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Allelism of endosperm balance number (EBN) in *Solanum acaule* Bitt. and other wild potato species

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Abstract The inheritance of endosperm balance number (EBN), a genetic, dose-dependent crossability system functioning in tuber-bearing *Solanum* (potato) species, was investigated for certain wild potato species having an EBN equal to one half of their ploidy. The EBN of *Solanum acaule*, a disomic 4(2EBN) South American species, was investigated by producing F₁ and F₂ hybrids with artificial 4x(2EBN) *S. commersonii*. This allowed assessment of recombination among the two genomes of disomic *S. acaule* and that of *S. commersonii*. When crossability of the hybrids with 1EBN, 2EBN and 4EBN standards was tested, no variation for EBN was detected. The apparent lack of recombination and segregation for EBN in these hybrids indicates that the genomes of *S. acaule* and *S. commersonii* carry EBN in a genetically-similar way. Combined with previous reports, these data indicate that the inheritance of EBN is similar in widely-separated taxa from South America and Mexico.

Key words Potato · *Solanum* · Germplasm · Crossability

Introduction

Johnston et al. (1980) developed a theory which states that crossability in tuber-bearing *Solanums* is governed largely by a genetic, dose-dependent phenomenon which they termed endosperm balance number (EBN). EBN values for the various potato species have been determined empirically by analyzing the success of crosses between EBN

standard testers and the species in question (Hanneman 1984).

A few potato species have been assigned an EBN equal to one-half of their ploidy. In their review of EBN and its evolutionary implications, Hawkes and Jackson (1992) indicate that these species are more primitive than the forms from which cultivated *S. tuberosum* originated. Thus, they may contain the extremes of genetic diversity available for conventional breeding. However, because they typically will not cross readily with *S. tuberosum* breeding stocks of the corresponding ploidy, enhancement has been problematic.

Most of these species occur in Mexico and Central America, but a few, like *S. commersonii* and *S. acaule*, are from South America (Hanneman and Bamberg 1986; Bamberg and Martin 1993). The degree of variation in the inheritance of EBN among and within their genomes has implications for their evolutionary relationships and the nature of their polyploidy. Interspecific combinations might also release recombinant types with higher EBNs, which would facilitate breeding.

Bamberg and Hanneman (1990) analyzed the crossing behavior of numerous combinations of diploid and tetraploid Mexican species and *S. commersonii*, all of which have an EBN equal to one-half of their ploidy. Since there was essentially no evidence of transgressive recombination, they concluded that EBN inheritance is allelic in all the constituent genomes tested. This study included species of the Series Longipedicellata, Pinnatisecta, and Commersoniana. Investigation of the inheritance of EBN in species where EBN equals one half of the ploidy is expanded here to include *Solanum acaule* ssp. *acaule* of the Series Acaulia.

While being distinct in appearance and origin, the South American *S. acaule* appears to be reproductively similar to the species of the Mexican Series Longipedicellata. All of these tetraploid species cross easily with most diploid species resulting in sterile triploids, while having a strong direct crossability barrier with 4x cultivated *S. tuberosum* (Rammana and Abdalla 1970; Vavilova 1973; Estrada 1980; Hanneman 1984). They have relatively-few func-

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tional 2n gametes (Swaminathan 1951; Hanneman 1984; Watanabe and Peloquin 1991; Watanabe et al. 1992), perhaps due to their high self fertility (Watanabe and Peloquin 1991). Past studies suggest that they are disomic polyploids (Mackey 1970; Everhardt and Rowe 1974; Hawkes 1979; Matsubayashi 1982, 1991; Singsit and Hanneman 1987).

S. acaule has many traits to contribute to potato breeding (Hanneman and Bamberg 1986), and various schemes have been proposed to overcome the EBN barrier (Estrada 1980; Brown and Adiwilaga 1990; Camadro and Espinillo 1990; Watanabe et al. 1992). The purpose of this study was to compare the inheritance of the EBN crossability system in *S. acaule* with that of *S. commersonii* in order to provide further insights into the nature of EBN and its potential impact on breeding schemes.

Materials and methods

The *Solanum* species germplasm used in this research was obtained from the Inter-Regional Potato Introduction Station, NRSP-6, Sturgeon Bay, Wisconsin.

Production of hybrids

A family of tetraploid *S. commersonii* derived from a colchicine-doubled individual of PI 243503 was used as the female parent (4x-cmm). This was judged to be an acceptable representation, since EBN appears to be invariable within species at a given ploidy. Four accessions of *S. acaule* were used as pollen parents to make 17 F₁ hybrids. One of these F₁ hybrids was also selfed to produce an F₂ family. Table 1a provides detailed identification of the hybrids tested for EBN.

EBN testing of the 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 stocks 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. Table 1b lists materials used as EBN testers.

Evidence of fertilization

Hybrids were intermated and selfed to establish their potential for seed production. EBN is a post-fertilization phenomenon, so pre-fertilization barriers must be eliminated from the data considered. Thus, pollen tube-growth was observed by fluorescent microscopy (Martin 1958). Styles were collected from test crosses no sooner than 48 h post-pollination. After at least 24 h of fixation in FAA, styles were rinsed in tap water, treated for 4 h in 8N NaOH, rinsed twice in tap water, stained for at least 4 h in aniline blue, flattened between slide and cover slip and observed at 365 nm.

Pollination and seed processing

Production of the hybrids and their testing took place in the field and greenhouse at the University of Wisconsin Peninsular Agricultural Research Station, near Sturgeon Bay. Pollen was collected in empty gelatin capsules by "buzzing" the anther cones with a hand-held, battery-powered vibrator. Capsules containing pollen were stored at

Table 1 Interspecific hybrids tested for EBN, and EBN standards used

a. Hybrids tested	No. individuals
F ₁ hybrids	
4x <i>S. commersonii</i> PI 243503 ^a × <i>S. acaule</i> PI 175395	5
4x <i>S. commersonii</i> PI 243503 ^a × <i>S. acaule</i> PI 195160	2
4x <i>S. commersonii</i> PI 243503 ^a × <i>S. acaule</i> PI 210029	7
4x <i>S. commersonii</i> PI 243503 ^a × <i>S. acaule</i> PI 210033	3
F ₂ hybrids (from a single selfed F ₁)	
4x <i>S. commersonii</i> PI 243503 ^a × <i>S. acaule</i> PI 210029	50
b. EBN standards used	Identifiers
1EBN	
cph = <i>S. cardiophyllum</i> bulk	PI 184762, 186548, 275212, 275213, 275214, 283062; WRF 276, 1276, 1277, 2436
cmm = <i>S. commersonii</i> bulk	PI 458317, 458319, 472833, 472834, 472835, 472836, 472837, 472838, 473408, 473410, 473411
2 EBN	
acl = <i>S. acaule</i> bulk	PI 472646, 310970, 435071, 472648, 246504
chc = <i>S. chacoense</i> bulk	PI 472823, 498318, 414143; WRF 317, 1275
4x-cmm = 4x <i>S. commersonii</i>	PI 243503 ^a
4EBN	
tbr = <i>S. tuberosum</i>	PI 161350, 161695, 245940, 246514, 280926 (ssp. <i>andigena</i>), cv Katahdin (ssp. <i>tuberosum</i>)

^a Population derived from a single chromosome-doubled individual

about 6 °C over anhydrous CaCl₂ in air-tight plastic containers. Pollen was usually collected fresh on the day of pollination. The decapitation technique of Peloquin and Hougas (1959) was used. Inflorescences were collected by severing the top 20–30 cm of the plant, trimming all axillary buds and all but two opposing leaves, and placing the stems in glass bottles filled with tap water. All opened buds and buds too small to be receptive were removed. Bottles were maintained in an air-conditioned greenhouse at 20–25 °C during emasculation, pollination, and fruiting. Pollination was done by dipping the stigma into the pollen in the gelatin capsules. Berries were collected no sooner than 4 weeks post-pollination and allowed to mature for 1–2 months in bags at room temperature before seeds were extracted.

The number of pollinations, fruit, and plump seeds were recorded. Plump seeds, which were potential evidence of transgressive EBN recombination, were planted and the resulting plants were visually judged for true hybridity. Root-tip chromosome counts were made to determine ploidy.

Table 2 Results of crosses between (4x *S. commersonii* × *S. acaule*) hybrids and EBN standard testers, and crosses among hybrids

Cross ^a	Poll. ^b (fert.)	Fruit	Plump seeds	Progeny ^c (ploidy)
F₁ hybrid test crosses				
___ × 1EBN cph	307 (+)	98	4	1 (4x)
1EBN cmm × ___	1,013 (+)	419	18	12 (4x)
___ × 2EBN chc (control)	317 (+)	206	4,502	Many
___ × 2EBN acl (control)	206 (+)	89	2,259	Many
___ × 2EBN 4x-cmm (control)	64 (+)	31	358	Many
2EBN chc × ___ (control)	1,012 (-)	58	33	-
___ × 4EBN tbr	328 (+)	177	200	66 (6x)
4EBN tbr × ___	1,211 (-)	113	45	23 (6x)
F₂ hybrid test crosses				
___ × 1EBN cph	163 (+)	74	14	9 (4x)
1EBN cmm × ___	919 (-)	413	2	0
___ × 2EBN chc (control)	193 (+)	90	1,663	Many
___ × 2EBN acl (control)	136 (+)	39	736	Many
___ × 2EBN 4x-cmm (control)	30 (+)	16	167	Many
2EBN chc × ___ (control)	965 (-)	21	4	-
___ × 4EBN tbr	172 (+)	92	65	14 (6x)
4EBN tbr × ___	637 (-)	16	0	0
Crosses among hybrids				
F ₁ hybrids × sib	117 (+)	68	1,132	Many
F ₂ hybrids × F ₂ bulk	45 (+)	26	462	Many
F ₁ hybrids selfed	610 (+)	326	15,000	Many

^a See Table 1 for key to species abbreviations

^b Number of pollinations. Fertilization: (+) indicates good pollen tube growth and assumed unlimited fertilization; (-) indicates inhibited pollen-tube growth and fertilization assumed to be limited

^c Control cross progeny: these plants were assumed to result from the union of n gametes from each parent. Germination percentages were above 80%. Many hybrids were readily obtained when fertilization was not limited

Results

EBN testing of hybrids

F₁ and F₂ hybrids exhibited a similar pattern of crossing to EBN standards. When these hybrids were crossed with diploid 1EBN standards, a few seeds and plants which appeared to be true hybrids resulted. These were all determined to be 4x. When used as females, hybrids crossed with 2EBN (control) testers produced relatively-abundant seeds. When hybrids were crossed with 4EBN testers, some seeds and apparent hybrid plants resulted in each cross direction. All were 6x. Interhybrid crosses and selfed hybrids typically produced abundant seeds. Table 2 provides details of these crossing results.

Assessing fertilization

Examination of pollen-tube growth suggests that fertilization was not limited when hybrids were used as females. When used as males, pollen-tube growth was poor in styles

of the 4EBN testers cv Katahdin and *S. andigena*, and the 2EBN tester *S. chacoense*. In contrast, germination and growth of hybrids' pollen in styles of the 1EBN tester *S. commersonii* (diploid) was normal.

Discussion

The crossing behavior of 4x *S. commersonii* × *S. acaule* hybrids clearly identifies them as invariable 2EBNs, since they crossed only to 2EBN testers. Some seeds and plants did result from crosses with 1EBN and 4EBN testers, but their ploidy revealed that the EBN requirement had been met by spontaneous chromosome (and EBN)-doubled (i.e., 2n) gametes. When these 4x(2EBN) hybrids were crossed to 2x(1EBN) testers to produce tetraploid offspring, the diploid must have contributed a 2x(1EBN) 2n gamete, equivalent to the normal n gamete of a 4x(2EBN) tester [expected to cross readily to the hybrids]. Cases in which the 4x(2EBN) hybrids crossed successfully to 4x(4EBN) testers resulted exclusively in 6x progeny. Here the hybrids themselves must have contributed 4x(2EBN) 2n gametes.

These would be equivalent to the normal n gametes of an $8x(4EBN)$ parent [expected to cross readily to the $4x(4EBN)$ testers].

Fertilization was not limiting when hybrids were used as females. Some crosses in which hybrids were used as males were not as informative with respect to EBN, since poor pollen-tube growth apparently limited seed set [see Table 2 pollinations marked as “(–)”. This was not due to any innate weakness of hybrid pollen, since F_1 hybrids readily self, and pollinations onto the 1EBN (diploid) *S. commersonii* testers exhibited exceptional pollen-tube growth (recall that *S. commersonii* is one of the constituents of the hybrids).

The simplest genetic interpretation of these results is that the EBN-determining alleles carried on the genome of *S. commersonii* and the two genomes of *S. acaule* are all allelic (and homozygous). Thus, no detectable segregation for EBN occurs in their gametes or progeny. From a breeding perspective this is unfortunate, since transgressive segregation which promoted direct crossing of this 2EBN germplasm with the 4EBN cultivated *tuberosum* breeding pool would have been welcomed.

For the sake of this discussion, potato species may be considered to have only two types of genomes with respect to EBN: 1EBN and 2EBN. These and their combinations account for all diploid and polyploid species: $2x(1EBN)$, $2x(2EBN)$, $4x(2EBN)$, $4x(4EBN)$, $6x(4EBN)$. The evidence provided here, combined with previous work (Bamberg and Hanneman 1990), shows that several apparently-disparate diploid and tetraploid taxa from Mexico and South America have genomes that are identical with respect to their 1EBN constitution. This may reflect their phylogenetic relationships. Hawkes and Jackson (1992) have interpreted the occurrence of 1EBN species in the light of previous theories of potato evolution and migration (Hawkes 1988, 1990). They suggest that 1EBN is the ancestral condition originating in Mexico and spreading to S. America. In South America, 2EBN forms evolved and flourished, spreading back to Mexico. This calls for relatedness of 1EBN taxa, which is generally supported (with some exceptions) by other phenotypic evidence.

Another possibility is that the change from 1EBN to 2EBN (and vice versa) has evolved several times. Ehlenfeldt and Hanneman (1988) postulated a system of three unlinked loci for which there exist two types of alleles, one having an EBN value twice the other. They used *S. commersonii*, the species shown here and in previous work (Bamberg and Hanneman 1990) to have the same inheritance of 1EBN as several Mexican species and *S. acaule*. Although this relatively-complex system would not seem likely to be subject to identical parallel evolution and reversion, perhaps a genetic mechanism exists which has allowed the change between the two basic states of 1EBN and 2EBN several times in the evolutionary development of the tuber-bearing *Solanum* species. Identification and manipulation of this mechanism, if it exists, could be quite useful for breeding, since the 1EBN genomes of whole species could be converted to parity with cultivated *S. tuberosum*. At present, the only direct way to circumvent this

EBN barrier is to circumvent dependence on endosperm – by embryo rescue (Watanabe et al. 1992) or protoplast fusion (Helgeson et al. 1993).

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