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Editing the Potato Genome with CRISPR/Cas: Prior Accomplishments and Future Plans

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INTRODUCTION

Plant biotechnology and agriculture have been completely transformed by genome engineering. Crop improvement is now easier, faster, and more precise than ever thanks to newly developed gene editing methods. Despite its status as a crop that contributes to global food security, potato has not benefited sufficiently from a wide range of these methods. Conventional breeding of this important crop is hindered by the unique genetic characteristics of cultivated potatoes, such as tetrasomic inheritance, high genomic heterozygosity, and inbreeding depression. As a result, genome editing gives potato trait improvement an excellent toolbox. Additionally, commercial varieties without transgenes can be engineered using specific transformation protocols. In the first part of this review, we talk about some of the things that haven't been done yet in potato genome editing and highlight some of the gaps in these efforts. Then, we present strategies for overcoming the technical difficulties associated with potato genome editing. Last but not least, we draw attention to some of the unexplored fields in the literature and discuss potato-specific genome editing applications. With 370 million tonnes produced annually, potato follows maize, wheat, and rice as the fourth most popular crop worldwide. However, consumption ranks third behind wheat and rice (FAO, 2019). Being plentiful in sugars, nutrients (C and B6) and minerals, potato assumes a vital part in human nourishment. According to Kraak (1992), starch in tubers is also used in food- and non-food-related industrial applications, such as the production of additives, paper, and textile products. Potato yield improvement over the past century has been marginal, in contrast to other major crops like maize, which benefit from approximately 1% genetic yield gain annually. This is due to the fact that traditional potato breeding is a slow process that is hindered by numerous factors. The majority of potatoes grown in cultivation are tetraploids with highly heterozygous genomes. In addition, according to Eggers et al., there are approximately fifty characteristics that affect a commercial cultivar's value. 2021). In addition to relatively long generational cycles and inbreeding depression, this genetic complexity, 2019a), which make it very hard to get the desired allelic combination in the offspring. Outcrossing wild cultivars with new traits can also result in the loss of allelic combination. Pests and pathogens like late/early blight, bacterial wilting, and a variety of potato viruses also cause yield losses in potato production. In general, all of these things, in addition to an ever-increasing global population and worsening climate, necessitate the application of novel and rapid breeding methods to engineer novel traits into potatoes.

Proof-of-Concept Studies Laid the Groundwork

First, transcription activator-like effector nucleases (TALENs) were used to edit the potato genome. The clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR associated protein (Cas) system, which was developed more recently. The primary reason for this is that TALENs necessitate specific protein engineering, which can be time-consuming and costly. CRISPR/Cas systems, on the other hand, are more adaptable, simple to design, and capable of multiplexing. Cas9 nuclease from Streptococcus pyogenes (SpCas9) and a chimeric single guide RNA (sgRNA) with a 20-nt protospacer that determines site specificity and a scaffold sequence necessary for Cas9 binding make up the most common CRISPR/Cas system. Cas9 is a sequence-specific nuclease (SSN) that inserts a double-stranded break (DSB) at the target site, typically three base pairs upstream of the protospacer adjacent motif (PAM) 5′-NGG-3′. There are two ways to fix this DSB: less common Homology Directed Repair (HDR) or more common non-homologous end joining (NHEJ) mechanism that frequently causes gene Knockout (KO) by inserting insertions or deletions (indels) at the target site. Plants have successfully utilized more advanced CRISPR/Cas methods, such as base editing, prime editing, and transcriptional activation/repression, in addition to traditional CRISPR/Cas9. Potato genome editing through RISPR/Cas9 was first documented in 2015 by two distinct studies. One review targeted StIAA2, a member of the auxin/indole-3-acetic acid family, and the other 2015) utilized either standard geminivirus T-DNA or modified geminivirus T-DNA to express genome editing agents and focused on the acetolactate synthase 1 (ALS1) gene. These two proof-of-concept studies laid the groundwork for subsequent efforts to edit the base and prime of the potato genome. Since then, potato has been subjected to a variety of CRISPR/Cas-mediated genome editing techniques to alter characteristics like tuber quality, resistance to abiotic stress, tolerance to herbicides, cooking properties, and self-compatibility. Because starch makes up 80% of the dry matter in

potato tubers, it is a significant source of calories and a plentiful resource for industrial applications. Amylose and amylopectin, which are both glucose chains that are linear and branched, make up its components. Amylose comprises of glucose particles basically connected through α-1,4 holding, though amylopectin additionally incorporates bunched stretching shaped by α-1,6 linkage. The ratio of amylose to amylopectin in tuber starch varies between cultivars, but is generally 1:4. Starch's chemical and physical properties are determined by this ratio, allowing it to be processed according to the intended use. For instance, amylose-free waxy starch is utilized as a coating, bulking, thickening, or bonding agent in the food industry and as a bonding agent in the paper industry. On the other hand, amylose-rich, digestion-resistant starches can be consumed as a healthier alternative to lower calorie intake, increase insulin resistance, and improve gut health.

Starch Granules

The gene for amylose synthesis, granule bound starch synthase 1 (GBSS1), has been the primary focus of CRISPR/Cas-mediated genome editing efforts to alter potato tuber starch quality. To cut down on the number of chemical and physical post-harvest treatments required to separate amylose and amylopectin, waxy phenotype tubers were created. This made the whole starch processing more economically and environmentally friendly. Due to technical considerations as well, GBSS1 has been an appealing target and model gene for numerous potato genome editing studies. Even though the waxy phenotype requires complete KO of all the alleles, it is still relatively simple to engineer this trait because, unlike many other starch-related genes, GBSS1 is the only isoform that controls it. In addition, amylose-free starch granules can be distinguished from wildtype granules, which exhibit a dark blue color due to the presence of amylose, by their red-brown color when stained with iodine.