Research on High-Throughput Seed Slicing Technology and Its Application in Crop Breeding: Postprint

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Abstract

In recent years, molecular marker-assisted breeding has been increasingly applied in crop breeding in China. Automated seed slicing machines represent critical equipment that influences breeding scale, and their associated technologies have advanced rapidly, promising to enhance China's crop breeding capabilities in the future. This article expounds upon the significance of automated seed slicing machines in crop breeding, examines their international and domestic development status and technical principles, and provides a detailed introduction to the technical features of China's first commercial seed slicing equipment for breeding purposes, thereby delineating the future development trajectory of such equipment in China.

Full Text

High-Throughput Seed Chipping Technology and Its Applications in Crop Breeding

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Abstract

Recent years have witnessed rapid development of molecular marker-assisted selection in China's crop breeding sector. Automatic seed chippers represent key equipment that constrains breeding scale and speed. Related technologies are advancing swiftly and promise to elevate China's crop breeding capabilities. This paper discusses the significance of automatic seed chippers in crop breeding, surveys current international and domestic development status and technical principles, and provides a detailed introduction to the technical characteristics of China's first commercial breeding seed chipping equipment, while identifying future development directions.

Keywords: automatic seed chipper, automatic seed picker, molecular marker-assisted selection, crop breeding

Introduction

China has a large population, and the crop seed industry is fundamental to ensuring national food security and supporting agricultural development, having become a strategic and core foundational industry. The development of crop breeding technology will enhance the competitiveness of agricultural products, accelerate agricultural modernization, and promote China's transformation from a major agricultural country to an agricultural powerhouse [**?**, **?**]. China's seed industry technology has developed rapidly in recent years, with molecular marker-assisted breeding beginning to be promoted and applied in crop breeding. This technology utilizes molecular marker genotyping results to select optimal progeny from tens of thousands of populations for breeding, shortening breeding cycles and expanding breeding scale within limited space, with higher selection efficiency in larger populations. However, as breeding scale expands, the efficiency and quality of sample collection for molecular detection has become a technical bottleneck for molecular-assisted breeding.

The traditional approach involves planting all seeds from breeding populations in experimental fields, collecting leaf samples from the field, and then conducting genotyping analysis to make selection decisions. This method suffers from large land requirements, time consumption, low efficiency, and high error rates. Moreover, over 80% of plants grown in field breeding populations have genotypes that do not meet expectations and will eventually be eliminated, severely wasting breeding resources and breeders' efforts. With the assistance of seed chippers, micro-sampling can be performed on seeds before planting using seed chipping technology. By analyzing molecular marker information from these micro-samples, the corresponding seed genotypes can be obtained. Only seeds with desired genotypes are then planted in the field, completing genotype selection before sowing. This dramatically reduces population planting scale, saves substantial field work, and allows breeders to focus more on selection for complex agronomic and yield traits.

Development Status of Seed Chipping Technology

The development of molecular breeding technology is driving the formation of new breeding models. Seed chipping technology is a critical technology in crop molecular breeding, where sampling quality and efficiency affect breeding scale [?, ?, ?]. International breeding companies recognized early on the application value of seed chipping equipment in crop molecular marker-assisted selection breeding and began developing related technologies. In 2007, U.S.-based Monsanto announced the application of seed chipping technology in corn and soybean breeding. It uses spectroscopy for seed orientation and laser or mechanical blades for precise kernel cutting, with chips collected in 96-well plates, processing tens of thousands of seeds per day [?]. The application of seed chipping technology increased the number of seed traits in multiple trait integration (MTI) breeding from 8 to 20 [?]. In 2008, Pioneer Hi-Bred International, Inc., a subsidiary of DuPont (hereinafter referred to as "Pioneer"), announced minimally invasive seed chipping technology (also called "laser-assisted seed selection"), which applies magnetic paint to the top surface of seeds for positioning, then uses laser beams to cut small amounts of endosperm tissue from the kernel tip, with sampled seeds maintaining germination viability [?, ?].

International Development Status

The manufacturing technology for high-throughput seed chippers was originally monopolized by international seed companies, which provided no services to external parties. The lag in chipping technology became a major obstacle to implementing molecular marker-assisted breeding in China. International seed giants highly value their monopoly on seed chipping technology and use it to restrict competitors from expanding breeding scale. They typically file patents immediately after developing seed chipping technologies and limit related R&D efforts. In 2012, Monsanto filed a patent infringement lawsuit against Pioneer's "laser-assisted seed selection" in the U.S. District Court for the Eastern District of Missouri. Although the two parties eventually reached a settlement, they continue to restrict further use of this technology [?]. Due to the first-mover advantage, monopoly position, and in-house development approach of international seed giants, no commercial seed chipping equipment has appeared on the international market to date.

Domestic Development Status

To break the monopoly of international seed giants on seed chipping technology, Chinese research institutions and seed companies began developing independent seed chipping equipment, including the Hefei Institutes of Physical Science, Chinese Academy of Sciences; Shenyang Institute of Automation, Chinese Academy of Sciences; Shenyang Ligong University; China Agricultural University; China Golden Marker; and Henan Agricultural University. Related technology R&D has progressed rapidly. In 2014, Li Lu et al. [?] invented a fully automatic seed chipper using laser for seed slicing. In 2015, Wang Xiqing et al. [?] invented a "high-throughput automated seed sampling and positive seed selection system and method," describing equipment and methods for automated seed sampling via laser cutting, which was authorized by the National Intellectual Property Administration. In the same year, Wei Yingzi et al. [?] invented a double-layer turntable-type laser automatic slicing sampler for breeding and obtained national patent authorization. In 2016, Yang Xiaodong [?] described methods for automated seed sampling using laser cutting in his thesis. Also in 2016, Li Huiqin et al. from Henan Agricultural University [?] invented an automatic corn breeding sampler and obtained national patent authorization. In 2017, Gu Kanfeng et al. [?] invented an automatic corn breeding sampling chipper capable of corn seed identification and positioning using neural network patterns, with laser cutting, and obtained Chinese patent authorization. Currently, China has submitted over 20 related patent applications for independently developed seed chipping technologies to the National Intellectual Property Administration, with more than 10 already granted. However, to date, only China Golden Marker has produced a mature, mass-producible high-throughput seed chipper.

Technical Principles and Equipment

High-Throughput Seed Chipping Technology

Molecular marker-assisted selection breeding utilizes the tight linkage between molecular markers and target trait genes to screen individuals with desired traits from breeding populations, effectively reducing breeding population size, shortening breeding cycles, and fundamentally solving problems of inaccurate trait identification, high costs, and long cycles in traditional breeding [?, ?, ?]. In the early 21st century, international seed companies such as Monsanto and Pioneer adopted new-generation single nucleotide polymorphism (SNP) molecular marker detection technology, which features rapid detection, low cost, and high throughput [?, ?]. As molecular detection technology developed and breeding scale expanded, population sampling has become the bottleneck in molecular marker-assisted selection breeding. Field leaf sampling suffers from low efficiency and high error rates, with over 80% of breeding populations planted in the field being discarded at the seedling stage, severely wasting breeding resources. Using seed chipping technology to perform micro-sampling on seeds before planting allows obtaining seed genetic information through sample analysis, after which only seeds with desired genotypes are planted in the field, greatly reducing population planting scale.

The combination of automated seed chipping technology with high-throughput molecular detection technology has formed a new breeding model that can select the optimal single seed from tens of thousands of kernels for breeding participation, exponentially improving breeding efficiency and scale [?, ?]. The massive numbers of seeds produced by scaled breeding populations contain very few premium seeds, requiring seed chippers with high automation to separate single seeds from large quantities; then perform accurate identification and precise cutting of specific parts on each single seed to ensure high post-sampling seed survival rates; and finally collect micro-samples and chipped seeds accurately in one-to-one correspondence to ensure genotype information obtained from samples matches corresponding seeds.

A typical fully automated seed chipping device mainly consists of six components: automatic feeding device, seed orientation recognition device, seed grasping and transfer device, sampling device, sample collection device, and control and information management system (Figure 2 [Figure 2: see original paper]). Under the command of information management control, the automatic feeding device separates single seeds from bulk seeds; after seed orientation recognition, seed direction is adjusted and transported to the sampling platform; then precise sampling is performed on the seeds, and the collection device gathers samples and minimally invasive seeds in a one-to-one correspondence [?, ?, ?, ?, ?, ?].

Equipment Components

(1) Automatic Feeding Device. The automatic feeding device bulk-loads seeds to be sampled and separates single seeds for transport to the seed recognition platform. Its primary function is single-seed separation, mainly achieved through rotary disc separation and vibrating disc separation methods. The rotary disc separation method uses rotating discs to separate single seeds in their grooves from bulk seeds [?, ?, ?], but this method is only suitable for relatively regular-shaped, large seeds such as corn and soybeans. The vibrating separation disc effectively solves the problem of seed shape diversity by using high-frequency vibration of a spiral disc to arrange bulk seeds in a single-layer planar state, then through spiral rotation of the disc, transforms seeds from planar arrangement to linear arrangement, gradually separating single seeds (Figure 3 [Figure 3: see original paper]) [?, ?, ?, ?].

(2) Seed Orientation Recognition Device. This device recognizes the orientation of single seeds to facilitate subsequent grasping, positioning, and precise cutting. The mainstream method calculates certain features of seed shape to determine orientation [?, ?]. This approach works mainly for regularly shaped seeds with distinct features but is unsuitable for irregularly shaped or feature-poor seeds such as kernels from corn ear tips or indica rice grains. The latest image intelligent recognition technology can solve this problem. Li Guangwei et al. [?] established a model using a neural network pattern recognition toolbox, achieving over 99% accuracy in corn seed pose recognition. China Golden Marker uses artificial intelligence recognition technology to identify indica rice grain orientation, also reaching 99% accuracy (internal data). Pioneer employs another method in corn laser chipping technology, applying iron-based powder to seed tips and using magnetic adsorption to directly adjust corn seed orientation [?].

(3) Seed Grasping and Transfer Device. This device grasps seeds with determined orientation and transfers them to the sampling device, adjusting seed direction during this process to facilitate precise sampling. For easily graspable seeds, clamp-like devices are generally used for direct grasping, while approximately elliptical seeds (such as corn and soybeans) typically use vacuum adsorption for seed pickup. Transfer methods generally employ conveyor belts or multi-axis robots.

(4) Sampling Device. This device fixes seeds and performs precise sampling. Fixing methods vary according to seed shape, with "V"-shaped devices effectively securing most seeds through compression. Sampling methods mainly include mechanical cutting and laser cutting, with different methods selected based on kernel texture and shape. The China Golden Marker corn seed chipper employs a composite mechanical sampling method, using a circular saw to cut 2/3 from below the seed tip, then performing a top-down punching cut to directly punch the seed sample into a funnel-shaped collection conduit. This composite cutting method both avoids damage to hard corn kernels from the enormous punching force of single punching methods and solves the problem of inconsistent sample chip landing positions after cutting with a single circular saw method [**?**].

(5) Sample Collection Device. This device collects and stores cut seeds and chip samples through pipelines. To accurately record the correspondence between samples and seeds, 96-well plates are commonly used for storage; to increase sampling throughput, automatic plate stacking is recommended. Additionally, a corresponding information management system must record the correspondence information between seeds and chips during collection to enable data traceability when needed.

(6) Control and Information Management System. The control system precisely coordinates all aspects of seed sorting, recognition, transport, cutting, and collection to ensure orderly sampling workflow, and additionally includes infrared monitoring, fault alarm, and other functions. The information management system records various information throughout the sampling process, such as fault information, collected sample storage information, post-chipping seed storage information, and sample-seed correspondence, ensuring traceability of sampling information.

Current Status and Future Prospects

At the 4th International Conference on Agricultural Genomics in 2017, China Golden Marker unveiled China's first commercial corn seed chipping and selection equipment [**?**], providing a complete technical solution for seed processing in corn molecular breeding. The system includes fully automated minimally invasive seed sampling equipment, seed selection equipment, and corresponding information management systems. The minimally invasive seed sampling equipment can perform batch sampling on seeds from each segregating generation without affecting seed viability. After obtaining genetic information through detection of micro-samples, the corn seed selection equipment automatically identifies and selects target seeds from massive populations based on genotype information. In the management system, minimally invasive seeds, chip samples, and genotypes are matched one-to-one, enabling tracking of each seedling planted in the field.

The fully automated seed chipping system, combined with high-throughput molecular detection technology, forms a complete technical solution for crop molecular-assisted breeding (Figure 1 [Figure 1: see original paper]), marking that China's molecular breeding has broken through the "bottleneck" in population sampling technology and target seed selection, reaching a new level.

Driven by market demand, multiple Chinese institutions have invested in seed chipper R&D, with commercial products already entering the market. However, compared with foreign seed giants, there remains a significant gap in usage experience and product maturity. Moreover, unlike foreign companies that develop for in-house use, China's commercial seed chippers require greater versatility, posing greater challenges. Currently, China Golden Marker, in collaboration with the Institute of Botany, Chinese Academy of Sciences, has successfully developed a corn seed chipper, with seed chippers for rice, wheat, and soybeans also under development and testing. We expect to launch commercial related chipping equipment in the near future to serve molecular marker-assisted breeding in China's seed industry. As China's crop molecular marker-assisted breeding technology develops and expands in application scope, seed chippers will have broad prospects in major crop breeding fields. Therefore, we must draw upon successful foreign experiences, adapt to China's national conditions, and develop products suitable for the Chinese market to promote the popularization and application of molecular marker-assisted breeding technology in China.

Author Biographies

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Note: Figure translations are in progress. See original paper for figures.