

GENOME EDITING THE WAY TO OVERCOME SELF-INCOMPATIBILITY IN DIPLOID POTATO BREEDING

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ABSTRACT

Potatoes are one of the world's most important food crops, but breeding them is not always easy. The majority of diploid potato clones (*Solanum* spp.) possess gametophytic self-incompatibility that is primarily controlled by a single multiallelic locus called the S-locus which is composed of tightly linked genes, S-RNase (S-locus RNase) and multiple SLFs (S locus F-box proteins), which are expressed in the style and pollen, respectively. Using S-RNase genes known to function in the Solanaceae gametophytic SI mechanism, we identified S-RNase alleles with flower-specific expression in two diploid self-incompatible potato lines using genome re-sequencing data. Recent advances in genome editing, especially using a Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9 (Cas9) approach, have provided a promising solution. By carefully targeting and modifying the genes responsible for self-incompatibility, researchers can make diploid potatoes self-compatible. This change allows plants to produce seeds through self-fertilization, speeding up breeding programs and making the development of disease-resistant, climate-tolerant, and higher-yielding potato varieties much more efficient.

Key words: Genome, potato, diploid, breeding

Introduction

The potato is one of the most consumed crops worldwide and plays a vital role in global food security. However, improving potatoes through breeding has always been a slow and complicated process. Most cultivated potatoes are tetraploid, which means they have four sets of chromosomes, and this genetic complexity makes breeding unpredictable. To simplify breeding, scientists often focus on diploid potatoes (with only two sets of chromosomes). But even diploid potatoes come with a major problem: self-incompatibility. This natural barrier prevents a plant from fertilizing itself, which limits the ability to create stable, uniform lines for future breeding. Genome editing especially CRISPR-Cas9, an advanced technology allows researchers to precisely target and “switch off” the genes responsible for self-incompatibility. In potatoes, the key players are S-RNase genes (which reject self-pollen) and related genes in the self-incompatibility pathway. By knocking out or modifying these genes, scientists can create self-compatible diploid potatoes. This means that a single potato plant can now fertilize itself and produce viable seeds without depending on another plant. The advantages of this breakthrough are huge. First, self-compatibility allows the development of inbred lines-plants that carry stable, uniform traits. Once these lines are established, breeders can cross them to create strong hybrid potatoes, similar to how hybrid maize or rice has transformed agriculture. This method makes breeding much faster and more predictable. Second, genome editing is precise and efficient, unlike traditional cross-breeding which can take many years and often transfers unwanted traits along with desired ones. Third, genome-edited potatoes could help farmers face real-world problems such as late blight disease, pests, and climate stress.

Background and significance: The potato (*Solanum tuberosum*) stands as the third most important food crop globally, playing a critical role in food security. However, breeding efforts have traditionally been slow and complex. This is largely because most cultivated potatoes are tetraploid, having four sets of chromosomes that makes inheritance patterns unpredictable. Diploid potatoes (two chromosome sets) offer

a simplified and more efficient breeding method. However, self-incompatibility (SI), a natural system preventing self-fertilization, hinders the creation of inbred lines needed for stable, uniform breeding programs (Ma *et al.*, 2021).

Why self-compatibility matters: In agriculture, breeders typically want plants to self-pollinate to fix desirable traits, like disease resistance, drought tolerance, or higher yield. Self-incompatibility acts as a “genetic lock,” forcing cross-pollination and delaying progress. Besides this, producing improved varieties requires lengthy, complex crossing schemes (Lee *et al.*, 2023)

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. 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 (Bethke *et al.*, 2022).

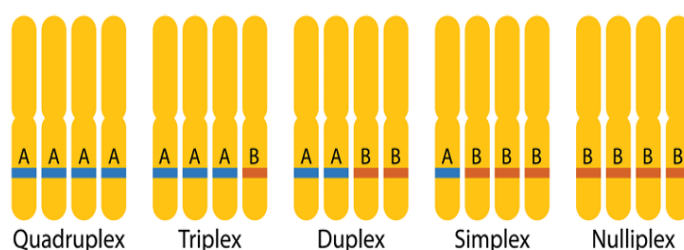


Fig. 1. Possible combinations of a biallelic trait in tetraploid potato

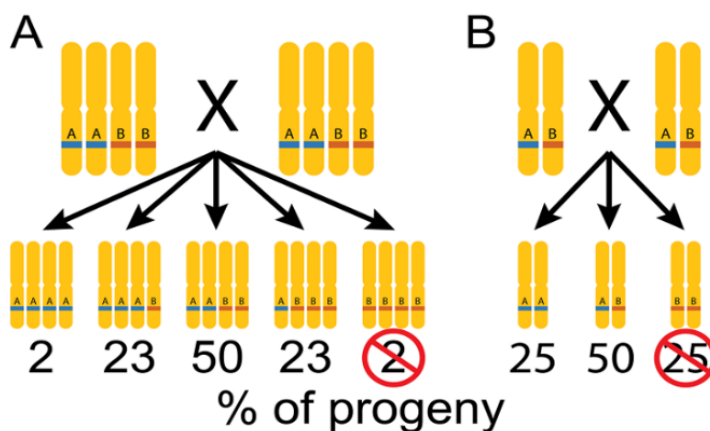


Fig. 2. Heterozygosity effect to remove undesirable traits in tetraploid (A) and diploid (B) progeny

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 (Fig-2A). 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 (Fig-2B) (Kardile *et al.*, 2022).

CRISPR-Cas9: Unlocking the genetic barrier

S-RNase Knockouts

The S-RNase gene plays a central role in rejecting self-pollen. CRISPR-Cas9 was used to knock out S-RNase in diploid potatoes, successfully creating self-compatible lines—plants that can self-fertilize and produce seeds without a partner (Ye, 2018). Additional studies using CRISPR targeted multiple conserved exons of S-RNase—such as the first and second exons—to disrupt several alleles (e.g., Sp5, St5, St6 or Sp3, Sp4). These interventions consistently yielded self-compatible lines, where truncated S-RNase proteins could no longer prevent self-pollen survival (Fig-3; A&B) (Chincinska, 2022).

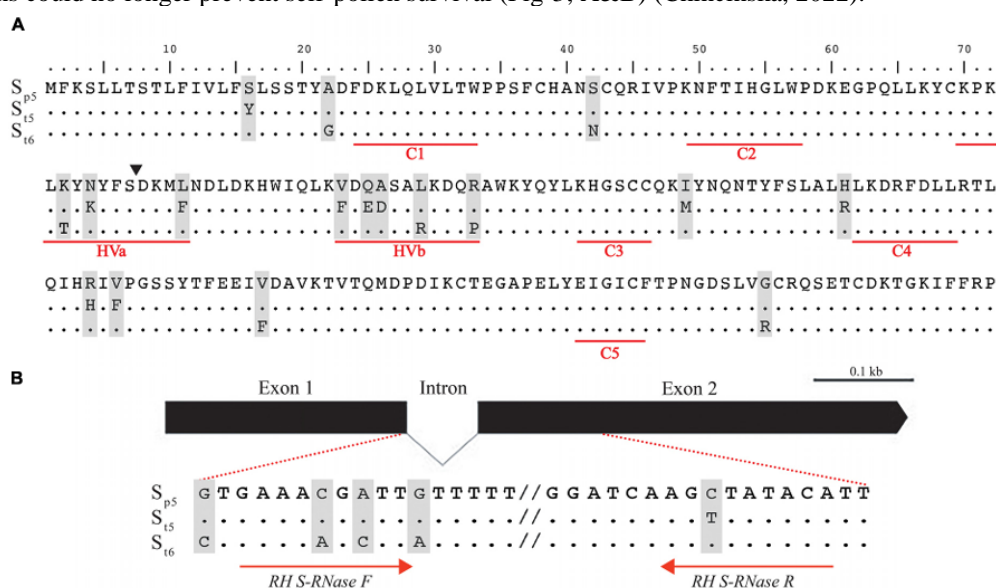


Fig. 3. S-RNase gene structure and allelic variants in diploid potato and related species; (A) S-RNase predicted amino-acid sequence alignment of the DM (Sp5) and RH (St5 and St6) alleles, (B) S-RNase gene structure

Broader context and practical advantages

- Converting potatoes into diploid seed-propagated inbred lines shortens breeding cycles and enhances genetic gain through heterosis-similar to successful hybrid systems in maize and rice (Lee *et al.*, 2023)
- Another advantage is the adoption of True Potato Seed (TPS) instead of pathogen-prone tubers-simplifying distribution and scaling, especially in resource-limited regions (Chincinska *et al.*, 2022).
- CRISPR-based editing also supports broader goals, like improving disease resistance, reducing anti-nutritional compounds, and adjusting starch or glycoalkaloid profiles (Chincinska *et al.*, 2022).

CLASSICAL breeding methods for diploid potatoes: Breeding diploid potatoes for clonal propagation can be conducted through the testing, selection, and vegetative multiplication of desirable clones from population improvement Breeding diploid potatoes for clonal propagation can be conducted through the testing, selection, and vegetative multiplication of desirable clones from population improvement schemes,

such as mass or family selection or from pair crosses between clones that complement each other for desirable traits. The new cultivar will have come from combining a gamete from its female parent with one from its male parent. For each of the 12 pairs of chromosomes, the female-derived chromosome will need to complement the male-derived chromosome for genetic loci at which they have different alleles. In the following simple example, small letters represent deleterious recessive alleles (Fig. 3; A-B).

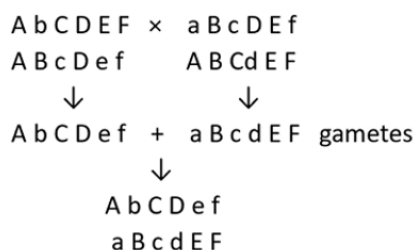


Fig-4(A): New cultivar from cross between two heterozygous diploid parents

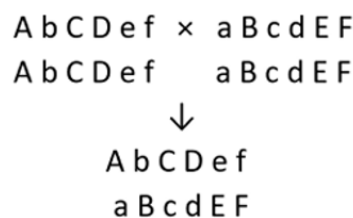


Fig-4(B): New diploid F1 hybrid cultivar from cross between two homozygous diploid parents

Thus, in the selected genotype, as in the parents, deleterious recessive alleles are accommodated without adverse effects through heterozygosis. Despite being homozygous for some deleterious alleles, the inbred parents must have sufficient vigor and fertility for the maintenance and production of their hybrid. The prerequisites for successful F1 hybrid breeding in plants are: i) the ability to produce seed by self-pollination; ii) the ability to produce homozygous inbred lines with acceptable vigor and fertility, or sufficiently homozygous inbred lines to produce an F1 hybrid of acceptable phenotypic uniformity; iii) the ability to produce sufficient inbred lines for combinations to be found that are superior to existing cultivars, and to achieve these over cycles of inbreeding and crossbreeding for continued progress; and iv) the ability to produce large quantities of F1 seed for growing the hybrids commercially.

Conclusion

Game changer for potato breeding, promises to deliver faster, more efficient, and more sustainable ways of producing one of the world's most important food crops. By unlocking self-fertilization, scientists are opening the door to stronger, healthier, and more productive potatoes that can meet the demands of a growing global population.

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