Theor Appl Genet (1991) 82:430–440

THEORETICAL AND APPLIED GENETICS

Somaclonal variation in tuber disc-derived populations of potato

I. Evidence of genetic stability across tuber generations and diverse locations *

R.C. Rietveld [1,], P.M. Hasegawa [2], and R.A. Bressan [2]**

[1] Rietveld Enterprises, Inc., P.O. Box 828, Wendell, ID 83355 USA
[2] Department of Horticulture, Purdue University, W. Lafayette, IN 47907 USA

Received July 6, 1990; Accepted January 23, 1991
Communicated by A.R. Hallauer

Summary. Approximately 1,600 potato (*Solanum tuberosum* L.) plants of the cultivar 'Superior' were regenerated *in vitro* from meristems adventitiously initiated on tuber disc explants. Direct regeneration from tuber disc cells, by passing a callus intermediary, is efficient and results in low frequencies of plants with gross phenotypic aberrations. The somaclonal plant population was statistically characterized in field plots over five asexual generations and in three diverse locations. When compared in advanced generations to a large population of control plants propagated from stem cuttings, the means of the somaclonal population were significantly different, often shifted in the desirable direction, for 16 of 22 horticulturally important traits. Somaclonal population variances statistically exceeded those of the controls for 13 of the 22 traits. Regressions between consecutive tuber generations and between locations or replications (blocks) within a generation were significant in the somaclonal population for all traits analyzed. In a few instances, significant control population regressions occurred that are interpreted to be the result of non-random, non-genetic factors primarily affecting control plants of low vigor. Selected somaclones exhibiting desirable alterations for yield, tuber number and shape, and vigor were stable over more than two consecutive asexual generations.

Key words: *Solanum tuberosum* L. – Adventitious regeneration – Heritability – Statistical analyses – Quantitative traits

* Research supported by a grant from NPI, 417 Wakara Way, Salt Lake City, UT 84108
** To whom correspondence should be addressed

Introduction

Much attention has been given to somaclonal variation as a tool for plant improvement (Larkin and Scowcroft 1981; Larkin and Scowcroft 1983; Evans et al. 1984). The potential of somaclonal variation may be the greatest in asexually propagated plants and in potato, in particular, due to difficulty with integrating desirable traits into polyploid genomes. There are reports indicating variability for traits including tuber, flower, leaf, and overall foliage characteristics in potato plants regenerated from various types of tissues (Secor and Shepard 1981; Thomas et al. 1982; Sree Ramulu et al. 1983; Wheeler et al. 1985; Austin et al. 1986; Evans et al. 1986; Rietveld et al. 1987).

Despite these encouraging reports, the use of somaclonal variation techniques has been very limited in potato breeding programs. In part this may be due to conflicting reports on its potential to improve commercially important characteristics such as yield and quality. Evidence indicating yield gains for somaclones (Secor and Shepard 1981; Evans et al. 1986) has been offset by reports of overall yield reduction of somaclones (Austin et al. 1986), lack of stability of putative high yielding somaclones in advanced tuber generations (Pavek and Corsini 1982), and suggestions that higher yielding somaclones may be bolters (Sanford et al. 1984). Also, only a few studies (Secor and Shepard 1981; Sree Ramulu et al. 1983) were conducted with initial somaclone population sizes near 1,000. Large populations are probably necessary to isolate specific somaclones that can be shown to be statistically superior to control plants under field conditions (Shepard 1979). Lastly, reports of stability across asexual generations and across diverse locations (Secor and Shepard 1981; Pavek and Corsini 1982; Sree Ramulu et al. 1984; Evans et al. 1986) are uncom-

mon and often inconclusive. In asexually propagated crops, stable transmission of a trait across at least two successive asexual generations is necessary to substantiate the genetic base of that trait (Scowcroft 1985).

We have developed an efficient method of adventitious regeneration of potato from tuber disc explants (Lam 1977; Jarret et al. 1980a; Jarret et al. 1981; Rietveld et al. 1987). Using this methodology, we regenerated a large population of somaclones from the locally popular cultivar 'Superior' (Rieman 1962). A smaller population of control plants was clonally propagated from lateral shoots so that statistical comparisons to the somaclones could be made on the population level. Furthermore, these populations were studied across five asexual generations, and the phenotypes were evaluated in three diverse locations. We present evidence of somaclonal variation for commercially important traits occurring at frequencies that should be useful for breeding purposes. Many somaclones exhibited phenotypic stability and maintained the horticulturally desirable characteristics inherent to 'Superior'.

Materials and methods

Tuber disc regeneration

Potato tubers (progeny of certified seed) of the cultivar 'Superior' were used as a source of disc explant material and were cultured onto a first medium as previously described (Jarret et al. 1980a, b; Rietveld et al. 1987). Shoot primordia appeared on the disc perimeters after 6–8 weeks, and the discs were transferred to a second medium containing gibberellic acid to elicit shoot elongation after 5–7 weeks (Jarret et al. 1981; Rietveld et al. 1987). Only one shoot, measuring 4 cm and possessing well-expanded leaves, was severed from a single initiation site on the disc to exclude the inclusion of clones resulting from the same meristem (Jarret et al. 1981). Shoots from all meristems were represented in the population to avoid selection of only the most vigorous shoots.

Shoots were cultured in 200-ml glass jars onto a third medium containing one-quarter strength MS salts and, in $mg\,l^{-1}$: *i*-inositol, 100; thiamine, 0.5; glycine, 2.0; pyridoxine, 0.5; nicotinic acid, 0.5; sucrose, 30,000; and Bacto-agar, 8,000 to elicit root initiation. After 1 week under low light (60 $\mu E\,m^{-2}\,s^{-1}$) at 26 °C the jars were placed under high intensity lighting (150 $\mu E\,m^{-2}\,s^{-1}$) at the same temperature for an additional 1–2 weeks.

Rooted plantlets were acclimated to ambient humidities under hydroponic conditions and low light (50–60 $\mu E\,m^{-2}\,s^{-1}$) using half-strength Hoagland's solution, pH 6.5 at 24 °C. After 1–2 weeks plantlets were transplanted into 5-cm square peat pots containing a standard greenhouse soil mix. After 1 week of partial shading, plantlets were allowed an additional 3 weeks of growth under full sun prior to field establishment.

Establishment of control plants

Terminal shoots, 6–8 cm in length, were severed from plants established from the same lot of tubers as used for production of adventitious shoots *in vitro*. These shoots were induced to form roots in 5-cm peat pots within 2 weeks on a mist bench in the greenhouse. Control plant establishment was coordinated with the development of the plants derived from tissue culture.

Field establishment and experimental design

Over 2,000 tuber disc-derived (hereafter termed treatment) and control potted plants were transplanted to the field in a repeated linear pattern of one control plant followed by five treatment plants. Within row plant spacing was 91 cm. Since control plants provide an estimate of environmental and any clonal variability, a smaller control population was established to allow the inclusion of the highest number of treatment plants. At maturity, tubers were collected from each plant, separately bagged, data collected, and the tubers were subsequently stored at 5 °C.

The initial planting was termed the A_0 generation (A for asexual propagation), and the tubers from this A_0 generation were used to establish the A_1 generation. Specific lines (i.e., individual plants from the A_0 generation were defined as putative lines) were chosen for inclusion in the A_1 generation by meeting the selection criteria presented in Fig. 1. The selected A_0 tubers were cut into seed pieces weighing 45 ± 3 g, allowed to suberize, and then planted at a 91 cm spacing and to a depth of 4–5 cm. To reduce heterogeneous field effects, up to 10 plants were established from each selected line, and these plants were divided equally between two completely randomized blocks.

Subsequent generation studies were designed and established as presented in Fig. 1. Tubers from the A_2 through the A_4 generations were collected, stored, and prepared for planting the following season using the methods described above. The only exception was the bulking of tubers harvested from the 20 plants in each block-replication in generations A_3 and A_4. In these instances collection and bagging of tubers from individual plants was not feasible because a closer plant spacing of 30 cm was used.

The proportion of lines infected with potato viruses X, Y, and leaf roll was estimated in the A_3 and A_4 generations. Leaves from one plant of each line in each of the two blocks were tested for the three viruses using the POTASCREEN™ ELISA assay available from Agdia (Elkhart, Ind., USA).

Collection and analysis of data

In the advanced generations 16 quantitatively measured traits and 6 ranked traits were used to characterize the lines (see Table 1). The characterization of earlier generations was based on fewer traits. All measured traits except main stem length were collected from every bagged sample. The main stem length measurement and all ranked traits were collected from a random sample representing 40% of all experimental units.

Results

Mean and variance differences on a population level

Block effects were not significant in A_1-IND-82, A_2-IDA-83, and A_2-IND-83 (data not shown). In A_3-IDA-84, a highly significant block effect was observed (data not shown), presumably due to a confounding seed piece origin effect since block A was derived from A_2-IDA-83 and block B from A_2-IND-83 (see Fig. 1). The three locations of the A_2 generation exhibited large mean differences for many traits (data not shown). For example, treatment population means for total tuber weight per plant were 727, 446, and 213 g in A_2-IDA-83, A_2-IND-83, and A_2-CAL-83, respectively. In A_3, the mean values of block A greatly exceeded those of block B for every trait except total tuber number. The block effect was not

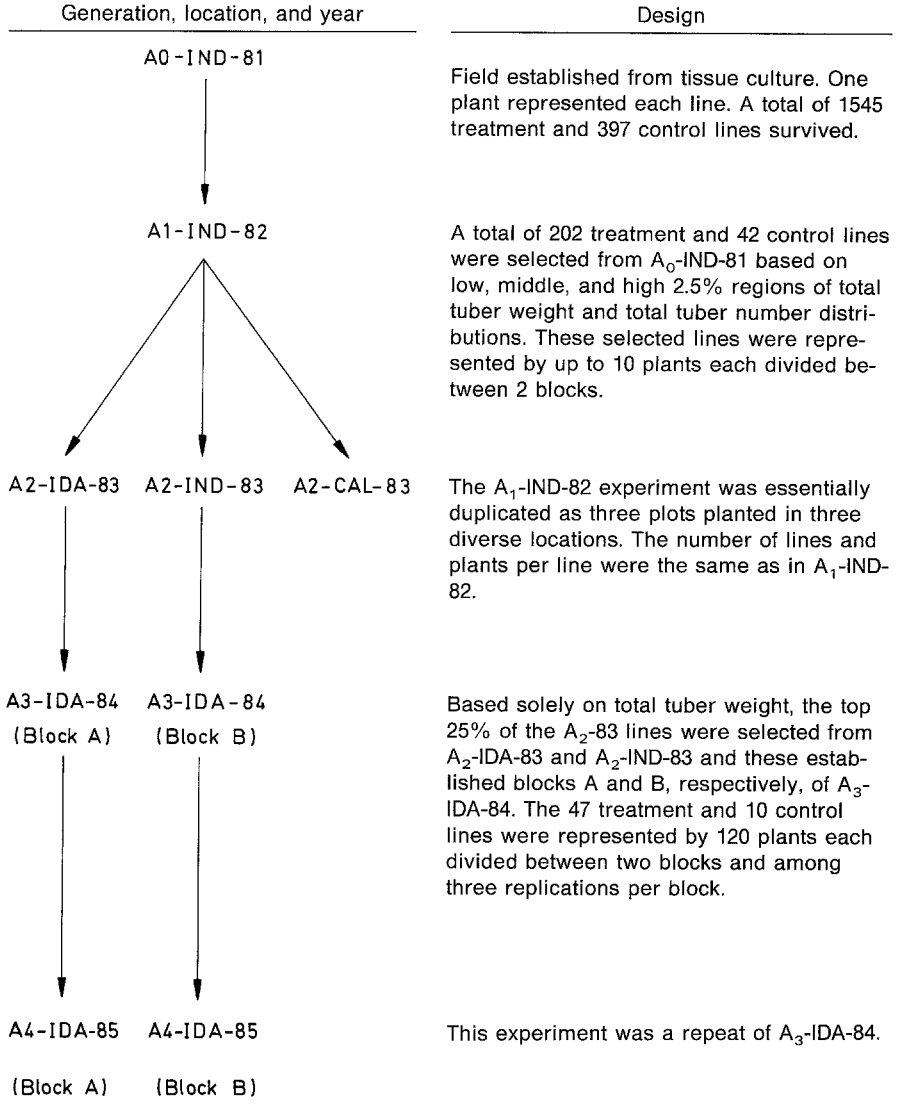


Fig. 1. Flow chart of asexual generation advancement. Asexual generations are represented by A_x, where x = consecutive generation number (initial generation of plantlets from tissue culture is A_0). Locations are abbreviated as follows: *CAL* El Centro, Calif., *IDA* Wendell, Idaho, *IND* LaCrosse, Ind.

significant in A_4-IDA-85; this apparent provenance effect was not stable for more than one tuber generation.

Means and variances of the treatment and control populations and the appropriate statistical tests (Neter et al. 1978) are presented in Table 2. Data collected from the asexual generation lineage passing through A_2-IND-83 and blocks B of A_3 and A_4 are not presented (see Fig. 1). The results of this lineage agree with the lineage passing through A_2-IDA-83, except the treatment means statistically exceed the control means for the traits of cull tuber number and weight in the A_2-IND-83 lineage (data not shown). A preponderance of treatment lines at the high end of the distribution region for several traits is reflected in significant variance differences (Fig. 2). In the A_3 and A_4 generations, additional traits were measured that were not included in the earlier generations (Table 3).

Line-to-line mean differences

On a population level the treatment and control populations were significantly different for most traits. More direct line-to-line comparisons between the two populations are presented in Table 4. Comparisons made in the A_4 generation between individual treatment lines and the extreme control lines or the 95% confidence intervals of the control lines substantiate the unidirectional shifts in the somaclones.

Individual A_4 treatment line means were statistically compared to control mean values using the Least Significant Difference (LSD) Test at the 5% level. The proportions of treatment lines significantly exceeding control mean values for tuber elongation ratio and total tuber number and weight were 0.92, 0.56, and 0.23, respectively (data not shown). When these statistical comparisons

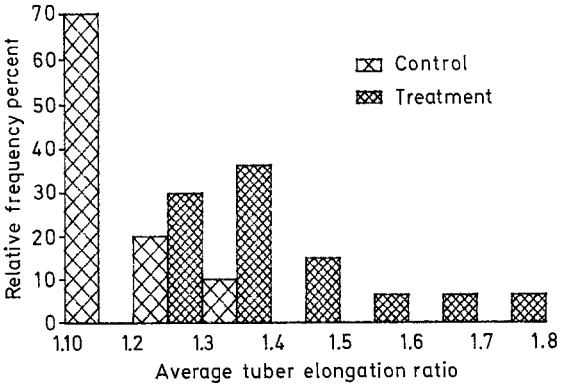
Table 1. Descriptions of ranked and measured traits

Ranked traits	*Rankings and description of ranks*
Plant maturity	1–3 (late to early maturing)
Flower number	0–2 (none present/few/many)
Plant vigor	1–4 (weak to very vigorous)
Leaf color	1–3 (pale green/normal/dark green)
Degree of branching	1–3 (single/few/many branches)
Time of blooming	1–3 (early to late blooming)
Measured traits	*Description of measurements*
Tuber elongation ratio	(Total length) (total width)$^{-1}$ for all U.S. 1 tubers
Total tuber number	Count of all tubers (per plant)
Total tuber weight	Weight in grams of all tubers (per plant)
U.S. 1 tuber number	Count of tubers 4.8–8.9 cm in diameter (per plant)
U.S. 1 tuber weight	Weight in grams of tubers 4.8–8.9 cm in diameter (per plant)
Cull tuber number	Count of cull tubers (per plant)
Cull tuber weight	Weight in grams of cull tubers (per plant)
Main stem length	Length in cm from soil surface to apex
Percent scald, growth cracks, second growth, and sun greening.	Each of these four traits is expressed as a percentage of cull tuber count
Percent hollow heart, internal necrosis, and vascular discolor;	Each of these three traits is expressed as a percentage of U.S. 1 tuber count
Specific gravity	Only for U.S. 1 tubers

were made relative to the highest control line, the proportions of significantly different treatment lines were 0.48, 0.10, and 0.02 for tuber elongation ratio and total tuber number and weight, respectively (data not shown).

Stability within distribution regions

Equal proportions of low, mean, and high performing lines (for the two traits total tuber weight and number) from the treatment and control A_0 populations were selected for inclusion in the A_1 generation (see Fig. 1). The performance of these selected lines in the next two generations is of interest because any stability within the distribution regions found for the treatment population but not found for the control population would support the hypothesis of stable genetic variability resulting from adventitious regeneration. Table 5 is a listing of the distribution region means for several traits in the A_1 and A_2 generations. Table 5 does not include the results of A_2-CAL-83, but the data were consistent with those data of the other two A_2 studies. Data of the A_3 and A_4 studies



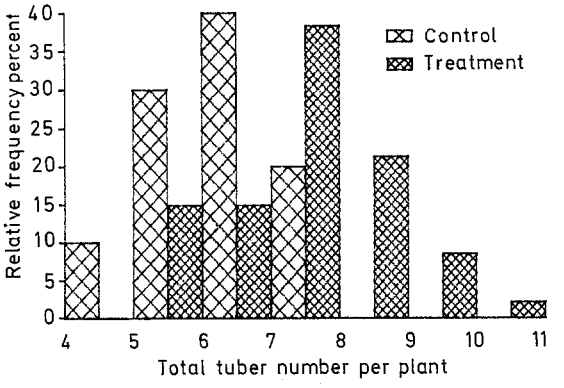


Fig. 2. Frequency distributions of the treatment and control populations for two traits in the A_3-IDA-84 generation

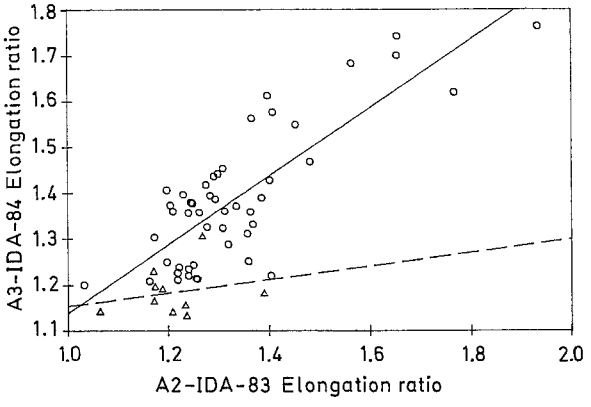


Fig. 3. Scatter plot of treatment (*circles*) and control (*triangles*) lines across two tuber generations for tuber elongation ratio. Regression line for the treatment population (*solid line*) is significant at $P \leq 0.001$. Regression line for the control population (*dashed line*) lacks significance at $P > 0.05$, and although drawn with positive slope, this slope is statistically indistinguishable from zero

Table 2. Mean and variance results for five generations

Trait	Generation, location and year	Treatment mean	Control mean	Mean difference		Variance difference		
				t-value	Significance	*F*-value	Significance	Direction[a]
Tuber elongation ratio	A_0-IND-81	1.396	1.241	23.2	***	1.64	***	T/C
	A_1-IND-82	1.510	1.341	11.9	***	2.43	***	T/C
	A_2-IDA-83	1.328	1.222	5.65	***	2.60	***	T/C
	A_3-IDA-84	1.385	1.184	7.31	***	8.44	***	T/C
	A_4-IDA-85	1.428	1.239	9.94	***	1.73	*	T/C
Total tuber number	A_0-IND-81	14.4	9.18	11.2	***	1.74	***	T/C
	A_1-IND-82	11.3	8.92	4.49	***	2.21	**	T/C
	A_2-IDA-83	9.14	7.84	3.59	***	1.44	?	T/C
	A_3-IDA-84	7.35	6.16	4.57	***	1.56	?	T/C
	A_4-IDA-85	7.21	6.07	4.53	***	1.21	NS	T/C
Total tuber weight	A_0-IND-81	530	683	8.42	***	1.19	*	T/C
	A_1-IND-82	1,300	1,180	1.64	*	1.43	?	T/C
	A_2-IDA-83	727	661	1.41	?	1.07	NS	T/C
	A_3-IDA-84	801	794	0.18	NS	1.98	?	C/T
	A_4-IDA-85	746	676	2.21	*	1.23	NS	T/C
U.S. 1 tuber number	A_1-IND-82	3.28	3.34	0.56	NS	1.25	*	T/C
	A_2-IDA-83	3.84	3.52	1.55	?	1.58	?	T/C
	A_3-IDA-84	1.65	2.48	5.84	***	1.09	NS	C/T
	A_4-IDA-85	1.52	1.96	4.10	***	1.38	NS	T/C
U.S. 1 tuber weight	A_1-IND-82	694	737	1.70	*	1.27	*	T/C
	A_2-IDA-83	816	787	0.54	NS	1.69	*	T/C
	A_3-IDA-84	313	478	5.86	***	1.35	?	C/T
	A_4-IDA-85	307	392	3.22	***	1.03	NS	C/T
Cull tuber number	A_3-IDA-84	0.411	0.334	1.47	?	1.79	?	T/C
	A_4-IDA-85	0.715	0.826	0.70	?	1.33	NS	C/T
Cull tuber weight	A_3-IDA-84	50.8	53.6	0.33	NS	1.01	NS	T/C
	A_4-IDA-85	62.8	65.4	0.17	NS	1.46	NS	T/C
Main stem length	A_1-IND-82	67.0	67.1	0.05	NS	1.45	**	T/C
	A_2-IDA-83	66.8	68.7	1.02	?	3.04	***	T/C
	A_3-IDA-84	52.2	50.4	1.25	?	1.67	?	C/T
	A_4-IDA-85	53.1	50.1	3.45	***	1.76	?	T/C
Maturity index	A_1-IND-82	1.9	2.0	2.34	**	1.67	**	T/C
	A_2-IDA-83	2.1	2.0	2.08	*	1.39	?	T/C
	A_3-IDA-84	2.6	2.9	3.72	***	5.46	**	T/C
	A_4-IDA-85	2.1	2.0	0.48	NS	9.97	***	T/C
Flower number index	A_1-IND-82	0.84	0.51	9.29	***	2.39	***	T/C
	A_2-IDA-83	0.66	0.31	5.01	***	2.11	**	T/C
	A_3-IDA-84	1.6	1.2	5.71	***	1.64	?	T/C
	A_4-IDA-85	1.1	0.61	4.86	***	1.18	NS	C/T

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ?, $0.05 < P < 0.25$ (questionable significance); NS, $P \geq 0.25$ (non-significance)
[a] T/C and C/T designate which population, treatment or control respectively, has the greater variance

were not included because selection employed between A_2 and A_3 eliminated the low and mean regions. Statistically significant disparity in the treatment population across the three distribution regions is noted for all the traits listed. Conversely, the three distribution region means of the control population do not significantly differ except in A_2-IND-83 for total tuber number and total tuber weight.

Correlations between generations

Significant correlations between generations were noted in all instances for the treatment population, and in contrast, poorer correlations existed in the control population (Table 6). An example of exceptional regression differences between the two populations is given in Fig. 3. In three instances of significant control correlations, ex-

Table 3. Mean and variance differences for additional traits

Trait	A_3-IDA-84				A_4-IDA-85			
	Mean difference[a]		Variance difference		Mean difference		Variance difference	
Plant vigor	*	T/C	?	C/T	***	T/C	?	T/C
Leaf color	**	T/C	NS	C/T	?	T/C	*	C/T
Degree of branching	?	T/C	?	T/C	*	T/C	?	T/C
Time of blooming	***	T/C	***	T/C	***	T/C	*	T/C
% Scald	?	T/C	**	T/C	***	C/T	*	T/C
% Growth cracks	*	T/C	**	T/C	–	T/C	–	T/C
% Second growth	NS	C/T	*	C/T	?	T/C	**	T/C
% Sun greening	NS	C/T	?	C/T	?	T/C	NS	T/C
% Hollow heart	NS	C/T	*	C/T	–	–	–	–
% Necrosis	?	T/C	**	T/C	NS	C/T	NS	T/C
% Vascular discolor.	*	C/T	**	C/T	NS	T/C	?	C/T
Specific gravity	**	T/C	NS	T/C	NS	C/T	NS	C/T

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ?, $0.05 < P < 0.25$ (questionable significance); NS, $P \geq 0.25$ (non-significance)

[a] T/C and C/T designate which population, treatment or control respectively, has the numerically larger mean or variance value. The dashes represent the situation of no lines having the specific phenotype for either or both populations

Table 4. Proportion of 47 total treatment lines numerically exceeding control lines. From the control population of 10 lines, the 95% confidence interval was calculated. The proportion of treatment lines which fall above and below this confidence interval are shown

Trait	Proportion of treatment lines in A_4-IDA-85			
	Above highest control line	Below lowest control line	Above control 95% C.I.	Below control 95% C.I.
Tuber elongation ratio	0.94	0.00	0.96	0.00
Total tuber number	0.44	0.00	0.69	0.04
Total tuber weight	0.21	0.04	0.56	0.15

Table 5. Means of distribution regions in A_1 and A_2. Total tuber weight and U.S. 1 tuber weight distributions are based on A_0-IND-81 total tuber weight data. Total tuber number and U.S. 1 tuber number distributions are based on A_0-IND-81 total tuber number data. Tuber elongation ratio distribution is based on A_0-IND-81 elongation ratio data. Means within a row followed by the same letter do not significantly differ at the $P = 0.05$ probability level using Newman-Keuls range test

Trait	Generation	Means and Newman-Keuls range test results					
		Treatment			Control		
		Low	Mean	High	Low	Mean	High
Tuber elongation ratio	A_1-IND-82	1.45bc	1.52b	1.62a	1.32c	1.33c	1.36c
	A_2-IND-83	1.40abc	1.41ab	1.47a	1.20c	1.25bc	1.21c
	A_2-IDA-83	1.29b	1.31b	1.47a	1.23b	1.19b	1.27b
Total tuber number	A_1-IND-82	12.7a	11.8a	10.2b	9.52b	9.17b	8.77b
	A_2-IND-83	8.48d	12.6c	17.8a	9.94cd	13.2bc	16.6ab
	A_2-IDA-83	7.75b	9.29ab	10.7a	6.81b	8.80ab	8.69ab
Total tuber weight	A_1-IND-82	1,100b	1,260ab	1,350a	1,130ab	1,260ab	1,230ab
	A_2-IND-83	273d	438bc	675a	306cd	543abc	644ab
	A_2-IDA-83	593b	776ab	852a	631ab	639ab	749ab
U.S. 1 tuber number	A_1-IND-82	4.21a	3.98a	3.22b	3.88a	3.28b	3.36ab
	A_2-IND-83	1.60a	1.99a	2.12a	1.79a	2.03a	2.11a
	A_2-IDA-83	2.07a	2.84a	2.46a	2.42a	2.87a	2.80a
U.S. 1 tuber weight	A_1-IND-82	631b	739ab	816a	795ab	806ab	804ab
	A_2-IND-83	231a	315a	313a	277a	293a	342a
	A_2-IDA-83	393b	512ab	516ab	475ab	642a	554ab

Table 6. Regressions between asexual generations. In those regressions marked with [a], the lower 20% of each population was excluded from regression analysis. In those compared generations having significant control population correlation coefficients, the slopes between the treatment and control populations do not significantly differ at $P<0.05$

Trait	Generations compared	Treatment population			Control population		
		R coefficient	Significance[b]	Slope	R coefficient	Significance	Slope
Tuber elongation ratio	A_0-IND vs A_1-IND	0.31	***	+0.26	0.13	NS	−0.10
	A_1-IND vs A_2-IDA	0.11	*	+0.15	0.22	*	+0.29
	A_2-IDA vs A_3-IDA	0.80	***	+0.75	0.23	NS	+0.15
	A_3-IDA vs A_4-IDA	0.40	**	+0.24	0.59	*	+0.64
Total tuber number	A_0-IND vs A_1-IND	0.30	***	−0.06	0.01	NS	−0.00
	A_1-IND vs A_2-IDA	0.32	***	+0.31	0.26	**	+0.32
	A_2-IDA vs A_3-IDA	0.19	?	+0.09	0.62	*	+0.22
	A_2-IDA vs A_3-IDA[a]	0.31	*	+0.13	0.62	?	+0.26
	A_3-IDA vs A_4-IDA	0.38	**	+0.31	0.63	*	+0.65
	A_3-IDA vs A_4-IDA[a]	0.32	*	+0.29	0.31	NS	+0.33
Total tuber weight	A_0-IND vs A_1-IND	0.19	*	+0.25	0.10	NS	+0.13
	A_1-IND vs A_2-IDA	0.28	***	+0.25	0.17	NS	+0.18
	A_2-IDA vs A_3-IDA	0.29	*	+0.09	0.56	*	+0.27
	A_2-IDA vs A_3-IDA[a]	0.36	*	+0.11	0.00	NS	+0.00
	A_3-IDA vs A_4-IDA	0.27	*	+0.31	0.54	?	+0.43

[b] See Table 3 for levels of significance

clusion of the lower 20% of each population distribution negated control correlation significance, but did not negate treatment correlation significance (Table 6, superscript a). The slope of a regression line that does not have a significant correlation coefficient is statistically indistinguishable from a line having zero slope (Neter et al. 1978). Therefore, in comparisons between the treatment and control populations having significant and lacking significant R coefficients, respectively, the treatment population slope is statistically greater than that of the control slope, regardless of the actual value of the latter. For the balance of the comparisons (i.e., where both populations show significant R coefficients and therefore non-zero slopes), the slopes do not significantly differ at $P \leq 0.05$.

Significant regression correlations between non-successive generations were less common. For tuber elongation ratio, significant regressions were observed in A_0 versus A_2 and A_0 versus A_3 for the treatment population only (data not shown). Total tuber number was significantly correlated in A_1 versus A_3 and A_2 versus A_4, and total tuber weight was correlated in A_0 versus A_4. These correlations were not significant in the control population (data not shown).

Correlations between replications of each generation

Significant correlations were detected between replications within a specific generation (Table 7). In the A_2 generation the replications compared were the three locations of California, Idaho and Indiana. In the A_3 and A_4 generations, the replications were blocks A and B that were established from A_2-IDA-83 and A_2-IND-83 tubers, respectively. This is an important analysis because a correlation between replications of specific lines separated spatially (i.e., different localities in A_2 and different areas of the same field in A_3 and A_4) is a strong indicator of stability. The means for most traits greatly differed among the A_2 locations and between the A_3 and A_4 blocks. In those instances where both populations showed significant R coefficients, the slopes were found to not significantly differ except in the A_3-IDA generation for tuber elongation ratio. For this occurrence, the treatment and control slopes of 1.09 and 0.49, respectively, differed significantly at $P \leq 0.05$ (Table 7).

Individual line stability

The stability of individual lines was studied by comparing lines that continued to rank in the top 20% for each trait across successive generations (Table 8). Across two, three, and four successive generations, each ending with A_4, the proportion of treatment lines observed in the top 20% of each generation for each trait statistically exceeded the expected proportions based upon random chance (Table 8). In the A_2 to A_4 generation span, at least one treatment line maintained stability in the top 20% of the distributions for each of the 13 traits examined (data not

Table 7. Regressions between replications of each generation. In the A_3 and A_4 generations replication (block) A originated from A_2-IDA-83 and B originated from A_2-IND-83. In those generations having significant control population correlation coefficients, the slopes between the two populations do not signficantly differ at $P<0.05$, except in the A_3-84 generation for tuber elongation ratio

Trait	Generation	Replications compared	Treatment population			Control population		
			R coefficient	Significance[a]	Slope	R coefficient	Significance	Slope
Tuber elongation ratio	A_2-83	CAL vs IDA	0.01	NS	+0.01	0.11	NS	+0.11
		CAL vs IND	0.05	NS	−0.04	0.03	NS	−0.02
		IDA vs IND	0.15	*	+0.13	0.03	NS	−0.03
	A_3-84	A vs B	0.73	***	+1.09	0.59	*	+0.49
	A_4-85	A vs B	0.45	***	+0.48	0.26	?	+0.25
Total tuber number	A_2-83	CAL vs IDA	0.13	**	+0.06	0.25	*	+0.11
		CAL vs IND	0.24	***	+0.07	0.33	**	+0.06
		IDA vs IND	0.29	***	+0.16	0.18	?	+0.07
	A_3-84	A vs B	0.43	**	+0.48	0.10	NS	−0.08
	A_4-85	A vs B	0.29	*	+0.39	0.13	NS	+0.12
Total tuber weight	A_2-83	CAL vs IDA	0.19	***	+0.06	0.34	**	+0.09
		CAL vs IND	0.25	***	+0.11	0.29	**	+0.09
		IDA vs IND	0.19	***	+0.25	0.15	?	+0.19
	A_3-84	A vs B	0.04	NS	+0.07	0.31	?	+0.28
	A_4-85	A vs B	0.19	?	+0.21	0.14	NS	+0.20

[a] See Table 3 for levels of significance

Table 8. Stability of individual lines in the top 20% of the distribution for each trait across asexual generations. There were 48 treatment and 10 control lines included in this analysis. The values in the expected column were calculated from probabilities due to random chance (i.e., $(0.2)^n$ where n = number of generations spanned)

Generations spanned	Total possible traits	Population	Number of lines for each trait in the top 20% of each generation (% of total possible)		Significance of difference between observed and expected proportions[a]
			Observed	Expected	
A_1–A_4	3	Treatment	3 (2.1%)	0.23 (0.16%)	*
		Control	0 (0.0%)	0.05 (0.16%)	NS
A_2–A_4	13	Treatment	24 (3.9%)	5.0 (0.80%)	***
		Control	1 (0.77%)	1.0 (0.80%)	NS
A_3–A_4	21	Treatment	131 (13%)	40 (4.0%)	***
		Control	15 (7.1%)	8.4 (4.0%)	?

[a] See Table 3 for levels of significance

shown). Across these same three generations, only one control line for just one trait, tuber elongation ratio, maintained stability. No treatment or control lines maintained stability in the top 20% of the population distributions across the five generations A_0 through A_4 for any trait (data not shown).

Virus incidence

Since virus infection may have been confounded with the various yield related traits, all lines in the A_3 and A_4 generations were assayed. Lines infected with potato viruses X (PVX), Y (PVY), and leaf roll (PLRV) comprised 73% or higher, 10% or lower, and 50% of both populations, respectively. For each of the three viruses, the proportions of positive lines were not significantly different between the treatment and control populations. The existence of treatment lines with putative virus resistance therefore cannot be experimentally supported. Also, differential virus resistance between the two populations is not presumed to be a component that caused the phenotypic differences observed between the populations. A significant negative correlation between total tuber weight and PVX was detected in both populations in the A_3 generation only (data not shown). Results of analysis of variance (data not shown) failed to show a

significant treatment by PVX interaction, thereby providing evidence that PVX affected the treatment and control populations uniformly. Correlations were not significant between total tuber weight and PVY or PLRV in either population or generation (data not shown).

Discussion

The utility of somaclonal variation to plant improvement will likely result from the ability to isolate, from well-established cultivars, variants with incremental improvements and without loss of horticultural quality (Evans et al. 1984). An adventitious regeneration procedure that does not involve a callus intermediary should be efficient and produce genetic diversity without gross aberrations. Severely aberrant genotypes are not useful because nearly all possess atypical ploidy levels (Sree Ramulu et al. 1983; Gill et al. 1986) and are not of commercial value (Shepard 1979). Direct regeneration from tuber disc explants has resulted in a much lower frequency of severely aberrant plants, 1.3%–1.6% (Rietveld et al. 1987). While the frequency of aberrant plants has been greatly reduced, the results demonstrate that potentially useful variation for horticulturally important phenotypes was inherent in these somaclones.

Most intriguing is the significance of mean differences (Tables 2 and 4) between the treatment and control populations for many traits. Selection that was imposed on the A_1 generation included equal numbers of lines from the low, mean, and high regions of the A_0 distributions of both populations. Therefore, the population means would not be expected to differ. The population means did differ, however, because the treatment population was enriched with individuals having skewed values for the various traits. The treatment population was comprised of lines that on average were higher yielding (excluding the result in A_0 due to the influence of regeneration and propagation artifacts (Cole and Wright 1967)), had greater flower numbers, and produced more tubers per plant that were significantly more elongated and of smaller size than those of the control population (Tables 2 and 4). Following selection that resulted in the A_3 generation, based solely on selecting the top 25% of A_2 lines for total tuber weight, the treatment population maintained those differences previously mentioned (Tables 2 and 4). This occurred although closer plant spacings were part of the A_3 and A_4 experimental designs (Caligari et al. 1985).

In this study unidirectional mean shifts, in more desirable directions, occurred for certain traits. Such shifts in the means have been attributed to the enrichment of the somaclonal population with plants having the bolter phenotype (Hooker 1981; Sanford et al. 1984). While varying degrees of the bolter phenotype are known to occur naturally at frequencies as high as 1.5×10^{-3}, the various pleiotropic effects are presumably due to a common attribute, which is a mutation that results in late maturity (Sanford et al. 1984). In this study maturity differences between treatment and control populations (Table 2) were significant in either direction (i.e., somaclones were earlier or later in maturity) or lacked significance altogether. A few very late maturing, bolter type lines were observed, but the frequencies were too low to account for the significant differences between populations.

Within the treatment population not all lines showed the mean shift response. While the total population mean shifted for the traits discussed above, a wide range of values was observed in the distributions of the early generations. This result is reflected in the frequency histograms (Fig. 2) and in the significant variance differences between the two populations (Tables 2 and 3). Upon selection for the top yielding lines establishing the A_3 generation, the variance differences for nearly all traits ceased to be significant. At this point the mean differences between the populations were enhanced for many traits, but the range of values in the treatment population was diminished. Presumably, selection against unaltered somaclonal lines, which would be indistinguishable from control lines, resulted in the selected treatment population having a significantly shifted mean but a variance statistically equal to that of the control population. These variances would principally reflect environmental effects.

In this study phenotypic stability has been demonstrated across generations and diverse locations (Tables 5–8). Evidence of stability in the control population also occurred (Tables 6 and 7), but this stability was clearly different from and of lower significance than that observed in the treatment population. Excluding the lower 20% of each population eliminated significant regression coefficients in the control, but not in the treatment population for the regression analyses between asexual generations (Table 6). Apparently, the low performing control lines as well as some treatment lines were more likely to establish similar low performing plants. In contrast to the treatment population, however, high performing control lines did not appear to result in high performing progeny. Therefore, the major difference between the treatment and control populations occurred at the high end of the distribution and not uniformly across the entire distribution (Table 5).

The influence of viruses might have been one explanation of the stability of low performing control and treatment lines. This was likely due to the establishment of both populations from potentially virus infected tuber progeny of a commercial production. At least for viruses PVX, PVY, and PLRV, this was not the explanation. Not only were differences lacking between the treatment and

control populations for infection frequency, but there was no greater incidence of these viruses in the low distribution region in the A_2 generation (data not shown). A second explanation of stability in the control population is clonal variation, which has been known to occur naturally within potato cultivars (McIntosh 1945; Bald and Oldaker 1950).

A provenance effect is a third and most likely explanation for successive generations of low yielding controls. This study provides strong evidence in support of a seed piece origin effect. Highly significant differences were observed between the two blocks in the A_3 generation for all traits except total tuber number. Blocks A and B were established from seed pieces grown the previous year in Idaho and Indiana, respectively (Fig. 1) – two locations that differed in productive capacity. Since identical lines were established in both locations from seed pieces originating from the A_1-IND-82 generation and since all seed pieces were uniformly sized, a provenance effect was clearly responsible. Initiation of the tuberization response, which affects overall yield potential, is strongly influenced by the physiological condition of the seed piece (Ewing 1985). Therefore, a provenance effect could explain how seed pieces from a control line that was subjected to a random stress in one generation could be expected to establish similar, low performing plants in a subsequent tuber generation. Provenance effects presumably occur only across two successive generations. Such non-genetic phenomena also must occur in the treatment population. But since the populations have been shown to statistically differ, additional variation showing stability of a genetic nature occurred only in the treatment population.

To summarize, evidence has been presented that demonstrates genetic variability results from the regeneration of potato plants from tuber disc explants. Direct adventitious regeneration bypassing callus was efficient, applicable to many cultivars (Jarret et al. 1980a), and produced a low frequency of aberrant plants (Rietveld et al. 1987) but a high frequency of potentially useful variability. Of the 22 traits examined in this study, certain traits were more variable than others. Of these more variable traits, potentially valuable unidirectional mean shifts often were observed. For the first time, a systematic study of stability has been conducted on a potato somaclonal population. Stability of a genetic nature across tuber generations and across diverse locations has been observed in the treatment population. A much lower stability of a non-random but presumably non-genetic nature occurred in the control population. The performance and stability of the proportion of treatment lines at the high end of the various distribution regions have been demonstrated. Future selections from these lines may well result in an improved cultivar of 'Superior' having greater commercial utility.

References

Austin S, Ehlenfeldt MK, Baer MA, Helgeson JP (1986) Somatic hybrids produced by protoplast fusion between *S. tuberosum* and *S. brevidens*: phenotypic variation under field conditions. Theor Appl Genet 71:682–690

Bald JG, Oldaker CEW (1950) Testing and maintenance of potato clones. Emp J Exp Agric 18:95–104

Caligari PDS, Brown J, Manhood CA (1985) The effect of varying the number of drills per plot and the amount of replication on the efficiency of potato yield trials. Euphytica 34:291–296

Cole EF, Wright NS (1967) Propagation of potato by stem cuttings. Am Potato J 44:301–304

Evans DA, Sharp WR, Medina-Filho HP (1984) Somaclonal and gametoclonal variation. Am J Bot 71:759–774

Evans NE, Foulger D, Farrer L, Bright SWJ (1986) Somaclonal variation in explant-derived potato clones over three tuber generations. Euphytica 35:353–361

Ewing EE (1985) Cuttings as simplified models of the potato plant. In: Li PH (ed) Potato physiology. Academic Press, Orlando, Fla., pp 153–207

Gill BS, Kam-Morgan LNW, Shepard JF (1986) Origin of chromosomal and phenotypic variation in potato protoclones. J Hered 77:13–16

Hooker WJ (1981) Compendium of potato diseases. The American Phytopathological Society, St. Paul, Minnesota

Jarret RL, Hasegawa PM, Erickson HT (1980a) Effects of medium components on shoot formation from cultured tuber discs of potato. J Am Soc Hortic Sci 105:238–242

Jarret RL, Hasegawa PM, Erickson HT (1980b) Factors affecting shoot initiation from tuber discs of potato (*Solanum tuberosum*). Physiol Plant 49:177–184

Jarret RL, Hasegawa PM, Bressan RA (1981) Gibberellic acid regulation of adventitious shoot formation from tuber discs of potato. In Vitro 17:825–830

Lam SL (1977) Plantlet formation from potato tuber discs in vitro. Am Potato J 54:465–468

Larkin PJ, Scowcroft WR (1981) Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor Appl Genet 60:197–214

Larkin PJ, Scowcroft WR (1983) Somaclonal variation and crop improvement. In: Kosuge T, Meredith CP, Hollaender A (eds) Genetic engineering of plants. Plenum Press, New York, pp 289–314

McIntosh TP (1945) Variations in potato varieties. Scott J Agric 25:125–132

Neter J, Wasserman W, Whitmore GA (1978) Applied statistics. Allyn & Bacon, Boston, Mass.

Pavek JJ, Corsini DL (1982) Field performance of clones from regenerated protoplasts of 'Russet Burbank'. Am Potato J 59:482

Rieman GH (1962) 'Superior': a new white, medium-maturing, scab-resistant potato variety with high chipping quality. Am Potato J 39:19–28

Rietveld RC, Hasegawa PM, Bressan RA (1987) Genetic variability in tuber disc-derived potato plants. In: Bajaj YPS (ed) Potato. Springer, Berlin Heidelberg New York (Biotechnology in agriculture and forestry, vol 3), pp 392–407

Sanford JC, Weeden NF, Chyi YS (1984) Regarding the novelty and breeding value of protoplast-derived variants of 'Russet Burbank' (*Solanum tuberosum* L.). Euphytica 33:709–715

Scowcroft WR (1985) Somaclonal variation: the myth of clonal uniformity. In: Hohn B, Dennis ES (eds) Genetic flux in plants. Springer, Berlin Heidelberg New York, pp 217–245

Secor GA, Shepard JF (1981) Variability of protoplast-derived potato clones. Crop Sci 21:102–105

Shepard JF (1979) Mutant selection and plant regeneration from potato mesophyll protoplasts. In: Rubenstein I, Gengenbach B, Green CE (eds) Emergent techniques for the genetic improvement of crops. University of Minnesota Press, Minneapolis, pp 185–219

Sree Ramulu K, Dijkhuis P, Roest S (1983) Phenotypic variation and ploidy level of plants regenerated from protoplasts of tetraploid potato (*Solanum tuberosum* L. cv. 'Bintje'). Theor Appl Genet 65:329–338

Sree Ramulu K, Dijkhuis P, Roest S (1984) Genetic instability in protoclones of potato (*Solanum tuberosum* L. cv. 'Bintje'): new types of variation after vegetative propagation. Theor Appl Genet 68:515–519

Thomas E, Bright SWJ, Franklin J, Lancaster VA, Miflin BJ (1982) Variation amongst protoplast-derived potato plants (*Solanum tuberosum* cv. 'Maris Bard'). Theor Appl Genet 62:65–68

Wheeler VA, Evans NE, Foulger D, Webb KJ, Karp A, Franklin J, Bright SWJ (1985) Shoot formation from explant cultures of fourteen potato cultivars and studies of the cytology and morphology of regenerated plants. Ann Bot 55:309–320