



Spray-Induced Gene Silencing Based on dsRNA: A Revolutionary Agricultural Technology for Rapid Creation of High-Amylose Functional Foods

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Abstract

Amylose, a key precursor of resistant starch, has garnered significant attention in functional food development due to its positive effects on blood glucose management and gut health. Traditionally, increasing amylose content in crops relied on time-consuming conventional breeding or genetic modification techniques that raise public concerns. This commentary aims to explore the significant potential of an emerging agricultural biotechnology—Spray-Induced Gene Silencing (SIGS) based on double-stranded RNA (dsRNA)—for the rapid improvement of crop nutritional traits, specifically for creating high-amylose starch crops as premium raw materials for slow-digesting carbohydrates and low-glycemic-index foods. This technology utilizes foliar application of dsRNA to rapidly, precisely, and in a non-transgenic manner, inhibit the expression of starch metabolism genes or its regulators, which offer a novel dietary solution to address the globally increasing challenges of diabetes and obesity.

Keywords: dsRNA; Spray-Induced Gene Silencing; High-Amylose Starch; Low-Glycemic-Index Food; Precision Nutrition

Introduction

Changes in global dietary patterns have made blood sugar-related health issues, such as diabetes and obesity, major public health challenges. Consuming low-Glycemic Index (GI) foods rich in Resistant Starch (RS) has been proven effective for blood sugar regulation and improving gut health [1, 2]. As detailed in the provided review, amylose content is positively correlated with resistant starch content [3]. Therefore, developing GI foods that can delay the postprandial rise in blood glucose is crucial. A key raw material for such foods is high-amylose starch in staple crops like

rice, wheat, and corn, as amylose is more resistant to breakdown by digestive enzymes compared to amylopectin, resulting in a more gradual release of glucose into the bloodstream.

Mechanism of amylose biosynthesis and regulation is gradually clear

Within the functional genomics research in cereal plants, it is concluded that the starch biosynthesis and regulation are gradually clear. In the cytosol, sucrose is hydrolyzed by sucrose synthase (SUS) to produce fructose and uridine diphosphate glucose

(UDPG), which are then converted to glucose-6-phosphate (G6P) and glucose-1-phosphate (G1P), respectively. G1P is subsequently catalyzed by ADP-glucose pyrophosphorylase (AGPase) to form ADP-glucose (ADPG) [4]. Upon entering the amyloplast, ADPG can be utilized in two distinct pathways: it can be catalyzed by soluble starch synthase (SSS), starch branching enzyme (SBE), and debranching enzyme (DBE) to form branched glucans, ultimately yielding amylopectin; alternatively, ADPG can be catalyzed by granule-bound starch synthase (GBSS) to form linear glucans, which then synthesize amylose, stored in the endosperm cells. For the regulation of amylose, the transcription factor OsbZIP58 from the bZIP family in rice can directly bind to the promoters of various starch biosynthesis-related genes, such as OsSSIa, OsWx, OsAGPL3, OsSBEI, OsSBEIIb, and OsISA2. OsbZIP58 promotes the expression of these genes, thereby influencing amylose synthesis [5]. Another type of transcription factor in rice, such as the OsNAC24-OsNAP complex, regulates starch synthesis by modulating the activity of the OsGBSSI and OsSBEI promoters. Mutation of OsNAC24 resulted in a decrease in amylose content in rice grains from 18.2% to 15.0% [6]. In maize, ZmZAT8 is a novel ABA-induced C2H2-type zinc finger transcription factor that enhances the expression of key genes involved in starch synthesis, such as Sh2, Bt1, and Wx, playing a regulatory role in starch synthesis in maize endosperm [7]. Subsequent research discovered that OsSGL (containing a DUF1645 domain) knockdown or mutation disrupts the starch biosynthetic pathway, leading to reduced levels of both starch and amylose in rice seeds [8].

SIGS technology had produced high-amylose crops

Traditionally, breeding high-amylose crops (e.g., corn) relied on time-consuming conventional breeding or permanent genetic modification. However, an innovative technology called SIGS is bringing disruptive changes to this field which relies on the functional identification of key genes in the amylose biosynthesis and regulation. The core of this technology involves spraying a formulated solution containing externally synthesized double-stranded RNA (dsRNA) targeting those key genes, such as the SBE gene or its regulators onto the leaves of crops (such as rice, wheat, potatoes) during critical growth stages like grain filling or tuber bulking. After the plants absorb these dsRNA molecules, they activate their own RNA interference (RNAi) machinery to specifically “silence” the expression of the SBE gene or regulators. This inhibits the synthesis of amylopectin, prompting the crops to accumulate a high proportion of amylose. In the practice, high amylose starch can be produced in cassava and rice, which are deficient of the SBEs function resulting higher amylose than the control [9, 10]. Besides, the double-stranded RNA gene-silenced form of TaSSI of wheat and OsSSI, OsCDPK1 of rice resulted in higher amylose content [11-13].

SIGS technology has advantages and challenge

The advantages of this technology for the field of food and nutritional science are clear. It enables targeted improvement of crop traits within a single growing season, bypassing the need for breeding cycles that take years or even decades, truly enabling “rapid creation.” Meanwhile, it also acts like “molecular scissors,” precisely regulating only the target gene without affecting other

desirable agronomic traits of the crop. Besides, since it does not alter the plant's own genetic material, its effects are confined to the treated generation. This may offer advantages in terms of regulatory pathways and consumer acceptance. Moreover, it can be applied to any existing high-yield, disease-resistant elite variety, immediately enhancing its nutritional and functional value. Looking ahead, dsRNA spray technology has the potential to become a powerful tool for precision nutrition and functional agriculture. By customizing agricultural products with specific health benefits, we can help the public manage blood sugar levels directly from the food source. Of course, large-scale application of this technology still faces challenges such as dsRNA stability, cost-effectiveness, and long-term environmental safety assessment, which are precisely the areas requiring focus in future interdisciplinary research.

Conclusion

Spray-induced gene silencing technology ingeniously combines genetic-level precision with field-operation flexibility and opens a promising new pathway for the rapid production of high-amylose starch crops and the advancement of low-glycemic-index foods. It is not intended to wholly replace the traditional methods but serves as a powerful complementary tool, collectively advancing “precision nutrition” agriculture. As dsRNA synthesis costs decrease and delivery systems improve, it represents a future trend of tighter integration between agricultural technology and human nutritional needs, and stands as a potential breakthrough in combating diet-related chronic diseases

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