Genetic Stocks Used for Potato Genome Sequencing

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Abstract

Potato is a highly heterozygous and tetraploid crop and therefore it was a major challenge to decipher the potato genome. This chapter highlights the developmental stories of the potato genetic stock used for the whole genome sequencing by the Potato Genome Sequencing Consortium (PGSC).

4.1 Introduction

As difficulties arose with sequencing attempts on heterozygous potato germplasm, assembly of a draft sequence of the potato genome was contingent upon the utilization of a completely homozygous cultigen in this highly heterozygous tetraploid ($2n = 4x = 48$) crop that declines rapidly on inbreeding. Cultivated potatoes all fall within *Solanum tuberosum* L. but have been divided taxonomically into indistinct Groups, including Group Tuberosum (tetraploid commercial cultivars grown throughout Europe and North America), and eight landrace populations grown in South America (Ajanhuiri Group, Andigenum Group, Chaucha Group, Chilotanum Group, Curtilobum Group, Juzepczukii Group, Phureja Group, and Stenotomum Group) (Huáman and Spooner 2002). Ploidy was thought to distinguish the Groups: Ajanhuiri, Phureja and Stenotomum were diploid; Juzepczukii and Chaucha were triploid; Tuberosum, Andigenum and Chilotanum were tetraploid; Curtilobum was pentaploid. However, exceptions to the ploidy classification were common. The similarity between Groups Tuberosum and Andigenum have been demonstrated graphically in two independent studies where diverse populations of tetraploid Andigenum landraces have been bred to resemble commercial potato cultivars through recurrent selection (Glendinning 1975; Huarte and Plaisted 1984). Spooner et al. (2007) later used simple sequence repeat (SSR) markers to try to distinguish a collection of 742 landraces and reclassified the previous eight landrace Groups into four species, with two Groups [Andigenum (now including Andigenum, Phureja, Stenotomum and Chaucha) and Chlotanum] within *S. tuberosum* and Groups Ajanhuiri, Juzepczukii and Curtilobum elevated to species. The

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Andigenum Group then encompassed the genetically indistinct diploids, triploids and tetraploids whereas the other more genetically distinct Groups and species retained the ploidy status of their previous Groups. So, potato presents a wealth of germplasm from primitive cultivars to advanced tetraploid commercial clones with little difference genetically among them, justifying the use of a primitive cultivar to represent the potato genome. For the sake of clarity, we will continue to use the now extinct Group Phureja designation in this review.

There are only a few reports of inbreeding in tetraploid Group Tuberosum germplasm. Krantz (1946) reported an extensive study of inbreeding through self-pollination in five different families where the average tuber yield of subsequent generations declined from 83% of the original plant material in the S_1 generation to 19% in the S_6 generation. The data for the S_6 generation were limited to only one of the five starting families due to a high proportion of weak plants that failed to flower in other families. The rapid decline of tetraploid potatoes after even a single generation of inbreeding has been confirmed in a wide range of germplasm, especially if starting with the most productive cultivars (Golmirzaie et al. 1998a, b; Hagberg and Tedin 1951). Many studies have been conducted on potato dihaploids, i.e., derivatives of tetraploid selections with the diploid chromosome number ($2n = 2x = 24$) obtained by prickle pollination (Uijtewaal et al. 1987a) or anther culture (Wenzel et al. 1979). Most dihaploids exhibit reduced vigor compared to the tetraploid progenitors, averaging only 50% of the yield and most do not shed functional pollen (Rokka 2009), limiting their utility in breeding. In an extensive study of 5377 dihaploids extracted from 31 different tetraploid clones, Hutten et al. (1995) found that 39% of a subset of the most vigorous did not tuberize and 32% did not flower. Although dihaploids have reduced heterozygosity compared to tetraploid cultivars, they still exhibit considerable heterozygosity and are therefore unsuitable nominees for sequencing as the differences in intergenic DNA on homologous chromosomes defied assembly using the sequencing platforms available in 2011. Reduction of dihaploids to the monoploid level ($2n = 1x = 12$) requires functional female gametes using prickle pollination or functional male gametes using anther culture, assuming that the genetic load were sufficiently light to obtain a viable monoploid genome. Uijtewaal et al. (1987b) obtained true monoploids (synonymous with monohaploids) from two different dihaploid families by prickle pollination; after chromosome doubling, these are the only reported truly homozygous plants obtained primarily from *Solanum tuberosum* Group Tuberosum germplasm. Yet, even in this case, a close look at the parental material reveals that Group Phureja diploids comprised either 3/8 (M9 family) or ½ (H78.01 family) of their composition (De Vries et al. 1987; Uijtewaal et al. 1987b). The monoploids and doubled monoploids obtained were extremely weak with little or no tuber set (Uijtewaal et al. 1987b). Because of the heavy genetic load of tetraploid Group Tuberosum germplasm revealed in these studies of chromosome reduction, it was an unlikely source of a suitable homozygous clone for sequencing. In any case this source of plant material was no longer available at the time when sequencing was first seriously envisioned.

Due to the lack of availability of a homozygous line derived from tetraploid Tuberosum germplasm, an alternative source of homozygous potato lines was necessary. Diploid accessions of Group Andigenum germplasm would be expected to carry a less crippling genetic load than tetraploids as they would have been subjected to more purifying selection through sexual propagation as well as reduced masking of deleterious alleles at the diploid compared to the tetraploid level. In order to demonstrate the possibility of homozygous potato germplasm, we embarked on a program starting in the 1980s to extract monoploids from diploid Phureja germplasm (Fig. 4.1). Our starting material was sexually propagated derivatives of the Phureja population bred for photoperiod adaptation to long days in North Carolina (Haynes 1972). We screened selected seedlings randomly from crosses among diplandrous ($2n$ pollen-producing) clones within

Fig. 4.1 Tubers of a diverse population of adapted Phureja

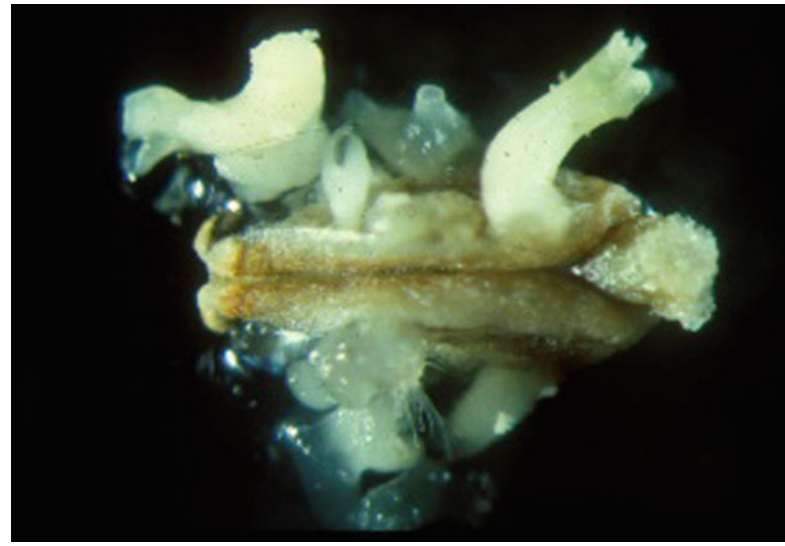

Fig. 4.2 Anther-derived embryos of diploid potato

a diverse population (Veilleux and Lauer 1981) for their ability to form embryos in anther culture (Fig. 4.2) using the protocol described by Wenzel et al. (1979). The rationale of using diplandrous clones was to derive monoploid plants from reduced pollen grains while retaining the ability of the resulting monoploids or their sexually derived hybrids to generate 2*n* pollen through fused or parallel spindles at the second division of microsporogenesis, thereby providing building blocks for sexual polyploidization in future applications. The corollary, however, was that the embryos generated from heterozygous 2*n* microspores in anther culture would be more vigorous and outcompete the relatively weak sought-after monoploid embryos from 1*n* microspores. Another complication was that diploids derived by anther culture would include heterozygous 2*n* pollen-derived clones as well as homozygous clones from spontaneously doubled 1*n* embryos or even 2*n* plants from somatic anther tissue. All diploids were routinely discarded as the effort of sorting homozygous from heterozygous clones using whatever marker

Fig. 4.3 Monoploid and isogenic doubled monoploid potato

system was in vogue at the time was deemed too great (Veilleux et al. 1995). Instead, verified monoploids were subjected to a leaf disc regeneration protocol (Hulme et al. 1992) resulting in diploidization (Fig. 4.3) either through regeneration from pre-existing endoreduplication in leaf explants or spontaneous endoreduplication during regeneration from callus (Paz and Veilleux 1999).

The first set of anther-derived embryos and plants derived from the adapted Phureja population was recovered from a single seedling that responded positively to anther culture (Veilleux et al. 1985). The homozygous plants, though weak compared to their heterozygous diploid anther donor, were sufficiently viable for greenhouse trials, field trials (Lough et al. 2001) and, once doubled (Fig. 4.3), crosses as stylar parents to various heterozygous pollinator plants (M'Ribu and Veilleux 1992). A modest effort was made to enlarge the germplasm base by screening for other anther culture-competent selections within the adapted Phureja seedling population to generate a more genetically diverse homozygous Phureja germplasm base (Johnson et al. 2001). Over the years, various efforts were made to improve the vigor of Phureja monoploids through somatic hybridization (Haynes 1972; Johnson et al. 2001; Lightbourn and Veilleux 2007) and outcrossing followed by re-extraction of monoploids (M'Ribu and Veilleux 1992; Paz and Veilleux 1997). The monoploids and doubled monoploids were maintained in vitro for many years at Virginia Tech and some were deposited in the Potato Gene Bank (http://www.ars-grin.gov/nr6/; most easily found by searching for germplasm developed by Veilleux) or provided to the International Potato Center (accession CIP 801092). One of the heterozygous anther donor clones, BARD 1-3, is also maintained at the US Potato Gene Bank as accession GS 224. As the response to anther culture was found to be a highly hereditable trait (Taylor and Veilleux 1992), seedling families obtained from crosses between anther-derived doubled monoploids and a range of heterozygous pollinators can be expected to respond to anther culture. Such seedling families have been generated at CIP, Virginia Tech and elsewhere. Likewise, tetraploid somatic hybrids derived by intermonoploid protoplast fusions (Lightbourn and Veilleux 2007) also respond positively to anther culture and represent heterozygous potato germplasm where all alleles would have passed through the monoploid sieve (Wenzel et al. 1979). One of these somatic hybrids is maintained by the US Potato Gene Bank as accession GS 220 (https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1648798). Hence, a limited variety of homozygous potato germplasm has been made

Fig. 4.4 Tubers of DM BARD 1-3 516 R44

available in recent years; most generated through haploid extraction with the aim of facilitating genetic studies rather than direct breeding applications.

When the Potato Genome Sequencing Consortium (PGSC) became frustrated with attempts to assemble the sequence of the heterozygous dihaploid potato clone, RH89-039-16, a search for homozygous potato germplasm that might be more amenable to sequencing using available Sanger, Roche 454 Pyrosequencing and Illumina Sequencing by Synthesis platforms was initiated. DM BARD 1-3 516 R44 (DM) was available at both Virginia Tech and CIP, facilitating its distribution to partner institutions in the PGSC. It had been extracted as one of many monoploids from heterozygous adapted Phureja clone BARD 1-3, then subjected to chromosome doubling by leaf disc regeneration (Paz and Veilleux 1999). The designation R44 is simply the 44th shoot regenerated that was later identified as a diploid. As with most selections of Phureja, DM prefers a cool season (22 °C days/16 °C nights); under these conditions, it will grow slowly but, once established, will flower (white flowers) and set fruit when pollinated by a fertile diploid potato selection. Although it produces stainable pollen, there are no reports of pollen fertility. It tuberizes after a few weeks, sooner if grown under a short 12 h photoperiod, later if grown under a long 16 h photoperiod. The tubers (Fig. 4.4) are fingerling, yellow fleshed, slightly and variably red-skinned and have poor keeping quality as they often exhibit tuber end rot even when still attached to the mother plant. Because of its homozygosity in intergenic as well as genic regions of the genome, the DM genome sequence was assembled rapidly and published in 2011 (The Potato Genome Sequencing Consortium 2011) where 86% of the 844 Mb genome was assembled and some 39,000 genes predicted. The genome assembly was later improved through marker analysis of a backcross population of DD x (DM x DD) where DD was a heterozygous clone of *S. tuberosum* Group Andigenum Goniocalyx cultivar group (Sharma et al. 2013).

4.2 Conclusion

As of March, 2017, the original publication of the DM sequence has been cited more than 500 times, providing a framework for studies of gene families (Charfeddine et al. 2015; Gao et al. 2016; Ma et al. 2016; Schreiber et al. 2014; Seo et al. 2016; Tang et al. 2016; Van Harsselaar et al. 2017), a scaffold for alignment of transcriptomic data (Campbell et al. 2014; Gong et al. 2015; Goyer et al. 2015; Liu et al. 2015; Morris et al. 2014; Tang et al. 2014) or a reference genome against which to discover genomic variation (Hardigan et al. 2016), to cite just a few. As sequencing platforms improve, the DM assembly will likely be supplanted by that of a more robust commercial potato line. In the meantime, it will have served its purpose to bring this genetically clumsy, yet important, crop into the genomic era.

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