中图分类号：S532　　**文献标识码**：A　　**文章编号**：1672-3635(2006)03-0170-06

综　　述

Use of the Endosperm Balance Number (EBN) in Potato Genetics and Breeding

Carputo Domenico[1]*, Jin Liping [2]

(1. Deptartment of Soil, Plant and Environmental Sciences (DISSPA), University of Naples "Federico II", Via Università 100, 80055 Portici, Italy;
2. Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China)

Abstract: Solanum species have a wide geographic distribution and show a large range of ecological adaptation, and thus have developed strong resistances to a wide range of pests, diseases and abiotic stresses. They represent unique material as a source of useful genes and allelic diversity for the tetraploid cultivated potato (Solanum tuberosum). Knowledge of the endosperm balance number (EBN) incompatibility system may be extremely useful for breeders in designing specific crosses schemes for potato breeding. EBN has been experimentally assigned to Solanum species after crosses with standard species whose EBN was arbitrarily established, and assuming the 2 ∶1 ratio as a prerequisite for normal endosperm development. The role of the EBN incompatibility system in potato breeding is strongly complemented by 2n gametes. Only 2n gametes can allow inter-EBN crosses and thus gene flow between sexually isolated species. This complementary role of EBN and 2n gametes not only facilitates gene introgression between inter-EBN species, but also maintains the ploidy integrity of the two parental species. Examples are given to illustrate how to use EBN in crossing schemes designed for 2x (2EBN) and 2x(1EBN) species. As increasing in the interest in exploiting exotic germplasm for breeding purposes, it is strongly believed that an understanding of EBN can be used for interspecific gene transfer in any crop species where an EBN-like system operates.

Key Words: Solanum species; isolation; hybridization; gene introgression

The genus Solanum consists of about 2 000 species. Among them, particularly important for the genetics and breeding of tetraploid ($2n=4x=48$) cultivated potato Solanum tuberosum are tuber-bearing species. There are more than 200 wild and cultivated potatoes, grouped in the section Petota, that includes subsections Estolonifera and Potatoe[1]. The species of the subsection Estolonifera are all diploid ($2n=2x=24$), and typically do not produce tubers. Species of subsection Potatoe form a polyploid series ranging from the diploid to the hexaploid ($2n=6x=72$) level, and have a minimal chromosome differentiation among taxa. Most of them (about 80%) are diploid and tuberize. Taxonomically, they are grouped in 16 series. Series Tuberosa contains the seven cultivated species (diploid S. tuberosum Group Stenotomum, S. tuberosum Group Phureja and S. ajanhuiri; triploid S. chaucha and S. juzepczukii; tetraploid S. tuberosum Group Andigena and Group Tuberosum; pentaploid S. curtilobum) as well as the wild species closely related to them[1].

Solanum species have a wide geographic distribution, ranging from the southern part of the United States to the southern Chile. The highest number of species per degree latitude is found from northcentral Peru to central Bolivia and in the central Mexican highlands[2]. All these species show a large range of ecological adaptation and thus have developed strong

---

Received date: 2006-01-06

Biography: Carputo Domenico(1963-), male, associate professor. Research area: potato breeding and genetics.

*Corresponding author: carputo@unina.it

resistances to a wide range of pests diseases, and abiotic stresses. They represent unique material as source of useful genes and allelic diversity for the tetraploid cultivated potato, whose genetic base is limited.

When designing breeding schemes for the exploitation of Solanum species through interspecific sexual hybridization, breeders should know that these species differ not only in the somatic chromosome number, but also in the endosperm balance number (EBN)[3]. The EBN represents a strong isolating mechanism that is more important than ploidy in determining the success of interploidy/interspecific crosses. Knowledge of the EBN incompatibility system may be extremely useful for breeders in designing specific crosses schemes for potato breeding. In this paper, we describe the EBN model and give examples of its use.

1 The EBN Model

According to the EBN model developed by Johnston et al. [3], each Solanum species has been assigned a number ranging from 1 to 4, the EBN. Diploid species can be 1 or 2 EBN, triploids 2EBN, tetraploids 2 or 4EBN, pentaploids and hexaploids 4EBN (Figure 1). The tetraploid cultivated S. tuberosum Group Tuberosum is 4EBN. According to the model, interspecific crosses are successful when in the hybrid endosperm there is a 2 ∶1 ratio between maternal EBN and paternal EBN. In all the other cases the endosperm degenerates after a few days from double fertilization. Figure 1 illustrates some examples of success/failure of interspecific and interploidy crosses. Three important aspects should be underlined: 1) Meiosis normally leads to the production of gametes with a reduction in chromosome number and EBN value; 2) Only when parents have same EBN crosses are successful; and 3) If parents have different EBN, crosses are successful if the parent with lower EBN produces 2n gametes.

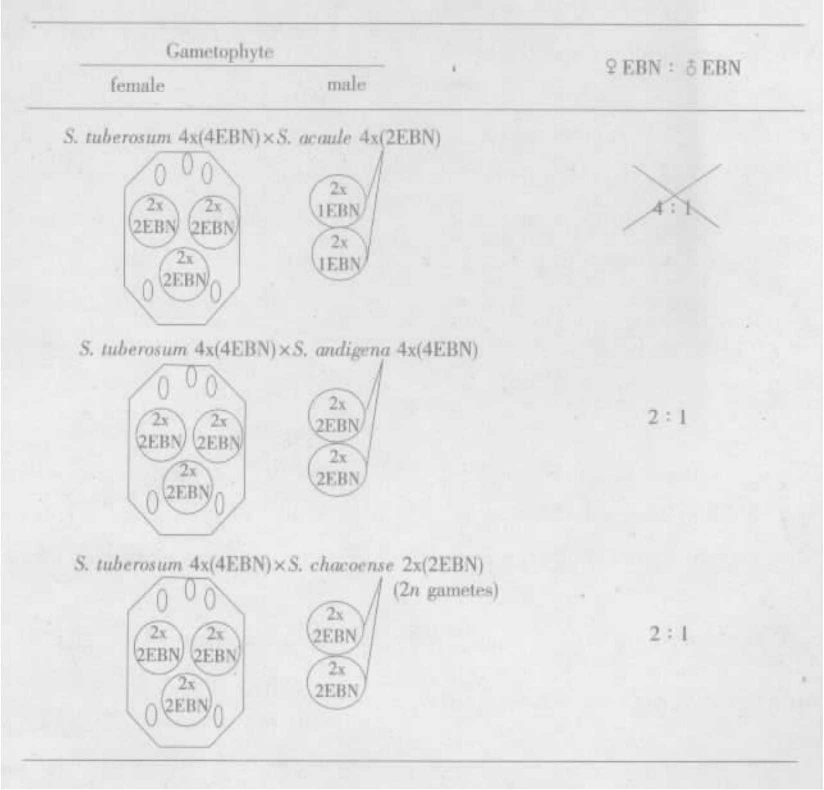

Fig. 1 The EBN model. Examples are given for three crosses involving 4x (4EBN) S. tuberosum as female parent and different male parents. Ploidy and EBN value of polar nuclei and egg cell of the female gametophyte, as well as ploidy and EBN value of the two sperm nuclei of the male gametophyte are shown. Only a 2 ∶1 maternal to paternal EBN ratio in the hybrid endosperm result in successful crosses

EBN has been experimentally assigned to Solanum species after crosses with standard species whose EBN was arbitrarily established, and assuming the 2 ∶1 ratio as a prerequisite for normal endosperm development. S. chacoense ($2n=24$) was first chosen as a standard species by Johnston and Hanneman[4] and given an EBN of 2. All species which resulted in successful crosses with S. chacoense were assigned an EBN of 2. 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. Johnston and Hanneman[4] assigned an EBN of 4 to S. tuberosum and hexaploids S. demissum and S. oplocense after they successfully crossed them with a colchicine-induced tetraploid 4EBN S. chacoense tester.

Some diploid species (S. brevidens, S. cardiophyllum, S. commersonii) strongly isolated from other diploids were also used by Johnston and Hanneman[5] to test the applicability of the EBN. It was found that S. cardiophyllum could be crossed with 2EBN diploid tester only after its chromosome number was doubled. Thus, it was assigned an EBN of 1, as were other species such as S. brevidens and S. commersonii.

Genetic studies by Ehlenfeldt and Hanneman[6] suggested that three unlinked loci with additive effects control the EBN. Camadro and Masuelli[7] developed a similar model to explain results from crosses between 2EBN S. acaule ($2n=48$) 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[8] confirmed that more than one gene and more than one chromosome are responsible for the genetic control of the EBN in Solanum and Datura.

2 The Complementary Role of EBN and 2n Gametes

The role of the EBN incompatibility system in potato breeding is strongly complemented by 2n gametes. Indeed, only 2n gametes can allow inter-EBN crosses and thus gene flow between sexually isolated species. Sexual polyploidization doubles both ploidies and EBN values, so that in crosses between a 2EBN diploid and a 4EBN tetraploid, the 2 ∶1 EBN requirement favors 2n gametes of the parent with lower EBN. It should be pointed out that this complementary role of EBN and 2n gametes not only facilitates gene introgression from diploids to tetraploids, but also maintains the ploidy integrity of the two parental species.

As known, 2n gametes are the result of modified meiosis typical of several Angiosperms[9]. Various meiotic mutations related to spindle formation and cytokinesis have been associated to 2n gamete production in potato. The main mechanism of 2n pollen formation is the parallel orientation of spindles at metaphase II of meiosis[10], whereas the most common mutation leading to 2n egg formation is the omission of second meiotic division[11]. Peloquin et al. reviewed the various meiotic anomalies of 2n gamete production in potato and their use for genetic studies and breeding purposes[9].

3 Use of EBN in Potato Breeding

Knowledge of EBN of Solanum species and of the 2 ∶1 requirement 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. Finally, the predictive value of EBN is useful for designing specific breeding schemes aimed at the exploitation of Solanum species which have developed reproductive isolating mechanisms. In the following part of the paper we will illustrate the use of EBN in crossing schemes designed for 2x (2EBN) and 2x (1EBN) species.

3.1 Exploitation of 2x(2EBN) species.

The most direct use of EBN is for the synthesis of highly heterozygous 4x(4EBN) hybrids through sexual polyploidization crossing schemes with 2x (2EBN) species (Figure 2). This approach represents the classical alternative to the traditional 4x ×4x crossing scheme for potato breeding. It first requires the production of haploids of common S. tuberosum cultivars.

Since S. tuberosum is 4x(4EBN), its haploids will be 2x(2EBN) and so can be easily crossed with 2x(2EBN) wild Solanum species to capture genes of interest and/or allelic diversity. Resulting hybrids are 2x(2EBN); they are selected for traits of interest as well as for 2n gamete production. Then, diploid hybrids are used in sexual polyploidization schemes to generate 4x(4EBN) progenies. Thus, the return to the 4x(4EBN) level of cultivated potatoes is quite fast. The significance of 2n gametes in these crossing schemes lies not only in the possibility of equalizing the EBN of parents, but also in their ability to transmit non-additive genetic effects (heterozygosity and epitasis) from the 2x parent to the 4x offspring.

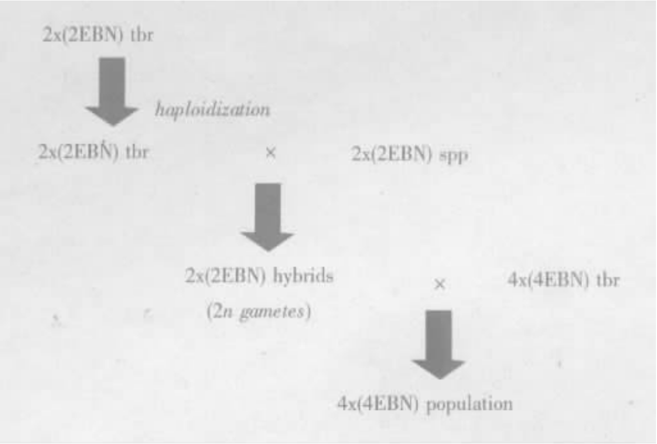

Fig. 2 Unilateral sexual polyploidization between S. tuberosum(tbr) and 2x(2EBN) Solanum species (spp).

Generally two types of polyploidization are used, unilateral sexual polyploidization (through 4x ×2x or 2x ×4x crosses), which requires functioning of either 2n pollens or 2n eggs, and bilateral sexual polyploidization (through 2x ×2x crosses), which requires functioning of both 2n pollen and 2n eggs. The choice of the breeding scheme to be used depends on the mode of 2n gamete formation present in the parent. However, 4x ×2x crosses are preferable to 2x ×4x crosses in that, compared to 2n eggs, 2n pollen transmits a much higher percentage of heterozygosity (80% vs. 40%). It should be pointed out that in unilateral sexual polyploidization crossing schemes triploids are rare due to the so called triploid block. For example, in a 4x(4EBN) ×2x(2EBN) cross, the union of n gametes from both parents would result in a triploid embryo and a 5x endosperm, with a 4 :1 female to male EBN ratio.

Several examples exist in the literature on the use of these sexual polyploidization approaches for the genetic improvement of the potato[12]. At the Institute of Vegetables and Flowers in Chinese Academy of Agricultural Sciences in Beijing, the genetic diversity obtained from the species of S. stenotomum, S. phureja, S. chacoense, S. sparsipilum, S. jamesii, S. microdonmum, and S. vernei have been incorporated into tetraploid cultivated varieties by unilateral sexual ploypoidization. A variety, Zhongda 1, with high dry matter content, regular tuber shape, low reducing sugar content and resistance to late blight was released and certificated at national level in 2005. Advanced clones of TD41-5, TD39-2, TD38-2, and TD41-6 from tetraploid-diploid progeny have been selected with high starch content or good chipping quality[13].

3.2 Exploitation of 2x(1EBN) species

Several noteworthy species of potential use for potato breeding are sexually isolated from S. tuberosum haploids due to EBN barriers. In particular, crosses between 2x(1EBN) S. bulbocastanum, S. cardiophyllum, S. brevidens, S. commersonii, S. brachistotrichum (among the other species) and 2x(2EBN) S. tuberosum haploids result in a 1 :1 or 2 :0.5 female to male EBN ratio following 2x(1EBN) ×2x(2EBN) and 2x(2EBN) ×2x(1EBN) crosses, respectively. Thus, the hybrid endosperm degenerate. Also in the case of 2x(1EBN) species, knowledge of EBN can be efficiently used to design crossing schemes aimed at equalizing the EBN of parents and at exploiting these species. Direct EBN manipulation, coupled with the use of 2n gametes, represents the simplest, most efficient, and reproducible method for the exploitation of 1EBN species.

The strategy that will be presented here is based on the production of the so called "ploidy bridges". The first bridge to produce is a triploid one (Figure 3). For this purpose, it is necessary to double the chromosome number of the 2x (1EBN) species, to produce 4x (2EBN) derivatives. This can be easily performed through either colchicine or in vitro regeneration from explants. The 4x (2EBN) derivatives produced can be crossed with 2x(2EBN) S. tuberosum haploids to produce triploid bridge. These have 2 genomes of the wild species, and 1 of S. tuberosum Therefore, their EBN value is 2 (0.5 + 0.5 + 1). If the 2x(1EBN) species produces 2n gametes, then triploids can be directly produced through 2x(1EBN) - 2x(2EBN) crosses, because 2n gametes equalize the EBN of parents. The second step in this scheme is the production of a pentaploid bridge through 3x (2EBN) ×4x (4EBN) S. tuberosum crosses. A necessary condition is that triploids must produce 2n gametes, which not only balance functional gametes of an odd ploidy parent, but also allow a compatible 2 ∶1 EBN ratio. Pentaploid bridges have 2 genomes of the wild species, and 3 of S. tuberosum Thus, their EBN value is 4 (0.5+0.5+1+1+1), and they can be easily backcrossed to 4x(4EBN) potatoes.

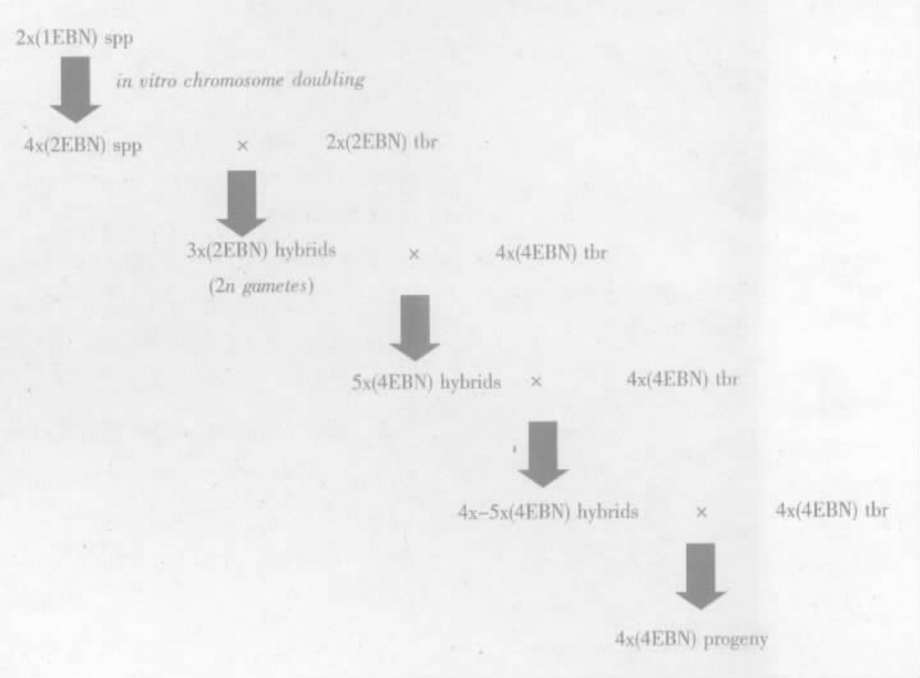

Fig. 3 The ploidy bridge approach to introgress useful genes/allelic diversity from 2x (1EBN) species (spp) into 4x (4EBN) S. tuberosum (tbr). If the 2x (1EBN) species produces 2n gametes, chromosome doubling is not necessary to produce triploid bridges

At the University of Naples, this ploidy bridge approach has been carried out to exploit 2x (1EBN) species S. commersonii[14]. This species, originating from Argentina and Uruguay, 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. Its EBN and ploidy were doubled through a tissue culture cycle and 4x(2EBN) S. commersonii was crossed to 2x (2EBN) S. phureja - S.tuberosum hybrids to produce the triploid bridges. Through the functioning of 2n eggs, 4EBN pentaploid hybrids were then generated following 3x(2EBN) ×4x (4EBN) crosses. As expected, pentaploid bridges were easily crossed with tetraploid varieties, giving rise to an aneuploid progeny[15]. This represented a skewed population, in that the somatic chromosome number of hybrids tended towards a low aneuploid level, and hybrids with 48 chromosomes were recovered[16]. The material developed showed introgression of useful traits from wild S. commersonii. Particularly interesting was the finding that in terms of resistance to low temperatures, triploids performed much better than pentaploids and aneuploid- tetraploid (Table 1). Thus, having more genomes from the resistant parent increased the capacity to withstand low temperatures, both in non acclimated and in acclimated conditions. However, within pentaploids and aneuploid-tetraploid hybrids with resistance up to -6℃ were found. The material developed is being evaluated for other traits of interest and also with AFLP markers, in order to select the hybrids combining useful traits with a wild genome content[17].

Table 1 Killing temperature in non acclimated (NAC) and acclimated (ACC) conditions of S. commersonii - S. tuberosum hybrids of 3x, 5x, and 4x-5x hybrid generations. The acclimation capacity is the difference between the killing temperature in acclimated conditions and the killing temperature in non acclimated conditions

Hybrid generation	Pedigree	Killing temperature(℃)		Acclimation capacity(℃)
		NAC	ACC	
3x	4x×2x	-4.2	-7.1	2.9
5x	3x×4x	-3.3	-4.6	1.3
4x-5x	5x×4x	-2.8	-4.2	1.4
S. commersonii	-	-5.8	-8.3	2.5
S. tubersoum	-	-3.3	-3.3	0.9

4 Conclusions

The EBN represents a powerful isolating mechanism in Solanum, and it may account for maintaining the genomic integrity of species. The concept of EBN as the effective ploidy of tuber and non-tuber bearing Solanum species has put interspecific hybridization on a more predictable and successful basis. Due to the growing interest in exploiting exotic germplasm for breeding purposes, we believe that an understanding of EBN can be used for interspecific gene transfer in any crop species where an EBN-like system operates, such as Impatiens, Lycopersicon, Avena, and Trifolium

References

[1] Hawkes J G. Origins of cultivated potatoes and species relationships [M] //Bradshaw J E, Mackay G R. Potato Genetics. Oxford, UK: CAB International, 1994: 3-42.

[2] Hijmans R J, Spooner D M. Geographic distribution of wild potato species [J]. Am J Bot, 2001, 88: 2101-2112.

[3] Johnston S A, den Nijs T M, Peloquin S J, et al. The significance of genic balance to endosperm development in interspecific crosses [J]. Theor Appl Genet, 1980, 57: 5-9.

[4] Johnston S A, Hanneman R E Jr. Support of the Endosperm Balance Number hypothesis utilizing some tuber-bearing Solanum species [J]. Am Potato J, 1980, 57: 7-14.

[5] Johnston S A, Hanneman R E Jr. Manipulations of Endosperm Balance Number overcome crossing barriers between diploid Solanum species [J]. Science, 1982, 17: 446-448.

[6] Ehlenfeldt M K, Hanneman R E Jr. Genetic control of Endosperm Balance Number (EBN): three additive loci in a threshold-like system [J]. Theor Appl Genet, 1988, 75: 825-832.

[7] Camadro E L, Masuelli R W. A genetic model for the endosperm balance number (EBN) in the wild potato Solanum acaule Bitt. and two related diploid species [J]. Sex Plant Report, 1995,8: 283-288.

[8] Johnston S A, Hanneman R E Jr. Genetic control of Endosperm Balance Number (EBN) in the Solanaceae based on trisomic and mutation analysis [J]. Genome, 1996, 39: 314-321.

[9] Peloquin S J, Boiteaux L, Carputo D. Meiotic mutants in the potato - valuable variants [J]. Genetics, 1999, 153: 1493-1499.

[10] Watanabe K, Peloquin S J. Cytological basis of 2n pollen formation in a wide range of 2x, 4x, and 6x taxa from tuber-bearing Solanum species [J]. Genome, 1993, 36: 8-13.

[11] Werner J E, Peloquin S J. Inheritance and two mechanisms of 2n egg formation in 2x potatoes [J]. J Hered, 1990, 81: 371-374.

[12] Ortiz R. Potato breeding via ploidy manipulations [J]. Plant Breed Rev, 1998, 16: 15-85.

[13] Jin L P, Qu D Y, Xie K Y, et al. Potato germplasm, breeding studies in China [C]. Proceedings of the Fifth World Potato Congress Kunming, China. Kunming: Yunnan Fine Arts Publishing House, 2004: 175-178.

[14] Carputo D, Barone A, Cardi T, et al. Endosperm Balance Number manipulation for direct in vivo germplasm introgression to potato from a sexually isolated relative (Solanum commersonii Dun.) [C]. Proc Natl Acad Sci USA, 1997, 94: 12013-12017.

[15] Carputo D. Cytological and breeding behavior of pentaploids derived from 3x ×4x crosses in potato [J]. Theor Appl Genet, 2003, 106: 883-888.

[16] Carputo D, Parisi M, Consiglio F, et al. Aneuploid hybrids from 5x - 4x crosses in potato: chromosome number, fertility, morphology and yield [J]. Am J Potato Res, 2003, 80: 93-101.

[17] Barone A, Sebastiano A, Carputo D, et al. Molecular marker-assisted introgression of the wild Solanum commersonii genome into the cultivated gene pool [J]. Theor Appl Genet, 2001, 102: 900-907.