

Reinventing Potato as a Diploid Inbred Line–Based Crop

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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.

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

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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 F₂ 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 F₂ 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.

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