

Euphytica (2008) 164:881–892  
DOI 10.1007/s10681-007-9641-x

# Hybridization between wild and cultivated potato species in the Peruvian Andes and biosafety implications for deployment of GM potatoes

Maria Scurrah · Carolina Celis-Gamboa ·  
Susana Chumbiauca · Alberto Salas ·  
Richard G. F. Visser

Received: 12 May 2007 / Accepted: 18 December 2007 / Published online: 15 January 2008  
© Springer Science+Business Media B.V. 2008

**Abstract** The nature and extent of past and current hybridization between cultivated potato and wild relatives in nature is of interest to crop evolutionists, taxonomists, breeders and recently to molecular biologists because of the possibilities of inverse gene flow in the deployment of genetically-modified (GM) crops. This research proves that natural hybridization occurs in areas of potato diversity in the Andes, the possibilities for survival of these new hybrids, and shows a possible way forward in case of GM potatoes should prove advantageous in such areas.

**Keywords** Hybridization · GM · Wild & cultivated potatoes · Biosafety · Center of origin

---

Maria Scurrah and Carolina Celis-Gamboa contributed equally to this manuscript.

---

M. Scurrah (✉) · S. Chumbiauca  
Ministry of Agriculture, Servicio Nacional de Sanidad Agraria (SENASA), Av. La Molina 1915, La Molina, Lima, Peru  
e-mail: [scurrah@gmail.com](mailto:scurrah@gmail.com)

C. Celis-Gamboa · R. G. F. Visser  
Laboratory of Plant Breeding, Wageningen University, P.O. Box 386, Wageningen 6700 AJ, The Netherlands

A. Salas  
CIP, P.O. Box 1558, Lima 12, Peru

## Introduction

The Genus *Solanum*, section Petota (tuber-bearing), includes about 187 species (Spooner et al. 2005) from which seven (Hawkes 1990) or nine (Ochoa 1990) are recognized as cultivated taxa. Wild and cultivated species are distributed from southwestern United States of America to southern Chile. They grow under highly diverse ecological conditions ranging from very dry to very wet and from sea level to more than 4,000 m. The worldwide cultivated potato (*Solanum tuberosum* L.) is a tetraploid species ( $2n = 4x = 48$ ), whereas its wild and cultivated relatives range from diploid to hexaploid species, with 12 as the basic chromosome number. Crossability between wild and cultivated potato depends on several pre- and post-fertilization events. Hybridization between different species is commonly prevented by stylar and ploidy barriers. In addition, the Endosperm Balance Number (EBN) is thought to play an important role in the success of interspecific hybridization (Cultivarston et al. 1980). The EBN is a number ranging from 1 to 4, which represents the “effective” ploidy level of *Solanum* species (Ehlenfeld and Hanneman 1988). However, the presence of  $2n$  gametes may allow a species to overcome stylar, ploidy and EBN barriers.  $2n$  gametes result from meiotic distortions affecting micro- or macro-sporogenesis (Peloquin et al. 1989; Ramanna 1979). In potato, wild species are scrutinized for their contribution to variability in traditional farmer’s fields and to current plant breeding efforts in

the search for traits that make potato cultivars tolerant or resistant to diverse diseases (Chen et al. 2003) and abiotic stresses such as drought and frost. The exploitation and contribution of wild species to the potato crop is possibly the most extensive of any crop (Ortiz 1998). The perceived risk of transgene escape in areas of natural diversity has triggered renewed interest in this area of research (Jackson and Hanneman 1999). Since crop improvement through interspecific hybridization between wild and cultivated *Solanum* species has been carried out under artificial conditions, the inverse situation, whether foreign genes deployed in transgenic potato could flow into cultivated and wild relatives under natural conditions needs more evaluation. Furthermore, the possible consequences of gene introgression of these foreign genes on both native cultivars and wild species need to be addressed in areas of diversity, where wild species are commonly found within and around cultivar mixtures grown together in subsistence farming. The objectives of this study were to: (1) quantify the crossing potential between bred-cultivars, native cultivars and wild species both in open pollination fields and in greenhouse crosses, (2) assess seed set, viability and hybridity of progeny, (3) identify pollinators and (4) evaluate progeny survival under wild and cultivated conditions.

## Materials and methods

### Open pollinated field experiments

To quantify the extent of natural cross pollination, 54 accessions/cultivars from 13 wild and cultivated *Solanum* species were planted in five localities distributed in the northern, central and southern region of Peru. Two plots were located in a farmer's field and the other three in experimental stations. Each plot included several cultivated and wild potato species, commonly found in the area. There were 10 plants for each entry/accession placed at random once in each row/block in a completely randomized block design. Plant distance was 0.35 m between plants in a row and 1 m between rows. The cultivated species included tetraploid 4EBN hybrid cultivars resulting from the cross between the tetraploid species *S. tuberosum* spp. *tuberosum* and ssp. *andigena*, (*adg*) and native cultivars from five species: tetraploid

(4 EBN) *S. tuberosum* spp. *andigena*, diploid (2 EBN) *S. phureja* (*phu*), *S. goniocalyx* (*gon*), *S. stenotomum* (*stn*) and pentaploid 4 EBN *S. curtilobum*, (*cur*). In every site two well known tetraploid cultivars, Revolución (male sterile showing flower abscission) and Yungay (fully fertile), were planted. Additionally, in each location the most commonly grown bred-cultivars and native cultivars were planted. Also seven wild species, hexaploid (4EBN) *S. albicans* (*alb*), *S. chomatophilum* diploid (2EBN) (*chm*), *S. acaule* tetraploid (2EBN) (*acl*), *S. bukasovii* diploid (2EBN) (*buk*), *S. raphanifolium* diploid (2EBN) (*rap*), *S. sparsipilum* diploid (2EBN) (*spl*) and *S. megistacrolobum* diploid (2EBN) (*mga*) were included, depending on location. These wild species are often found within and around cultivated potato fields (Hijmans 2002; Ugent 1970; Linder 1987). This article only shows the results from the open pollinated field in Puno, since it has the largest genetic diversity, both in number of species/accessions and differences in chromosome number. This site was at the Instituto Nacional de Investitaacion Agraria, (INIA) Experimental Station Salcedo, outside the city of Puno at 3,800 m a.s.l. and 15.82° South. The cultivated and wild entries grown in this site are given in Table 1. Tubers for all the improved cultivars and wild species, including seeds from *S. chomatophilum*, were kindly provided by the International Potato Center (CIP), whereas from the native cultivars, seed tubers were obtained from CIP or from local markets.

### Greenhouse crosses

Parallel to the open pollination plots crosses between the same entries were carried out under greenhouse conditions at CIP experimental station in Huancayo. All the flowers from five plants per entry were emasculated before hand pollination.

### Quantification of flowers, berries and seeds

In the open pollination field experiment, the number of open flowers and berries present in each plant was recorded every 4 days. Upon maturity, the berries from each plant were harvested and identified with the field number of the mother plant from which they were collected. Similarly, the number of pollinated flowers and the number of berries resulting from the

**Table 1** Cultivated and wild *Solanum* species grown in the open pollinated field trial in Puno

Cultivated species	Cultivar name	Code	Ploidy (EBN <sup>a</sup> )
<i>S. phureja</i> 1	Chaucha Colorada	phu1	2x (2EBN)
<i>S. andigena</i> 5	Qompis	adg5	4x (4EBN)
<i>S. andigena</i> 6	Yana Imilla	adg6	4x (4EBN)
Hybrid 1 <sup>b</sup>	Revolución	hyb1	4x (4EBN)
Hybrid 2	Yungay	hyb2	4x (4EBN)
Hybrid 6	Andina	hyb6	4x (4EBN)
<i>S. stenotomun</i> 1	Chuco pitiquiña	stn1	2x (2EBN)
<i>S. curtilobum</i>	Shiri redonda	cur	5x (4EBN)
Wild species	CIP code	Code	Ploidy (EBN <sup>a</sup> )
<i>S. acaule</i> 1	HHCH 5075	ac11	4x (2 EBN)
<i>S. acaule</i> 2	OCH 11990	ac12	4x (2 EBN)
<i>S. acaule</i> 3	SS 7253	ac13	4x (2 EBN)
<i>S. acaule</i> 4	HAM 2	ac14	4x (2 EBN)
<i>S. bukasovii</i> 2	OCH 2032	buk2	2x (2 EBN)
<i>S. bukasovii</i> 6	OCHS 15618	buk6	2x (2 EBN)
<i>S. bukasovii</i> 7	HHCH 5064c	buk7	2x (2 EBN)
<i>S. bukasovii</i> 8	HJT 1407	buk8	2x (2 EBN)
<i>S. sparsipilum</i> 1	CUS 94-02	sp11	2x (2 EBN)
<i>S. sparsipilum</i> 2	OCH 2064a	sp12	2x (2 EBN)
<i>S. sparsipilum</i> 3	SS 7213	sp13	2x (2 EBN)
<i>S. sparsipilum</i> 4	OCHS 16147	sp14	2x (2 EBN)
<i>S. megistacrolobum</i> 1	HHCH 4589	meg1	2x (2 EBN)
<i>S. megistacrolobum</i> 2	HHCH 5058	meg2	2x (2 EBN)
<i>S. megistacrolobum</i> 3	HHCH 4853	meg3	2x (2 EBN)
<i>S. megistacrolobum</i> 4	OCH 11941a	meg4	2x (2 EBN)

<sup>a</sup> Endosperm balance number

<sup>b</sup> Tetraploid cultivars hybrids of *S. tuberosum* spp. *tuberosum* and spp. *andigena*

crosses made under greenhouse conditions were recorded. Seeds were extracted and counted to obtain the average number of seeds per berry. The percentage of germination was determined from the samples taken from each seed batch for morphological and molecular characterization.

#### Hybridity of seed produced from greenhouse crosses

A sample of 100 seeds from each cross combination was planted in plastic trays and from each seedling an

AFLP fingerprint was generated to compare it with that of the two parental species as described previously by Celis et al. (2004). In addition, 10 plants from each cross combination made under greenhouse conditions were grown in plastic pots and their morphological characteristics were compared with those of the parental species.

#### Hybridization frequency between *Solanum* species grown in Puno

The AFLP fingerprint of each entry present in the field plot was generated and compared with the banding pattern of each seedling from a 100-progeny sample. The seed samples were taken from 40 selected plants of 143 plants which set seeds in Puno. From this comparison, the most likely male parent was identified.

#### DNA isolation

To isolate DNA from the parental plants, a sample of fresh young leaves was collected from each entry growing in the field and in the greenhouse, placed in a paper bag and dried at 36°C during 48 h. DNA was isolated using a CTAB protocol (Bernatzky and Tanksley 1986) with modifications, including a reduction in both the amount of leaf tissue and the volume of the extraction buffer to fit in a 2 ml conical tube. To isolate DNA from the progenies, seeds were planted in plastic trays filled with soil and DNA was isolated from 3-week-old seedlings, using the same extraction protocol.

#### AFLP fingerprinting

From the 20 AFLP primer combinations most commonly used in potato (potatodbase at <http://www.potatodbase.dpw.wau.nl/tabletst.htm>), 12 were tested with the 54 accessions. We found that the primer combination E35/M48 (EACA/MCAC) gave the best discrimination between and within species (and accessions) based on the unique AFLP banding pattern generated by the presence/absence combination of 203 bands (markers). The AFLP procedure was performed as described by Vos et al. (1995). AFLP

markers were visually scored as presence or absence of the bands to generate the molecular fingerprint of 20,790 plants from field and greenhouse experiments (Celis et al. 2004).

#### Identification and monitoring of pollinator insects

Insects visiting potato flowers were observed in five experimental plots the following year. Three were located in Junín, at Espital (3,420 m), La Victoria (3,250 m) and Pachapaqui (3,550 m). The other two plots were in Puno (Salcedo, 3,820 m) and Cusco (Andenes, 3,600 m). In each location two native and two hybrid cultivars (*S. tuberosum* spp. *tuberosum* × ssp. *andigena*) were planted. The four cultivars were planted in two replicated blocks and randomized within each block. Each plot was 4 rows with 1 m between rows and 0.35 m between plants in a 5 m long row. The native cultivars were Amarilla Peruana (*S. gonioalix*,  $2n = 24$ , abundant crimson flowers) and Qompis (*S. andigena*,  $2n = 48$ , less abundant white flowers). The bred-cultivars were Yungay ( $2n = 48$ , less abundant pink flowers) and Revolución ( $2n = 48$ , male sterile with scarce white flowers). Once a week in February and twice a week in March, flying insects visiting potato flowers were counted during walks done around the perimeter of the experimental area every hour, during a 10-min period, from 07:00 to 18:00 h. Specimens were collected and sent for identification to Dr. Claus Rasmusen at the University of Illinois.

#### Survival of seed produced in the open pollinated experiments

In June 2002, 21 samples of 20 berries each were used to assess germination of seeds and survival of the resulting seedlings. The berries came from plants setting seeds in three of the open pollinated field experiments, based on seed abundance. Thirteen plants with abundant berries were chosen from Puno, five from Colpar and three from La Victoria. The experimental plot was located in La Victoria (CIP's Experimental Station near Huancayo, in Junín). The berry samples were randomized and planted in rows with spacing of 0.35 m between berries and 1 m between rows and 5 cm depth. No further

intervention was made. Quantification of emerging seedlings was carried out once a week from December, the beginning of the rainy season, until March 2003. During the following growing season (December–March 2004), the plot was monitored again to quantify the number of emerging plants either from tubers produced the previous year or from the berries buried in 2002.

#### Selection of F1 hybrids by a farmer

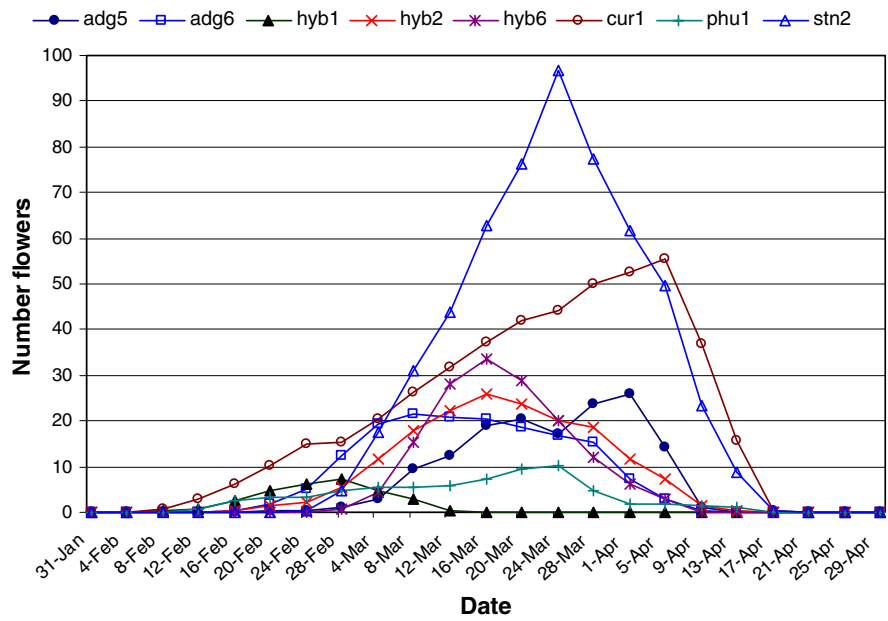
From each one of the 65 progenies obtained from greenhouse crosses between wild and cultivated species, 10 seedlings were grown for morphological comparison. From each plant a tuber was taken to form a tuber family. Sixty-five tuber families were given to a farmer in Colpar (Junín) to be grown along with his potato crop. At harvest, he and his family choose those plants that according to their criteria had good plant and tuber characteristics that would make them worth keeping.

## Results

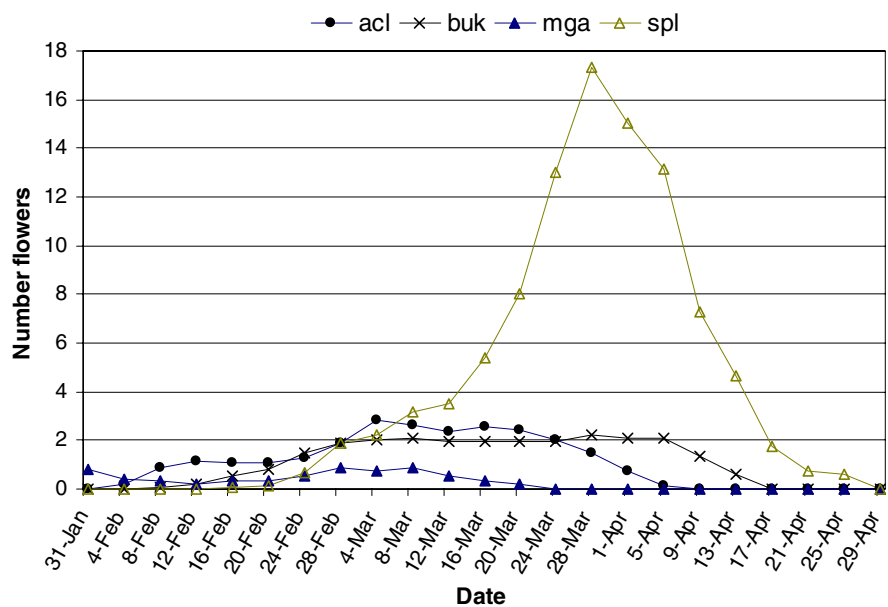
#### Production of flowers, berries and seeds in field

Among the cultivated species (Fig. 1), *S. stenotomun* (stn) had the highest number of flowers per plant (97), followed by *S. curtlobum* (cur) (56) whereas *S. phureja* (phu) had the lowest number (10). Among the hybrid cultivars, Andina (hyb 6) had the highest (34) and the male sterile cultivar Revolution the lowest number of flowers per plant (7). These flowers aborted and no berries were produced at any of the five sites. The two cultivars from *S. andigena* (adg) had a slightly lower number of flowers per plant (26 and 21) than the hybrids. In contrast, the wild species overall produced a lower number of flowers (Fig. 2) than the cultivated species. *S. sparsipilum* had the highest (17) and *S. megistacrolobum* (mga) had the lowest number of flowers per plant (1). Flowers were observed during a period of 84 days. The wild species flowered for longer periods (52–68 days) than the cultivated species (52–60 days). During 32 days, open flowers from all species overlapped in the field. The cultivated species produced more flowers than the wild

**Fig. 1** Average number of flowers per date produced by the cultivated species in Puno



**Fig. 2** Average number of flowers per date produced by the wild species in Puno. *S. acaule* (acl); *S. bukassovi* (buk); *S. megistacrolobum* (mga) and *S. sparsipilum* (spl)



species, which was reflected in the total number of berries per plant (Table 2). The number of seeds per berry was also higher in the cultivated species, except for *S. acaule* which came close (170) to the hybrids (196). Furthermore, the cultivated species produced four times as many berries (44.6 vs. 11.7) and four times as many seeds per plant across all sites.

Seed production from greenhouse crosses

A total of 65 cross combinations were obtained from 1996 flowers of which 29 or 45% of the seed lots resulted from cultivated × wild pollen parents and 36 or 55% from the wild female parents × cultivated pollen parents. The majority of the berries 420 or 60% came from wild females and cultivated males,

**Table 2** Seeds production from wild and cultivated species grown in Puno

	Species	Ploidy	Plants <sup>a</sup>	Berries/ <sup>b</sup> plant	Seeds/berry (SD)	Seeds/plant	Total seed
Wild	acl	4x-2EBN	33/40	13	165	2,159	70,560
	buk	2x-2EBN	15/30	5	35	271	4,070
	spl	2x-2EBN	24/30	27	35	1,442	34,605
	meg	2x-2EBN	5/20	2	13	20	200
	<b>16<sup>c</sup></b>		<b>77/120</b>	<b>11.7</b>	<b>59.8 (58)</b>	<b>548</b>	<b>109,435</b>
Cultivated	stn	2x-2EBN	10/10	75	58	4,290	42,900
	phu	2x-2EBN	9/10	43	60	6,843	27,331
	adg	4x-4EBN	18/20	27	127	3,498	62,960
	hyb	4x-4EBN	18/30	54	196	10,525	197,855
	cur	5x-4EBN	10/10	24	20.5	292	29,191
	<b>8<sup>d</sup></b>		<b>65/80</b>	<b>44.6</b>	<b>92.3 (33)</b>	<b>5,542.1</b>	<b>360,237</b>
Total	<b>24</b>		<b>142/200</b>	<b>28.7</b>	<b>82.3</b>	<b>3,229.2</b>	<b>430,797</b>

Bold character represents: Total and averages of wild total and averages of cultivated overall total

<sup>a</sup> Number of plants setting seed/total number of plants

<sup>b</sup> Mean number of berries/plant

<sup>c</sup> Number of accessions

<sup>d</sup> Number of varieties

however the average number of seeds per berry from this group was half (53.2) as those from the cultivated mothers (98.4).

In six different crosses, flowers were pollinated but no seed was obtained. In five cases very few seeds were obtained, mostly in the case of hybrids or *Andigena* crossed with wild species (Table 3). Here only the crosses that were represented in the Puno open pollinated field are reported.

#### Hybridity of seed produced from greenhouse crosses

From 24 cross combinations of which 50% are cultivated × wild (Table 3), six crosses were between different ploidy levels with aborted berries or berries were formed but had no seeds (parthenocarpic), while four progeny were obtained across the ploidy barrier and the seed were confirmed as hybrid. Of 12 cultivated females × wild males seven had hybrid progeny, four aborted and had no seed and one showed all the seeds to be selfed. On the other hand, out of 12 wild females × cultivated pollen parents, eight had hybrid progeny, one aborted and three showed the progeny to be selfed. Large differences were found in the rate of hybrid progeny between reciprocal crosses. Thus, in the

cross adg (4x, 4EBN) × acl (4x, 2EBN), 100% of the progeny was hybrid but in the reciprocal acl × adg 0% was hybrid.

Except for the cross adg × spl (4x × 2x), the cross combinations cultivated × wild were more successful in producing hybrids than the reciprocal wild × cultivated crosses. On the other hand, in crosses with the same ploidy, all the progeny was hybrid regardless of the direction of the cross, except for the *S. acaule* case mentioned above. *S. acaule* is known for its high rate of selfing (Ugent 1981), showing that in forced crosses timely emasculation for this species is difficult. In the six crosses where very few seed was obtained AFLP data are not available, but visual data suggests hybrids were obtained.

#### Hybridization under field conditions

Table 4 shows results of AFLP analysis of seeds obtained from the field grown plants grown in Puno. A total of 470 hybrids were identified from 1,130 seedlings from three wild species as mother plants: *S. sparsipilum*, *S. bukasovii* and *S. acaule*. The most successful pollen receptor was *S. sparsipilum*, with the highest percentage of hybrids (59%), followed by

**Table 3** Percentage of hybrids identified by AFLP from 24 cross combinations made under greenhouse conditions involving the cultivated and wild species grown in Puno

Cultivated species	Cross combination (♀ × ♂)	Ploidy	br/fl %	sd/br	% Germination	Seedlings tested by AFLP	% Hybrid
<i>S. stenotomum</i>	stn × mga	2x × 2x	10/18	38	48	48	100
	stn × spl	2x × 2x	20/33	60	80	160	100
	spl × stn	2x × 2x	28/32	22	97	97	100
<i>S. phureja</i>	phu × buk	2x × 2x	25/41	91	89	441	100
	buk × phu	2x × 2x	10/34	264	84	251	100
	phu × spl	2x × 2x	23/34	79	91	364	100
	spl × phu	2x × 2x	23/29	311	84	142	100
	acl × phu	4x × 2x	6/7	103	91	181	100
	phu × acl	2x × 4x	0/14	0	–	–	–
	meg × phu	2x × 2x	7/7	47	0.5	1	100
	<i>S. andigena</i>	adg × buk	2x × 4x	62/81	25	88	407
buk × adg		2x × 4x	70/114	0.8	90	10	100 <sup>a</sup>
adg × acl		4x × 4x	3/20	86	95	161	100
acl × adg		4x × 4x	18/28	33	95	95	0
adg × spl		4x × 2x	12/14	35	97	122	21
spl × adg		2x × 4x	59/85	8.2	58	93	100
<i>S. tuberosum</i> spp. <i>tuberosum</i> × spp. <i>andigena</i>	hyb × acl	4x × 4x	1/3	0	–	–	–
	acl × hyb	4x × 4x	4/24	110	74	74	0
	hyb × buk	4x × 2x	29/89	221	90	270	0
	buk × hyb	2x × 4x	9/103	0	–	–	–
	hyb × spl	4x × 2x	13/47	0	–	–	–
	spl × hyb	2x × 4x	13/85	5	83	75	100
<i>S. curtilobum</i>	cur × acl	4x × 5x	0/11	0	–	–	–
	acl × cur	5x × 4x	4/24	110	98	98	0

Number of berries formed from the total number of pollinated flowers (br/fl), number of seeds per berry (sd/br), percentage of germination and number of seedlings tested by AFLP

<sup>a</sup> Identified as hybrids based on plant and tuber morphology

*S. bukasovii* (36%) while *S. acaule* had a very low percentage of hybrid progeny (4%). The cultivated species identified as pollen donors in order of success were *S. phureja* (46.5%), *S. stenotomum* (21.4%), hybrids (19.7%) and *S. andigena* (5.9%). No natural hybrids of *S. acaule* with cultivated diploids *S. phureja* or *S. stenotomum* were found. Furthermore, the cultivated tetraploid species were identified as pollen donors of progeny on both diploid and tetraploid wild species, whereas only diploid wild species were successfully pollinated by the diploid cultivated species.

When the progeny of field grown cultivated species were analyzed for success of viable progeny from wild pollen parents the order of success is as follows: *S. stenotomum* 63%, *S. phureja* 31%, hybrids

4% and *S. andigena* 2%. The most successful pollen parents identified were: *S. bukasovii* (53%) mostly on diploid species, *S. sparsipilum* with 44% and again *S. acaule* as a far third with only 2.8% and only on tetraploid species.

#### Identification of insect pollinators in the experimental plots

Twelve insect species were identified visiting potato flowers in the different sites. Only *Bombus funebris* was present at all sites. In Puno eight insect species were observed (Table 5), the black Andean bee (*Lonchopria* spp. (Colletidae) provided 36.9% of the visits, followed by *Bombus opifex* with 34.7% and

**Table 4** Percentage of progeny from open pollinated field trial in Puno identified as hybrids between wild and cultivated plants based on AFLP analysis

Cultivated species	Wild species as male parent					Total progeny tested	No. of hybrids	% Hybrid
	Acaule (4x-2EBN) (%)	Bukasovii (2x-2EBN) (%)	Sparsipilum (2x-2EBN) (%)	Total progeny tested	No. of hybrids			
(a) Female parent								
<i>Stenotomum</i> (2x-2EBN)	1.4	18.9	42.6	461	226	62.9		
<i>Phureja</i> (2x-2EBN)	0	30.9	0.3	169	112	31.2		
<i>Andigena</i> (2x-2EBN)	0.6	0.3	1.4	435	8	2.3		
Hybrids (4x-4EBN)	0.8	2.8	0	551	13	3.6		
Total				1616	359	22		
Cultivated species as male parent								
(b) Female parent								
<i>S. acaule</i>	0	0	2.9	221	10	4.4		
<i>S. bukasovii</i>	6.5	27.2	2.4	340	124	36.4		
<i>S. sparsipilum</i>	21.4	19.3	14.4	569	337	59.2		
Total				1130	470	41.2		

*B. funebris* with 4.4% of the visits. The highest number of flower visits per day was registered in Puno (505) followed by Espital (107). Most of the insect visits took place between 9 a.m and 2 p.m when the temperature was between 15 and 27°C. The native cultivar Amarilla Peruana (*S. goniocalyx*) which produced the largest amount of flowers also had the highest number of insect visits. The hybrid cultivar Yungay and the native cultivar Qompis (*S. andigena*) produced a lower number of flowers but both set abundant seed. Revolucion registered the lowest number of visits (except for hoverfly on its leaves), and again set no berries or seed.

#### Survival of seed produced in the open pollinated experiments

During the 2003–2004 growing season, a total of 312 seedlings emerged from 39 out of 420 berries buried. In this trial berries from the cultivated species produced more seedlings than the berries from the wild species. Drought, frost and late blight affected the plants alternatively which never reached the flowering stage. However, between 60–70% set small tubers, from which 39 new plants (12%) emerged the following year (2004–2005) mostly from the cultivated species and 11 new seedlings emerged from a berry produced by Camotillo (gon-3). Again in the second year all plants died before flowering due to frost, when the tallest plant was about 12-cm height.

#### Selection of F1 progeny by a farmer

We were interested to know whether hybrids between wild and cultivated species would be selected by farmers. A complete set of tuber families from all crosses was planted with a farmer who was asked to select only those that he would be interested in continuing to grow. At harvest, farmers chose 21 out of 917 plants present in the field, based on their own criteria about yield and tuber characteristics. The selections turned out to be mostly selfings from *S. andigena* (16), but five were hybrids showing that it is possible that closely related species growing around farmer's fields can hybridize and progeny could be selected.



**Table 5** Average number of daily visits made by pollinator insects observed in five field plots, located in Junin, Cusco and Puno

Pollinator insect	Order (Family)	Junin			Cusco	Puno
		La Victoria 3,250 m	Espital 3,420 m	Pachapaqui 3,550 m	Andenes 3,600 m	Salcedo 3,820 m
<i>Astylus</i> sp.	Coleóptera (Melyridae)		59	3	20	
<i>Anthophora</i> sp.	Himenóptera (Apidae)					14
<i>Apis mellifera</i> *	Himenóptera (Apidae)	1	2	2		
<i>Bombus baeri</i>	Himenóptera (Apidae)					8
<i>Bombus funebris</i>	Himenóptera (Apidae)	5	9	20	7	29
<i>Bombus opifex</i>	Himenóptera (Apidae)					246
<i>Lonchopria</i> sp.	Himenóptera (Colletidae)		32			240
<i>Megachile</i> sp.	Himenóptera (Megachilidae)				3	11

\* *Apis mellifera* is not a vibratile insect and potatoes do not offer nectar. Honey bees rarely visit potato flowers and may rarely feed on pollen if the pores are wide open

## Discussion

To quantify the ease of successful crossability among 24 accessions from eight *Solanum* species commonly found in the potato fields from Puno, plants obtained from forced crosses made under greenhouse conditions were analyzed with AFLP to verify their hybrid nature. From 24 forced cross combinations analyzed, 15 progenies were hybrid, four were selfings of the female parent and in five cases no seeds germinated. Contrasting results, depending on the direction of the cross, were obtained between *Solanum* species differing in chromosome number. For instance, from two crosses involving *S. andigena* (4x) or the tetraploid hybrid varieties (*S. tuberosum* spp. *tuberosum* × spp. *andigena*) as the female parent and *S. sparsipilum* (2x) as the male parent, very few or no hybrids were obtained. However, when the direction of the cross was reversed, all the crosses were successful in producing hybrids. Data from the field, however, suggests that there is not a one-way crossing barrier, but rather the propensity of some flowers to open when already fertilized which makes the crossing difficult. In a field, once flowers open, they readily accept pollen from other sources and hybrid seed is found in smaller proportions than selfed seeds. In contrast, all the progenies from crosses involving diploid wild and cultivated species (2x × 2x) were hybrids. The high rate of success in obtaining hybrids between these large number of species and accessions, despite of the low number of flowers pollinated (1–20), is strong evidence of the relative ease with which hybridization could take place

between cultivated and wild tuber-bearing *Solanum* species commonly found in Peruvian potato fields.

In this study the close phylogenetic relationships between wild and cultivated species (Ugent 1970; Hawkes 1990) was reflected in the percentage of successful crossability (seeds per berry) between wild and cultivated species, 67 and 58% for the crosses cultivated × wild and wild × cultivated, respectively. These values were very high compared with those obtained by Jackson and Hanneman (1996), who using 47 wild species from the Series Tuberosa, distributed in Mexico and South America and five cultivated species from South America, found 20 and 16%, respectively. Although they did not confirm the hybrid nature of the resulting seed, they evaluated the production of 2n gametes in most of these species, in order to find an explanation for the production of seed between species with EBN and ploidy number barriers. They found a measurable amount of 2n pollen grains in at least one accession per species. In this study, we confirmed the hybrid nature of the resulting seed by AFLP analysis of a large number of seedlings per cross combination. We also found that despite EBN and ploidy differences, hybrids were produced.

Several studies have shown that traditional potato fields in Perú are grown in highly variable mixtures of cultivars and species although the predominant species is the tetraploid *S. tuberosum* spp. *andigena* (Johns and Keen 1986; Zimmerer and Spooner 1991; Quiros et al. 1990). In general, tetraploid species are highly self-compatible in contrast with diploid

species, which are mainly cross-pollinated due to self-incompatibility. However, both groups are very fertile. Among the most important factors influencing gene flow under field conditions are plant proximity, overlapping of the flowering period and activity of insect pollinators.

The distribution of the plants in the field is as important as the overlapping of the flowering period, favoring crossing between and within potato species. The obvious indication that sexual reproduction is taking place under field conditions is the high occurrence of fruits (berries) on both wild and cultivated species. Synchrony of flowering time is essential for ensuring out-crossing and the amount of hybrid seed produced between wild and cultivated species will depend on the extent of the overlapping period. In this study, during 32 days a variable number of open flowers were observed on each wild and cultivated potato species grown in Puno. This long period of flowering overlap favored gene flow, since from 24 possible cross combinations between three wild and four cultivated potato species, hybrid plants belonging to 20 cross combinations were identified by AFLP. This occurred even though the tall cultivated species often overshadowed the small wild species perhaps influencing the lower number of flowers registered for the wild species, indicating that in such trials the space between plants should be increased to around 0.5 m rather than the 0.35 m that we used.

Even if wild and cultivated species were in close proximity in the field and there was overlap of the flowering period, gene flow could only occur if insect pollinators were present in the field and moving between species. Studies on the pollination biology of potato (Buchmann et al. 1977; Sanford and Hanneman 1981) showed that vibratile pollination insects are important in releasing pollen from the poricidal anthers. In this study, we evaluated the role of pollinator insects in the hybridization events identified between wild and cultivated potato species under field conditions. The presence and behavior of flying insects during flowering was monitored in five sites, and 12 insect species were identified. In all sites, *Bombus funebris* was observed visiting flowers from the different cultivars, supporting previous findings about the role of bumblebees (Hymenoptera) as active pollinators of potato flowers (Johns and Keen 1986). In Puno, hybrid progeny resulting from 20

different cross combinations, involving three wild and four cultivated potato species, were identified by AFLP. Since distance between plants ranged between 0.35 and 8.00 m, it is highly probable that pollinator insects were the pollen carriers between species. In Puno, *Lonchropia* spp., *Anthopora* spp. and three *Bombus* species were frequently observed foraging on the potato flowers. These insects have been associated with hybridization events between wild and cultivated potato species (Johns and Keen 1986). Since more than one potato species was identified as pollen donor of the hybrid seed produced on individual plants from both, cultivated and wild species, it is evident that pollinator insects were moving between species. Furthermore, differences within progenies in the proportion of hybrids fathered by the various potato species could be due, among other factors, to differences in foraging cues offered by the flowers from each species, influencing the wandering of pollinators among plants.

Propagation of new potato genotypes may be favored in a cultivated field, which provides more protection for seed and clone survival. Under traditional potato farming in the Andes, there is a traditional 7-year cycle where potato is followed by 3–4 years of other crops and 2 or more years of fallow (Mayer 1980; Thurston 1991). Potato berries fall to the ground when mature and the cultivation cycle maintains a disturbed environment suitable for seed germination (Johns and Keen 1986; Zimmerer and Spooner 1991). We found that under conditions of undisturbed soil by agricultural practices (equivalent to the fallow period under crop rotation), seeds from less than 1% of the buried berries germinated and produced some tubers in the first year, from which the following year a few plants emerged but died early in the season. Thus, seedlings resulting from hybridization under normal freeze and drought conditions have little chance, without human intervention through agricultural practices, but may survive under favorable conditions.

Incorporation of new genotypes resulting from hybridization, into the cultivated gene pool is dependent on farmer intervention, who will harvest the tubers and selected among them the seed material for the following year, keeping these hybrids if it is advantageous (Jackson et al. 1980; Ugent 1970; Brush et al. 1981). Since tuber shape and color are important tuber characteristics in the native

classification of potatoes, they are reflected in the many names given to native cultivars. Importance of a rich diversity in color and shape, characteristic of native varieties (Quiros et al. 1990), was reflected in the selection made by a farmer, among the resulting tubers from sexual seed. The farmer chose tubers with different shapes and colors as good candidates for his seed stock. New genotypes resulting from gene flow between wild and cultivated species would be initially incorporated into the gene pool to be planted in the next growing season (Jackson et al. 1980; Ugent 1970).

Hybridization between wild and cultivated species is a widespread phenomenon in potato and it is the basis for the intense and long-lived debate about the origin and classification of potato species (Ugent 1970; Hawkes 1990; Huaman and Spooner 2002; Spooner et al. 2005). Introgression of genes from wild to cultivated species could enable crops to adapt better to biotic and abiotic factors, whereas gene flow from cultivated to wild species is responsible for the origin of conspecific weeds accompanying crop plants.

*Solanum tuberosum* spp. *andigena*- is the most ubiquitous species grown in traditional potato farming, accounting for more than two thirds of the planted potatoes (Brush et al. 1981; Quiros et al. 1990). However in recent years, tetraploid hybrid cultivars (*S. tuberosum* spp. *tuberosum* × *adg*) are being used more often as cash crop displacing native cultivars. Thus, it is likely that if a transgenic potato were released in central Andes, it would be a *S. adg* or a hybrid cultivar. Other cultivated species such as the diploids *S. stenotomum* and *S. phureja*, although grown in lower proportion have a wide geographical distribution (Hijmans 2002). In this study we found that 22% of the sampled seed produced by the cultivated species were hybrids fathered by the wild species. The majority of the hybrids (94%) were identified within the progeny of *S. stenotomum* and *S. phureja* and only 6% in the progeny of *S. andigena* and the hybrid cultivars. On the other hand, 30% of the sampled progeny produced on the wild species were hybrids fathered by the cultivated species. From these, *adg* and the hybrid cultivars fathered 26% of the hybrids in contrast with 74% by *S. stenotomum* and *S. phureja*. Thus, under field conditions, gene flow involving both wild and cultivated potato species took place in both directions, but in slightly higher proportion when the cultivated species were

the pollen donors. Given the concern about the perceived risk of gene flow from a transgenic potato cultivar into its wild relatives in regions of high genetic diversity, measures to minimize the risk must be taken. Among these, the use of sterile cultivars with scarce flower production and lacking seed production such as *Revolución*, could minimize the risk of gene flow.

**Acknowledgements** This research was financed by the International Cooperation Program for Development from the European Union (ICFP599A4PR01). We are grateful to CIP and INIEA for the greenhouse and experimental fields facilities and staff that collaborated; to Eutropio Contreras and his family and Raul Canto for helping with planting, monitoring and evaluations of these trials on growing and selecting the plant material. We are also very grateful to Dr. Claus Rasmusen from the University of Illinois for identification of insect pollinators.

## References

- Bernatzky R, Tanksley SD (1986) Methods for detection of single or low copy sequences in tomato on Southern blots. *Plant Mol Biol Rep* 4(1):37–44
- Brush SB, Heath JC, Huaman Z (1981) Dynamics of Andean potato agriculture. *Econ Bot* 35(1):70–88
- Buchmann SL, Jones CE, Colin LJ (1977) Vibratile pollination of *Solanum douglasii* and *Solanum xanthii* (Solanaceae) in Southern California. *The Wasman J Biol* 35:1–25
- Celis BC, Scurrah M, Cowgill S, Chumbiauca S, Green J, Franco J, Main G, Kiezebrink D, Visser R, Atkinson HJ (2004) Environmental biosafety and transgenic potato in a centre of this crop's diversity. *Nature* 432:222–225
- Cultivarston SA, den Nijs APM, Peloquin SJ, Hanneman RE Jr (1980) The significance of genetic balance to endosperm development in interspecific crosses. *Theor Appl Genet* 57:5–9
- Chen Q, Kawchuk LM, Lynch DR, Goettel MS, Fujimoto DK (2003) Identification of *Lte Blfht*, Colorado potato beetle and blackleg resistance in three Mexican and two South American wild @x (IEBN) *Solanum* species. *Am J Potato Res* 80:9–19
- Ehlenfeldt MK, Hanneman RE Jr (1988) Genetic control of Endosperm Balance Number (EBN): three additive loci in a threshold-like system. *Theor Appl Genet* 75:825–832
- Hawkes JG (1990) The potato: evolution, biodiversity and genetic resources. Smithsonian Institution Press, Washington, DC, 259 pp
- Hijmans RJ (2002) Assessing the risk of natural gene flow between wild and genetically modified cultivated potatoes: taxonomic and geographic considerations. Report preparad for Servicio Nacional de Sanidad Agraria (SENASA) Ministerio de Ag, Peru
- Huaman Z, Spooner DM (2002) Reclassificatio of landrace populations of cultivated potatoes (*Solanum* sect. *Petota*). *Am J Bot* 89(6):947–965

- Jackson SA, Hanneman RE Jr (1996) Potential gene flow between cultivated potato and its wild tuber-bearing relatives: implications for risk assessment of transgenic potatoes. In: 1996 Proceedings biotechnology risk assessment symposium. [on-line]. Information Systems for Biotechnology (ISB), Virginia, USA <http://www.isb.vt.edu/brarg/brasym96/jackson96.htm>
- Jackson SA, Hanneman RE Jr (1999) Crossability between cultivated and wild tuber- and non tuber-bearing solanums. *Euphytica* 109:51–67
- Johns T, Keen S (1986) Ongoing evolution of the potato on the altiplano of western Bolivia. *Econ Bot* 40(4):409–424
- Linder CR (1987) Diversity within traditional potato agriculture in the Peruvian Andes and its relevance to crop evolution. M-S. Thesis Cornell University
- Mayer E (1980) Land use in the Andes with special mention of potatoes. Cip, 80 pp
- Ochoa CM (1990) The potatoes of South America: Bolivia. Cambridge University Press, Cambridge, UK
- Ortiz R (1998) Potato breeding via ploidy manipulations. *Plant Breed Rev* 16:15–86
- Peloquin SJ, Yerk GL, Werner JE, Darro E (1989) Potato breeding with haploids and 2n gametes. *Genome* 31:1000–1004
- Quiros CF, Brush SB, Douches DS, Zimmerer KS, Huestis G (1990) Biochemical and folk assessment of variability of Andean cultivated potatoes. *Econ Bot* 44(2):254–266
- Ramanna MS (1979) A re-examination of the mechanisms of 2n gamete formation in potato and its implications for breeding. *Euphytica* 28:537–561
- Sanford JC, Hanneman RE (1981) The use of bees for the purpose of inter-mating in potato. *Am Potato J* 58:481–485
- Spooner DM, McLean K, Ramsay G, Waugh R, Bryan GJ (2005) A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proc Natl Acad Sci* 102:14694–14699
- Thurston D (1991) Andean potato culture: 5,000 years of experience with sustainable agriculture In: Zehnder GM, Powelson ML, Jansson RK, Raman KV (eds) *Advances in potato pest biology and management*. The American Phytopathological Society (APS Press), St. Paul, Minnesota (USA), ISBN 0-89054-164-7. 1994, pp 6–13
- Ugent D (1970) The potato. *Science* 170:1161–1166
- Ugent D (1981) Biogeography and origin of *Solanum aculeolatum*. *Phytologia* 48(1):85–95
- Vos P, Hogers R, Blaker M (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* 23:4407–4414
- Zimmerer KS, Spooner DM (1991) Geographical approaches to crop conservation: the partitioning of genetic diversity in Andean potatoes. *Econ Bot* 45(2):176–189