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SYMPOSIUM

Diploid Potatoes as a Catalyst for Change in the Potato Industry

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Abstract
In response to increasing interest in diploid potato (*Solanum tuberosum*) breeding and the production of diploid inbred hybrid potato varieties, the Breeding and Genetics section of the Potato Association of America (PAA) organized a symposium on diploid breeding that took place during the 2021 PAA annual meeting. Proceedings from that symposium are documented in this manuscript. Speakers from academia, government and industry presented their unique perspectives. Presentations covered a wide range of topics. Potential advantages of diploid breeding were introduced, and reasons to be skeptical about diploid breeding were highlighted. The impact that diploid breeding might have on the potato seed industry was discussed. Advantages for genetics research were emphasized. Aspects of tomato breeding and production were reviewed and considered as potential models for diploid potato breeding and production activities. Lastly, an industry-centered view of diploid potato breeding was provided. Taken together, these presentations are a snapshot of how diploid potato breeding was viewed in the moment, a vision for how diploid breeding might be implemented, and a thoughtful reflection on how diploid breeding and inbred hybrid varieties might change the potato variety development process and impact the potato industry.

Resumen
En respuesta al creciente interés en el mejoramiento de la papa diploide (Solanum tuberosum) y la producción de variedades híbridas de papa consanguínea diploide, la sección de Mejoramiento y Genética de la Asociación Norteamericana de la Papa (PAA) organizó un simposio sobre el mejoramiento de diploides que tuvo lugar durante la reunión anual de la PAA 2021. Las actas de ese simposio están documentadas en este manuscrito. Oradores de la academia, el gobierno y la industria presentaron sus perspectivas únicas. Las presentaciones cubrieron una amplia gama de temas. Se mencionaron ventajas potenciales del mejoramiento de diploides, y se destacaron las razones para ser escépticos sobre el mejoramiento de diploides. Se discutió el impacto que el mejoramiento de diploides podría tener en la industria de semillas de papa. Se enfatizaron las ventajas para la investigación genética. Se revisaron los aspectos del mejoramiento y producción de tomate y se consideraron como modelos potenciales para las actividades de mejoramiento y producción de papa diploide. Por último, se proporcionó una visión centrada en la industria del mejoramiento de papa diploide. En conjunto, estas presentaciones son una instantánea de cómo se vio el mejoramiento de la papa diploide en el momento, una visión de cómo se podría implementar el mejoramiento diploide y una reflexión bien pensada sobre cómo el mejoramiento diploide y las variedades híbridas endogámicas podrían cambiar el proceso de desarrollo de variedades de papa e impactar a la industria.

Keywords Inbred hybrid breeding · True potato seed · Potato seed certification · Potato genetics and genomics · Sustainable potato production

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Introduction

This collection of symposium papers is adapted from a transcript of talks given at the 2021 annual meeting of the Potato Association of America. It has been edited for clarity and grammar. We have tried to preserve the "speaking-style" of the presenters to make the topics accessible to readers who are not experts in potato breeding and genetics.

An Overview of Diploid Potatoes and a Brief Refresher on Potato Genetics

Presenter: Dennis Halterman, Research Geneticist, USDA.

Ploidy is a Determinant of Genetic Complexity

The ploidy of an organism tells us how many copies of each chromosome are present. A haploid has one copy, a diploid has two, a triploid has three, a tetraploid has four, and so forth. Cultivated potato is tetraploid. It has four copies of each chromosome and therefore four copies of each gene. Diploid potato has two copies of each chromosome and therefore two copies of each gene. Why is this important? Most potato traits are determined by genes at specific locations on a chromosome. In organisms with multiple copies of each chromosome, such as diploid or tetraploid potato, there may be different variants of these genes. These variants are called alleles. If the alleles of a gene are the same on each copy of a chromosome, the individual plant is considered homozygous for that gene. If the alleles are different, it is heterozygous for that gene.

For simplicity, consider a gene that has two alleles, as illustrated in Fig. 1. Tetraploid individuals can be homozygous for one allele, or for the other. Alternatively, they can be heterozygous and have one, two or three copies of either allele. Thus, there are five possible combinations of two alleles for a gene in a tetraploid individual (Fig. 1). When there are more than two alleles of a gene, the number of possible allelic combinations increases. Approximately 20,000 genes in an individual clone of cultivated potato are heterozygous (Hirsch et al. 2013). This is not surprising, since increasing heterozygosity was a goal of potato breeders (Mendoza and Haynes 1974). When two heterozygous plants are crossed, the progeny contain one of many allelic combinations at each gene where one of the parents is heterozygous. As a result of this extensive underlying genetic variation, the progeny exhibit extensive variation in multiple traits. Superior individuals are those that, by chance, have favorable combinations of alleles for hundreds if not thousands of genes. Such individuals are rare and this makes generating and identifying them a monumental challenge. This approach of searching for rare individuals in large populations is the basis of the tetraploid potato breeding system (Jansky and Spooner 2018). Conversely, when two plants that are homozygous at all genes are crossed, all of the progeny are identical because the same combination of alleles is present in all offspring. The ability to produce uniform offspring is the basis of inbred hybrid breeding.

Inbred Lines are Made from Self-Compatible Individuals

The most common way to generate plants that are homozygous at all, or nearly all, genes is to inbreed them. Plants are self-pollinated by collecting pollen from one flower and using it to pollinate a flower on the same plant. This process is repeated for several generations and in each subsequent generation, the amount of homozygosity increases. That is, with increasing frequency, only a single allele is present at each gene. For diploid individuals, five to six generations are typically needed to reach a threshold of about 90% homozygosity, and this is considered the goal to develop suitable inbred lines for hybrid breeding (Jansky et al. 2016; Zhang et al. 2021).

Inbreeding is conceptually simple, but there are operational challenges. For example, most diploid potatoes are self-incompatible, i.e. self-pollination results in either no fruit or fruit with no seeds (Swaminathan and Howard 1953; Hawkes 1958; Hawkes 1990; Zhang et al. 2019). Research is beginning to reveal how self-compatibility is determined in potato and some of the important genes involved in this process have been identified (Hosaka and Hanneman 1998a,

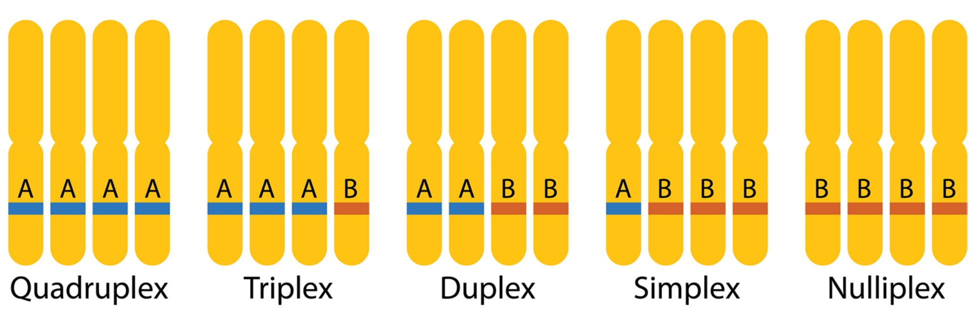

Fig. 1 Possible combinations of a biallelic trait in tetraploid potato

b; Kao and McCubbin 1996; Dzidzienyo et al. 2016). One of the first steps in producing parents for diploid potato breeding is incorporating genes that promote self-compatibility, such as the self-incompatibility inhibitor gene *Sli* (Hosaka and Hanneman 1998a, b).

Heterozygous Plants Have Numerous Undesirable Alleles

Alleles of a gene may contribute positively to a potato clone, or they may not have an observable effect. Alternatively, some alleles cause undesirable defects or production problems (Zhang et al. 2019). As an example, imagine you have two alleles of a gene (Fig. 2). Allele 'A' provides a nice tuber shape. Allele 'B' causes the tubers to have an undesirable pear shape. In this example, whenever an 'A' allele is present the tubers have a nice tuber shape because the 'A' allele is dominant over the 'B' allele. Most of the individuals in this population have a nice tuber shape because they have at least one 'A' allele. Only a few individuals have the undesirable pear shape and they are likely to be discarded by a breeding program. However, most progeny in this population will be heterozygous for the 'B' allele and their progeny will continue to segregate for pear shape when they used as parents in the future.

In tetraploid potato, it is extremely difficult to remove deleterious alleles from breeding lines (Jansky and Spooner 2018). They are carried silently from generation to generation at heterozygous loci. When an individual that carries one, two or three copies of the deleterious allele is used as a parent in a breeding program, rare offspring that are homozygous for that allele will express the undesirable trait and can be discarded. Since deleterious alleles are scattered throughout the genome, the proportion of discarded individuals can be very large. Unfortunately, removing those individuals does not alleviate the problem. Most of the offspring retained by the breeding program will be heterozygous for numerous undesirable recessive alleles and will pass them on to the next generation.

In diploid breeding, with only two alleles at each locus, plants that are homozygous for a deleterious recessive allele occur at greater frequency than in tetraploid breeding and are easier to remove from the breeding population. To use the previous example, if two individuals heterozygous for the deleterious tuber shape gene are crossed, one quarter of the offspring will be homozygous for the deleterious allele and can be discarded (Fig. 2B) compared to only 2% in the tetraploid population. Equally important, one quarter of the offspring will have no copies of the deleterious allele and will breed true for attractive tuber shape. At the diploid level, it is also easier to select for desirable traits that are only expressed when two recessive alleles are present. Examples include certain glycoalkaloids that confer insect resistance (Sanford et al. 1996; Ronning et al. 1999; Yencho et al. 2000) and genes required to give a deep yellow or orange color to the tuber flesh (Wolters et al. 2010).

Potential Benefits of Diploid Potatoes

Breeding with diploid potato is expected to be a more efficient and effective process than tetraploid breeding (Jansky et al. 2016). Breeding cycles to develop improved varieties are estimated to be one to three years instead of the current five to eight years (Jansky et al. 2016). It will be easier to introduce traits from wild potato relatives, many of which are diploid (Bethke et al. 2017, 2019). Equally

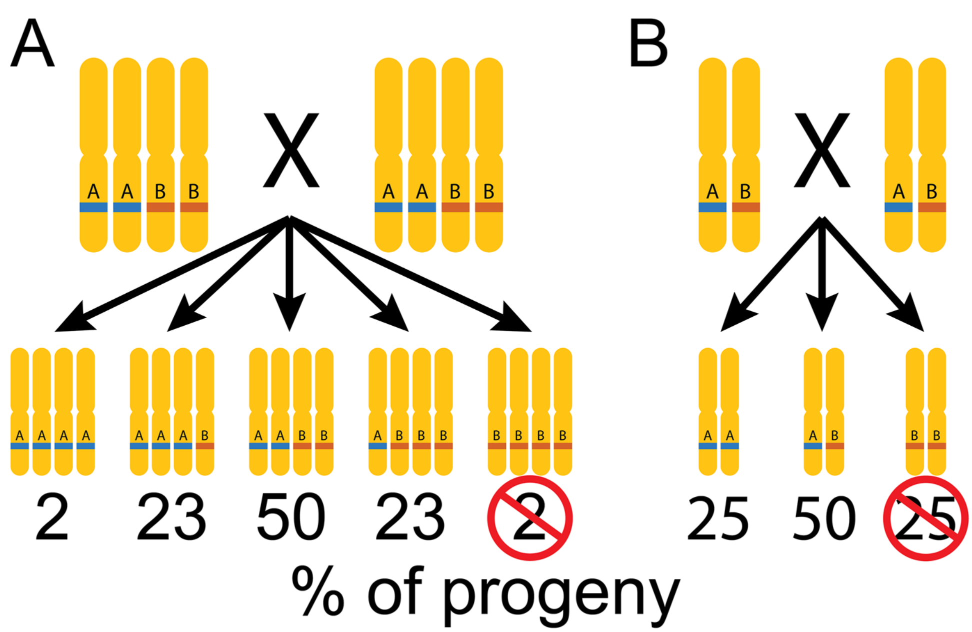

Fig. 2 Effect of heterozygosity on the ability to remove undesirable traits in tetraploid (**A**) and diploid (**B**) progeny. The progeny exhibiting the undesirable recessive trait are those homozygous for the 'B' allele and these can be removed easily from the breeding system (indicated by the circle with a diagonal line through it)

important, not only will it be easier to introduce beneficial traits like those for disease resistance, but it will be easier and much faster to remove problematic alleles that currently plague the breeding process (Jansky et al. 2016; Zhang et al. 2021). We will be able to use genomic tools and gene editing more efficiently with diploid rather than tetraploid potato. Since the genome size is reduced by half, sequencing data are more informative and allelic variation is easier to deal with. And because the entire breeding process is more efficient, we anticipate that new varieties will reflect a more rapid rate of genetic gain. Finally, true potato seed will increase seed availability to growers in a shorter amount of time.

A Skeptics View of Diploid Breeding

Presenter: Paul Bethke, Research Plant Physiologist, USDA.

Do you believe everything you hear? The answer is probably "No". We know from experience that the revolution often does not lead to substantive change, that the paradigm shift often leaves us exactly where we started, that things that seem too good to be true are probably too good to be true (Simmonds 1991). We will begin by discussing diploid breeding from a skeptic's point of view. A skeptic surely has many questions. Why is diploid breeding a better approach than tetraploid breeding? Why did we not think of this before? Why now? Will it work? Who will use it? Who will make money from it? Some of these questions have answers, or partial answers. Some do not have answers and may not for many years. Undoubtedly there are important questions we have not asked yet.

An Outlandish Proposition

Let us begin by stating the obvious. Converting the world's third most important food crop from tetraploid to diploid is quite an outlandish proposition. There is essentially no precedent for this. Oddly enough, there is precedent for going the other way. Many horticultural crops have used conversion from diploid to tetraploid to increase the size of organs such as flowers and fruits, or to make them more resistant to stress (Sattler et al. 2016). We also start with, perhaps, some large assumptions about why we are currently growing tetraploid potatoes. One relates to the history of potato as it moved from South America to Europe. Cultivated diploid and tetraploid potatoes were developed in South America, but the potato that became a commercial crop throughout the world is tetraploid (Hardigan et al. 2017; Gutaker et al. 2019). We might assume that this outcome was just chance. If we could return to the fifteenth century and start over, maybe modern potato would be diploid. Or we might assume that conditions under which tetraploid potato was selected were substantially different from conditions today. Therefore, if we started making selections again with the same mix of South American germplasm, we might be as likely to end up with diploid potato as tetraploid potato. We also might assume that by using genomic technologies and molecular tools, we can create diploid potatoes that are as good or superior to tetraploid potatoes, even tetraploids improved using the same approaches. Each of these assumptions allow us to believe that there is nothing fundamentally superior about tetraploid compared with diploid potato.

A Clear Goal, But How Far away?

So far, the discussion has been about diploid potatoes, but that is not really the end goal. The goal is diploid hybrid potato varieties, produced by crossing inbred parents, and propagated from true potato seed (Jansky et al. 2016). That is the long-range goal. By making it specific, by stating it clearly, it sounds achievable. But we know from experience that being able to clearly describe a desired outcome does not make it happen. A very specific goal is 'peace and prosperity for everyone', but there are many roadblocks in the way that have kept us from achieving that goal. Likewise, before we have successful diploid hybrid potato varieties, many different things must happen: the genetics of diploid potato have to come together in just the right way; technologies for producing hybrid potatoes and true potato seed have to come together; and procedures for commercialization need to be established. We have a goal, but it is right for a skeptic to wonder if the goal is so far off, 10 years, 20 years, "a while", that the goal will always stay on the horizon, like a mirage - we make progress, but we never get there.

Why Have Diploid Potatoes Become a Hot Topic?

There are questions a skeptic might ask. For example, why has this not been done before if it is such a great idea? The answer to this question is based, in part, on how previous observations were interpreted. There was a belief that you could not make vigorous inbred lines. That is certainly true for tetraploid potato (Krantz 1946; Simmonds 1997). The time it takes to make an inbred tetraploid potato is probably as long as the career of a potato breeder; it takes decades. Diploid potatoes, like the wild species relatives of potato from which cultivated potato was derived, were thought to be self-incompatible (Cipar et al. 1964). You could not make inbred lines with diploid potatoes because they were obligate out crossers.

There was also a belief that a greater number of alleles conferred advantages to the crop (Bonierbale et al. 1993). Therefore, four copies of each chromosome should have advantages over two copies. Think for example of a disease resistance trait. If, as usual, there is genetic variation in the population of organisms that cause disease, then four alleles for a resistance gene targeted to that disease may provide more complete protection than two alleles.

Finally, there was a longstanding belief that tetraploid potatoes yield more than diploid potatoes (Mendoza and Haynes 1974; Muthoni et al. 2019; Maris 1990). The data in Fig. 3 are quite provocative. Potato breeders might be familiar with this study (Uijtewaal et al. 1987), but it is unlikely to be familiar to many others. On the very bottom of the photograph in Fig. 3 is M9, which is a diploid potato. M9 was used to produce a series of monoploids. Those are the three lines indicated by the cards on the left. Then the single copy of each chromosome in these monoploids was doubled and doubled again. The result was monoploid (x), diploid (2x) and tetraploid (4x) lines that all had exactly the same alleles in exactly the same order. The only difference was ploidy. In this study, as the potatoes went from monoploid to diploid to tetraploid, tuber size increased, and yield went up. It may be important to note that the monoploid tubers were very small because of the severe inbreeding depression that occurred when the monoploid was produced. The data suggest, however, that there may be advantage to being tetraploid.

Another question a skeptic might ask is, why now? Why is this the time when we are suddenly going to shift our thinking and move away from tetraploid potatoes towards diploid potatoes? The one thing we can say for sure is that it is not because people today are smarter than they were in the past. There is plenty of evidence to show that is not true (Hegelund et al. 2021; Bratsberg and Rogeberg 2018). What has changed? Part of the answer is we have learned more about self-compatibility. We have developed some genetic tricks that allow for self-compatibility to be manipulated that we did not have before (Jansky et al. 2014; Ye et al. 2018; Enciso-Rodriguez et al. 2019; Ma et al. 2021). That really is an important part of the answer. We also have some very recent, intriguing data on yield and size that suggests that diploid potatoes can make big potatoes (Alsahlany et al. 2021), and that yields might be comparable to those of tetraploid potatoes (Marand et al. 2019). In general, however, those data have not been from apples-to-apples comparisons. They have compared an unselected or early generation breeding line to a variety. The variety, of course, has high yield but it also has been selected for numerous other traits; the breeding line has often been selected for just a few traits or maybe just for yield. Finally, it goes without saying that new tools for molecular genomics and genetics raise the possibility that we can do things with potato, especially diploid potato, now that we have not been able to do before.

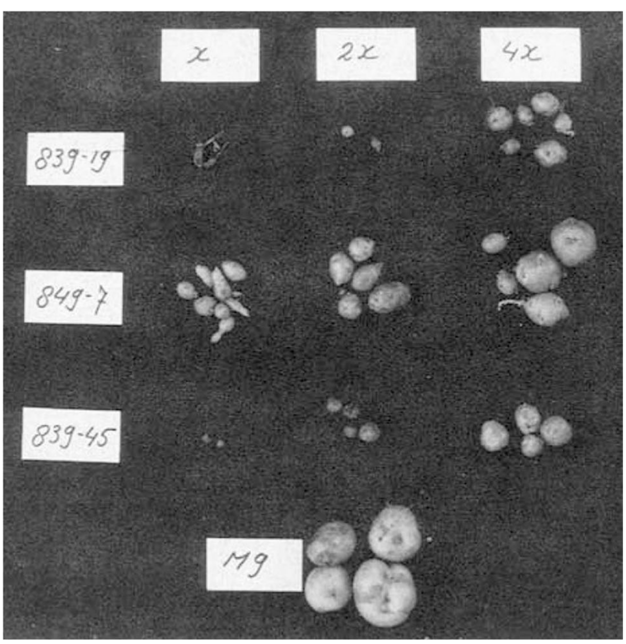

Fig. 3 Tuber production per plant of the heterozygous diploid line M9, three monoploids (x) derived from M9, mitotically doubled diploid (2x) lines and twice-doubled tetraploid (4x) lines produced from each monoploid. Figure originally published by Uijtewaal et al. 1987. Reprinted with permission

Potential Advantages of Diploid Potatoes

Even a skeptic can see clear advantages to diploid potatoes. There is no doubt that diploid potato will be a boon for potato research. This is not a new idea. Diploid potatoes have been used for genetics research for decades (see for example De Jong 1981; De Jong and Burns 1993; Douches and Freyer 1994; Thill and Peloquin 1994). Still, diploid potatoes coupled with computational biology and gene editing creates extraordinary opportunities for learning about how potato works, for answering some of the basic questions about potato. That research, however, is different from developing diploid hybrid potato varieties.

Diploid hybrid potato varieties are likely to have certain advantages over tetraploid varieties. They do not need to be maintained in tissue culture and can be saved as seed. That is a huge advantage. Tissue culture is tedious, expensive, and prone to contamination and failure. Seeds are easy to store. A tremendous amount of potato seed can be stored in a common freezer. If the power goes out or the freezer stops working, it is okay. You wait until the power comes back on or the freezer is fixed and then turn it back on. Seeds are not as vulnerable as tissue culture plantlets to environmental perturbations.

Rapid multiplication of new varieties is likely to be a lot easier with diploid hybrid varieties than with tetraploid varieties. And it is going to be easier to incorporate some traits by conventional breeding, especially dominant single gene traits such as PVY resistance (Fulladolsa et al. 2015; Nie et al. 2016; Slater et al. 2020). Indeed, even a skeptic might agree that if diploid potato varieties are commercialized, they will all be resistant to PVY.

Problems We Can Foresee

A skeptic also recognizes that there are clear disadvantages to diploid hybrid varieties. For instance, producing the seeds is labor intensive (Almekinders et al. 2009; Pallais 1991). The best method we have is to pollinate flowers by hand (Malagamba et al. 1983). That is not particularly high throughput, but it is the method used by the tomato industry which means that it is feasible on a commercial scale. There are diseases of seeds that we have not had to think about in the past that may generate concerns in the future. More generally, current regulations related to seed production, seed movement across international borders, and phytosanitary regulations related to true potato seed quality are probably inadequate.

Unknowns Abound

There are many unknowns. Consider one that seems like a detail but is very important. The cost of seed is unknown. Profitability of diploid potato production depends on the cost of seed. Figure 4 shows pelleted potato seeds. Similar seeds could be the foundation of every new hybrid diploid potato variety that goes into the ground. How much it costs to produce those seeds is going to depend, in part, on where they are produced. Are they going to be produced overseas, or are they going to produced domestically? Many scenarios for the use of diploid potato varieties posit that we may be planting seeds in greenhouses, taking transplants to the field, collecting seed tubers, and then multiplying those seed tubers for one or more generations to bring down the cost per seed piece. The number of field years required under this scenario is unknown, but we do know that there are disadvantages to using more field generations to decrease cost. In particular, there are concerns about greater accumulation of disease with increased time in the field.

There is another unknown that is going to be a concern for the industry. Who will control the varieties? Currently in the U.S., there are potato breeding programs in the public and private sectors. A private breeding company has complete control over its varieties. As such, a hybrid potato variety developed by a private breeding company could be discontinued at any time and may be lost forever if the parents are not maintained. Public breeding programs might have a greater incentive to maintain varieties as a service to their constituents, but there will be logistical challenges involved with maintaining numerous hybrid lines and their inbred parents. It is not clear how public breeding programs will manage those challenges.

There is also a question about how public potato breeding efforts will interdigitate with private potato breeding efforts. Currently, individual parents are not particularly valuable as breeding lines and are freely shared among breeding programs. As programs start developing

Fig. 4 True potato seed (**A**), and true potato seed pelleted for uniformity and ease of handling (**B**)

inbred lines suitable for producing hybrids, those lines will become more valuable as they are improved. Inevitably, there will be disincentives for sharing germplasm. We do not know how this change will affect the exchange of ideas, technologies, and breeding stocks between public and private potato breeding programs in the future.

Finally, a skeptic is aware of another potentially significant unknown. If you have followed recent trends in agriculture, you know there has been a consolidation of the seed business so that a few companies control a large share of the market (Howard 2009). We might ask if potato production based on hybrid seed will follow the same path. If so, growers may have fewer options for seed and potato breeders will start to worry about access to germplasm (Luby and Goldman 2016; Kotschi and Horneburg 2018).

Are Hybrid Diploid Potatoes Economically Viable?

Finally, commercial successes of diploid potatoes is not guaranteed. We know that the same rules that apply to our current tetraploid varieties are going to apply to diploid varieties. New varieties must be superior to existing varieties to succeed. The marketplace is unlikely to care if a variety is diploid or tetraploid. What will matter is how well a variety grows, stores, ships, processes and sells.

Profit matters. From the developer of the variety, to the entity that sells the potatoes, to the final customer, everybody along the line has to make a profit in order for the system to work. For diploid varieties, the profitability equation changes at certain places along the line of production, and it is worth taking a brief look at those changes.

True potato seed licenses and royalties must cover the cost of variety development. That is certainly going to be true in the private breeding sector and is likely to be approximately true in the public sector. Profit from seed is the price of the seed times the volume of seeds sold, minus the expenses. If you are a skeptic, you are likely to believe that the price of seed will go up. Why would someone delivering a superior product sell it for less than existing products? But price does have limitations based on what the market will bear. Another way to increase revenue and cover expenses is to increase sales volume. That might mean looking at global markets rather than just regional markets. In this regard, big players in the industry may have advantages over smaller players. How do you minimize expenses for seed development? That turns out to be a very interesting question. In public breeding programs, many of the expenses are offset. Public breeding programs receive grants from government agencies, use laboratory, greenhouse and field facilities at subsidized rates, and some salaries are paid by universities or the government. Private breeding companies do not have these advantages, but they do have the ability to reduce expenses because people who work for them are dedicated to one thing - producing new varieties. Individuals working at public breeding programs are often working on multiple tasks, producing new varieties but also doing research on potato genetics, teaching students, and doing outreach to their stakeholder community.

Growers must make a profit. You cannot sell a variety where the growers lose money. What determines profit for a grower? It is yield times price, minus expenses. The biggest driver in that equation is yield. Nothing pays better than yield, so yield of new varieties is going to have to be high. Expenses are an interesting part of the equation for diploid potato varieties. Seed costs are likely to be higher. That is the skeptics view at least, which means that input costs may need to go down. The promise of diploid potato varieties is that they may reduce the need for inputs. But the reality is going to depend on the performance of the varieties under reduced input management conditions.

Do You Believe?

There are numerous promises made by those promoting diploid hybrid potatoes. Varieties will come to market quicker. Seed will have less disease. Diploid varieties will have increased resistance to disease, pests, and stress. Varieties will be improved through incremental change rather than by starting over. It sounds wonderful. So, here is the question: Should a skeptic believe it? And here is the answer: Believe it when you see it, just like you do for everything else.

These are some of the things that a skeptic will want to see before believing that diploid hybrid potato is really the way of the future. The first is higher yield potential. Genetic potential for yield of potatoes has barely increased during the last 100 years of potato breeding (Douches et al. 1996). If diploid potatoes live up to their promise, we should see an increase in yield potential comparable to that observed for almost every other crop. In short order, marketable yields should go up 10 to 20%.

Second, diploid varieties should maximize economic yield with fewer inputs. Specifically, one might look for substantial reductions in fumigation and fungicide applications. Growers use a lot of fungicides to control late blight. They would rather not do this because fungicides are expensive and may have unintended environmental consequences. Genetic control of late blight has been demonstrated for potatoes containing a late blight resistance gene inserted using biotechnology (Halterman et al. 2016; Byarugaba et al. 2021). If you can make improvements by introducing a gene using biotechnology, you can also do it by introducing the gene through genetics if you are working at the diploid level. Look for late blight resistant varieties that allow growers to use less fungicides and save money.

Finally, a skeptic might look for higher quality potatoes. Three well known quality defects of potato are black spot bruise, sugar ends, and scab. We have lived with these for so long that we are not surprised when they show up. But that does not have to be the case. We know how to make potatoes less susceptible to black spot bruise (González et al. 2019; Hara-Skrzypiec et al. 2018). We know how to make potatoes that are highly resistant to sugar ends (Zhu et al. 2014). And we have demonstrated the ability to transfer scab resistance from one breeding line to another (Jansky et al. 2019). Diploid breeding will be a success when it is used to greatly reduce these long-standing quality defects. Scab resistance, a visual defect that has plagued every market class for as long as we have been growing potatoes, may be the golden ticket. When potato breeders can, with some facility, move scab resistance into inbred lines and develop hybrids that are scab resistant, then even a skeptic might believe.

Ketchup and Fries or Chips and Salsa. Can Tomato Teach Us Anything About Breeding and Production of a True-seed Propagated Potato?

Presenter: David M. Francis, Professor- tomato breeding and genetics, The Ohio State University.

As the title suggests, just as ketchup is a good complement to fries, an awareness of tomato breeding, seed, and production systems may benefit those thinking about transitioning potato from a tuber seed propagated crop to a true seed propagated crop. My point of view is that of a tomato breeder. I won't advocate for true seed and diploid potato breeding. I won't be overly skeptical, but I will point out areas of concern and potential growth for the potato industry.

Converting potato from a tetraploid tuber propagated crop to a diploid true-seed propagated crop has implications for breeding programs and production systems. Prior to the 1970's tomato was produced from inbred "open-pollinated" varieties. The introduction of hybrid varieties facilitated the combination of traits, protection of germplasm, and eventually improved yield. Arguably these changes also facilitated market and production system diversification. Production changed from a direct seeded crop to a transplanted crop as a response to changes in seed prices. Growing seedlings and transplanting required a greenhouse industry and suitable equipment all of which have continued to change and improve. Seed production shifted overseas due to the cost of labor involved in producing hybrids. These changes also created exposure and mechanisms for pathogen migration and created a need for seed testing and seed trade regulations. Research conducted under 'Potato 2.0' will not only help evaluate the potential for true-seed potato, it will also create an opportunity to imagine how disruptive technologies may re-shape or even create new markets for the potato industry. Tomato seed industry and production practices provide lenses to imagine how this future might develop.

Why Hybrids?

The primary driver for introducing hybrids in vegetable crops was originally to protect proprietary germplasm, that is, inbred lines. There were, of course, other reasons for the transition. One of those was to combine traits. Dominant disease resistance traits, for example, can be combined easily in hybrids. Creating hybrids also recreates the heterozygosity that is lost with inbreeding, which may be advantageous for yield. Tomato hybrids currently have a slight advantage in yield over inbred lines (Fig. 5), though measurable heterosis as seen in hybrid corn is not observed. It was not the yield advantage that drove hybrid production in most vegetable crops. The driver toward hybrids was protecting germplasm and combining traits. Research programs are investigating true seed potato all over the world (Zhang 2021). It is appropriate to ask whether the potato industry, in the U.S. and beyond, is prepared to embrace diploid breeding and subsequent hybrid production.

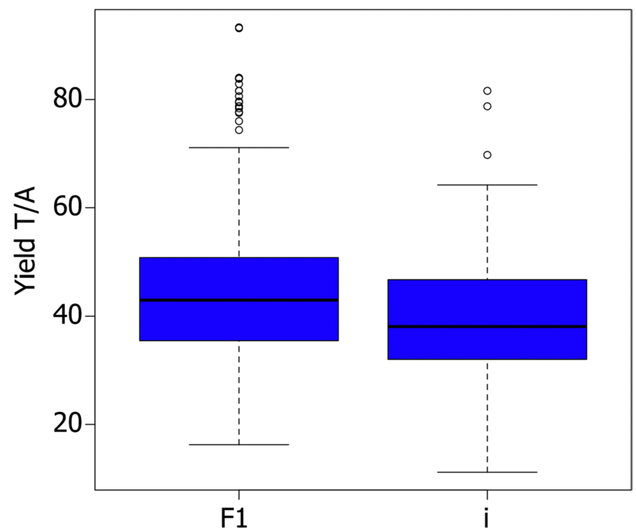

Fig. 5 Yield advantage of F1 hybrids relative to inbred (i) lines in tomato

Opportunities for More Rapid Genetic Gain

One exciting consequence of true seed production is that there are possibilities to accelerate breeding. Figure 6 illustrates what can happen with back crossing. Back crossing is common in tomato but is not done with tetraploid potato. A new resistance gene, as an example, can be very quickly driven into a high-quality inbred genetic background using back crossing supplemented with genomic information. Figure 6 shows the distribution of progeny from a first back cross between an elite recurrent parent and a donor of a novel gene. On average, progeny contain 75% of the recurrent parent genome. However, the percentage of the parent genome in individuals is distributed around that mean of 75%. Some individuals have more, and some have less. Therefore, in a single back cross we can select individuals that have a greater proportion of the recurrent parent than expected at BC_2. Those individuals are highlighted in red in Fig. 6. The approach can be extended by using molecular markers spread throughout the genome. With background selection and backcross breeding, a breeding team can introduce new resistance genes (Bernal et al. 2020) or quality traits (Orchard et al. 2021) into an existing line very efficiently. The pace of genetic improvement can be outstanding. Genomic selection can also be dovetailed very nicely into a diploid breeding program. There are opportunities to accelerate breeding when we are working with an inbred diploid crop.

Using Wild Germplasm for Variety Improvement

Diploid breeding has allowed tomato breeders to rapidly identify and incorporate into cultivated tomato valuable

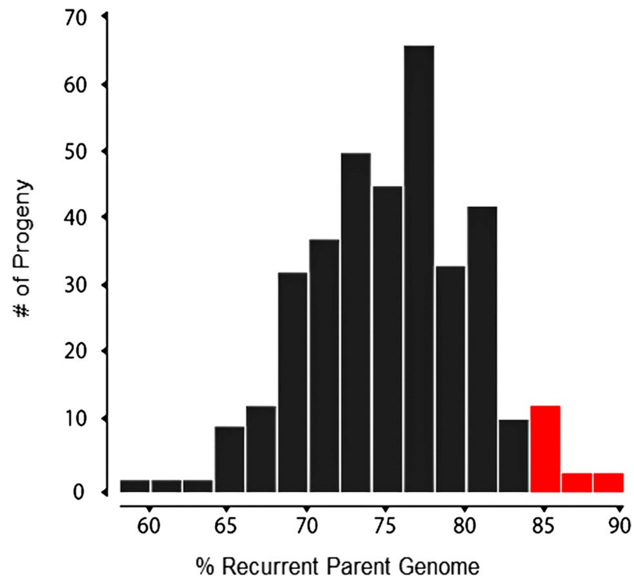

Fig. 6 Distribution of recurrent parent genome in backcross progeny. The percent recurrent parent genome is estimated from Single Nucleotide Polymorphisms distributed across the 12 chromosomes of tomato. On average, progeny in a population possess 75% recurrent parent at the BC_1 generation. Individuals containing the equivalent of BC_2 or BC_3 can be identified (>87.5% of the recurrent parent) accelerating the process of introducing novel genes into elite backgrounds

traits from a broad range of germplasm resources (Yang and Francis 2006). Tomato breeders have been using wild species relatives of tomato for over 100 years (United States Department of Agriculture 1919). Wild species have contributed resistance to numerous viruses, fungi and bacteria (e.g. Alexander 1934; Watts 1947). Virtually all major resistance genes have come out of wild germplasm, not out of cultivated varieties. They have also been important for resistance to soil-borne diseases. While we tend to think of the wild relatives as being important for disease resistance, they have contributed much more than that. The genes that allowed for differentiation between fresh market and processing tomatoes, for example, came largely from the wild relative *S. pimpinellifolium*. Genes from that wild species drove market differentiation.

Genetic Diversity as a Driver for Market Differentiation

Genetic diversity in tomato has increased due to breeding (Sim et al. 2011; Blanca et al. 2015). Two important facts emerge from the analysis of genetic variation in contemporary tomato. First, that the narrow base of the vintage (landrace or heirloom) varieties has been expanded dramatically by plant breeding. Second, that we now have distinct germplasm groups. Tomato uses several production systems. Processing tomatoes are grown in beds and the vines lay on the ground. Plants are staked for fresh market in open field production. In greenhouses, the indeterminate vines may be grown hydroponically for nine months or more. This kind of differentiation in the production system is reflected in the products that come out of those systems and the genotypes that are grown. Tomato market niches include the whole peel and paste industries. A ketchup tomato is not the same as a whole peel tomato. In the fresh market, there are round, Roma, and cherry as examples of three of the main categories. In greenhouse production, there is market differentiation into beefstake, cluster, cherry (both round and grape), and Roma. There are also rootstock breeding programs. These niches are defined by fruit shape, size, color, and most importantly, end use quality. Differentiation in production systems and market niches has gone hand in hand with the differentiation in tomato germplasm that occurred as a result of plant breeding. Although processing tomato breeding programs and fresh market programs share pedigrees, breeding has driven our end-products and plant architecture into separate niches. I would expect that breeding for end use is going to drive similar genetic differentiation in diploid potato.

Diploid Breeding Will Encourage Private Breeding Efforts

True seed potato breeding is going to drive potato breeding from the public sector towards the private sector. It took several decades for this change in tomato breeding to occur. You could argue that it took a half a century. For potato, there is not going to be an immediate change but there will be a slow and steady change.

Growing a Diploid Potato Crop

There are several possible scenarios or schemes for implementing true breeding seed into a production system. One way is to create inbred seed that is direct seeded to produce a crop. For potato, another scenario is that true breeding seed could be used to produce seed tubers. Those seed tubers would then be used to establish the commercial crop the following year. I think it is more likely that you are going to see something like this. A true breeding inbred parent is crossed to another true breeding parent to create hybrid seed. That seed is planted in a greenhouse to produce seedlings, and those seedlings are transplanted into the field to produce the commercial crop.

The Logistics of Hybrid Seed Production

The infrastructure for hybrid seed production exists for other crops and adapting systems to potato will be an important factor determining seed price and quality. A brief description of the process used for tomato seed production may be informative. Pollen is collected from the male parent. The female parent is emasculated and pollen is transferred to the stigma. Flowers that are not pollinated are removed to prevent a mixture of self and hybrid seed. It is common for self-contamination to occur in hybrids. Thus, hand pollination requires quality control and manual labor. Because the process is labor intensive, tomato seed production is done overseas where labor costs are low. The overseas production of hybrid seed introduces new concerns such as seed-borne diseases. In turn, the existence of seed-borne diseases leads to issues of seed testing, and a seed market that is subject to perturbation. Those perturbations relate not only to seed production and disease incidence but to politics as well. Additional testing requirements imposed by a government agency may prevent seed from being shipped. For emerging disease concerns, those requirements may come online before there are labs set up and diagnostic tests for the disease.

Seed Production Requires Dedicated Infrastructure

Since tomato is a wet seed crop, removing the seeds from the fruit begins with a chopping and grinding step. This step ranges in scale from handwork to mechanized. Mesh or sieves are used to separate large chunks of fruit from the seeds. The seeds fall into a catch basin and then go into a secondary sieve. For commercial production, where kilograms of seed are produced, specialized equipment is required for each of these steps. The seed are then treated with acid or bleach to remove germination inhibitors and clean seed-borne pathogens from the surface. Seed are then dried. At this point there may be additional steps to remove fine hairs from the seed to prevent clumping and facilitate handling at planting. There is often a secondary heat treatment aimed at removing seed-borne pathogens. Seed needs to be tested for germination percentage, purity, and pathogens. There are several commercial labs that test for viral and bacterial pathogens including Tobacco mosaic virus, Tomato Brown Rugose Virus, *Pseudomonas syringae* pathovar tomato, *Xanthamonas* species and *Clavibacter*. Finally, the seed is usually coated. The infrastructure for seed testing and regulations that allow shipping seed across international borders exist for other crops, but do not currently exist for potato. Potato is going to require similar infrastructure to extract true seed on a commercial scale and the industry needs to be thinking strategically about what pieces to the seed supply puzzle need to be put in place.

There is a range of cost for tomato hybrid seed, from less than four cents per seed up to 50 cents per seed. The high price range is often for rootstock seed used in the greenhouse industry. The lower-end price range is for processing tomato seed where the cost of seed accounts for $500 to $800 per acre of production. In contrast, hydroponically grown greenhouse tomatoes, may have well over $10,000 an acre invested in just seed.

Agronomic Practices Will Expand and Evolve

True seed is going to impact the industry at multiple stages in the production cycle. Consider the agronomics of tomato production. Seed are not directly seeded into the field. They are seeded into flats in a greenhouse at high density. Seedlings in the flats are then transplanted into the field using mechanical transplanters. Nobody uses single row transplanters anymore except for research plots. At a minimum, the industry is planting three rows at a time. If you search the internet for tomato transplanter and add Ferrari to that, you are going to see an example of a highly robotic transplanter. The team of people who used to work on a transplanter is being replaced by one person whose job is making sure the flats move into the robot correctly. Transplants drive innovation in greenhouse and field production methods. Agronomists need to optimize production in both environments for different market classes and multiple locations. As an example, in Michigan, Ohio, and Indiana, we see tomatoes on single row, on raised beds, on flat, and on double row. For potato, a lot of research will be needed to optimize production and to maximize yield. It is probably not going to be the same as when starting with a tuber.

A Parting Thought

What I have tried to convey are some of the issues that are going to come up with diploid breeding and hybrid diploid potato varieties. Converting potato from a tetraploid tuber propagated crop to a diploid true seed propagated crop has the potential to drive innovation and infrastructure development. For those of you that are excited by change, this is an exciting time.

Seed Potato and Potato Seed Production

Presenter: Amy O. Charkowski, Professor of Plant Pathology, Colorado State University.

The Seed Potato System and its Strengths

I have found that even people who have worked with potato for years may not have spent much time thinking about seed potato production. It may be worthwhile, therefore, to begin by describing the general scheme of how potatoes are produced commercially in the US. Varieties are maintained in tissue culture. Those tissue culture plants are multiplied, first in tissue culture, then by planting into potting mix or a hydroponic system in a greenhouse. Mini tubers are harvested and stored for several months before they are planted into the field for the first field generation of tuber seed production. Those potatoes are harvested, stored again over the winter, and may be increased for another two to five years in the field. Finally, once the amount of seed has increased enough, it is sold to a commercial grower who will produce potatoes for consumption. It takes four to seven years and a lot of planning to get from tissue culture to table.

I like to represent this process as an inverted or an upside-down pyramid for a couple of reasons (Fig. 7). One is that the width of each level of this pyramid represents how many people and farms are involved in each step. There are very few seed farms, and at each subsequent level the number of people and the number of acres increases. The height of each box represents the number of years that these steps might take. It is a complex system with multiple steps and many players.

Why do we produce seed potatoes this way? Why make it so complicated? We have tomato, which is very closely related to potato, as an example of an alternative approach. Tomato uses true seed not vegetative cuttings. Why do we use a more complex system for potato? The system that we use currently in the US had its origins in 1913. It was initiated in New York and Wisconsin, based on seed systems in Europe. What they were aiming for was varietal purity and freedom from disease. In 1913 they were not exactly sure what diseases they were trying to control. That was about the time that pathology was getting started as a scientific discipline. But this concept of trying to have varietal purity and potato free from disease is still the goal today.

The seed system works very well for what it was designed to do. For example, yields have increased fourfold or more for potato, and about half of that is due to healthy seed and the other half to improved chemicals, not so much to improved varieties. Some important diseases have become rare. We do not see bacterial ring rot very often anymore. At times in US history, that disease was seen in 25% of the seed potato lots (Frost et al. 2013). We also do not see potato spindle tuber viroid very much anymore, and that used to be a very common problem (Frost et al. 2013). These improvements are not because the plants are resistant, it is simply because our seed system is able to exclude the diseases.

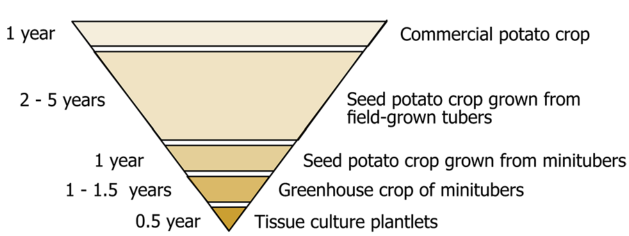

Fig. 7 Diagram, illustrating the tuber seed production system that starts with tissue culture plantlets. On the right is the step taken to produce potatoes and on the left is the approximate amount of time needed to complete each step

Weaknesses of the Seed Potato System

There are problems with the seed system that it was not designed to address. The first is that it is not very flexible. We have a fairly slow increase in seed. As a result, we find ourselves in tissue culture labs and greenhouses, trying to guess how many potatoes of which variety the industry is going to need five years from now. There are a lot of opportunities for something to go wrong when it takes five years or seven years to increase the seed supply. For example, we see a lot of herbicide injury on seed potatoes. We see mutations in tissue culture that cause varieties to go off-type. When that happens, you throw out the whole early generation seed lot. We see problems with storage, anything from low oxygen, to fires, to heating, to disease. There is a lot of risk going from tissue culture plants to selling seed potatoes.

Another significant issue with our current system is that there are many diseases spread by the tubers or by soil that are not managed by seed potato certification. Unfortunately, these are diseases for which there is no useful resistance. Some recent examples are tobacco rattle virus, which is spread by nematodes, and potato mop top virus, which is spread by *Spongospora subterranea*. Both are soil-borne diseases and are spread by the soil that adheres to tubers and on the tubers themselves. Seed certification does not have good management for these problems.

There is also a huge amount of hand labor associated with seed potato production. Essentially, there is no automation in early generation seed production. The tissue culture plants are cut by hand. When you look at the greenhouses with hydroponics systems, those tubers are harvested by hand twice a week. Every single tuber grown in a greenhouse is touched at least once in the harvest process. There is a lot of sanitation that is done by hand. There is testing required to produce a really healthy vegetative crop like potato. The leaves for testing are collected by hand, they are processed by hand; there is essentially no automation. Even in early generation production, the tubers may be cut by hand and placed by hand on the planters. I do not know of another major crop where we have as much hand labor as seed potato production.

Another big problem with the way we produce seed is that we have little flexibility over how it is stored. Seed potatoes can only be stored for one crop year. You can store them through the winter, but you cannot store them through another winter. To maintain varieties, you have to maintain them in tissue culture. Again, it is important to really pay attention to how the tissue culture plantlets are being stored. At best, they can only be stored in culture for a few years before they need to be re-established from fresh cuttings. All the while, there is a risk of mutation in those stored varieties.

There is a very strict quarantine on seed potatoes with our current system because of concerns over importing significant diseases that will be difficult to remove from this vegetatively propagated crop. The United States imports seed tubers from Canada and nowhere else. Because of our production system, seed potato can only be grown effectively in northern states with cold winters. As a result, we have a small area for seed potato production that is not close to substantial commercial potato acreage.

Some have noted that heterozygous tetraploid potatoes could be better for disease resistance than diploid potatoes. In practice, however, they are not. As an example, we can look at PVY resistance. We have at least three dominant genes for PVY resistance, and they are not widely used in potato, in large part because potato breeding is so complicated. We are not very efficient at moving disease resistance into the crop compared to the efficiency with which we get new diseases.

A final challenge with the current seed potato system is that it is very difficult to export the system itself. It works well in areas where it was developed over the past century, but it is difficult to take that system and move it elsewhere. As a result, healthy seed potatoes are hard to access in large parts of the world. There are not technical reasons for why the system cannot be exported effectively, it has more to do with the need to transfer and establish the entire system.

What to Think About When Thinking About Potato Seed

Given these many limitations, could true potato seed (botanical seed) be used to produce potatoes? I tend to be an optimist and am probably too optimistic often, but I am really excited about the possibilities. When I was at a field day in the San Luis Valley, I had a strong reminder about what a production system really means and how every little part of how we produce potato has been optimized. Changing to this new system could mean changing everything. For now, there are so many unknowns. We do not understand what the economics will be, or the business models. We do not know what kind of automation there might be, or what phytosanitary system we need. We do not even really know where all the steps might occur. Mindful of these unknowns, I will end with a few guesses.

With true seed production, we tend to see the seed market controlled by large companies like Syngenta and BASF. There has been substantial consolidation and control of seed systems for these crops by a few companies. Vegetative crops like potato, however, are controlled primarily by family farms, in partnership with universities, or are very vertically integrated like we see with PepsiCo. We could expect that if we move to true potato seed production in potato, there will be a shift that makes the business model more like that for tomato.

It is very possible we will see true potato seed production in areas where we don't have tuber seed production. As an example, BASF recently opened a tomato seed production facility in Ethiopia. Because hand labor is required and is a significant expense, seed production moves to where labor is less expensive. With changes in location, I anticipate we will see changes in pathogens. We would expect that diseases we do not see very much anymore, like potato spindle tuber viroid, would come back. It is efficiently transmitted through true seed. I would expect to see additional pathogens. I do not think that we can predict which ones, but we can look at what's in tomato and see the potential for similar diseases. I have always been fascinated by the diseases that we do not see frequently in potato. For example, we do not see as much *Pseudomonas*, *Xanthomonas*, or *Clavibacter* as you might expect to see based on what we see in tomato. If we move to a diploid system with true seed, I expect we will see more of these pathogens, particularly if we start producing seed in new locations. I would also expect to see new diseases.

What about the amount of hand labor? I have produced many tubers both from tissue culture and from true seed. I cannot make any guesses at the cost, other than I can tell you that I would rather do it from true seed. The amount of hand labor required for tissue culture is significant. With tissue culture plants, you have to do something every three weeks to get those plants to increase. With seed, you have to do something once.

From looking at tomato and other crops, it looks like the potential for automation is much greater with a true seed crop than with the way we currently produce vegetatively propagated potato.

Storage and transportation are important considerations for potato seed. Mini tubers can be stored for a year and a half, at best, and from six mini tubers you get six plants. Fruit may contain 300–400 seeds and those seeds can be stored for years. The ability to store true potato seed, I believe, would improve food security for those who depend on potato, and it is certainly a lot easier to transport true seed than tuber seed.

How might the cost of production change? Again, it is really hard to predict for the system as a whole. You can figure out the cost of a true potato seed, and of the tissue culture plants, and mini tubers, and tuber seed potatoes, but there are so many ways of looking at cost and benefit that we really do not know how aggregate costs will change, since we do not know what the varieties will be. We can predict there might be less soil damage because we will have to increase generations less often than with true seed. We might eventually have less pesticide use, which would increase safety. Less hand labor would mean increased safety. We probably would have more global spread of plant disease and that would be a big cost. If the seed system becomes controlled by a handful of entities, I would expect a loss of income in rural areas, and we might have increased food insecurity. It is just really hard to know the answers to basic socioeconomic questions.

My personal hope is we will see a wider range of varieties in production. I have been envious of the tomato industry because of the huge number of varieties compared to what we have in potato. We could have that diversity in potato but the system does not currently allow for it.

The Velocity of Change

I want to end with a couple of parting thoughts. One is that even really, really good ideas can take a long time to come to fruition. Recently, virus resistant GMO pinto beans were put on the market in Brazil. This project was led for many years by Josias Faria and his colleagues at Embrapa. He came to Wisconsin to learn how to do this. I was an undergraduate at the time and I washed dishes for him. This project started in the late 1980s. By 1992, he had virus resistant transgenic beans that could cut crop losses in Brazil by 40%. It took from 1992 to 2021 to get them on grocery store shelves. The system was not ready for genetically modified beans, even though they are really good and it was a really good idea. I have also been thinking about my time as an undergraduate and what my parents thought it was important for me to know. They wanted me to know how to use a slide rule and how to dial a rotary phone. My mother was devastated that I did not take a typing class because, how could you possibly be successful without that? At that time I was probably renting some of my first videos at Blockbuster. Systems can change, really, really fast sometimes. We do not know where we are with diploid potato. Is it going to be like those pinto beans, where it took 30 years to get a really good plant in the field? Or is it going to be like cell phones, where it happened almost overnight?

Genetic and Biotech Research Based on Diploid Potato

Presenters: Jiming Jiang and David Douches, Michigan State University.

The skeptics and doubters have highlighted some of the many unknowns related to diploid potato breeding. When we consider the question of how diploid potato will influence potato research, the answers are much firmer and there are fewer doubts. We will discuss what we can do in genetic and biotech research with diploid potato that we cannot do in tetraploid potato.

Genetic Mapping Then and Now

The first genetic linkage map of potato was published in 1988 (Bonierbale et al. 1988). That was a milestone paper and a milestone year. The work was essentially a Ph.D. thesis by Merideth Bonierbale at Cornell University. She retired from CIP a few years ago, after a very impactful career. It is no surprise that the first map was developed from a population based on diploid rather than tetraploid potatoes. The mapping population used was derived from a cross between a *S. phureja* female and a *S. tuberosum* haploid x *S. chacoense* hybrid as the male parent. The genetic markers used in the mapping were based on a technology called Restriction Fragment Length Polymorphism (RFLP). This was the first generation of marker technology developed in late 1980s, but a lot of people have forgot about it since then. The map was based on a set of 135 tomato genomic and cDNA markers, because Cornell was the center of tomato genetic research at the time, led by Dr. Steve Tanksley. I give full credit to Merideth for developing the first potato genetic map, but we cannot forget the contribution from Christiane Gebhardt. She published a very similar paper in TAG in 1989 (Gebhardt et al. 1989). Merideth was lucky to have access to the genetic resources from tomato, but Christiane had to develop everything de novo in potato which took a tremendous amount of work.

Why wasn't this groundbreaking research done with tetraploid potato? The answer is that it would be much more challenging, and much more complicated, to do similar work with tetraploid potato. I think my USDA and University of Wisconsin colleague John Helgeson was one of the very first to attempt genetic mapping in tetraploid potatoes using RFLP markers (Williams et al. 1993). In this research, John and his co-workers used RFLPs to determine the segregation pattern of *S. brevidens* chromosomes in the progeny of a somatic hybrid, which was created between a *S. tuberosum* line fused with *S. brevidens* and then backcrossed with cv. Katahdin. The DNA blot data were much more complicated than those with diploid potatoes. It was not easy to score a particular band on the blots. Most DNA markers produced multiple bands on DNA blots from each of the tetraploid progenitors. Many of the markers were either not polymorphic or too polymorphic to score in the population. It turned out to be very difficult to do such RFLP-based genetic mapping with tetraploids, and very few labs have attempted to do this.

Fast forward to more recent genetic mapping efforts. Alex Marand was a graduate student in my lab when Shelley Jansky and I decided to map traits in a diploid population that Shelley developed based on two of her favorite clones. The female, US-W4, is a haploid clone derived from a University of Minnesota breeding line, and the male is the very famous M6 self-compatible *S. chacoense* clone. Alex and I discussed how we should genotype the population. At the time, we could have used an established single nucleotide polymorphism (SNP) array with a few thousand markers. But Alex and I decided to go for a more difficult route. We decided to sequence every one of the 110 clones in the population to create as many markers as possible. This approach was still quite challenging five or six years ago when we started the project, and we knew it was going to be a lot of computational work to find those markers and map them. In the end, we identified almost four million markers, most of which were SNPs but a few were indels. We were able to generate a high-resolution map and used it to map the positions of nearly 630 meiotic crossovers at an average resolution of five kilobases (kb) (Marand et al. 2017). We were able to map a yield QTL to a 256 kb region. This QTL accounted for almost 30% of the variation in yield in the population (Marand et al. 2019). It was amazing that some of the clones in this population yielded so well, and the tubers were as large as 'Atlantic'. Figure 8 shows two of the clones in this population. Tubers of clone 65 are similar in size to tubers from wild species relatives of potato. The tubers of clone 92 are large enough that you might not believe they came from a diploid potato.

In 2021, back-to-back papers were published in Nature Communications that described the cloning of the famous S-locus inhibitor (*Sli*) gene (Eggers et al. 2021; Ma et al. 2021) that was originally identified by Yoshi Hosaka (Hosaka and Hanneman 1998a, b). *Sli* is located on chromosome 12, in a region with severe segregation distortion. In the mapping and cloning of this gene, researchers had to deal with this specific genetic challenge. The two labs used slightly different strategies for fine mapping. Either way, it was an amazing accomplishment, and again it was done in diploid potato because it is much easier to do the relevant genetic analysis than with tetraploid potato.

My first take home message regarding genetic analysis in potato: diploid-based genetic tools will allow us to identify genes associated with complex potato traits such as yield and self-compatibility, even in regions of chromosomes that do not follow the rules of Mendelian genetics. This simply has not been possible with tetraploid-based materials.

Biotechnology as a Tool to Improve Potato Varieties

When I talk to students, I compare potato with wine grapes. The wine grape industry has some very famous, favorite varieties, such as Chardonnay and Cabernet Sauvignon, that it has been growing for hundreds of years. The wine industry loves these varieties and does not want to change them. Potato is like that in many ways. In the US, we have been growing our most famous variety, Russet Burbank, for over 100 years. The industry does not necessarily want to replace Russet Burbank, because it makes a french fry highly desired by the quick-serve restaurants. But it is not an ideal variety to grow because it is susceptible to many diseases and quality defects. One dream is to improve an existing variety like Russet Burbank by modify a single gene without altering anything else. For example, improving its late blight resistance just by adding or modifying one gene. Can we really modify a single gene or a single trait in Russet Burbank without causing any other alterations in the rest of the genome? The simple answer is that it is theoretically possible, but in practice, it is nearly impossible. Why is that? In brief, Russet Burbank has four copies of each gene. Currently, there are no efficient techniques that will allow us to modify all four copies at one time without adding additional genes or without making genetic or epigenetic modifications to the rest of the genome.

As an example, Dave Douches developed the Kalkaska variety. It is a very good chipping variety with very high resistance to common scab, and he recently created an RNA interference (RNAi) silencing line that targets the vacuolar invertase gene (*VInv*). The *VInv* gene plays a major role in cold-induced sweetening (Bhaskar et al. 2010). Silencing of this gene allows you to chip Kalkaska directly from 4˚C storage. There is just one issue. This is not the situation I just described, where you modify the *VInv* gene and do not touch anything else. In this instance, the silencing construct is still present in the DNA of the Kalkaska silencing line. As a result, there are strict regulations that govern how and where it is grown and if it can be used for food. It is hard to predict how long it will take to go through all the regulations

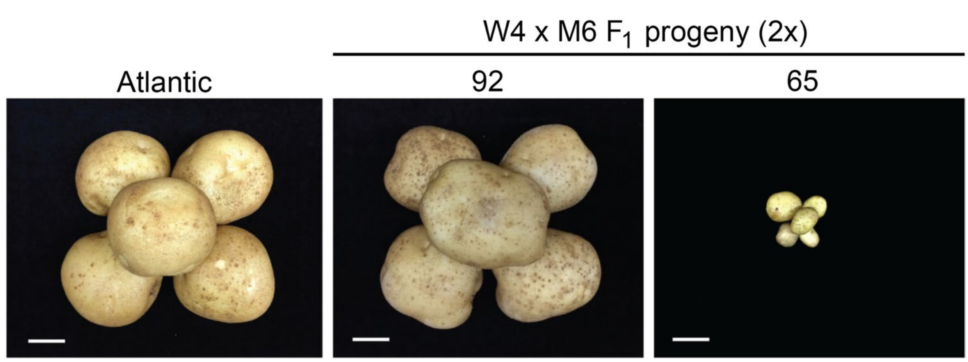

Fig. 8 Tubers of tetraploid cultivar Atlantic and F1 diploid lines 92 and 65. Scale bar = 2.54 cm (1 inch). Images were published by Marand et al. (2019), with permission from Genetics

in order to grow this line, which is obviously better than the original Kalkaska.

The research community is very excited about a new technology called gene editing (Tiwari et al. 2022). It is also called CRISPR-Cas methodology. Two scientists won the Nobel Prize last year for this technology (The Nobel Prize 2020). This technology will allow us to precisely modify a specific gene in potato. It requires only two components to modify a gene. One is called the guide RNA and the other is the Cas-9 protein. The best analogy is that the guide RNA is like a flashlight that is used to locate the correct position in the potato genome. The Cas-9 protein is like a scissors that cuts the DNA. The flashlight will guide the system to the specific gene and the scissors will make a cut in the DNA. The potato cell knows that each of the DNA cuts needs to be repaired. Many of the repairing events will revert to the original version, so there's no change in the DNA. But sometimes the cell will make a mistake during the repair. For example, a couple of base pairs may be lost or added. Those changes may be enough to cause a mutation of the gene or cause an alteration of gene expression. This process is becoming increasingly more precise. We are moving toward being able to modify any base pair in the genome. We can add one, we can delete one, we can replace one.

What is the problem of using this technology with tetraploid potato? It is exactly the same problem with using an RNAi silencing construct. The Cas-9 protein will remain in the genome unless you can segregate it out by backcrossing, which would alter the genetic identity of the original cultivar. In the past few years, there have been exciting papers showing that CRISPR/Cas-9 can be used to engineer a potato trait. As an example, the Douches lab was able to successfully engineer the self-compatibility of potato (Enciso-Rodriguez et al. 2019). Many diploid potatoes and most dihaploids generated from tetraploid potato are self-incompatible. That creates a problem for diploid breeding. It turned out, however, that mutating the S-locus RNase genes in diploid potato can turn a self-incompatible potato into a compatible potato (Enciso-Rodriguez et al. 2019). Figure 9 shows data from two different diploid lines, DRH-195 and DRH-310. Neither produces fruit when self-pollinated. CRISPR Cas-9 was used to knock out S-locus RNase genes in these lines. Theoretically, you only need to make a small deletion in the S-locus RNase gene for it to become non-functional. Gene editing made those two lines self-compatible. They produced fruits and seeds when self-pollinated (Fig. 9). More importantly, approximately one quarter of the progenies segregated away Cas-9, because only one copy was present in the original edited line.

My second take home message regarding genetic engineering in potato: CRISPR Cas-9-based tools and inbred diploid potato will allow us to precisely edit any gene. We will be able to add a single base pair, or delete a single base pair, or replace a single base. We probably are not there yet, but we are moving in that direction. I am very confident that it is going to work in the future. However, it is nearly impossible, at least right now, to edit all copies of a single gene in tetraploid potato and, at the same time, not make any other changes to the rest of the genome.

With Great Power Comes Great Responsibility

Presenter: Joshua Parsons, R&D Associate Principal Scientist at Frito-Lay.

The View from Industry

I will try to give as broad a perspective as I can to represent all of the potato industry. By industry I mean breeders, growers, manufacturers, and others. People who are involved in potatoes from breeding to table but who are not funded by a public institution.

Industry is in charge of getting good quality potatoes to consumers. This has always been true, but the needs of the industry globally are changing, and they are changing

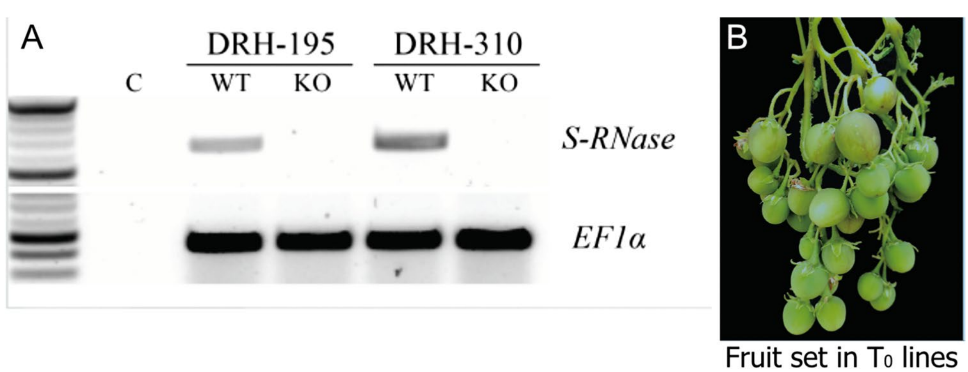

Fig. 9 **A** DNA blot showing use of CRISPR Cas-9 to produce RNase knockout (KO) lines from wild type (WT) diploid lines DRM-195 and DRH-310. **B** fruit from a RNase KO line following self fertilization

more rapidly than they have before. What we see is changing climate, changing demographics, changing economics, changing consumer trends, new production regions, emerging pests and diseases, restrictive chemical use, and more. These changes are overlaid with companies' sustainability goals, which are oftentimes extremely ambitious. These factors all influence the potato supply chain and it is important to industry to stay on top of these changes. When I talk to my global groups, I hear that their supply regions are changing, consumer preferences are changing, and they are changing fast. We need to respond more quickly to rapidly changing needs, and I think this is felt across all of industry, and not just with PepsiCo.

What is the industry perspective on diploid potato? When I say diploid potato breeding, what I really mean is a diploid inbred hybrid system because it is really the inbred hybrid that makes it all work. When industry looks at diploid potatoes, specifically at the inbred hybrid system, what we see is possibility for improvement. Tetraploid breeding is fine, we have had progress for a while, and there have been some great varieties. There has been varietal turnover, as much as people like to mention that Russet Burbank is still the most popular potato in the US. But what industry sees in diploids is the opportunity for investment and return on investment that will directly correlate to their needs.

In this rapidly changing world, I think we are going to see industry groups coming together to partner with breeders to take on very specific challenges. We are going to have new opportunities to solve problems together. Imagine there is a grower group that is confronting a pest or an emerging disease problem in their region. They have a diploid variety Z that they really truly love, and this new problem affects their production. What we could see is those growers pooling their resources and contracting with a local breeder. They are going to say we need resistance to problem disease Y. The breeder could do a literature search, find out there is resistance to Y in specific wild species relatives of potato and request the appropriate accessions of those species from a potato genebank. That resistance is backcrossed into variety Z to get variety Z + in a relatively short amount of time, but only if they are working with parents that are inbred diploids. With tetraploids, this is a 20- to 30-year time scale at a minimum, and you do not just end up with Z+, you end up with a completely new variety.

Industry sees a lot of opportunities with diploids, and they are not just with pests and diseases. There are opportunities for optimizing manufacturing performance, optimizing consumer preference attributes, or shifting varieties based on consumer trends. We might see starch manufacturers, for instance, interested in breeding varieties that express specific starch profiles. This is already happening. Some are working at the tetraploid level but more at the diploid level where companies are working together to solve perceived manufacturing problems or explore opportunities with diploid potatoes. With diploid potatoes, the potential opportunities seem greater and solutions to problems definitely seem more achievable.

Industry overall is cautiously optimistic or even boldly optimistic about diploid breeding, depending on who you are. You can get really excited about diploids when you see breeding progress happen. Figure 10 illustrates breeding progress expected from diploid and tetraploid potatoes. We are somewhere on the lower left part of that graph with diploid potatoes. We can argue about if we are farther or closer to the tipping point where breeding progress with diploid potatoes catches up with that of tetraploid potatoes. We can argue about what the axes are, what the scales are, what the slopes of the lines are, but at the end of the day, diploid breeding is more efficient than tetraploid breeding. When we hit that cross-over point and diploids switch to being on the top, there will be a lot more interest again.

There is already a lot of excitement in the diploid inbred hybrid system, and there is only going to be more once we have line-of-sight to varieties that are advantageous. Yield is definitely going to be an attribute of diploid lines that is crucially important, especially with sustainability goals coming into play. Water use efficiency, nutrient use efficiency, and disease resistance are likely to be highly advantageous. When a diploid variety has some combination of benefits that is better than the current tetraploids, then you will sort of tip the breeding system over the edge. I think it is going to be a while, but there is absolutely an opportunity for that.

Options for Diploid Potato Production

What is a diploid system going to look like and who is it going to affect? I will describe several possibilities, but I would like to emphasize that there may not be many changes for most growers. Realistically, the commercial crop is

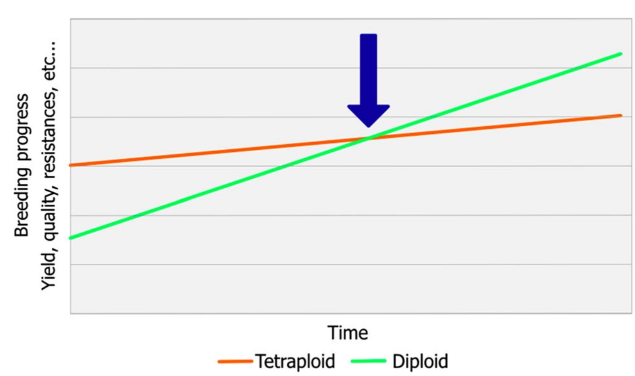

Fig. 10 Expected improvement of potato over time with tetraploid and diploid breeding systems

probably going to be grown the same for a long time. I will also emphasize that at the end of the day, commercial growers will get good varieties, the manufacturers will get good varieties, and consumers will get good potatoes.

Breeding is going to be more effective with diploid potatoes and the mechanics of breeding will change profoundly. Production of nuclear seed and early generation seed tubers are probably the two places where we will see the most disruption beyond breeding. It is quite likely, however, that the first way we will handle a successful diploid potato variety could be scenario zero (Table 1). That is a scenario where we identify a clone within a diploid hybrid population, enter it into tissue culture, and use our existing seed system to generate seed tubers. Scenario zero does not take advantage of true seed, but it does take advantage of our existing ability to identify superior clones. Scenario zero is attractive before we have really inbred parents and understand how those work best together. If partially inbred parent by partially inbred parent makes a pretty uniform hybrid, and we select superior individuals from those hybrids to clone, that might be the first rung on the commercialization ladder.

An alternative is scenario one where hybrid true seed is produced, and transplants are put in the field. Seed tubers are harvested, and increased for one or two field generations prior to planting the commercial crop. I think this is where the industry will operate for a while based on the differences in vigor I see between seed tubers and transplants.

There are many people who think that eventually we are going to be transplanting the commercial crop, which I will call scenario two, or direct seeding under scenario three. There are a lot of details to figure out before we can make those scenarios work. If we look at where we are today and what we know about potatoes, we are very comfortable with scenario zero and we can figure out scenario one fairly easily. Scenarios two and three are going to require a large change in investment strategy. We are going to need greenhouses to generate thousands of acres of transplants. We are going to need significant infrastructure for true seed production and handling, and we are going to need seedling vigor which is a trait that we have not bred for yet.

The industry is cautiously optimistic that diploids can help us produce better potatoes, more sustainably. Most people are interested in trying to figure this out however we can, but with scenario one, two, or three, or even zero, it is going to be an economic decision. The system that that is adopted by industry is going to be the one that makes everybody money and provides quality potatoes to the consumer.

Challenges That Must be Addressed

There are significant challenges that we need to overcome in diploid inbred hybrid breeding. There are new traits we need to work on. We need to establish new diploid germplasm, and we need to learn how to breed potato at the diploid level. Fertility is one trait that we have largely ignored as tetraploid breeders but is ultra-important at the diploid level. We need self-compatibility, and we have to be able to produce inbred lines. Those lines must have enough vigor to economically produce hybrid seeds through cross pollination. After that, the industry will need to figure out how to produce and grow transplants really well. Some of this we can do concurrently, but we need line-of-sight to relatively good hybrids before we can make much progress with agronomic trials, such as deciding to plant one-row or two-row beds. Maybe you plant seven-row beds and harvest it all at one time. These are the kinds of questions that we will have to address. There is a lot of opportunity to explore new approaches.

To leverage the full benefits of diploid hybrids, we will need procedures for shipping true seed safely and efficiently across international borders. Existing seed systems for tomato, pepper, and eggplant provide models for how to do this. To give an example of scale, all the seed for tomato production is produced outside of the United States. International movement of seed increases the risk of new disease concerns. We need to plan for that. But that should not

Table 1 Four scenarios showing ways that diploid breeding may be incorporated into the production of clones, transplants or true potato seed used for the commercial crop

Year	Scenario 0	Scenario 1	Scenario 2	Scenario 3
1	Superior hybrids as tissue culture (or true seed)	Hybrid true seed produced	Hybrid true seed produced	Hybrid true seed produced
2	Mini-tuber production	Transplants to field tuber	Transplanted commercial crop	Direct-seeded commercial crop
3	Field tuber production	Field tuber production		
4	Field tuber production	Field tuber production		
5	Field tuber production	Commercial crop		
6	Field tuber production			
7	Commercial crop			

prevent us from establishing a framework for moving seed internationally. We will need that ability to fully leverage the diploid system. We can live in scenario zero for as long as it is the most efficient approach. Eventually, however, we are going to want to significantly increase the transfer of true potato seed across borders. Most countries currently have restrictions in place that make transferring the required volume of seed impossible. We will need a risk-based approach that measures levels of diseases, or involves sampling for pathogens, or certification of the facility where the seed comes from, or other precautions that reduce the risk associated with global seed shipments. Such a system will allow us to transfer genetics to breeders, cross license inbred lines, and produce true seed at a low cost. There is a lot of work to do in this area and it has to be done cooperatively. Industry needs to participate because they are likely to do the most shipping. Universities will need to be involved, because they have expertise in pathology and entomology that will be needed to develop the risk-based approach that we need. Governments clearly need to be involved because they write and enforce the regulations.

Moving Forward is a Shared Responsibility

With all of the promise of diploid potatoes, with all of the genetic power behind diploid breeding, comes great responsibility. Now there is investment and interest, and as a potato community we need to deliver on the diploid ask. Specifically, the first step is with the breeders. If we do not have good diploid varieties, we cannot figure out appropriate agronomic practices for the diploid system, and we cannot figure out how to process them, etc. In the big picture, it is a breeding problem first. But there is plenty of space right now for everybody to participate in developing a diploid production system.

We are going to have to work together. There are too many questions floating around and PepsiCo, for example, cannot do it alone. UW-Madison cannot do it alone. Michigan State cannot do it alone. We have got to work together to solve complex, interdisciplinary problems, especially those addressing the value of true seed as a means of transferring genetics within the breeding community and to the field. There is a lot of research to be done in that space.

Overall, industry has a positive view of diploid potatoes. Despite that, a shift to diploid potatoes is not something we are going to decide to do overnight. The graph in Fig. 10 gives an idea of what we can expect from diploid and tetraploid breeding. People ask: how long will it be until diploid potatoes are competitive with tetraploid potatoes? Is it a 20-year, 30-year or 10-year outcome? Some think it is shorter than 10 years. Time will tell, but to get there we need to work together and there is a lot of room to work in pre-competitive space.

I will end with a call to action. We should band together as a potato community and solve some of the problems that are too big for any one group. The good news is that this is already happening in some areas. The Specialty Crops Research Initiative project on diploid potato breeding (Potato 2.0 2022) is a really good example of this. It is a USDA-funded project that is working to establish the foundation for diploid potato breeding across public breeding institutions in the US. PepsiCo is contributing funds to this effort, to put our money where our mouth is. We have been talking about diploid potatoes for a long time. Our intent is to get a critical mass of material and knowledge, so that multiple programs can incorporate diploid breeding into their activities.

Another example comes from Europe. Holland Innovative Potato is a group of 13 companies that collaborate with public researchers in the Netherlands (Holland Innovative Potato 2022). They target pre-competitive projects that are bigger than any one company can undertake on their own. They are able to use private institute money and matching funds from the government to solve problems. They work within a framework where a standard body of private industries is involved, and projects are pitched to the industry. All the intellectual property agreements are done in advance and are ready to go for partnership. The system allows a lot of really good research to happen quickly in a way that is advantageous to all the participants. By bringing everybody together, research projects become very attractive to government funding agencies. They have assurance that the public institutions are doing research that private industry wants, and they see that that reach of public funds is extended with private funds.

One other example is the potato pan genome project, which was coordinated by a private company. That company started working with a group in the Netherlands to sequence some potato varieties and asked if other people were interested in joining the effort. Public researchers in North America got on board and now they are sharing the sequence information so that everybody has a better pan genome to use (Hoopes et al. 2022). This project was not funded by a specific grant. The public breeders pooled their resources to participate. Again, this is a great example of collaboration and thinking outside the box.

I am going to finish with some concrete action items. I will begin with a list for those in industry: (1) Participate in the conversation about diploid potatoes. It is not just a fringe discussion with odd professors and crazy hair. This is something that is going mainstream. The breeding companies are investing a lot of money in diploid potatoes. Many people in industry are interested. Diploid potatoes will happen; it is only a matter of time. Those conversations will help people get ready for change; (2) Let people know what your concerns are, because we must address those. Seed certification is something that continually comes up as we have these conversations. How do you certify true seed? How do you ensure that there is variety purity and quality? These are questions we need to put on a list somewhere so that we know as a community that we address them satisfactorily; (3) As part of the conversation, think about how diploids could affect your business. Be forward thinking about how you might work with a diploid potato system. How can you transition or how can you participate? (4) Consider investing in the diploid space. Invest with field space, storage capability, manufacturing runs, whatever you can. It does not have to be dollars; it can be in-kind. In-kind contributions are often inconvenient, but they are hugely beneficial for public breeders and public researchers. Being able to do trials on farms in real life conditions is a huge advantage to breeders and agronomists. Participate in the diploid space when possible and be open to collaborate. There is a lot of work to do.

The list for public researchers looks very similar to the industry one except, as an industry representative, I will tell you to ask the industry for support. Yes, ask for dollars. Also, ask for field space, for manufacturing plant runs as you get diploid varieties that you can bulk, request space on growers' fields for agronomy studies, do a storage study, talk to a manufacturer, try to get into a fry plant or chip plant, ask for feedback from the industry.

Diploid potatoes are a great opportunity, and genetics are a powerful tool for change. We need to work together to exploit the potential of diploid potato for the industry and for consumers worldwide. There is too much to do for one group to do it alone.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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