Chapter 7
Genetics and Cytogenetics of the Potato

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Abstract Tetraploid potato (*Solanum tuberosum* L.) is a genetically complex, polysomic tetraploid ($2n = 4x = 48$), highly heterozygous crop, which makes genetic research and utilization of potato wild relatives in breeding difficult. Notwithstanding, the potato reference genome, transcriptome, resequencing, and single nucleotide polymorphism (SNP) genotyping analysis provide new means for increasing the understanding of potato genetics and cytogenetics. An alternative approach based on the use of haploids ($2n = 2x = 24$) produced from tetraploid *S. tuberosum* along with available genomic tools have also provided means to get insights into natural mechanisms that take place within the genetic load and chromosomal architecture of tetraploid potatoes. This chapter gives an overview of potato genetic and cytogenetic research relevant to germplasm enhancement and breeding. The reader will encounter findings that open new doors to explore inbred line breeding in potato and strategic roads to access the diversity across the polyploid series of this crop's genetic resources. The text includes classical concepts and explains the foundations of potato genetics and mechanisms underlying natural cytogenetics phenomena as well as their breeding applications. Hopefully, this chapter will encourage further research that will lead to successfully develop broad-based potato breeding populations and derive highly heterozygous cultivars that meet the demands of having a resilient crop addressing the threats brought by climate change.

7.1 Introduction

The most grown potatoes are tetraploid ($2n = 4x = 48$), but farmers in the Andes grow diploid ($2n = 2x = 24$), triploid ($2n = 3x = 36$), and pentaploid ($2n = 5x = 60$) cultivars (Watanabe 2015). The basic chromosome number of these tuber-bearing

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Solanum species is 12. Diploid cultivars along with tetraploid cultivars are used in potato breeding through ploidy manipulations with haploids and 2*n* gametes (or gametes with the sporophytic chromosome number), and chromosome engineering using aneuploidy (Ortiz 1998). Improvement of cultivated potato is challenged by its high heterozygosity (Bradshaw et al. 2006; Hirsch et al. 2013) and complex polysomic tetraploid inheritance (Howard 1970; Ortiz and Peloquin 1994; Ortiz and Watanabe 2004). The tetraploid *Solanum tuberosum* has four homologues, which include 12 unique chromosomes each, thus showing tetrasomic inheritance (Bradshaw 2007). Epistasis and heterozygosity are key for succeeding in 4*x* potato breeding because multi-allelic quantitative trait loci showing high-order genic interactions, while additivity also contributes to quantitative traits with high heritability.

The genetics of tetrasomic potato depends on four sets of homologous chromosomes (instead of two as in diploid potato). Three genotypes (*AA*, *Aa*, *aa*) are expected after selfing a heterozygous diploid (*Aa*), while the selfing of a comparable tetraploid (*AAaa*) gives five different genotypic classes in its offspring: *AAAA* (quadruplex), *AAAa* (triplex), *AAaa* (duplex), *Aaaa* (simplex), and *aaaa* (nulliplex). Double reduction—related to tetrasomic inheritance—occurs when two chromosomes in a gamete derive from two sister chromatids; i.e., the sister chromatids end in same gamete. Quadrivalent formation, a single crossing over between the centromere and the locus to allow sister chromatids to attach to two different centromeres, that these centromeres with sister chromatids move to the same pole in anaphase I, and sister chromatids go the same pole in anaphase II are necessary for double reduction. The probability of double reduction to occur is noted as α, which is equalto $\frac{qea}{2}$, where q is the quadrivalent frequency, e is the frequency of equational separation that depends on the gene–centromere map distance, and a is the frequency of non-disjunction $(\text{often}\frac{1}{3})$. Chromosome segregation arises when $\alpha \neq 0$,thus indicating that a locus of interest lies close to the centromere; while if $\alpha = \frac{1}{7}\text{or}\frac{1}{6}$ then chromatid segregation or maximal equational division (MED), respectively, occurs. MED is rarely found because of the requirements for its occurrence (Burham 1984). DNA-aided marker analysis confirmed the occurrence of double reduction and that it increases with distance from the centromeres (Bourke et al. 2015). As noted by Gálvez et al. (2017), the potato reference genome and transcriptome, research on both gene expression and regulatory motif, plus resequencing and SNP genotyping analyses, provide new means for increasing the understanding of potato genetics. For example, DNA resequencing allows assembling a genome reference for each cultivar or landrace, which may provide useful knowledge regarding structural differences between the various potato groups. In this regard, the resequencing of diversity panel including wild species, landraces, and cultivars demonstrated that a limited gene set accounts for early improvement of the potato cultigen, while distinct loci seems to be involved on the adaptation *S. tuberosum* group Andigenum (upland potato) and *S. tuberosum* groups Chilotanum

and Tuberosum (lowland potato) populations (Hardigan et al. 2017). Signatures of selection in genes regulating pollen development/gametogenesis reduced fertility. Introgression of truncated alleles of wild species, particularly *S. microdontum*, was noted in long day cultivars, thus showing how wild tuber-bearing *Solanum* species are key sources of variation for breeding.

7.2 Haploids and Disomic Inheritance

Tetraploid potato shows significant inbreeding depression (De Jong and Rowe 1971) because polyploidy and heterozygosity mask deleterious recessive mutations and buffer genomic imbalance (Comai 2005; Henry et al. 2010; Tsai et al. 2013). These characteristics led to an alternative breeding approach based on the use of haploids ($2n = 2x = 24$) produced from tetraploid *S. tuberosum*. The homozygosity/heterozygosity of methylated DNA may be, however, involved in inbreeding depression/heterosis in self-compatible diploid potatoes because DNA methylation may suppress gene expression (Nakamura and Hosaka 2010).

The induction of haploid plants is generally referred to as "haploidization." There are two main pathways by which haploid formation can be induced in potato: androgenesis and gynogenesis. Androgenesis is through in vitro culturing of whole anthers or free microspores on a nutrient rich medium to induce plantlet regeneration from single gametic cells or haploid calli (Veilleux 1996), while gynogenesis is haploidization via the "maternal" or seed parent's genome. Potato haploids are routinely obtained by gynogenesis, a process in which specific *S. tuberosum* Group Phureja ($2n = 2x = 24$) selections, known as "haploid inducers," contribute the paternal gametes for pollination of the desired haploid progenitor. The formation of a haploid embryo begins when the egg is either induced into parthenogenesis (Hermsen and Verdenius 1973) or when the zygote experiences spontaneous abortion of the pollen donor's set of chromosomes (Clulow et al. 1991). Evidence for the latter has been the identification of Phureja-specific molecular markers in aneuploids ($2n = 2x + 1 = 25$) among the offspring of some haploid induction crosses (Clulow et al. 1991; Clulow and Rousselle-Bourgeois 1997; Samitsu and Hosaka 2002; Straadt and Rasmussen 2003; Ercolano et al. 2004). Ortiz et al. (1993a) suggested that the genetics of the ability to induce haploids is relatively simple.

Putative haploids are identified firstly by the lack of a dominant morphological marker for anthocyanin pigmentation on developing embryos (embryo spots) or on seedling shoots (nodal bands). This marker that allows early haploid selection is present in homozygosis in certain pure *S. tuberosum* Group Phureja clones or has been bred to homozygosity in its derived hybrids. The ploidy of the resultant seedlings is confirmed by counting chromosomes in mitotically dividing root cells (Sopory 1977), counting chloroplasts in stomatal guard cell pairs (Singsit and Veilleux 1991) or through flow cytometric analysis (Owen et al. 1988). The haploid-inducing clones often used owing to their relatively superior haploid-induction frequency and homozygosity for the seed marker "embryo-spot" are the following

Table 7.1 Haploid induction ability of "IVP 35," "IVP 101," and "PL-4"

Character[a]	"IVP 35"	"IVP 101"	"PL-4"
Number of haploids per 100 berries	69	62	96
Number of haploids per 1000 seeds	52	85	103

[a]Pooled data per haploid inducer from 13 seed parents

Group Phureja clones: "IVP 35," "IVP 48", and "IVP 101" (Ross 1986). "IVP 101" has been derived from the cross [(G609 × "IVP 48") × ("IVP 10" × "IVP 1")]. G609 is a haploid from Group Tuberosum cultivar "Gineke" that combines its own haploid induction ability with a high degree of male fertility, profuse flowering, and vigor.

The efficiency of haploid production is determined by both, production ability of the tetraploid seed parent and induction ability of the diploid pollinator (Hougas et al. 1964; Frandsen 1967; Hermsen and Verdenius 1973). However, interaction between seed parents and pollinators were also noted (Frandsen 1967). Despite this interaction, haploid induction ability of "IVP 101" has proved to be higher than "IVP 35" and "IVP 48" (Hutten et al. 1993). By the end of the 1990s a promising haploid inducer named "PL-4" (CIP596131.4) was selected at the International Potato Center (CIP, Lima, Perú) as a transgressive genotype from the cross between "IVP 35" × "IVP 101" due to its highest haploid inducer ability, degree of flowering, shedding and pollen viability relative to its parents (M. Upadhya and R. Cabello, CIP, unpublished data). Historical data accumulated from 2001 to 2009 from haploid induction crosses between 37, 4*x* breeding lines with both "IVP 101" and "PL-4" showed that the latter produced twice the amount of seeds without embryo spot (putative haploids) of "IVP 101." A more comprehensive study to determine the haploid inducer ability of "PL-4" relative to their parents was performed during 2015 and 2016 at CIP. This involved haploid induction of 13, 4*x* breeding clones with the three haploid inducers. Haploid confirmation was made by counting chloroplasts in stomatal guard cell pairs and flow cytometric analysis in seedlings grown from seeds without embryo spot. "PL-4" produced a significantly higher number of haploids than its parents. Meanwhile, "IVP 101" outperformed "IVP 35" in number of haploid per 1000 seeds (Table 7.1). There were also differences in haploid production ability between seed parents (Table 7.2). Two breeding clones, CIP 300056.33 and CIP 392820.1, showed the highest number of haploids.

7.2.1 Further Research and New Evidence on Haploid Origin

Previous research in potato haploids originated by gynogenesis detected aneuploids ($2n = 2x + 1 = 25$ and $2n = 2x + 2 = 26$) instead of the expected 24-chromosome karyotypes with concurrent appearance of Group Phureja-specific molecular markers (Clulow et al. 1991, 1993; Waugh et al. 1992; Wilkinson et al. 1995; Clulow and Rousselle-Bourgeois 1997; Ercolano et al. 2004). Moreover, there was one case in which translocation of a Group Phureja chromosomal segment to the Group Tuberosum genome was detected by genomic in situ hybridization (GISH; Wilkinson

Table 7.2 Haploid production ability of 13, 4*x* breeding clones from CIP potato breeding program

CIP-number[a]	Breeding code	Number of haploids per 100 berries	Number of haploids per 1000 seeds
CIP 300056.33	LR00.014	141	169
CIP 300072.1	LR00.022	68	101
CIP 300093.15	LR00.027	82	58
CIP 301023.15	C01.020	40	63
CIP 388615.22	C91.640	75	141
CIP 388676.1	Y84.027	40	32
CIP 390478.9	C90.170	2	2
CIP 390637.1	93	76	107
CIP 391931.1	458	11	17
CIP 392780.1	C92.172	9	21
CIP 392820.1	C93.154	258	175
CIP 397073.16	WA.104	39	30
CIP 397077.16	WA.077	100	68
Average		72	76

[a]Pooled data per breeding clone from three haploid inducers

et al. 1995). The cause, frequency, and nature of these introgressions remain unknown, though they may affect performance of the haploids (Allainguillaume et al. 1997). A similar phenomenon has been observed in CenH3-based haploid induction in *Arabidopsis thaliana* (Ravi and Chan 2010; Ravi et al. 2014; Tan et al. 2015). In this system, mis-segregation of the haploid inducer chromosomes leads to genome elimination. In a fraction of the haploid progeny, one or few of the haploid inducer chromosomes were retained, resulting in aneuploid progeny. This DNA introgression was identified readily by low-pass sequencing and single nucleotide polymorphism (SNP) analysis (Tan et al. 2015). These findings added evidence that DNA introgression from a haploid inducer is expected to involve large contiguous segments, and often whole chromosomes. Lately, K.R. Amundson et al. (unpublished) surveyed a haploid segregating population for aneuploids by low-pass sequencing. The population was previously developed at CIP for tetraploid genetic mapping of a major gene controlling *Potato leaf roll virus* (PLRV) resistance in the Group Andigena cultivar "Alca Tarma" (Velásquez et al. 2007). They identified 19 haploids (11.4%) that displayed elevated relative sequence read coverage of a single chromosome consistent with 25-chromosome karyotypes in root tip metaphase spreads in these putatively aneuploid clones. By sequencing parental genotypes to higher depth (40–66×) and identifying homozygous SNP between "Alca Tarma" and either of the two haploid inducers, "IvP-101" or "PL-4," plus assuming to have sired each haploid (Velásquez et al. 2007), they found nearly 0% haploid inducer

SNP for all chromosomes of these aneuploids. Thus, the additional chromosomes observed likely did not originate from the haploid inducer genome, but were maternally inherited. This lack of Group Phureja SNP in haploids was previously reported in another study that employed DNA markers (Samitsu and Hosaka 2002). The production of aneuploid gametes is a common property of polysomic polyploids (Comai 2005), and "Alca Tarma" was not an exception. Admusson et al. (unpublished) concluded that for haploid inducers "IvP-101" and "PL-4," either the mechanism of haploid induction does not involve egg fertilization or genome elimination in "Alca Tarma" was very efficient.

7.3 Relevance of Haploids in Plant Breeding and Genetics

Haploids showed disomic inheritance, which means that each chromosome paired with its homolog, thus providing means for simplifying genetic research in potato. They can also be efficiently used for research on chromosome pairing and natural mutation accumulated at the tetraploid level. Initially, potato haploids were envisioned as a tool to simplify the breeding of *S. tuberosum* cultivar production by reducing tetraploid germplasm to a diploid breeding level (Chase 1963). A second early reason for the production of haploids was to acquire a "genetic bridge" between the various genomes of *Solanum* species. Ploidy barriers between the cultivated and wild *Solanum* species could be circumvented by crossing haploids to the wild diploid *Solanum spp*. and novel hybrid germplasm incorporated back into tetraploid breeding programs through $4x \times 2x$ crosses using $2n$ gamete formation, or by colchicine-doubling of the novel diploid hybrid (Ross 1986). In addition to their use in breeding, diploid potato hybrids represent a powerful tool for genetic analysis due to its much simpler segregation ratios compared to tetraploid cultivated potatoes (Ortiz and Peloquin 1994). Thus, diploid potato has been used to determine the inheritance of economically important traits such as tuber shape (De Jong and Burns 1993; Van Eck et al. 1994b), tuber flesh and skin pigmentation (De Jong 1987; Van Eck et al. 1994a), and tuber skin texture (De Jong 1981). The genetic basis of some physiological mutants has also been analyzed with the use of diploids (De Jong et al. 1998, 2001). Haploids have been convenient for trait mapping (Kotch et al. 1992; Pineda et al. 1993; Freyre et al. 1994; Simko et al. 1999; Naess et al. 2000; Velásquez et al. 2007) and in the development of an online catalogue of amplified fragment length polymorphisms (AFLP) covering the potato genome (Rouppe van der Voort et al. 1998). On the other hand, many breeders have extracted haploids from superior parents and also maintain a diploid gene pool composed of hybrids between haploids and diploid wild species carrying specific quality and host plant resistance genes not found in cultivars (Carputo and Barone 2005; Ortiz et al. 2009).

Currently, there are some ongoing efforts towards genetically restructuring potato as a diploid inbred line-based crop (Jansky et al., 2016; Lindhout et al. 2011). Here, the vision is a diploid potato crop composed of a series of inbred lines that

capture the favorable genetic diversity available in the potato cultigen. This diploid genepool with a broad suite of traits represent a valuable stock for fixing desirable gene combinations and realized breeding gains. Last but not least, haploids have been regarded as a tractable ploidy state for copy number variation (CNV) analysis in tetraploid potatoes as these variants are meiotically transmissible and diploid gametes can likely shield deficiencies such as recessive lethal and dosage sensitive loci (Comai 2005; Lovene et al. 2013; Henry et al. 2015). CNV is defined as stretches of DNA from 1 kilobase (kb) to several megabases (Mb) that display different copy numbers in populations (Feuk et al. 2006). Analyses of multiple genotypes in *Arabidopsis* and maize suggest that CNV may play a significant role in phenotypic diversity and hybrid heterosis in plant species (Swanson-Wagner et al. 2010; Cao et al. 2011). Moreover, they can affect phenotype impacting important agronomic and host plant resistance traits (Maron et al. 2013; Díaz et al. 2012; Zhu et al. 2014; Cook et al. 2012). Direct CNV detection in tetraploid potato is challenging. The dosage increase associated with a duplication is subtler in tetraploids (25% increase) than in diploids (50% increase), and high heterozygosity impedes haplotype assembly (Potato Genome Sequencing Consortium 2011). As a consequence, CNV analysis is often limited to few loci of interest and is costly to be practical in breeding programs. Hence, sampling the gametophyte genome of tetraploids by ploidy reduction through haploidy is an alternative approach.

7.4 2*n* Gametes

Gametes with the sporophytic chromosome number should be named as 2*n* gametes and not as "unreduced" gametes as wrongly dubbed. They result from pre-meiotic, meiotic, or post-meiotic abnormalities during gametogenesis. The modes of formation are pre-meiotic doubling, first division restitution (FDR), chromosome replication during meiotic interphase, second division restitution (SDR), post-meiotic doubling, and apospory (diploid sac formed from nucellus or integument cell). FDR and SDR mechanisms are the most common for 2*n* pollen and 2*n* egg formation in potato (Ortiz 1998). Heterozygous 2*x* parents transmit 80% and 40% of their heterozygosity to their 4*x* hybrid offspring after sexual polyploidization with FDR or SDR 2*n* gametes, respectively.

The parallel orientation of the spindles in the second meiotic division accounts frequently for FDR 2*n* pollen, while omission of the second division after a normal first division seems to be often involved in SDR 2*n* eggs. The abnormal meiosis leading to these 2*n* gametes are under the genetic control of recessive mutants: *ps* for 2*n* pollen and *os* for 2*n* egg, both of which appear to be ubiquitous in *Solanum* species. The finding of genes whose mutations led to a high frequency of 2*n* gametes in the model plant species *Arabidopsis thaliana* provided further means for understanding their formation in plants (Brownfield and Kölher 2011). It appears to be very likely that a mechanism related to a loss of protein function leads to the formation of 2*n* gametes.

The frequency of 2*n* gametes may be affected by incomplete penetrance and variable expressivity, which is under minor modifier genes and influenced by plant age and the environment. Phenotypic recurrent selection could be effective for increasing the frequency of FDR 2*n* pollen, while recurrent selection with progeny testing may raise SDR 2*n* egg expressivity.

There are synaptic mutants affecting gametogenesis in haploids, *Solanum* species and haploid-species hybrids. They may cause poor pairing, reduced chiasma, or both, thus reducing recombination. For example, the synaptic mutant sy_3—found in Group Phureja–haploid hybrids—along with *ps* produces FDR 2*n* pollen without crossing over (FDR-NCO), while the desynaptic mutant *ds-1* generates sterile *n* eggs and fertile FDR 2*n* eggs owing to a direct equational division of univalent chromosomes at anaphase I, i.e., pseudohomotypic division. Desynaptic gametes may transfer about 95% of the 2*x* genotype to their 4*x* hybrid offspring.

7.5 Cytoplasm Diversity and Male Sterility

There are six distinct cytoplasmic genome types in potato, namely, M, P, A, W, T, and D. Many clones bred worldwide show a genetic bottleneck in cytoplasmic diversity due to the continuous use of cytoplasmic-based male sterility "lineages" derived from *S. demissum* or *S. stoloniferum* that are often used as sources of host plant resistance. For example, T (45%), D (38%), and W (11%) are the most frequent types in CIP bred germplasm (Mihovilovich et al. 2015); while the most popular among EU cultivars and breeding clones are T (59%), D (27%), and W (12%) (Sanetomo and Gebhardt 2015), and cultivars and breeding lines from Japan plus a sample of landraces and foreign cultivars show 73.9% T, 17.4% D, and 2.4% W (Hosaka and Sanetomo 2012).

Cytoplasmic factors and nuclear alleles are involved in indehiscence, shriveled microspores, sporad formation, anther-style fusion, ventral-styled anthers, and thin anthers (Grun et al. 1977). Group Andigena and its ancestors, Group Stenotomum and Phureja, share most plasmon factors, of which many differ from those found in Group Tuberosum. Hence, cytoplasmic-genetic male sterility is often noted in hybrid offspring among some tuber-bearing *Solanum* species because interactions between sensitive factors in the cytoplasm of one species and nuclear genes from the other species. Hybrids derived from crossing Group Tuberosum haploids as female and Group Phureja or Stenotomun as males are very often male sterile, but the reciprocal cross show male fertile offspring. Male sterility ensues from the interaction of a dominant gene (*Ms*) from the Group Phureja or Stenotomun with Group Tuberosum sensitive cytoplasm. Diploid (2EBN) wild species such as *S. chacoense, S. berthaultii,* and *S. tarijense* do not carry genes that interact with the Group Tuberosum cytoplasm as shown by the high male fertile hybrid offspring between them.

The frequency of male fertile offspring in hybrids between Group Tuberosum and groups Stenotomum or Phureja may vary because some tetraploid cultivars bear

a dominant male fertility restorer (*Rt*) gene (Iwanaga et al. 1991b). The *Ms* and *Rt* genes, which are independently inherited, are very distal from the centromere, thus showing both loci chromatid segregation (Ortiz et al. 1993b). The *Rt* gene allows to partially bypass male sterility using crossbreeding, e.g. by crossing Group Tuberosum haploids bearing *Rt* with 2× species carrying *Ms* because ½ (*rt*/*rt* × *Ms*/*ms*) ¾ (*Rt*/*rt* × *Ms*/*ms*) or 100% (*Rt*/*Rt* × *Ms*/*Ms* or *Rt*/*Rt* × *Ms*/*ms*) of the resulting hybrid offspring will be male fertile.

7.6 Self-Incompatibility and *s* Locus Inhibitor Mechanism

Diploid potatoes and their related wild tuber-bearing *Solanum* species are self-incompatible due to a gametophytic self-incompatibility (GSI) system controlled by the interaction of a pollen *S* gene with pistil *S* gene(s). GSI inhibits fertilization by self-pollen or pollen from closely related (sibling) plants (Hanneman Jr 1999). In this system, compatibility is controlled by the *S* locus, which consists of two closely linked genes: *S-RNase* and *S-Locus F-box* (*SLF/SFB*) that control the female and male specificity, respectively. S-RNase-based self-incompatibility systems are widespread mechanisms for controlling selfing (Hancock et al. 2003). *S* locus variants, currently known as S-haplotypes, determine self-incompatible pollen rejection when there is a match between the single S-haplotype in the haploid pollen and either of the two haplotypes in the diploid system. S-RNases are the determinants of *S*-specificity in the pistil and act in pollen recognition as well as in direct pollen growth inhibition by degrading pollen RNA in incompatible pollinations (McClure et al. 1990). A distinct S-RNase protein is expressed from each functional S-haplotype and upon recognition the protein enters intact pollen tubes retaining its potentially cytotoxic enzyme activity (Gray et al. 1991). Conversely, pollen RNA is stable in compatible pollinations as interaction between S-RNase and *SLF* confers resistance to the cytotoxic effects of S-RNase (Fig. 7.1; Golz et al. 2001). The *SLF/SFB* is a family of F-box protein genes whose most well-known role is ubiquitin-mediated protein degradation. Pollen modifier genes encoding proteins that form complexes with SLF provides for ubiquitylation and degradation of non-self S-RNase as a necessary step to overcome the cytotoxicity of S-RNase. On the other hand, self S-RNases fail to bind productively and thus escape degradation (Hua et al. 2008; Zhang et al. 2009).

Modifier genes encoding putative pistil self-incompatibility factors were found in potato wild relatives. *HT-A* and *HT-B* are two similar genes expressed in the genus *Solanum*, but only *HT-B* has shown to be strongly suppressed in self-incompatibility breakdown in *S. chacoense.* HT-B proteins appear to be degraded in pollen tubes after compatible pollination, while this is not the case in incompatible pollen tubes where substantial amounts of HT-B reactive protein were found (Fig. 7.1; Goldraij et al. 2006).

Self-compatibility can be obtained by converting a self-incompatibility diploid (e.g., S_1S_2) to a tetraploid (de Nettancourt 1977). Here the defect occurs only in the pollen due to the so-called heteroallelic pollen (HAP) effect. Thus, $S_1S_1S_2S_2$ pistils

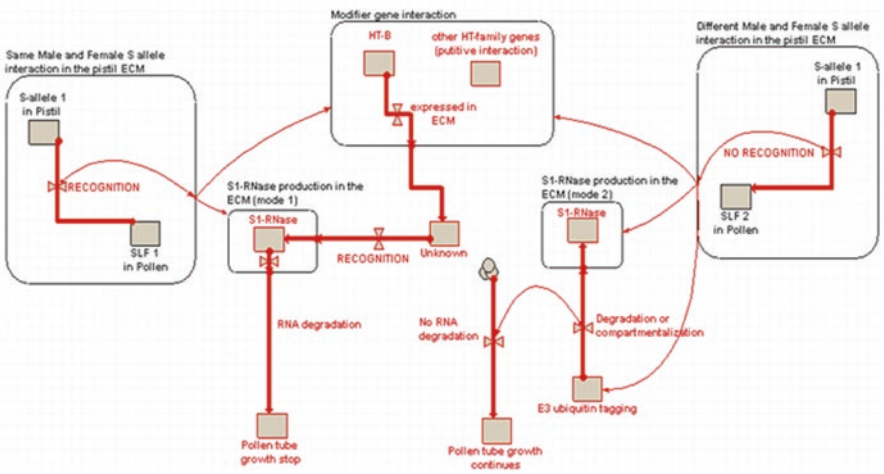

Fig. 7.1 A model for S-RNase-based self-incompatibility. When the pollen tube enters the extracellular matrix (ECM) the proteins HT-B, S-RNase, and 120K (not represented) are expressed. On a positive recognition of self-pollen (or a self *SLF* gene) the pollen tube RNA is broken down by the production of S-RNase and fertilization is unlikely. In the opposite reaction, when an unrecognizable match is made, S-RNase is degraded after being tagged by an E3 ubiquitin ligase complex. (Courtesy: Dr. Philippe Kear, International Potato Center)

reject S_1- and S_2-pollen normally, but diploid pollen is not rejected. Self-incompatibility breakdown only occurs in the HAP case, S_1S_2. Pollen *S* functions to provide resistance to S-RNase. Self-compatible variants have often been described among genotypes of self-incompatible potato species, such as, *inter alia*, *S. chacoense*, *S. kurtzianum*, *S. neohawkesii*, *S. pinnatisectum*, *S. raphanifolium*, *S. sanctaerosae*, *S. tuberosum* Groups Phureja and Stenotomum (Cipar et al. 1964), and *S. verrucosum* (Eijlander 1998). SC variants were also noted in haploids ($2n = 2x = 24$ chromosomes) from Group Tuberosum (De Jong and Rowe 1971; Olsder and Hermsen 1976) and in hybrids between Group Phureja and haploids ($2n = 2x = 24$ chromosomes) of Group Andigenum (Cipar 1964).

Self-compatible variants in *S. chacoense* show a single dominant gene "*Sli*" that is expressed in a sporophytic fashion (Hanneman 1985; Hosaka and Hanneman 1998a). The existence of self-incompatible progeny segregating from *S. chacoense* selfed plants having *Sli* gene in a heterozygous condition shows that fully functional S-haplotypes are transmitted through pollen even when the *Sli* factor is not. *Sli* was mapped to the end of chromosome 12 (Hosaka and Hanneman 1998b). Since the *S* locus has been localized on chromosome 1 (Gebhardt et al. 1991; Jacobs et al. 1995; Rivard et al. 1996), it is evident that the *Sli* gene is independent of the *S* locus. *Sli* can be regarded as a dominant gain-of-function (GOF) pollen-part mutant (PPM) that interacts in some way with the GIS system in pollen and results in self-compatible plants. This pollen side gene may inhibit S-RNase uptake, break down

the pollen-stigma recognition system, overcome the cytotoxic activity of S-RNase independent of its pollen *S*-genotype or through the action on a non-S-specific factor (Hosaka and Hanneman 1998b; McClure et al. 2011). Likewise, self-compatible variants in *S. verrucosum* (*ver*) has been assumed to be a pistil side nonfunctional S-RNase haplotype (*Sv* allele) that allows *ver* plants be pollinated by its own pollen as well by the pollen of other self-incompatible potato species (Eijlander 1998). Absence of pistillate S-RNases seems to be a characteristic feature of this species (Makhan'ko 2011).

7.6.1 Self-Compatibility in Breeding

Most cultivated tetrasomic polyploid or self-incompatible diploid potatoes have not realized breeding gains due to low recombination, long generation cycles, polyploidy, inbreeding depression, and poor adaptation of wild potato germplasm (Visser et al. 2009; Lindhout et al. 2011). New breeding methods that involve the development of diploid inbred lines in potato were proposed as a strategy to address many perceived limitations faced by potato breeders (Birhman and Hosaka 2000; Phumichai et al. 2005). In the last few years a trend emerged in a group of potato breeders to reconsider the crop as a diploid species composed of a series of inbred lines that capture the favorable genetic diversity available in cultivated and wild potatoes. Inbreeding due to selfing may be efficient for organizing the whole gene pool into various favorably interacting and stable epistatic systems (Allard 1999).

The self-incompatibility inhibitor (*Sli*) gene opens new doors to explore inbred line breeding in potato (Lindhout et al. 2011). Highly inbred *S. chacoense* lines such as M6, which has been self-pollinated for seven generations, are vigorous and fertile (Jansky et al. 2014). CIP has incorporated *Sli* into diploid cultivars of *S. tuberosum* groups Stenotomum and Phureja and selected a panel of 20 *Sli* bearing self-compatible hybrids to provide a more desirable self-compatibility source than wild *S. chacoense* for the development of inbred lines. These self-compatible hybrids denoted BSLi, are being used to incorporate novel diversity from wild species and take full advantage of modern genetics and genomics tools to generate inbred genetic resources such as recombinant inbred lines (RILs), for fundamental gene discovery and gene mapping.

Self-compatibility has been identified in five diploid cultivated potatoes of *S. tuberosum* Phureja (phu) and Stenotomum (stn) Groups held in CIP's genebank. Selfing three of these self-compatible diploid cultivars; i.e., CIP705468 (goniocalyx), CIP703320 (stn), and CIP701165 (stn) yielded progenies that segregated for self-compatible and self-incompatible individuals. The segregation ratio 2 self-compatible: 1 self-incompatible was significantly skewed from the expected ratio of 3 self-compatible: 1 self-incompatible for a mutant factor in heterozygosis because of the small population size ($N < 60$) analyzed in each selfed cultivar. Self-compatible plants due to pistil side mutations that compromised S-RNase or HT factors produce only self-compatible plants. The same is true for GOF mutations of the *SLF* gene

(McClure et al. 2011). On the other hand, self-compatible plants with a *Sli* mutation in heterozygocity produce 3 self-compatible: 1 self-incompatible plants regardless of its haplotype condition in the *SLF* locus; i.e., *SxSx*, *SxSy*, *SySy*. Hence, the presence of self-compatible plants in the offspring of self-compatible 2*x* cultivars suggests a dominant pollen-side mutation similar to the *Sli* gene since this is the only scenario that yields self-incompatible offspring. Further research will be required to elucidate whether this *Sli*-like phenotype is a novel pollen-side GOF factor or *Sli* gene variant. Whatever its nature, self-compatible 2*x* cultivars would provide a more desirable self-compatible source than *S. chacoense* as they will avoid the undesirable linkage drag associated with the use of a wild species in the development of 2*x* inbred lines.

7.6.2 Interspecific Crosses and Incompatibility

Interspecific reproductive barriers (IRBs) complicate using wild germplasm species for crop improvement (Zamir 2001; Jansky et al. 2013). Therefore, the rich trait diversity available in CIP's extensive collection of tuber-bearing *Solanum* accessions requires overcoming IRBs to be introgressed into cultivated *Solanum* taxa. These barriers include incompatibility between pollen and pistil, male sterility resulting from interactions between nuclear and cytoplasmic genes, and endosperm failure (Camadro et al. 2004). Post-zygotic IRBs are due to EBN incompatibilities that lead to endosperm failure, whereas pre-zygotic IRBs are typically associated with pollen tube growth inhibition (Camadro and Peloquin 1981; Fritz and Hanneman 1989; Novy and Hanneman 1991; Camadro et al. 1998; Erazzú et al. 1999; Hayes et al. 2005).

Genetic and molecular research shows that some self-incompatibility factors also function in prezygotic IRBs. However, self-incompatibility and IRBs differ in terms of specificity and the precise factor requirements. IRBs show broad specificity, and a single S-RNase can cause rejection of pollen from species or groups of species (Murfett et al. 1996; Tovar-Méndez et al. 2014). *S-RNase* and *HT* genes dual roles in self-incompatibility and interspecific pollen rejection points out pleiotropic effects and hence linkage between these two mechanisms.

IRB mechanisms' complexity is such that multiple redundant mechanisms can contribute to interspecific incompatibility, even between a single pair of species (Murfett et al. 1996; McClure et al. 2000). This may complicate experiments because defects in one rejection mechanism do not necessarily result in compatibility. For example, HT-proteins previously found implicated only in S-RNase-dependent self-incompatibility and IRBs have also been involved in S-RNase-independent pollen rejection in tomato (McClure et al. 2011).

Selective pressures like reproductive assurance make self-incompatible to self-compatible mating system transitions (MSTs) common in nature (Barrett 2002; Goldberg et al. 2010; Goldberg and Igic 2012). Self-compatibility has not been extensively investigated in the potato clade and few self-compatible species have

been recognized. Loss of S-RNase function is a common route to self-compatibility. This is the case of self-compatible variants of *S. verrucosum* in which pollen tubes of other potato species, including those having 1EBN can grow without inhibition in their pistils reaching ovules in great quantity (Hermsen and Ramanna 1976; Makhan'ko 2011). Furthermore, dominant gain-of-function (GOF) pollen-part mutants such as *Sli*, which results in self-compatible variants in *S. chacoense*, increase seed set in interspecific crosses to *S. pinnatisectum* in addition to suppressing self-incompatibility (Sanetomo et al. 2014).

Phumichai et al. (2006) were able to introduce after crossing the *S*-locus inhibitor gene (*Sli*), which can inhibit gametophytic self-incompatibility, in diploid potatoes and alter self-incompatible to self-compatible plants, into 32 diploid genotypes. *Sli* has been also successfully introduced to diploid cultivars from *S. tuberosum* Phureja and Stenotomum Groups using SC *S. chacoense* variants as male parents at CIP. Assuming that this pollen side mutant acts either breaking down pollen-stigma recognition system or overcoming S-RNAse cytotoxic activity (Hosaka and Hanneman 1998b), then a change in interspecific compatibility may occur after crossing *Sli*-bearing self-compatible hybrids as pollen parents and self-incompatible sources of late blight resistance from wild *S. piurae* and *S. chiquidenum* species. Previous attempts at CIP to cross these wild species with 2*x S. tuberosum* cultivars produced very few seeds with *S. chiquidenum* and no seed set at all with *S. piurae*. Embryo rescue was often required to save the few hybrids produced from *S. chiquidenum*. Linkage between self-incompatibility and IRBs results in MSTs with significant implications in germplasm enhancement programs particularly when decisions between different crop wild relatives have to be made in interspecific crosses.

7.7 Unilateral Compatibility

This is a very common IRB pattern that refers to crosses that are compatible in only one direction (Lewis and Crowe 1958). Most IRBs conform to the self-incompatible × self-compatible rule, in which pollen from the self-compatible species is rejected on pistils of related self-incompatible species but the reciprocal pollination is compatible (Nathan Hancock et al. 2003; Bedinger et al. 2011). In potato only some IRBs conform to this rule (Hermsen and Ramanna 1976; Eijlander et al. 2000). For example, crosses between the self-compatible 2*x* (1EBN) species *S. pinnatisectum* and the 2*x* (1EBN) self-incompatible species *S. cardiophyllum* were successful only when self-compatible *S. pinnatisectum* was used as the female parent (Chen et al. 2004). A similar pattern was observed in crosses between the self-compatible 2*x* (1EBN) species *S. commersonii* and the self-incompatible 2*x* (2EBN) species *S. chacoense* (Summers and Grun 1981). Although it is not absolute, the consistency of the "self-incompatible × self-compatible" rule suggests a link between inter- and intraspecific pollen rejection. The suggested linkage is that the *S*-locus controls unilateral incompatibility as well as self-incompatibility. Conversely, there are many exceptions to the self-incompatible × self-compatible rule in potato such

as the occurrence of both unilateral and bilateral self-incompatible × self-incompatible conflicts (Camadro et al. 1998, 2004; Kuhl et al. 2002; Raimondi et al. 2003). Genetic systems entirely independent of the *S*-locus have been proposed to explain cross incompatibility, which is a term used to emphasize potato clade cross distinctive complexity. Cross incompatibility includes both post- and pre-zygotic mechanisms.

An important phenomenon worth mentioning is compatibility encountered in crosses between self-compatible *S. verrucosum* plants (2*x*, 2EBN) and a wide range of accessions of various 1EBN potato species. Success on producing these novel sexual hybrids was achieved with several 1EBN diploid species, such as *inter alia*, *S. bulbocastanum*, *S. pinnatisectum*, *S. polyadenium*, *S. commersonii*, and *S. circaeifolium* (Yermishin et al. 2014). Absence of S-RNases in pistils in self-compatible *S. verrucosum* allowed growth of pollen tubes from these 1EBN wild species and fertilization of egg cells. In addition, "rescue pollination" was used to improve hybridization effectiveness. "Rescue pollination" also known as "double pollination" is a technique that reduces premature fruit drop and involves the application of pollen from the incompatible species, followed a day or 2 later by that of a compatible species, denoted as "mentor pollen" (Singsit and Hanneman Jr 1990). The "mentor pollen" fertilizes several ovules, stimulating the development of fruit and pollen tubes from the incompatible parent to reach the ovules and effect fertilization (Singsit and Hanneman Jr 1990; Yermishin et al. 2014). Pollen of Group Phureja pollinators are used as "mentor pollen" because of their typical dominant seed spot marker, so its offspring can be visually identified and eliminated (Brown and Adiwilaga 1991; Iwanaga et al. 1991a).

A feature of inter-EBN interspecific hybridization using self-compatible *S. verrucosum* as "bridge species" is male sterility in most resulting hybrids (Abdalla and Hermsen 1972–Abdalla and Hermsen 1973; Yermishin et al. 2014). Cytoplasmic male sterility factors (CMS) from *S. verrucosum* have been assumed to interact with dominant nuclear genes from the 1EBN male parents, resulting in male sterility of hybrids. CMS has also been suggested to account for male sterility of hybrids produced when 2*x* (2EBN) Group Tuberosum haploids were used as male parents in crosses with cultivated 2*x* (2EBN) Phureja and Stenotomum Groups (Grun et al. 1962; Ross et al. 1964; Carroll 1975) as well as with a wide range of wild species (Hermundstad and Peloquin 1985; Tucci et al. 1996; Santini et al. 2000). Male fertile hybrids obtained when the haploids were the male parent corroborated this assumption (Tucci et al. 1996; Novy and Hanneman 1991). Consequently, this type of barrier can be overcome by carrying out reciprocal crosses.

Male sterility of hybrids from *S. verrucosum* did not represent a drawback since crosses with Tuberosum haploids were successful when the (*S. verrucosum* × 1EBN) hybrids were used as females (Yermishin et al. 2014). However, male fertile Tuberosum haploids are not widely available (Makhan'ko 2008). Breeders may overcome this drawback by extracting haploids from 4*x S. tuberosum* cultivars or breeding clone bearing the male fertility dominant restorer gene *Rt* that gives fertility to plants that contain the dominant male sterility gene *Ms* in the presence of sensitive cytoplasm (Iwanaga et al. 1991b). Selection of Group Tuberosum haploids

carrying a restorer of fertility (*Rt*) gene can be used to pollinate (*S. verrucosum* × 1EBN) hybrids and produce male fertile hybrids for further backcrossing with cultivated potato.

An alternative appealing approach for creating opportunities to solve the problem of the prezygotic interspecific incompatibility with a number of 1EBN wild species was proposed by Polyukhovich et al. (2010). These investigators transferred the nonfunctional S-RNase *Sv* haplotype from self-compatible *S verrucosum* directly to Group Tuberosum haploids using some rare pollen receptive haploids. Further development of homozygote *SvSv* hybrids were achieved after selfing or sib-mating of F_1 self-compatible hybrids with high functional pollen fertility. These *SvSv* hybrids were identified based on their good penetration of pollen tubes in the styles of 1EBN *S. bulbocastanum* and *S. pinnatisectum* species. These *SvSv* Tuberosum hybrids may produce fertile hybrids in direct crosses with 1EBN wild diploid potato species.

According to available knowledge, 2*x* (2EBN) self-compatible *S. verrucosum*, which is used as a "bridge species", provides potato breeders an ideal route in germplasm enhancement aimed at introgressing valuable genes from 1EBN wild diploid *Solanum* species into breeding populations. Efficiency of hybrid production using self-compatible *S. verrucosum* is greater than other methods based on ploidy manipulations such as somatic hybridization or embryo rescue, which require considerable experience and expenditure of time and resources (Jansky 2006).

Group Tuberosum haploids are the most promising recipients for introgression of novel genes for traits of interest from diploid wild species germplasm (Peloquin et al. 1989; Jansky et al. 1990). Smaller population size in comparison with tetraploids is needed for selecting recombinants that meet breeding requirements due to their disomic inheritance. Accumulation of desirable genes and elimination of undesirable ones flows faster at this ploidy level. In addition, the presence of naturally occurring meiotic mutations in the potato gene pool, which leads to the production of 2*n* gametes, allows chromosome doubling for the transfer of valuable gene combinations to the tetraploid level (Carputo et al. 2000; Yermishin et al. 2014).

7.8 Endosperm Balance Number (EBN) and Interspecific Reproductive Barriers

Various genetic mechanisms account for the success or failure of seed development in flowering plants, which undergo double fertilization during sexual reproduction (Kinoshita 2007). Failure for producing triploids after tetraploid × diploid crosses—also known as "triploid block"—led to studying as a reproductive barrier the endosperm, which provides nourishment to the seed embryo. A first concept was to consider a 2:3:2 ploidy balance between maternal tissue, endosperm and embryo but further research demonstrated that normal endosperm development depends on having a 2:1 maternal to paternal genome dosage in the endosperm (Ehlenfeldt and

Ortiz 1995 and references therein). This endosperm dosage system for both intra- and inter-specific crossing seems to be multigenic in *Solanum* species, in which is known as the endosperm balance number (EBN) that also explains some aspects of species evolution therein. For example, Ortiz and Ehlenfeldt (1992) indicated the role of EBN in the origin of both diploid and polyploid potato species or how it becomes a hybridization barrier for speciation among sympatric *Solanum* species with same ploidy.

The EBN is a unifying concept that may predict endosperm function in intraspecific, interploidy, and interspecific crosses in potato and wild crop relatives (Johnston et al. 1980). Each species carries an EBN value that is constant across interspecific crossing, thus determining the effective ploidy in the endosperm, which must be in a 2 maternal:1 paternal ratio that is a necessary for successful endosperm development. The EBN, which is in itself an arbitrary value, is given to a species based on its crossing behavior with known EBN standards. The EBN does not reflect directly the ploidy of a species (Hanneman Jr 1999). For example, there are 2*x* (1EBN), 2*x* (2EBN), 3*x* (2EBN), 4*x* (2EBN), 4*x* (4EBN), 5*x* (4EBN), and 6*x* (4EBN) *Solanum* species.

The EBN concept was useful to elucidate the nature of the pollinator effect in haploid extraction (Peloquin et al. 1996). Haploid embryos appear to be associated with hexaploid endosperms as a result of having the union of 2-chromosome sets from the pollinator with the polar nuclei and lack of fertilization of the egg, thus having a 2 maternal: 1 paternal EBN in the endosperm that normal seed development requires.

The "triploid block" is a reproductive barrier resulting from endosperm malfunction due to the epigenetic phenomenon of genomic imprinting (Ehlenfeldt and Ortiz 1995; Köhler et al. 2009). Evidence shows that the endosperm dosage systems are imprinted within the gametes, thus the same gene being functionally different in maternal and paternal chromosomes. The maternally and paternally imprinted genes often carry a DNA methylation or histone modification in their vicinity (Kinoshita 2007). The position of these epigenetic modifications determines how the gene will be expressed; i.e., either from the maternal or paternal inherited allele (Köhler et al. 2012). Maternally imprinted genes appear to repress endosperm proliferation, which seems to be promoted by paternally imprinted genes. Abnormal endosperm development results from the imbalance of these imprinted genes. This "parental conflict" is consistent with the differential maternal and paternal genome effects in interploidy mating (Köhler et al. 2009). Products of imprinted PcG genes such as *Medea* (*MEA*) and *Fertilization Independent Seed2* (*FIS2*) could be limiting factors affecting endosperm and seed development. For example, FIS PcG proteins repress fertilization-independent seed formation and restrict endosperm proliferation (Köhler and Makaverich 2006).

The EBN provides useful prior knowledge for germplasm transfer from 2*x* (1EBN), 2*x* (2EBN), 4*x* (2EBN) and 6*x* (4EBN) species into 4*x* (4EBN) potato (Ortiz and Ehlenfeldt 1992). For example, chromosome engineering using 4*x* (2EBN) and 2*x* (2EBN) addition lines will allow to introduce specific chromosomes bearing the target allele into the 4*x* cultigen pool for further use in breeding.

Likewise, 2*x* chromosome addition lines from may result using 2*x* (1EBN) and 2*x* (2EBN) *Solanum* species. The first step will be to get a 3*x* (2EBN) interspecific hybrids after crossing both 2*x* species and thereafter using these hybrids by crossing them with 2*x* (2EBN) species to obtain 2*x* (2EBN) chromosome addition lines, which can be further used for breeding at 2*x* level. Haploids from 5*x* (4EBN) clones may be another method for producing 2*x* (2EBN) offspring with traits from the 2*x* (1EBN) parent because haploid extraction will allow screening for the right balance in the endosperm.

7.9 Trait Genetic Research: A Summary Prior to DNA Markers

Swaminathan and Howard (1953) provided the first summary of the inheritance of most important traits in potato, which was further updated by Howard (1960, Howard 1970), Bradshaw and Mackay (1994 and chapters therein), and Tiemens-Hulscher et al. (2013). Table 7.3 give some details about trait genetics and gene symbols as noted in some of these publications.

Further research using the candidate gene approach led to identifying diagnostic DNA-based markers for genes involved, *inter alia*, in quantitative resistance to late blight (*R1* gene family in chromosome V) or cyst nematode (major QTL in same resistance "hot spot" on potato chromosome V), and both chip color (e.g., co-localized with a cold-sweetening QTL in chromosome IX and other sugar QTL in chromosome X) and tuber starch content in tetraploid potato cultivars (Gebhardt et al. 2007). Other annotated loci in the potato genetic map are *Y* (yellow flesh color) in chromosome III, *H3* of *Gpa4* in chromosome IV, Rx_2 (extreme resistance to *Potato Virus X*) and Nx_{tbr} (hypersensitivity to *Potato Virus X*) in chromosome V, Nx_{phu} in chromosome IX, *Ro* (tuber shape) and anthocyanin (including flower and skin color) in chromosome X, Ry_{sto}, and *Ry-adg* in chromosome XI, and both *Gpa 2* (resistance to potato cyst nematode) plus Rx_1 in chromosome XII.

7.10 Cytogenetics for Crossing, Scaling Up and Down Ploidy, and Chromosome Engineering

Germplasm enhancement (mistakenly replaced by some as pre-breeding, Ortiz 2002) is the early component of sustainable plant breeding that includes identifying a useful character, "capturing" its genetic diversity, and putting those genes into a "usable" form (Peloquin et al. 1989). Potato provides an interesting example of using crop wild relatives in germplasm enhancement. The wild species *S. demissum* (6*x*, 4EBN), *S. stoloniferum* (4*x*, 2EBN), and *S. vernei* contributed host plant resistance genes to late blight, *Potato Virus Y*, and cyst nematode, respectively; while

Table 7.3 Gene symbols and their genetics of key traits for potato breeding

Trait	Gene symbol	Genetics (disomic inheritance unless indicated otherwise)
Plant growth type		Upright dominant to prostrate, and intermediate procumbent recessive to upright but unknown to prostrate
Dwarfism		Recessive phenotype producing compact, dark green, rosette plant
Flower color	*D* red	*F* involved in anthocyanin expression in flowers
	P blue	Purple = *D_P_F_*, Red to rose *D_ppF_*, Blue = *ddP_F_*, White = *D_P_ff* or *ddppF_*
Skin color	*D* red	*I* engaged in anthocyanin expression in tuber skin
	P blue	Purple = *D_P_I_*, Red *D_ppI_*, Blue = *ddP_I_*, White to yellow to brown = *D_P_ff* or *ddppF_*
Flesh color	*Y* yellow, *y* white	Yellow caused by carotenoids dominant to white
	Or allele at *Y* locus	Orange depends on two genes: one determining production and other (recessive) accounting for accumulation of zeazhantin. Orange dominant to yellow
	D red	Purple or red due to anthocyanins regulated by *B*.
	P blue	Purple = *D_ P_ B_*, Red = *D_ pp B_*, Blue = *dd P_ B_* (2*x*)
Tuber color pattern		Splashed and spotted only in eyes while speckled (dominant) reverses; i.e., eyes are either white or yellow
Tuber shape	*Ro* round	Round (*Ro__*) dominant to long (*roro*)
	ro long	
Russet skin		Three independent loci having an additive effect to each other; i.e., *AABBCC* show more russet skin that *AaBbCc*
Stem pubescence		Single gene, being pubescent dominant to glabrous stem
Early maturity		Earliness due to dominant allele with additive effects; i.e., duplex (*AAaa*) earlier than simplex (*Aaaa*) in 4*x*
Tuber dormancy		Dominant early sprouting with short dormancy
Tuberization under long days		High heritability but influenced by day length, light intensity, and temperature

(continued)

Table 7.3 (continued)

Trait	Gene symbol	Genetics (disomic inheritance unless indicated otherwise)
Chip color		3-Gene hypothesis for both reversion resistance (ability to produced light-colored chips after harvest or short storage) and reconditioning (controlled tuber warming after storing fall crop for 4–6 months) to eliminate reducing sugars: A dominant allele in each of three loci for good chipping, being one or two loci common to both traits
Late blight resistance	12 known *R* genes derived from *S. demissum, Rpi-blb3* from *S. bulbocastanum*, and *Rpi-abpt* derived from quadruple hybrid involving *S. acaule*, *S. bulbocastanum*, plus groups Phureja and Tuberosum	Dominant *R* for race-specific host resistance corresponding to virulence genes in oomycete *Phytophthora infestans* (*R8* and *R9* seem to be "durable")
		Many genes are involved in partial host plant resistance that slow down the development of all *Phytophthora infestans* races, thus being likely more durable than race-specific resistance
Early blight resistance		High heritability for partial resistance, thus additivity being the most important gene action
Potato leaf roll virus resistance	N_L for hypersensitivity	Dominance of resistance but major gene does not provide enough durable resistance to a cultivar
Potato virus X resistance	Rx_i for extreme resistance, while Nx_i controls hypersensitivity	Dominant inheritance
Potato virus Y resistance	Ny_{chc}, Ny_{dms}, $Ry_{sto}{}^{n1}$ and $Ry_{sto}{}^{n2}$ gives hipersensitivity, whereas *Ry*-adg, *Ry-chc*, *Ry-hou* and *Ry-sto* are for extreme resistance	Dominant extreme resistance from *S. stoloniferum*, *S. hougasii,* and Group Andigena, while multigenic for Group Phureja
Potato virus A	*Na* protects for infection through hypersensitivity	
Wart resistance		Dominant alleles in two loci necessary for resistance
Verticillium wilt		2 genes
Bacterial wilt resistance		3–4 dominant genes required
Black leg and bacterial soft rot resistance		Minor genes with additive effects increase resistance
Potato tuber moth resistance		Simple inheritance due to additivity

(continued)

Table 7.3 (continued)

Trait	Gene symbol	Genetics (disomic inheritance unless indicated otherwise)
Root-knot nematode resistance		High narrow-sense heritability for resistance in Group Phureja (susceptible)—*S. sparsipilum* (likely three major complementary genes in heterozygous state) segregating population, but reciprocal effects suggest maternal effects of *S. sparsipilum* or cytoplasmic-nuclear gene interaction
Potato cyst nematode resistance	*H1* (from Group Andigena), *H2* (from CPC2602), *Fa* and *Fb* (from *S. spegazzinii*), plus *B* and *C* from *S. verneii* provide host plant resistance against *Globodera rostochiensis*, while *Gpa2*, *Gpa5*, *GPa1, H2,* and *H3* of *Gpa4* give resistance against *G. pallida*	*H1* gives host plant resistance against Ro-1 and Ro-4 pathotypes

processing quality was derived from S. *chacoense* (2*x*, 2EBN) in various cultivars (Jansky et al. 2013).

Introgression and incorporation are the two approaches for using wild species in plant breeding (Simmonds 1993). Introgression refers to transferring one or a few alleles from wild germplasm to breeding populations that lack them, while a large-scale program for developing breeding populations using wild germplasm to broaden its genetic base is known as incorporation. Figure 7.2 illustrates both in potato breeding. "Bridge" species, double pollination, embryo rescue, 2*n* gametes, and the EBN knowledge allows using chromosome engineering, while haploids, wild 2*x* (2EBN) species, and 2*n* gametes are used in ploidy manipulations.

CIP released in the mid-1990s diploid bred-germplasm generated from using haploids from tetraploid cultivars and breeding clones, plus diploid landraces and wild species (Watanabe et al. 1994). These genetic resources are of value for potato breeding due to their genetic diversity, crossability with the 4*x* cultigen pool facilitated by 2*n* gametes (mostly FDR 2*n* pollen), and high host plant resistance to pathogens and pests derived mostly from wild relatives. Ploidy manipulations at CIP led to the transmission of host plant resistance to cyst and root-knot nematodes, bacterial wilt, early blight, and potato tuber moth, as well as producing high-yielding 4*x* (4EBN) breeding clones also having yield stability over environments (Ortiz et al. 1994). Burundi released in the 1990s the tetraploid potato cultivar "Nemared," which derived from this diploid breeding population (Fig. 7.3), because of both its host plant resistance to root knot nematode and desired agronomic traits.

Potato, as shown above, is the model crop species for germplasm enhancement of polysomic polyploids. Crop wild relatives and landraces are the diversity sources, while haploid derived from the tetraploid cultigen pool "capture" this diversity after crossing them with the former. The haploid-species hybrids producing 2*n* gametes

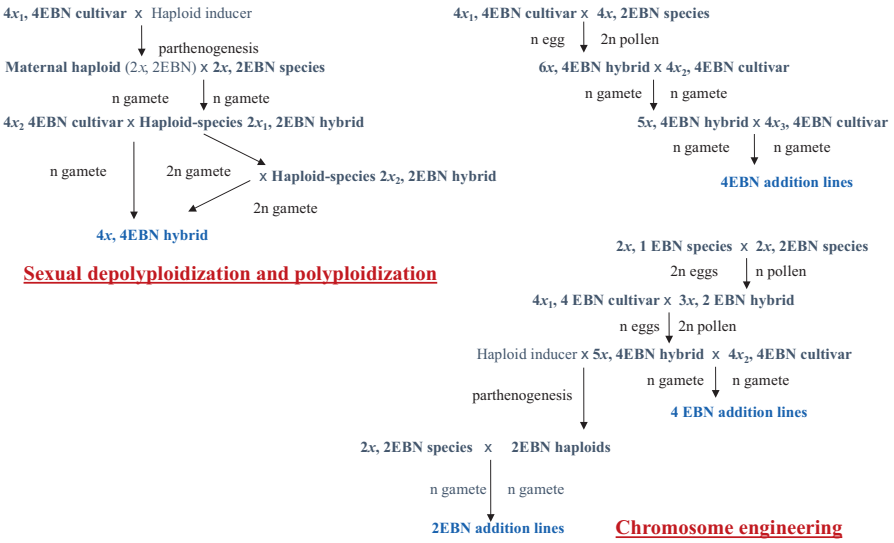

Fig. 7.2 Germplasm enhancement approaches in potato breeding: introgression through chromosome engineering and incorporation using sexual depolyploidization and polyploidization

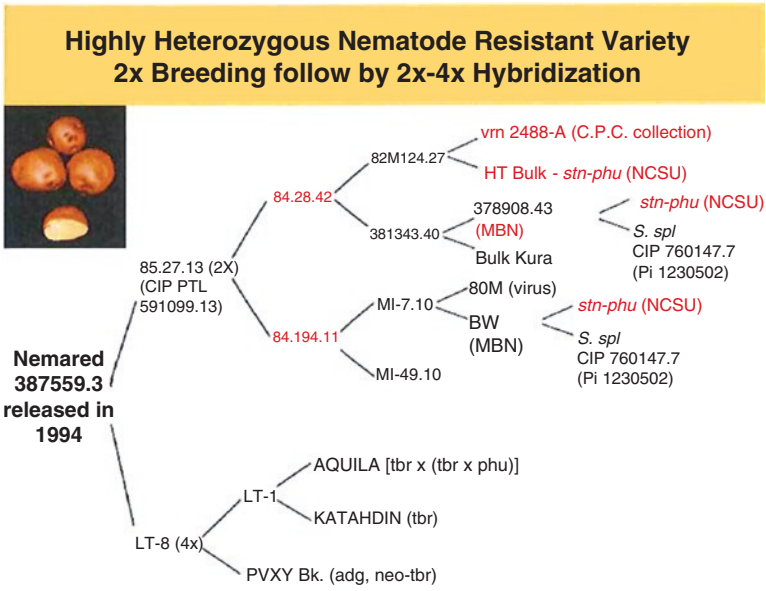

Fig. 7.3 Pedigree of tetraploid cultivar potato "Nemared" resulting from ploidy manipulations

transfer this diversity to the tetraploid breeding pool through sexual polyploidization in which the EBN ensures the resulting ploidy of the hybrid offspring.

7.11 Concluding Remarks

Despite its the biological characteristics which made genetic improvement more complex in potato than in other crops, potato breeders have at their disposal such a powerful and effective approach as the unique ability to conduct across ploidy and species-wide crosses in order to introgress relevant genetic variation from its genetic resources into potato breeding programs. Progress in the field of cytogenetics of potato enables a more effective transfer of relevant genetic variation from wild *Solanum* accessions kept in genebanks or through in situ approaches. The increasing demands to develop resilient potato varieties able to withstand the threats brought by climate change, as well as the substantial progress recently achieved with the development of potato hybrid cultivars at the 2*x* level, underpin the increasing contribution of cytogenetics to the genetic improvement of the potato crop. The increasing availability of vast amounts of genomic information, as well as the continuous reduction of expenses associated with the sequencing of whole genomes are expected to further increase the contribution of potato cytogenetics, in terms of accelerating the development of varieties which provide not only superior adaption to changing production environments but also increased food and nutrient security to the millions of people to whom potato represents a staple crop.

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