin et al. 1989 a). This can have negative consequences, especially in the 4*x* potato, since total tuber yield has been reported as predominantly controlled by non-additive genetic effects (Mendoza and Haynes 1974 b; Mendiburu et al. 1974; Mendiburu and Peloquin 1977 b).

In this context, the unilateral sexual polyploidization (USP) with 4*x*-2*x* crosses (Mendiburu and Peloquin 1976) offers an alternative scheme to the traditional 4*x*-4*x* method in potato breeding programs. In this 2n-pollen-based strategy, the 4*x* parent is an adapted cultivar (or an advanced selection) and the 2*x* parent is a haploid Tuberosum-diploid species hybrid. The 2x male parent is a 2n-pollen-producer by either first-division restitution with crossing over (FDR-CO) or without crossing over (FDR-NCO) (Hermundstad and Peloquin 1987; Peloquin et al. 1989 b). Through an abnormal meiotic process, conditioned by the presence of the mutant allele *ps* ('parallel spindles'), a diploid FDR-CO clone will produce 2n-pollen grains able to transmit approximately 80% of the heterozygosity to the offspring and with a large proportion of epistatic (interlocus) interactions (Hermsen 1984; Barone et al. 1995). Through an association of two meiotic mutant alleles, *ps* and *sy-3* (= synaptic 3), FDR-NCO gametes will transmit almost 100% of the heterozygosity and epistasis found in the 2*x* parent to the progeny (Peloquin 1983). Therefore, both types of FDR 2n-gametes are considered unique breeding tools, since they can transfer not only additive effects but also dominance and epistatic effects (Tai 1994).

The potential value of the USP breeding scheme for potato improvement has been previously demonstrated by the often high degree of heterosis for tuber yield observed in several crosses involving Phureja-haploid Tuberosum and haploid Tuberosum-*S. chacoense* hybrids (Bani-Aameur et al. 1991; Tai 1994; Clulow et al. 1995). However, one of the most attractive features of the USP strategy has yet to be extensively evaluated: the possibility of obtaining heterotic clones with a combination of desirable quantitative traits using relatively small 4*x*-2*x* populations (Ortiz et al. 1991; Peloquin and Ortiz 1992). Handling small clonal populations (with a high frequency of potentially superior clones during the selection stage) has obvious practical advantages in a plant-breeding perspective. In the traditional 4*x*-4*x* scheme only a very small fraction of a large initial seedling population remains at the stage of evaluation as potential cultivars (Simmonds 1969; De Jong et al. 1981).

Presently, standard selection programs in potato breeding include simultaneous evaluation of a large collection of traits (De Jong and Tarn 1984; Douches et al. 1996). Therefore, a more-efficient breeding scheme would be the one in which a large proportion of the segregating clones contains a desirable combination of multiple traits. The present work provides additional evidence that the USP scheme can be an efficient strategy for multitrait selection in potato using relatively small populations of hybrid clones.

Materials and methods

Plant material

A total of 840 clones (from 42 families with 20 plants each) obtained from a diverse range of 4*x*-2*x* crosses were grown on the UW-Lehah Stark Potato Breeding Farm at Rhinelander-Wisconsin (Wis). Some of the 4*x* parents used in these 4*x*-2*x* crosses were cultivars and/or advanced clones of North American origin: 'New Haig', 'Superior', 'New Superior', 'W 231', 'W 639', 'W 785'. Two European cultivars, 'Desiree' and 'Spunta', were also used as 4*x* parents. The diploid parents were nine 2n-pollen-producer clones formed by either the FDR-CO or the FDR-NCO mechanism. These 2*x* clones were Phureja-haploid Tuberosum FDR-CO ('H-2', 'H-3', 'H-4', 'H-7', 'M-5', 'L-10', 'L-11', and 'J'); Phureja-haploid Tuberosum FDR-NCO ('SY-8'), and haploid Tuberosum-*S. chacoense* FDR-CO ('T-704') hybrids.

Selection criteria and selection intensity for the second clonal generation

A total of 168 out of 840 clones (four clones per family with an intensity of selection of 20% within families) were selected based on general tuber appearance (GTA) in one replication of each of the previous set of three experiments (Buso 1986). The tubers of these clones were maintained separately. Later, another selection was done for total tuber yield (TTY) based on a subjective scale from 1 = low to 5 = high yield. Ninety six out of 168 clones were maintained for field evaluation (i.e., a selection intensity of 57.14%). In this later selection, the parentage of the clone was not taken into consideration.

Field assays

All 96 selected clones and the eight original 4*x* parents were evaluated at two locations: Hancock-Wis (UW Research Station) and Rhinelander-Wis (UW-Lelah Stark Potato Breeding Farm). A randomized complete block design with two replications (four hills/plot) was used in each location. At Hancock, the spacing was 0.91 m by 0.30 m between and within rows, respectively. At Rhinelander, the spacing was 0.91 m by 0.41 m between and within rows, respectively. Planting and harvesting dates were April 27 and September 25 for Hancock and May 24 and September 24 for Rhinelander, respectively. All harvesting was done by hand. The plots were irrigated three times a week at Hancock. At Rhinelander, the plots were only rainfed, with no supplementary irrigation being provided during the entire course of the experiment.

Trait assessment

Each entry (original 4*x* parents and selected hybrid clones) was evaluated at both locations (Hancock and Rhinelander) for the following five traits. (1) Total tuber yield (TTY). Evaluation was performed with selected tubers (only those over 2.5 cm diameter were weighed). Plot averages (expressed in kg/hill) were used for analysis. (2) General tuber appearance (GTA). This trait was evaluated visually at harvesting using a semi-quantitative scale from 1 = poor to 5 = excellent, with 0.5 increments. GTA assessment reflects the overall number, size, color, uniformity, and shape regularity of tubers. (3) Haulm maturity (HM). This trait was estimated as the number of days from the planting date until the date when 50% of the plants in the plot were completely senesced. (4) Plant vigor (PV). This trait was assessed visually based on the vine appearance using a scale from 1 = poor to 5 = very good. (5) Eye depth (ED). This was scored visually, on a per-plot basis, using a scale from 1 = shallow to 5 = very deep.

Analysis of variance

The source of variation among clones with 103 degrees of freedom (*df*) was subdivided into: (1) among clones from 4*x*-2*x* crosses with 95 *df*; (2) among 4*x* parents with 7 *df*, and (3) a contrast between the two group means. This contrast is a measure of the superiority or inferiority of the selected clonal population over the group of 4*x* parents. The source of variation among clones from the 4*x*-2*x* crosses was further subdivided into the following components: among clones from the 4*x* parents of North American origin (74 *df*), and among clones from the 4*x* parents of European origin (20 *df*), as well as a contrast between the two groups.

Genotype by environment interaction

A combined analysis of variance considering both locations was carried out. If the error variances of analysis in each environment were heterogeneous, the mean square of the pooled error was still used to test the clone by location interaction and its components. However, the degrees of freedom of the *F*-test for clone by environment interaction was that observed in the experiment with the largest mean square (MS) error according to Cochran and Cox (1957).

Selection using independent culling levels for TTY and GTA

A frequency distribution of clones was carried out by considering two traits: TTY and GTA. The frequency of clones with TTY significantly above the mean of the 4*x* parents was estimated at each location. For GTA, the frequency of clones with no significant difference from the 4*x* parents was estimated at each location. The frequency estimations were done using as criteria the 4*x*-parent group mean plus the Least Square Difference (LSD) at the 5% level, or the 4*x*-parent group mean minus the LSD at the 5% level, for TTY and GTA, respectively.

Results and discussion

Degree of heterosis and performance of 4*x*-2*x*-derived clones for total tuber yield

The means and ranges of TTY for the group of 96 experimental clones and the group of eight 4*x* parents at both locations are presented in Table 1. The contrast clones vs. 4*x* parents was significant at both locations (Tables 2 and 3). The estimated heterosis values for TTY were 48% and 27.8% over the mean of the 4*x* group at Hancock and Rhinelander, respectively. The population of experimental clones also had a wider TTY range when compared with the 4*x* group at both locations (Table 1). This wide TTY range probably reflects the lack of selection effectiveness for yield from single hills in the first clonal generation (Maris 1966; Kichefski et al. 1976; Tai and Young 1984).

The range of trait expression is also a meaningful measure when comparing a group of genotypes from an asexually propagated crop such as potato. A single superior genotype for TTY once identified can be immediately multiplied. In this regard, the best experimental clone yielded 101.6% and 63.2% over the best 4*x* parent at Hancock and Rhinelander, respectively (Tables 4 and 5). The hybrid clones responded well to the very favorable growing conditions in Hancock. In the more stressful environment of Rhinelander, where there was no irrigation, the best clone from the 4*x*-2*x* crosses still yielded 63.2% more than the most-productive 4*x* parent. Drought is an important abiotic stress that depresses yield in potato (Wissar and Ortiz 1988). The Rhinelander results were significant, since none of the entries (experimental clones or 4*x* parents) had been selected for drought tolerance.

The heterosis values of the clones for TTY were similar to previous studies comparing 4*x*-2*x* versus

Table 1 Means and ranges of total tuber yield (kg/hill) and general tuber appearance of tetraploid parents and experimental clones (4*x*-2*x* clones) derived from crosses using 4*x* parents from either USA or European origin, at Hancock and Rhinelander-Wisconsin

Genotype	# Entries	Total tuber yield (kg/hill)		General tuber appearance[a]	
		Mean	Range	Mean	Range
Hancock					
4*x*-2*x* clones	96	2.34	0.94–3.89	2.99	1.00–4.50
4*x*-2*x* clones (USA-origin)	75	2.28	0.94–3.89	2.88	1.00–4.25
4*x*-2*x* clones (European-origin)	21	2.53	1.48–3.55	3.38	2.25–4.50
4*x* parents	8	1.58	0.99–1.93	3.81	3.00–4.50
Rhinelander					
4*x*-2*x* clones	96	1.15	0.49–2.04	2.97	1.50–4.50
4*x*-2*x* clones (USA-origin)	75	1.14	0.49–2.04	2.91	1.50–4.25
4*x*-2*x* clones (European-origin)	21	1.19	0.88–1.65	3.18	1.50–4.50
4*x* parents	8	0.90	0.26–1.25	3.97	3.50–4.75

[a] General tuber appearance: based on a scale from 1 = poor to 5 = excellent

Table 2 Analysis of variance of total tuber yield, haulm maturity, plant vigor, eye depth and general tuber appearance of 4*x*-parents and 96 experimental 4*x*-2*x* clones obtained from crosses using 4*x* parents of either USA or European origin, Hancock-Wisconsin

Source of variation	*df* [a]	Mean squares				
		Total tuber yield	Haulm maturity	Plant vigor	Eye depth	General appearance
Blocks	1	7.031	0.011	0.077	0.043	4.043
4*x*-2*x* clones and 4*x* parents (i.e., all entries)	103	1.155**	0.724**	0.485**	0.886**	1.036**
4*x*-2*x* clones	95	1.149**	0.730**	0.453*	0.920*	0.966**
4*x*-2*x* clones (USA-origin)	74	1.208**	0.805*	0.473*	1.003**	0.886**
4*x*-2*x* clones (European-origin)	20	0.880**	0.469**	0.395	0.395	0.914**
4*x*-2*x* clones (USA) vs 4*x* (European)	1	2.099**	0.383	0.122	5.225**	7.859**
4*x* parents	7	0.188	0.500*	0.857**	0.143	0.705
4*x*-2*x* clones vs 4*x* parents	1	8.578**	1.772**	0.923°	2.845**	10.002**
Error	103	0.246	0.220	0.271	0.267	0.439
CV (%)		21.76	15.26	14.87	31.13	21.70

°,*,** Significant at the 10%, 5% and 1% levels, respectively
[a] *df* = degrees of freedom

Table 3 Analysis of variance of total tuber yield, haulm maturity, plant vigor, eye depth and general tuber appearance of 4*x*-parental clones and 96 experimental 4*x*-2*x* clones obtained from crosses using 4*x* parents of either USA or European origin, Rhinelander-Wisconsion

Source of variation	*df* [a]	Mean squares				
		Total tuber yield	Haulm maturity	Plant vigor	Eye depth	General appearance
Blocks	1	1.898	0.019	8.889	3.769	0.059
Clones and 4*x* parents (i.e., all entries)	103	0.179**	0.765**	0.916**	0.628**	1.077**
4*x*-2*x* clones	95	0.169**'	0.774**	0.921**	0.642**	0.979**
4*x*-2*x* clones (USA-origin)	74	0.188**	0.878**	1.028**	0.738**	0.962*
4*x*-2*x* clones (European-origin)	20	0.103*	0.424**	0.545°	0.264	0.977
4*x*-2*x* clones (USA) vs 4*x* clones (European)	1	0.072	0.045	0.532	4.833**	2.367*
4*x* parents	7	0.203**	0.705**	0.705°	0.107	0.444
4*x*-2*x* clones vs 4*x* parents	1	0.954**	0.337	1.963*	2.963**	14.769**
Error	103	0.057	0.165	0.355	0.362	0.590
CV (%)		21.07	12.2	16.92	39.08	25.23

°,*,** Significant at the 10%, 5% and 1% levels, respectively
[a] *df* = degrees of freedom

4*x*-4*x* progenies (Sanford and Hanneman 1982; Tai 1994). The high-parent heterosis values for TTY are striking when compared with results obtained with the traditional 4*x*-4*x* breeding scheme (even when 4*x* parents with a broad genetic base were used). For example, non-selected F_1 hybrids between the *S. andigena* (Andigena) population and Tuberosum had 17% more TTY than non-selected clones obtained from intra-Tuberosum crosses (Cubillos and Plaisted 1976). In this case, the Andigena population had five cycles of recurrent selection for adaptation to North temperate growing conditions. Similarly, Tarn and Tai (1977) reported 15% heterosis over a 4*x*-4*x* population for TTY, when five Tuberosum clones were crossed with five adapted Andigena parents. Even though the 4*x* parents used in the present study were highly selected for several traits, including TTY, is very unlikely that the clonal population obtained by intercrossing them would have a superior heterosis value to that observed here with the progenies from 4*x*-2*x* crosses. In fact, previous reports with the conventional 4*x*-4*x* crosses have shown that the clones obtained, on average, are not superior to the parent in yield (Maris 1966; Quin and Peloquin 1973; Tarn and Tai 1983).

The sample of 4*x*-2*x* clones evaluated at the second clonal generation might be considered almost as a random sample from the first clonal generation and, consequently, the seedling population for TTY. This can be assumed as true, especially because of the unreliability of early selection for TTY and the low intensity of selection applied under our experimental conditions (57%). Thus, it appears that the original population of seedlings generated by 4*x*-2*x* breeding scheme had a high frequency of high-yielding clones. Related to this point, it must be emphasized that the population size from which the high-yielding experimental clones were derived was relatively small (840 individuals). In this regard, the results reported here are in agreement with

Table 4 Pedigree and characteristics of the 20% higher-yielding 4*x*-2*x* clones grouped by origin of their 4*x* parents as well as the original 4*x*-parent group evaluated at Hancock. For trait evaluation see Materials and methods

Pedigree	Means				
	Total tuber yield	General tuber appearance	Plant vigor	Plant maturity	Eye depth
4*x*(USA)-2*x* clones					
[New Haig × L-11] (#1)	3.89	3.50	4.00	3.50	1.00
[New Haig × M-5] (#1)	3.75	3.00	4.00	3.25	2.50
[New Haig × M-5] (#4)	3.69	3.25	4.00	3.00	3.00
[New Haig × L-11] (#3)	3.60	2.50	4.50	5.00	2.00
[Superior × L-11] (#3)	3.58	3.00	4.00	4.00	2.00
[W231 × H-4] (#3)	3.52	3.50	3.50	2.75	1.50
[W639 × L-11] (#2)	3.32	3.75	3.50	3.25	1.00
[New Haig × M-5] (#2)	3.32	2.00	4.00	4.00	2.50
[W785 × H-7] (#2)	3.29	3.50	3.50	3.25	2.00
[New Haig × H-7] (#4)	3.23	1.50	5.00	4.50	3.00
[New Haig × H-4] (#2)	3.23	1.50	3.50	3.00	3.50
[W231 × L-11] (#1)	3.23	2.25	3.50	4.25	1.00
[New Superior × L-11] (#4)	3.21	3.50	4.50	4.50	1.00
[W639 × J] (#2)	3.18	3.00	3.00	2.75	2.50
[New Superior × L-11] (#2)	3.14	3.00	4.00	3.00	1.00
4*x*(Europe)-2*x* clones					
[Desiree × H-3] (#4)	3.55	4.25	3.50	3.50	1.00
[Desiree × H-3] (#3)	3.55	4.00	3.50	3.50	1.00
[Spunta × L-11] (#4)	3.46	2.25	4.00	3.75	1.50
[Spunta × L-11] (#2)	3.38	2.50	3.50	3.00	1.50
[Spunta × H-3] (#1)	3.06	3.50	3.50	3.00	1.00
4*x*-parents					
New Haig (USA)	0.99	3.00	3.00	3.00	1.50
Superior (USA)	1.59	3.50	2.50	2.50	1.50
New Superior (USA)	1.93	4.30	4.00	3.50	1.50
W231 (USA)	1.67	3.00	3.00	2.50	1.00
W639 (USA)	1.79	4.50	3.00	2.50	1.50
W785 (USA)	1.70	4.30	2.50	2.50	1.00
Spunta (Europe)	1.25	3.80	4.00	3.30	1.00
Desiree (Europe)	1.68	4.30	4.00	3.30	1.00

previous reports with distinct 4*x*-2*x* populations evaluated in a large range of environments (De Jong et al. 1981; Concilio 1985; Buso 1986).

An increase in the frequency of high-yielding clones following 4*x*-2*x* (FDR-CO/FDR-NCO) crosses could be explained by an increase in the frequency of individuals with an intra-locus interaction of high order in the population. In fact, intra-locus interaction of a high order has been considered as one of the fundamental factors controlling yield in polysomic polyploids (Mendiburu et al. 1974; Mendoza and Haynes 1974 b). In addition, this high frequency of high-yield clones is also the expected outcome according to theoretical models, where higher frequencies of triplex, duplex, and monoplex genotypes will be observed in the 4*x*-2*x* progenies when compared with that of the progenies from conventional 4*x*-4*x* crosses (Tai 1994).

Performance for TTY of clones grouped by the 4*x* parent

Each tetraploid parent gave rise to a variable number of clones that were higher yielding than their parent at both locations (Table 6). Even though each parent was not crossed with the same set of 2*x* clones, these frequencies may indicate where to concentrate efforts in an applied breeding program. Tetraploid parents such as 'New Superior', 'New Haig', 'Spunta' and 'Desiree', with high frequencies of high-yielding clones, would be the parental materials of choice to obtain larger seeding populations if another cycle of selection were to be initiated. The presence of higher frequencies of high-yielding clones in crosses involving European cultivars was a somewhat expected outcome. The 2*x* Phureja-haploid Tuberosum clones used here originated from interspecific crosses involving only North American cultivars as a Tuberosum background. Our results suggest that even small levels of genetic diversity via unrelated Tuberosum 4*x* parents could increase TTY performance in 4*x*-2x crosses. The importance of using genetically unrelated parents in potato breeding has been emphasized in the literature (Mendiburu et al. 1974; Mendoza and Haynes 1974 a; Peloquin 1983).

The introgression of genetic diversity using 2*x* clones with 2n-pollen production by mechanisms akin to FDR may also play a key role in the observed increase in the yield ability of the hybrid clones. In FDR gametes, a large fraction of the original heterozygosity and epistasis is transmitted to the progeny without

Table 5 Pedigree and characteristics of the 20% higher yielding 4*x*-2*x* clones grouped by origin of their 4*x* parents as well as the original 4x-parent group evaluated at Rhinelander. For trait evaluation see materials and methods

Pedigree	Means				
	Total tuber yield	General tuber appearance	Plant vigor	Plant maturity	Eye depth
4*x*(USA)-2*x* clones					
[New Haig × L-11] (#4)	2.04	2.50	4.50	4.00	2.50
[New Haig × M-5] (#4)	1.82	2.75	4.00	4.00	2.50
[New Superior × L-11] (#1)	1.70	2.50	4.00	4.00	2.50
[New Haig × M-5] (#1)	1.65	2.50	5.00	4.00	2.50
[W785 × H-4] (#1)	1.65	4.00	4.50	4.00	1.00
[New Superior × H-3] (#1)	1.59	1.50	4.00	3.50	2.00
[W639 × H-3] (#1)	1.56	3.00	5.00	4.00	1.50
[W231 × H-4] (#1)	1.54	3.50	3.50	3.00	1.50
[W231 × H-4] (#3)	1.54	3.00	3.50	3.00	2.50
[Superior × H-7] (#2)	1.50	2.50	3.50	4.00	3.00
[New Haig × H-4] (#1)	1.42	2.50	4.00	4.00	2.00
[New Haig × H-4] (#3)	1.40	2.50	4.50	4.00	3.00
[W231 × L-10] (#4)	1.39	4.00	3.50	3.00	1.00
[W639 × J] (#2)	1.37	2.50	4.00	3.00	2.50
[W231 × H-3] (#3)	1.37	4.00	2.50	3.00	1.00
4*x*(Europe)-2*x* clones					
[Desiree × H-3] (#4)	1.65	4.00	3.50	4.00	1.00
[Spunta × H-3] (#2)	1.59	4.00	3.50	3.00	1.00
[Desiree × SY-8] (#2)	1.56	3.00	3.50	3.50	2.00
[Desiree × H-3] (#1)	1.48	2.50	4.00	3.50	1.50
[Desiree × H-3] (#3)	1.37	2.75	4.00	4.00	1.50
4*x*-parents					
New Haig (USA)	1.08	3.50	3.50	3.00	1.50
Superior (USA)	0.91	4.00	3.00	2.00	1.00
New Superior (USA)	1.08	4.50	3.50	3.50	1.00
W231 (USA)	0.74	4.00	3.00	3.00	1.00
W639 (USA)	1.14	4.80	4.00	3.00	1.00
W785 (USA)	0.74	4.00	3.00	3.00	1.00
Spunta (Europe)	0.26	3.50	2.00	4.00	1.00
Desiree (Europe)	1.25	3.50	3.50	3.50	1.50

Table 6 Means and ranges of total tuber yield –TTY- (kg/hill) of experimental clones, grouped by the 4*x* parent and 4*x* parent means at Hancock (H) and Rhinelander (R)-Wisconsin

4*x* parent	4*x*-parent means		4*x*-2*x* clones						
	H	R	# Clones	TTY means		TTY ranges		Percent over 4*x* parent mean + LSD (5%)	
				H	R	H	R	H	R
W231 (USA)	1.67	0.74	15	2.05	1.19	0.94–3.52	0.74–1.54	20.0	46.7
W639 (USA)	1.79	1.14	7	2.15	1.15	1.05–3.32	0.83–1.56	28.6	0.0
W785 (USA)	1.70	0.74	16	2.00	1.01	0.96–3.29	0.49–1.65	12.5	25.0
Superior (USA)	1.59	0.91	8	2.13	1.15	1.33–3.58	0.85–1.50	25.0	12.5
New Superior (USA)	1.93	1.08	7	2.58	1.24	1.33–3.21	0.82–1.70	42.8	28.6
New Haig (USA)	0.99	1.08	22	2.65	1.17	1.33–3.89	0.68–2.04	72.7	13.7
Spunta (Europe)	1.25	0.26	10	2.44	1.17	1.48–3.46	1.00–1.59	50.0	100.0
Desiree (Europe)	1.68	1.25	11	2.62	1.21	1.59–3.55	0.88–1.65	45.5	0.0

disruption by meiosis. As a consequence, the recovery of polyploidy in the progeny per se can allow the establishment of novel intra- and inter-locus interactions. In this context, the increased yielding ability could be explained by the transmission the original intra-locus interactions from the 2*x* parent, undisturbed through the mode of 2n gamete formation, and the formation of new and favorable epistatic interactions in the hybrid progeny.

Several reports have indicated that non-additive genetic variance is the preponderant factor in determining TTY in tetraploid potato (Plaisted et al. 1962;

Mendiburu et al. 1974; Mendoza and Haynes 1974 b; Mendiburu and Peloquin 1977). Subsequent experiments, using yield components, indicated that the TTY increase in potato following 4*x*-2*x* crosses was due to an increase in the total number of tubers with a slight decrease in tuber weight (De Jong and Tai 1977). In addition, it was apparent that the heterozygosity/epistasis hypothesis and the yield-component hypotheseis could not be considered as mutually exclusive (De Jong and Tai 1977). However, regardless of the mechanism involved, in a practical potato breeding perspective there is a clear advantage to the fact that this crop is vegetatively propagated. In this particular case, all types of genetic effects (i.e., additive, digenic, trigenic, tetragenic and epistasis) can be fixed and, superior clones can be selected regardless of the magnitude of each individual effect.

Genotype by environment interaction for TTY

The number of clones with TTY significantly above their 4*x* parents was variable for each female parent at each location. These differences can be explained by the presence of large G × E interaction, which is a common feature influencing TTY expression in potato (Simmonds 1969, 1981; Tai and Young 1972; Mendoza and Saywer 1985). Indeed, a highly significant G × E interaction for the experimental clones was found when a combined analysis of variance for TTY for both locations was carried out (Table 7). However, the G × E interaction was not observed when only the 4*x* parents were considered. The source of variation [(clones vs 4*x* parents) × location] being highly significant, indicates that the differences of the clonal and 4*x* parent populations for TTY will be variable depending upon the location.

The presence of G × E interaction indicates that selection of the most suitable clone will be effective only if performed at the specific target environment. However, some clones with a potentially broad range of adaptation ('individual buffering') were identified in our study. For example, clone #4 from the cross ('New Haig' × 'M-5'] yielded more than the respective 4*x* parent in each location (Tables 4 and 5). However, in general, it was found that the experimental clones had a high yield in more-favorable (E # 1) and a lower yield in less-favorable (E # 2) environments, but almost always had a higher yield than the 4*x* parents did in both locations (Tables 4 and 5). Stability of performance and the ability to respond to a good environment are among the basic requirements necessary for a successful potato cultivar (Tai 1971; Simmonds 1981; Tai and Young 1984).

Performance of 4*x*-2*x* clones for GTA

The means and ranges for GTA are presented in Table 1. The group of 4*x* parents had a 27% and a 34% higher GTA mean score than the mean of the clonal populations at Hancock and Rhinelander, respectively. These differences were highly significant at both locations (Tables 2 and 3). Thus, the parents on average had tubers of better appearance than the hybrid clones. However, this was not an unexpected outcome. The fact that the 4*x* parents are cultivars or advanced clones should be taken into accout to explain these differences in GTA scores. The 4*x* parents represent an elite group of cultivars and clones, highly selected for this trait.

It was interesting to note that experimental clones with a genetic contribution from European 4*x* cultivars had, as a group, significantly higher GTA scores in both locations when compared with clones derived from crosses with USA parents (Table 1). It seems that European cultivars will have priority when establishing a 4*x*-2x breeding program where a high GTA score is a crucial consideration. Some sophisticated markets have very restricted tuber appearance standard (e.g., shallow eyes, medium-large tubers, uniform tuber size

Table 7 Combined analysis of variance of total tuber yield and general tuber appearance of experimental clones from 4*x*-2*x* crosses and tetraploid parents, at Hancock and Rhinelander

Source of variation	*df* [a]	Mean squares	
		Total tuber yield	General tuber appearance
Location	1	137.115	0.005
Blocks/location	2	4.602	2.051
Clones	103	0.881**	1.511**
4*x*-2*x* clones	95	0.856**	1.318**
4*x* parents	7	0.258	0.847
4*x*-2*x* clones vs 4*x* parents	1	7.626**	24.539**
Clones vs location	103	0.453**	0.601**
4*x*-2*x* clones vs location	95	0.461**	0.627**
4*x* parents vs location	7	0.133	0.303
(4*x*-2*x* clones vs 4*x* parents) vs location	1	1.905**	0.262
Pooled error	206	0.152	0.257

[a] *df* = degrees of freedom

Table 8 Means and ranges general tuber appearance -GTA- of experimental clones, grouped by their 4*x* parents and 4*x*-parent means, at Hancock (H) and Rhinelander (R), Wisconsin

4*x* parent	4*x*-parent mean		4*x*-2*x* clones						
	H	R	# Cones	GTA means		GTA ranges		% not significant different 4*x*-parent	
				H	R	H	R	H	R
W231	3.00	4.00	15	2.90	3.10	2.00–3.75	2.00–4.00	100.0	80.0
W639	4.50	4.75	7	3.25	2.93	1.50–3.75	2.50–3.50	71.4	14.3
W785	4.25	4.00	16	2.88	2.97	1.00–4.25	1.50–4.00	56.3	81.3
Superior	3.50	4.00	8	2.84	2.78	2.25–4.00	2.00–400	100.0	87.5
New Superior	4.25	4.50	7	2.89	2.68	2.00–3.50	1.50–3.50	57.1	42.9
New Haig	3.00	3.50	22	2.76	2.85	1.50–3.50	1.50–4.25	90.9	95.5
Spunta	3.75	3.50	10	3.28	3.28	2.25–4.25	1.50–4.50	90.0	90.0
Desiree	4.25	3.50	11	3.48	3.09	2.50–4.50	2.50–4.00	81.8	100.0

and shape). In addition, from the farmer's perspective, genetic material with this type of visual attribute can result in improved profit levels.

GTA scores according to the 4*x* parents

The means and ranges of GTA grouped by the 4*x* parent at both locations are presented in Table 8. In the GTA case, the percentage of clones not significantly different from the 4*x* parent was considered. This would be the case when the breeder would like to have a new clone that has at least as good a GTA as the 4*x* parent). This seems to be the situation in the majority of breeding programs, where the GTA value is the determinant factor for the commercial acceptance of the cultivar. Variable frequencies of clones without a difference from the 4*x* parents were observed in both locations (Table 8). G × E interaction could be responsible for this discrepancy (Table 7).

The GTA means of clonal groups from European parents were consistently over 3, a value that has been considered a limiting level for the selection of this trait in breeding programs (Buso 1986). A GTA score of 3 was the lowest among 4*x* parents, at both locations. Over 80**%** of clones from European 4*x* parents would be selected in the clonal population for being not significantly different from the respective parent. This high frequency was consistent regardless of the location.

Genotype by environment interaction for GTA

Similar to TTY, the presence of significant G × E interaction for GTA (Table 7) gives an indication of how difficult the selection process, will be based upon evaluation at one single location. It is probable that in a way similar to the 4*x* parents, some of the clones could have no G × E interaction for this trait (Table 7). However, as a group, it seems that the superiority of the 4*x* population over the experimental clones is not influenced by the location where the evaluation was performed. This is a plausible interpretation for the lack of significance for the source of variation (clones vs 4*x* parents) × location.

The lower means for GTA in the clonal population may be due to the presence of Phureja germplasm in one of the parents of the 2*x* hybrids used in the 4*x*-2*x* crosses. This diploid cultivated Group has, in general, some undesirable tuber traits (small-sized tubers, large tuber number, very deep eyes, raised internodes) that are known to be transmitted to the 4*x* progeny (De Jong and Tai 1977; Schroeder 1983). Another reason would be the possible low reliability of selection for this trait in the first clonal generation, as previously observed (Maris 1966). In the latter case, selection for GTA would increase the clonal population mean. A factor that should be taken into account is that the 2*x* parents were not selected for GTA or any tuber-quality trait before crossing. Previous results indicated that the 2*x*-parent mean for average tuber weight and eye depth was correlated with the family mean after 4*x*-2*x* crosses (Schroeder 1983). Therefore, a selection of 2*x* hybrid clones for these traits would probably have the positive effect of increasing the family mean for GTA. Plaisted and Cubillos (1972) indicated that an increase of average tuber size could be possible in 4*x*-4*x* crosses by selecting at least one of the parents for large tuber size. Thus, a selection for GTA at both 2*x* and 4*x* levels would increase the frequency of clones with superior GTA scores in the progeny of 4*x*-2*x* crosses. On the other hand, 2*x* clones with deep eyes, and consequently low GTA scores, could not be completely eliminated from the crossing plan if other traits of importance are present in these parents. As an example, 'H-7', a very deep-eyed 2*x* clone, was able to transmit high yield ability to several clones in its progenies with

Table 9 Means and ranges of haulm maturity, plant vigor, and eye depth of experimental clones from 4*x*-2*x* crosses and tetraploid parents, at Hancock and Rhinelander, Wisconsin

Type	# Entries	Haulm maturity[a]		Plant vigor[b]		Eye depth[c]	
		Mean	Range	Mean	Range	Mean	Range
Hancock							
4*x*-2*x* clones	96	3.10	2.00–5.00	3.50	2.50–4.50	1.69	1.00–3.50
4*x* parents	8	2.75	2.25–3.50	3.25	2.50–4.00	1.25	1.00–1.50
Rhinelander							
4*x*-2*x* clones	96	3.34	2.00–5.00	3.55	1.50–5.00	1.57	1.00–3.00
4*x* parents	8	3.63	2.00–4.00	3.25	2.00–4.00	1.13	1.00–1.50

[a] Haulm maturity: based on a scale from 1 = senesced to 5 = still flowering
[b] Plant vigor: based on a scale from 1 = poor to 5 = very good vigor
[c] Eye depth: based on a scale from 1 = shallow to 5 = very deep

GTA scores over three. In fact, 2 out of 11 'H-7'-derived clones at Hancock and 5 out of 11 at Rhinelander were in that combined category (i.e., high yield + a GTA score over three). However, as expected, the general tendency of better-looking tubers coming from the 2*x* parent with smooth, good-sized tubers is what is noted when other diploid clones are considered. As an example, 7 out of 13 and 12 out of 13 clones were selected having a GTA score of over three at Hancock from clones with 'L-10' and 'SY-8', in their pedigree, respectively. These 2*x* clones have tubers with medium to large sizes and shallow eyes. For Rhinelander, 83% and 85% of the clones assessed from those two 2*x* parents were selected because they had GTA scores over three.

Variability for haulm maturity, plant vigor and eye depth

Analyses of variance indicated that there is variation for all five traits among clones derived from 4*x*-2*x* crosses (Tables 2 and 3). This implies that the selection of clones combining desirable attributes for all five traits would be feasible. The observed mean and phenotypic ranges for HM, PV, and ED are in accordance with this notion (Table 9). In fact, the performance of the top 20% high-yielding clones gives additional support to this view, since clones with high TTY and GTA values also displayed high values for PV as well as low to intermediate HM and ED values (Tables 4 and 5).

Efficiency of multitrait selection using independent culling levels for TTY and GTA

Modified independent culling levels would better describe the type of multitrait selection used in practical potato breeding programs (Simmonds 1981). Here, individuals that perform better than a certain level for each trait are selected; those with a performance below this level for one or more traits are rejected, even if they were very desirable for the other trait. TTY and GTA are major foci of practical breeding programs to discard clones at the selection stage.

The frequency distribution of clones considering simultaneously TTY and GTA was impressive in both Hancock (Fig. 1) and Rhinelander (Fig. 2). If both traits were considered in selection at the 4-hill stage, high frequencies of clones would be retained for evaluation in subsequent cycles of propagation and for selection for other traits. With two culling levels set at a TTY over or equal to the 4*x*-parent group mean and a GTA equal or above 3, about 56% and 48% of the clonal population would be retained, respectively, for Hancock and Rhinelander. These population fractions are much higher than those reported using the conventional 4*x*-4*x* breeding scheme (Cubillos and Plaisted 1976) where 4.1**%** of a Tuberosum-Andigena hybrid population was identified as having an acceptable tuber yield and tuber shape. In addition, with intra-Tuberosum crosses about 17% of the clones would be

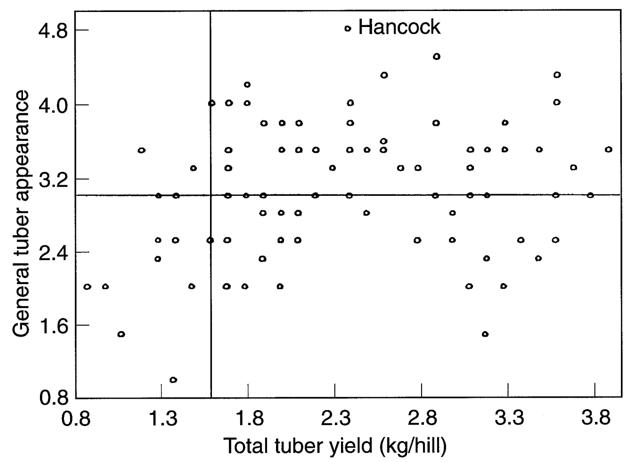

Fig. 1 Frequency distribution of 96 4*x*-2*x* clones based on total tuber yield (kg/hill) and general tuber appearance (from 1 = poor to 5 = excellent), at Hancock. *Vertical line* = 4*x* parent mean (1.59 kg/hill). *Horizontal line* = general tuber appearance = 3.0

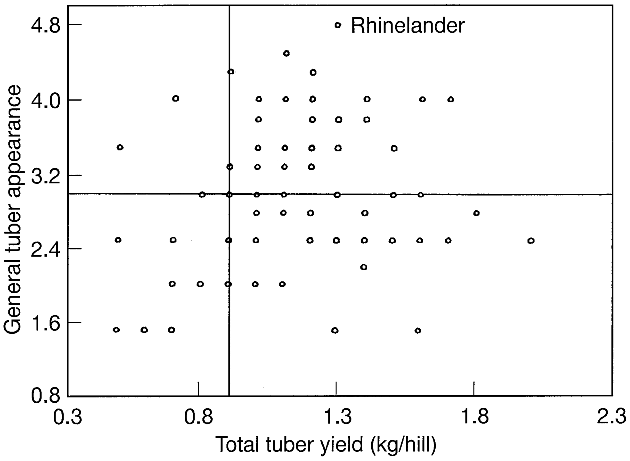

Fig. 2 Frequency distribution of 96 4*x*-2*x* clones based on total tuber yield (kg/hill) and general tuber appearance (from 1 = poor to 5 = excellent), at Rhinelander. *Vertical line* = 4*x* parent mean (0.90 kg/hill). *Horizontal line* = general tuber appearance = 3.0

retained based upon the same set of criteria (Cubillos and Plaisted 1976).

Conclusions

Our results indicated that the USP breeding scheme with 4*x*-2*x* crosses (Mendiburu and Peloquin 1977 a) is a viable alternative for a 4*x*-4*x* breeding scheme. The value of the USP breeding scheme has been previously demonstrated by the high degree of heterosis for total tuber yield observed in several crosses involving potato cultivars and Phureja-haploid Tuberosum hybrids and/or Phureja clones (for a review see Tai 1994; Ortiz and Peloquin 1994). Another attractive feature of the USP breeding scheme, also clearly demonstrated here, is the possibility of obtaining superior clones with high-yielding ability in combination with other quality traits based upon selection in relatively small 4*x*-2*x* populations (Peloquin and Ortiz 1992). This feature can maximize the resources available for breeding programs with obvious positive consequences in reducing the cost of cultivar development and simultaneously improving levels of performance for important agronomic traits when compared with the presently available 4*x* cultivars.

References

Bani-Aameur F, Lauer FI, Veilleux RE, Hilali A (1991) Genomic composition of 4*x*-2*x* potato hybrids: Influence of *Solanum chacoense*. Genome 34:413–420

Barone A, Gebhardt C, Frusciante L (1995) Heterozygosity in 2n gametes of potato evaluated by RFLP markers. Theor Appl Genet 91:98–104

Buso JA (1986) Evaluation of families and clones from the 4*x*-2*x* breeding scheme in potato. PhD dissertation, University of Wisconsin – Madison, Wisconsin, USA

Clulow SA, McNicoll J, Bradshaw JE (1995) Producing commercially attractive, uniform true potato seed progenies: the influence of the breeding scheme and parental genotype. Theor Appl Genet 90:519–525

Cochran WG, Cox GM (1957) Experimental design, 2nd edn. John Wiley & Sons, New York

Concilio L (1985) Evaluation of yield and other agronomic characteristics of TPS families and advanced clones from different breeding schemes. MS Dissertation, University of Wisconsin – Madison, Wisconsin, USA

Cubillos AG, Plaisted RL (1976) Heterosis for yield in hybrids between *S. tuberosum* ssp. *tuberosum* and *tuberosum* spp. *andigena*. Am Potato J 53:143–150

De Jong H, Tai GCC (1977) Analysis of tetraploid-diploid hybrids in cultivated potatoes. Potato Res 20:111–121

De Jong H, Tarn TR (1984) Using germplasm in potato breeding in Canada. Canada Agric 3:12–15

De Jong H, Tai GCC, Russel WA, Johnston GR, Proudfoot KG (1981) Yield potential and genotype-environment interactions of tetraploid-diploid (4*x*-2*x*) potato hybrids. Am Potato J 58:191–199

Douches DS, Maas D, Jartrzebski KR, Chase RW (1996) Assessment of potato breeding progress in the USA over the last century. Crop Sci 36:1544–1552

Hermsen, JGTh (1984) Mechanisms and genetic implications of 2n-gamete formation. Iowa State J Res 58:421–434

Hermundstad SA, Peloquin SJ (1987) Breeding at 2*x* level and sexual polyploidization. In: Jellis GJ, Richardson DR (eds) The production of new potato varieties: technological advances. Cambridge University Press, Cambridge, UK, pp 197–210

Kichefski DF, Quin AA, Peloquin SJ (1976) The effecti veness of selection during early clonal generations in varietal breeding. Am Potato J 53:370–371

Maris B (1966) The modifiability of characters important in potato breeding. Euphytica 15:18–31

Mendiburu AO, Peloquin SJ (1976) Sexual polyploidization and de-polyploidization: some terminology and definitions. Theor Appl Genet 48:137–143

Mendiburu AO, Peloquin SJ (1977 a) Bilateral polyploidization in potatoes. Euphytica 26:573–583

Mendiburu AO, Peloquin SJ (1977 b) The significance of 2n gametes in potato breeding. Theor Appl Genet 54:177–180

Mendiburu AO, Peloquin SJ, Mok DWS (1974) Potato breeding with haploids and 2n gametes. In: Kasha KJ (eds) Haploids in higher plants: advances and potential. Gueph University Press, Gueph (Ontario), Canada, pp 249–258

Mendoza HA, Haynes FL (1974 a) Genetic relationship among potato cultivars grown in the United States. HortScience 9:328–330

Mendoza HA, Haynes FL (1974 b) Genetic basis of heterosis for yield in autotetraploid potato. Theor Appl Genet 45:21–25

Mendoza HA, Saywer RL (1985) The breeding program at the International Potato Center (CIP) In: Progress in plant breeding. Russel GE (ed) Butterworth, UK, pp 117–137

Ortiz R, Peloquin SJ (1994) Use of 24-chromosome potatoes. In: Bradshaw JE, Mackay GR (eds) Potato genetics. CAB International, Wallingford, UK, pp 133–154

Ortiz R, Peloquin SJ, Freyre R, Iwanaga M (1991) Efficiency of the 4*x*-2*x* breeding scheme in potato for multitrait selection and progeny testing. Theor Appl Genet 82:602–608

Peloquin SJ (1983) Genetic engineering with meiotic mutants. In: Mulcahy DL, Ottaviano E (eds) Pollen: biology and implications for plant breeding, Elsevier Science Publishing Inc, Amsterdam, pp 311–316

Peloquin SJ, Ortiz R (1992) Techniques for introgressing unadpated germplasm to breeding population In: Stalker HT, Murphy JP (eds) Plant breeding in the 1990s. CAB International, Wallingford, UK, pp 485–507

Peloquin SJ, Yerk GL, Werner JE (1989 a) Ploidy manipulations in the potato. In: Adolph KW (ed) Chromosomes: eukaryotic, prokaryotic, and viral, vol. II. CRC Press, Boca Raton, Florida, USA, pp 167–178

Peloquin SJ, Yerk GL, Werner JE, Darmo E (1989 b) Potato breeding with haploids and 2n gametes. Genome 31 : 1000–1004

Plaisted RL, Cubillos A (1972) Selection of parents on the basis of components of yield. Am Potato J 49 : 182–186

Plaisted RL, Sanford L, Federer WT, Kerh AE, Peterson LC (1962) Specific and general combining ability for yield in potatoes. Am Potato J 39 : 185–197

Quin AA, Peloquin SJ (1973) Use of experimental tetraploids in potato breeding. Am Potato J 50 : 415–420

Sanford JC, Hanneman Jr RE (1982) A possible heterotic threshold in the potato and its implication for breeding. Theor Appl Genet 61 : 151–159

Schroeder SH (1983) Parental value of $2x$, 2n pollen clones and $4x$ cultivars in $4x \times 2x$ crosses in potato. PhD dissertation, UW-Madison, Madison, Wisconsin, USA

Simmonds NW (1969) Prospects of potato improvement. Ann Rep Scot Plant Breed Sta 48 : 291–299

Simmonds NW (1981) Principles of crop improvement. Longman, London

Tai GCC (1971) Genotypic stability analysis and its application to potato regional trails. Crop Sci 11 : 184–190

Tai GCC (1994) Use of 2n gametes. In: Bradshaw JE, Mackay GR (eds) Potato genetics. CAB International, Wallingford, UK, pp 109–132

Tai GCC, Young DA (1972) Genotypic stability analysis of eight potato varieties tested in a series of ten trails. Am Potato J 49 : 138–150

Tai GCC, Young DA (1984) Early generation selection for important agronomic characteristics in a potato breeding population. Am Potato J 61 : 419–439

Tarn TR, Tai GCC (1977) Heterosis and variation of yield components in F_1 hybrids between Group Tuberosum and Group Andigena potatoes. Crop Sci 17 : 517–521

Tarn TR, Tai GCC (1983) Tuberosum × Tuberosum and Tuberosum × Andigena potato hybrids: comparison of families and parents, and breeding strategies for Andigena potatoes n long-day temperate environments. Theor Appl Genet 66 : 87–91

Wissar R, Ortiz R (1988) Mejoramento de papa en el CIP por adaptacion a climas calidos tropicales. Research Guide of International Potato Center No. 22, Lima, Peru