Estimation of Outcrossing Rates in Andigena Cultivars: Implications in Breeding TPS Cultivars

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ABSTRACT

Studies were carried out to determine the outcrossing rate of four Andean potato cultivars, Solanum tuberosum ssp. andigena. An anthocyanin spot on the embryo (seed-spot) was used as a morphological marker. Clearly distinguishable markers which can be scored early in the life cycle of seedling progenies are most suitable for outcrossing studies. The use of seed spot represents a tremendous savings in time and resources needed to determine the outcrossing rate.

Single inflorescences of the test varieties were place in an apiary paired with the seed spot donors. The estimates therefore, represent outcrossing between inflorescences. The lowest rates observed were 5% and the highest 100%.

The effects of mixed selfing and random-mating on heterozygosity in autotetraploid populations will be discussed.

Introduction

In the Andean Cordillera, the place of origin of the cultivated potato, cultivars exist in a polyploid series ranging from diploid (2n = 24) to pentaploid (2n = 60). Cultivated diploids are hemaphroditic and self-incompatible, while tetraploids, Andigena, (2n = 48) are self-compatible. Although different forms of gynodioecy are especially frequent in long-day adapted Tuberosum cultivars, that evolved from Andigena originally, Andigena cultivars are mostly male and female fertile.

There is abundant observation that bumblebees are the predominant agents of pollination (Glendinning, 1976). Berry set is rare without some kind of manipulation if plants are protected from bumblebees, such as in a greenhouse situation (Plaisted, 1980).

The floral morphology suggests that the proportion of fertilizations arising from selfing could be high due to the close juxtaposition of dehisced anther pores and receptive stigma in the flower.

The purpose of this paper is to present estimates of outcrossing rates of several Andigena cultivars obtained by use of a seedspot genetic marker. The importance of outcrossing rate will be discussed in terms of the effects on inbreeding levels in autotetraploid populations. Application of this relationship to an alternative scheme for breeding potato populations for utilization as true seed cultivars will be explored.

Materials and Methods

Genetic Stocks: Two cultivars of *Solanum tuberosum* ssp. *andigena* possessing "seedspot" phenotype, i.e. anthocyanin pigmentation visible on the hypocotyl region of the embryo, were selected as a "donors" of the genetic marker. Three Andigena cultivars whose true seed was free of this marker were selected as "recipients."

Experimental set-up: Inflorescences were cut from field grown plants when one to three flowers had anthesed. Recipient inflorescences were paired with donor inflorescenses and placed in milk bottles. The milk bottles were placed at a distance of five meters apart in an apiary of honeybees *Apis mellifera* L. at 3,300 m elevation in the Mantaro Valley of the Departament of Junin in Peru. The distance between the recipient and donor inflorescences in each bottle was approximately 5 cm.

Scoring: The presence or absence of seedspot was scored by observing at 10x magnification with flourescent light transmitted from below, through the translucent seed coat. Before scoring a careful screening of seed was carried out to insure that only seeds with fully developed embryos were scored. This prevented overestimation of the proportion of spotless individuals through scoring of apparently normal seeds, lacking embryos, as spotless phenotype. Deformed and opaque, usually dark brown, seed coats were naturally not considered for scoring.

Controlled pollinations, including all combinations of donor and recipients and self-pollination of recipients, were made to provide estimates of the proportion of the progeny expected to show seedspot upon 100% outcrossing. All pistillate parents were emasculated first and pollinated in an insect-free screenhouse to prevent accidental selfing or outcrossing to unknown genotypes.

The percent outcrossing, t, was calculated by:

$$\%t = \frac{D_i}{P_i} \times 100$$

where D_i is the proportion of individuals with seedspot phenotype in the progenies of recipient i from the outcrossing experiment and P_i is the proportion of individuals with seedspot in the progenies of recipient i from the controlled crosses. This is a modification of the dominant-recessive technique upon which Jain (1979) commented. The donors are not homozygous dominant as will be reported below. Thus, P_i represents the phenotypic proportions expected with full outcrossing. The estimates of standard deviation were calculated as indicated by Jain (1979).

Results: Controlled Crosses

The proportions of progenies from controlled crosses with seedspot is shown in Table 1. The progenies of crosses between unpigmented recipients and pigmented donors showed seedspot in differing proportions. Donor clone OCH 6579 when crossed with OCH 7525 produced progenies with 46.2% seedspot. When crossed with 702316 and CUP 183 the progenies showed 41.2 and 41.4%, respectively. The donor clone 702156 showed a much lower proportion of expression than OCH 6579, producing

32.5, and 26.2% embryos with seedspot when crossed with OCH 7525 and 702316, respectively. In addition, the utility of OCH 6579 as a marker clone is greater because the spotted phenotype is much more clearly defined with a darker, larger spot. The cultivar 702156 produces a less distinct, fainter purple spot which is difficult to score. The percent seedspot shown in Table 1 was converted to a proportion by dividing by 100. The resulting P_i values were used to calculate outcrossing rates for each combination of donor and recipient.

Results: Outcrossing Estimates

Pollinators: Although the inflorescences were placed in a honeybee apiary, effective extraction of pollen or transfer of pollen to the stigma of flowers by honeybees, was never observed. This agrees with the observations of Sanford and Hanneman (1981) that honeybees appear to be totally unadapted for pollination of flowers of cultivated potato. Occasionally worker bees would land on flowers but did not demonstrate any organized pollination activity. An unidentified "bumblebee" did enter the apiary and worked the flowers as is typically seen in potato fields of male fertile cultivars in the area. The area immediately surrounding the apiary was planted for table stock with two different male sterile Peruvian Tuberosum-Andigena hybrid cultivars (cv. Revolucion and cv. Mariva).

Table 1. Percent seedspot in progenies of controlled crosses between donors and recipients.

	% Seedspot	N
OCH 7525 x	0	100
OCH 7525 x OCH 6579	46.2	186
OCH 7525 x 702156	32.5	166
702316 x	0	100
702316 x OCH 6579	41.2	223
702316 x 702156	26.2	221
CUP 183 x	0	100
CUP 183 x OCH 6579	41.4	244

The estimates range from 13.2 to 44.9% (see Table 2). There is some difference between the two donors but the estimates for recipient 702316 are very close.

Discussion

The question of whether cultivated tetraploid potato is a predominantly self-pollinated crop is essentially a point requiring quantitative answers. Glendinning (1976) reported estimates ranging from 14 to 30% with a mean of 20% in Neo-tuberosum. Peloquin reported a range of from 10 to 40% in breeding clones of cultivated potato (CIP, 1980). In tetraploid and hexaploid wild species, Camadro and Peloquin (1982) reported maximum estimates of 50 and 65% outcrossing, respectively.

Table 2. Estimates of outcrossing rates in Andigena cultivars.

	Outcrossing Rate $\hat{\%}_t \pm \hat{\sigma}_{\hat{\%}t}$	N
OCH 7525 x 702156	27.3 ± 7.6	304
OCH 7525 x OCH 6579	44.9 ± 3.9	751
702316 x 702156	13.2 ± 9.9	145
702316 x OCH 6579	15.2 ± 4.4	272
CUP 183 x OCH 6579	41.0 ± 7.4	259

The outcrossing estimates presented here range from 13 to 45%. Taking an average value of 36% for cultivar OCH 7525, of 14% for 702316 and the single estimate of 41% for cultivar CUP 183, the average over three clones is 31%. This lies within the range of all three above mentioned reports. Based on the very small sample, these estimates should only be regarded as preliminary.

Considering the present estimates and those available from the cited literature, it is not strictly correct to call the cultivated potato a selfing species in regards to its sexually reproductive phase. The effect of a mixture of selfing and random-mating in hemaphroditic plant species is a reduction of heterozygocity below that which would exist in a random mating population assuming no selective advantage of heterozygotes. However, as long as some random-mating occurs, the population will not become completely homozygous. Wrights inbreeding coefficient, F, the probability that two alleles taken at random are identical by descent, is a useful parameter for comparing the equilibrium levels of inbreeding with different rates of outcrossing. Bennet (1968) derived an equation for the coefficient of inbreeding at equilibrium for partial selfing, $F_\infty = (S + 2\alpha)/4 - 3S + 2\alpha$, for autotetraploid species where α is the coefficient of double reduction and S is the probability of selfing. Assuming $\alpha = 0$ the result is $F_\infty = S/4\text{-}3S$. In Table 3 the values of the inbreeding coefficient at equilibrium are shown for a range of outcrossing percentage, $\%t = 100 \times (1\text{-}S)$. If 30% is taken as a benchmark value for potato, it can be seen that F_∞ is .368. This is certainly less inbreeding than would be expected from full selfing, and in fact, on a population basis this value would allow for considerable heterozygosity and thereby genetic variability. The progenies of certain crosses of present day North American cultivars would have coefficients of inbreeding close to this value: Cobbler x Earlaine, F = .344; and Triumph x La Soda, F = .313 (Mendoza and Haynes, 1974).

Implications of Present Data for Breeding Potato for Cultivation from True Seed

Starting in the People's Republic of China 20 years ago and now under intensive study at the International Potato Center, the culture of potato as a crop established from seedlings derived from true seed is of great interest. While previously potato cultivars were single clones, already at this writing several true seed cultivars are available from private seed firms. The genetic impact of mating system now takes on increasingly significant role because the breeding of potato cultivars can be conducted by several new alternative routes.

There are essentially three approaches at first glance. First, seed lots obtained from open-pollinated berries may be used as cultivars. Regardless of outcrossing rate seed balls obtained from large fields of a single clone are genetically selfed.

A partial polycross system might be imagined where cultivars consist of open-pollinated seed lots obtained from single clones where pollen from other clones would have been available. In this case, OP seed would consist of a mixture of progenies from selfing and outcrossing to various clones. The actual amount of inbreeding would be highly dependent on the actual arrangement of parental clones in the field and the outcrossing rate.

Table 3. Values of inbreeding coefficient at equilibrium, F_∞, on autotetraploid population with mixed selfing and random mating (outcrossing), $0 \leq \%\ t \leq 100$, $0 \leq F_\infty \leq 1$.

%t	F_∞
0	1.000
1	0.96
5	0.83
10	0.69
20	0.50
30	0.37
40	0.27
50	0.20
60	0.14
70	0.10
80	0.06
90	0.03
95	0.01
100	0

Second, simple hybrids derived from controlled crossing of two clones or a simple bulk produced by crossing a single pistillate parent with a composite pollen from two or more staminate parents may be developed as cultivars. This approach could take advantage of pretested superior progenies and would maximize heterozygosity.

A third approach, that of a synthetic, would allow a small farmer to propagate TPS cultivars through true seed for a number of generations. A synthetic is a cultivar consisting of two or more parents comprising an S_0 generation which are randomly mated to form the S_1 generation. Subsequent generations are produced through natural pollination means. The population size is very small in the S_0 and virtually infinite in size in the S_1 and future generations. The quantity of true seed available is rapidly increased in the S_2 and S_3 generations and further, if necessary. Seed increase is inexpensive as hand pollination is not needed. The performance of the successive S generations may change. A specific generation may be used as a cultivar or all generations after which sufficient seed is available. For example, successive generations may be planted in successive crop years: crop year 1→S_1, crop year 2→S_2, crop year 3→S_3. Busbice (1969) described the progress of inbreeding, expressed as Wright's inbreeding coefficient, F, in autotetraploid synthetic varieties where the mating system is mixed selfing and random mating.

The key initial variables are F_o, the average level of inbreeding in the initial parents, r_o, the average level of coancestry, and n, the number of parents. From these, F_1, and r_1, are calculated for the S_1 generation.

$$r_1 = \frac{1}{n} \frac{1 + 3 F_o}{4} + \frac{n - 1}{n} \quad r_o$$

$$F_1 = \frac{1}{3} \; 2 \; r_o \; + \; F_o$$

A recurrence equation for F_t in succeeding generations was derived

$$F_t = \frac{s}{6} \quad \frac{2 \; (1 - s)}{3} r_1 \; \frac{1 - \frac{s}{2} + \frac{1}{3}^{\,t-1}}{1 - \frac{s}{2} + \frac{1}{3}} \quad + \frac{s}{2} + \frac{1}{3}^{\,t-1} F_1$$

where s is the probability of selfing. From this relation the equilibrium inbreeding coefficient is:

$$F_\infty = r_1 + \; s(1 - r_1) \; / \; s + 4 \; (1 - s)$$

(Busbice, 1969).

Table 4 shows the relationship of outcrossing to the equilibrium value of F for synthetics originating from two, four, eight, and sixteen parental clones. The assumptions here are that the parental clones are not inbred ($F_o = 0$) and they are unrelated ($r_o = 0$). Outcrossing rates shown cover the range of 0 to 50% only.

For a given value of outcrossing, the inbreeding coefficients decrease as the number of parents increase. For a given number of parents, the inbreeding coefficient decreases with increasing outcrossing rate. An eightfold increase in the number of parents results in a 16% decrease in inbreeding coefficient at equilibrium for 30% outcrossing. But, a 20% percent decrease in the inbreeding coefficient at equilibrium is achieved for a synthetic originating from just two parents if the outcrossing is raised from 30 to 40%, an increase of one-third.

It is difficult to choose large numbers of parents with high general combining abilities. The reduction in inbreeding is not as dramatic with an increase in the number of parents as with an increase of outcrossing rate.

The nature of genetic variability in outcrossing rates remains as yet undefined. The goal of selecting for higher outcrossing appears possible based on the variability in estimates already reported in the literature and those presented here. Continued selection in a breeding program may be impractical owing to the difficulty of rapidly screening clonal lines for outcrossing rate. The seedspot marker could alleviate this problem, however.

The present discussion of inbreeding coefficient suggests that synthetics based on as few as two non-inbred, unrelated clones will accumulate relatively little inbreeding if the outcrossing rates are maintained between 30 and 40%. Therefore, it would seem that the objective of providing TPS cultivars which can be multiplied sexually for a number of generations without deleterious accumulation of inbreeding can be achieved in synthetics originating from as few as two parents as long as outcrossing rates are maintained higher than 30%. This value is well within the range of the heretofore reported estimates. More attention should be placed on outcrossing rate if it is contemplated that TPS cultivars will be multiplied by small farmers to provide an inexpensive continuous nondegenerating seed supply.

It is likely that certain agroecosystems would require that farmers be capable of producing their own true seed in successive seasons. Purchase of hybrid seed could increase costs too much. Only acceptable levels of inbreeding will accumulate if the outcrossing rates are sufficiently high. This may also provide a means of increasing quantities of seed available from simple hybrids (two parents) where seed quantity may be the main factor limiting commercial exploitation of superior progenies.

The increase in the inbreeding coefficient over successive cycles of multiplication with natural pollination is shown in Figure 1. Starting with a cross of two unrelated noninbred clones how much seed could be produced in two further cycles of multiplication? If the multiplication rate is 1,000 seeds/plant in the field, then 1,000 S_1 seeds would yield 100,000,000 seeds in the S_3. If an outcrossing rate of 30% had been maintained during these two cycles, the inbreeding coefficient would have reached 0.3. If we assume that 150,000 seeds/ha are required then enough seed would be available to plant 67,000 hectares. If we had started with 100,000 seeds we could plant 6,700,000 hectares with the S_3 seed. The decline in performance of TPS populations that is expected to occur with inbreeding will be less with higher outcrossing rates. Efforts to select for and maintain the highest possible outcrossing rate are therefore justified. At the present time the difficulty of producing sufficient quantities of TPS appears to be a serious obstacle to wider applications of this new technique for growing the potato crop.

Table 4. Coefficient of inbreeding at equilibrium in autotetraploid synthetic with differing numbers of noninbred, unrelated parental clones in relation to percent outcrossing (%t) during successive sexual multiplication.

	Number of initial parental clones			
%t	2	4	8	16
0	1.00	1.00	1.00	1.00
10	.73	.71	.70	.70
20	.56	.53	.52	.51
30	.45	.41	.39	.38
40	.36	.32	.30	.28
50	.30	.25	.22	.21

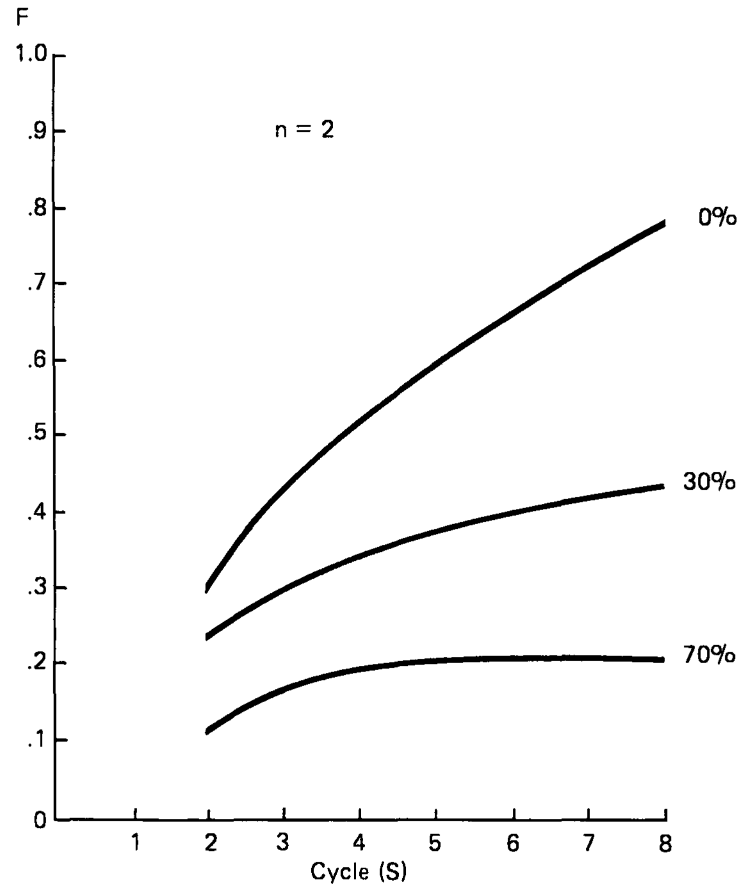

Figure 1. Inbreeding coefficient over cycles of multiplication for different outcrossing rates (%). The S_0 consists of two nonimbred, unrelated parents.

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