HYBRIDIZATION BETWEEN GP. TUBEROSUM HAPLOIDS AND 1EBN WILD POTATO SPECIES

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Abstract

Diploid wild potato species, classified as 1EBN (Endosperm Balance Number), do not cross with tetraploid or diploid forms of *S. tuberosum* Gp. Tuberosum. The crossing of 2n pollen producing 1EBN clones as well as chromosome-doubled 1EBN clones with Gp. Tuberosum haploids was initiated to overcome this hybridization barrier. The screening of eleven 1EBN species, *S. brachistotrichum*, *S. bulbocastanum*, *S. cardiophyllum*, *S. chancayense*, *S. commersonii*, *S. etuberosum*, *S. fernandezianum*, *S. jamesii*, *S. mochicense*, *S. pinnatisectum* and *S. trifidum*, resulted in the identification of 2n pollen producers in eight of the species examined. Direct hybridization with Gp. Tuberosum haploids utilizing the above-mentioned crossing scheme was successful with *S. chancayense* and *S. commersonii*. The hybrids obtained, however, were male and female sterile. Abnormal microsporogenesis in the hybrids was postulated to be the result of an interaction between Gp. Tuberosum cytoplasm and nuclear genes contributed by the 1EBN male parent. Analyses of the growth of 1EBN species' pollen tubes in the stigma/style of Gp. Tuberosum haploids were also conducted. Interspecific incompatibilities were observed with the most severe forms found with the use of species in the Series Etuberosa. The use of the Endosperm Balance Number theory can aid in designing crosses which overcome barriers to successful endosperm development. Sterilities in the hybrids obtained as well as interspecific incompatibilities will also need to be addressed, however, before 1EBN species germplasm can be successfully utilized for the improvement of the cultivated potato.

Compendio

Especies silvestres diploides de papa, clasificadas como 1EBN (Número de Balance del Endosperma), no se cruzan con las formas tetraploides o diploides de *S. tuberosum* Gp. Tuberosum. Se inició el cruzamiento de clones 1EBN productores de polen 2n, al iguao que el de clones 1EBN con cromosomas doblados, con haploides del Gp. Tuberosum, para eliminar esta bar-

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rera a la hibridación. La evaluación y selección de once especies 1EBN, *S. brachistotrichum, S. bulbocastanum, S. cardiophyllum, S. chancayense, S. commersonni, S. etuberosum, S. fernandezianum, S. jamesii, S. mochicense, S. pinnatisectum* y *S. trifidum*, dió por resultado la identificación de productores de polen 2n en ocho de las especies examinadas. La hibridación directa con haploides del Gp. Tuberosum utilizando los sistemas de cruzamientos mencionados anteriormente fue exitosa con *S. chancayense* y *S. commersonii*. Sin embargo, los híbridos obtenidos fueron totalmente estériles. Se supone que la microesporogenesis anormal en los híbridos es el resultado de una interacción entre el citoplasma del Gp. Tuberosum y los genes nucleares pertenecientes al progenitor masculino 1EBN. Se condujeron también análisis del crecimiento de los tubos polínicos de las especies 1EBN en el estigma/estilo de los haploides del Gp. Tuberosum. Se observaron incompatibilidades interespecíficas con las formas más severas encontradas con el uso de especies en la Serie Etuberosa. El uso de la teoría del Número de Balance del Endosperma puede servir para diseñar cruzamientos que superen las barreras para el desarrollo exitoso del endosperma. Sin embargo, la esterilidad de los híbridos obtenidos, al igual que las incompatibilidades interespecíficas, necesitarán también ser corregidas antes que el germoplasma de especies 1EBN pueda ser exitosamente utilizado para el mejoramiento de la papa cultivada.

Introduction

While the majority of diploid wild potato species hybridize readily with *Solanum tuberosum* Gp. Tuberosum haploids (8, 15), some diploid species, categorized as 1EBN (Endosperm Balance Number), do not cross with tetraploid or diploid forms of Gp. Tuberosum (4, 12). Further attempts at introgressing desirable traits from these "difficult" species into a potato breeding program have centered upon the utilization of bridge species such as *S. acaule* or *S. verrucosum* (1, 3, 6, 7).

The failure to obtain direct hybridization between these 1EBN species and Gp. Tuberosum may be explained on the basis of the Endosperm Balance Number (EBN) theory. As proposed by Johnson *et al.* (10), the EBN theory maintains that it is not the actual ploidy levels of the species that are important for determining the success or failure of a cross. Instead, a 2:1 ratio of maternal:paternal EBN in the developing endosperm would result in viable seed, while any deviation from this ratio would deleteriously affect endosperm differentiation and ultimately seed development.

With regard to the 1EBN species, their crossing with tetraploid or diploid forms of Gp. Tuberosum was largely unsuccessful due to an inherent inability to achieve the desired 2:1 EBN ratio. Hypothetically, the realization of this 2:1 EBN ratio would be possible in crosses between Gp. Tuberosum haploids and 2n pollen producing or chromosome-doubled

1EBN plants. Such a crossing scheme was instituted, the results of which are presented in this paper.

Materials and Methods

2n Pollen Screening

Forty plant introductions (P.I.'s) representing 11 1EBN diploid *Solanum* species (Table 1) were screened for 2n pollen production during the winter-spring of 1987. Plant material was obtained from the Inter-Regional Potato Introduction Project (IR-1) Sturgeon Bay, Wisconsin and grown under greenhouse conditions at Madison and Arlington, WI.

Two or three open flowers were collected from individual plants. Pollen was then bulked from the flowers of each individual and stained with a drop of glycerol-acetocarmine. Prepared slides were then observed with a light microscope under 150x magnification. 2n pollen percentages were then calculated for individual plants after randomly observing approxi-

TABLE 1.—*1EBN plant material screened for 2n pollen production.*

SPECIES	P.I.[1]	SPECIES	P.I.
S. brachistotrichum (bst*)	255530	*S. etuberosum* (etb)	245924
	283095		245939
	320265		
		S. fernandezianum (frn)	320270
S. bulbocastanum (blb)	275187		473463
	255516		
		S. jamesii (jam)	255536
S. cardiophyllum (cph)	186548		275169
	275212		275262
	275214		275266
	275215		458423
	275216		458425
	283062		458428
	283063		
	347759	*S. mochicense* (mcc)	283114
S. chancayense (chn)	338615	*S. pinnatisectum* (pnt)	230489
	442699		275232
	498411		275233
			275234
S. commersonii (cmm)	243503		
	458318	*S. trifidum* (trf)	255536
	458319		255539
	320267		283104
			498303

[1]Plant Introduction number.

* = species abbreviations from (9).

mately 200 pollen grains. Those clones with 2n pollen percentages of 1% or greater were classified as 2n pollen producers. Pollen diameters varied between and within species, but in general, 2n pollen was 1.2-1.3x the diameter of 1n pollen (14).

Crosses and Horticulture

Following the identification of 2n pollen producing plants (Table 2), crosses were conducted between these selected individuals and *Solanum tubero-*

TABLE 2.—*1EBN species clones utilized: A) as male parents in spring and summer-1987 crosses and B) in pollen tube analyses.*

1EBN Species	Clone Designation	P.I.[1]	A. Season		B. Pollen Tube Analyses
			Spring	Summer	
S. bulbocastanum	243511-4*	243511		x	
	243511-9*	"		x	
	EBN-2*	347757		x	x
	EBN-3*	"		x	
S. cardiophyllum	768-G*	255519		x	x
	283062 #27	283062	x	x	x
	25-1	347759	x	x	x
S. chancayense	29-1	338615	x	x	x
	29-3	"	x	x	x
	29-8	"	x	x	x
S. commersonii	3-10	243503		x	x
	(10 x 6)*	"		x	x
	618-5**	"		x	
	39-20	320267	x	x	
	4-21	458319	x	x	x
	320267 x 4x243503 #4*	Hybrid	x	x	x
S. jamesii	36-6	275169		x	
	36-14	"		x	x
	37-7	275266	x	x	
	37-10	"	x	x	
S. mochicense	mcc-16	283114	x	x	x
S. pinnatisectum	770-8*	184764		x	x
	771-5*	"	x		
S. trifidum	111-8	255539	x	x	
	112-5	283104	x	x	
	283104*	283104		x	x

[1]Plant Introduction number.
*Chromosome-doubled clone, *i.e.* 2n = 4x(2EBN).
**Colchicine treated—not verified as to ploidy level.

sum Gp. Tuberosum haploids which were utilized as the female parents. Chromosome-doubled clones (2n=4x=2EBN) of several 1EBN species were also included as male parents in the crosses (Table 2).

A. *Spring 1987.* Preliminary crossings were conducted under greenhouse conditions utilizing eight haploid clones of Gp. Tuberosum (Table 3). Emasculated flowers of the haploids were pollinated utilizing pollen collected from mature, open flowers of selected 2n pollen producing plants as well as pollen from two tetraploid clones (2n=4x=48) of *S. commersonii* and *S. pinnatisectum*, respectively (Table 2).

B. *Summer 1987.* A larger crossing program was undertaken during the summer of 1987 at the University of Wisconsin Agricultural Research Station, Sturgeon Bay, WI utilizing the cut-stem technique (13). Fifteen Gp. Tuberosum haploids (Table 3) were used as female parents in crosses with 2n pollen producers and chromosome-doubled clones of several 1EBN species (Table 2). Crossings were conducted in an air-conditioned greenhouse with stems obtained from field grown Gp. Tuberosum haploids.

Fruit were allowed to develop on the plant for at least 30 days following pollination. Seed extraction was performed on harvested fruit which

TABLE 3.—*S. tuberosum Gp. Tuberosum haploids utilized as female parents in: A) Spring and summer 1987 crosses and B) Pollen tube analyses of 1EBN male parent clones.*

Haploid	A. Crosses		B. Pollen Tube Analyses	Tetraploid Parent
	Spring	Summer		
US-W 4657			x	B96-56
US-W 527		x		Cherokee
US-W 551		x		Chippewa
US-W 1817		x		Chippewa
US-W 1818		x		Chippewa
US-W 2673		x		Chippewa
US-W 10,614		x	x	Chippewa
US-W 1		x	x	Katahdin
US-W 3573	x			Katahdin
US-W 357		x		Merrimack
US-W 621	x	x	x	Merrimack
US-W 2838	x		x	Merrimack
US-W 3817		x	x	Merrimack
US-W 3956		x		Merrimack
US-W 10,349		x	x	Merrimack
US-W 4639			x	Minn. 64.57-56
US-W 2179		x	x	Minn. 113-1
US-W 709	x			W 231
US-W 730	x	x	x	W 231
US-W 973	x	x	x	W 231
US-W 1887	x		x	W 231
US-W 2850	x			W 231

had been allowed to ripen an additional four weeks after removal from the plant. To facilitate germination, seeds were soaked for 24 hours in a vial containing 1500 ppm gibberellic acid. Seeds were then planted in the greenhouse.

Cytological Techniques

A. *Root Tip Chromosome Counts*—Root tips were collected in the morning and placed in vials containing 8-hydroxyquinoline (0.29 g/liter) for 4-5 hours. Root tips were then transferred to a 3:1 (ethanol:acetic acid) fixative, where they were held for at least 24 hours. Prior to examination, root tips were hydrolyzed in 1N HCl at 60°C for 10 minutes, and then transferred to vials containing distilled water. Root tips were then macerated in a drop of 1% carmine in 45% acetic acid. A trace of iron acetate was added to the mixture on the slide to enhance the contrast of the chromosomes. A coverslip was then added; the mixture was squashed, and the prepared slide was warmed over a flame and examined.

B. *Bud Analyses*—Meiotic analyses and chromosome counts were performed with flower buds fixed in either 6:3:2 (ethanol:chloroform:45% propionic acid saturated with iron acetate), or 3:1 (ethanol:acetic acid). Buds were fixed in 6:3:2 for 24-48 hours and stored in a refrigerator. The buds were then transferred into 95% ethanol and kept until examined. Buds fixed in 3:1 were treated for approximately 24 hours after which they were stored in a solution of 70% ethanol at room temperature until examination. Anthers were squashed in 1% acetocarmine for observation as described for root tips.

Pollen Tube Analyses

A. *Materials—1EBN Male Parents*—Pollen collected from 2n pollen producing or chromosome-doubled clones of 1EBN *Solanum* species was used to pollinate 12 Gp. Tuberosum haploids (Table 3). An effort was made to use as many of the haploids originally used in the summer crossings as possible. In some cases, however, haploids which flowered well in the field did not perform as well under greenhouse conditions, and no pollinations were obtained. Pollen used in early analyses had been collected during the previous summer and frozen for 3-4 months. Later pollinations were conducted using fresh pollen collected from the male clones.

B. *Methods*—Pistils were collected from Gp. Tuberosum haploids approximately 48 hours after pollination and fixed in FAA (1 part formalin: 8 parts 80% ethanol: 1 part glacial acetic acid). After being held in FAA for at least 24 hours, the pistils were then rinsed in tap water and placed in 8N NaOH for 17-19 hours. Pistils were then rinsed in tap water for a minimum of one hour, after which they were put in an aniline blue solution (0.05% aniline blue in 0.1N K_2PO_4) for a minimum of four hours. The style and one-fourth of the ovary of each treated flower was then

mounted on a slide with 2-3 drops of aniline blue solution. A cover slip was added and the pistils were slightly squashed. The prepared slide was then observed on a Zeiss Universal microscope equipped with an ultraviolet light source (Zeiss HBO 50W high-pressure mercury lamp), a G365 excitation filter with a dichroic reflector (FT460), and a LP520 barrier filter. Photographs of pollen tubes were taken using a Zeiss MC63 photomicrographic camera with Ektachrome 160 film.

Results

2n Pollen Screening

The results of screening plants of 40 accessions representing eleven 1EBN potato species for 2n pollen production are given in Table 4. 2n pollen production was present in eight of 11 species examined. The absence of 2n pollen producers in three of the species screened, does not, however, preclude the possibility of 2n pollen formation within these species. Poor flowering among several of these 1EBN species often limited the number of plants which could be screened, thereby reducing the probability of identifying 2n pollen producers. Twelve percent or more of 2n pollen producers were found in several of the 1EBN species, from which those plants with 3% or greater 2n pollen were selected for future crossings to Gp. Tuberosum haploids.

Crossing Results

Spring 1987— Seven hundred and nine pollinations were made on eight Gp. Tuberosum haploids, the results of which are summarized in Table 5. In addition to the 12 diploid, 2n pollen producers utilized as male parents,

TABLE 4.—*2n pollen screening of 1EBN species.*

Species	# of P.I.'s Evaluated	# of Plants Screened	# of 2n Pollen Producers*	% 2n Pollen Plants of Total Screened
S. brachistotrichum	3	16	2	12.5
S. bulbocastanum	2	9	0	0
S. cardiophyllum	8	75	9	12.0
S. chancayense	3	38	6	15.8
S. commersonii	4	50	2	4.0
S. etuberosum	2	12	0	0
S. fernandezianum	2	21	0	0
S. jamesii	7	108	14	13.0
S. mochicense	1	22	2	9.1
S. pinnatisectum	4	18	1	5.6
S. trifidum	4	23	2	8.7

*1% or greater 2n pollen.

TABLE 5.—*Results of crossings between Gp. Tuberosum haploids and 2x (1EBN) species and chromosome-doubled clones—spring of 1987.*

Species#	Male Parent Designation	2n Pollen %	Results: (poll./fruit/seed)
chn	29-1	15%	88/0/0
	29-3	3%	37/0/0
	29-8	9%	39/1/16
cmm	39-20	7%	18/0/0
	4-21	5%	97/0/0
	320267 × 4x243503 #4*	n.a.	35/0/0
cph	283062 #27	10%	15/0/0
	25-1	11%	81/0/0
jam	37-7	6%	16/0/0
	37-10	12%	54/0/0
mcc	mcc-16	7%	104/0/0
pnt	771-5*	n.a.	32/0/0
trf	111-8	18%	84/0/0
	112-5	3%	9/0/0

#Species abbreviations (9): chn=*S. chancayense*, cmm=*S. commersonii*, cph=*S. cardiophyllum*, jam=*S. jamesii*, mcc=*S. mochicense*, pnt=*S. pinnatisectum*, trf=*S. trifidum*.
*Indicates chromosome-doubled clone 2n=4x(2EBN).

two chromosome-doubled clones were also included in the crossing program. The inclusion of chromosome-doubled clones in the spring and summer crossing programs was done in order to allow for possible hybrid production in certain 1EBN species where no 2n pollen producers were identified. In other 1EBN species, where 2n pollen producers were found, doubled clones were utilized to broaden the parental base, and hopefully increase the likelihood of obtaining hybrids.

As is indicated in Table 5, the results of these crossings were not encouraging in terms of hybrid seed production. One fruit was, however, retained upon a clone of US-W 1887 which had been pollinated by a 2n pollen producing *S. chancayense* clone designated as 29-8. From this fruit, 16 seeds were extracted of which eight germinated to produce vigorous plants.

Summer of 1987—Utilizing 15 different Gp. Tuberosum haploids as females, 6026 pollinations were performed. The results of the crossings are summarized in Table 6. An increased fruit retention was observed when compared to the earlier spring crosses, but parthenocarpy is promoted by the cut-stem technique. Upon completion of seed extraction, it was found that the majority of harvested fruit were parthenocarpic, with few seed being obtained.

The seeds extracted were immediately planted in the greenhouse after treatment with gibberellic acid. From those seed which germinated, thirteen plants were obtained, all having the tetraploid, *S. commersonii* clone (10x6)

TABLE 6.—*Results of summer-1987 crossings of 2x(1EBN) species and chromosome-doubled clones to Gp. Tuberosum haploids.*

Species+	Male Parent	Results: (poll./fruit/seed)
blb	243511-4*	59/3/0
	243511-9*	234/20/0
	EBN-2*	134/10/0
	EBN-3*	98/11/0
chn	29-1	526/36/8
	29-3	191/18/0
	29-8	551/39/4
cmm	4-21	260/33/0
	3-10	96/12/1
	(10 × 6)*	397/37/47
	618-(5)**	63/21/1
	320267 × 4x243503 #4*	149/14/0
	39-20	107/12/1
cph	283062 #27	357/25/0
	25-1	298/35/0
	768-G*	428/40/2
jam	36-6	52/2/0
	36-14	445/41/2
	37-7	116/13/0
	37-10	296/21/0
mcc	mcc-16	578/52/3
pnt	770-8*	339/35/0
trf	111-8	91/5/0
	112-5	44/4/0
	283104*	117/14/0

*Chromosome-doubled clone 2n = 4x(2EBN).
**Colchicine-treated—not verified as to ploidy level.
+*Species abbreviations* (9): blb = *S. bulbocastanum*, chn = *S. chancayense*, cmm = *S. commersonii*, cph = *S. cardiophyllum* jam = *S. jamesii*, mcc = *S. mochicense*, pnt = *S. pinnatisectum*, trf = *S. trifidum*.

as the male parent. One additional plant was also obtained from a cross with a 2n pollen producing *S. chancayense* clone (29-8) as the male parent.

Description of Hybrid Plants:

Haploid Gp. Tuberosum-S. chancayense Hybrids: A total of nine haploid Gp. Tuberosum-*S. chancayense* (tbr-chn) hybrids were obtained. Based upon the Endosperm Balance Number (EBN) theory, it was expected that any hybrids obtained from intercrossing Gp. Tuberosum haploids with 2n pollen producers or chromosome-doubled forms of 1EBN species would be triploid. Root tip analyses confirmed that the plants obtained were triploid.

Assessed under screenhouse conditions, the hybrids were vigorous plants with upright growth habits and leaflets which were larger than those seen for either parent (Fig. 1). Hybrid leaves also displayed the pale-green

FIG. 1. F_1 hybrid from the cross US-W 1887 × *S. chancayense* clone 29-8.

coloration of the male parent but nine leaflets, rather than the 5-7 leaflets of *S. chancayense* were typical. Under greenhouse conditions, limited tuberization was obtained among the hybrids, but it is doubtful whether field-grown plants would tuberize prior to killing frosts.

1. *Male Fertility*: The mature flowers of the hybrids looked relatively fertile with plump, normal-looking anthers which appeared to have dehiscent tips. No pollen grains (not even abortive types) were observed, however. Examination of microsporogenesis revealed few apparent meiocytes. Microspore mother cells, though in generally small numbers, were evident even in relatively mature buds. These sporocytes appeared to have never initiated the meiotic process or had arrested meiosis in early prophase (Fig. 2).

2. *Female Fertility*: Utilizing pollen obtained from the cultivar, Katahdin, as well as pollen from Gp. Tuberosum haploids US-W 1, US-W 1887,

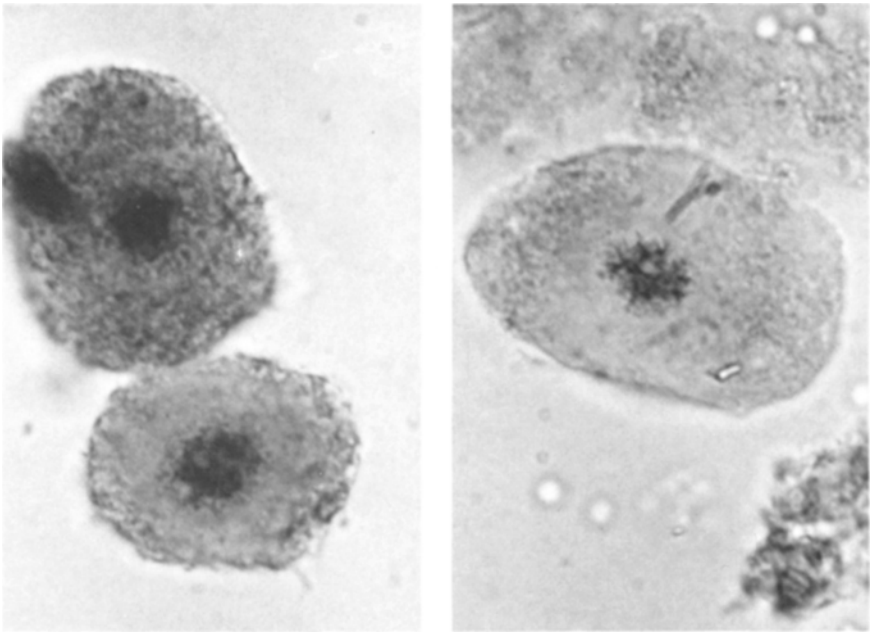

FIG. 2. Sporocytes found in large buds of haploid Gp. Tuberosum-1EBN species hybrids (1250x). Gp. Tuberosum-*S. chancayense* (left). Gp. Tuberosum-*S. commersonii* (right).

and US-W 2838, pollinations were performed upon the tbr-chn hybrids. A total of 77 pollinations were performed on six of the nine hybrids with no berry set occurring.

Haploid Gp. Tuberosum-S. commersonii hybrids: Thirteen hybrid plants were obtained from crossing Gp. Tuberosum haploids with a tetraploid clone of *S. commersonii* designated (10x6). This *S. commersonii* clone had been synthesized from the crossing of two diploid *S. commersonii* plants presumed to have been synaptic mutants. The tetraploid nature of (10x6) was attributed to the union of 2n gametes.

The F_1 plants were successfully obtained from three Gp. Tuberosum haploids utilized as the female parent in crosses with clone (10x6). The three Gp. Tuberosum haploids were US-W 621 (3 hybrids), US-W 973 (4 hybrids), and US-W 10,349 (6 hybrids). As was the case with the tbr-chn hybrids, these F_1 offspring were also expected to be triploids. Root tip chromosome counts verified that the ploidy level for all but one hybrid was indeed triploid. The death of one hybrid occurred before an accurate count of its chromosomes could be conducted.

Like their *S. commersonii* parent 10x6, the hybrid plants exhibited a low-growing, rosette type of growth in their early stages of development. As the plants matured, however, elongation of the stem occurred, and the plants

displayed a growth habit analogous to that observed for pot-grown Gp. Tuberosum haploids (Fig. 3). Two of the hybrids, however, retained a compact growth habit, characterized by short internodes with no stem elongation occurring. Leaf morphology of the hybrids reflected the genetic influence of the *S. commersonii* male parent, with obvious similarities evident when leaves are compared (Fig. 4).

Tuberization among the hybrids within the greenhouse environment was generally good, especially in relation to the tuberization observed for the Gp. Tuberosum-*S. chancayense* hybrids.

1. *Male Fertility*: Anthers of the hybrid flowers were generally malformed and a pale-greenish yellow color, but a few individuals displayed normal-appearing anthers. All hybrids which flowered, however, were found to be male sterile. Analysis of microsporogenesis (10/13 hybrids) revealed a situation analogous to what was found for the tbr-chn hybrids (Fig. 2). Bud analyses showed the presence of small numbers of apparent microspore mother cells which appeared to have had the meiotic process blocked in early prophase or had never apparently initiated the meiotic process. Three hybrids did however, produce meiocytes which had progressed to later meiotic stages; generally early metaphase to the telophase I stage.

2. *Female Fertility*: To evaluate female fertility, 141 pollinations were performed upon six tbr-cmm hybrids. The pollen utilized in the crosses was from the tetraploid cultivar Katahdin or from four Gp. Tuberosum haploids—US-W 730, US-W 1887, US-W 2179, and US-W 2838. No berry set was obtained.

Interspecific Incompatibility

The results obtained from crossing Gp. Tuberosum haploids with 2n pollen producers or tetraploid forms of several 1EBN species were puzzling. The majority of seed that was obtained (88%) was from the use of the *S. chancayense* 2n pollen producers 29-1 and 29-8, as well as the tetraploid *S. commersonii* clone (10x6). Many male parents from other 1EBN species had comparable or higher percentages of 2n pollen or were chromosome-doubled clones, yet no seed was obtained when they were used in crosses. It was suspected that interspecific incompatibilities might be responsible for the observed crossing results.

Analyses of 1EBN species pollen tube growth down styles of Gp. Tuberosum haploids was initiated to confirm whether incompatibility was present. The results are listed in Table 7. As had been suspected, stylar barriers to normal pollen tube growth were observed in several instances.

Discussion

Utilizing the Endosperm Balance Number theory, it was hypothesized that the use of 2n pollen producers or chromosome-doubled forms of 1EBN

FIG. 3. Hybrids obtained from crossing tetraploid *S. commersonii*, (10×6), with female parents US-W 621 (left), US-W 973 (center) and US-W 10,349 (right).

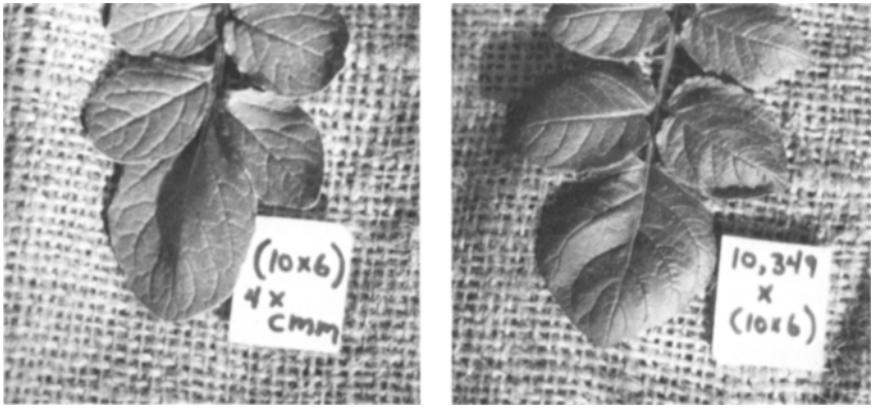

FIG. 4. Leaves of *S. commersonii* (10×6) (left), and US-W 10,349 × (10×6) (right).

species in crosses with Gp. Tuberosum haploids as females would result in a desired 2:1 EBN ratio within the endosperm. Development of the hybrid seed would hypothetically proceed normally with triploid hybrids eventually being obtained. These hybrids or their chromosome-doubled forms

TABLE 7.—*Pollen tube growth of 1EBN male parental clones in stigma/style of Gp. Tuberosum haploids.*

Male Parent	Description[1] a	b	c	d	#
S. bulbocastanum					
EBN-2					
US-W 4657	1	1	-	-	1
US-W 1	-	4	-	-	-
US-W 2838*	-	1	1	-	-
US-W 10,349	1	2	-	-	1
US-W 730*	4	2	-	-	3
S. cardiophyllum					
25-1					
US-W 4657	-	-	3	-	1
US-W 4639	2	1	-	-	-
US-W 730*	-	-	-	5	-
US-W 973	-	2	-	-	1
283062 #27					
US-W 4657	-	1	1	-	-
US-W 1	2	2	-	-	-
US-W 2179	-	2	2	-	2
768-G					
US-W 10,614*	-	2	-	-	-
US-W 730*	-	-	1	-	-
US-W 973*	1	-	-	-	-
S. chancayense					
29-1					
US-W 4657	1	-	-	-	-
US-W 1	-	1	3	-	-
US-W 10,349	2	-	-	-	-
29-3					
US-W 2838*	-	-	-	1	1
US-W 730*	-	-	1	1	-
US-W 1887*	-	-	1	2	1
29-8					
US-W 2179	-	1	2	-	-
US-W 973	-	-	-	4	-
S. commersonii					
4-21					
US-W 4657	-	-	2	-	-
US-W 2838	-	-	-	6	-
US-W 730	-	-	-	2	4
US-W 1887	-	-	-	1	-
(10 × 6)					
US-W 2838	-	-	-	2	-
US-W 973	-	-	-	5	-
320267 × 4x243503 #4					
US-W 2838	-	-	-	2	-
US-W 730	-	-	-	4	1
US-W 973	-	-	-	4	1
S. jamesii					
36-14					
US-W 4657	-	1	1	-	-
US-W 1	5	-	-	-	-
US-W 2179	2	-	-	-	1
S. mochicense					
mcc-16					
US-W 4657	3	-	-	-	-
US-W 1	-	-	-	4	-
US-W 2838*	-	-	1	-	-
US-W 2179	-	-	2	-	2
US-W 730*	-	1	-	2	-
US-W 973*	5	-	-	-	-
S. pinnatisectum					
770-8					
US-W 4657	2	-	-	-	-
US-W 10,614	-	3	-	-	-
US-W 621	-	2	-	-	2
US-W 3817	4	-	-	-	-
US-W 2179	2	-	-	-	-

[1]*Description indicates growth of pollen tube in each style examined:*
a = pollen tube growth stopped in upper ¼-⅓ of style.
b = pollen tube growth stopped in upper ½ of style.
c = pollen tube growth stopped in lower ⅔-¾ of style.
d = complete pollen tube growth though the style and into the ovary.
# = Poor staining or lack of pollen grains for accurate analysis.
*Pollen from male parent had been frozen.

TABLE 7.— *continued*

Male Parent	Description[1] a	b	c	d	#	Male Parent	Description[1] a	b	c	d	#
						S. trifidum					
S. commersonii						*283104*					
3-10						US-W 4657	-	3	5	-	-
US-W 4657	-	2	-	1	1	US-W 2838*	-	-	-	1	3
US-W 730	-	-	-	-	3	US-W 3817	-	2	1	-	-
US-W 973	-	-	2	-	1	US-W 2179	-	-	5	-	-
						US-W 730*	-	-	-	2	1
						US-W 973*	-	-	-	6	1

[1]*Description indicates growth of pollen tube in each style examined*:
a = pollen tube growth stopped in upper ¼-⅓ of style.
b = pollen tube growth stopped in upper ½ of style.
c = pollen tube growth stopped in lower ⅔-¾ of style.
d = complete pollen tube growth though the style and into the ovary.
# = Poor staining or lack of pollen grains for accurate analysis.
*Pollen from male parent had been frozen.

could then be utilized in further crosses to Gp. Tuberosum cultivars, thereby allowing for the introgression of the 1EBN germplasm into a potato breeding program.

The screening of 11 1EBN species for 2n pollen producers to be utilized as male parents in the above-outlined crossing scheme was conducted. As had been observed within other potato species (16, 17), 2n pollen production was found in eight of the eleven species examined. In several of these 1EBN species, it was found that greater than 10% of the total plants screened were 2n pollen producers. The largest percentage of 2n pollen producers was found in the South American species *S. chancayense* (15.8%).

Crosses utilizing 2n pollen producers and chromosome-doubled 1EBN clones as males and Gp. Tuberosum haploids as females resulted in the production of nine Gp. Tuberosum haploid-*S. chancayense* (tbr-chn) hybrids and 13 Gp. Tuberosum haploid-*S. commersonii* (tbr-cmm) hybrids. Utilizing the EBN theory as a predictive tool, it was expected that any hybrids obtained would be triploid. Root tip analyses confirmed that the nine tbr-chn hybrids and 11 of the tbr-cmm hybrids were indeed triploid. Accurate chromosome counts were not obtained for two of the tbr-cmm hybrids, but they also were presumed to be triploids. These results demonstrate the usefulness of the EBN theory in designing a crossing scheme which allows for hybrid production.

Hybrid Male Fertility

Male sterility was encountered in both the tbr-chn and tbr-cmm hybrids. Poor fertility was expected owing to the triploid nature of the

hybrids. Mature anthers of both groups of hybrids did not, however, shed even abortive types of pollen grains. This finding led to the suspicion that something other than the triploid nature of the hybrids was responsible for the observed male sterility.

Analyses of microsporogenesis in the majority of tbr-chn and tbr-cmm hybrids revealed abnormalities in the early stages of the meiotic process. Closely analogous to the "sporad" form of genic-cytoplasmic male sterility observed by Grun and Aubertin (5), the rare microspore mother cells observed apparently had never initiated the meiotic process or development had stopped in early prophase of the first meiotic division. These inferences were made based upon the observation of the above-mentioned sporocytes in large buds where one would expect to see meiocytes and/or microspores. Hybrids were also observed with lesser degrees of meiotic abnormalities, with meiosis proceeding to later stages. Ultimately, however, abnormal microsporogenesis was observed in all hybrids analyzed with the possible exception of one tbr-cmm hybrid (designated #12) with US-W 973 as the female parent. This hybrid appeared to have normal early phases of meiosis. It was not possible to evaluate later stages of meiosis, however, due to premature bud abscission.

The abnormal microsporogenesis observed among the tbr-chn and tbr-cmm hybrids seems most likely due to an interaction between Gp. Tuberosum cytoplasm and nuclear genes contributed by the 1EBN male parent. Differences in expressivity of the genic-cytoplasmic trait would explain the progression of microsporogenesis to later meiotic stages in certain Gp. Tuberosum haploid-1EBN hybrids.

Interestingly, of the 12 1EBN species currently recognized, three are tuber-bearing species with origins in South America, those being *S. chancayense*, *S. commersonii* and *S. mochicense*. Is it just coincidental that two of these three species produced hybrids with similar male sterility problems, or is this to be expected when South American 1EBN tuber-bearing potato species are used as males in crosses with Gp. Tuberosum haploids? It would be interesting to obtain and evaluate the male fertility of Gp. Tuberosum haploid-*S. mochicense* hybrids, in order to answer the above-mentioned supposition.

Hybrid Female Fertility

Female fertility was also poor among the tbr-chn and tbr-cmm hybrids. Crosses were made utilizing Gp. Tuberosum haploids and cultivars as the male parents. No berry set was obtained, although 77 pollinations were performed upon six tbr-chn hybrids, and 141 pollinations were made upon six tbr-cmm hybrids. Stylar analyses did not identify any barriers to pollen tube growth. The majority of pollinations performed utilized pollen derived from tetraploid cultivars. It was felt that if 2n egg production was

present among the hybrids, a 2:1 endosperm ratio would be possible through the fertilization of the 6x(4EBN) central cell by a 2x(2EBN) sperm nuclei.

Possible explanations for the lack of fruit retention could relate to Dionne's (2) finding that following interspecific crosses, often fertilization occurs, but the ovary does not develop normally. A more plausible explanation is that few viable megagametophytes would be expected due to the triploid nature of the hybrids, and their low frequency could seriously affect the chance of fertilization and subsequent fruit retention upon the plant.

Interspecific Incompatibility

Interspecific incompatibility was found to be present in Gp. Tuberosum haploid × 1EBN species crosses. Pollen tube inhibition in styles of Gp. Tuberosum haploids was observed with the use of certain male clones of the species, *S. bulbocastanum*, *S. cardiophyllum*, *S. chancayense*, *S. jamesii*, *S. mochicense*, *S. pinnatisectum* and *S. trifidum*. Within these species, pollen tube growth was often stopped in the upper ¼-½ of the style, although less severe restrictions to pollen-tube growth were also observed for these clones. Clones from the species *S. commersonii* showed little or no incompatibility problems. Why additional seed was not obtained from the utilization of 2n pollen producers of *S. commersonii* is not readily clear. Low female fertility among the female Gp. Tuberosum haploids used and/or pollen certation (differential competitive ability among pollen grains) are possible explanations.

Incompatibility appeared to be influenced by the Gp. Tuberosum haploid utilized as the female. This observation is clearly evident if one looks at the incompatibility results for the male clones 25-1 (*S. cardiophyllum*), mcc-16 (*S. mochicense*), and 283104 (*S. trifidum*) (Table 7). With some Gp. Tuberosum haploids, these male clones showed severe to moderate cessation of pollen-tube growth, while in the styles of other haploids, no apparent stylar barriers were observed.

Such a maternal influence on pollen tube growth was also observed when Gp. Tuberosum haploids were pollinated with bulked pollen from certain 1EBN species accessions (Data not reported). The exceptions being *S. brevidens* and *S. fernandezianum*, which displayed consistent cessation of pollen tube growth in the upper ¼-⅓ of the style, regardless of the Gp. Tuberosum haploid utilized as the female. Such severe interspecific incompatibilities displayed by these two Series Etuberosa species can be explained on the basis of the "unilateral incompatibility" proposal (11), whereby pollen from a self-compatible species will be inhibited in the styles and/or stigma of self-incompatible species.

Interspecific incompatibilities between certain 1EBN species and Gp. Tuberosum haploids pose an additional problem in the successful hybridization between these potato species. A possible means of solving this

dilemma might be to conduct preliminary crossings with the 1EBN species of interest and several different Gp. Tuberosum haploids. Stylar analyses could then hopefully identify haploids which show little or no inhibition of pollen tube growth. These "chosen" haploids could then be used for further crossings. Another possible method for overcoming incompatibility barriers might be to utilize Swaminathan's (15) technique of applying nutrient medium to the cut surface of the style prior to pollination, thereby allowing for improved pollen tube growth. Lastly, chromosome-doubled or 2n egg producing 1EBN clones could be utilized as females in crosses with Gp. Tuberosum male fertile haploids. This cross might not show the incompatibility problems encountered with its reciprocal.

The Endosperm Balance Number theory was instrumental in the implementation of a crossing scheme which allowed for direct hybridization between Gp. Tuberosum haploids and 1EBN species. Hybrid sterilities thought to relate to genic-cytoplasmic interactions, as well as interspecific incompatibilities between Gp. Tuberosum and several 1EBN species were encountered in this study. These barriers will need to be confronted and surmounted if 1EBN germplasm is to be successfully utilized for the improvement of the cultivated potato.

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