



## Challenges and prospects of endosperm balance number in potato (*Solanum tuberosum*) improvement

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### ABSTRACT

Endosperm Balance Number (EBN) is a genome-specific ploidy, which is not necessarily equivalent to the chromosome ploidy. The EBN is the 'effective ploidy' that determines crossability in *Solanum* species. It varies from 1 to 4 and the two *Solanum* species are crossable if they have the same EBN so that maternal to paternal ratio of EBN in the endosperm is 2:1. However, a number of significant exceptions to this rule have been observed in potato (*Solanum tuberosum* L.). EBNs have been experimentally assigned to a number of *Solanum* species after crosses with standard species whose EBN was arbitrarily established. Neither specific genes nor the molecular basis of EBN have been established in potato, but 2-3 unlinked loci with equal additive effects are known to determine the EBN in *Solanum* species. EBN acts as a powerful isolating mechanism in the sexual reproduction of *Solanum* species. EBN incompatibility played an important role in the speciation of polyploids from diploids, complementing the role of 2n gametes in the polyploidy evolution of potato species. EBN has great predictive value for planning interspecific crosses in potato. EBN of a species can be modified through sexual or somatic polyploidization. This has facilitated the transfer of useful disease and pest resistance genes from 1 EBN and 2EBN wild species to 4EBN cultivated potatoes. Besides direct, indirect gene transfer through bridge species from otherwise incompatible *Solanum* species has been accomplished for improving cultivated potatoes. The concept, inheritance and role of EBN in speciation, and its prospects in potato varietal improvement are reviewed in this paper. The problems associated in transfer of useful traits from wild species to cultivated potatoes due to EBN barriers are also highlighted.

**Key words:** 2n gametes, Interspecific crosses, Ploidy manipulation, Potatoes, Seed development, *Solanum* species and Speciation

It is well known that in Angiosperms double fertilization occurs; one sperm nucleus fuses with the egg to generate a zygote, the other fuses with the polar nuclei of the central cell of the female gametophyte to form the primary endosperm nucleus. The endosperm is major food and feed source, and a unique and fundamental tissue in Angiosperms. It provides food materials for the developing embryo and, in many cases, for the young seedlings. Furthermore, the endosperm is extremely important in relation to seed formation or failure in interploid and interspecific crosses, in that endosperm breakdown is usually the main cause of seed failure after double fertilization (Brink and Cooper 1947).

Over the years many studies have been carried out to explain the basis of normal seed development. Early hypothesis suggested a need for a particular balance of chromosome sets between fundamental parts of the developing seed (i.e. maternal tissue, embryo and endosperm) for normal seed growth (Boyes and Thompson 1937, Valentine 1956). The central cell of the female

gametophyte has two exact duplicate sets of chromosomes of the egg. Since the male nuclei delivered to the central cell and the egg are the same, the nuclear constitution of the endosperm differs from embryo only in having one extra set of the maternal chromosomes: thus, development of the endosperm is dependent on the same genes as its accompanying embryo but in different doses, i.e. 2 maternal : 1 paternal ploidy ratio.

### Seed development and EBN in potato

A number of significant exceptions to the rule of a required 2 maternal : 1 paternal endosperm ploidy ratio in potatoes were observed. During studies on haploid extraction in potatoes through 4x-2x crosses, Wangenheim *et al.* (1960) found that normal endosperm development occurred with various balances in the chromosome sets of the above mentioned tissues. For example, 4x (2n = 4x = 48) *Solanum acuale* yields aborted seed when crossed with the cultivated potato, 4x *S. tuberosum* Group Tuberosum (Wangenheim 1955). Yet, *S. acuale* crosses readily with 2x (2n = 2x = 24) haploids extracted from Gp. Tuberosum, producing seeds with normally developed endosperm (Irikura 1968). In the 4x *S. acuale* × 4x Gp. Tuberosum cross, the maternal:

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paternal ploidy ratio in the endosperm is 4:2 (=2:1), yet the endosperm aborts, while it develops normally in the 4x *S. acuale* × 2x Gp. Tuberosum haploid cross where the maternal:paternal ploidy ratio is 4:1. Mexican tetraploid species of series Longipedicellata (*S. fendleri*, *S. hjertingii*, *S. paptia*, *S. polytrichon*, and *S. stoloniferum*) cross readily to many of the South American diploid species (Ramanna and Abdalla 1970, Johnston and Hanneman 1980, Matsubayashi 1982), where the endosperm ploidy ratio is 4:1 or 2:2 in reciprocal crosses. If series Longipedicellata 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 (Swaminathan 1951, Lamm 1953). Most diploid species from Mexico do not cross readily with diploids from South America where the requirement of a 2:1 endosperm ploidy ratio is met (Lee and Cooper 1958, Dionne 1963, Hermsen and Taylor 1979, Amaya and Matsubayashi 1981). 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 ratio were not 2:1 (Johnston and Hanneman 1980). This suggested that endosperm development could depend upon its own constitution, and not on that of the embryo or the maternal tissue.

To explain failures in both intraspecific and interspecific crosses in *Solanum*, Johnston *et al.* (1980) developed a model which relies on a balance of qualitative genetic factors (Endosperm Balance Numbers EBNs), and not chromosome sets for endosperm development. The EBN concept proposes that species can be assigned “effective ploidies” (EBN numbers). According to this hypothesis, each species has a genome-specific ploidy level, the EBN, which is not necessarily equivalent to the chromosome ploidy. The EBN varies from 1 to 4 in *Solanum* species. The chromosome 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. Triploids are 2EBN and pentaploids and hexaploids are 4EBN (Hanneman 1994). Doubling of chromosome number also doubles the EBN. The EBN must be 2:1 maternal to paternal ratio in the hybrid endosperm for normal development of endosperm and, consequently, of the hybrid embryo. Thus, successful crosses occur only when the male and female gametes have the same EBN.

#### Assignment of EBN

EBNs have been experimentally assigned to a number of *Solanum* species after crosses with standard species whose EBN was arbitrarily established, and assuming the 2:1 ratio as prerequisite for normal endosperm development. *S. chacoense* (2n = 24) was first chosen as a standard species by Johnston and Hanneman (1980) and given an EBN of 2. All species which resulted in successful crosses with *S. chacoense* were assigned an EBN of 2. A colchicine produced autotetraploid of *S. chacoense* was assigned an

Table 1 Assignment of endosperm balance numbers (EBN) to unknown species by crosses with known EBN standards

Unknown EBN	Standard EBN	Result	Conclusion
4x A	× 2x (2EBN)	Failed	
	× 4x (4EBN)	4x	A is 4x (4EBN)
4x B	× 2x (2EBN)	3x	
	× 4x (4EBN)	Failed	B is 4x (2EBN)
4x C	× 2x (2EBN)	Failed	
	× 4x (4EBN)	Failed	Inconclusive
4x C <sup>a</sup>	× 2x (2EBN)	3x	
	× 4x (4EBN)	Failed	C is 2x (1EBN)

<sup>a</sup> Colchicine-doubled

EBN of 4 and this was further used as a standard for assigning EBN to other species as shown in Table 1 (Ehrlenfeldt and Ortiz 1995).

In fact when species with the same EBN are crossed, the maternal to paternal EBN ratio in the developing endosperm will always be 2:1, regardless of the paternal ploidies and the direction of the cross. Following these test crosses, a number of species including diploids (e.g. *S. verrucosum* and *S. boliviense*), and tetraploids (*S. acuale* and *S. stoloniferum*) have been assigned an EBN of 2. In the same study, Johnston and Hanneman (1980) assigned an EBN of 4 to *S. tuberosum* and hexaploids *S. demissum* and *S. oppoense* after they successfully crossed them with a colchicine-induced tetraploid 4EBN *S. chacoense* tester. Diploid species (*S. brevidens*, *S. cardiophyllum*, *S. commersonii*) strongly isolated from other diploids were also used by Johnston and Hanneman (1982) to test the applicability of the EBN. It was found that *S. cardiophyllum* could be crossed with 2EBN diploid standards only in the artificially produced 4x form or through the function of 2n gametes (unreduced gametes with sporophytic chromosome number). Thus, it was assigned an EBN of 1, as were *S. brevidens* and *S. commersonii*. Hawkes and Jackson (1992), Ochoa (1992), and Hanneman (1994) have contributed to assigning an EBN to large number of *Solanum* species. EBNs for most of the species have been listed by Spooner and Salas (2006) in their chapter on ‘Structure, biosystematics and genetic resources of potato’ published in “Handbook of potato production, improvement and postharvest management” (Gopal and Khurana 2006). The most common ploidy and EBN combinations include 6x (4EBN); 4x (4EBN)(including Tuberosum Group); 4x (2EBN); 2x (2EBN)(including most wild species); and 2x (1EBN). Species from Mexico typically have EBNs that are one-half of their ploidy {e.g. 2x (1EBN), 4x(2EBN)}.

Johnston and Hanneman (1980) proposed that in crossability experiments for determination of EBN, a minimal information is required on 1) 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; and 4) the ploidy of the offspring, especially from interploidy crosses. They

considered a cross successful if it averaged ten or more plump seeds/fruit and the chromosome count and/or morphology of the offspring indicated hybridity.

#### *Inheritance of EBN*

Some attempts to understand the genetic system behind the EBN hypothesis were made by Ehlenfeldt and Hanneman (1988a) through the analysis of a complete diallel of exceptional *S. commersonii* – *S. chacoense* (1½ EBN) hybrids and backcrosses to parental species. They postulated that within a species EBN is under the control of three unlinked loci with equal additive effects, and with an equal gene value. Their results also indicated that the three genes involved are homozygous in each species. Under this additive model, genetic recombination in the hybrids may produce gametes with various EBN gene values. Gametes with equal EBN gene values from both parents will give the required 2:1 EBN ratio in the endosperm and thus the offsprings will be normal. Camadro and Masuelli (1995) developed a similar model to explain the results from crosses between 2EBN *S. acuale* (2n = 48) and either 1EBN *S. commersonii* (2n = 2x) or 4EBN *S. gourlayi* (2n = 48). However, they hypothesized that the EBN of these species is under the control of two, and not three, independent loci, with alleles in homozygosity. Johnston and Hanneman (1996) confirmed that more than one gene and more than one chromosome are responsible for the genetic control of EBN in *Solanum*. They used a partial trisomic series to see if the addition of any single chromosome could alter the EBN, but it was found to be ineffective in modifying EBN expression. If EBN loci are additive as hypothesized by Ehlenfeldt and Hanneman (1988a), there might be duplicate loci.

Bamberg and Hanneman (1990) analyzed the crossing behaviour of numerous combinations of diploid and tetraploid Mexican species and the South American species *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. Similar conclusion was drawn by Bamberg (1994) for the EBN constitution of two South American species *S. acaule* and *S. commersonii*, as there was apparently lack of recombination and segregation for EBN in hybrids of these species. Thus inheritance of EBN is similar in widely separated taxa from South America and Mexico.

Neither specific genes nor the molecular basis of EBN has been established in potato (Sanetomo *et al.* 2011). Scotti *et al.* (2007) reported significant association between mitochondrial DNA patterns and EBN in wild potato species. Recently, in an attempt to investigate the molecular bases of post-zygotic hybridization barriers in interspecific crosses between the wild potato species *S. acuale* and *S. commersonii*, Cornejo *et al.* (2012) reported a possible role for the differentially expressed sequences in cytokinesis, cell cycle, secondary and hormonal metabolism, biodegradation and transport. They suggested that collapse

of the embryo and endosperm in incompatible crosses may be related to alterations in cell cycle and cytokinesis.

#### *EBN and speciation*

EBN acts as a powerful isolating mechanism in the sexual reproduction of *Solanum* species. Differences in EBN create effective incompatibility barriers between sympatric species, leaving their genotypic integrity intact. EBN incompatibility may have played an important role in the speciation of polyploids from diploids, complementing the role of 2n gametes in the polyploidy evolution of potato species.

In their review of EBN and its evolutionary implications, Hawkes and Jackson (1992) indicated that diploid 1EBN 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. They proposed taxonomic and evolutionary implications of EBN in potato. According to them, 1 EBN condition in Mexican primitive species is associated with their white stellate flowers, and that this condition is also found in species from South America with flowers of same colour and shape. The evolution of a rotate corolla seems to be correlated with 2 EBN. They postulated that the 2 EBN state arose as reproductive isolating mechanism in South America. Some recent studies (Wang *et al.* 2008, Jansky *et al.* 2009, Cai *et al.* 2011, Sliwka *et al.* 2012) have shown that 1EBN species of potatoes carry novel resistance genes imparting higher level of resistance to certain diseases and pests than those in 2 or 4 EBN species.

#### *EBN and 2n gametes*

den Nijs and Peloquin (1977) proposed an evolutionary scheme where n and 2n gametes link together all ploidy levels, thus overcoming the ploidy barriers and providing the opportunity for gene flow throughout sympatric species with different chromosome numbers. Indeed sexual polyploidization double both ploidies and EBN values, so that in crosses between a 2EBN diploid and a 4EBN tetraploid, the 2:1 EBN requirement favours 2n gametes of the parent with a lower EBN. Only 2n gametes can allow inter-EBN crosses, and thus gene flow, between species. This complementary role of EBN and 2n gametes has an important meaning not only because it facilitates gene introgression from diploids to tetraploids, but also because it maintains the ploidy integrity of the two parental species. Under the EBN hypothesis *S. tuberosum* may have risen from the fusion of 2n gametes of 2EBN diploid species. Hawkes (1990) reported that it is likely that the two diploid species involved were *S. stenotomum* and *S. sparsipilum*, both 2EBN.

#### *Predictive value of EBN*

Barring presence of stylar or other pre-fertilization barriers, the establishment of EBNs makes it possible to logically predict the success or failure of crosses between

*Solanum* species of interest. In addition, it allows the prediction of the ploidy and EBN of the offspring, even when previous crossability data are not available. The predictive value of EBN is also useful for designing breeding schemes and for the exploitation of species which have developed reproductive isolating mechanisms.

A list of successful interspecific crosses produced through EBN (ploidy) manipulations is given in Table 2.

For example, *S. commersonii* ( $2n = 24$ , 1EBN) has several valuable traits, including resistance to low temperature stress and cold acclimation capacity, resistance to pathogens and pests, and a high dry matter content of tubers (Hanneman and Bamberg 1986). Knowledge of its EBN and the occurrence of  $2n$  gametes have been used to manipulate whole sets of chromosomes for direct germplasm transfer from *S. commersonii* to 4EBN *S. tuberosum* (Carputo *et al.*

Table 2 Successful interspecific crosses produced through ploidy manipulations for EBN

Female parent	Male parent	Strategy	Reference
phu <sup>1</sup> 2x (2EBN)	tbr 2x (2EBN)	Male parent produced by haploid extraction	Ross <i>et al.</i> (1964)
phu 2x (2EBN)	tbr 2x (2EBN)	Male parent produced by haploid extraction	McHale and Lauer (1981)
tbr 2x (2EBN)	phu, stn 2x (2EBN)	Female parent produced by haploid extraction	Carroll (1975)
tbr 2x (2EBN)	ber, blv, buk, can, chc, ifd, ktz, mcd, rap, sct, spl, spg, tar 2x (2EBN)	Female parent produced by haploid extraction	Hermundstad and Peloquin (1985, 1986)
tbr 2x (2EBN)	buk, can, grl, ifd, ktz, mlt, spl, spg, vrn, ver 2x (2EBN)	Female parent produced by haploid extraction	Yerk and Peloquin (1989)
tbr 2x (2EBN)	chc, phu, stn, vrn 2x (2EBN)	Female parent produced by haploid extraction	Rousselle-Bourgeois and Rousselle (1992)
tbr 2x (2EBN)	buk, can, grl, mlt, spl, vrn 2x (2EBN)	Female parent produced by haploid extraction	Watanabe <i>et al.</i> (1995)
tbr 2x (2EBN)	ber, buk, spl 2x (2EBN)	Female parent produced by haploid extraction	Serquen and Peloquin (1996)
tbr 2x (2EBN)	phu, sct 2x (2EBN)	Female parent produced by haploid extraction	Tucci <i>et al.</i> (1996)
cmm 2x(1EBN)	chc 2x (2EBN)	2n eggs	Ehlenfeldt and Hanneman (1988)
acl 4x (2EBN)	tbr 4x (4EBN)	2n eggs	Camadro and Espinillo (1990)
phu 2x (2EBN)	tbr 4x (4EBN)	2n eggs	Hayes and Thill (2002)
ktz 2x (2EBN)	tbr 4x (4EBN)	2n eggs	Raimondi and Camadro (2003)
acl 4x (2EBN)	etb-pnt(doubled) 4x (2EBN)	Somatic doubling of male and 2n eggs	Chavez <i>et al.</i> (1988)
chc 2x (2EBN)	cph, cmm 2x (1EBN)	2n pollen	Johnston and Hanneman (1982)
sto 4x (2EBN)	tbr 2x (2EBN)	2n pollen	Brown (1988), Brown and Adiwilaga (1990)
tbr 4x (4EBN)	phu 2x (2EBN)	2n pollen	Hayes and Thill (2002)
ver 2x (2EBN)	chn, cph, cmm, trf 2x (1EBN)	2n pollen	Jansky and Hamernik (2009)
fen, hjt, plt, pta, sto, 4x (2EBN)	tbr 2x (2EBN)	2n pollen in 3x offspring	Adiwilaga and Brown (1991)
tbr 2x(2EBN)	chn 2x (1EBN)	2n pollen	Novy and Hanneman (1991)
tbr 4x(4EBN)	ktz 2x(2EBN)	2n pollen	Raimondi and Camadro (2003)
acl doubled 8x (4EBN)	tbr 4x (4EBN)	Somatic doubling of acl	Lamm (1953), Kameraz <i>et al.</i> (1978)
ver 2x (2EBN)	cph doubled 4x (2EBN)	Somatic doubling of cph	Johnston and Hanneman (1982)
brd doubled 4x (2EBN)	chc 2x (2EBN)	Somatic doubling of brd	Johnston and Hanneman (1982)
cmm doubled 4x (2EBN)	acl, fen, hjt, pta, sto, 4x (2EBN)	Somatic doubling of cmm	Bamberg <i>et al.</i> (1994)
cmm doubled 4x (2EBN)	phu-tbr 2x (2EBN)	Somatic doubling of cmm	Carputo <i>et al.</i> (1995, 1997)

1 Species abbreviations (Spooner and Hijmans 2001): acl: *S. acaule*; ber, *S. berthaultii*; blv, *S. boliviense*; brd, *S. brevifolium*; buk, *S. bukasovii*; can, *S. canasense*; chc, *S. chacoense*; chn, *S. chancayense*; cmm, *S. commersonii*; cph, *S. cardiophyllum*; etb, *S. etuberosum*; fen, *S. fendleri*; grl, *S. gourlayi*; hjt, *S. hjertingii*; ifd, *S. infundibuliforme*; ktz, *S. kurtzianum*; mcd, *S. microdontum*; mlt, *S. multidissectum*; phu, *S. tuberosum* Phureja group; plt, *S. polytrichon*; pnt, *S. pinnatisectum*; pta, *S. papita*; rap, *S. raphanifolium*; sct, *S. sanctae-rosae*; spl, *S. sparsipillum*; spg, *S. spegazzinii*; sto, *S. stoloniferrum*; stn, *S. stenotomum* Tuberosum group; tar, *S. tarijense*; tbr, *S. tuberosum* Tuberosum group; trf, *S. trifidum*; ver, *S. verrucosum*; vrn, *S. vernei*

1995, 1997). Knowledge and manipulation of EBN served also for germplasm introgression to potato from 2EBN tetraploid species of the series *Acaulia* (Watanbe *et al.* 1992) and the series *Longipedicellata* (Adiwilaga and Brown 1991). Inter-EBN introgression by *in vivo* manipulation was used to transfer genes from diploid *S. commersonii* and *S. brevidens* to *S. chacoense* and *S. gourlayi* ( $2n = 2x = 24$ , 2EBN) background (Peloquin *et al.* 1989, Masuelli *et al.* 1992) demonstrating an indirect means to infuse 1EBN species into *Tuberosum* via “bridge” species. Indirect germplasm transfer through bridge species also has been accomplished by Adiwilaga and Brown (1991) in the 4x-2EBN species *S. acaule* and by Jansky and Hamernik (2009) in the 2x-1EBN species *S. cardiophyllum*, *S. chancayense*, *S. commersonii*, and *S. trifidum*. Indirect transfer is less effective when compared with direct transfer, in that it has limited genetic efficiency due to the higher percentage of unadapted germplasm transmitted. In addition, the breeding programme followed is longer and more time consuming due to the number of bridge crosses and backcrosses involved. Johanston and Hanneman (1982) produced a 3x hybrid between colchicine induced 4x(2EBN) *S. brevidens* (a non-tuber bearing species) and a weedy South American tuber-bearing species, 2x(2EBN) *S. chacoense*, utilizing the knowledge of EBN. The use of 2n gametes from the triploid allowed the unique opportunity for transferring exotic germplasm from series *Etuberosa* to Gp. *Tuberosum* material. Using a synaptic mutant (*sy-2*) of 2x (1EBN) *S. commersonii* Dun., Ehlenfeldt and Hanneman (1988b) demonstrated the transfer of alleles across EBN levels for the recovery of both qualitative and quantitative traits from 1EBN species. The value of sexual polyploidization as a tool for potato breeding has been extensively reviewed by Peloquin and Ortiz (1992). A review by Jansky (2006) on overcoming hybridization barriers in potato provides further insight into predictive value of EBN in potato.

#### Exceptions to EBN hypothesis

Despite the apparent predictive nature of the EBN hypothesis, exceptions to the 2:1 EBN requirements between different potato species, both within and between ploidy levels have been reported in the literature. For example, Tarn and Hawkes (1986) produced triploid hybrids from crosses between tetraploid *S. commersonii* (2EBN) and diploid *S. commersonii* (1EBN). Even though according to the EBN hypothesis only tetraploid offspring are expected, Chavez *et al.* (1988) obtained tetraploid and hexaploid hybrids by crossing *S. acuale* (2EBN) with a tetraploid *S. etuberosum-S. pinnatisetum* hybrid (2EBN). Also deviating from the EBN model is the production of inter-EBN hybrids between *S. commersonii* (1EBN) and *S. chacoense* (2EBN) (Ehlenfeldt and Hanneman 1988a), *S. circaefolium* (1EBN) and the *tbr* haploid (2EBN) (Louwes *et al.* 1992), *S. acuale* (2 EBN) and *S. gourlayi* (4EBN) (Camadro and Masuelli 1995); and *S. stoloniferum* (2EBN) and *tbr* (4EBN) (Janssen *et al.* 1997). Hayes *et al.* (2005) observed that crosses were not successful between *S. cardiophyllum* and *S.*

*pinnatisetum*, two Mexican species having same ploidy and EBN (2x, 1EBN). The success of a cross may also be genotype specific. In 4x -2x crosses between *S. tuberosum* subsp. *tuberosum* and diploid hybrids, Hanneman and Peloquin (1968) confirmed the existence of a ‘triploid block’ (Marks 1966). When similar crosses were carried out between *S. tuberosum* subsp. *andigena* and primitive diploid cultigens such as *S. stenotomum* in the Andes, a higher frequency of triploid progeny were recovered (Jackson *et al.* 1978). It has also been reported by Chavez *et al.* (1988) that the EBN hypothesis cannot explain the success of crosses between complex hybrids.

Different hypothesis can explain the success or failure of inter-EBN crosses. Pre- or post-zygotic barriers may prevent the formation of seed even when EBN condition is fulfilled. Crosses fail either because the pollen does not germinate on the stigma, or pollen tubes do not reach the ovary. This is certainly the case in crosses at the tetraploid level between *Longipedicellata* species (2EBN) and *S. tuberosum* (4 EBN) (Clugston 1988). Panahandeh *et al.* (2008) reported that crosses between an interspecific tetraploid hybrid (from 2 EBN *S. stoloniferum* × 4EBN *S. tuberosum*) with 2 and 4EBN testers failed due to the pollen-pistil incompatibility. Post-zygotic barrier in potatoes of which EBN is the major factor may be due to abortion of embryo, endosperm, or both tissues, sterility, and hybrid weakness and breakdown in segregating generations (Camadro *et al.* 2012).

The other most obvious reason is that 2n gametes occurred, and equalized the parental EBNs. Following various 2x-2x inter-EBN crosses, triploid hybrids were produced through the function of 2n gametes from the lower EBN parent (Ehlenfeldt and Hanneman 1988b, Masuelli *et al.* 1992). When 2n gametes do not account for the exceptions to the EBN ratio requirement, other hypotheses have been postulated. For example, Hermsen (1987) suggested that the different degree of compatibility found between species and the genotypes can be explained by assuming that EBN is a quantitative, rather than a qualitative factor. Camadro and Masuelli (1995) and Masuelli and Camadro (1997) explained triploid hybrids production from inter-EBN crosses between *S. acuale* ( $2n = 48$ , 2EBN) and *S. commersonii* ( $2n = 24$ , 1EBN) in terms of segregation of the EBN loci. They also hypothesized that EBN could be part of a more complex system of interspecific barriers acting at pre- and post-zygotic levels. Johnston and Hanneman (1995) hypothesized an incomplete penetrance of the EBN ratio requirement, so that random environmental events may allow an occasional endosperm to develop normally. They also maintained that 2:1 EBN ratio requirement may not be as leaky as it appears, and that the exceptions found may be manifestations of unusual fertilization and mitotic events. For example, due to unusual fertilization, two pollen tubes may fertilize the same ovule, delivering two generative nuclei to the central cell and one to the egg. In 4x (4EBN) – 2x (2EBN) crosses it would give a 3x embryo and a 6x endosperm with a balanced EBN

ratio. Alternatively, it is possible that mitotic abnormalities occur after inter-EBN crosses, and that loss or gain of chromosomes during the proliferation of the endosperm cells restores a 2:1 EBN balance.

In conclusion, an understanding of the EBN system would help potato breeders in transferring useful genes from a large array of unexploited wild *Solanum* species to cultivated potatoes for developing improved potato varieties carrying resistance to a number of diseases and pests. Allele mining has proved wild species particularly 1EBN species of potatoes as a rich source of novel resistance genes (Wang *et al.* 2008, Jansky *et al.* 2009, Cai *et al.* 2011, Sliwka *et al.* 2012), which can be transferred to the cultivated potatoes using the EBN concept reviewed in this paper.

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