Theor Appl Genet (1986) 72:346–352

An interpretation of genomic balance in seed formation in interspecific hybrids of *Solanum* *

J. A. Smith [1] **and S. L. Desborough**

[1] Department of Genetics, University of California-Berkeley, Berkeley, CA 94720, USA
[2] Department of Horticultural Science and Landscape Architecture, University of Minnesota, St. Paul, MN 55108, USA

Received December 5, 1985; Accepted January 5, 1986
Communicated by H. F. Linskens

Summary. To investigate the mechanisms of seed failure in intraspecific and interspecific crosses of *Solanum* two diploid, *S. commersonii* and Group Phureja, and one tetraploid species, *S. acaule,* species were crossed and the seeds were analyzed for embryo and endosperm development. Many seeds of certain crosses observed seven days after pollinations were found to contain abnormal embryos and degenerating endosperms. In some cases seeds contained an embryo with no endosperm, or an endosperm with no embryo. Other interspecific crosses which were predicted to fail actually produced seeds with normally developed embryos and endosperms. To further characterize the intra- and interspecific embryos and endosperms the nuclear DNA was measured. There are several ways to explain the ploidy levels of embryos and endosperms among the crosses, the occurrence of unreduced gametes in some cases and genomic instability in other cases. The latter resulted in chromosome loss at meiotic and mitotic divisions. Genomic balance in interspecific seeds is critical to both embryo and endosperm development.

Key words: Interspecific crosses – *Solanum* – Embryo development – DNA quantity

Introduction

The formation of the seed is fundamental to the study of plant genetics. In normal seeds, after double fertilization, the zygote enters a quiescent phase while the endosperm begins rapid development. The zygote's first division may not occur until after the endosperm has completed six to eight divisions. In seeds destined to abort, abnormal cell divisions in the endosperm have been observed and the rate of endosperm growth was inhibited (Brink and Cooper 1939, 1944, 1947; Cooper and Brink 1945). To explain the production of viable seed Muntzing (1933) suggested the ploidy ratio of seed tissue was required to be: 2 maternal : 3 endosperm : 2 embryo. Many exceptions to this rule have been reported, especially in plants able to form unreduced gametes. Seeds were formed when maternal, endosperm and embryo tissues had ploidy ratios of 2 : 3 : 3 and 2 : 3 : 4. Howard (1939) and Stephens (1942) implied that among species of plants there were different genetic strengths which controlled the outcome of interspecific pollinations. These ideas were largely ignored until Valentine, later joined by Woodell, began a series of investigations with *Primula* species (Valentine 1947, 1952, 1955, 1956; Woodell and Valentine 1960; Woodell 1960a, b). They were convinced the maternal tissue behaved as if it were "incompatible" with the embryonic tissue. There was some evidence that a ploidy ratio of 2 maternal to 3 endosperm was highly correlated with the largest viable seeds. However, the numbers were not reliable enough to predict successful crosses. To examine ploidy relationships, Woodell and Valentine (1960) used colchicine to derive tetraploids from diploid species which had failed to form viable seeds when crossed with one another. Hybrid and the reciprocal crosses were made between diploids and the derived tetraploids. The only viable seeds were from tetraploid progeny presumably obtained from unreduced gametes from diploid parents. They did not obtain the expected triploid progeny. Their work and that of others led Marks (1966) to propose the concept of "triploid block" as an explana-

* Scientific Journal Series Article No. 14636 of the Minnesota Experiment Station

tion for the lack of hybrid seed formation. He crossed wild diploid *Solanum* species with tetraploid cultivars and concluded there were genomic barriers responsible for the selective breakdown of pentaploid endosperms associated with triploid embryos formed in tetraploid-diploid crosses.

These results focused interest on the genomic constitution of the endosperm itself. Normally endosperm is initiated by the fusion of the female central cell (2n) and one sperm (1n). Johnston and Hanneman (1982) found certain diploid species could only form hybrids with other diploids when a colchicine-doubled plant was used in the cross. In successful seeds this plant would contribute four genomes of maternal DNA to the endosperm, instead of the usual two. The reciprocal cross was also successful when the colchicine-doubled plant contributed two genomes of paternal DNA, instead of the usual one, to the endosperm.

These observations led to the proposal of the endosperm balance theory of Johnston et al. (1980) which combined the concept of genic balance and the prerequisite of endosperm development for the formation of *Solanum* seeds. They proposed that the balance of genomes in the endosperm determined the potential for successful seed formation in interspecific crosses. *Solanum* species could be assigned endosperm balance numbers (EBN) of 1, 2, 3 or 4. Plants were selected from each EBN category and species with undetermined EBN number were crossed with them. Crosses were usually only successful within one category and the species could be assigned this EBN number. By using the EBN system predictions can be made concerning which species should form normal seeds when crossed with one another; the predictive value of the EBN system has met with very few expections.

Formation of the endosperm as an absolute prerequiste for normal embryo formation can be questioned, however some dependence of the embryo on the endosperm for nutrients is apparent and the endosperm is formed prior to cellular division in the embryo (Wangenheim et al. 1960). Hybrid embryos have been rescued from abortive seeds and grown to healthy mature plants with the use of in vitro techniques (Laibach 1925, 1929; Smith 1944; Davies 1960). Lin (1975) noted that maize embryo development was normal even in those kernels with highly deformed endosperms. Sheridan (1982) used translocation strains of maize crossed with a line heterozygous for a lethal gene and demonstrated that the embryo genotype controlled its development even when endosperm was completely absent.

This study records early developmental events in seed formation within interspecific crosses of three species of *Solanum*. Crosses were made which could be predicted to either succeed or fail on the basis of the EBN system. *S. acaule* (2n = 48) and *S. tuberosum* Group Phureja (2n = 24) have an EBN of 2 and *S. commersonii* (2n = 24) has an EBN of 1 (Johnston et al. 1980; Hanneman 1984). Embryos and endosperms were scored by estimates of their cell numbers after pollinations to establish whether they had normal or abnormal development. Seeds were dissected and embryos and endosperms were measured by a microspectrophotometric technique for their nuclear DNA content. It was possible to examine cellular developmental and ploidy level of the embryos and endosperms to help understand the influence of the endosperm balance number.

Materials and methods

Plants

Solanum tuberosum Group Phureja seeds were obtained from Dr. F.L. Haynes, Dept. of Plant Sciences, North Carolina State. *S. acaule* (P.I. 435071) and *S. commersonii* (P.I. 243503) seeds were obtained from the Inter-Regional collection at Sturgeon Bay, WI. All plants were grown as seedlings in the greenhouse at St. Paul, MN. Seedlings were transplanted to the experimental fields at Grand Rapids, MN during the second week of June in 1982 and 1983.

Pollen tube analyses

Hybrid crosses and their reciprocals were done both with plants flowering in the greenhouse and in the field. Pollinations were done on buds which had been emasculated one day prior to flower opening. Pollen was collected mechanically from mature flowers of each species on the day of its use. Five flowers from each cross were collected at 24, 48 and 72 h after pollination and placed into FPA (5% formalin, 5% propionic acid and 90% ethyl alcohol). After fixation, styles and ovules were softened overnight with 8N NaOH. Tissues were rinsed 3X with DDW and placed in 1% aniline blue prepared according to Kho and Baer (1968). After 4 h in aniline blue, style and ovules were placed on microscope slides and pressed with a coverslip. Pollen tubes which has been stained with aniline blue fluoresced yellow when illuminated with an excitation wavelength of 365 nm by an Osram HBO 200 W high pressure mercury vapor lamp. Pollen tube growth was measured with an ocular micrometer in an 8X objective of a light microscope.

Crosses for developmental scores

Crosses were made with plants of the three species growing both in the greenhouse and field. Immature flower buds were emasculated one day prior to opening and tagged. The following day fresh pollen was collected and pooled from plants of each species. Flowers of each plant were divided so that some were dusted with pollen from its own species and others with pollen from the different species. Fruits which developed were collected at daily intervals from 5 to 30 days postpollination. The developmental stages of the crosses are given in Table 1 (P = Phureja, C = *S. commersonii* and A = *S. acaule*).

Embryo scoring and endosperm measurements

The stages of *Solanum* embryo development have been previously described by Bhaduri (1936); he assigned numbers to stages of embryo development. The number 20 was given to embryos at the earliest heart stage. When the length of the cotyledons was equal to the length of the embryonic axis, the embryo was a torpedo and given the number 30. As the cotyledons continued to grow and elongate, the tips would begin to curve. The embryo was given the number 40 when curvature was definite.

In the normal seed the number of endosperm cells determined the dimensions of the embryo sac. Endosperm development was scored by measuring the long and short axis of the embryo sac with an ocular micrometer. Area of the elipse was calculated; this area of the embryo sac was divided by the average area occupied by an endosperm cell to give the equivalent number of endosperm cells. This value was intended to be used as a unit of comparison and not as an absolute measurement of the numbers of endosperm cells present.

Table 1. Major developmental stages in seed formation of intraspecific and interspecific seeds of *Solanum* (see "Materials and methods" for abbreviations)

Development stage	Postpollination time in hours or days					
	P×P	C×C	A×A	C×P	A×P	A×C
Pollen tubes reach ovarian cavity	24	25	30	24	42	48 h
Four to eight cell globular embryo	7	7	7	7	8	8 d
Cotyledons initiated heart stage	16	18	12	–	19	15
Cotyledon length equal to axis length torpedo stage	24	25	20	–	27	22
Curvature of cotyledons mature embryo	30	30	25	–	37	30
No. of observations	265	83	200	164	204	298
% with no embryo	0.4	3.6	1.0	34	2.0	3.7

Ovule clearing technique

A clearing technique was developed for *Solanum* seeds by Stelly (1984). Briefly, this technique was used as follows. Fruits were collected and placed into FPA. After 24 h of fixation, the ovary wall was dissected and seeds attached to the placenta were exposed to the clearing solution. Tissues were processed in 30 min intervals by serial dehydration with graded alcohol concentrations to 95% alcohol; then were placed in 0.1% Chlorazal Black E in 95% ethyl alcohol (Stelly, personal communication), held under vacuum for 15 min, and rinsed in 95% alcohol to remove excess dye. Finally they were given 30 min treatments with increasing concentrations of methyl salicylate up to 100%.

Microspectrophotometry

Slides with thin smears from freshly collected heparinized chicken red blood cells (CRBC) were prepared and stored in the cold with dessicant. Embryos and endosperms were taken from 16 to 18-day old seeds of each type and put into fixative. For DNA measurements a small area in the CRBC smear was cleaned and wet with fixative; macerated embryo or endosperm cells were pressed with a coverslip and the slides were placed on dry ice. The coverslip was removed and the slide was put into fixative. Slides with the CRBC smear for reference nuclei and either the embryo or endosperm cells were stained with Feulgen (Berlyn and Niksche 1976).

Quantification of DNA

DNA measurements were done with a Zeiss MPM01K microspectrophotometer and a PM-2 Indicator using a 40X objective to observe embryo and endosperm nuclei. CRBC were used as standard reference nuclei values were adjusted to have 5.3 pg DNA per nucleus (Berlyn and Miksche 1976; Phillips et al. 1983). Mitotic figures observed in root tip preparations were measured at metaphase (4C) or anaphase (2C). Interphase nuclei of the diploid embryos were expected to range in DNA content between 2C and 4C and of the triploid endosperm from 3C to 6C because the nuclei would be in G_1, S or G_2 phases of the cell cycle. Likewise the tetraploid embryos were expected to range between 4C and 8C and the hexaploid endosperm nuclei from 6C to 12C.

The interspecific hybrid embryo and endosperm nuclei were examined for the amount of DNA in their nuclei. The theoretical C values were taken to be the average of both parental root tip amounts.

The statistical comparison of the amount of DNA to the theoretical ploidy level number was the t-distribution (Dixon and Massey 1957).

Results

To identify cases of seed failure which were due to arrested pollen tube growth, all possible combinations of the three species were compared (Table 1). Pollen tubes of parental crosses reached ovules 24 to 30 h after pollination. Certain interspecific crosses failed to form seed because pollen tube growth was stopped in the styles. Pollen tubes of *S. acaule* were inhibited when they grew in styles of *S. commersonii* or *S. tuberosum* Group Phureja. *S. commersonii* pollen tubes were inhibited in the styles of Phureja. Phureja pollen tubes in styles of *S. acaule* or *S. commersonii* reached the ovules 24 or 48 h after pollination. *S. commersonii* tubes reached *S. acaule* ovules in 48 h. Pollen tubes of Group Phureja reached *S. commersonii* ovules in 24 h. Consequently, the interspecific crosses of *S. commersonii* × Group Phureja, *S. acaule* × Group Phureja and *S. acaule* × *S. commersonii* were analyzed for embryo and endosperm development.

To characterize the ploidy level of the embryo and endosperm the DNA amounts were compared to those of the root tip nuclei of each species.

DNA measurements were made with Phureja (P) root tips and the 2C value of 5.73 ± 72 pg was determined. The P × P embryo nuclei means were 7.18, 10.02 and 11.71 which correspond to 2.5C, 4C and 4C (Ta-

Table 2. Average DNA means for nuclei from embryos and endosperms of intraspecific crosses

Group Phureja × Group Phureja					
Root 2C = 5.73 ± 0.72 $n = 8$					
		n		t	*P*
Embryo	10.02 ± 2.67	8	vs 4C	1.52	NS
	11.71 ± 2.64	10	vs 4C	1.23	NS
	7.18 ± 0.75	7	vs 2.5C	0.8	NS
Endosperm	27.88 ± 6.76	7	vs 8C	1.80	NS
S. commersonii × *S. commersonii*					
Root 2C = 5.18 ± 1.25 $n = 8$					
Embryo	4.47 ± 0.98	11	vs 2C	2.52	0.025**
	4.63 ± 0.92	12	vs 2C	2.19	0.05*
	7.36 ± 2.56	8	vs 3C	0.44	NS
	10.56 ± 2.92	8	vs 4C	0.65	NS
Endosperm	8.38 ± 2.79	8	vs 3C	0.04	NS
	9.15 ± 1.72	9	vs 3C	1.28	NS
	11.89 ± 4.69	8	vs 4C	0.40	NS
S. acaule × *S. acaule*					
Root 4C = 8.62 ± 2.82 $n = 8$					
Embryo	12.91 ± 4.52	13	vs 6C	0.02	NS
	5.55 ± 1.81	13	vs 2C	6.12	0.005**
Endosperm	13.07 ± 3.98	7	vs 6C	0.09	NS
	27.63 ± 6.36	9	vs 12C	0.84	NS

ble 2) and the P × P endosperm was 27.88 which corresponds to 8C.

DNA amounts were determined with *S. commersonii* (C) root tips and the 2C value was 5.18 ± 1.25. The C × C embryo means were 4.47 and 4.63 which are less than the 2C amount and 7.3 and 10.56 which correspond to 3C and 4C amounts. The means of the endosperm nuclei were 8.38, 9.15 and 11.89; these correspond to 3C and 4C amounts (Table 2).

DNA content of *S. acaule* (A) root tip anaphase figures were measured and found to be 8.62 ± 2.82; this is the 4C amount since this species is tetraploid. Most A × A embryo nuclei had a 6C mean of 12.91. One embryo contained cells with a mean of 5.55 which is close to the 2C amount. The A × A endosperm means were 13.07 and 27.63 which correspond to the expected 6C and 12C values (Table 2).

In the diploid crosses of *S. commersonii* (EBN 1) × Group Phureja (EBN 2) only 5 to 10% of the ovules began enlarging after pollination. Enlarging seeds were either empty, or contained an embryo with no endosperm, or an endosperm with no embryo. The 2C value expected for diploid C × P embryo is 5.46; no means observed fit the 2C value (Table 3). The mean of 9.18 corresponds to a 3C value and 10.62 and 10.04 correspond to 4C values. The higher C × P means of 13.48 and 19.87 correspond to 5C and 7C values. Two low means of 2.27 and 3.60 do not differ from 1C values. Twin embryos were observed which had means of 6.68 and 7.96, these correspond to 2.5C and 3C. The C × P endosperm nuclei are expected to range from 3C to 6C values if normal fertilization occurs between these diploids. The mean DNA amounts were 9.14 and 9.16 which were 3C values, 14.03 and 15.02 which were 5C values and 16.01 which was a 6C value (Table 3).

The crosses of the tetraploid *S. acaule* and diploid Group Phureja were expected to form normal triploid embryos and pentaploid endosperms because they were both in the 2EBN category. The 3C value (2C of A and 1C of P) was expected to be 7.17 and range to 6C of 14.34 (Table 3). The embryo DNA was observed to be

Table 3. Average DNA means for nuclei from embryos and endosperms of interspecific crosses

S. commersonii × Group Phureja					
Theor. 2C value = 5.46					
		n		t	*P*
Embryo	9.18 ± 2.15	8	vs 3C	1.31	NS
	10.62 ± 1.94	9	vs 4C	0.45	NS
	10.04 ± 1.16	5	vs 4C	1.67	NS
	13.48 ± 4.62	8	vs 5C	0.10	NS
	19.87 ± 3.86	7	vs 7C	0.54	NS
	2.27 ± 1.27	11	vs 1C	0.97	NS
	3.60 ± 1.52	10	vs 1C	1.01	0.05*
	6.68 ± 2.12^	15	vs 2.5C	0.13	NS
	7.96 ± 2.44^	9	vs 3C	0.27	NS
	[^twin]				
Endosperm	9.14 ± 1.87	4	vs 3C	1.11	NS
	9.16 ± 3.75	11	vs 3C	0.87	NS
	14.03 ± 3.73	8	vs 5C	0.30	NS
	15.02 ± 4.58	11	vs 5C	0.97	NS
	16.01 ± 4.84	14	vs 6C	0.27	NS
S. acaule × Group Phureja					
Theor 3C value = 7.17					
Embryo	6.54 ± 0.45	4	vs 3C	0.70	NS
	6.99 ± 0.85	6	vs 3C	0.54	NS
	10.86 ± 2.08	15	vs 4C	0.19	NS
Endosperm	19.55 ± 4.65	13	vs 9C	1.53	NS
	23.70 ± 5.22	18	vs 9C	1.77	NS
	31.10 ± 6.47	6	vs 12C	0.91	NS
S. acaule × *S. commersonii*					
Theor. 3C value = 6.90					
Embryo	6.21 ± 1.85	17	vs 3C	1.54	NS
	11.77 ± 2.81	7	vs 6C	1.34	NS
	5.04 ± 1.14	5	vs 2C	0.27	NS
Endosperm	12.89 ± 3.03	20	vs 6C	1.34	NS
	16.48 ± 4.09	6	vs 7C	0.19	NS
	19.53 ± 5.88	11	vs 9C	0.68	NS
	21.57 ± 6.13	26	vs 9C	0.72	NS
	25.46 ± 2.73	6	vs 12C	1.92	NS
	25.92 ± 5.38	8	vs 12C	0.88	NS
	29.45 ± 9.71	8	vs 12C	1.85	NS

Table 4. Developmental scores for embryos and endosperms of intra- and interspecific crosses of three species

	Days post pollination		
	7	14	20
Intraspecific			
Embryo			
Group Phureja × Group Phureja	5	12	22
S. commersonii × *S. commersonii*	4	9	18
S. acaule × *S. acaule*	6	15	30
Endosperm			
Group Phureja × Group Phureja	50	100	250
S. commersonii × *S. commersonii*	30	100	240
S. acaule × *S. acaule*	100	220	500
Interspecific			
Embryo			
S. commersonii × Group Phureja[a]	5	9	shriveled
S. acaule × Group Phureja	6	15	25
S. acaule × *S. commersonii*	6	14	22
Endopsperm			
S. commersonii × Group Phureja[b]	55	125	250
S. acaule × Group Phureja	75	175	210
S. acaule × *S. commersonii*	60	165	240

[a] Seeds contained embryos but no endosperms
[b] Seeds contained endosperms but no embryos

6.54 and 6.99 in agreement with the 3C value and 10.86 which was not different from 4C. The endosperm 5C to 10C values expected should range from 12.55 to 25.10. The observed endosperm values were 9C for 19.55 and 23.70 and a 12C mean of 31.10 (Table 3). The embryos and endosperms in seeds of A × P progressed normally though all stages of development.

The crosses of *S. acaule* × *S. commersonii* were expected to fail; however, these seeds had normal embryos and endosperms. Triploid embryos were expected to contain 3C (2A + 1C) to 6C amounts of DNA of 6.9 to 13.8 and pentaploid endosperm nuclei, 5C to 10C, of 11.65 to 23.29. Both 3C, 6.21, and 5C, 11.77, embryo means were observed (Table 3). A third mean of 5.04 fit the expected value of a 2C *S. commersonii* diploid. Four endosperm means observed were calculated to be 6C, 7C and 9C; three means, 25.46, 25.92 and 29.45 did not differ from 12C values (Table 3).

The development of embryo and endosperm in hybrid seeds was recorded at different times after pollination. The embryos were classified by their various stages and the endosperms by measurements of their cell estimates (Table 4).

When the developmental scores for the embryos and endosperms in the diploid crosses were compared there was very little difference in either embryos or endosperms of the Group Phureja × Group Phureja or *S. commersonii* × *S. commersonii* (Table 4). The tetraploid crosses of *S. acaule* × *S. acaule* had more advanced embryos, and the relative number of endosperm cells was nearly double that of the diploid intraspecific crosses (Table 4). The triploid embryo scores of *S. acaule* × Phureja or *S. commersonii* were very similar (Table 4). The endosperms of these tetraploid × diploid crosses were calculated to have 6C to 12C DNA values, in contrast to the 5C to 10C expected. The day 20 developmental scores were not as large as those in the intraspecific *S. acaule* crosses, but at day 14 the endosperms were somewhat larger than the diploid crosses. Ideally the day 20 endosperms would have scores of 375.

Discussion

The developmental scores of the embryos from the intraspecific diploid crosses were similar; both the Phureja and *S. commersonii* crosses had embryos at the heart stage by 15 to 20 days postpollination. Embryos of the tetraploid intraspecific *S. acaule* cross were at the torpedo stage by this same time.

There was obvious breakdown of the interspecific *S. commersonii* × Group Phureja embryos by day fourteen. *S. acaule* crossed with either Phureja or *S. commersonii* both had embryos at the heart stage by day twenty. Because *S. commersonii* had an EBN number of 1 the presence of these embryos was an unexpected

observation. We did expect *S. acaule* to be capable of producing seed with Phureja because they both have an EBN of 2 (Hanneman 1984).

Examination of the cellular development of the endosperms indicated that triploid endosperm contained about half as many cells from 7 to 20 days after pollination when compared to pentaploid or hexaploid endosperm. This trend was also observed in the interspecific crosses of *S. acaule* by Phureja or *S. commersonii.*

Thus, the interspecific seeds of *S. commersonii* × Group Phureja were frequently observed to contain an abnormal embryo with few or no endosperm cells. Another type of seed was found with a normally developed endosperm tissue and no embryo at all. In none of the 164 seeds examined was there a normally developed embryo and endosperm.

The question to be answered is: How does one deduce underlying mechanisms of seed failure by comparing the developmental observations to the nuclear DNA measurements?

DNA measurements on the nuclei of Phureja × Phureja embryo cells ranged from 2.5 to 4C, these are in the expected range for diploid embryos. DNA values found in nuclei of *S. commersonii* × *S. commersonii* were more variable. All crosses with these parents also had a significantly higher rate of embryo lethality (Table 1). The endosperm formed in both of these intraspecific diploid crosses contained nuclear DNA within the expected 3C to 6C values.

Some reports state that *S. commersonii* has a highly variable chromosome number within the same plant (Olah 1938). This species has been reported to have both diploid and triploid forms (Rybin 1933; Correll 1962). Olah demonstrated that this species has a high rate of chromosome loss during mitotic divisions.

Embryo cells of *S. acaule* × *S. acaule* contained DNA quantities which fall within the 4C to 8C expected range, except in one case in which an embryo contained an average of 2C content of DNA. This embryo was probably derived as a maternal haploid (2n = 24).

Abnormal embryos dissected from the *S. commersonii* × Phureja seeds contained a large range of DNA values. The very low DNA values could be a result of chromosome elimination and/or haploid embryo formation. The 5C and 7C DNA values suggest that gametes with a higher ploidy than n = 12 were formed by *S. commersonii* and/or Group Phureja.

Endosperm tissue which formed in seeds with no embryo may represent a more stable genetic balance. The central cell comprised of two maternal genomes normally is fused with the sperm nucleus to form the endosperm. The two genomes of *S. commersonii* may balance the Group Phureja genome adequately so that abnormal mitotic divisions and chromosome loss does not occur. The companion embryo of these seeds must die after the first few cell divisions following fertilization.

The *S. acaule* × Phureja crosses were expected to have normal development since each are 2 EBN. The DNA range of triploid embryos would be 3C to 6C; both 3C and 4C were found. The companion endosperm DNA values would be 5C to 10C and two observations of 9C DNA estimates of endosperm nuclei were found. A third nucleus had 12C which could have been the result of an unreduced male gamete from Phureja. The cultivar 'Acaphu' (2n = 48) was selected by Estrada (1984) from a cross of *S. acaule* × *S. phureja* when an unreduced male gamete functioned.

Finally the seeds formed by *S. acaule* × *S. commersonii* were expected to fail since these species belong to EBN 2 and 1. Observations of developing embryos and endosperms clearly establish that normal seeds were formed. DNA measurements of embryonic nuclei correspond to nuclei with 24 chromosomes, this suggests that many of the embryos are maternal haploids. The embryo which had an average of 6C DNA may have been a product of a 2n gamete from the *S. commersonii* pollen parent. DNA measurements of endosperm nuclei correspond to what would result if the endosperm was formed by fusion of two gametes of the female of *S. acaule* and two gametes of the *S. commersonii.* This indicates that fertilization is somehow distorted in this cross such that both male gametes frequently fuse with the central cell.

In conclusion, it is apparent that many complex events are working in concert to contribute to seed failure. Part of the story must lie in the characteristic instability which has been documented in the *S. commersonii* genome. In addition, most potato species seem to be capable of forming plants which are of unusual ploidies. Since our ability to characterize chromosome karyotypes is limited, our certainty about the chromosome constitution of resulting interspecific crosses must be questioned. The formation of seed is not proof of interspecific hybrid genome composition. The potential rewards for producing useful hybrids between various species of *Solanum* are great. An understanding of seed failure among interspecific crosses may contribute to this goal.

References

Berlyn GP, Miksche JP (1976) Botanical microtechniques and cytochemistry. Iowa State Univ Press, Ames, Iowa

Bhaduri PN (1936) Studies on the embrogeny of the *Solanaceae.* Bot Gaz 98:238–295

Brink RA, Cooper DC (1939) Somatoplastic sterility in *Medicago sativa.* Science 90:545–546

Brink RA, Cooper DC (1944) Hybrids between *Hordeum jubatam* and *Secale cereale* failed as seeds early in development due to endosperm breakdown. J Hered 35:67–75

Brink RA, Cooper DC (1947) The endosperm in seed development. Bot Rev 13:423–541

Cooper DC, Brink RA (1945) Seed collapse following matings between diploid and tetraploid races of *Lycopersicon pinpinelifolium*. Genetics 30:376–400

Correll DS (1962) The potato and its wild relatives. Texas Res Found, Renner TX

Davies DR (1960) The embryo culture of interspecific hybrids of *Hordeum*. New Phytol 59:9–14

Dixon NJ, Massesy FJ Jr (1957) Introduction to statistical analysis. McGraw-Hill, New York

Ehlenfeldt MK, Hanneman RE Jr (1984) The use of endosperm balance number and 2n gametes to transfer exotic germplasm in potato. Theor Appl Genet 68:155–161

Estrada RN (1984) Acaphu: a tetraploid fertile breeding line, selected from an *S. acaule*×*S. phureja* cross. Am Pot J 61:1–7

Hanneman RE Jr (1984) Assignment of endosperm balance numbers (EBN) to the tuber-bearing *Solanum* species. Res Prog Rep to NCR-84, Pot Gen Tech Comm, Dec

Howard HW (1939) The size of seeds in diploid and autotetraploid *Brassica oleracea* L. Genetics 38:325–340

Johnston SA, den Nijs T, Peloquin SJ, Hanneman RE (1980) The significance of genic balance to endosperm development in interspecific crosses. Theor Appl Genet 56:293–297

Johnston SA, Hanneman RE Jr (1982) Manipulations of endosperm balance number overcome crossing barriers between diploid *Solanum* species. Science 217:446–448

Kho YO, Baer J (1968) Observing pollen tubes by means of fluorescence. Euphytica 17:298–302

Laibach F (1925) Das Taubwerden von Bastardsamen und die künstliche Aufzucht früh absterbender Bastardembryonen. Z Bot 17:417–459

Laibach F (1929) Ectogenesis in plants. J Hered 20:201–208

Lin B-Y (1975) Parental effects on gene expression in maize endosperm development. PhD Thesis, University of Wisconsin

Marks GE (1966) The enigma of triploid potatoes. Euphytica 15:205–209

Muntzing A (1933) Studies on meiosis in diploid and triploid *Solanum tuberosum*. Hereditas 17:223–245

Olah L von (1938) Cytological investigations on the genus *Solanum* Sect. *Tuberarium* III. *S. commersonii* Dun. and some of its hybrids. Z Vererbungsl 74:228–241

Phillips RL, Wang AS, Kowles RV (1983) Molecular and developmental cytogenetics of gene multiplicity in maize. In: Gustafson JP (ed) Stadler Symp No 15. University of Missouri, Columbia MO

Rammanna MS, Hermsen JGTh (1971) Somatic chromosome elimination and meiotic chromosome pairing in the triple hybrid 6X-(*Solanum acaule*×*S. bulbocastanum*)×2X-*S. phurja*. Euphytica 20:470–481

Rowe PR (1974) Parthenogenesis following interspecific hybridization. In: Kasha KJ (ed) Haploids in higher plants. University of Guelph, Guelph, Canada

Rybin VA (1933) Cytological investigation of the South American cultivated and wild potatoes and its significance for plant breeding. Bull Appl Bot, Genet Plant-Breed, Leningrad, Ser 2:3–100

Sheridan WF (1982) Maize developmental mutants. J Hered 73:318–329

Smith PG (1944) Embryo culture of a tomato species hybrid. Proc Am Hortic Sci 44:413–416

Smith JA, Desborough SL (1985) Characterization of proteins extracted from seeds of *Solanum* species (in preparation)

Stelly DM, Peloquin SJ, Palmer RG, Crane CF (1984) Mayer's hemalum-methyl salicylate: A stain-clearing for observations within whole ovules. Stain Technol 59:155–161

Stephens SG (1942) Colchicine produced polyploids in *Gossypium*. 1. An autotetraploid Asiatic cotton and certain of its hybrids with diploid species. J Genet 47:272–295

Valentine DH (1947) Studies in British *Primulas*. 1. Hybridization between Primrose and Oxlip (*Primula vulgaris* Huds. and *P. elatior* Schreb.). New Phytol 46:229–253

Valentine DH (1952) Studies in British *Primulas*. 3. Hybridization between *Primula elatior* L. Hill and *P. veris* L. New Phytol 50:383–399

Valentine DH (1955) Studies in British *Primulas*. 4. Hybridization between *Primula vulgaris* Huds. and *P. veris* L. New Phytol 54:70–80

Valentine DH (1956) Studies in British *Primulas*. 5. The inheritance of seed compatibility. New Phytol 55:305–318

Woodell SRJ (1960a) Studies in British *Primulas*. 7. Development of seed from reciprocal crosses between *P. vulgaris* Huds. and *P. veris* L. New Phytol 59:302–313

Woodell SRJ (1960b) Studies in British *Primulas*. 8. Development of seed from reciprocal crosses between *P. vulgaris* Huds. and *P. veris* L. and *P. elatior* (L.) Hill: and between *P. veris* L. and *P. elatior* (L.) Hill. New Phytol 59:314–325

Woodell SRJ, Valentine DH (1960) Studies in British *Primulas*. 9. Seed incompatibility in diploid-autoploid crosses. New Phytol 59:282–294

Wangenheim KH von, Peloquin SJ, Hougas RW (1960) Embryological investigations on the formation of haploids in the potato (*Solanum tuberosum*). Z Vererbungsl 91: 391–399