

# Reinventing Potato as a Diploid Inbred Line–Based Crop

Shelley H. Jansky,\* Amy O. Charkowski, David S. Douches, Gabe Gusmini, Craig Richael, Paul C. Bethke, David M. Spooner, Richard G. Novy, Hielke De Jong, Walter S. De Jong, John B. Bamberg, A. L. Thompson, Benoit Bizimungu, David G. Holm, Chuck R. Brown, Kathleen G. Haynes, Vidyasagar R. Sathuvalli, Richard E. Veilleux, J. Creighton Miller, Jr., Jim M. Bradeen, and Jiming Jiang

## ABSTRACT

The third most important food crop worldwide, potato (*Solanum tuberosum* L.) is a tetraploid outcrossing species propagated from tubers. Breeders have long been challenged by polyploidy, heterozygosity, and asexual reproduction. It has been assumed that tetraploidy is essential for high yield, that the creation of inbred potato is not feasible, and that propagation by seed tubers is ideal. In this paper, we question those assumptions and propose to convert potato into a diploid inbred line–based crop propagated by true seed. Although a conversion of this magnitude is unprecedented, the possible genetic gains from a breeding system based on inbred lines and the seed production benefits from a sexual propagation system are too large to ignore. We call on leaders of public and private organizations to come together to explore the feasibility of this radical and exciting new strategy in potato breeding.

S.H. Jansky, USDA–ARS Vegetable Crops Research Unit, Dep. of Horticulture, Univ. of Wisconsin, 1575 Linden Dr., Madison, WI; A.O. Charkowski, Dep. of Plant Pathology, Univ. of Wisconsin, Madison, WI; D.S. Douches, Dep. of Plant, Soil, and Microbial Sciences, Mich. State Univ., East Lansing, MI; G. Gusmini, PepsiCo, St. Paul, MN; C. Richael, Simplot Plant Sciences, Boise, ID; P.C. Bethke and D.M. Spooner, USDA–ARS Vegetable Crops Research Unit, Dep. of Horticulture, Univ. of Wisconsin, Madison, WI; R.G. Novy, USDA–ARS Small Grains and Potato Germplasm Research Unit, Aberdeen, ID; H. De Jong, Agriculture and Agri-Food Canada, Fredericton, New Brunswick, Canada (retired); W.S. De Jong, School of Integrative Plant Sciences, Cornell Univ., Ithaca, NY; J.B. Bamberg USDA–ARS, Dep. of Horticulture, Univ. of Wisconsin, Madison, WI, and US Potato Genebank, Sturgeon Bay, WI; A.L. Thompson, Dep. of Plant Sciences, North Dakota State Univ.; B. Bizimungu, Agriculture and Agri-Food Canada, Fredericton, New Brunswick, Canada; D.G. Holm, Dep. of Horticulture and Landscape Architecture, Colorado State Univ., San Luis Valley Research Center, Center, CO; C.R. Brown, USDA–ARS, Prosser, WA; K.G. Haynes, USDA–ARS, Beltsville, MD; V.R. Sathuvalli, Dep. of Crop and Soil Science, Oregon State Univ., Hermiston Agricultural Research and Extension Center, Hermiston, OR; R.E. Veilleux, Dep. of Horticulture, Virginia Tech, Blacksburg, VA; J.C. Miller, Jr., Dep. of Horticultural Sciences, Texas A&M Univ., College Station, TX; J.M. Bradeen, Dep. of Plant Pathology, Univ. of Minnesota, St. Paul, MN; J. Jiang, Dep. of Horticulture, Univ. of Wisconsin, Madison, WI. G. Gusmini is an employee of PepsiCo, Inc.; the views expressed in this presentation are those of the author and do not necessarily reflect the position or policy of PepsiCo Inc. C. Richael is an employee of Simplot Plant Sciences; the views expressed in this presentation are those of the author and do not necessarily reflect the position or policy of Simplot Plant Sciences. Received 3 Dec. 2015. Accepted 25 Jan. 2016. \*Corresponding author (shelley.jansky@ars.usda.gov; shjansky@wisc.edu).

**Abbreviations:** CIP, International Potato Center; ILs, introgression lines; RILs, recombinant inbred lines; TPS, true potato seed.

Published in *Crop Sci.* 56:1412–1422 (2016).  
doi: 10.2135/cropsci2015.12.0740

© Crop Science Society of America | 5585 Guilford Rd., Madison, WI 53711 USA  
This is an open access article distributed under the CC BY-NC-ND license  
(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

CAN potato (*Solanum tuberosum* L.) be converted from an asexually propagated tetraploid crop into an inbred seed-propagated diploid one? This was the fundamental question posed by a group of public and private sector scientists who met at the University of Wisconsin–Madison on June 25, 2015. The consensus of the group was that the benefits of working with diploid inbred lines are too substantial to be ignored any longer. We must consider the possibility of reinventing the potato crop at the diploid level to take full advantage of the modern genetics and genomics tools available to improve gain from selection. We envision breeding potato using the suite of technologies that have been developed for diploid seed propagated crops but are not readily transferable to a tetraploid clonal breeding program.

### History of Converting Potato to a True Seed Crop

The potato crop is typically grown from “seed potatoes,” which can be whole tubers or tuber pieces with eyes (nodes) from which plants emerge. The term true potato seed (TPS) is used to describe sexual seeds produced in berries, as opposed to asexual seed potatoes. At the International Potato Center (CIP), research into converting the potato from a vegetative clonal crop to a botanical seed crop was intensive from 1977 to 2000. The primary motivation was to avoid the disadvantages of propagating the crop using a modified stem (a tuber) with a short preservation time and the potential to accumulate systemic diseases when propagated vegetatively over field generations. General observations that tetraploid cultivars were higher yielding than diploid cultivars drove the bulk of the research into TPS arising from biparental crosses between tetraploids. Research efforts focused on the genetics and physiology of traits essential for successful TPS-based cultivars, such as heterosis, uniformity, cytoplasmic male sterility, combining ability, disease resistance, and seedling vigor (Kidane–Mariam et al., 1985b; Golmirzaie et al., 1994; Muthoni et al., 2013). However, direct-seeded TPS produced lower yields than crops started from tubers. This led many agronomists to use TPS to generate seed tubers, which were then planted as the commercial crop in the following year. These seed tubers were nearly pathogen free, providing a better hygienic status than locally produced clonal cultivars—especially important in developing countries without the resources and technologies for the production of pathogen-free-certified clonal seed. However, TPS did not revolutionize potato production, as was predicted by the International Potato Center (Almekinders et al., 2009). Breeders working with TPS often selected the best plants and treated them as clonal cultivars in future endeavors. While the conversion of potato to a diploid TPS crop was not contemplated, the knowledge generated by CIP’s research endeavors can provide the foundation for TPS production efforts at the diploid level.

### What Are the Drivers for This Conversation?

It has long been assumed that the tetraploid state is optimal for superior agronomic performance in cultivated potato. However, most cultivars in Europe, North America, and Asia may be tetraploid because of a shared history rather than genetic superiority. The potatoes that were originally brought to Europe and eventually transported to North America and Asia originated in southern Chile, where landraces are typically tetraploid (Ames and Spooner, 2008). Throughout the remainder of South America, including the center of origin in Peru, landraces of *S. tuberosum* range in ploidy from diploid to tetraploid, with no obvious phenotypic distinctions among the levels (Dodds, 1965; Spooner et al., 2007). In our experience, diploid wild potato relatives and their hybrids with cultivated potato often produce plants that are as vigorous as potato cultivars.

The assumption that tetraploid potatoes are higher yielding than diploid potatoes is based in large part on the untested hypothesis that four allelic variants per locus contribute to yield superiority (Bani–Aameur et al., 1991; De Jong and Tai, 1991; Werner and Peloquin, 1991; Buso et al., 1999, 2000). However, in a study comparing tetraploid three-way hybrids with two-way hybrids, the three-way hybrids were never higher yielding (Sanford and Hanneman, 1982). This suggests that increased levels of heterozygosity do not necessarily translate to improved yield. In addition, recent genomics data indicate that, at least at the single nucleotide level, tri-allelic and tetra-allelic loci are rare (Uitdewilligen et al., 2011; Hirsch et al., 2013). In fact, the contribution of multiple alleles per locus to yield superiority must be questioned in light of the observation that diploids have been repeatedly shown to have high yield potential (Rowe, 1967a; Carroll, 1982; Maris, 1990; Hutten, 1994; De Maine, 1996; Simmonds, 1997).

The most significant hindrance to breeding gain in potato at the tetraploid level is likely the abundance of deleterious alleles that can be tolerated within a polyploid genome. If this is the case, then one would predict that a high proportion of inferior genetic combinations would result when crosses are made between heterozygous tetraploid parents. Such observations are commonplace in traditional potato breeding programs, where 90% or more of progeny from crosses between elite lines are discarded because they are not superior to their parents for the vast array of characteristics required for a potato cultivar. This problem was clearly identified by Allard (1999) who stated that “nearly all vegetatively propagated plants are open-pollinated, and highly heterozygous. . . . [W]hen they reproduce sexually, the extensive segregation and recombination that occur during sexual reproduction lead to very large numbers of novel genotypes, nearly all of which are inferior to the parental genotype. Thus, improvement tends to be slow in nature and in cultivation.” Thus, the ideal breeding system for potato can be envisioned as one

in which genetic diversity for favorable alleles is maintained but diversity for deleterious alleles or allelic combinations is greatly reduced. This can be accomplished through self-pollination, as “inbreeding (especially selfing) is capable of binding the entire genotype together, including favorably interacting alleles of different loci located on different chromosomes. Hence, inbreeding by selfing is expected to be particularly efficient in organizing the entire gene pool into multiple favorably interacting and stable epistatic systems” (Allard, 1999). While genetic gains are more easily realized at the diploid level, new breeding technologies that allow genotypic recurrent selection, along with selection for combining ability, will also be likely to allow breeders to produce superior hybrids at the tetraploid level. While the reinvention of the potato crop will be facilitated greatly by a reduction in ploidy, it will also benefit from the production of inbred lines to systematically assemble desirable combinations of genes and exploit heterosis. The creation of homozygous sets of additive loci through recombination and inbreeding is important for genetic gain (Fasoula and Fasoula, 2002). Efforts to self-pollinate potato at the tetraploid level were initiated in the mid-twentieth century in response to the success of the inbred-hybrid methods developed in maize (*Zea mays* L.) (Shull, 1909; Krantz, 1924, 1929; Guern, 1940). Low yield and poor fertility were observed in  $S_5$  and  $S_6$  generations, so this effort was terminated. In another line of research, attempts to develop tetraploid TPS-based cultivars (described above) revealed that one to two generations of self-pollination does not always result in inbreeding depression (Kidane-Mariam et al., 1985a; Golmirzaie et al., 1998). However, the approach to homozygosity in autotetraploids is much slower than in diploids (Haldane, 1930). Consequently, these efforts to self-pollinate tetraploid potato clones never actually tested the value of creating inbred lines to eliminate deleterious alleles and fix desirable ones.

Reinventing the potato as a diploid crop based on inbred lines will enable researchers to accelerate progress toward understanding the genetics of yield and quality traits. Diploid potatoes will make full use of computational genomics tools developed for field crops, and inbred lines will facilitate the mapping of genetic loci for important agronomic traits. In addition, diploid inbred potatoes, in combination with functional genomics data, may be valuable in elucidating the molecular bases of heterosis. Finally, the methodologies for mutant screens developed in other model plant species are impractical with clonally propagated tetraploid potatoes but can be implemented readily with an inbred diploid potato line (Duangpan et al., 2013).

## What Will This New Breeding System Look Like?

We envision potato as a diploid crop composed of a series of inbred lines that capture the favorable genetic diversity available in cultivated potato. Some of these lines will be fixed for major genes that control skin color and texture, flesh color, starch content, and tuber shape (De Jong, 1981, 1991; De Jong and Burns, 1993; Zhang et al., 2009). Crosses between inbreds within each market class (red, white, russet, specialty) will combine the myriad traits necessary for success in potato production, such as time to senescence, tuber yield, flesh texture, tuber composition, disease resistance, and maintenance of tuber quality in storage. In other crops, the assignment of inbreds to heterotic pools has been important in breeding for higher yield (Li et al., 2010; Fischer et al., 2010; Reif et al., 2010; Riedelsheimer et al., 2012; Suwarno, 2014). This strategy would likely lead to significant yield gains in potato as well.

Since potato is a clonally propagated crop, partial inbreds are likely to be components of the breeding system, especially in the near term when the vigor of inbred germplasm is likely to be lower than that of parental lines and hybrids. Hybridization of partial inbreds could lead to the selection of clones that are asexually propagated as cultivars using methods of traditional potato breeding. Such an approach would benefit from the reduction in deleterious alleles that is expected to occur as individual lines approach homozygosity. However, there are several advantages to ultimately generating inbred lines maintained as TPS rather than tubers. True potato seed can be easily shared among research programs, each of which is likely to collect phenotypic data on a different set of traits. Phenotypic data are cumulative for each inbred line and can be aligned with genotype data. Using TPS for this kind of research eliminates the burden that is currently placed on individual breeding programs to vegetatively maintain many genotypes either in tissue culture, where mass propagation is costly, or as seed tubers, which may carry viruses that have accumulated during prior growing seasons and can seriously influence measured phenotypes. True potato seed of inbred lines can also be stored in a freezer almost indefinitely yet are readily available for use (Simmonds, 1963; Howard, 1975).

Seed tubers can carry a multitude of pathogens. For example, new strains of potato virus Y have spread throughout the United States on infected seed tubers. Similarly, *Phytophthora infestans*, the causal agent of late blight, has moved around the world on infected seed tubers (Ristaino, 2002; Pule et al., 2013). True potato seed does not carry any economically important fungal, oomycete, or bacterial pathogens nor major potato viruses (Simmonds, 1997). It is important to note, though, that potato spindle tuber viroid and some minor viruses are carried in TPS. In addition to tuber-borne pathogens, soil

adheres to tubers so seed tubers pose a high risk for the spread of soil-borne potato pathogens, such as potato cyst nematode or *Verticillium dahliae*, and soil-borne pathogens that infect common rotation crops, such as soybean cyst nematode. Consequently, TPS will have significant positive impacts on seed production and disease control and will go far to prevent the spread of established and emerging pathogens in potato (Muthoni et al., 2013).

Finally, TPS can be rapidly increased for agronomic and storage trials when a potential new hybrid cultivar is identified. The multiplication rate of potato tuber seed is 1:10 per generation, while a single plant can produce thousands of TPS in a matter of months. How efficiently inbred lines will produce hybrid seed is a matter of speculation, but there is at present no reason to believe that TPS production will be substantially less than that observed in existing germplasm. In contrast, it takes several years and greater financial resources to increase seed tubers for field trials and commercial evaluations. In addition, while it is difficult to overcome dormancy in tubers, TPS dormancy is easily overcome using gibberellic acid (Simmonds, 1964; Jansky et al., 2012). To be clear, though, at least in the foreseeable future, the potato crop we envision will be planted from tubers. However, the generation of these tubers will begin with TPS rather than tissue culture plantlets.

### What Challenges Do We Face?

It is impossible to know whether the diploid strategy is feasible until it is tested on a large scale, which will require significant resources and buy-in from the potato-breeding community. However, an alternative way to view the situation is to ask whether incentives exist to continue the status quo. In conventional tetraploid breeding programs, the four sets of 39,000 genes in potato are randomly reassorted in each generation. There is no mechanism to identify and remove deleterious alleles nor to fix desirable alleles or epistatic combinations. Again, genomics data are providing a picture that contradicts conventional beliefs. A century of potato breeding should have increased heterozygosity if that is important for cultivar success. However, heterozygosity in modern cultivars is no different than that of older cultivars that were created 10 to 15 generations ago (Hirsch et al., 2013; Vos et al., 2015). This is likely because of the relatively narrow genetic base of cultivated potato in Europe and North America as a result of bottlenecks during its introduction from South America.

Efforts have been made to improve yield by introducing new genetic diversity in tetraploid breeding programs. Hybridization with diploid and tetraploid cultivated relatives has produced plants with enhanced vigor and yield (Hanneman and Peloquin, 1969; Plaisted, 1973; Hoopes et al., 1980; De Maine et al., 2000). However, late maturity and commercially unacceptable tuber type has limited the success of this approach. When using exotic

germplasm as a source of genetic diversity for heterosis, then, it is important to carefully select the donor parents. Selection for adaptation in diploid wild potato relatives before hybridization with cultivated potato is not effective (Jacobsen and Jansky, 1989). Consequently, selection will need to be performed in hybrids between exotic and cultivated potatoes (Watanabe et al., 1995; Santini et al., 2000). It is important to consider, though, that heterozygosity at some loci may be more important than overall heterozygosity (Bonierbale et al., 1993).

It is difficult to displace existing potato cultivars with new ones (Jansky et al., 2014a). Indeed, the average age of major North American cultivars is 38 yr, based on NASS data and cultivar release dates. The cultivar Russet Burbank occupies 23% of Canadian commercial potato acreage and 20% certified seed potato acreage in the United States, even though it is 114 yr old. While many factors affect adoption and success of new cultivars, at least some of the challenges are due to a lack of new gene combinations that are clearly superior to those in existing cultivars.

In the transition to a diploid crop, it will be important to consider the effect of the ploidy reduction on agronomic traits. As discussed above, it is often difficult to distinguish between tetraploid and diploid plants on the basis of vine and tuber appearance. Comparisons between diploids and their somatically doubled counterparts, though, have shown that diploids grow faster and mature earlier than tetraploids (Rowe, 1967b; Maris, 1990). Both of these features would be desirable. Diploids typically produce smaller cells than tetraploids. We do not know how this might affect starch granule size, tuber sugar content, potato texture, and other quality characteristics, but methods to test all of these parameters are available.

We have an exciting opportunity to revolutionize potato research and the potato industry. Undoubtedly, we are proposing to disrupt a system that has been in place for over a century. This new system, however, should be more nimble and responsive to changes in market demands and production environments. It will allow breeders to adopt the new breeding strategies developed in other crops. New genes can be stacked into well-established inbred lines and seed increase can occur in months rather than years. An inbred line-based breeding system also provides the possibility for small farmers in both developed and developing countries to create their own custom cultivars and grow them using locally produced TPS.

### What Questions Must We Answer as We Develop This New System?

*What can be gained by moving to the diploid level?* The genetic challenges of working at the tetraploid level are significant and this impedes breeding progress. Many of these challenges are greatly reduced when working with diploid material. Population sizes needed for mapping can be

much smaller in diploids than in autotetraploids (Little, 1945, 1952). Genes that produce easily scored dominant phenotypes in diploids may become incompletely dominant at the tetraploid level. Expected genetic ratios can be determined more easily for diploids than polyploids. In tetraploids, gene dosage effects may influence phenotype, and epistatic interactions are complex. Because of these complexities, “genetic resolution is commonly lost in polysomic polyploids and qualitative traits blur into quantitative traits” (Sanford, 1983). It is often difficult to identify major genes in polyploids. It is even more difficult to map and develop molecular markers for those genes. In fact, while other breeders routinely use markers for early generation selection, there are few markers used routinely in potato breeding programs.

Another advantage of diploid breeding is that genomics resources developed for other diploid species can be used directly. Genomics analysis with tetraploids often requires extensive modification of software or workaround solutions that are cumbersome and require specialized expertise. Most SNP analysis platforms cannot capture more than two alleles at a locus, making it a challenge to identify multiallelic loci. In addition to the technical challenges associated with molecular marker analyses at the tetraploid level, more read depth is required to obtain whole genome coverage in tetraploids compared with diploids.

One of the most significant benefits of working at the diploid level is that it is possible to breed for and fix traits under recessive genetic control, whereas it is nearly impossible to fix recessive alleles at the tetraploid level. In addition, large numbers of plants must be evaluated when screening for recessive traits in a germplasm collection (Bamberg and del Rio, 2004). One example of a recessive trait is deep yellow flesh, which results from the recessive allele of the zeaxanthin epoxidase gene (*zep1*) (Wolters et al., 2010). Assuming the desirability of high levels of xanthophylls such as zeaxanthin (a phytonutrient for eye health), the task of producing nulliplex *zep1* is improbable in a tetrasomic breeding system and difficult to track in view of lack of an easily measurable phenotype. Only 3% of progeny from a cross between two duplex parents is expected to be nulliplex (McCord et al., 2012). Other examples of desirable recessive traits include acyl sugar droplets on glandular trichomes, which contribute to broad spectrum insect resistance (Schilmiller et al., 2012), and the production of toxic leptine glycoalkaloids, which provide insect resistance in leaves but are not expressed in tubers (Ronning et al., 1998). Efforts to breed for these traits at the tetraploid level have largely been abandoned in the past because of the challenges associated with fixing recessive alleles.

*How will we manage genetic diversity?* There are approximately 100 wild relatives of potato, many of which are sexually compatible with cultivated potato, especially at the diploid level (Spooner et al., 2014). Hybrids between

diploid wild and cultivated potato are often surprisingly well-adapted and agronomically acceptable (Hermundstad and Peloquin, 1986; Yerk and Peloquin, 1989; Jansky et al., 2004). In diploid breeding efforts, it will be important to produce wild × wild, wild × cultivated, and cultivated × cultivated hybrids. Both discovery and introgression populations should be created. We will be combining genomes that may never have been together before, so it is impossible to predict the types of epistatic interactions that will be generated or the linkages that will be disrupted. Hybrid breakdown in F<sub>2</sub> populations from interspecific hybrids has been observed in potato and other plants (Hawkes, 1990; Li et al., 1997; Johansen-Morris and Latta, 2006). Unthrifty plants in the F<sub>2</sub> generation likely result from the disruption of adapted complexes in the two parental species. On the other hand, introgression of wild species' chromosomal regions into cultivated genomes has uncovered genes that contribute to quality and yield (Eshed and Zamir, 1995; Monforte et al., 2001; Fridman et al., 2004). Ultimately, the production of near-isogenic lines and other types of introgression lines are likely to identify genomic contributions from wild species that improve yield, disease and pest resistances, and tuber quality (Bernacchi et al., 1998; Monforte et al., 2001; Huang et al., 2003; Stevens et al., 2007). In tomato (*Solanum lycopersicum* L.), introgression lines developed by the Zamir lab led to significant advances in understanding the genetic basis of agronomically important traits (Eshed and Zamir, 1995; Eshed et al., 1996; Fridman et al., 2000, 2004; Ronen et al., 2000). Similar germplasm could be developed in potato.

Eventually, hybrid production in potato may be supported by a cytoplasmic-genetic male sterility system. Cytoplasmic-genetic male sterility has been reported in a number of potato interspecific hybrids (Dionne, 1961; Grun et al., 1962; Abdalla and Hermsen, 1973; Hermundstad and Peloquin, 1985; Phumichai and Hosaka, 2006). Fertility restorer genes are also found in potato germplasm (Iwanaga et al., 1991; Tucci et al., 1996). Cybrid production using protoplast fusion between male sterile cytoplasmic sources and male fertile cultivars has been used to change male fertile potato cultivars to male sterile ones without altering the nuclear genome as a step in developing TPS parents (Perl et al., 1990). Alternatively, as male fertility genes in diploids are mapped and sequenced, it may be possible to use gene-editing techniques, such as CRISPR/Cas9, to create male sterile plants for use as female parents in hybrid production (Belhaj et al., 2015).

*What are the challenges associated with moving toward the inbred-hybrid system?* The obvious practical limitation is that existing breeding programs do not have the necessary personnel, facilities (e.g., greenhouse and field space), or monetary resources to continue feeding the tetraploid cultivar development pipeline while developing a

parallel program based on releasing diploid inbred lines or  $F_1$  hybrids. Abandonment of tetraploid cultivar development is not warranted until the proposed merits of diploid breeding can be explored and validated as a feasible alternative. Since tetraploid cultivated potato is self-compatible, one suggestion is to create inbred lines at the tetraploid level, and this is being attempted in some programs. However, as discussed above, the approach to homozygosity is slow at the tetraploid level. In addition, unless the tetraploids carry dominant self-incompatibility inhibitor alleles, which are difficult to select at the tetraploid level, they will likely lose their ability to self-pollinate as they approach homozygosity. In tetraploids, pollen tube growth is inhibited when pollen is homozygous for S alleles but not when it is heterozygous (Lewis, 1943, 1947). Apparently, heterozygous pollen tubes do not elicit an incompatibility reaction (Levin, 1983).

Instead of expecting each existing cultivar development program to initiate a diploid breeding program, another option is to plan a transition from the polyploid to the diploid breeding system as a global community and dedicate some programs, perhaps government programs and/or private companies, entirely to developing the diploid system. The latter option could be complicated by proprietary considerations. A third option is to advocate for the creation of a few new programs for a finite period of time to create diploid germplasm and breeding methodologies and to quantify the potential for making more rapid genetic gain. While these options are being considered, North American breeders are moving forward to develop diploid resources that can be used by the breeding community. For example, several breeders are extracting dihaploids from superior parents. The collection of dihaploids that will be generated can be used to advance diploid breeding efforts across programs.

*What are the genetic impediments to the development of inbred lines?* In potato, it is relatively easy to generate dihaploids from tetraploid cultivars. Dihaploid potatoes can be generated through anther culture or through pollination by specific “pollinator” lines of *S. tuberosum* Group Phureja. (Hougas et al., 1958; Peloquin et al., 1996). However, potato dihaploids exhibit inbreeding depression, including poor vigor and low fertility (Peloquin and Hougas, 1960). Most dihaploids are male sterile, but many are female fertile. The frequent expression of male sterility at the diploid level will be a challenge for diploid breeding, at least initially. Dihaploids readily hybridize with many wild diploid *Solanum* species (Jansky et al., 1990; Watanabe et al., 1995). Some breeding programs maintain a diploid gene pool, often composed of hybrids between dihaploids and diploid wild species carrying specific quality and disease resistance genes not found in cultivars. The number of these lines is small, however, and they have been developed with the intention of introgressing those

traits into tetraploid potato via  $2n$  gametes (Carputo and Barone, 2005; Ortiz et al., 2009). Selection for the broad range of traits required in a new cultivar has not been a priority in these breeding programs.

Diploid potatoes are self-incompatible because of a genetically controlled gametophytic incompatibility system. However, the identification of a dominant self-incompatibility inhibitor (*Sli*) in the sexually compatible wild species *S. chacoense* opens new doors to explore inbred line breeding in potato (Hosaka and Hanneman, 1998). Self-compatibility has also been found sporadically in other wild and cultivated potato species, leading to the possibility of multiple genetic sources of self-compatibility (Cipar et al., 1964; De Jong and Rowe, 1971). Highly inbred *S. chacoense* lines, such as M6, which has been self-pollinated for seven generations, are vigorous and fertile (Jansky et al., 2014b). Consequently, another long-held concept, that homozygosity implies low vigor, is not always true.

As with other allogamous plants that have been forced to self-pollinate, inbreeding depression occurs when diploids are self-pollinated in potato (De Jong and Rowe, 1971; Phumichai et al., 2005; Lindhout et al., 2011). Reductions in vigor are observed, but the main challenge is maintaining male fertility. In early generations of selfing, inbreeding depression is likely due in part to the expression of deleterious recessive alleles. However, in our experience, male sterility in  $F_4$  and  $F_5$  populations derived from interspecific  $F_1$  hybrids is still a barrier. This may be due to hybrid breakdown, as described above. Consequently, while biological species barriers do not exist between potato and many of its wild relatives, genetic interactions may impact our ability to develop inbred lines from interspecific hybrids. Backcross breeding to introgress small chromosomal regions from wild species into a cultivated background may be a more effective strategy.

Anther culture (androgenesis) provides an alternative to selfing for the development of inbred lines of potato and has provided the only currently available completely homozygous diploid potato lines derived from adapted selections of cultivated diploid land races of *S. tuberosum* Group Phureja (Veilleux, 1990). Such a doubled monoploid (DM1-3) enabled the sequencing of the potato genome (Potato Genome Initiative, 2011). Androgenesis bypasses self-incompatibility and does not require completely functional gametes to generate a monoploid plant; hence the doubled monoploids that have been generated to date could be used as female parents in hybrid schemes, but they lack male fertility (Paz and Veilleux, 1999). Although its application is limited to germplasm responsive to the technique, androgenesis still promises to deliver new inbred lines, as germplasm carrying desirable traits can be introgressed into responsive clones. Thus, anther culture is another key to germplasm development in the conversion of potato to a diploid crop.

*What are the potential impediments to commercializing inbred lines or  $F_1$  hybrids in potato production systems?* If we envision eventually propagating cultivars from TPS, it will be important to begin seed physiology studies now. Because potatoes have historically been propagated by tubers, research efforts have not focused on optimizing fertility, seed set, and seed germination. It will be necessary to understand how to generate an abundance of seeds quickly and efficiently. In addition, it will be important to determine the best methods for generating large quantities of first generation tubers from TPS. Nutrient film technique, a hydroponic system currently used to grow mini tubers from tissue culture plantlets, could be used to generate mini tubers from TPS. The benefits of this system include simplicity, low water requirements, a protected environment that results in disease-free tubers, and easy adaptability to season and to either urban or rural environments. Alternatively, a bed system could be implemented for such a purpose.

*Which inbred genetics resources should be developed initially?* Recombinant inbred lines (RILs) provide a resource for fundamental gene discovery and gene mapping (Broman, 2005; Kump et al., 2011; Barrière et al., 2012). We have already made good progress toward the production of RILs. The  $F_2$  population from the inbred lines DM1-3 and M6 is powerful for mapping, since every allele can be traced back to a grandparent. In fact, 10 major genes have been mapped in that single population (Endelman and Jansky, 2016). Recombinant inbred lines derived from that  $F_2$  population will be even more valuable for mapping, since it will be possible to track smaller chromosomal fragments after six cycles of recombination. Currently, we are using dominant self-incompatibility inhibitors from wild potato relatives to allow us to generate RILs. The diploid inbred wild species clone M6 is the main source of self-compatibility in the first phase of inbred line development. By necessity, then, wild species will be the foundation of the first cycle of RILs.

The production of introgression lines (ILs) will be the next step in our plan to reinvent potato at the diploid level. As described above, introgression lines will be especially useful for breeding because they provide a mechanism to track the contributions of wild genomes in a cultivated background. The current limitation in potato is that we lack self-compatible inbred lines of cultivated potato. However, we are using multiple strategies, including monoploid development and self-pollination, to generate these lines. We currently have  $S_5$  plants derived from a self-compatible *S. tuberosum* dihaploid.

Both RILs and ILs are likely to yield unexpected findings, and those “surprises” are critical to both the fundamental understanding of the genetic basis of agronomically important traits and to breeding applications. With inbreds, it will be easy to use accelerated backcross methods to introgress major genes, such as R-genes for disease resistance, and

to stack genes with an additive effect. Pyramiding late blight genes by crossing heterozygous diploids has been shown to delay disease development (Tan et al., 2010). In addition, it may be possible to stack several genes into one recombination unit using emerging gene editing techniques.

*What is needed in a model system for root and tuber crops?* We have learned much about basic biology from model plants, such as *Arabidopsis*, maize, rice (*Oryza sativa* L.), tomato, and *Medicago* (Meissner et al., 1997; Arabidopsis Genome Initiative, 2000; Bell et al., 2001; Zhao et al., 2007; Coudert et al., 2010). However, no well-developed model plant produces fleshy rhizomes, tap roots, or tubers. In addition, important starchy diploid crops, such as cassava, sugar beet, and sweet potato, are genetically complex, making it difficult to investigate fundamental questions, such as how asexual propagules form and how they tolerate pests and disease. Because of this, other researchers have proposed the development of inbred lines in cassava and diploid cultivars in sugar beet to take advantage of the benefits outlined in this paper (Fénart et al., 2008; Ceballos et al., 2015). Inbred diploid potato lines have the potential to serve as a valuable model for tuber development, for carbohydrate storage in starchy vegetables, and for understanding the biology of pests and diseases that affect storage organs of plants.

## CONCLUSIONS

We are at a crossroads for revolutionizing potato breeding. Genomic selection, gene editing, transformation, and hybrid breeding can all be used for targeted improvements of inbred diploids. These approaches will rely on gene discovery efforts that are enabled by the development of inbred lines that incorporate germplasm from cultivated species and wild potato relatives. Diploid inbred line breeding in potato is being tested by the private sector in Europe (Lindhout et al., 2011). A concerted effort of the public and private research community in North America is needed to mobilize resources, focus activities, and develop a long-term programmatic approach to transforming the potato industry, while developing the genetic knowledge and breeding platforms that will enable breakthrough progress in the near future. We call on leaders of public and private organizations to come together to explore the feasibility of this radical and exciting new strategy in potato breeding.

## References

- Abdalla, M., and J. Hermsen. 1973. An evaluation of *Solanum verrucosum* Schlecht. for its possible use in potato breeding. *Euphytica* 22:19–27. doi:10.1007/BF00021551
- Allard, R.W. 1999. History of plant population genetics. *Ann. Rev. Genet.* 33:1–27.
- Almekinders, C.J.M., E. Chujoy, and G. Thiele. 2009. The use of true potato seed as pro-poor technology: The efforts of an international agricultural research institute to innovating

- potato production. *Potato Res.* 52:275–293. doi:10.1007/s11540-009-9142-5
- Ames, M., and D.M. Spooner. 2008. DNA from herbarium specimens settles a controversy about origins of the European potato. *Am. J. Bot.* 95:252–257. doi:10.3732/ajb.95.2.252
- Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408:796–815. doi:10.1038/35048692
- Bamberg, J.B., and A.H. del Rio. 2004. Hypothetical obscured recessive traits in tetraploid *Solanum* estimated by RAPDs. *Am. J. Potato Res.* 81:45. doi:10.1007/BF02870198
- Bani-Aameur, F., F.I. Lauer, R.E. Veilleux, and A. Hilali. 1991. Genomic composition of 4x-2x potato hybrids: Influence of *Solanum chacoense*. *Genome* 34:413–420. doi:10.1139/g91-063
- Barrière, Y., V. Méchin, B. Lefevre, and S. Maltese. 2012. QTLs for agronomic and cell wall traits in a maize RIL progeny derived from a cross between an old Minnesota13 line and a modern Iodent line. *Theor. Appl. Genet.* 125:531–549. doi:10.1007/s00122-012-1851-5
- Belhaj, K., A. Chaparro-Garcia, S. Kamoun, N.J. Patron, and V. Nekrasov. 2015. Editing plant genomes with CRISPR/Cas9. *Curr. Opin. Biotechnol.* 32:76–84. doi:10.1016/j.copbio.2014.11.007
- Bell, C.J., R.A. Dixon, A.D. Farmer, R. Flores, J. Inman, R.A. Gonzales, M.J. Harrison, N.L. Paiva, A.D. Scott, J.W. Weller, and G.D. May. 2001. The Medicago Genome Initiative: A model legume database. *Nucleic Acids Res.* 29:114–117. doi:10.1093/nar/29.1.114
- Bernacchi, D., T. Beck-Bunn, D. Emmatty, Y. Eshed, S. Inai, J. Lopez, V. Petiard, H. Sayama, J. Uhlig, D. Zamir, and S. Tanksley. 1998. Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L. pimpinellifolium*. *Theor. Appl. Genet.* 97:170–180. doi:10.1007/s001220050882
- Bonierbale, M., R.L. Plaisted, and S. Tanksley. 1993. A test of the maximum heterozygosity hypothesis using molecular markers in tetraploid potatoes. *Theor. Appl. Genet.* 86:481–491. doi:10.1007/BF00838564
- Broman, K.W. 2005. The genomes of recombinant inbred lines. *Genetics* 169:1133–1146. doi:10.1534/genetics.104.035212
- Buso, J.A., L.S. Boiteux, and S.J. Peloquin. 2000. Heterotic effects for yield and tuber solids and type of gene action for five traits in 4x potato families derived from interploid (4x-2x) crosses. *Plant Breed.* 119:111–117. doi:10.1046/j.1439-0523.2000.00037.x
- Buso, J.A., L.S. Boiteux, G.C.C. Tai, and S.J. Peloquin. 1999. Chromosome regions between centromeres and proximal crossovers are the physical sites of major effect loci for yield in potato: Genetic analysis employing meiotic mutants. *Proc. Natl. Acad. Sci. USA* 96:1773–1778. doi:10.1073/pnas.96.4.1773
- Carputo, D., and A. Barone. 2005. Ploidy level manipulations in potato through sexual hybridization. *Ann. Appl. Biol.* 146:71–79. doi:10.1111/j.1744-7348.2005.04070.x
- Carroll, C.P. 1982. A mass-selection method for the acclimatization and improvement of edible diploid potatoes in the United Kingdom. *J. Agric. Sci. Cambridge* 99:631–640. doi:10.1017/S0021859600031312
- Ceballos, H., R.S. Kawuki, V.E. Gracen, G.C. Yencho, and C.H. Hershey. 2015. Conventional breeding, marker-assisted selection, genomic selection and inbreeding in clonally propagated crops: A case study for cassava. *Theor. Appl. Genet.* 128:1647–1667. doi:10.1007/s00122-015-2555-4
- Cipar, M.S., S.J. Peloquin, and R.W. Hougas. 1964. Variability in the expression of self-incompatibility in tuber-bearing diploid *Solanum* species. *Am. Potato J.* 41:155–162. doi:10.1007/BF02855317
- Coudert, Y., C. Périn, B. Courtois, N.G. Khong, and P. Ganet. 2010. Genetic control of root development in rice, the model cereal. *Trends Plant Sci.* 15:219–226. doi:10.1016/j.tplants.2010.01.008
- De Jong, H. 1991. Inheritance of anthocyanin pigmentation in the cultivated potato: A critical review. *Am. Potato J.* 68:585–593. doi:10.1007/BF02853712
- De Jong, H. 1981. Inheritance of russeting in cultivated diploid potatoes. *Potato Res.* 24:309–313. doi:10.1007/BF02360368
- De Jong, H., and V.J. Burns. 1993. Inheritance of tuber shape in cultivated diploid potatoes. *Am. Potato J.* 70:267–284. doi:10.1007/BF02849314
- De Jong, H., and P.R. Rowe. 1971. Inbreeding in cultivated diploid potatoes. *Potato Res.* 14:74–83. doi:10.1007/BF02355931
- De Jong, H., and G. Tai. 1991. Evaluation of potato hybrids obtained from tetraploid-diploid crosses. I. Parent-offspring relationships. *Plant Breed.* 107:177–182. doi:10.1111/j.1439-0523.1991.tb01204.x
- De Maine, M.J. 1996. An assessment of true potato seed families of *Solanum phureja*. *Potato Res.* 39:323–332. doi:10.1007/BF02357936
- De Maine, M.J., A.K. Lees, D.D. Muir, J.E. Bradshaw, and G.R. Mackay. 2000. Long-day-adapted Phureja as a resource for potato breeding and genetic research. *Potato, Glob. Res. Dev.* 1:134–137.
- Dionne, L.A. 1961. Cytoplasmic sterility in derivatives of *Solanum demissum*. *Am. Potato J.* 38:117–120. doi:10.1007/BF02870217
- Dodds, K. 1965. The history and relationships of cultivated potatoes. In: J. Hutchinson, editor, *Crop Plant Evolution*. Cambridge Univ. Press, Cambridge, UK. p. 123–141.
- Duangpan, S., W. Zhang, Y. Wu, S. Jansky, and J. Jiang. 2013. Insertional mutagenesis using *Tnt1* retrotransposon in potato. *Plant Physiol.* 163:21–29. doi:10.1104/pp.113.221903
- Endelman, J.B., and S.H. Jansky. 2016. Genetic mapping with an inbred line-derived F<sub>2</sub> population in potato. *Theor. Appl. Genet.* doi:10.1007/s00122-016-2673-7
- Eshed, Y., G. Gera, and D. Zamir. 1996. A genome-wide search for wild-species alleles that increase horticultural yield of processing tomatoes. *Theor. Appl. Genet.* 93:877–886. doi:10.1007/BF00224089
- Eshed, Y., and D. Zamir. 1995. An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. *Genetics* 141:1147–1162.
- Fasoula, V.A., and D.A. Fasoula. 2002. Principles underlying genetic improvement for high and stable crop yield potential. *F. Crop. Res.* 75:191–209. doi:10.1016/S0378-4290(02)00026-6
- Fénart, S., J.-F. Arnaud, I. De Cauwer, and J. Cuguen. 2008. Nuclear and cytoplasmic genetic diversity in weed beet and sugar beet accessions compared to wild relatives: New insights into the genetic relationships within the *Beta vulgaris* complex species. *Theor. Appl. Genet.* 116:1063–1077. doi:10.1007/s00122-008-0735-1
- Fischer, S., A.E. Melchinger, V. Korzun, P. Wilde, B. Schmiedchen, J. Möhring, H.-P. Piepho, B.S. Dhillon, T. Würschum, and J.C. Reif. 2010. Molecular marker assisted broadening

- of the Central European heterotic groups in rye with Eastern European germplasm. *Theor. Appl. Genet.* 120:291–299. doi:10.1007/s00122-009-1124-0
- Fridman, E., F. Carrari, Y.-S. Liu, A.R. Fernie, and D. Zamir. 2004. Zooming in on a quantitative trait for tomato yield using interspecific introgressions. *Science* 305:1786–1789. doi:10.1126/science.1101666
- Fridman, E., T. Pleban, and D. Zamir. 2000. A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. *Proc. Natl. Acad. Sci. USA* 97:4718–4723. doi:10.1073/pnas.97.9.4718
- Golmirzaie, A., P. Malagamba, and N. Pallais. 1994. Breeding potatoes based on true seed propagation. In: J. Bradshaw and G. MacKay, editors, *Potato genetics*. CAB International, Wallingford, UK. p. 499–512.
- Golmirzaie, A.M., R. Ortiz, G.N. Atlin, and M. Iwanaga. 1998. Inbreeding and true seed in tetrasomic potato. I. Selfing and open pollination in Andean landraces (*Solanum tuberosum* Gp. Andigena). *Theor. Appl. Genet.* 97:1125–1128. doi:10.1007/s001220051000
- Grun, P., M. Aubertin, and A. Radlow. 1962. Multiple differentiation of plasmons of diploid species of *Solanum*. *Genetics* 47:1321–1333.
- Guern, A. 1940. On self pollinated strains of potatoes. *Proc. Leningr. Acad. Agric. Sci. USSR* 7:29–36.
- Haldane, J. 1930. Theoretical genetics of autopolyploids. *J. Genet.* 22:359–372. doi:10.1007/BF02984197
- Hanneman, R.E.J., and S.J. Peloquin. 1969. Use of *Phureja* and haploid to enhance the yield of cultivated tetraploid potatoes. *Am. Potato J.* 46:436.
- Hawkes, J.G. 1990. *The potato: Evolution, biodiversity, and genetic resources*. Smithsonian Institution Press, Washington, DC.
- Hermundstad, S.A., and S. Peloquin. 1985. Male fertility and 2n pollen production in haploid-wild species hybrids. *Am. J. Potato Res.* 62:479–487. doi:10.1007/BF02854521
- Hermundstad, S.A., and S.J. Peloquin. 1986. Tuber yield and tuber traits of haploid-wild species F<sub>1</sub> hybrids. *Potato Res.* 29:289–297. doi:10.1007/BF02359958
- Hirsch, C., C. Hirsch, K. Felcher, J. Coombs, D. Zarka, A. VanDeynze, W. DeJong, R. Veilleux, S. Jansky, P. Bethke, D. Douches, and C. Buell. 2013. Retrospective view of North American potato (*Solanum tuberosum* L.) breeding in the 20th and 21st centuries. *G3 Genes Genomes Genet.* 3:1003–1013.
- Hoopes, R., R. Plaisted, and A. Cubillos. 1980. Yield and fertility of reciprocal-cross *Tuberosum*-*Andigena* hybrids. *Am. Potato J.* 57:275–284. doi:10.1007/BF02855305
- Hosaka, K., and R.E. Hanneman. 1998. Genetics of self-compatibility in a self-incompatible wild diploid potato species *Solanum chacoense*. 1. Detection of an S locus inhibitor (*Sli*) gene. *Euphytica* 99:191–197. doi:10.1023/A:1018353613431
- Hougas, R.W., S.J. Peloquin, and R.W. Ross. 1958. Haploids of the common potato. *J. Hered.* 49:103–106.
- Howard, H.W. 1975. The prolonged storage of true seeds of potatoes. *Potato Res.* 18:320–321. doi:10.1007/BF02361736
- Huang, X.Q., H. Cöster, M.W. Ganai, and M.S. Röder. 2003. Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 106:1379–1389.
- Hutten, R. 1994. Basic aspects of potato breeding via the diploid level. Ph.D. thesis, Wageningen Univ., Wageningen, the Netherlands. 93 pp.
- Iwanaga, M., R. Ortiz, M.S. Cipar, and S.J. Peloquin. 1991. A restorer gene for genetic-cytoplasmic male sterility in cultivated potatoes. *Am. Potato J.* 68:19–28. doi:10.1007/BF02893338
- Jacobsen, T.L., and S.H. Jansky. 1989. Effects of pre-breeding wild species on tuberization of *Solanum tuberosum* haploid-wild species hybrids. *Am. J. Potato Res.* 66:803–811. doi:10.1007/BF02853961
- Jansky, S., P. Bethke, and D. Spooner. 2014a. Yield gains in potato: Contributing factors and future prospects. In: S. Smith, B. Diers, B. Carver, and J. Specht, editors, *Yield gains in major U.S. field crops*. Crop Science Society of America, Madison, WI. In press.
- Jansky, S., Y. Chung, and P. Kittipadukul. 2014b. M<sub>6</sub>: A diploid potato inbred line for use in breeding and genetics research. *J. Plant Reg.* 8:195–199. doi:10.3198/jpr2013.05.0024crg
- Jansky, S., G. Davis, and S.J. Peloquin. 2004. A genetic model for tuberization in potato haploid-wild species hybrids grown under long-day conditions. *Am. J. Potato Res.* 81:335–339. doi:10.1007/BF02870179
- Jansky, S., A. Hamernik, and X. Cai. 2012. Rapid cycling with true potato seed. *Seed Sci. Technol.* 40:43–50. doi:10.15258/sst.2012.40.1.05
- Jansky, S., G. Yerk, and S. Peloquin. 1990. The use of potato haploids to put 2x wild species germplasm into a usable form. *Plant Breed.* 104:290–294. doi:10.1111/j.1439-0523.1990.tb00438.x
- Johansen-Morris, A.D., and R.G. Latta. 2006. Fitness consequences of hybridization between ecotypes of *Avena barbata*: Hybrid breakdown, hybrid vigor, and transgressive segregation. *Evolution* 60:1585–1595.
- Kidane-Mariam, H.M., G.C. Arndt, A.C. Macaso-Khwaja, and S.J. Peloquin. 1985a. Comparisons between 4x × 2x hybrid and open-pollinated true-potato-seed families. *Potato Res.* 28:35–42. doi:10.1007/BF02357568
- Kidane-Mariam, H.M., H. Mendoza, and R. Wissar. 1985b. Performances of true potato seed families derived from intermating tetraploid parental lines. *Am. Potato J.* 62:643–652. doi:10.1007/BF02853473
- Krantz, F. 1924. Potato breeding methods. *Minnesota Agric. Ext. Stn. Tech. Bull.* 25:3–32.
- Krantz, F.A. 1929. Potato breeding methods II. Selection in inbred lines. *Minnesota Agric. Ext. Stn. Tech. Bull.* 58:3–26.
- Kump, K.L., P.J. Bradbury, R.J. Wisser, E.S. Buckler, A.R. Belcher, M.A. Oropeza-Rosas, J.C. Zwonitzer, S. Kresovich, M.D. McMullen, D. Ware, P.J. Balint-Kurti, and J.B. Holland. 2011. Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nat. Genet.* 43:163–168. doi:10.1038/ng.747
- Levin, D.A. 1983. Polyploidy and novelty in flowering plants. *Am. Nat.* 122:1–25. doi:10.1086/284115
- Lewis, D. 1943. Physiology of incompatibility in plants. III. Autopolyploids. *J. Genet.* 45:171–185. doi:10.1007/BF02982933
- Lewis, D. 1947. Competition and dominance of incompatibility alleles in diploid pollen. *Heredity* 1:85–108. doi:10.1038/hdy.1947.5
- Li, Z., S.R.M. Pinson, A.H. Paterson, W.D. Park, and J.W. Stansel. 1997. Genetics of hybrid sterility and hybrid breakdown in an intersubspecific rice (*Oryza sativa* L.) population. *Genetics* 145:1139–1148.
- Li, J., B. Schulz, and B. Stich. 2010. Population structure and genetic diversity in elite sugar beet germplasm investigated with SSR markers. *Euphytica* 175:35–42. doi:10.1007/s10681-010-0161-8

- Lindhout, P., D. Meijer, T. Schotte, R.C.B. Hutten, R.G.F. Visser, and H.J. Eck. 2011. Towards F<sub>1</sub> hybrid seed potato breeding. *Potato Res.* 54:301–312. doi:10.1007/s11540-011-9196-z
- Little, T. 1945. Gene segregation in autotetraploids. *Bot. Rev.* 11:60–85. doi:10.1007/BF02861164
- Little, T.M. 1952. Gene segregation in autotetraploids II. *Bot. Rev.* 24:318–339. doi:10.1007/BF02872438
- Maris, B. 1990. Comparison of diploid and tetraploid potato families derived from *Solanum phureja* × dihaploid *S. tuberosum* hybrids and their vegetatively doubled counterparts. *Euphytica* 46:15–33. doi:10.1007/BF00057615
- McCord, P., L. Zhang, and C. Brown. 2012. The incidence and effect on total tuber carotenoids of a recessive zeaxanthin epoxidase allele (*Zep1*) in yellow-fleshed potatoes. *Am. J. Potato Res.* 89:262–268. doi:10.1007/s12230-012-9250-7
- Meissner, R., Y. Jacobson, S. Melamed, S. Levyatuv, G. Shalev, A. Ashri, Y. Elkind, and A. Levy. 1997. A new model system for tomato genetics. *Plant J.* 12:1465–1472. doi:10.1046/j.1365-313x.1997.12061465.x
- Monforte, J., E. Friedman, D. Zamir, and S.D. Tanksley. 2001. Comparison of a set of allelic QTL-NILs for chromosome 4 of tomato: Deductions about natural variation and implications for germplasm utilization. *Theor. Appl. Genet.* 102:572–590. doi:10.1007/s001220051684
- Muthoni, J., H. Shimelis, and R. Melis. 2013. Alleviating potato seed tuber shortage in developing countries: Potential of true potato seeds. *Aust. J. Crop Sci.* 7:1946–1954.
- Ortiz, R., P. Simon, S. Jansky, and D. Stelly. 2009. Ploidy manipulation of the gametophyte, endosperm and sporophyte in nature and for crop improvement: A tribute to Professor Stanley J. Peloquin (1921–2008). *Ann. Bot. (Lond.)* 104:795–807. doi:10.1093/aob/mcp207
- Paz, M., and R. Veilleux. 1999. Influence of culture medium and in vitro conditions on shoot regeneration in *Solanum phureja* monoloids and fertility of regenerated doubled monoloids. *Plant Breed.* 118:53–57. doi:10.1046/j.1439-0523.1999.118001053.x
- Peloquin, S.J., A.C. Gabert, and R. Ortiz. 1996. Nature of “pollinator” effect in potato (*Solanum tuberosum* L.) haploid production. *Ann. Bot. (Lond.)* 77:539–542. doi:10.1006/anbo.1996.0064
- Peloquin, S.J., and R.W. Hougas. 1960. Genetic variation among haploids of the common potato. *Am. Potato J.* 37:289–297. doi:10.1007/BF02855072
- Perl, A., D. Aviv, and E. Galun. 1990. Protoplast-fusion-derived CMS potato cybrids: Potential seed-parents for hybrid, true-potato-seeds. *J. Hered.* 81:438–442.
- Phumichai, C., and K. Hosaka. 2006. Cryptic improvement for fertility by continuous selfing of diploid potatoes using *Sli* gene. *Euphytica* 149:251–258. doi:10.1007/s10681-005-9072-5
- Phumichai, C., M. Mori, A. Kobayashi, O. Kamijima, and K. Hosaka. 2005. Toward the development of highly homozygous diploid potato lines using the self-compatibility controlling *Sli* gene. *Genome* 48:977–984. doi:10.1139/g05-066
- Plaisted, R. 1973. Components of yield in potato crosses involving andigena and tuberosum germplasm. *Am. Potato J.* 50:336. doi:10.1007/BF02851772
- Potato Genome Initiative. 2011. Genome sequence and analysis of the tuber crop potato. *Nature* 475:189–195. doi:10.1038/nature10158
- Pule, B.B., J.C. Meitz, A.H. Thompson, C.C. Linde, W.E. Fry, S.D. Langenhoven, K.L. Meyers, D.S. Kandolo, N.C. Van Rij, and A. McLeod. 2013. *Phytophthora infestans* populations in central, eastern and southern African countries consist of two major clonal lineages. *Plant Pathol.* 62:154–165. doi:10.1111/j.1365-3059.2012.02608.x
- Reif, J.C., S. Fischer, T.A. Schrag, K.R. Lamkey, D. Klein, B.S. Dhillon, H.F. Utz, and A.E. Melchinger. 2010. Broadening the genetic base of European maize heterotic pools with US corn-belt germplasm using field and molecular marker data. *Theor. Appl. Genet.* 120:301–310. doi:10.1007/s00122-009-1055-9
- Riedelshimer, C., A. Czedik-Eysenberg, C. Grieder, J. Lisek, F. Technow, R. Sulpice, T. Altmann, M. Stitt, L. Willmitzer, and A.E. Melchinger. 2012. Genomic and metabolic prediction of complex heterotic traits in hybrid maize. *Nat. Genet.* 44:217–220. doi:10.1038/ng.1033
- Ristaino, J.B. 2002. Tracking historic migrations of the Irish potato famine pathogen, *Phytophthora infestans*. *Microbes Infect.* 4:1369–1377. doi:10.1016/S1286-4579(02)00010-2
- Ronen, G., L. Carmel-Goren, D. Zamir, and J. Hirschberg. 2000. An alternative pathway to beta-carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. *Proc. Natl. Acad. Sci. USA* 97:11102–11107. doi:10.1073/pnas.190177497
- Ronning, C., L. Sanford, R. Kobayashi, and S. Kowalski. 1998. Foliar leptine production in segregating F<sub>1</sub>, inter-F, and back-cross families of *Solanum chacoense* bitter. *Am. J. Potato Res.* 75:137–143. doi:10.1007/BF02895848
- Rowe, P.R. 1967a. Performance and variability of diploid and tetraploid potato families. *Am. Potato J.* 44:263–271. doi:10.1007/BF02862526
- Rowe, P.R. 1967b. Performance of diploid and vegetatively doubled clones of Phureja-haploid Tuberosum hybrids. *Am. Potato J.* 44:195–203. doi:10.1007/BF02893091
- Sanford, J.C. 1983. Ploidy manipulations. In: J.N. Moore and J. Janick, editors, *Methods in fruit breeding*. Purdue Univ. Press, Purdue. p. 100–123.
- Sanford, J., and R. Hanneman. 1982. A possible heterotic threshold in potato and its implications for breeding. *Theor. Appl. Genet.* 61:151–159. doi:10.1007/BF00273884
- Santini, M., E.L. Camadro, O.N. Marcellán, and L.E. Erazzú. 2000. Agronomic characterization of diploid hybrid families derived from crosses between haploids of the common potato and three wild Argentinean tuber-bearing species. *Am. J. Potato Res.* 77:211–218. doi:10.1007/BF02855788
- Schillmiller, A.L., A.L. Charbonneau, and R.L. Last. 2012. Identification of a BAHD acetyltransferase that produces protective acyl sugars in tomato trichomes. *Proc. Natl. Acad. Sci. USA* 109:16377–16382. doi:10.1073/pnas.1207906109
- Shull, G. 1909. A pureline method of corn breeding. *Am. Breeders Assoc. Rep.* 5:51–59.
- Simmonds, N. 1963. Experiments on the germination of potato seeds. I. *Potato Res.* 6:45–60. doi:10.1007/BF02364634
- Simmonds, N.W. 1964. The genetics of seed and tuber dormancy in the cultivated potatoes. *Heredity (Edinb)* 19:489–504. doi:10.1038/hdy.1964.56
- Simmonds, N. 1997. A review of potato propagation by means of seed, as distinct from clonal propagation by tubers. *Euphytica* 40:191–214.
- Spooner, D.M., M. Ghislain, R. Simon, S.H. Jansky, and T. Gavrilenko. 2014. Systematics, diversity, genetics, and evolution of wild and cultivated potatoes. *Bot. Rev.* 80:283–383. doi:10.1007/s12229-014-9146-y

- Spooner, D.M., J. Núñez, G. Trujillo, M.D.R. Herrera, F. Guzmán, M. Ghislain, J. Nunez, M. del Rosario Herrera, and F. Guzman. 2007. Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. *Proc. Natl. Acad. Sci. USA* 104:19398–19403. doi:10.1073/pnas.0709796104
- Stevens, R., M. Buret, P. Duffé, C.C. Garchery, P. Baldet, C. Rothan, M. Causse, and P. Duffé. 2007. Candidate genes and quantitative trait loci affecting fruit ascorbic acid content in three tomato populations. *Plant Physiol.* 143:1943–1953. doi:10.1104/pp.106.091413
- Suwarno, W.B. 2014. The usefulness of molecular markers approach for developing heterotic groups in maize. *J. Trop. Crop Sci.* 1:4–10.
- Tan, M.Y.A., R.C.B. Hutten, R.G.F. Visser, and H.J. van Eck. 2010. The effect of pyramiding *Phytophthora infestans* resistance genes *RPI-mcd1* and *RPI-ber* in potato. *Theor. Appl. Genet.* 121:117–125. doi:10.1007/s00122-010-1295-8
- Tucci, M., D. Carputo, G. Bile, and L. Frusciante. 1996. Male fertility and freezing tolerance of hybrids involving *Solanum tuberosum* haploids and diploid *Solanum* species. *Potato Res.* 39:345–353. doi:10.1007/BF02357938
- Uitdewilligen, J., A.-M. Wolters, V. der Vlies, R. Visser, and H. Van Eck. 2011. SNP and haplotype identification from targeted next-generation re-sequencing in a set of 83 potato cultivars. 18th Trienn. Conf. Eur. Assoc. Potato Res. p. 97.
- Veilleux, R. 1990. *Solanum phureja*: Anther culture and the induction of haploids in a cultivated diploid potato species. In: Y. Bajaj, editor, *Biotechnology in agriculture and forestry 12: Haploids in crop improvement I*. Springer-Verlag, New York. p. 530–543.
- Vos, P.G., J.G.A.M.L. Uitdewilligen, R.E. Voorrips, R.G.F. Visser, and H.J. van Eck. 2015. Development and analysis of a 20K SNP array for potato (*Solanum tuberosum*): An insight into the breeding history. *Theor. Appl. Genet.* 128:2387–2401. doi:10.1007/s00122-015-2593-y
- Watanabe, K.N., M. Orrillo, S. Vega, M. Iwagana, R. Ortiz, R. Freyre, G. Yerk, S.J. Peloquin, and K. Ishiki. 1995. Selection of diploid potato clones from diploid (haploid × wild species) F<sub>1</sub> hybrid families for short day conditions. *Breed. Sci.* 45:341–347.
- Werner, J.E., and S.J. Peloquin. 1991. Significance of allelic diversity and 2n gametes for approaching maximum heterozygosity in 4x potatoes. *Euphytica* 58:21–29. doi:10.1007/BF00035336
- Wolters, A.-M.A., J.G.A.M.L. Uitdewilligen, B.A. Kloosterman, R.C.B. Hutten, R.G.F. Visser, and H.J. van Eck. 2010. Identification of alleles of carotenoid pathway genes important for zeaxanthin accumulation in potato tubers. *Plant Mol. Biol.* 73:659–671. doi:10.1007/s11103-010-9647-y
- Yerk, G.L., and S.J. Peloquin. 1989. Evaluation of tuber traits of 10, 2x(2EBN) wild species through haploid × wild species hybrids. *Am. Potato J.* 66:731–739. doi:10.1007/BF02896829
- Zhang, Y., C.S. Jung, and W.S. De Jong. 2009. Genetic analysis of pigmented tuber flesh in potato. *Theor. Appl. Genet.* 119:143–150. doi:10.1007/s00122-009-1024-3
- Zhao, K., M.J. Aranzana, S. Kim, C. Lister, C. Shindo, C. Tang, C. Toomajian, H. Zheng, C. Dean, P. Marjoram, and M. Nordborg. 2007. An *Arabidopsis* example of association mapping in structured samples. *PLoS Genet.* 3:71–84. doi:10.1371/journal.pgen.0030004