SUPPORT OF THE ENDOSPERM BALANCE NUMBER HYPOTHESIS UTILIZING SOME TUBER-BEARING *SOLANUM* SPECIES[1]

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Abstract

The normal development of the endosperm appears to depend on a ratio of two female genomes to one male genome in most angiosperms. However, successful crosses between some tuber-bearing *Solanum* species do not conform to this ratio. An endosperm balance number (EBN) hypothesis has been proposed to reconcile the two female genomes: one male genome ratio proposal and the anomalous crossing behavior of some species. Crosses performed to test the EBN hypothesis are reported in this paper. The results support the hypothesis and demonstrate its usefulness in predicting the success or failure of a cross and the ploidy of the offspring. A format for conducting and reporting crossing experiments is proposed that facilitates the comparison of results of different experiments.

Resumen

El desarrollo normal del endosperma, parece depender en la proporción de dos genomios provenientes de la planta hembra y uno de la planta macho; sin embargo, cruzamientos entre algunas especies tuberíferas de *Solanum* se han conseguido y no conforman dicha proporción. Se ha propuesto la hipótesis de factores de balance en el endosperma (EBN) para reconciliar la proporción de genomios 2:1 (hembra:macho) y el comportamiento anormal en cruzamientos de algunas especies. Cruzamientos de prueba de dicha hipótesis se reporta en el presente artículo y los resultados obtenidos concuerdan con ella, demostrando su utilidad en la predicción de éxito o fracaso del cruzamiento, asimismo, el nivel de ploidía de la progene. Un esquema para conducir y reportar experimentos de cruzamientos es propuesto facilitando la comparación de resultados de diferentes experimentos.

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Introduction

The successful development of the seed in almost all angiosperms depends on a functional endosperm (4). Interploidy crosses (4, 5, 8, 10, 29), even between diploids and their autotetraploids (1, 9, 15, 20), usually do not yield offspring of intermediate ploidy. This has been attributed to the failure of the endosperm to develop normally, resulting in the death of the embryo (3, 4, 20, 22, 23, 25, 26).

There have been several proposals to explain seed failure in interploidy crosses (17, 23, 25, 26, 27, 28). Lin (13) has shown that interploidy crosses of maize fail because they deviate from a two:one ratio of female:male genomes in the endosperm. Our interpretation (14) of the embryological work of v. Wangenheim *et al.* (26) suggests that this is also true in *Solanum tuberosum* L.

However, the endosperm ratio rule for intraspecific, interploidy crosses is not directly applicable to all interspecific, interploidy crosses in the tuber-bearing *Solanum* species. These exceptional crosses include several 4x Mexican species and 4x *Solanum acaule,* which readily form triploids when crossed with some diploids (8, 12, 19, 23, 24, 25), as well as 6x *Solanum demissum* which forms pentaploids when crossed with tetraploids (11, 12).

Johnston, *et al.* (14) have proposed an Endosperm Balance Number hypothesis (EBN) to reconcile the rule of two female genomes:one male genome ratio with the anomalous crossing behavior of some of the *Solanum* species. Under this hypothesis an EBN is arbitrarily assigned to one species. Other species are assigned EBNs on the basis of their crossing behavior with the species used as a standard. A successful endosperm results only if the cross has a two female:one male *EBN* ratio. Crossing data in the literature provide some information, but cannot be used to critically test the EBN hypothesis because data relating to seed quality, seed set, occurrence of fertilization, or ploidy of the offspring either are not reported or can not be compared. For this reason a format to be followed in crossing experiments is outlined here.

In this experiment several species and species hybrids were intercrossed. The results of these crosses have been used to test the EBN hypothesis.

Materials and Methods

Table I shows the plants used in the crosses. All except the ones noted were obtained from the Inter-Regional Potato Introduction Station at Sturgeon Bay, Wisconsin. The plants were grown in the field at Sturgeon Bay. Crosses were done with the cut-stem technique (18). All pollinations were done on emasculated buds 1-2 days before opening. At least three styles of each cross were fixed two or three days after pollination and were stained with aniline blue; the pollen tubes were observed under ultra-violet light (16) to determine if fertilization could have occurred.

TABLE 1. — *Description of tuber-bearing* Solanum *materials used in the crosses.*

Materials	Ploidy	Description
S. chacoense (chc)[1]	2x	P.I.s 209411, 217451
S. verrucosum (ver)	2x	P.I.s 161173, 275255
S. megistacrolobum (mga)	2x	P.I.s 435077,435078
S. boliviense (blv)	2x	P.I. 435072
S. infundibuliforme (ifd)	2x	P.I.s 435075, 435076
S. acaule (acl)	4x	P.I.s 210029, 435070, 435071
S. stoloniferum (sto)	4x	P.I.s 161158, 195164
S. chacoense	3x	From 4x x 2x cross[2]
S. chacoense	4x	P.I.s 209411-C.1, C-3[2]
Gp. Andigena (adg)	4x	F_1s of P.I. 320326 x 347773
S. gourlayi (grl)	4x	P.I.s 435073, 435074
Gp. Tuberosum (tbr)	4x	A derivative of Sebago[2]
WRF 1321	5x	(8x *S. stoloniferum* x Merrimack) x Wis AG231=WRF 1321
S. demissum (dms)	6x	P.I.s 160221, 195165
S. oplocense (opl)	6x	P.I.s 435079, 435080
S. acaule ssp. *albicans*	6x	P.I. 230494
2082	6x	(*S. stoloniferum* x Gp. Stenotomum) CT(6x) x Self, Dionne s.n. (2)=P.I. 435083

[1]Abbreviations according to: Simmonds, N.W. 1963. Abbreviations of Potato Names. Eur Potato J 6: 186-190.
[2]Plants courtesy S.J. Peloquin.

Plump seeds extracted from the resulting fruit were immediately treated with gibberellic acid (1500 ppm for 24 hours). A maximum of ten randomly chosen plants from each cross was grown. Chromosome counts (aceto-carmine squash of root-tips) were done on at least four of these to prove hybridity in interploidy crosses.

Results and Discussion

The following minimal information is considered necessary by the authors to derive useful information from a crossing experiment: 1) the criterion for judging the seed to be viable (i.e., plumpness, germinability, or size); 2) the number of viable seeds/fruit; 3) in a cross that failed, determination if fertilization occurred or at least if pollen tubes reached the ovary; 4) the ploidy of the offspring, especially from interploidy crosses.

The results of the crosses in this experiment are summarized in Table II. A cross is indicated as successful (+) if it averaged ten or more plump seeds/fruit and the chromosome count and/or morphology of the offspring

TABLE 2. —*Results of intercrossing some tuber-bearing Solanums of various ploidy levels and various Endosperm Balance Numbers (EBN).*

	♀ \ ♂	2EBN 2x chc	2EBN 2x ver	2EBN 2x mga	2EBN 2x blv	2EBN 2x ifd	2EBN 4x acl	2EBN 4x sto	3EBN 3x chc	4EBN 4x chc	4EBN 4x adg	4EBN 4x grl	4EBN 4x tbr	4EBN 5x 1321	4EBN 6x dms	4EBN 6x opl	4EBN 6x acl	4EBN 6x 2082
2EBN	2x chc[1]	/	*				*	+		–	–				*	*	*	*
2EBN	2x ver	+	/		+		+	–		–	–			*	–	–	–	–
2EBN	2x mga	*		/			–			–	–	–				–	–	
2EBN	2x blv	–			/		*			–	–	–				–		
2EBN	2x ifd	+				/	*					–				–		
2EBN	4x acl	+	*	+		+	/	*		*	–	–		*	–		*	
2EBN	4x sto	–	*	*			–	/		–	–				–	–	–	–
3EBN	3x chc	+							/	+								
4EBN	4x chc	–	*	–			*	*		/	+			*	*	+	*	*
4EBN	4x adg	–	*	–		*	*	*		*	/	+			*	+	*	*
4EBN	4x grl	–		–		–	*				+	/				–		
4EBN	4x tbr												/		+	+		
4EBN	5x 1321	+	*	–			*	*		+	+			/	–	+		
4EBN	6x dms	–	*	–		–	–	–		+	+	+			/	+	+	+
4EBN	6x opl	–	*	–		–	*	*		*	*	+			*	/	*	*
4EBN	6x acl	–	*							+	–						/	
4EBN	6x 2082	–	*				*	–		–	+				*	+	+	/

\+ = 10 or more plump seeds/fruit.

– = Either fruit had aborted seeds, or examination of the styles showed many pollen tubes reached the ovary.

* = At least ten pollinations were made but pollen tubes did not effect fertilization.

[1]Abbreviations according to: Simmonds, N.W. 1963. Abbreviations of Potato Names. Eur Potato J 6:186-190.

indicated hybridity. A cross is indicated as a failure (−) if fruit had aborted seeds or, in crosses where no fruit was set, the styles showed many pollen tubes had reached the ovary.

If the triploid and pentaploid crosses are not considered, Table II can be divided into four sections on the basis of the presence or absence of successful crosses (Table III). The crosses of sectors I and IV in Table III

TABLE 3. — *A summary of the successes and failures of crosses of tuber-bearing Solanums in Table 2.*

	Diploids, 4x *S. acaule*, 4x *S. stoloniferum*	Tetraploids and Hexaploids
Diploids, 4x *S. acaule*, 4x *S. stoloniferum*	+ I	− II
Tetraploids and Hexaploids	− III	+ IV

can form functional endosperms; the crosses of sectors II and III apparently cannot. Clearly, no particular female:male genome ratio in the endosperm can be associated with all successful crosses.

Under the EBN hypothesis an arbitrary number is assigned to a species that is chosen as a standard. It is the EBNs which must be in a 2:1 ratio in the endosperm. Then, any species producing plump seeds when crossed with the standard species is assigned the same EBN.

For example, if 2x *S. chacoense* was assigned an EBN of 2, then all species with which it successfully crossed, and others crossing successfully with them, would also be assigned an EBN of 2 (sector I). Thus, 4x *S. acaule* and 4x *S. stoloniferum* would have an EBN of 2. Colchicine-induced, tetraploid *S. chacoense* would have an EBN of 4 (twice that of 2x *S. chacoense)* as would any plant with which it successfully crossed. Thus, all hexaploids tested would be assigned an EBN of 4 as well as all tetraploids except *S. acaule* and *S. stoloniferum*. Table IV lists the genomic and the EBN ratios for various crosses.

Five tests demonstrate the consistency of the EBN hypothesis.

1) None of the plants tested can be assigned two different EBNs. This also indicates non-complementation of the factors determining the EBN of different species.

2) Each species or hybrid tested with an EBN less than its ploidy level probably has genome differentiation. Therefore, the 2EBN tetraploids and the 4EBN hexaploids may be expected to show disomic inheritance. *S.*

acaule and *S. stoloniferum* apparently have disomic inheritance (7, 21). *S. demissum* has bivalent pairing and may have disomic inheritance (2). The mode of inheritance in the other hexaploids is not known.

TABLE 4. — *Parental ploidy levels, parental Endosperm Balance Numbers (EBN), and the resultant ploidy ratios and EBN ratios in the endosperm of each combination of crosses among tuber-bearing Solanums.*

♀ \ ♂	2x,2EBN	4x,2EBN	4x,4EBN	6x,4EBN
2x,2EBN	2:1/2:1	2:2/2:1	2:2/2:2	2:3/2:2
4x,2EBN	4:1/2:1	4:2/2:1	4:2/2:2	4:3/2:2
4x,4EBN	4:1/4:1	4:2/4:1	4:2/4:2	4:3/4:2
6x,4EBN	6:1/4:1	6:2/4:1	6:2/4:2	6:3/4:2

The ratios for a cross signify: female:male ploidy ratio/female:male EBN ratio.

3) The synthetic hexaploid, (PI 435083), was created by colchicine treatment of a triploid from a *S. stoloniferum* x Gp. Stenotomum cross. Therefore, under the EBN hypothesis, it should have an EBN of 4. This is also the value assigned from the crossing data.

4) The triploid *S. chacoense* was assigned an EBN of 3. If the factors determining the EBN were on one or a few chromosomes, one might expect these chromosomes to segregate randomly at meiosis. This triploid would then be expected to cross with both 2EBN and 4EBN plants. This was found (Table II). By the same argument, one would expect the pentaploid, WRF 1321, to cross with both 2EBN and 4EBN plants. This was also found (Table II).

5) An occasional plump seed occurred in crosses that would be predicted to fail by the EBN ratios. These seeds were germinated; the plants were grown, and their ploidy level was determined (Table V). In most cases the ploidy level could be explained by the functioning of a 2n gamete to produce a 2:1 EBN ratio. For example, in the cross 6x (4EBN) *S. demissum* x 4x (2EBN) *S. stoloniferum,* 2n pollen apparently functioned to give a 2:1 endosperm ratio and a septaploid (7x) plant.

Clearly, a 2:1 EBN ratio is a necessary but not sufficient criterion for the success of a cross. There were seed abortions in crosses between plants which gave a 2:1 EBN ratio in the endosperm (Table II). Also, in many instances the crosses failed because the pollen tubes did not reach the ovary.

The results of these crosses support the EBN hypothesis (14), thus extending the 2:1 ratio rule to interspecific crosses in the tuber-bearing Solanums. Using existing crossing data it is possible to assign EBN numbers to most of them. This new characterization can be used to predict the

TABLE 5. — *Crossing data and chromosome number for potato plants from interploidy crosses that usually failed.*

Cross	No. of plump seeds	No. of fruit	Plump seeds/fruit	No. of pollinations	Chromosome number of offspring
2x blv[1] x 4x adg	1	2	0.5	8	48
2x blv x 4x grl	1	2	0.5	8	48
2x ifd x 4x grl	1	5	0.2	13	36
4x chc x 2x chc	2	12	0.2	12	36
4x chc x P-35[2]	4[3]	3	1.3	17	24
5x 1321 x 6x dms	1	2	0.5	9	64 ± 1
6x dms x 4x sto	1	6	0.2	8	84
6x dms x 2x ifd	1	6	0.2	12	60
6x opl x 2x ifd	1	9	0.1	50	48
6x 2082 x 2x P-35	1	3	0.3	8	60

[1]Abbreviations according to: Simmonds, N.W. 1963. Abbreviations of Potato Names. Eur Potato J 6:186-190.
[2]P-35 is a *S. tuberosum* Gp. Phureja pollinator used to extract haploids. It is homozygous for the dominant gene – seed spot.
[3]No seed spot.

success or failure of a specific cross. The EBN hypothesis complements our knowledge of the functioning of 2n gametes in interploidy crosses and evolution in potato (6) and may have application to other species (14). The genetic and physiological factors underlying the EBNs remain to be discovered.

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