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Editorial

Molecular Genetics, Genomics, and Biotechnology in Crop Plant Breeding

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Abstract: A diverse set of molecular markers techniques have been developed over the last almost 40 years and used with success for breeding a number of major crops. These have been narrowed down to a few preferred DNA based marker types, and emphasis is now on adapting the technologies to a wide range of crop plants and trees. In this Special Issue, the strength of molecular breeding is revealed through research and review papers that use a combination of molecular markers with other classic breeding techniques to obtain quality improvement of the crop. The constant improvement and maintenance of quality by breeding is crucial and challenged by a changing climate and molecular markers can support the direct introgression of traits into elite breeding lines. All the papers in this Special Issue "Molecular genetics, Genomics, and Biotechnology in Crop Plant Breeding" have attracted significant attention, as can be witnessed by the graphs for each paper on the Journal's homepage. It is the hope that it will encourage others to use these tools in developing an even wider range of crop plants and trees.

Keywords: genomic selection; mutants; ddRAD sequencing; genotyping-by-sequencing; CRISPR/Cas9 site directed mutagenesis; genome-wide association scan; genetic modification; F1 hybrids; QTL

1. Introduction

The availability of genome sequences for major crop plants have opened up new possibilities for combining genotyping and phenotyping to make crop improvements, while more powerful statistical methods are being developed that allow for the identification of the underlying genes of quantitative traits. Genomic prediction has been successfully used in animal breeding and is now also increasingly being used in plant breeding [1]. Biometric statistics also support gene discovery when genome-wide markers are combined with phenotyping in large breeding nurseries or collections. Furthermore, next-generation sequencing and site-directed mutagenesis allow for some of the original ideas explored by biotechnology in crop plants to be revisited and more precise solutions to be pursued.

There has been a desire to combine genetics and the knowledge of plant nutrition, but the precise phenotyping that is required of a large number of plants from different environments and growth seasons still represents a major challenge in the improvement of nutrient use efficiency. With the introduction of DNA sequencing in the early 1980s, the genetic transformation of important crop species, the development of polymerase chain reaction (PCR)-based methods for marker-assisted selection, and next-generation sequencing has allowed for the cost-effective development of markers for orphan crops.

Other topics include the development of crops for food, feed, fuel, and fun, with the last possibly including ornamentals, along with the removal of anti-nutritional factors or improvements to the health properties of the harvested crop. This Special Issue presents a selection of research papers and

evaluates the experience acquired over the past 35 years of molecular genetics and biotechnology in crop plants, plus new research and methods.

Wheat is one of the crops that feeds the world and, in addition to grain yield, quality traits for making bread, pasta, and noodles have always been a target for breeding. Germination of mature seeds before harvest, based on weather conditions, results in poor baking quality and is referred to as pre-harvest sprouting (PHS). Resistance to PHS is controlled by many genes and their interaction with the environment is revealed in a comprehensive review of 236 papers [2]. Seed dormancy is the major genetic factor controlling PHS resistance and is controlled by QTLs located on all 21 chromosomes of hexaploid wheat. The roles of flavonoids, alpha-amylase, the plant hormone abscisic acid, and gibberellin signal pathways are reviewed. It is argued that considerable research is still needed, however, eight genes have been identified by comparative genomics, transcriptomics, and map-based cloning.

There is also a requirement for a biotechnological and digital revolution in plant breeding in order to develop climate-smart crops [3]. By surveying the literature on genetic tools developed to support crop improvement since 2000, the authors found relatively few studies that included climate change as a target. Interestingly, mutations have been used consistently over the years and the bibliometric search also highlights key papers based on citations that could be of interest.

Genomic selection for the improvement of barley and wheat is now routinely used by breeding companies alongside conventional strategies [4]. These cereals are both bred as spring type and winter type requiring vernalization, and for barley also as 2-row and 6-row spike types. Both are used as food and feed, and hence breeding for quality relates to baking and pasta quality and malting for beer and whisky.

This Special Issue of Agronomy shows, through a number research papers and reviews, that existing tools are being used and new ones are being developed to assist breeding, not only in the major crops but also in species that attract less attention.

2. Quality Traits, Yield, and Mutations in Breeding

Cytoplasmic male sterility (CMS) has been studied and explored in more than 150 plant species and hybridology involves research on different aspects of hybridization. The heterosis effect (e.g., F1 offspring) is superior to both parent lines in terms of yield, the size of fruits, or other attractive attributes. Sunflower production may be restricted to a narrow climatic zone; however, its oil content and fatty acid composition makes it an attractive oil crop. Seed production of sunflower hybrids all over the world is based on the extensive use of *H. petiolaris* PET1 CMS combined with Rf1 gene F1 hybrid seeds [5]. Using a genome-wide association scan (GWAS) for the fertility restorer gene PET1, its location has been narrowed down to a chromosomal segment of approximately 7 Mb containing 21 candidate genes, all except one, belonging to the pentatricopeptide gene family. The study identified the branching locus that provided a longer flowering time on linkage group 10 and Rf1 on linkage group 13, which is in agreement with previous publications by several other researchers.

In rice, the hulls open on the flowering date for a short period of just 40 to 90 minutes to allow fertilization, and then close again. This mechanism helps control self-fertilization in cereal crop plants in general. The morphology of the spikelet during this period is well characterized, but the genes involved are MADS-box genes, and the non-open hull (*noh1*) rice mutant identified by marker-assisted cloning is used to identify the structural gene [6]. The authors included three figures that effectively illustrate the morphology of spikelets and the comparative time-course of flowering. The *NOH1* gene was mapped to a chromosomal region of 60 kb, containing nine genes on rice chromosome 1.

Breeding for unwanted or anti-nutritional factors such as tannins has been undertaken in faba bean (*Vicia faba* L.) where two mutations *zt-1* and *zt-2* each control zero tannin seeds [7]. Faba bean breeding has attracted growing interest as a protein crop for temperate agroclimatic zones and as a source for plant-based protein food. These two recessive genes also promote a white flower phenotype, with the seed coat of all-white flowering varieties found to be free of tannins. Condensed tannins have

negative effects on the use of faba beans for food because they give an astringent taste, decrease the efficiency of food utilization, and are linked to low-protein seeds. The authors successfully developed markers linked to the recessive *zt-1* gene for use in selection against tannins in a breeding program.

Since the 1920s, when a brown midrib (*bmr*) phenotype was identified in a maize breeding nursery and increased digestibility in cattle was known to be related to lignin content. Much later, the mutated gene(s) were identified and confirmed to be key genes in the mono-lignol biosynthetic pathway [8]. This was an extensive review of *bmr* mutants in the C4 photosynthesis crops of maize and sorghum and similar mutations in the C3 plants rice, barley and wheat. With the knowledge acquired over decades of agronomic performance and an overview of genetically-modified crops regulated in lignin biosynthesis together with cloned mutant genes, the time has come to adopt new site-directed mutagenic approaches.

3. New Breeding Technologies

Occasionally the term canola is used synonymously with rapeseed, but strictly speaking. it as one of the successes of the larger Canadian rapeseed low acid breeding programs in the 1970s, which was obtained by mutational breeding. The same ideotype was subsequently obtained using genetic modification. *Brassica napus* L. has been modified genetically over a number of years with success. It would seem that rapeseed is a crop plant that is easily modified, which can be explained by the easy transfer of knowledge from the Arabidopsis model plant to rapeseed.

The objectives of rapeseed improvement have been to increase the seed oil content and changes in oil composition. This has been achieved by conventional breeding and by genetic modification of single genes. Here, it was shown that by using the soybean transcription factors *GmDof4* and *GmDof11* (DNA binding with one finger) in rapeseed, *FAB2* and *FAD2* genes in the biosynthesis of fatty acids can be modified, resulting in an increase in the healthy oleic acid content [9]. *Agrobacterium*-mediated transformation of the 'Yangyou' variety was used, which already has a double low phenotype. There are 134 Dof genes in *B. napus*, and it appears that soybean *GmDof11* and *GmDof4* target specific genes in *B. napus*. The authors provide a detailed assessment of lines obtained by site-directed mutagenesis and discuss ways to introduce these onto the market.

There is a great deal of cultural interaction involved in eating rice, so the goal of a 2% increase in yield per annum in order to meet the target for food supply in 2050 might be a bold one. It may come at the expense of cultural associations with rice consumption such as aroma, texture after cooking, and palatability. This study [10] reviewed the current status for *Wx* and *TGW* in indica and japonica rice types. TGW6 (purine acetic acid-glucose hydrolase) is one cloned out of nine genes related to rice grain weight (GW) traits. Loss of function phenotype increases seed length and GW and leads to a 15% increase in rice production [10]. The paper showed that the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) site-directed mutagenesis combined with hybrid rice breeding speeds up the time it takes to improve maintainer lines and that rice hybrid breeding is the key to achieving target traits quickly. Off-target analysis was performed and it generally appears that as a start, 40–50 mutant lines should be obtained for breeding purposes. In the T3 generation, a pollen fertility test showed that the CRISPR/Cas9 mutation did not affect the fertility of maintainer lines. To reduce the breeding cycles to develop glutinous rice lines, the mutant glutinous maintainer lines (males parent) developed were used to hybridize with the CMS line 209A (female parent) to produce F1 hybrids, and the F1 hybrids were then backcrossed with mutant lines. The tissue culture can itself introduce variation, hence more lines and backcrossing are needed to overcome these shortcomings.

Metabolic profiling of phloem exudates has been developed as a biochemical marker to discriminate between wheat varieties [11]. The paper presented the use of advanced instrumentation for direct injection mass spectrometry (DIMS) through electrospray ionization time-of-flight (ESI-TOF-MS), which offers a rapid method to obtain an initial metabolic profile of samples. It was chosen as an approach for profile analysis of phloem exudate samples in this proof-of-concept study. Principal

component analysis provided strong evidence that cultivars can be distinguished from each other and between quality groups.

Universal protocols do not always adapt well to non-model species [12], therefore the authors optimized ddRADseq (restriction site-associated DNA sequencing) in *Eucalyptus dunnii* Maiden as a lower-cost option. For *Eucalyptus*, several genotyping platforms based on single nucleotide polymorphism (SNP) array are available. They proposed that the optimized protocol can easily be applied to any plant species. The combined or individual use of two protocols (P1 for setting up in a low number of samples and P2 for scaling up the number of samples) show the benefits of similar reported protocols, but reduce the drawbacks. Furthermore, the advantages of RADseq-derived methods such as *de novo* marker discovery and removal of ascertainment bias in new germplasm, may make ddRADseq technology one of the most promising genotyping approaches in future.

4. Abiotic Stress: Drought

More than 60% of food production is based on rain-fed agriculture, making it sensitive to annual fluctuations in climate [13]. This calls for genetic improvements for drought tolerance. Drought is a complex quantitative trait controlled by the interaction of genes at many levels. In its introduction, the paper reviewed some of the constraints and challenges faced when breeding drought-tolerant wheat and the limited success in correlating molecular data obtained under controlled conditions with field conditions. Differential expressed genes in drought-tolerant 'Jimai No. 47' and drought-sensitive 'Yanzhan No. 4110' wheats in the field under irrigated and drought-stressed conditions identified 377 genes that overlapped potential drought-responsive genes, enriched in signaling transduction and MAP (mitogen-activated protein) kinase activity. RNA editing sites were identified in both genotypes, thus RNA editing should be considered as a mechanism in drought response in wheat. RNA editing takes place during transcription, providing post-transcriptional modification of genes, and has also been shown under stress responses. Targets were identified in untranslated regions regions as well as single nucleotide editing potential in coding sequences, which introduces changes of amino acid where C to T mutation in the codons was found to be the most common.

The low-cost and easy-to-use PCR-based simple sequence repeat (SSR) makers showed its efficiency in the study of genetic diversity in landraces of *Prunus salicina* Lindl in the Paraná River Delta in Argentina, which has a particularly harsh agro-ecosystem, especially regarding water stress [14]. These neutral markers were found to be adequate for population genetic studies and cultivar identification. They also assessed the SSR flanking genome regions (25 kb) in silico to search for candidate genes related to stress resistance or associated with other agronomic traits of interest. Interestingly, at least 26 of the 118 detected genes seemed to be related to fruit quality, plant development, and stress resistance. This study suggests that the molecular characterization of specific landraces of Japanese plum that have been adapted to extreme agroecosystems is a useful approach for localizing candidate genes that are potentially of interest for breeding purposes.

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