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Genetic control of Endosperm Balance Number (EBN): three additive loci in a threshold-like system

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Summary. The genetic control of Endosperm Balance Number (EBN) was investigated by a complete diallel of four exceptional diploid *Solanum commersonii-S. chacoense* hybrids (1½ EBN) and backcrosses to their species parents, *S. commersonii* (1 EBN) and *S. chacoense* (2 EBN). Crosses in which the female parent had a higher EBN value than the male, *S. chacoense* (2 EBN) × F_1 (1½ EBN) and F_1 (1½ EBN) × *S. commersonii* (1 EBN), produced viable seed to aborted seed ratios of 1:1.1 and 1:1.3, respectively, and had average to small sized viable seed. Crosses in which the female parent had a lower EBN value than the male, *S. commersonii* (1 EBN) × F_1 (1½ EBN) and F_1 (1½ EBN) × *S. chacoense* (2 EBN), produced viable seed to aborted seed ratios of 1 : 7.9 and 1 : 6.7, respectively, and had average to large sized viable seeds. The results of these crosses appear to be consistent with the relative EBN values of the male and female parent. A model is proposed for the system regulating endosperm development. The assumptions of this model are: (1) three unlinked loci control the system; (2) the loci are homozygous within a species; (3) the genes have additive effects and are of equal strength within a species; (4) the genes within *S. chacoense* have twice the effect with respect to endosperm regulation as those within *S. commersonii*; and (5) a slight excess maternal dosage will produce the qualitative effect of small but viable seed. This model, in which quantitative genes operate in a dosage dependent system bears many similarities to classical, threshold-type genetic models.

Key words: Endosperm Balance Number (EBN) – Potato – *Solanum* – Speciation

Introduction

The Endosperm Balance Number (EBN) system of the tuber-bearing *Solanums* (Johnston et al. 1980) describes the crossing behavior of *Solanum* species with regard to endosperm development. It provides a rationale for understanding the success or failure of both inter- and intraspecific crosses through its interpretation of hybrid endosperm development. This interpretation is based on the hypothesis of a requirement of a 2 : 1 maternal to paternal EBN dosage in the endosperm for normal endosperm development. The EBN hypothesis was inspired by the work of Lin (1975, 1984) who demonstrated, in maize, a requirement for a 2 : 1 maternal to paternal genomic dosage for normal endosperm development. A 3 : 1 ratio gave miniature but viable kernels. Johnston et al. (1980) expanded upon Lin's work to hypothesize, in potatoes, the existence of endosperm factors (EBNs) rather than entire genomes, which must be in a 2 : 1 balance for successful endosperm development. Much circumstantial evidence supports this hypothesis, and 2x(1 EBN), 2x(2 EBN), 4x(2 EBN), 4x(4 EBN) and 6x(4 EBN) species have been identified (Hanneman 1982; Johnston and Hanneman 1980, 1982). Despite the success of this system in predicting the outcome of crosses, its genetic control is unknown.

The genetic study of this system is a difficult one hampered by the fact that generally the most instructive plants, in terms of resolving its control, are not produced because of endosperm inviability. Ideally for genetic analysis, an inter-EBN hybrid needs to be

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produced and its crossing and segregation behavior observed. Since species are assumed to be homozygous for their EBN values, and since EBN appears to be controlled by nuclear genes, segregation should follow Mendelian genetics. Two closely related species, 2x(1EBN) *S. commersonii* and 2x(2EBN) *S. chacoense* are good candidates for this type of experiment.

This report details the production of rare 2x (1½ EBN) hybrids from *S. commersonii* and *S. chacoense* for the study of the EBN system and the subsequent determination of its genetic control.

Materials and methods

The materials used are listed in Table 1.

Crosses were carried out in any of three manners: (1) on plants in insect-free screenhouses at the University of Wisconsin Agricultural Research Station, Sturgeon Bay, Wisconsin; (2) on cut stems (Peloquin and Hougas 1959) in air-conditioned greenhouses at the same location, or (3) on plants in a University of Wisconsin greenhouse at Madison, Wisconsin. The flowers of all plants were emasculated at the bud stage.

Fruit was allowed to mature on the plant or cut-stem for at least 30 days after pollination. At maturity, fruit was harvested and allowed to ripen a minimum of 4 weeks prior to seed extraction. Seeds were treated with 1,500 ppm gibberellic acid for 24 h to break dormancy and give more uniform germination. Seeds from the interspecific crosses were germinated in petri dishes and transferred to a potting mixture. Root tips were taken from potted plants and ploidy determinations were made. Nineteen diploid hybrids were entered into a preliminary crossing block to assess male fertility and fruit set. Male fertility was assessed by examining pollen stainability with acetocarmine. Fruit set was assessed by a series of crosses with the parental species. Each diploid hybrid was used as a female in a minimum of 20 crosses with both *S. commersonii* and *S. chacoense.* On the basis of male fertility and fruit set, the four clones F, X, EE and RR were selected for use in a complete diallel crossing block including the species parents. Meiotic chromosome counts were made on each selected clone prior to entry into the diallel to assure that plants were euploid.

A minimum of 35 pollinations were made for each of the 30 cross combinations possible in a 6×6 diallel, excluding selfs. The ten largest fruit from each cross were selected for evaluation of seed set and development. The characteristics for evaluating seed development were: (1) the ratio of viable seed to aborted seed for each cross, and (2) the size of the viable seed. The separation of viable seed from aborted seed was done on a visual basis after preliminary tests to correlate visual assessment of seed condition with germination were done. A seed was considered abortive if no endosperm development was seen beyond a very early stage or if a mature sized seed/ovule was completely collapsed with no outward sign of being viable. Plump seeds, no matter how small, were considered viable.

Ploidy determinations were generally done on root-tips. Root tips were collected in the morning and pre-treated in an 8-hydroxyquinoline solution (0.29 g/l) for approximately 4 h. Root tips were then transferred to 3:1 (ethanol:acetic acid) and fixed at least 24 h. For examination, root tips were hydrolyzed in 1N HCl at 60 °C for 10 min, rinsed in distilled water for a minimum of 5 min and squashed in a solution of 1% carmine in 45% acetic acid to which a small amount of iron acetate was added.

Meiotic studies and exact chromosome counts were done using buds. Buds were fixed in 6:3:2 (ethanol:chloroform: 45% propionic acid saturated with iron acetate) for 24–48 h and then transferred to 90% ethanol. Anthers were squashed in 1% acetocarmine for examination.

Table 1. Materials used in EBN genetics in *Solanum*

Designation	Description	Source
2x cmm	*S. commersonii*	
	ssp. *commersonii* P.I.s 243503, 320267	IR-1
	ssp. *malmeanum* P.I. 320269	IR-1
	ssp. *malmeanum* × ssp. *commersonii* P.I.s 320269 × 320266	Author
	ssp. *commersonii* × ssp. *malmeanum* P.I.s 320267 × 320269	Author
2x chc	*S. chacoense* P.I.s 230580, 230582	IR-1
2x cmm × chc		
F	*S. commersonii* P.I. 320267 clone 8 × *S. chacoense* P.I. 230582	Author
X	(*S. commersonii* P.I.s 320269 × 320266, clone 8) × *S. chacoense* P.I. 230582	Author
EE	(*S. commersonii* P.I.s 320269 × 320266, clone 6) × *S. chacoense* P.I. 230582	Author
RR	(*S. commersonii* P.I.s 320267 × 320269, clone 10) × *S. chacoense* P.I. 230582	Author

Results and interpretations

Exceptional diploid F_1 hybrids were generated through 2x × 2x *S. commersonii-S. chacoense* crosses. Approximately 950 such pollinations were performed with *S. commersonii* as the female. About 150 pollinations were performed using *S. chacoense* as the female; however, stylar blockage of pollen tubes resulted in very low fruit set. The fruit set in *S. commersonii* × *S. chacoense* crosses was satisfactory; however, the endosperm barrier between these two species drastically limited viable seed development. From 1100 pollinations, 54 seeds were produced which gave rise to plants. Sixty-three percent of these plants (34 plants) were triploid, arising presumably from 2x(1EBN) gametes from *S. commersonii* uniting with normal 1x(1EBN) gametes from *S. chacoense.* A single monoploid (2%) was found from a cross of *S. chacoense* × *S. commersonii.* The remaining 35% (19 plants) were diploid. Among these 19 plants, 5 *S. chacoense*-like and 3 *S. commersonii*-like plants were found. These plants did not appear to be hybrid and are presumed to be apomicts or contaminants. Eleven plants appeared to be true F_1 hybrids, morphologically intermediate between the two species parents and markedly different from the two other groups of plants recovered.

Preliminary evaluations of fertility and fruit set of the 11 diploid *S. commersonii-S. chacoense* hybrid clones (inter-clonally and with parent species) led to the selection of four clones, F, X, EE and RR, which were crossed in a complete diallel.

The results of diallel crosses are present in Table 2 and Fig. 1. The trends seen in these data are as follows:

(1) $F_1 \times F_1$ crosses: moderate seed set with segregation for seed size ranging from large to small; plump seed to aborted seed ratio of 2.5 : 1

(2) $F_1 \times$ cmm: seed set similar to sib-matings with seed small to average sized; viable seed to aborted seed ratio of 1 : 1.3

(3) cmm $\times F_1$: very low seed set, but the developed seeds average to large sized; viable seed to aborted seed ratio of 1 : 7.9

(4) $F_1 \times$ chc: low seed set with average to large sized seeds; viable seed to aborted seed ratio of 1 : 6.7

(5) chc $\times F_1$: low to moderate seed set with average to small sized seed; viable seed to aborted seed ratio of 1 : 1.1.

Variation in absolute seed number appears affected by both plant genotype and stylar barriers. Attempts were made to examine seed for discrete seed size classes; however, variation for seed size appeared to be continuous in all of the crosses. Figure 2 illustrates seed types in three of these crosses. Reciprocal F_1-species crosses differed markedly in their results (Fig. 1), whereas non-reciprocal pairs of crosses, in one case cmm (1EBN) × F_1 (1½EBN) and F_1 (1½EBN) × chc (2EBN), and in the other case chc (2EBN) × F_1

Table 2. Seed set, seed abortion, seeds per fruit and viable seed to aborted seed ratios in 2x F_1 *S. commersonii–S. chacoense* EBN genetics diallel

Cross[a]	No. of fruit	No. seeds	No. aborted seeds	Seeds/fruit	Ratio of viable seeds to aborted seeds
F×X	3	44	15	14.7	2.5:1
×EE	4	49	10	12.2	
×RR	7	201	48	28.7	
X×F	10	533	270	53.3	
×EE	10	542	198	54.2	
×RR	10	837	518	83.7	
EE×F	10	288	65	28.8	
×X	10	144	76	14.4	
×RR	10	417	93	41.7	
RR×F	10	330	120	33.0	
×X	10	217	60	21.7	
×EE	10	279	68	27.9	
F×cmm	10	150	90	15.0	1:1.3
X×cmm	10	592	728	59.2	
EE×cmm	10	88	185	8.8	
RR×cmm	10	385	594	38.5	
F×chc	10	64	210	6.4	1:6.7
X×chc	10	166	1,064	16.6	
EE×chc	10	69	303	6.9	
RR×chc	10	88	1,010	8.8	
chc×F	10	222	160	22.2	1:1.1
×X	10	197	108	19.7	
×EE	10	66	157	6.6	
×RR	10	211	355	21.1	
cmm×F	22	17	216	0.8	1:7.9
×X	19	15	40	0.8	
×EE	23	11	82	0.5	
×RR		–	–	–	

[a] cmm = *S. commersonii*, chc = *S. chacoense*, F, X, EE and RR are 2x F_1 *S. commersonii* × *S. chacoense* hybrids

	F1	cmm	chc
F1	large to small 2.5v : 1a	average to small 1v : 1.3a	average to large 1v : 6.7a
cmm	average to large 1v : 7.9a	____	____
chc	average to small 1v : 1.1a	____	____

Fig. 1. Seed sizes and observed viable seed to aborted seed ratios in EBN genetics diallel crosses. Note: cmm = *S. commersonii*, chc = *S. chacoense*, F_1 = 2x *S. commersonii* × *S. chacoense* hybrids, v = viable seeds, a = aborted seed

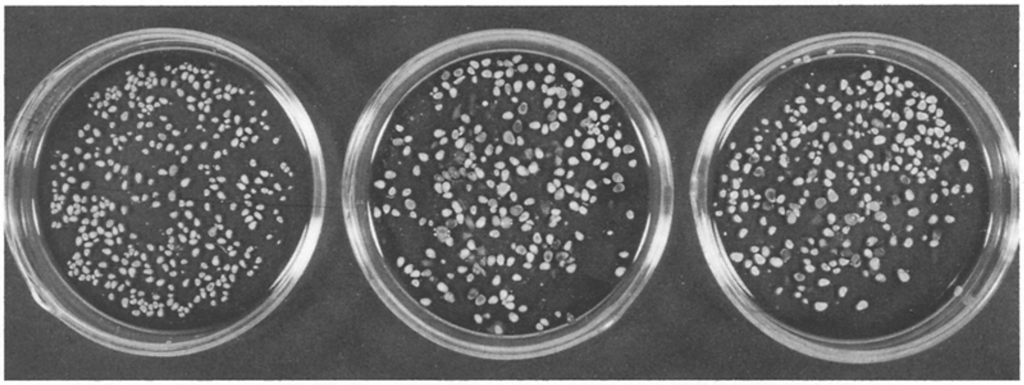

Fig. 2. Viable and aborted seed from $F_1 \times$ *S. commersonii*, $F_1 \times F_1$ and $F_1 \times$ *S. chacoense* (*left to right*). Aborted seeds are dark or very small and pale. $F_1 \times$ *S. commersonii* crosses have mostly small but viable seeds. $F_1 \times F_1$ crosses have a range of viable seed sizes and both large and small aborted seeds. $F_1 \times$ *S. chacoense* crosses have average to large sized viable seeds and many large and small aborted seeds

	Female EBN > Male EBN	Female EBN < Male EBN
Cross (adjacent pairs)	chc : F_1 : cmm	cmm : F_1 : chc
EBNs	2 : 1 1/2 : 1	1 : 1 1/2 : 2
Seed size	average to small	average to large
Approximate viable(v): aborted (a) seed ratio	1v : 1a	1v : 7a

Fig. 3. Seed size and viable seed to aborted seed ratios in crosses with respect to EBN. Note: cmm = *S. commersonii*, chc = *S. chacoense*, F_1 = 2x *S. commersonii* × *S. chacoense* hybrids, v = viable seeds, a = aborted seed

(1½EBN) and F_1 (1½EBN) × cmm (1EBN), were very similar.

These results may be understood in terms of relative EBN values of the parents and the direction in which the cross was performed (Fig. 3). In the first pair of crosses the female has a lower EBN (i.e., 1EBN × 1½EBN and 1½EBN × 2EBN, respectively). In the second pair of crosses, this situation was reversed and the female has a higher EBN (i.e., 2EBN × 1½EBN and 1½EBN × 1EBN, respectively). The results reflect the relative EBN relationships of the parents involved.

In consideration of specifics, two factors have a significant bearing on the interpretation of the EBN diallel data: the unequal viable to aborted seed ratios in reciprocal crosses (approximately 1:1 versus 1:7) and the major variations in seed size. The differences in viable to aborted seed ratios appear to be a directional effect related to which parent is male and which is female within any given cross, yet it does not appear to be a cytoplasmic effect since the same female may give different results depending upon the pollen source. The effect is most likely due to dosage effects in the endosperm. Likewise, seed size, especially the occurrence of decidedly small seed in crosses in which the females have higher EBN values, appears related to endosperm dosage. This effect was also seen in $F_1 \times F_1$ crosses, but was unseen in crosses in which the female had lower EBN values.

These observations, together with the knowledge of endosperm dosage effects compiled by Lin (1975, 1984) in maize, allow us to make some critical assumptions in developing a model to explain EBN. The crucial observations made by Lin were that seeds with an excess female dosage in the endosperm progressed further than seeds with an excess male dosage and that a 3:1 dosage in maize endosperm resulted in "miniature" but viable seed phenotypes. Results of crosses in potato are clearly consistent with this. Crosses in which the female parent has the higher EBN result in a high number of viable seed, many having a small phenotype.

To build a genetic model consistent with the facts known about the EBN system in potato and the genomic dosage effects observed in maize endosperm, five minimal assumptions were made after preliminary evaluation:[1] (1) the EBN system between *S. commersonii* and *S. chacoense* is controlled by three loci; (2) the loci involved are unlinked; (3) each gene within

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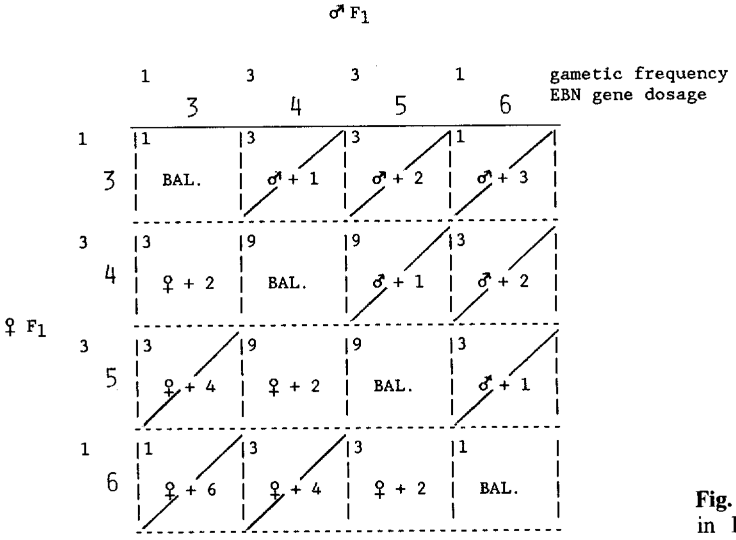
[1] See Ehlenfeldt (1984) for the construction and evaluation of several alternative models



Cross	Predicted viable seed to abortive seed ratios	Observed viable seed to abortive seed ratios
F_1 x F_1	35v : 29a	2.5v : 1a
F_1 x cmm (first vertical column)	4v : 4a	1v : 1.3a
F_1 x chc (last vertical column)	1v : 7a	1v : 6.7a

Fig. 4. Punnett square representing endosperm condition in $F_1 \times F_1$ *S. commersonii-S. chacoense* hybrids under a three gene model and summary of predicted and observed viable (v) seed to abortive (a) seed ratios in $F_1 \times F_1$, $F_1 \times$ *S. commersonii* (cmm) and $F_1 \times$ *S. chacoense* (chc) crosses. Note: Punnett square denotes relative EBN gene dosages in endosperm. Offspring with a 2 : 1 maternal to paternal dosage (3/3, 4/4, 5/5 or 6/6) are considered balanced (BAL.) Offspring with a 4/3, 5/4 or 6/5 ratio are labelled ♀+2, those with a 3/4, 4/5 or 5/6 ratio are labelled ♂ + 1, etc. *Boxes with a slash* denote abortive seed

a species has an identical EBN value and an additive effect; (4) the genes within *S. chacoense* are twice as effective with respect to endosperm development as those within *S. commersonii* (i.e., each gene in *S. commersonii* has an EBN gene value of 1 and each gene in *S. chacoense* has an EBN gene value of 2), and (5) a slight excess EBN gene dosage on the female side (i.e., in excess of 2 : 1 in the central cell) produces small but viable seed.

Under these assumptions, any 2x *S. commersonii-S. chacoense* hybrid is composed of three alleles with an EBN gene value of 1 (*S. commersonii* genes) and three alleles with an EBN gene value of 2 (*S. chacoense* genes). Under an additive assumption, recombination during meiosis between these loci will produce a variety of gametic EBN values ranging from those equivalent to gametes of *S. commersonii* (1 + 1 + 1) to those identical in value with those of *S. chacoense* (2 + 2 + 2). The segregation of these gametic values can be predicted by the coefficients of a binomial expansion of the form $(a + b)^3$, in which a and b represent the EBN alleles of *S. commersonii* and *S. chacoense*. This expansion yields a 1 : 3 : 3 : 1 distribution of gametes with values of 3 (= 1 + 1 + 1), 4 (= 1 + 1 + 2), 5 (= 1 + 2 + 2) and 6 (= 2 + 2 + 2).

The distribution of gametic types generated from these expansions can be used to construct a frequency distribution Punnett square to represent endosperm development in diallel crosses (Fig. 4). To simplify the determination of the outcome of these crosses, progeny in the Punnett square are represented only in terms of endosperm constitution. Any offspring receiving gametes with equal EBN gene values from both parents (i.e., 3/3, 4/4, 5/5 or 6/6) will have a 2 : 1 ratio in the endosperm and are listed as "balanced". Offspring receiving a single extra EBN gene dose from the female parent will have two extra doses in the central cell and subsequent endosperm (i.e., 4/3, 5/4 or 6/5). The designation for this endosperm type is "♀ + 2". Similar notations may be derived for endosperms with other excess female or male dosages (i.e., 5/3 or 6/4 = ♀ + 4; 3/4 = ♂ + 1). Only the "balanced" type and "♀ + 2" type endosperms give rise to viable seeds under the model assumptions; the former produces normal type seeds and the latter small or miniature type seeds. All other EBN dosage types result in abortive seed.

Figure 4 lists $F_1 \times F_1$, $F_1 \times$ *S. commersonii* and $F_1 \times$ *S. chacoense* crosses and their viable seed to aborted seed expectations along with the observed ratios from the EBN diallel crosses. The pooled observations for $F_1 \times$ *S. commersonii* and $F_1 \times$ *S. chacoense* crosses approximate the predictions of the model, although discrepancies exist when viewing individual crosses. More seriously, the $F_1 \times F_1$ crosses exhibit a ratio of 2.5

Table 3. Chi-square values for EBN genetic crosses based on expected ratios (35:32:8) of seed set between sib-matings and species backcrosses. Note: F, X, EE and RR are 2x F_1 *S. commersonii* × *S. chacoense* hybrids; cmm = *S. commersonii;* chc = *S. chacoense*

Cross	Seeds per fruit		χ^2	*P*
	Observed	Expected		
F × F_1	21.0	19.8	1.384^{ns}	0.501
F × cmm	15.0	18.1		
F × chc	6.4	4.5		
X × F_1	63.7	65.1	0.231^{ns}	0.891
X × cmm	59.2	59.5		
X × chc	16.6	14.9		
EE × F_1	28.3	20.5	9.288**	0.010
EE × cmm	8.8	18.8		
EE × chc	6.9	4.7		
RR × F_1	27.5	34.9	3.018^{ns}	0.221
RR × cmm	38.5	31.9		
RR × chc	8.8	8.0		

viable seed : 1 aborted seed in the pooled data where a ratio of 35 viable seed : 29 aborted seed (1.2 : 1) is expected (Fig. 4, lower).

If the EBN model derived from backcross data is correct, the observed values for $F_1 \times F_1$ crosses are biased by a general underestimation of the number of aborted seed. This underestimation, along with seed abortion due to factors other than endosperm failure, undoubtedly biases the ratios seen in both backcrosses and sib-matings. This bias may be resolved by comparing the crosses on a seed set basis only. If the ratios of viable (v) to aborted (a) seed of the model are converted to ratios of viable seed to the total (t = aborted + viable) number of developing seed, the predictions of the model compare favorably to the original gross observations of seed set.

$F_1 \times F_1$	35/29 (v/a) expectation becomes 35/64 (v/t)
$F_1 \times$ cmm	1/1 (v/a) expectation becomes 32/64 (v/t)
$F_1 \times$ chc	1/7 (v/a) expectation becomes 8/64 (v/t)

In the original observations, seed set was seen to be similar in $F_1 \times F_1$ and $F_1 \times$ cmm crosses and significantly lower in $F_1 \times$ chc crosses. The converted model predicts relative proportions of 35/64 and 32/64 for the $F_1 \times F_1$ and $F_1 \times$ cmm crosses, respectively, and 8/64 for the $F_1 \times$ chc cross, thus showing good overall agreement with the observed seed set.

The proportionality between the three crosses in the model, 35 : 32 : 8, was used to generate expected values and to test the fit of the actual seed set data to the three locus EBN model (Table 3). Using only viable seed set data, the comparison allows for testing of this model without regard to errors in the estimation of the number of aborted seed.

For the clones observed the chi-square values were non-significant in three of the four cases (clones F, X and RR). Non-significant chi-square values support the three locus model. For clone EE, the chi-square was significant; however, observation of the seed set values for this clone revealed that the major discrepancy occurs in the cross EE × cmm. The other two crosses have reasonable values.

Discussion

In the wild, endosperm failure may serve as an isolation mechanism between species. *S. commersonii* and *S. chacoense* normally produce viable hybrids only when *S. commersonii* is functionally tetraploid. This condition is satisfied by somatic doubling or by the production of 2n gametes yielding 2x(1 EBN) = 24 gametes. A gamete of this kind, fertilized by a 1n = 1x (1 EBN) = 12 gamete from *S. chacoense* yields a 2n = 3x (2 EBN) = 36 hybrid associated with an endosperm having a 2 : 1 maternal to paternal EBN dosage regardless of direction of cross. Diploid hybrids are not expected to be formed under the expectations of the EBN system.

The diploid F_1 *S. commersonii* × *S. chacoense* hybrids found arose in both *S. commersonii* and *S. chacoense* cytoplasms and different possible explanations exist for their formation. The hybrids in an *S. commersonii* cytoplasm may have arisen through irregularities of the central cell, particularly proliferation or endomitosis of nuclei in the central cell allowing a 2 : 1 balance to occur upon fertilization, coupled with the fertilization of a normal egg cell. This is analogous to the indeterminate gametophyte system in maize (Kermicle 1971). That gene (*ig*), when carried by the female gametophyte, conditions irregularities in the division of the egg and/or central cell and permits abnormal fertilizations to occur.

In maize, the production of unexpected triploids in crosses of 2x *ig/ig* maize with 4x *Ig/Ig/Ig/Ig* maize have been observed (Kermicle 1971). This kind of fertilization is analogous to those that produced the 2x(1½ EBN) F_1 hybrids when *S. commersonii* was the female.

The seven F_1 diploids in *S. chacoense* cytoplasm most likely arose by multiple fertilization of the *S. chacoense* central cell and single fertilization of the *S. chacoense* egg by sperm nuclei from more than one *S. commersonii* pollen grain. A similar phenomenon has been suggested to occur in maize (Sarkar and Coe 1971; Johnston 1980) and is fully compatible with the EBN theory requiring a 2 : 1 balance in the endosperm.

The non-hybrid *S. chacoense*-like types arose only from crosses in which *S. chacoense* served as the female; thus, it is most probable that these plants were the result of pollen contamination by *S. chacoense* pollen. Yet, since a monoploid of *S. chacoense* was produced, it is possible that all of the diploids arose from abnormal fertilization events and from the spontaneous doubling of monoploid egg cells. The vigor of the plants produced, however, argues against this. Another, less likely, possibility is parthenogenetic seed development.

The non-hybrid *S. commersonii* types, like the *S. chacoense* types, may also have resulted from contamination. This is plausible because in these cases *S. commersonii* was the female parent. Central cell irregularities and female parthenogenesis, however, are also possible explanations. In maize, monoploid production has been observed in 2x × 2x crosses when only the central cell is fertilized by a sperm nucleus from a regular 1x male gamete, leaving the egg cell unfertilized (Sarkar and Coe 1966). In *S. commersonii*, the fertilization of a $2n = 2x(1\mathrm{EBN}) = 24$ megaspore mother cell and $4n = 4x(2\mathrm{EBN}) = 48$ central cell could produce the 2x *S. commersonii* types observed upon pollination by *S. chacoense*. Parthenogenesis is also a stronger possibility in *S. commersonii* than in *S. chacoense*. Some clones of *S. commersonii* have a very strong tendency to set parthenocarpic fruit, and this may serve as an added stimulus to occasional parthenogenetic seed development.

The resolution of the EBN genetic system is largely based on inferences regarding gene action in the production of different sized seed drawn from maize research. That altered seed size may be associated with excess female endosperm EBN gene dosage in potato (as it is for excess female genome dosage in maize) is a crucial concept in clarifying the EBN genetic system. Yet, while this study draws important inferences from maize, it does not rely on the seed size concept to evaluate the successful dosage combinations; ultimately the analysis rests upon seed set data alone. The effects observed on a genomic basis in maize are mirrored on a genic basis in potatoes. In our study of hybrids between tuber-bearing Solanums, different genomes allowed the fine structure of the system to be observed on a genic basis in a system which follows all of the restrictions observed in the genomic system.

Within the three locus model the EBN genes appear quantitative in nature due to their additivity; however, the system as a whole exhibits a unique balance of quantitative and qualitative action because it is a system that is more ratio dependent than dosage dependent. The genes are quantitative, but as a result of their operation within the constraints of a maternal to paternal balance in the endosperm, their effects are highly qualitative. This kind of gene action is similar to that associated with "threshold" characteristics, (i.e., quantitative genes producing qualitative effects). The difference in this case is that the requirement for an actual balance in the endosperm produces an effect more like a "window", in which a certain level of gene action on both the female and male side must not only be achieved, but a certain level must not be exceeded. Only a 2 : 1 balance of EBN values in the endosperm produces fully normal development. In $F_1 \times F_1$ matings, this balance may be derived through any of four EBN gene dosage combinations (6/3, 8/4, 10/5 or 12/6). A single extra maternal gene dose produces seeds of reduced size. Two extra maternal doses result in seed abortion. The addition of any extra paternal EBN gene dose causing a deviation from the 2 : 1 ratio also results in seed abortion.

Another level of gene regulation, imprinting, must also be addressed in discussing endosperm developmental control. Imprinting refers to the effect of parental source in the differential function of genes (Crouse 1960). Imprinting has been documented in maize (Kermicle 1975) and has been shown to occur in the development of maize endosperm (Lin 1982). In the EBN system imprinting also seems to occur, and is demonstrated by similar total gene dosages producing different effects if their male and female components are different. It is likely that the maternal and paternal EBN genes are identical since the genes have similar segregation ratios regardless of whether a plant is used as a male or a female. Yet, functionally, the maternal and paternal genes behave differently and require a specific balance if their interaction is to produce normal seed. Hence, in the three locus model both 8/4 and 6/6 endosperm combinations have the same total gene dosage, but only the 8/4 ratio will produce viable seed. The 6/6 combination is totally inviable. The elucidation of the mechanism of imprinting is another step, above and beyond Mendelian genetics, that will be necessary to fully understand this system.

This attempt to determine the genetic basis of EBN is a start to understanding the basis of inter- and intraspecific endosperm function in potatoes. On an evolutionary basis, three homoeologous loci suggest that single EBN gene differences may exist in wild *Solanum* species and that these differences may serve as speciating mechanisms between divergent populations. Such single gene differences may be detectable in reciprocal interspecific crosses through diminished seed size and unilateral crossability. These differences, if found, may require a reappraisal of some aspects of speciation in

Solanum. Additionally, the fact that the EBN differences between *S. commersonii* and *S. chacoense* exist as alterations in the "strength" of genes at three loci suggests that speciation via EBN in *Solanum* may operate in a far more complex manner than just single gene changes. It carries implications of a concerted duplication of genes without a concomitant doubling of genomes, or at the very least a difference in the level of regulation of these genes. The elucidation of this system will allow more detailed studies of endosperm function and development to be done. Moreover, because endosperm development is important in the production of many crop plants, this and other studies of the genetic control of endosperm development may have far reaching importance in the utilization of both wild and genetically altered germplasm in many crop species.

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