OUTCROSSING RATE IN CULTIVATED AUTOTETRAPLOID POTATO

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Abstract

Outcrossing rates in potato were measured in an open-pollination field experiment at a location in the Peruvian Andes. Embryo spot, a dominant genetic seed marker, was used to measure outcrossing rate. The source of the dominant marker, the donor, was interplanted among recipient clones lacking the marker (recessive genotype), for which outcrossing was to be measured. Eight out of the 17 clones tested were advanced breeding clones which had wild species cytoplasm. Seven out of eight of these had outcrossing rates above 0.75, showing complete male sterility in one case and partial male sterility in six cases. Among the other clones tested, which were male-fertile, outcrossing estimates ranged from 0.10 to 0.74. Wide variability in outcrossing rate in autotetraploid potato suggests that it should be possible to select for higher outcrossing rate in production of open-pollinated true seed populations of potato for genetic or commercial purposes.

Compendio

En un experimento de campo con polinización libre de papa, se midieron las tasas de intercruzamiento en un lugar de los Andes peruanos. La mancha embrional, un marcador genético dominante de la semilla, fue utilizado para medir la tasa de intercruzamiento. La fuente del marcador dominante, el donante, fue interplantada entre los clones recipientes carentes del marcador (genotipo recesivo), para los cuales se tenía que medir el intercruzamiento. Ocho de los 17 clones probados eran clones avanzados de mejoramiento que tenían citoplasma de especies silvestres. Siete de los ocho tuvieron tasas de intercruzamiento mayores de 0.75, mostrando esterilidad masculina completa en uno de los casos y parcial en los otros seis. Entre los otros clones probados, que eran macho-fértiles, los estimados de intercruzamiento variaron de 0.10 a 0.74. Una gran variación en la tasa de intercruzamiento en papas autotetraploides sugiere que podría ser posible seleccionar para una tasa alta de intercruzamiento en la producción de poblaciones de semilla sexual de papa de polinización libre, para propósitos genéticos o comerciales.

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Accepted for publication June 21, 1993.

ADDITIONAL KEY WORDS: *Solanum tuberosum* ssp. *tuberosum*, *Solanum tuberosum* ssp. *andigena*, cytoplasm, true potato seed, synthetics, inbreeding.

Introduction

Potato (*Solanum tuberosum* L.) is customarily propagated clonally in its center of origin in South America as well as in the areas of the rest of the world to which it has been introduced. Modern efforts aimed at genetic improvement through sexual crosses predominantly involve controlled biparental matings or use of pollen bulks on single female parents. There are few references to breeding with natural or open-pollination as the mating procedure (9, 15). This type of mating offers little advantage when a breeding program is composed of vegetatively propagated clones as is the case for most potato improvement research. Therefore, the question of outcrossing rate in the presence of natural pollen vectors has had minor importance.

Vegetative propagation is, however, undesirable where the seed of commerce is already infected or easily contaminated with systemic pathogens. These problems are most acute in situations where lack of suitable storage facilities or difficulty of transport result in the bulky tuber seed being difficult to maintain or obtain. Most systemic pathogens are not transmitted through true seed (11). Establishing ware potato or seed potato fields from true seed has merit in agroecological zones where the hygiene of tuber seed is poor and the cost of producing or importing clean stock is the main barrier to yield increase.

Cultivation of potato from a sexually produced seed of commerce requires consideration of systems for seed production (3). Seed production options include the following: 1) open-pollinated seed collected from single clonal varieties (selfed seed), 2) biparental F_1 hybrids derived from controlled crosses, and 3) synthetics involving two or more parents that have been multiplied several cycles under open-pollination (2). One of the variables that will have an impact on the genetic structure of these populations is outcrossing rate. Diploid cultivated potato species (*S. phureja* Juz. et Buk., *S. stenotomum* Juz. et Buk., and *S. ajanhuiri* Juz. et Buk.) have a gametophytic self-incompatibility system while tetraploid potato is self-compatible (12). Thus the question of outcrossing rate is of greatest interest in the autotetraploid *Solanum tuberosum.* Potato flowers lack nectaries and are not attractive to the honeybee (*Apis mellifera* L.), but they are visited by many wild bee buzz-pollinators, in particular those in the genus *Bombus* (2, 19). Under field conditions male fertile potato varieties attract wild pollinators and abundant fruit may be produced. The presumption exists that potato is predominantly a selfing species (6, 9).

The purpose of this experiment was to place various autotetraploid potato (*Solanum tuberosum*) genotypes in a field setting where natural bee pollinators could effect pollination and estimates of outcrossing rate could be made. This report presents estimates of outcrossing rates in a number of tetraploid clones. Some of the clones possess forms of male sterility that appear to enhance the outcrossing rate.

Materials and Methods

To ascertain the proportion of seed that resulted from outcrosses versus selfs, it was necessary to have a dominant marker gene supplied by a "donor." The proportion of this marker would be scored in the progeny of the "recipient" genotypes, which lack the marker. Seventeen genotypes of autotetraploid potato were planted as recipient genotypes in a completely randomized design. Recipient clones alternated with the donor, *Solanum tuberosum* ssp *andigena* Hawkes clone OCH 6579 (obtained from Dr. Z. Huaman, International Potato Center, Lima, Peru), which harbors embryo spot marker. Final spacing between plants was 1 m. Embryo spot was previously described in cultivated diploid potato breeding materials (10, 12). It is visible in mature seeds when examined under 10X magnification as a purplish spot located on the hypocotyl of the embryo in the seed. Embryo spot marker has the advantage that its presence can be scored without germinating the seed. Presence of embryo spot is controlled by two independent complementary loci, *P* and *B*. The *P* locus controls the presence of the anthocyanin delphinidin in flowers and tubers, visible as blue coloration. The allele B^d of the *B* locus is responsible for localization of pigmentation in the cotyledonary node of the embryo (embryo spot), at the base of the petiole, in the floral abscission layer and on the eyebrow of the tuber. Controlled crosses between OCH 6579 and recipient clones were made in a pollinator-free greenhouse employing emasculation to prevent selfing. Emasculated flowers of recipient clones pollinated with a collected bulk of recipient clone pollen produced seed.free of embryo spot. Fruit clusters from single inflorescences were obtained from recipient plants located in a randomized complete block design, where one plant of each of the 17 genotypes was randomly planted in each of 10 blocks. Multiple inflorescences (fruit clusters) were obtained from single plants.

The genetic background of clones contained in Table 1 is presented here. Clone #1 resulted from a breeding program to introduce immunity to potato virus Y into cultivated potato from the wild species *Solanum stoloniferum* Schlechtd. et. Bche., which is also the source of the cytoplasm of clone #1. The Mexican variety Atzimba (#2) resulted from a breeding program utilizing resistance to late blight from *Solanum demissum* Lindl. (J. Niederhauser, personal communication). In both cases the wild species were pistillate parents in the initial hybrids, and the cytoplasm remained unchanged in the remaining introgression cycles due to unidirectional crosses. Clones #3 through #8 are progenies of various pollen sources with cv. Atzimba as the pistillate parent. The four clones #4, #5, #6, and #7 are full sibs from a single biparental mating. The nuclear composition of the clones #2 through #8 in the presence of *S. demissum* cytoplasm and clone #1 with *S. stoloniferum* cytoplasm would be considered to be *S. tuberosum* ssp. *tuberosum* taxonomically due to tolerance of long daylength and typical ssp. *tuberosum* foliage traits. Clones #9, #10, and #11 are *Solanum tuberosum* ssp. *tuberosum* having no wild species cytoplasm. The above mentioned clones were obtained from various breeding projects of the International Potato Center. Clones #12 through #17 are accessions of *Solanum tuberosum* ssp. *andigena* maintained in the World Collection of the International Potato Center.

The planting was made at 3,300 m elevation above sea level at the La Victoria-EPSA field site of the International Potato Center in the Mantaro Valley of the Peruvian Andes. Planting was performed in December, flowering took place in February and March, and fruits were harvested in April. Pollen of the recipient clones was examined by staining with acetocarmine-glycerol (1% carmine w/v, in equal parts 45% acetic acid and glycerol) (14) and was observed under 400X magnification. Berries were refrigerated at 4 C to prevent overripening and rotting while seed extraction was carried out. Two hundred seeds from each inflorescence were scored for seedspot marker, or less if fewer seeds were available. Number of seeds per berry was derived from a count of the total number of seeds. The outcrossing rate was estimated using the "dominant marker donor-recessive recipient procedure" reviewed by Jain (13). Therefore, $t = H/p$, where t is the outcrossing rate expressed as a proportion ($0 \leq t \leq 1$), H is the proportion of seeds of recipient clones with embryo spot, and p is the estimate of frequency of embryo spot genotype in the pollen pool. The estimate of variance of the outcrossing estimate is $\sigma^2_t = (t/[(a+b)P])[(1-P)/(1-q)]$ where a and b are the number of progeny with and without the embryo spot marker, respectively, and $q=1-p$. The estimate of p was taken from the proportion of seed possessing embryo spot in fruits resulting from open-pollination of clone Y245.7, a known male-sterile attractive to bumblebees (5). Since Y245.7 was presumed to have $t = 1.0$, its proportion of seed with seedspot markers, 0.281, was used as the estimate of p, the frequency of embryo spot genotype in the pollen pool.

Results

Seedlots produced from the controlled crosses of donor by recipients produced uniformly 42% of seeds with embryo spot. This percentage of seed spot would be produced by a genotype that is duplex at one locus and simplex at the other. Either of the genotypes, $B^dB^db^db^dPppp$ or $B^db^db^db^dPPpp$, would produce this percentage of seed spot when crossed with $b^db^db^db^dpppp$. The absence of blue pigment in flowers and tubers of recipients assures that their genotype was *pppp*, but the recipient clones may harbor a B^d allele with no detectable associated phenotype. The uniform segregation proportion of 42% seed spot in the controlled crosses negates this possibility. All recipients must be $b^db^db^db^d$. A two-way analysis of variance of the proportion of seed spot with genotypes and blocks as sources of variation revealed that "genotypes" differed significantly ($F_{(16,110)} = 10.64$, $\underline{P} <$

0.001), while "blocks" was not a significant source of variation ($F_{(9,110)}$ = 1.24, $\underline{P} < 0.28$). The recipient clones are listed in Table 1. The capacity of the breeding clone Y245.7 (#1) to produce seed despite its male sterility has been previously noted (5). Its pollen consisted of unseparated tetrads. The attached grains were typically aborted, although infrequently one grain of the tetrad contained cytoplasm and stained uniformly with acetocarmine. Attempts at self-pollination were unsuccessful. The pollen of Atzimba (#2) appeared to be functional when stained with acetocarmine, but it was rarely successful as a male parent in crosses made in this study. Abundant seed was set when this clone was used as a pistillate parent. The nature of sterility in Atzimba is unknown, but has been designated as "reduced pollen function sterility" by Atlin (unpublished manuscript). An additional report of this type of sterility (4) described a potato clone with nonfunctional but highly stainable pollen. Atzimba showed an outcrossing rate of 0.90 in Table 1. Interestingly, five of six sexual progeny (#3, #4, #5, #6, and #7) that have Atzimba as the maternal parent have t-values of 0.75

TABLE 1.—*Outcrossing rate,* $\hat{t}$, *standard deviation* $\hat{\sigma}_{\hat{t}}$ *and sample size, N, of 17 autotetraploid clones as estimated scoring embryo spot in true seeds.*

I.D.	Recipient Clone	Cytoplasm	$\hat{t}$	$\hat{\sigma}_{\hat{t}}$	N
Solanum tuberosum ssp. *tuberosum*					
1	Y245.7	sto	1.00	0.03	10,554
2	Atzimba (Atz)	dem	0.90	0.01	42,611
3	Atz x P_1	dem	0.84	0.03	7,997
4	(Atz x P_2).1\[1]	dem	0.83	0.03	6,517
5	(Atz x P_2).2\[1]	dem	0.77	0.03	6,890
6	(Atz x P_2).3\[1]	dem	0.96	0.04	6,068
7	(Atz x p_2).4\[1]	dem	0.75	0.05	2,737
8	Atz x P_3	dem	0.12	0.01	5,835
9	78C11.5	nw	0.10	0.01	18,480
10	XY13.15	nw	0.51	0.01	28,812
11	EU/B.1302 X DT0.28	nw	0.70	0.04	4,413
Solanum tuberosum ssp. *andigena*					
12	702316	nw	0.74	0.02	11,618
13	OCH 7525	nw	0.44	0.01	24,357
14	CUP 183	nw	0.39	0.02	9,423
15	OCH 10211	nw	0.33	0.02	8,152
16	700229	nw	0.27	0.01	17,782
17	OCH 9286	nw	0.21	0.03	2,730

[1] Clones #4, #5, #6, and #7 are full sibs of the cross Atzimba x P_2.
sto = *Solanum stoloniferum* cytoplasm
dem = *Solanum demissum* cytoplasm
nw = no wild species cytoplasm

or higher. This suggests a possible maternally inherited factor influencing pollen function and resulting in high outcrossing rate, however the lack of reciprocal crosses leaves this a debatable question. The self-sterility is not complete as some selfing is evident in all cases. Clone #8 indicates that there may be nuclear factors which suppress this putative partial self-sterility as its t-value places it in a predominantly selfing category.

The six clones representing a sample of the ssp. *andigena* (#12 through #17) had t-values ranging from 0.21 to 0.74. The mean for the group is 0.40. The t-values of three ssp. *tuberosum* clones lacking wild species cytoplasm (#9, #10, and #11) ranged from 0.10 to 0.70. In Figure 1 the relationship of the number of seeds per berry and outcrossing rate is plotted. The significant correlation coefficient indicates that seed production per berry decreases as outcrossing rate increases. The coefficient of determination ($r^2 = 0.64$) reveals that 64% of the total variation can be attributed to this covariation.

Discussion

Glendinning (9) estimated an outcrossing rate, t, between 0.14 and 0.30 for *S. tuberosum* ssp. *andigena* populations. Brown and Huaman (6)

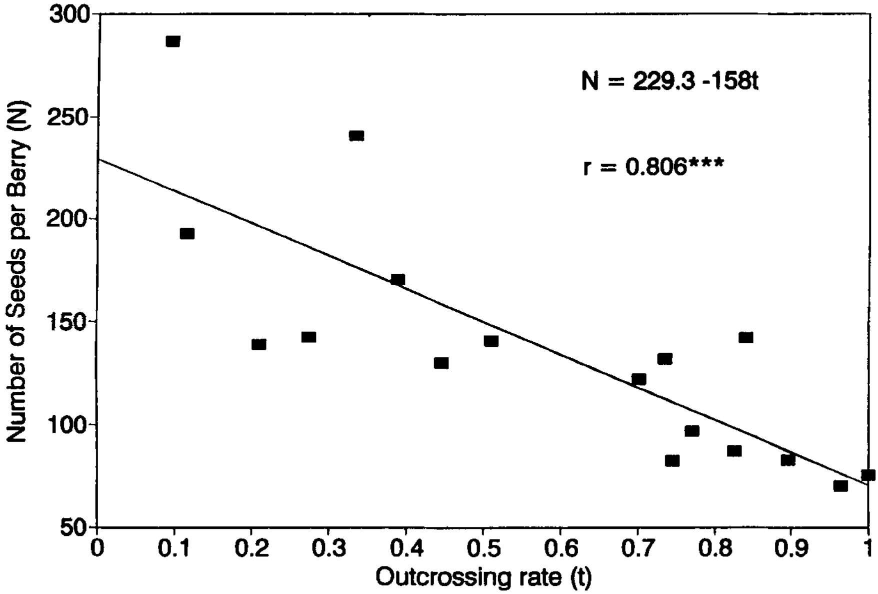

FIG. 1. Relationship of outcrossing rate, t, and seed production (seeds per berry) in potato in a field open-pollination environment.

obtained estimates from specific ssp. *andigena* clones that ranged between 0.13 and 0.45. The ssp. *andigena* clones measured in this study extend the range of values above those previously recorded and provide a higher estimate of the mean than these previous studies. Camadro and Peloquin (8) estimated outcrossing rates of 0.78 and 0.99 for polyploid self-fertile accessions of wild species *S. gourlayi* Hawkes and *S. oplocense* Hawkes, respectively. Rabinowitz and coworkers (16) found that *S. stenotomum* cultivars and wild *S. sparsipilum* (Bitt.) Juz. et Buk. produce true seed that is 95% interspecific hybrid in composition when interplanted under natural field conditions in the Andes. Self-pollination is not at issue in this work as the two species are self-incompatible diploids. This work illustrates, nonetheless, the high degree to which pollen can be transported between unrelated individuals by native "buzz" pollinators. Tynan *et al.* (18) estimated movement of an inserted gene controlling herbicide resistance by a "donor-recipient" method. Progeny were scored using herbicide as a selection agent. Although outcrossing rates were not calculated, it was noted that gene migration of the donor declined greatly at distances greater than 1 m from the source.

The negative correlation between outcrossing rate and seed set may have several explanations. One possibility is that the seed production of a particular genotype is partially limited by viability of its own (self) pollen. When self pollen is partially or totally dysfunctional, the seed set is limited by the rate at which functional pollen from another source is supplied by pollinators. Furthermore, where self pollen is totally functional, seed set is independent of pollinator delivery of additional functional pollen. If pollinator-delivered pollen is limiting, then seed production in the higher ranges of outcrossing would be reduced. Alternately, one might investigate if some degree of female sterility is associated with the male sterilities studied here. Clearly the population dynamics of "buzz" pollinators is one of the variables that can affect outcrossing rates between locations and years.

It appears that complete and partial sterilities raise outcrossing to the high levels present in certain advanced breeding materials that have wild species cytoplasms. This study showed that the tetrad sterility of clone #1, "reduced pollen function sterility" of Atzimba (#2) and five out of six of its progeny were associated with reduced seed per berry. In the other group of fully self-fertile clones, a broad range of outcrossing was also found. The mechanism(s) responsible for this are likely to involve protandry or protogyny possibly acting in concert with relative positioning of the stigma and stamens at anthesis. It is a common observation that male-fertile potato does not produce fruits in the absence of native pollinators or mechanical manipulation in the field or greenhouse. Vibration of flowers at anthesis by pollinator or artificial means is necessary for fruit set even in the case of full male-fertility.

It has been suggested that autotetraploid potato is an inbreeding species, and that successive generations of natural pollination will lead to an increase in homozygosity (9). This study found outcrossing rates that span the entire range of possible rates. Outcrossing in excess of t = 0.5 was measured in 10 of the 17 clones assayed in this study. Outcrossing in the upper range may prevent accumulation of unacceptably high levels of inbreeding. The evolution of inbreeding in populations mating with a mixture of selfing and outcrossing has been elucidated by Busbice (7). For the purpose of discussion, the hypothetical relationship between inbreeding coefficient and outcrossing rate in a synthetic population is diagrammed in Figure 2 using Busbice's equations. The curves show the progress of inbreeding in an autotetraploid synthetic derived from two (N=2) noninbred, unrelated parents. The inbreeding coefficient (F) represents the probability that two alleles chosen at random are identical by descent. It is assumed that the Syn 1 generation is created by a controlled cross between the two parents without selfing, and that mating leading to production of

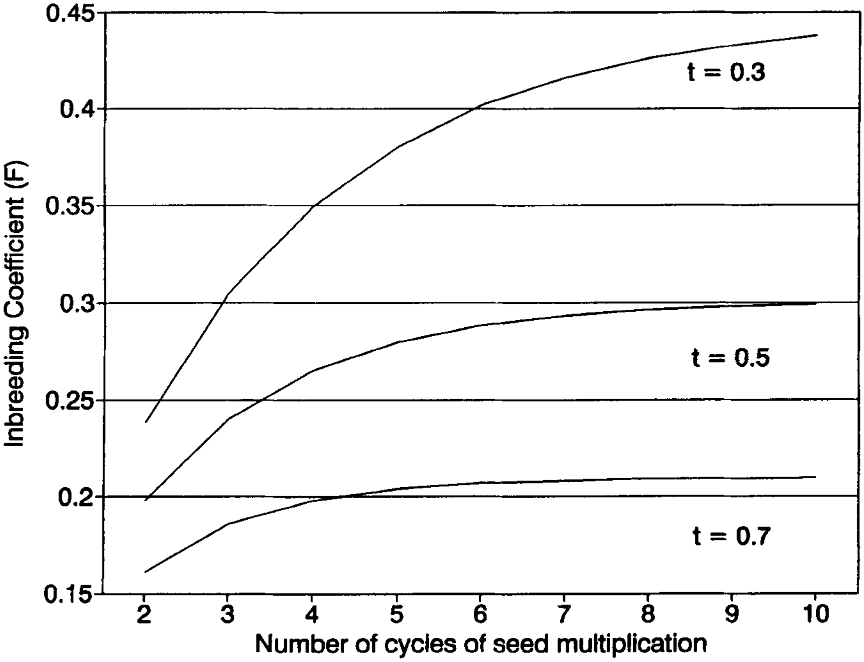

FIG. 2. Change of inbreeding coefficient (F) in an autotetraploid synthetic derived from two (N=2) noninbred, unrelated parents with three outcrossing rates (t). Syn X refers to cycle of multiplication. Thus Syn 0 refers to the parents, Syn 1 to the F_1 progeny and Syn 2 through Syn 10 to the successive sexual progeny generations resulting from the designated outcrossing rates.

populations of the Syn 2 and future cycles is by open-pollination, random mating and selfing in proportions denoted by the variable t. It is noteworthy that by Syn 6 the synthetic with t = 0.7 is approaching asymptotically an inbreeding equilibrium that is approximately ½ that of the synthetic with t = 0.3. The population with higher outcrossing would maintain much greater heterozygosity over the cycles of multiplication. Atlin has discussed (1) and documented (2) the effects of inbreeding on yield performance of potato populations produced from true seed. Lower inbreeding coefficients, below F = 0.2, seem to have relatively little effect while populations with values above F = 0.3 showed tuber yield reductions that can reach 30%. Shonnard and Peloquin (17) have shown that in inbred potato populations, plants have much lower open-pollinated fruit production. Compared to the noninbred initial hybrid population OP fruit production per plant was reduced by 78% for the S_1 generation and by 92% in the S_2 generation. This was accompanied by similar reductions in flowering, plant vigor, pollen stainability, and tuber yield. It would appear that the inbred progeny of self-fertilization contribute relatively less to succeeding generations than less inbred progeny of outcrossing.

The results of this study suggest that outcrossing in autotetraploid potato has a broad range. It might be possible to assemble parents with high outcrossing rates as the initial parents of synthetics. The tendency of higher outcrossing rates to be associated with lower seed set might lead, however, to directional selection toward decreased outcrossing over several cycles of multiplication by open-pollination. Nonetheless, for the purpose of true seed production, starting with parents with higher outcrossing rates may keep inbreeding at low levels and prevent drastic performance decreases for a number of cycles of seed increase.

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