



Use of Molecular Markers to Identify Genes Associated with Seed Longevity

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Seed deterioration while in storage has been a recurring problem in modern day agriculture. Seeds viability is an important factor to ensure agriculture and food security, from on-farm storage of paddy to dense germplasm storage in gene banks. The physiology of seed deterioration has a complex interaction between polygenetic pathway and environmental factors. Advances in modern genomic techniques such as SSRs, SNPs, GWAS have allowed the mapping of QTLs and the identification of longevity genes. The applications of these molecular markers are discussed and targets for genetic loci associated with longevity in major field crops are identified.

Keywords: Seed longevity, seed deterioration, molecular markers, qualitative trait loci, marker assisted selection, genome wide association studies.

Introduction

Seed longevity is a complex polygenetic trait; hence it is not easy to select for the trait. In addition to genetics, environmental factors at seed development, moisture at harvest and drying speed are important in seed performance. Traditional seed longevity study is carried out by multilocation / multi-year testing of natural seed ageing or by accelerated ageing by heating and humidifying. These experiments are time-consuming and tend to be unpredictable, using large quantities of seeds. However, molecular markers overcome these difficulties and provide reliable results based on identifying beneficial allele in seeds. The shift to high density SNPs has enabled seed storage quality traits to be precisely located instead of basic SSRs. The genetic targets for longevity of seeds include free radicals scavenging, induction of heat shock proteins, restoration of membrane lipids and stabilization of DNA and RNA. Marker assisted selection (MAS) technique can enable screening of thousands of seeds in a short time without physical seed aging. Seeds are amazing instances of organic engineering. During dormancy, they package up the full genetic information of a plant in a safe, portable way that is able to withstand harsh cold, long dry spells, or extended seed bank periods. The longevity of a seed, which is the length of time a seed will germinate, is a basic seed trait for commercial seed growers, breeding organizations and gene bank managers. The improved longevity of seeds means that field emergence is reliable, replanting of gene bank stocks is less frequent and more expensive, and farmers are less likely to suffer heavy replant losses. Although of paramount importance in agriculture, the storage ability of seeds is one of the most complex traits in plant biology. It acts as a polygenetic trait influenced by a system of physiological components, maternal environment at seed development stage, post-harvest drying, storage humidity and temperature fluctuation. The standard approach for predicting the storability is based on multi-year natural storage experiments or accelerated aging experiments under stress conditions. These physical tests are valuable, but are extremely labor intensive and time consuming, and they require loss of valuable seed reserves.

Molecular Markers

Molecular markers are sequence-specific labels in the DNA – genomic markers that allow us to analyse the genome without waiting years for physiological measurements. In the past two decades, marker technologies have advanced from the early Simple Sequence Repeats (SSRs) to high density Single Nucleotide Polymorphism (SNP) arrays and high produced sequencing technologies. Plant researchers can shed light on the complex DNA landscape that gives seeds their toughness by mapping the genes that influence storage performance, or Quantitative Trait Loci (QTL) and Genome-Wide Association Studies (GWAS). Scientists have identified several candidate genes through marker identification that play important roles in protecting the embryo against the effects of drying and storage, such as by detoxification of the reactive oxygen species (ROS); heat shock protection; cell wall integrity; and DNA repair processes. At present, marker-assisted selection (MAS) can be used to select for thousands of lines for longevity-associated alleles at a very early stage of the vegetative plant growth phase, without waiting for seed set and physical ageing. High-resolution molecular markers will be an essential tool to develop stress-tolerant resilient seed that will be stable for agricultural security in global climate change.

The biochemical mechanisms of seed deterioration

In a physiological sense if seeds are viable they are able to retain their ability to germinate for a period of time under specific storage conditions. The older the seeds the more a series of biochemical pathways leading to cell degradation are activated:

- Induction of oxidative stress: ROS cause damage to lipids of cell membranes, denature proteins, and cause double-strand breaks in DNA.
- Leakage of sugars and electrolytes: There is a leakage of sugars and electrolytes as a result of degradation of the cell membrane, which reduces the germination vigor.
- Denaturation of enzymes: Antioxidant and other enzymes are denatured or protein aggregates are formed. Hormonal changes: Absence of normal dormancy and germination cycles due to changes in the ratio of abscisic and gibberellic acids.
- Changes in epigenetics: DNA methylation and histone acetylation. Varieties differ in their production of protective compounds and the resistant varieties produce more of them initially.

Methods to discover genes

One of the techniques used is Quantitative Trait Loci Mapping; using genetically contrasting parent genotypes to develop biparental mapping populations which segregate for high or low seed viability.

GWAS: GWAS is a method that can map natural variation that affects storage performance in diverse germplasm panels. The integration of the multi-omics (genomic, transcriptomic and metabolomic techniques) is an important step to confirm the functionality of the storage performance genes.

Candidate Gene Approach: Candidate genes of superoxide dismutase (SOD), catalase (CAT), LEA proteins, heat shock factors and DNA ligases are studied.

Marker Assisted Selection (MAS)

This technology is being used in regular breeding programs to test for desired alleles in samples of tissues. Identifying genes associated with longevity led the groundwork for practical crop enhancement. With Marker-Assisted Selection (MAS), breeders usually assess thousands of breeding lines in lab settings using fast DNA tests. Instead of keeping seed stocks for extended periods to determine the longevity of the seed, researchers determine if target DNA markers are present which are associated with longevity genes. If the target marker is present it is a confirmation that the desired protective allele is present. This approach significantly reduces breeding timelines, and enables the stacking (pyramiding) of several longevity QTLs in high-value commercial rice, wheat, maize and oilseed lines. This

marker-based method will accelerate breeding programmes, so that future cultivars will not only yield high, but be stress-tolerant to biotic stress and have good seed storage.

Markers and Genes of Longevity in Major Crops (e.g. Rice and Soyabean)

In field crops, a number of significant sites and genes have been delineated over the decades of mapping.

Rice (*Oryza sativa*)

Rice (*Oryza sativa*) Important QTLs discovered using SSR and SNP markers on chromosomes 9 and 3 respectively are labeled as loci qSS-9 and qSS-3.

The gene OSALDH7 on chromosome 5 encodes an aldehyde dehydrogenase enzyme responsible for the clearance of lipid peroxidation products which are said to be increased in old age.

Genes sLOX3 and OsLOX2: Genes for membrane lipid-degrading enzymes, selection of low-activity alleles prevents membrane demolition.

Gene OsSDR: a short-chain dehydrogenase/reductase gene typically associated with sugar metabolism during seed maturation required for desiccation tolerance.

Soybean (*Glycine max*)

Soybean (*Glycine max*) Correlated markers Satt371, Satt453, and Satt618 were SSR markers associated with seed coat properties related to water and oxygen uptake during storage.

Gene Glyma20g27840: Mapping using GBS-SNPs and found to encode LEA protein which is responsible for preventing protein aggregation during dry storage.

Tocopherol Transfer Protein (TTP): An important gene that encodes the enzyme responsible for synthesizing vitamin E and which is also a major lipid anti-oxidant in oil rich seeds.

Challenges in applying Markers

The following are some of the key issues with the longevity markers:

- Genotype-by-Environment (G×E) Interaction: the markers mapped in one climatic zone may not work in other climatic zones, due to the differences in climatic and soil conditions before harvesting.
- Epistatic Interactions: Interaction between multiple genes can influence the predicted expression of the phenotypic effects of QTLs in a particular genotype.
- Cost of Genotyping: High throughput genotyping infrastructure can be very costly, and may not exist for small breeding stations in underdeveloped areas.

Some of the most recent technologies that are continually being developed to drive the gene discovery and deployment process forward are CRISPR/Cas9-based editing and genomic selection algorithms.

Conclusion

The molecular markers have turned the concept of seed longevity from a physiological phenotype to a classic genetic target. The use of genomic selection in combination with field breeding will accelerate the development of crop varieties with improved seed shelf life, which will help to ensure agricultural resilience and sustainable seed systems.

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