Theor Appl Genet (1990) 80:161–166

Allelism of Endosperm Balance Number (EBN) in Mexican tuber-bearing *Solanum* species *

J.B. Bamberg [1] and R.E. Hanneman, Jr. [2]

[1] USDA, ARS, Inter-Regional Potato Introduction Station, Sturgeon Bay, WI 54235, USA
[2] USDA, ARS, Department of Horticulture, University of Wisconsin, Madison, WI 53706, USA

Received November 17, 1989; Accepted April 3, 1990
Communicated A.R. Hallauer

Summary. Endosperm Balance Number (EBN) is a genetic, dose-dependent crossability system functioning in tuber-bearing *Solanum* species. Each species has been assigned 1EBN, 2EBN, or 4EBN. Species thus designated cross only within their EBN group. Doubling of chromosome number also doubles the EBN. The ploidy: EBN ratio is not consistent among *Solanum* species. Some diploids are 2EBN while others are 1EBN. Some tetraploids are 4EBN while others are 2EBN. Species from Mexico typically have EBNs that are one-half of their ploidy [e.g. 2x(1EBN), 4x(2EBN)]. Hybrids of Mexican species and a South American species, 2x(1EBN) *S. commersonii*, and its 4x(2EBN) colchicine derivative were made and crossed to 1, 2, and 4EBN standard testers to determine the relationship of the genetic organization of EBN among and within these species. Diploid hybrids crossed only to 1EBN standard testers. Hybrids of 4x(2EBN) *S. commersonii* and 4x(2EBN) Mexican species crossed almost exclusively to 2EBN standard testers. Complex tetraploid hybrids involving *S. commersonii*, *S. stenophyllidium* (a Mexican diploid), and Mexican tetraploids of series Longipedicellata also crossed only to 2EBN testers. The apparent lack of recombination and segregation for EBN in these hybrids indicates that the genomes of the Mexican diploid and tetraploid species carry EBN in a way genetically similar to that of the South American species *S. commersonii*.

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

* Cooperative investigation of the U.S. Department of Agriculture, Agricultural Research Service and the Wisconsin Experiment Station. Supported in part by the USDA/Cooperative States Research Service Competitive Grant No. 83-CRCR-1-1253

Introduction

Johnston et al. (1980) developed a theory which holds that crossability in tuber-bearing Solanums is governed largely by a genetic, dose-dependent phenomenon which they termed Endosperm Balance Number (EBN). EBN is similar to crossability theories which require certain maternal to paternal ploidy contributions in the various seed tissues, except that all species do not have the same EBN-to-ploidy ratio. EBN values for the various potato species have been determined empirically by analyzing the results of crosses between standard testers and the species in question (Hanneman 1984).

The normal ratio of maternal-to-paternal genomes in the endosperm is 2:1 for successful crosses between parents of the same ploidy. As stated by Ramanna and Hermsen (1971), "The basic thought . . . is the experience that in several cases equal ploidy levels of parents promote crossability." There are significant exceptions to the rule of a required 2 maternal:1 paternal endosperm ploidy ratio in potatoes, however. For example, Mexican tetraploid species of series Longipedicellata (*S. fendleri*, *S. hjertingii*, *S. papita*, *S. polytrichon*, and *S. stoloniferum*) cross readily to many of the South American diploid species (Johnston and Hanneman 1980; Matsubayashi 1982; Ramanna and Abdalla 1970; Toxopeus 1960), where the endosperm ploidy ratio is 4:1 or 2:2 in reciprocal crosses. Crossing is very difficult between these tetraploid species and 4EBN standards (such as 4x *S. tuberosum*), where this ratio is 4:2=2:1 (Hanneman 1984; Ross 1966). If series Longipedicellata (LON) species are first colchicine doubled to the 8x level, crosses to 4x *S. tuberosum* Group Tuberosum are successful despite an 8:2 or 4:4 maternal:paternal endosperm ploidy ratio (Lamm 1953; Swaminathan 1951). Most diploid species from Mexico do not cross readily with diploids from

Table 1. Interspecific hybrids tested for EBN and their pedigrees

Hybrid[a]	No. clones	Pedigree[b]
Diploids		
cmm × bst	4	PI 243503 × (PI 283095 + 320265 + WRF 1271)
cmm × cph	9	unknown
cmm × pnt	2	PI 243503 × PI 186554
cmm × sph	5	PI 458319 × PI 255529
Tetraploids (4x cmm × LON) F_2		
4x cmm × fen	8	PI 243503 × (PI 275156 + 275157 + 275158 + 275162 + 275163 + 283101)
4x cmm × hjt	1	PI 243503 × (PI 186559 + 251063 + 251065 + 275174 + 283103)
4x cmm × pta	5	PI 243503 × (PI 265895 + 275227 + 275740 + 275741 + 283143)
4x cmm × sto	5	PI 243503 × (PI 160224 + 186544 + 275244 + 310964 + 338617)
(LON × 4x cmm) F_1		
fen × 4x cmm	many[c]	PI 275158 × PI 243503
hjt × 4x cmm	many	PI (251063 + 251065) × PI 243503
sto × 4x cmm	10	PI (310964 + 338617) × PI 243503
(4x cmm × LON) F_2		
4x cmm × fen	many	selfs of F_1s above
4x cmm × hjt	many	selfs of F_1s above
4x cmm × pta	many	selfs of F_1s above
Complex hybrids		
4x (cmm × sph) × fen	many	4x (PI 458319 × 255529) × PI 275156
4x (cmm × sph) × hjt	many	4x (PI 458319 × 255529) × PI 283103
4x (cmm × sph) × sto	many	4x (PI 458319 × 255529) × PI 186544

[a] Abbreviations: bst = *S. brachistotrichum*, cph = *S. cardiophyllum*, cmm/4x cmm = *S. commersonii* (diploid/tetraploid), fen = *S. fendleri*, hjt = *S. hjertingii*, LON = series Longipedicellata species, pta = *S. papita*, pnt = *S. pinnatisectum*, sph = *S. stenophyllidium*, sto = *S. stoloniferum*. All of these species except *S. commersonii* are Mexican species

[b] "(___ + ___ + ___)" indicates bulked pollen of the listed accessions

[c] "many" indicates that at least 50 clones were tested (individually or in bulk)

South America where the requirement of a 2:1 endosperm ploidy ratio is met (Amaya and Matsubayashi 1981; Dionne 1963; Hermsen and Taylor 1979; Lee and Cooper 1958; Toxopeus 1960). However, when the Mexican diploid *S. cardiophyllum* was colchicine doubled to the tetraploid level and crossed with South American diploids, these 2x × 4x or 4x × 2x crosses produced relatively abundant triploids though the endosperm ploidy ratios were not 2:1 (Johnston and Hanneman 1980).

These phenomena can be explained if crossability is conditioned by the "effective ploidy" (i.e., EBN) and not the actual ploidy of the parents (Johnson et al. 1980). This concept is reflected in the assignment of EBN, since the EBN : ploidy ratio is not consistent among all species. If species of the same EBN are mated, however, the EBN ratio in the endosperm will be 2:1, regardless of the ploidy of the parents or the direction of the cross.

Mexican diploid and tetraploid species represent a relatively untapped reservoir of germplasm of potential breeding value (Hermsen 1980; Toxopeus 1964), and are the most primitive of the potatoes (Hawkes 1958). Their poor crossability to cultivated forms of South American origin can explained by a difference in ploidy : EBN ratio, which thwarts intraploidy crosses and results in odd-ploidied hybrids.

This research was undertaken to investigate the genetic organization of EBN in Mexican potato species by attempting to detect recombination for EBN in hybrids involving them and the South American diploid, 2x(1EBN) *S. commersonii*.

Materials and methods

Production of hybrids

Diploids. Reciprocal crosses were made to obtain F_1 and F_2 hybrids of South American 2x(1EBN) *S. commersonii* and 2x(1EBN) Mexican species. Nine *S. commersonii* × *S. cardiophyllum* diploid F_1 hybrids were also obtained from M. K. Ehlenfeldt (then a graduate student in our program).

Tetraploids. Crosses were made to obtain F_1 hybrids of 4x(2EBN) (colchicine-doubled) *S. commersonii* × 4x(2EBN) Mexican species of series Longipedicellata (LON) and reciprocals. F_1 hybrids in which 4x *S. commersonii* was the male parent were selfed to obtain F_2 clones. A colchine-doubled diploid F_1 hybrid of 2x (1EBN) *S. commersonii* × 2x (1EBN) *S. stenophyllidium* was produced and evaluated with EBN testers. Test crosses of this clone with series Longipedicellata species resulted in com-

plex (3-way) tetraploid hybrids, which were also evaluated through EBN test crosses. Table 1 lists all of the hybrids tested and their pedigrees.

EBN testing of hybrids

The evaluation of hybrids for determination of their EBN involved performing "control" crosses (crosses to testers with the same EBN as that of the hybrids' parents) and "test" crosses (crosses to testers with EBN differing from that of the hybrids' parents).

Certain species and accessions have been identified as being EBN standards by virtue of their thoroughly established EBN value, fertility, and lack of reproductive abnormalities, which would confound the interpretation of EBN. These standards were used as much as possible for EBN test parents and reciprocal test crosses were usually made. Crossing experience has indicated that these species are completely consistent in crossing behavior with respect to EBN, and therefore bulks of unrecorded PI numbers within these species were sometimes used. Table 2 lists the EBN testers used to evaluate the hybrids. The particular tester(s) used within an EBN group was governed by their availability when the hybrids were ready for crossing.

Styles were collected from test crosses no sooner than 48 h post-pollination and fixed in FAA, a 1:7:1 solution of formalin, 95% ethanol, and glacial acetic acid, respectively. Lack of stylar inhibition of fertilization was confirmed by observing pollen tube growth with fluorescence microscopy (Martin 1958). After 24 h of fixation, styles were rinsed with tap water, treated for 4 h with 8 *N* NaOH, rinsed two times with tap water, and stained for at least 4 h in aniline blue. Styles were flattened under a cover glass and observed with a fluorescent microscope at 365 nm. Pollinations that apparently did not result in fertilization were not included in the tabulated results.

The number of pollinations, fruit, and plump seeds were tabulated. Plump seeds, resulting from crosses in which the EBN tester had a different EBN than that of the hybrids' parents, were grown and visually assessed for signs of true hybridity, and the somatic chromosome number was determined by counting the chromosomes in root-tip cells. Obvious cases of inadvertent selfing or other mistakes were removed from the tabulations. Root tips were treated for 4 h in 8-hydroxyquinoline (0.29 g/l), fixed in 3:1 (ethanol:acetic acid), hydrolyzed at 60 °C in 1 *N* HCl for 10 min, and rinsed with tap water. Root tips were then mascerated on a microscope slide in acetocarmine stain and squashed.

Techniques used

Pollination and seed processing. Most of the production and testing of hybrids took place at the University of Wisconsin Agricultural Research Station, Sturgeon Bay, WI. Pollen was collected into empty gelatin capsules by "buzzing" the anther cones with a hand-held, battery-powered doorbell vibrator. Capsules containing pollen were stored at about 6 °C over anhydrous $CaCl_2$ in closed plastic containers. Pollen was usually collected fresh before each day's crossing.

The decapitation technique of Peloquin and Hougas (1959) was employed. Inflorescences were collected by severing the plant about 20–30 cm below the cyme, trimming all axillary buds and all but two opposing leaves. All opened buds and all buds judged too small to be receptive (3 or more days from opening) were removed. Bottles were maintained in a shaded, air-conditioned greenhouse at about 20°–25 °C throughout pollination and fruiting. Buds were usually emasculated immediately after collection of stems, and pollination was usually done the day of stem collection. Pollination was accomplished by dipping the stigma into pollen contained in a gelatin capsule.

Table 2. 1, 2, and 4 EBN testers used to determine the Endosperm Balance number of the hybrids

Species[a]	Accession/Cultivar
1EBN testers	
cph	PI 279272, 283062, 347759, bulk[b]
cmm	PI 243503, 320269, 458319
mcc	PI 365344
pnt	PI 275235
2EBN testers	
chc	PI 209411, 217451, 230582, 414144, 414153, bulk
LON	fen PI 275156, hjt PI 283103, sto PI 186544
phu	bulk
stn	bulk
4EBN testers	
adg	PI 347773, bulk
chc (4x)	PI 230582
tbr	cultivars Butte, Fortuna, Hudson, Katahdin, Norland, Superior

[a] Abbreviations: adg = *S. tuberosum* Group Andigena, cph = *S. cardiophyllum*, cmm = *S. commersonii*, chc = *S. chacoense*, fen = *S. fendleri*, hjt = *S. hjertingii*, LON = series Longipedicellata, mcc = *S. mochicense*, phu = *S. tuberosum* Group Phureja, pnt = *S. pinnatisectum*, stn = *S. tuberosum* Group Stenotomum, sto = *S. stoloniferum*, tbr = *S. tuberosum* Group Tuberosum

[b] Bulk = bulk of unrecorded PI numbers

Berries were collected from the cut stems at least 4 weeks after pollination, and were allowed to mature for 1–2 months prior to seed extraction. Seeds were counted to determine the relative success of each type of cross. Those "seeds" that were flat (underdeveloped) and chaffy and judged to be unable to germinate were not included in tabulated results.

Results

EBN testing of hybrids

Diploids. Diploid F_1 hybrids between *S. commersonii* and Mexican diploid species crossed only to 1EBN testers. One thousand two hundred twelve pollinations were made yielding 286 fruit and 827 seeds. Numerous pollinations with 2EBN testers (2,047) and 4EBN testers (411) failed to yield a single plump seed (Table 3a).

Tetraploids. Nineteen tetraploid F_1 hybrids (4x *S. commersonii* × series Longipedicellata species) were crossed with EBN standard testers (Table 3b). Few seeds resulted, aside from those of the 2EBN (control) tester pollinations. Three triploids resulted from 12 fruit from 419 pollinations with 1EBN testers; 2,093 seeds resulted from 113 fruit from 685 pollinations with 2EBN testers, and no seeds resulted from 7 fruit from 884 pollinations with 4EBN testers.

Reciprocal F_1 (series Longipedicellata species × 4x *S. commersonii*) hybrids were also crossed to EBN standard testers (Table 3c). Like their reciprocals, these hybrids crossed best to 2EBN (control) testers. Seven

Table 3. Results of crosses between hybrids and EBN testers

EBN testcross	Poll.	Fruit	Plump seeds	No. of progeny (ploidy)[a]
(a) Diploid F_1 hybrids: 2x(1EBN) *S. commersonii* × 2x(1EBN) Mexican diploid species				
× 1EBN and recip. (control)	1,212	286	827	–
× 2EBN and recip.	2,047	166	0	0
× 4EBN and recip.	411	13	0	0
(b) (4x *S. commersonii* × series Longipedicellata species) tetraploid F_1 hybrids				
× 1EBN and recip.	419	12	3	3 (3x)
× 2EBN and recip. (control)	685	113	2,093	–
× 4EBN and recip.	884	7	0	0
(c) (Series Longipedicellata species × 4x *S. commersonii*) tetraploid F_1 hybrids				
× 1EBN and recip.	258	7	0	0
× 2EBN and recip. (control)	403	49	458	–
× 4EBN and recip.	430	68	61	0
(d) Selfs of (4x *S. commersonii* × series Longipedicellata species) tetraploid F_1 hybrids				
× 1EBN and recip.	1,698	26	1	0
× 2EBN and recip. (control)	698	193	444	–
× 4EBN and recip.	3,090	659	224	66 (6x)
(e) A doubled diploid hybrid: 4x (*S. commersonii* × *S. stenophyllidium*)				
× 1EBN and recip.	146	56	0	0
× 2EBN and recip. (control)	60	17	785[b]	–
× 4EBN and recip.	188	30	0	0
(f) Complex (3-way) tetraploid hybrids: 4x (*S. commersonii* × *S. stenophyllidium*) × series Longipedicellata species				
× 1EBN and recip.	367	57	0	0
× 2EBN and recip. (control)	739	55	323	–
× 4EBN and recip.	812	107	7	7 (6x)

[a] Control cross progeny: these plants were assumed to result from the union of n gametes from each parent. Germination percentages were not precisely measured but a high percentage of each type of control seeds germinated

[b] These became the complex hybrids tested in part f

parthenocarpic fruit resulted from 258 crosses with 1EBN testers, and 68 fruit containing 61 inviable seeds resulted from 430 crosses with 4EBN testers. In contrast, 458 seeds with a high percent germination resulted from 403 pollinations with 2EBN testers.

When selfs of F_1 hybrids between 4x(2EBN) *S. commersonii* and 4x(2EBN) series Longipedicellata species were crossed with 1EBN testers, only one seed that failed to germinate was produced from 26 fruit resulting from 1,698 pollinations. Crosses with 2EBN (control) testers resulted in 444 seeds in 193 fruit from 698 pollinations. Two hundred twenty-four seeds yielded 66 hexaploid seedlings from 659 fruit resulting from 3,090 pollinations with 4EBN testers (Table 3d).

A colchicine-induced tetraploid clone of 2x(1EBN) *S. commersonii* × 2x(1EBN) *S. stenophyllidium* crossed successfully only with 2EBN (control) testers, producing over 13 seeds per pollination and 46 seeds per fruit (Table 3e). Only parthenocarpic fruit resulted from crosses with 1EBN or 4EBN testers although 146 and 188 pollinations were done, respectively.

Complex (3-way) hybrids of 4x (*S. commersonii* × *S. stenophyllidium*) × series Longipedicellata species like other tetraploid hybrids, crossed best to 2EBN (control) testers. Seven hundred thirty-nine pollinations yielded 55 fruit and 323 seeds. In contrast, no seeds resulted from 367 pollinations with 1EBN testers. Seven seeds were obtained from 107 fruit resulting from 812 pollinations with 4EBN testers, but each of these produced hexaploid seedlings (Table 3f).

Discussion

There are several possible approaches by which germplasm of species in the 2x(1EBN) and 4x(2EBN) groups might be integrated with that of 4EBN cultivars. The most obvious is to raise these species' EBN via colchicine doubling or 2n gametes. These methods have proved difficult for Mexican species, however. Mexican 2x(1EBN) species used as pollen parents do not cross to 2x(2EBN) *S. tuberosum* Group Tuberosum haploids via 2n gametes (Novy 1988), nor do 2n gametes readily facilitate crossing of 4x(2EBN) species with 4x(4EBN) *S. tuberosum* Group Tuberosum (Hanneman 1984; Ross 1966). Colchicine doubling of Mexican 2x(1EBN) species to te-

traploids does not typically promote easy crossing to 2EBN testers (Bamberg 1988). Swaminathan (1951) discovered, however, that 4x(2EBN) Mexican species, when colchicine doubled to octoploids, produced relatively abundant hexaploid hybrids with 4x(4EBN) *S. tuberosum* Group Tuberosum.

Triploid hybrids from 4x(2EBN) × 2x(2EBN) crosses selected for 2n gamete production are also a feasible method by which to make 4x (2EBN) germplasm crossable to 4EBN-cultivated materials (Brown 1988). Similarly, a triploid derived from a colchicine-doubled 2x(1EBN) species has been used to transfer 1EBN germplasm to 4EBN cultivars via 2n gametes (Ehlenfeldt and Hanneman 1984).

Another method of overcoming EBN crossing barriers in these species is to do great numbers of pollinations to obtain anomalous hybrids. Ehlenfeldt and Hanneman (1988) overcame the EBN barrier between the species 2x(1EBN) *S. commersonii* and 2x(2EBN) *S. chacoense* by this method. F_1 hybrids backcrossed with relative ease to the 2EBN parent, indicating that *S. commersonii* germplasm had been made accessible to the 2EBN group.

In each of the schemes cited above, the ultimate goal is to separate the desirable traits of the Mexican species from their undesirable EBN. To do so, one must produce hybrids segregating for both the desirable traits and EBN. A simple alternative for accomplishing this is theoretically possible through intraploidy, intra-EBN hybrids if all species (or populations) with the same EBN value are not genetically identical for EBN. The most simple illustration of this would involve hybrids between two diploid 2EBN species that carry EBN differently at two loci. The first might have genotype *11 00* (homozygous 1EBN at the first locus and homozygous 0EBN at the second locus), and the second with genotype *00 11* (homozygous 0EBN at the first locus and homozygous 1EBN at the second locus). F_1 hybrids would be *10 01*, and would produce some *11* = 2EBN gametes, which would be successful in crosses with a 4EBN species. This transgressive recombination for EBN would allow hybrids between two 1EBN species to produce n gametes that are 1EBN (required for crossing to 2EBN stocks), or hybrids between two 2EBN species to produce n gametes that are 2EBN (required for crossing to 4EBN stocks, as in the example above). The crossability of such intra-EBN hybrids would indicate not only their usefulness in a breeding scheme, but would also provide basic information with respect to the allelism of EBN in the contributing species.

Although there is no certain way to predict which species might carry EBN differently, it is reasonable to assume that species that are more evolutionarily distinct would be most likely to exhibit this type of genetic divergence. Thus, the South American species 2x(1EBN) *S. commersonii* and its colchicine-induced 4x(2EBN) form would seem to be good candidates for hybridization with 2x (1EBN) and 4x (2EBN) Mexican species, respectively. Similarly, Mexican species appear to be a relatively primitive and diverse group (Hawkes 1958) and, as such, the EBN carriage among these species might also vary. The Mexican species of series Longipedicellata are disomic tetraploids and therefore might carry EBN differently on their genomes.

Hybrids of the type described above were synthesized with relative ease since they were derived from intra-EBN crosses. Because these were also intraploidy crosses, the F_1s had even ploidy numbers and sufficient fertility to allow the production of advanced, complex hybrids. EBN test crosses revealed very little evidence of segregation for EBN in the gametes of any of these hybrids. Hybrids between 2x(1EBN) *S. commersonii* and 2x (1EBN) Mexican species crossed only to 1EBN testers (Table 3a).

Hybrids between 4x *S. commersonii* and series Longipedicellata species behaved analogously, crossing only as 2EBN. Three seeds did result from crosses to 1EBN testers (Table 3b). These seeds were triploids from a 4x(2EBN) × 2x(1EBN) cross, so apparently resulted from the union of two n gametes. If these seeds were promoted by recombination for EBN in the tetraploid hybrid parent, however, it is difficult to explain why such recombination was not evident in the reciprocal tetraploid hybrids (Table 3c) or selfs of F_1 hybrids (Table 3d). The reciprocal crosses produced no seeds when crossed with 1EBN testers and only inviable seeds when crossed with 4EBN testers (Table 3c).

In contrast to diploids, in some heterozygous tetraploids, combinations of alleles may occur in the gametes of selfs of F_1s that do not occur in gametes of the F_1s themselves (disregarding double reduction). Thus, any true recombination made evident in the gametes of F_1 hybrids would be expected to be even more evident in certain self progeny of the F_1s. Unfortunately, selfs of F_1 tetraploid hybrids crossed exclusively as 2EBN (Table 3d), with only one inviable seed resulting from numerous 1EBN test crosses. Sixty-six hexaploid progeny resulted from crosses with 4EBN testers. These presumably resulted from the functioning of 2n eggs and therefore do not indicate recombination for EBN.

The crossability of a colchicine-doubled diploid hybrid was completely analogous to that of the diploid hybrids. Diploids crossed only to 1EBN testers (Table 3a), and this tetraploid crossed only to 2EBN testers, confirming the conclusion that no recombination for EBN occurred between the genomes of the parental species (Table 3e).

The remaining comparison that could be made between the EBN carriage in these three groups of species was between Mexican diploids and Mexican tetraploids. Complex hybrids involving *S. commersonii* (South Amer-

ican diploid), *S. stenophyllidium* (Mexican diploid), and series Longipedicellata species (Mexican tetraploid) served this purpose. These hybrids carried parts of each of the four genomes of the contributing parents. EBN test crosses indicated that these hybrids crossed exclusively as 2EBN (Table 3f). No seeds were produced with 1EBN testers. Like *S. commersonii* × series Longipedicellata F_1 hybrid selfs (Table 3d), these tetraploids produced hexaploid offspring when crossed with 4EBN testers. The fact that seedlings produced from matings of 4x hybrids with 4EBN testers were usually hexaploid indicates that 2n eggs, and not recombination for EBN, had satisfied the EBN requirement for seed production.

The results of EBN test crosses did not reveal recombination of EBN genes in the gametes of hybrids of 2x(1EBN) *S. commersonii,* 2x(1EBN) Mexican species, or disomic 4x(2EBN) series Longipedicellata (Mexican) species. Although it is possible that recombination was thwarted by a suppression of chromosome pairing and crossing-over, the most obvious conclusion is that similar or identical EBN alleles are fixed at the same loci on each of the constituent chromosome sets of the parental species. This would suggest that EBN genes have been conserved in these diverse species such that crossability to higher EBN may only be accomplished via 2n gametes (raising the ploidy of the gamete). Although crossability to species of higher EBN via 2n gametes was not the focus of this study, the success of these crosses nonetheless indicates a practical avenue for the incorporation of these species' germplasm into cultivated forms.

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