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Discovery of a synaptic mutant in potato haploids and its usefulness for potato breeding

M. Iwanaga
International Potato Center (CIP), P.O. Box 5969, Lima, Perú

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Summary. A synaptic mutant was found in haploids (2n = 2x = 24) extracted from the Mexican potato variety 'Atzimba' (2n = 4x = 48). The mutant is inherited as a simple Mendelian recessive, designated *sy4*. Meiotic abnormalities of the mutant during microsporogenesis include: poor synapsis at pachytene; high frequency of univalents at diakinesis; elongated and curved spindles and univalents being scattered over the spindles at metaphase I and anaphase I; abnormal chromosome distribution at anaphase I; and production of sterile pollen, presumably due to unbalanced chromosome complement. The expression of *sy4* in megasporogenesis was also detected. The *sy4* mutant is very useful for potato breeding when combined with another meiotic mutant, parallel spindles (*ps*), because haploids homozygous for *sy4* and *ps* produce fertile 2n pollen which transmit almost intact genotypes of the parents to the progenies. Thus, the meiotic mutants provide a powerful breeding method for maximizing heterozygosity and epistasis. They can also provide a very efficient method of transferring diploid germplasm, which has desired characteristics efficiently combined at the 2x level, to tetraploids. Many haploids have been identified with 2n pollen production by *ps* alone or by *sy4* and *ps*, vigorous growth and good flowering, and a high level of resistance to late blight. The importance of a further search for meiotic mutants and their use for breeding is discussed.

Key words: Potato – Haploids – Synaptic mutant – Parallel spindles – 2n gametes

Introduction

The terms asynapsis and desynapsis have been used to describe pairing failure during meiotic prophase. Mutant genes that influence the initial pairing of chromosomes are referred to as asynaptic, whereas those that alter the maintenance of pairing between synapsed chromosomes are designated as desynaptic. To differentiate between these two conditions, detailed analyses of early prophase stages are required in conjunction with analyses of later stages of the first meiotic division. This distinction, although clearly important, is often difficult to make in practice since early prophase stages of meiosis are often indistinct and unresolvable in many species. Thus, Riley and Law (1965) proposed an alternative term, synaptic, to describe the activities of major genes that influence the extent of meiotic pairing.

Three factors provide unusual opportunities for potato improvement: 1) The wild and cultivated tuber-bearing relatives of the potato represent a large source of valuable germplasm, which is valuable in providing both specific desirable traits, such as disease and insect resistance, and for broadening the genetic base. 2) Haploids (2n = 2x = 24) of cultivars (2n = 4x = 48) can be readily obtained and used (Hougas and Peloquin 1958). They offer the advantages of simpler inheritance patterns (disomic vs. tetrasomic) and, more important, a direct approach to germplasm transfer from the numerous 24-chromosome, tuber-bearing relatives of the potato. They also provide us with a unique means of capturing genetic diversity. 3) The discovery of meiotic mutants that give rise to 2n gametes (gametes with the same chromosome number as the parent) provides unique and exciting opportunities to increase yield and genetic diversity. It is estimated that 2n gametes formed by First Division Restitution (FDR) transfer intact 80% of the genotype of diploids to their tetraploid progeny in 4x–2x and 2x–2x crosses (Peloquin 1979). The meiotic mutant, parallel spindles (*ps*), is an FDR mechanism that accomplishes this transfer in the production of 2n pollen (Mok and Peloquin 1975).

The potato breeding approach using species, haploids, and 2n gametes has become more significant since the use of a synaptic mutant combined with parallel spindles was proposed by Okwuagwu and Peloquin (1981). A synaptic mutant affects synapsis in microsporogenesis, resulting in mainly univalents at metaphase I and high male sterility. However, when a synaptic mutant is combined with the parallel spindles mutant, fertile 2n pollen is produced. The genetic significance of this combination of meiotic mutants is that it makes possible an increased level of heterozygosity that can be transmitted to the offspring of the diploids. If a synaptic mutant is completely asynaptic (i.e., no pairing and no crossing over), a truly exceptional opportunity is possible – transmitting 100% of the heterozygosity and epistasis of the parent to the offspring. Even though a synaptic mutant is only partially asynaptic, a higher transmission rate of the heterozygosity of the diploids than that of FDR 2n pollen produced by normal parallel spindles can be achieved. Thus, the meiotic mutants provide a powerful breeding method that maximizes heterozygosity and epistasis, and transfers diploid germplasm, which has desired characteristics efficiently combined at the 2x level, to tetraploids.

A new synaptic mutant was found in haploids extracted from the Mexican variety 'Atzimba'. This paper describes cytology and inheritance studies of the new mutant, which is combined with *ps*, also found in haploids. A discussion on the use of this mutant in potato breeding is also presented.

Materials and methods

Extraction of haploids from 'Atzimba' was done by 4x–2x crosses, using a diploid clone, IvP35, which was reported to be a good haploid inducer with an embryo spot marker (Hermsen and Verdenius 1973). The seeds obtained from the crosses were checked for embryo spot. Observation of general morphology of seedlings and counting of chloroplast number of stomata were used to eliminate triploid and tetraploid seedlings grown from embryo-spotless seeds. More than 500 haploid seedlings were grown in pots and used for cytological and crossing studies.

Pollen samples were prepared by the method of Marks (1954) and checked for pollen stainability and 2n pollen production. Microsporogenesis was studied by normal aceto-carmine squash, and megasporogenesis by paraffin sections using the staining method of Gerlach (1969).

Results

Studies of pollen samples identified three interesting groups of haploids with different types of pollen. The first group of haploids had high stainability and 2n pollen production (Fig. 1 a), which was due to parallel orientation of spindles at metaphase and anaphase II. The second group had very low stainability, and pollen size was highly variable (Fig. 1 b). In the third group, all stainable pollen grains were of 2n size, and pollen grains of other sizes were nonstainable (Fig. 1 c). Cytological analysis of microsporogenesis of the last two haploid groups revealed that meiotic abnormalities due to poor synapsis were responsible for producing the two types of pollen.

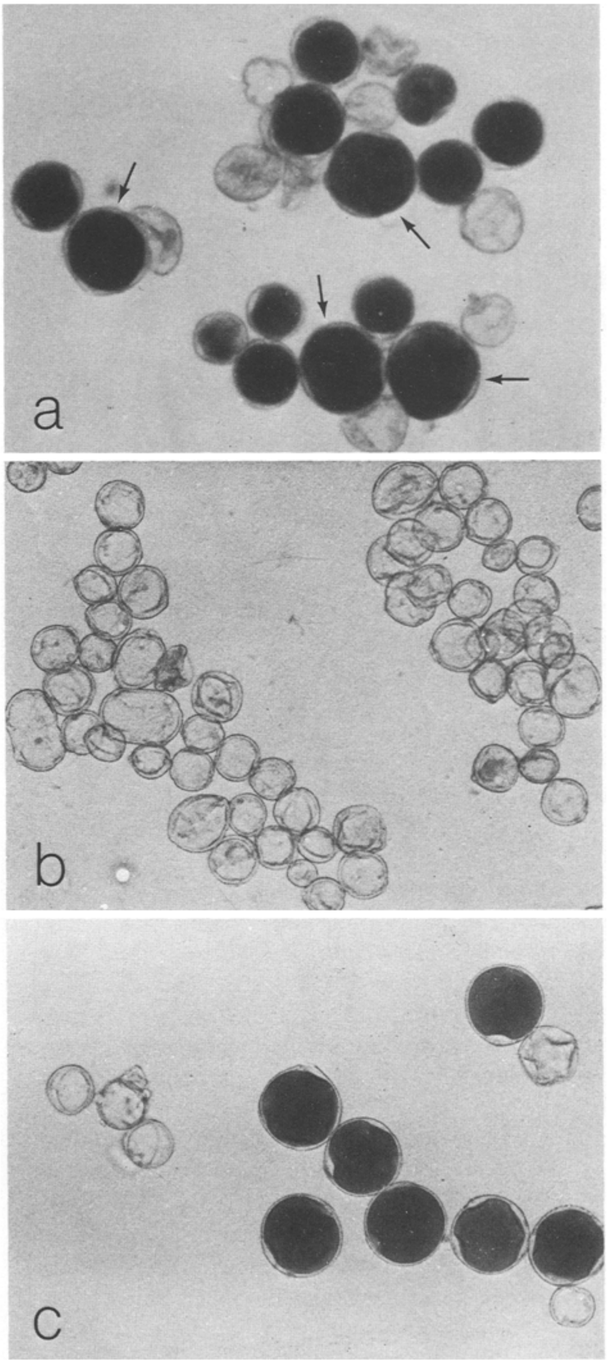

Fig. 1 a–c. Three types of pollen grains found in the haploids. **a** High stainability and 2n pollen grains (*arrows*) along with normal size pollen; **b** sterile pollen grains of different sizes; **c** all stainable pollen grains are of 2n size

Cytological description of the synaptic abnormalities

1 Pachytene. In this stage, the degree of pairing varied among the cells (Fig. 2 a), and there were some cells without any indication of pairing failure. In other cells

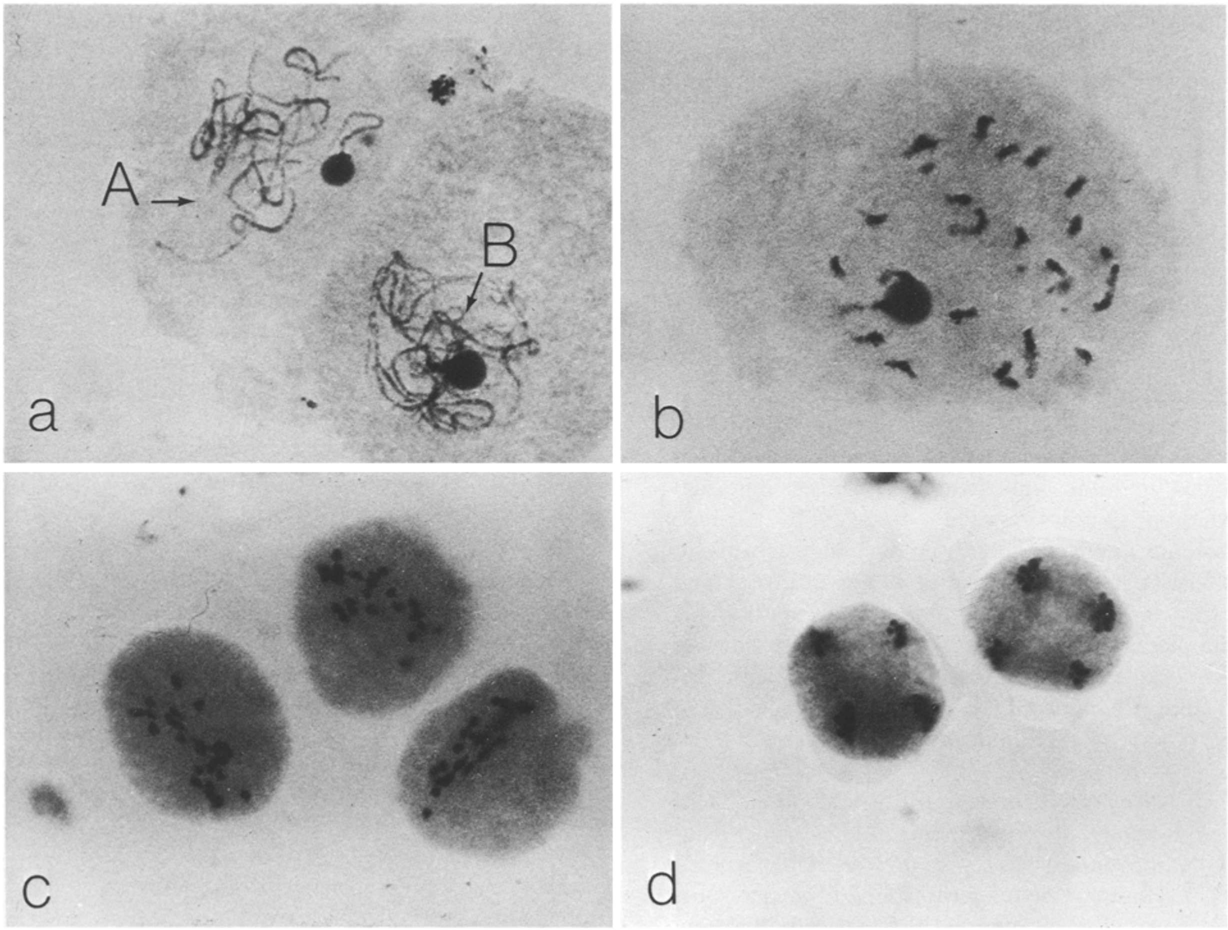

Fig. 2a–d. Microporogenesis in *sy4* plants. **a** Pachytene stage showing one cell (*A*) with normal pairing and the other (*B*) with poor pairing; **b** mostly univalents at diakinesis; **c** meiocytes with many univalents at metaphase I; **d** anaphase II showing parallel orientation of spindles

some chromosomes were completely paired and other chromosomes had unpaired portions. Often chromosomes were paired in the middle but unpaired toward the ends. Because of the inherent difficulties of pachytene analysis in potatoes, the cells were simply classified into five levels of pairing in order to get some estimate of degree of asynapsis (Table 1). Of 49 meiocytes studied, 90% had some degree of pairing failure.

Table 1. Classification of meiocytes into 5 levels of pairing at pachytene

Level of pairing	No. of meiocytes
(1) Normal pairing	5 (10%)
(2) No pairing in a few chromosomes	20 (40%)
(3) No pairing in many chromosomes	16 (32%)
(4) Some pairing in a few chromosomes	8 (16%)
(5) No pairing	0 (0%)
	49

Although it is clear that the mutant has an asynaptic effect (reduction of chromosome pairing and subsequent reduction of crossing-over), exact quantification of degree of the asynapsis was technically impossible.

2 Diakinesis. Most chromosomes were present as univalents at this stage (Fig. 2b). Average frequencies of univalents and bivalents of 59 cells, which were analyzed in a single plant, were 18.27 and 2.86, respectively. Almost all of the bivalents had only one chiasma.

3 Metaphase I and anaphase I. Metaphase I and anaphase I could not be clearly distinguished from one another, as there was no clear equatorial orientation of univalents, which remained scattered in the spindles (Fig. 2c). The spindles were elongated and often curved. Distribution of univalents to each pole seemed to be at random, resulting in abnormal chromosome number distribution at the end of anaphase I. Lagging chromosomes were common, but no indication of precocious chromatid separation of univalents was observed.

4 Second division. a) Plants with nonstainable pollen of varied size: second division of this group of plants was normal except for irregularities probably due to disturbance at the first division.

b) Plants with stainable 2n-size pollen and nonstainable pollen of other sizes: in this group of plants, many meiocytes had unusual orientation of spindles at metaphase II and anaphase II. Instead of being oriented at 60°, the spindles had parallel orientation (Fig. 2d). The result was production of dyads with presumably unreduced chromosome number. Thus, abnormal orientation of spindles resulted in recovery of balanced chromosome number and subsequent male fertility in the otherwise male sterile plants. It was also observed that expression of parallel orientation of spindles was not complete. Meiocytes with normal orientation of spindles resulted in production of sporads with different sizes, which would develop into sterile pollen because of abnormal chromosome complement.

5 Megasporogenesis. Female sterility was observed in the synaptic plants during crossing studies, which suggested that the mutant was also expressed in megasporogenesis. Preliminary cytological study of megasporogenesis in two clones supported the expectation. Many abnormalities, common in synaptic mutants, were observed which included elongated spindles, many univalents being scattered over the spindle, and abnormal chromosome distribution.

Inheritance of the synaptic mutant and estimated genotypes of Atzimba for the synaptic mutant and parallel spindle mutant (ps)

A cytological study of the first meiotic division of 53 haploids extracted from 'Atzimba' resulted in identification of 28 normal and 25 mutant plants. This fits well to a 1 : 1 segregation, which indicates that the mutant is a single recessive and that 'Atzimba' is simplex for the mutant. It is proposed that the symbol *sy4* (synaptic mutant No. 4) be used to designate the controlling synaptic abnormalitiy in the haploids. Thus, the genotype of 'Atzimba' is *Sy4sy4sy4sy4,* and 50% of the haploids from 'Atzimba' are *Sy4sy4* (normal) and 50% are *sy4sy4* (synaptic mutant).

A meiotic mutant, *ps,* is responsible for the parallel orientation of spindles at metaphase II and anaphase II and subsequent production of 2n pollen (Mok and Peloquin 1975). Therefore, haploid plants producing 2n pollen by parallel spindles should be *psps.* From an analysis of 94 haploids, a ratio of 44 (normal) : 49 (parallel spindles) was observed, which fits well the expected ratio of 1 : 1 in haploids extracted from a simplex (*Pspspsps*) tetraploid. Thus, the genotypes of 'Atzimba' are expected to be *Sy4sy4sy4sy4* and *Pspspsps.*

Discussion

Nature of sy4

1 Inheritance. Koduru and Rao (1981) listed synaptic mutants that have been reported in 126 species belonging to 93 genera of higher plants. In most of the synaptic mutants investigated, monogenic recessive inheritance was observed. The *sy4* mutant also showed monogenic recessive inheritance. This hypothesis is further supported by the finding of synaptic mutant haploids extracted from two hybrid tetraploids whose female parent was Atzimba (Iwanaga, unpublished data). Three synaptic mutants have been reported in potatoes. Since *sy4* was found in a different genetic background than those of the earlier mutants, it is assumed that *sy4* is a different mutant. It has not been determined, however, if *sy4* is allelic to any of the mutants.

2 Genetic consequence of sy4. The disturbance of chromosome pairing observed at pachytene clearly indicates that *sy4* reduces chromosome pairing and subsequent genetic recombination to a certain degree. It is important to characterize the extent of this reduction because it determines the gametic array and level of heterozygosity to be transmitted to the progeny. The following questions need to be studied: 1) Does *sy4* affect recombination rates equally in all chromosomes? 2) What is the exact level of reduction of recombination rate? 3) Do genetic backgrounds of *sy4* mutants affect the action of *sy4?* and 4) Do nongenetic factors affect expression of *sy4?* It is clear that all four questions cannot be answered by cytological study alone. Along all chromosome arms, many marker genes with known location are necessary to answer the questions.

Production of 2n pollen by *ps* in the haploids makes it possible to do gene centromere mapping by 4x–2x matings (Mendiburu and Peloquin 1979). Use of electrophoretic variants as new markers would facilitate the mapping study (Mok and Peloquin 1982) and subsequent analysis of the genetic consequence of *sy4.*

Combined use of sy4 and ps for potato breeding

The *sy4* mutant alone is not useful for potato breeding because it causes sterility. Abnormal chromosome distribution at anaphase I caused by *sy4* normally results in production of sterile pollen with unbalanced chromosome complement. When *sy4* is combined with *ps*, it produces fertile pollen. Figure 3 illustrates four types of microsporogenesis: 1) normal, 2) *psps*, 3) *sy4sy4*, and 4) *sy4sy4, psps*. First, normal microsporogenesis results in production of a tetrahedron with each microspore having 12 chromosomes. A *psps* plant has parallel orientation of spindles at metaphase II and anaphase II, which causes production of a dyad with 24 chromosomes. In a *sy4sy4* plant, poor synapsis at prophase I results in abnormal chromosome distribution at anaphase I, such as 15-9 instead of normal 12-12 as illustrated in the figure. Two microspores with 15 chromosomes and the other two with 9 chromosomes are produced at the end of second division: all four are sterile because of unbalanced chromosome complements. Finally, a mutant plant homozygous for both *sy4* and *ps* has abnormal chromosome distribution at anaphase I as do *sy4* plants. However, parallel orientation of spindles at metaphase II and anaphase II due to *ps* produces a dyad, each having 24 (15 plus 9) chromosomes and normal fertility. Expressivity of *ps* is not complete and a certain portion of meiocytes do not have parallel spindles, which results in production of meiocytes with unbalanced chromosome sets. Thus *sy4sy4, psps* plants have stainable 2n pollen as well as nonstainable pollen with different sizes reflecting different chromosome number included in the pollen. The result is that the only functional pollen produced by the *sy4sy4 psps* plant is 2n.

Table 2. Average transmission rates of heterozygosity as a function of chiasma frequency

Class	Chiasmata/ meiocyte	Average heterozygosity (%)[a]
I	18	82
II	15	85
III	12	88
IV	9	91
V	6	94
VI	3	97
VII	0	100
Tetrasomic inheritance		62[b]

[a] Each value refers to the average percentage of heterozygosity which is transmitted to the 4x progeny from a highly heterozygous diploid

[b] This value refers to the average percentage of heterozygous loci in 2x pollen produced by normal tetrasomic inheritance of a tetraploid which is duplex for all loci

The advantage for breeding using *sy4* is determined by frequency of crossing-over. Thus, expected rates of heterozygosity to be transmitted were calculated as a function of frequency of chiasma (i.e., frequency of crossing-over), assuming that normal diploid potatoes (2n = 2x = 24) have six bivalents with two chiasmata and six with only one chiasma: 18 chiasmata per meiocyte in total (Table 2). After normal pairing (i.e., 18 chiasmata/meiocyte), 82% of the heterozygosity of the parent can be transmitted to 4x progeny via 2n pollen by *ps* when crossed with tetraploids (Case I). This value is much better than that of normal tetra-

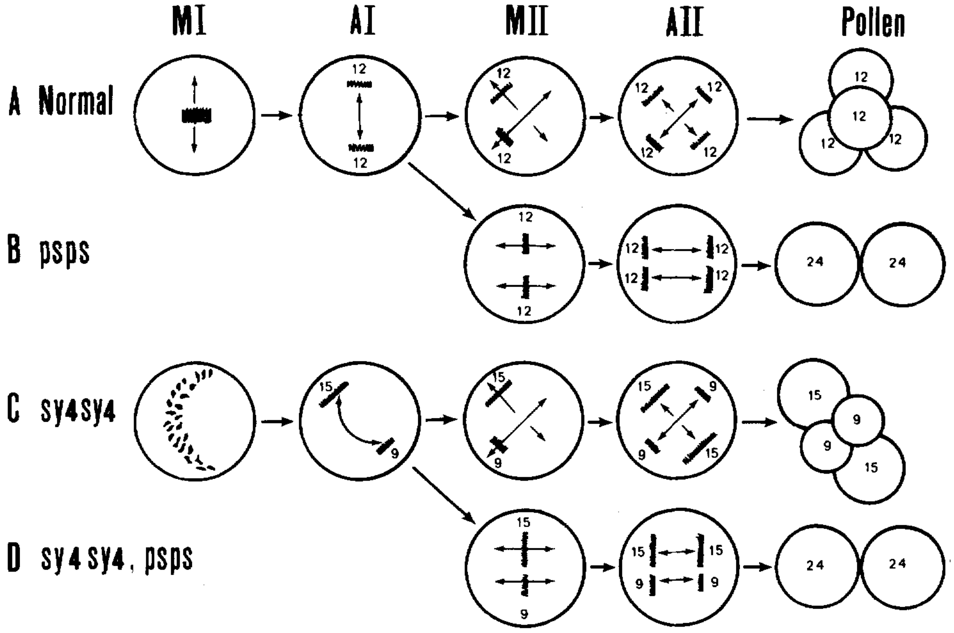

Fig. 3. Sequence of microsporogenesis in four different genotypes

somic inheritance, since only 62% of the heterozygosity of a tetraploid, duplex for all loci, can be transmitted by normal tetrasomic inheritance. If there is no crossing-over (Case VII) due to complete asynapsis, 100% transmission is obtained. This means that 2n pollen produced without crossing-over is genetically identical to the parent. Any reduction of chiasma frequency due to partial asynapsis (Case II–VI) can result in increasing the transmission rate. What would be the expected transmission rate for *sy4*? If the chiasma frequency observed at diakinesis of *sy4* (i.e., about 3 chiasmata/meiocyte) represents the real frequency of crossing-over, 97% transmission of heterozygosity is achieved in *sy4* mutants. It seemed, however, that at pachytene, which is the most crucial stage for estimating frequency of crossing-over, there was more chromosome association than at diakinesis. Probably, some paired chromosomes became unpaired during diplotene and diakinesis; thus the real transmission rate of *sy4* would be lower than 97%. Nevertheless, *sy4* is useful in increasing the transmission rate.

The combined use of the mutants has immediate application not only for clonal selection but also for use of true potato seed (TPS) for ware potato production. The use of TPS instead of traditional seed tubers for commercial potato production has been a major research objective at the International Potato Center (CIP) and other institutions (Accatino 1979; Li and Shen 1979; Mendoza 1979; Peloquin 1979; Upadhya 1979). One of the potential disadvantages of TPS would be phenotypic segregation of TPS progeny. The problem can be minimized by using the mutants, because segregation of the progeny from 4x–2x (*sy4sy4, psps*) matings should be much less than that of regular 4x–4x progeny, due to heterozygous but homogeneous 2n pollen. Since the mutant plants produce only functional 2n pollen, higher seed set is expected from 4x–2x (*sy4sy4, psps*) crosses than from 4x–2x (*psps*) crosses, which is an additional advantage of using *sy4*.

It is possible to get the nuclear equivalent of somatic cell hybridization by sexual union of female and male gametes which are produced by meiotic mutants (Peloquin 1983) – genetically identical 4x hybrids with maximum heterozygosity can be produced by 2x–2x crosses. Two types of unrelated 2x hybrids are needed to derive maximum benefits from this approach. One 2x hybrid must produce highly heterozygous male gametes that are all of the same genotype. The combination of a synaptic mutant with complete asynapsis and *ps* accomplishes this goal. The other 2x hybrids must produce a high frequency of 2n eggs, all of the same genotype and highly heterozygous. This high frequency would occur either through apospory – the development of the female gametophyte from a somatic cell – or via a meiotic sequence, with no crossing-over followed by FDR (Iwanaga and Peloquin 1979, 1982). In both types of 2x hybrids, all of the 2n pollen and 2n eggs would have the same genotypes as their respective sporophytes. It is important to point out that this sexual approach using meiotic variants probably could be accomplished in less time and for a fraction of the research investment needed to obtain similar results with somatic cell fusion (Peloquin 1982).

It seems that synaptic mutants are rather common in potatoes: Iwanaga and Peloquin (1979) first reported a synaptic mutant (*sy1*) and emphasized the importance of a further search for meiotic mutants for potato breeding. Since then, three new synaptic mutants, *sy2* (Johnston et al. 1981), *sy3* (Okwaugwu and Peloquin 1981) and *sy4* (present report) have been reported. It is worth mentioning that all the mutants were discovered accidentally. Therefore, it is reasonable to assume that an intensive search for synaptic mutants would provide fruitful discoveries. A synaptic mutant with stable and complete asynapsis is highly desirable.

Finding the *ps* gene in the haploids was not unexpected. Most tuberosum cultivars are either simplex (*Pspspsps*) or duplex (*PsPspsps*), and gene frequency of *ps* in tuberosum cultivars as a group is estimated to be as high as 0.69 (Iwanaga and Peloquin 1979). Thus, many haploids extracted from these tetraploids are expected to produce 2n pollen by *ps*, if they are male fertile.

Use of the haploids for breeding

It was fortunate to find two meiotic mutants (i.e. *ps* and *sy4*) together in haploids with immediate breeding value. Pérez-Ugalde and Peloquin (1967) reported that 'Atzimba' haploids showed high levels of field resistance to late blight (*Phytophthora infestans*) in the Toluca Valley, in Mexico, where the sexual cycle of *P. infestans* and heavy epiphytotics occur. The majority of 'Atzimba' haploids were attractive, vigorous, flowered profusely, and hybridized with other 24-chromosome selections. The high level of resistance has been confirmed by further evaluation over six seasons in the Toluca Valley (Villarreal and Rivera, personal communication).

Our studies at CIP also support the report described above. Among the more than 4,000 haploids obtained at CIP, the Atzimba haploids are outstanding in plant vigor. Moreover, they flower well even under the short-day conditions in Peru, which facilitates crossing work. Preliminary evaluation of the haploids by artificial inoculation in an environment-controlled greenhouse and by natural infection in a CIP field in Peru also showed that some of the haploids with 2n pollen production have a level of resistance that is the highest among the haploids extracted from many late blight resistance cultivars (Henfling et al., unpublished data).

Selected haploids are being intermated to get a 2x population with increased level of resistance and with 2n pollen production by *ps* alone or by *ps* and *sy4*. They will be crossed with 4x female parents to transfer the high level of resistance, selected efficiently at the 2x level, to 4x progeny. Furthermore, male fertile haploids are being crossed with other selected diploids and haploids to combine resistance and 2n pollen production.

References

Accatino P (1979) Agronomic management in the utilization of true potato seed: preliminary results. In: International Potato Center Report of the Planning Conference on the production of potatoes from true seed. Manila, Philippines, pp 61–99

Bedi AS, Smale P, Burrows P (1979) Experimental potato production in New Zealand from true seed. In: International Potato Center Report of the Planning Conference on the production of potatoes from true seed. Manila, Philippines, pp 100–116

Gerlach D (1969) A rapid safranin-crystal violet-light green staining sequence for paraffin sections of plant materials. Stain Technol 44:210

Hermsen JGTh, Verdenius J (1973) Selection from *Solanum tuberosum* group Phureja of genotypes combining high-frequency haploid induction with homozygosity for embryo-spot. Euphytica 22:244–259

Hougas RW, Peloquin SJ (1958) The potential of potato haploids in breeding and genetic research. Am Potato J 35:701–707

Iwanaga M (1982) Potato breeding for combined resistance to tropical pests by the ploidy level manipulation approach: progress in extraction of haploids with male fertility and 2n pollen. In: 6th Symp Int Soc Tropical Root Crops (in press)

Iwanaga M, Peloquin SJ (1979) Synaptic mutant affecting only megasporogenesis in potatoes. J Hered 70:385–389

Iwanaga M, Peloquin SJ (1982) Origin and Evolution of cultivated tetraploid potatoes via 2n gametes. Theor Appl Genet 61:161–169

Iwanaga M, Peloquin SJ (1983) Genetic and cytological evidence for FDR and SDR 2n egg formations in diploid potatoes. Euphytica

Iwanaga M, Peloquin SJ (1983) Apospory-implications for potato research. Euphytica

Johnston SA, Hanemann RE Jr, Rhude RW (1981) Identification of an asynaptic variant in *Solanum commersonii.* Am Potato J 58:506

Koduru PRK, Rao MK (1981) Cytogenetics of synaptic mutants in higher plants. Theor Appl Genet 59:197–214

Li CH, Shen CP (1979) Production of marketable and seed potatoes from botanical seed in the People's Republic of China. In: International Potato Center Report of the Planning Conference on the production of potatoes from true seed. Manila, Philippines, pp 21–28

Marks GE (1954) An acetocarmine glycerol jelly for use in pollen fertility counts. Stain Technol 29:277

Mendiburu AO, Peloquin SJ (1979) Gene-centromere mapping by 4x–2x matings in potatoes. Theor Appl Genet 54: 177–180

Mendoza HA (1979) Preliminary results on yield and uniformity of potatoes grown from true seed. In: International Potato Center Report of the Planning Conference on the production of potatoes from true seed. Manila, Philippines, pp 156–172

Mok DWS, Peloquin SJ (1975) Three mechanisms of 2n pollen formation in diploid potatoes. Can J Genet Cytol 17:217–225

Mok IG, Peloquin SJ (1982) Sexual polyploidization and protein diversity in potatoes. Am Potato J 59:480

Okwuagwu CO, Peloquin SJ (1981) A method of transferring the intact parental genotype in the offspring via meiotic mutants. Am Potato J 58:512–513

Peloquin SJ (1979) Breeding method for achieving phenotypic uniformity. In: International Potato Center Report of the Planning Conference on the production of potatoes from true seed. Manila, Philippines, pp 151–155

Peloquin SJ (1982) New approaches to breeding for the potato of the year 2000. In: Proc CIP Decennial Anniversary, research for the potato in the year 2000, pp 32–34

Pérez-Ugalde G, Peloquin SJ (1967) Potato haploids with field resistance to late blight. Am Potato J 44:339–340

Riley R, Law CN (1965) Genetic variation in chromosome pairing. Adv Genet 13:57–107

Upadhya MD (1979) Potential for potato production from true seed under developing countries' conditions. In: International Potato Center Report of the Planning Conference on the production of potatoes from true seed. Manila, Philippines, pp 12–20