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POTATOES & PESTS PAPAS

Actionable Science Against Nematodes

Figure 1. Cornell Potato Breeding Program harvest and selection

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Breeding For Nematode Resistance

One of the objectives of the PAPAS project is to develop resistant cultivars to several nematodes that attack potatoes. Like most things in life, it is a task easier said than done. The true challenge in potato breeding lies in potato's high level of heterozygosity. This means, for most genes in potato, that there are multiple variations of each gene. Potato's genetic complexity is further amplified because most cultivated potatoes are tetraploid; for each gene, in any given potato, there could be two, three or even four different versions of the gene present.

This exceptionally high level of genetic diversity leads to vastly different offspring when any two potatoes are crossed through pollination. Much of potato breeding entails sifting through the genetic variability of a population to identify a suitable clone for release as a cultivar.

With little control over how traits are transmitted following the intercrossing of two parents, potato breeding can be envisioned as a lottery. Each hybrid potato clone or "ticket" has the potential to be a successful cultivar with the desired combination of traits the breeder is seeking to combine. However, as more traits are added to the list of the "ideal potato", the probability of finding such a clone gets smaller and smaller. In practice a breeder must actively weigh the importance of traits as they make selections.

The time from cross to cultivar release typically spans from 10-15 years, depending on the institution and specific potato clone. The process involves extensive testing and evaluation of new breeding clones relative industry standards to continuously narrow down the number of clones being considered as new cultivars, as illustrated in Figure 3.

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Starting the Breeding Process

Breeding begins with the selection of parents for crossing. The choice of parents depends on the specific objectives for the potato cultivar being developed. In the case of the PAPAS project, the primary aim is to breed nematode-resistant cultivars. This necessitates that at least one parent has resistance to the targeted nematode species.

Nematode resistance genes can be broadly categorized into two types: those that confer high resistance and those that confer partial resistance. High resistance is preferred as it ensures that a clone can greatly reduce nematode populations and produce an acceptable crop. However, high resistance genes are not available for all potato nematodes, or the high resistance may reflect multiple genes working in tandem, which must again be brought together in progenies to ensure the high levels of resistance are again expressed. In such cases, breeders often have no choice but to use genes that confer partial resistance, which allow some nematode reproduction, but not as much reproduction as would occur on a fully susceptible cultivar. How breeding programs utilize these different types of genes will be discussed in the *Evaluating Resistance to Nematodes* and *Comparing Breeding Programs* sections.

After selecting the parents for hybridizing, one is designated as "male", and will contribute pollen, while the parent that will receive pollen is designated as "female". Deciding which parent to use as males and females depends on factors such as whether it can produce pollen (surprisingly enough, many potatoes cannot), its rate of flower abortion, and past performance as either a male or female parent. Pollen is carefully collected from the male parent and applied to the flowers of the female parent. Successful pollination results in the development of potato berries, which look like little green tomatoes, each containing many botanical seeds, oftentimes referred to as "true potato seed". The seeds in potato berries are initially dormant. This dormancy can be broken by storing the seeds for a year, or by treating seeds with gibberellic acid.

Figure 2. Cornell Potato Breeding Program's pollination greenhouse.

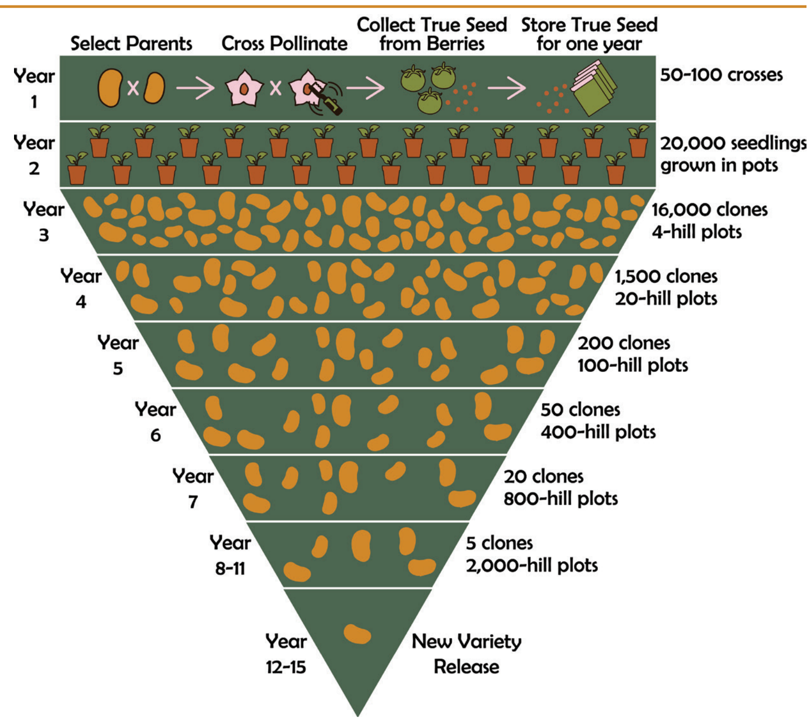

Figure 3. General breeding scheme for the Cornell Potato Breeding Program. Quantity of clones and plots sizes may vary between programs.

Comparing Nematode Resistance Breeding Programs in the US

Sagar Sathuvalli
Breeder at Oregon State University

Main Nematode of Concern:
Columbia root knot nematode (*Meloidogyne chitwoodi*)

Main Market Class of Program:
French Fries (Russets)

Existence of Nematode Resistance Breeding Part of the Program:
30 years

Personal Experience Breeding Against Nematodes:
11 years

Number of Resistant Varieties Released by the Program:
0 varieties for *M. chitwoodi*

Challenges:
Combining resistance with high yielding and processing traits

Helpful Tools for the Program:
Marker Assisted Selection

Source of Resistant Germplasm:
Wild species from the Genebank

Demand for Resistant Variety:
Very high

Rich Novy
USDA-ARS Breeder in Idaho

Main Nematode of Concern:
Pale Potato Cyst Nematode (*Globodera pallida*)

Main Market Class of Program:
Russets

Existence of Nematode Resistance Breeding Part of the Program:
16 years

Personal Experience Breeding Against Nematodes:
16 years

Number of Resistant Varieties Released by the Program:
0 varieties for *G. pallida*
2 varieties for *G. rostochiensis*

Challenges:
Bringing polygenic resistance into the russet market class alongside processing attributes

Helpful Tools for the Program:
Marker Assisted Selection

Source of Resistant Germplasm:
Varieties from other regions including Europe, New Zealand and South America

Demand for Resistant Variety:
Very high in the APHIS regulated acreage of Idaho

Walter De Jong
Breeder at Cornell University

Main Nematode of Concern:
Golden Potato Cyst Nematode (*Globodera rostochiensis*)

Main Market Class of Program:
Chipstock (Round whites)

Existence of Nematode Resistance Breeding Part of the Program:
~80 years

Personal Experience Breeding Against Nematodes:
24 years

Number of Resistant Varieties Released by the Program:
26 varieties for *G. rostochiensis*

Challenges:
Learning to prioritize quality traits over nematode resistance otherwise a clone won't be successful

Helpful Tool for the Program:
Having a nematologist who is willing to screen large numbers of candidate varieties each year

Source of Resistant Germplasm:
Cornell Released Varieities and Breeding Lines

Demand for Resistant Variety:
Very high for affected growers

Headshots from Lynn Ketchum, John O'Connell, and Pia Spychalla

see **Comparing Programs cont.** Page 6

Selecting in the Field

After seed dormancy has broken, seeds are sown to produce potato seedlings. Each of which is genetically unique and distinctly different from either parent. The greenhouse-grown seedlings produce the first tubers in the breeding process, and the tubers are typically small, as breeders grow the seedlings in small pots. Little or no selection occurs in the seedling generation since the growing conditions are not representative of a farmer's field.

The following year the potatoes move into the field segment of the breeding pipeline. Methods for the first field year vary among programs depending on available field space, resources, and goals of the program. For instance, at Cornell, four tubers are planted from each clone to allow for a reasonable assessment of the uniformity, size and shape of each potato clone, whereas other programs typically plant only one tuber per clone if they are selecting for highly heritable traits like skin russeting. Selections made at this stage primarily focus on traits that can be evaluated by eye, including agronomic traits such as maturity, relative yield, tuber appearance, and active selection against external tuber defects.

As a cohort of potato clones progress through additional years of field evaluation, the number of unique clones decreases while the amount of potatoes harvested and data collected from each clone increases. Each step in the breeding scheme collects more data about each surviving clone relative to cultivars currently grown by industry, and breeders use that data to make additional rounds of decisions.

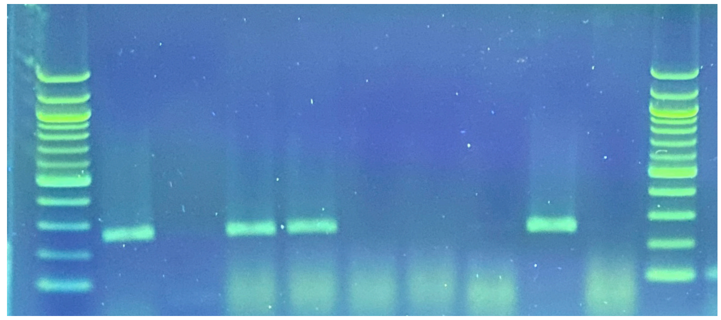

Figure 5. DNA marker analysis. The presence of a band indicates a positive result for a resistance marker.

Evaluating Resistance to Nematodes

In later generations, selection decisions incorporate additional traits, like nematode resistance.

In the fourth year, clones are screened with DNA markers to determine if they are likely to contain desired resistance genes. These markers are closely linked to specific genes known to confer resistance. In a typical marker assay the polymerase chain reaction (PCR) is used to test whether a specific DNA sequence associated with resistance is present in a potato clone. The presence of a marker strongly suggests the potato clone will have resistance against the targeted nematode, but there's always a small possibility that although the marker is present, the potato is still susceptible, since markers can become separated from resistance genes by recombination during meiosis.

Thus, while DNA markers serve as a useful initial screening tool, a bona-fide resistance assay is necessary for confirmation. Conducting a nematode resistance assay is a labor-intensive process demanding significant time and resources. This assay requires a strong collaboration with a nematologist to screen potentially hundreds of clones on an annual basis to precisely quantify the resistance of each potato clone.

Figure 4. Tubers of a potato clone selected in the Cornell Potato Breeding Program.

Other Traits Matter Too

While resistance to nematodes is a PAPAS project goal, the success of a potato cultivar hinges on a multitude of agronomic traits. Simply having resistance is insufficient if the potato clone lacks traits that appeal to growers and consumers. Therefore, extensive data is collected on other parameters including fry color for chips and/or French fries, marketable yield, tuber size profile, specific gravity (a measure of starch content), frequency of internal and external defects, and others. Ultimately, to be successful, a potato clone must align with market preferences.

Potato clones that appear to meet market criteria after several rounds of evaluation are promoted to elite clone status. Elite clones then undergo evaluation in national trials conducted across different breeding programs, on-farm trials with growers and processors/fresh pack collaborators in the breeder's region, large-scale storage trials, and additional assessments.

Varietal Release

When an elite potato clone gains significant demand or is deemed worthy by the breeder to become a cultivar, it is given a varietal name. The chosen name should be unique from all already existing cultivars worldwide to prevent confusion since potatoes are globally traded and grown.

Breeders apply for Plant Variety Protection (PVP) on most new potato cultivars nowadays, a process that requires demonstrating the new cultivar is distinct from all other varieties, uniform and stable. If PVP is granted, the institution involved in the new cultivar's development (such as a university) is entitled to collect royalties, which in turn helps to fund the continued breeding and development of future cultivars.

If not already done, the potato clone, that is now a new cultivar, will also go through a virus eradication procedure and be placed into tissue culture for storage and propagation. This allows for the production of certified seed for use by industry.

The amount of time it takes to develop resistant cultivars for each nematode varies, depending on factors such as the breeding program's history of working against the pest, available resources, how many genes are needed to provide high resistance against the nematode, how well adapted the resistance gene donors are, and the difficulty of breeding an agronomically acceptable clone suitable for the needs of the potato industry. For a detailed comparison of three different breeding programs working towards nematode resistance, refer to page 3 and 6.

To learn more about the breeding process, follow the "Selecting for Success" blog post series on the PAPAS website at potatonematodes.org for more in-depth explanations and photographs of each step of the breeding scheme.

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Figure 6. Breeder Walter De Jong and field technician Matt Falise make selections in the field.

Figure 7. Potato clones being fried for fry color evaluation.

Figure 8. New York cooperative extension vegetable specialist Margie Lund helps plant elite clones in an on-farm trial for muck soil.

Comparing Programs cont.

Each breeding program funded by the PAPAS project is dedicated to developing nematode resistant potato cultivars. But how each is approaching the development of resistant cultivars a program is contingent on various factors.

To start, the germplasm utilized to create resistant potato clones differs significantly among the breeding programs. Cornell University, with about 80 years of experience in breeding against *G. rostochiensis*, has resistant germplasm adapted to the northeastern US which can continuously be used as parents in new crosses.

On the other hand, the programs at Oregon State University (OSU) and USDA-ARS Idaho have comparatively shorter breeding histories against their respective nematodes, necessitating the use of resistant germplasm that is not well adapted to their environments, and which typically lack the attributes unique to the russet market class.

Because breeders outside of North America been working for many decades to develop resistance to *G. pallida*, the USDA-ARS Idaho breeding program has made crosses with resistant cultivars from Europe, South America, and New Zealand. Although the foreign cultivars are much more adapted for modern cultivation than any resistant wild species, the growing conditions these cultivars were selected for are vastly different from Idaho. In addition, resistant germplasm typically is round, used for fresh consumption (with poor processing attributes), and has no russet skin. So the USDA-ARS Idaho breeding program has been crossing these resistant cultivars with russet potatoes. The program also performs subsequent modified backcrossing with russets to pyramid *G. pallida* resistance genes coupled with the shape, skin russeting, and processing attributes that typify the russet market class.

Meanwhile, OSU faces the challenge of a nematode, *M. chitwoodi*, for which no commercial cultivars with resistance have ever been developed. This has forced them to turn to wild species. Any cross with a wild species inevitably introduces several undesirable traits into the offspring such as high glycoalkaloid levels, poor fry quality, and sticky stolons. To maintain resistance while eliminating the unwanted traits, several cycles of backcrossing to commercial cultivars are required. Moreover, crosses with wild species often introduce male sterility. If a resistant clone is male sterile, it can only be used as a female parent. Two male sterile parents can never be crossed with each other, which limits crossing options.

Furthermore, the genetics of resistance vary for each nematode. The most common pathotype of *G. rostochiensis*, Ro1, can be well controlled with a single dominant gene (*H1*). However, a new pathotype, Ro2, discovered in New York in 1995, overcomes H1, so that the Cornell program is now working to bring in additional resistance genes, like *Grp1*, to help manage it.

Currently, all known resistance genes against *G. pallida* confer only partial resistance to the pathotypes present in Idaho. In order to develop a cultivar that has a higher, useful level of resistance, a breeder has to stack resistance genes. This means a breeder needs to incorporate two or more resistance genes into a genetic background that is agronomically acceptable. A task that is difficult to achieve with the high level of heterozygosity inherent to potato.

While there is single dominant resistance gene effective against *M. chitwoodi*, its efficacy against the dominant pathotype in the Columbia basin Oregon and Washington, Race 1_{Roza}, is limited. This has forced researchers to return to screening wild species for a new resistance gene to introgress into cultivated potato for Race 1_{Roza} control.

In conclusion, breeders and their staff are diligently striving to develop cultivars tailored to their regions. However, patience is essential as breeders sift through tens of thousands of clones trying to identify one that is not only nematode resistant but also has the appropriate processing and market traits for their targeted market class.

Figure 9. Onion bags filled with a potato clone selection.

Meet the Graduate Student: Pia Spychalla

Pia's upbringing on a potato farm in Northern Wisconsin helped her find her passion for potatoes at an early age. Following her undergraduate studies at the University of Wisconsin – Madison, she started her Ph.D. in plant breeding at Cornell under the guidance of potato breeder Walter De Jong.

As part of her Ph.D., Pia is overseeing the *G. pallida* resistance breeding portion of Cornell's program, since hands-on experience is vital when training to become a potato breeder. Having shadowed Walter and field technician Matt Falise during the past two field seasons, she has started grasping the fundamentals of selection, but there is still a lot to learn.

Pia's research focuses on testing the hypothesis that stacking two partial *G. pallida* resistance genes will result in higher resistance levels compared to each individual gene. This hypothesis has previously been tested with other combinations of *G. pallida* resistance genes. In the upcoming summer of 2024, she plans to screen her population with DNA markers to identify clones with both, one, or none of the target genes. Collaborating with nematologist Louise-Marie Dandurand at the University of Idaho, they will assess the resistance levels of these clones. This research will help inform breeders working to develop potato cultivars resistant against *G. pallida*.

In addition to her research pursuits, Pia has an interest in science communication and is minoring in the field. She has created content for the PAPAS project by writing a blog series and photographing the breeding process. This newsletter is a brief overview of what she hopes to cover in the series and includes photographs she has taken of the Cornell Potato Breeding Program.

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Figure 10. Potato berries forming from a pollination

Figure 12. Cornell Potato Breeding Program planting.

Figure 13. Walter De Jong and Matt Falise laughing at the excessive amount of tubers stuck to the vines.

Figure 14. Cornell Potato Breeding Program harvest.

Our next newsletter will elaborate on other project goals!
For more information, check our website: www.potatonematodes.org