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BREEDING CROP PLANTS USING MOLECULAR GENETICS, GENOMICS, AND BIOTECHNOLOGY

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Abstract *Over the previous nearly 40 years, several different kinds of molecular markers techniques have been developed and successfully applied to breeding several important crops. Now that they have been reduced to a few favoured DNA-based marker types, the focus is on modifying the technologies to work with various crop plants and trees. This Special Issue features research and review papers that highlight the power of molecular breeding by combining genetic markers with other conventional breeding techniques to improve crop quality. The ongoing improvement and upkeep of quality through breeding is challenged by a changing climate and the use of molecular markers to enable the direct introgression of traits into elite breeding lines. Precise plant phenotyping in various environments and seasons is necessary for increased fertilizer efficiency, but this was previously costly. It is now less expensive thanks to genetic engineering, DNA sequencing, and PCR-based marker-assisted selection. New techniques like next-generation sequencing can target crop development that responds to climate change.*

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Introduction

Major crop plants' genome sequences are now available, creating new opportunities for combining genotyping and phenotyping to improve crops (Mir et al., 2019). Furthermore, better statistical techniques are being created to determine the Genes that underlie quantitative features. (Mackay et al., 2009). Given its successful application in animal breeding, genomic prediction is beginning to be used more often in plant breeding. When phenotyping in large breeding nurseries or collections is combined with genome-wide markers, biometric statistics can aid in identifying novel genes (Mackay et al., 2019). Moreover, site-directed mutagenesis and next-generation sequencing enable the investigation of more exact solutions and reexamining some of the original concepts investigated by biotechnology in agricultural Plants (Sahu et al., 2020).

The goal has been to integrate genetics with the understanding of plant nutrition; nevertheless, improving the efficiency of nutrient utilization still faces significant challenges due to the exact phenotyping that must be done on a huge variety of plants across different growing seasons and environments. The creation of affordable markers for orphan crops has been made possible by advancing polymerase chain reaction (PCR)-based marker-assisted selection techniques and genetic transformation of important crop species; the early 1980s saw the debut of DNA sequencing and next-generation sequencing (Cheng et al., 2017). The production of crops for food, fuel, entertainment, and feed—the latter of which may include ornamentals as well as the elimination of anti-nutritional elements and enhancements to the health benefits of the harvested crop are other subjects (Singh et al., 2023). One of the world's staple commodities, wheat has traditionally been the main goal of breeding initiatives in addition to grain output because it produces high-quality noodles, pasta, and bread. Harvest-premature sprouting is the term used to describe the germination of ripe seeds before harvest, depending on the weather, which leads to poor baking quality (Krishnappa et al., 2022). A thorough analysis of 236 publications reveals that numerous genes regulate resistance to PHS and how they interact with the environment (Kulwal et al., 2004). The primary genetic component governing PHS resistance is seed dormancy, regulated by QTLs found on each of the 21 chromosomes of hexaploid wheat (Ali et al., 2019). The functions of gibberellin signal pathways, alpha-amylase, flavonoids, and the plant hormone abscisic acid are discussed (Tuan et al., 2018). Despite the argument that more research is necessary, eight genes have been found using map-based cloning, transcriptomics, and comparative genomics (Wang et al., 2011).

Developing climate-smart crops also requires advancing plant breeding through biotechnology and digital means. The authors found that only a few addressed climate change when they compiled the corpus of papers on genetic technologies developed to support crop improvement since 2000 (Zenda et al., 2021). It's interesting to note that mutations have been utilized consistently, and the bibliometric search also identifies important articles based on potentially interesting citations (Hong et al., 2019). Breeding businesses commonly use genomic selection in addition to conventional tactics for barley and wheat improvement (Heffner et al., 2009). These cereals are bred as 2-row and 6-row spike types for barley and spring and winter forms that require vernalization. Since they are both used as food and feed, malting for beer and whisky and baking for pasta are related to breeding for quality (Rasmussen, 2020). Through various research papers and reviews, this Special Issue of Agronomy demonstrates how new and current tools are being developed to support breeding in key crops and species that receive less attention.

Breeding's Quality Traits, Yield, and Mutations

More than 150 plant species have been the subject of cytoplasmic male sterility (CMS) studies, and hybridology examines several facets of hybridization (Irina and Evgeny, 2020). The fruits of the heterosis effect (F1 offspring, for example) are larger, yield more, and have other desirable qualities above those of either parent line (Fortuny et al., 2021). Even though sunflower production is limited to a small climate zone, sunflower oil's high oil content and makeup of fatty acids make it a desirable oil crop (Regitano Neto et al., 2016; Sarwar et al., 2022). Global sunflower hybrid seed production relies heavily on combining Rf1 gene F1 hybrid seeds and *H. petiolaris* PET1 CMS (Meena et al., 2022). A chromosomal stretch of about 7 Mb comprising 21 potential genes—all but one belonging to the pentatricopeptide gene family—was found during a genome-wide association scan (GWAS) for the fertility restorer gene PET1. This has allowed researchers to pinpoint its position. The analysis identified the branching locus that gave linkage groups 10 and 13 a longer blooming period and Rf1, respectively, consistent with several other researchers' earlier findings. When rice reaches flowering, the hulls open for forty to ninety min. to get fertilized, after which they close once more. In general, this mechanism aids in the regulation of self-fertilization in cereal crop plants (Jennings, 1979). The spikelet's morphology at this time is well-characterized, however, the genes involved are MADS-box genes, and the structural gene is found in the rice mutant known as non-open hull (noh1) discovered by marker-assisted cloning (Rasmussen, 2020). Three illustrations provided by the authors depict the shape of spikelets and the varying timespan of blossoming. On rice chromosome 1, the NOH1 gene was located within a 60 kb chromosomal region that housed nine genes (Brunelle, 2018).

Often referred to as the wide bean, fava bean, or faba bean (*Vicia faba* L.), the breeding has been done to target undesirable or anti-nutritional components like tannins; Zt-1 and Zt-2 are two mutations that each regulate zero tannin seeds. (Zanotto, 2018). Breeding faba beans has gained popularity as a plant-based protein food source and as a protein crop for temperate agroclimatic zones (Vandenberg and Khazaei, 2023). The seed coats of all-white flowering types have been discovered to be devoid of tannins, suggesting that these two recessive alleles also contribute to the white floral phenotype (Berry, 1969). Because they have an astringent taste, reduce the effectiveness of food usage, and are associated with low-protein seeds, condensed tannins harm the use of faba beans as food (Reddy et al., 1985). Through the effective development of markers connected to the recessive zt-1 gene, the authors could select against tannins in a breeding program (Zanotto, 2018). Since the discovery of the brown midrib (bmr) phenotype in a maize breeding nursery in the 1920s, higher digestibility in cattle has been linked to lignin concentration (Rasmussen, 2020). The altered gene(s) were found and shown to be important genes in the mono-lignol biosynthesis pathway much later (Dixon and Srinivasa Reddy, 2003). This was a thorough analysis of BMR mutants found in the C4 photosynthetic crops, sorghum and maize, and related mutations found in the C3 plants, barley, wheat, and rice (Rasmussen, 2020). The time has come to implement novel site-directed mutagenesis techniques armed with decades of agronomic performance knowledge, an overview of genetically modified crops regulated in lignin production, and cloned mutant genes.

Innovative Breeding Techniques

Although the term "canola" is sometimes used interchangeably with "rapeseed," it refers to one of the products of mutational breeding, which was employed in the bigger Canadian rapeseed low acid breeding initiatives of the 1970s (Daun et al., 2015). Through genetic alteration, the identical ideotype was later achieved. For several years, successful genetic modifications have been made to *Brassica napus* (Hu et al., 2021). The facile transfer of knowledge from the Arabidopsis model plant to rapeseed explains why it appears that rapeseed is a crop plant that is easily manipulated (Zhu et al., 2016). Changing the oil composition and raising the seed oil content have been the goals of rapeseed improvement (Hu et al., 2021). Traditional breeding and genetic manipulation of individual genes have been used to accomplish this (Ashraf and Akram, 2009). Here, it was shown that using the soybean transcription factors GmDof4 and GmDof11 (DNA binding with one finger) in rapeseed can change the FAB2 and FAD2 genes involved in the manufacture of fatty acids to increase the quantity of beneficial oleic acid. With the use of agrobacterium-mediated transformation, the double low phenotypic "Yangyou" variety was used.(Rasmussen, 2020). Out of the 134 Dof genes in *B. napus*, soybean GmDof11 and GmDof4 seem to target particular genes in the bacteria. (Pandita et al., 2022). The writers offer a thorough evaluation of the lines produced by site-directed (Fanucci and Cafiso, 2006). The authors address strategies for bringing site-directed mutagenesis lines to the market and thoroughly evaluate these lines (Eriksson et al., 2019).

The objective of a 2% annual yield increase to fulfil the food supply target 2050 may be risky, given the significant cultural interaction involved in rice consumption (Hubert et al., 2010). It might be sacrificed in favor of rice's cultural connotations, which include aroma, cooked texture, and palatability (Aguilar, 2005). The current state of Wx and TGW in *indica* and *japonica* rice varieties was reviewed in this work (Feng et al., 2023). Out of nine genes linked to rice grain weight (GW) features, only one has been cloned, and that gene is TGW6 (purine acetic acid-glucose hydrolase) (Han et al., 2018). The loss of function trait causes a 15% increase in rice output by increasing seed length and GW (Yan et al., 2011). The study demonstrated that hybrid rice breeding is essential to rapidly obtaining goal features. Site-directed mutagenesis using the clustered, regularly interspaced short palindromic repeats (CRISPR/Cas9) speeds up improving maintainer lines (Romero and Gatica-Arias, 2019). Based on the results of the off-target analysis, it seems that 40–50 mutant lines should be obtained initially for breeding reasons (Manghwar et al., 2020). A pollen fertility test conducted on the T3 generation revealed that the CRISPR/Cas9 mutation did not affect maintenance line fertility (Han et al., 2018). The mutant glutinous maintainer lines aim to shorten the breeding cycles required to produce glutinous rice lines (Han et al., 2018). The male mutant glutinous maintenance lines were used to hybridize with the female CMS line 209A to produce F1 hybrids, which were subsequently backcrossed with mutant lines to shorten the breeding cycles required to develop glutinous rice lines. More lines and backcrossing are required to get around these drawbacks because tissue culture can introduce variation on its own (Brown and Thorpe, 1995).

Phloem exudate metabolic profiling has been created as a biochemical marker to distinguish across wheat cultivars (Basile et al., 2018). The combination of electrospray ionization time-of-flight (ESI-TOF-MS) with sophisticated instrumentation for direct injection mass spectrometry (DIMS) was discussed in the paper (Ibáñez et al., 2015). This technique provides a quick way to get a sample's first metabolic profile. This proof-of-concept investigation selected it as a method for profile analysis of phloem exudate samples (Gao et al., 2021). Strong evidence that cultivars may be discriminated from one another and between quality categories was found using principal component analysis (Kher et al., 2006). Because restriction site-associated DNA sequencing, or ddRADseq, is not always well adapted to non-model species, the scientists optimized ddRADseq in *Eucalyptus dunnii* Maiden as a less expensive alternative (Aguirre et al., 2019). Numerous genotyping systems utilizing single nucleotide polymorphism (SNP) arrays are accessible for Eucalyptus (Grattapaglia et al., 2011). They suggested that any plant species may implement the optimized methodology. Utilizing the two procedures (P1 for setting up a small number of samples and P2 for scaling up the number of samples) in combination or separately demonstrates the advantages of related stated protocols while mitigating their disadvantages. Furthermore, ddRADseq technology may be one of the most promising genotyping strategies due to the benefits of RADseq-derived techniques, including de novo marker discovery and eliminating ascertainment bias in novel germplasm (Aguilar, 2005).

Abiotic Stress: Drought

Rain-fed agriculture, which accounts for more than 60% of food production, is highly susceptible to yearly variations in climate (Mongi et al., 2010). This necessitates genetic advancements for resistance to drought (Ashraf, 2010). A complicated quantitative trait, drought is regulated by several gene interactions (Ali et al., 2017; Ali et al., 2015; Bhargava and Sawant, 2013). The paper began by reviewing some of the limitations and difficulties encountered in breeding drought-tolerant wheat, as well as the patchy results of establishing a correlation between controlled molecular data and field circumstances (Langridge and Reynolds, 2021). Under irrigated and drought-stressed field conditions, the differential expression of genes in drought-tolerant "Jimai No. 47" and drought-sensitive "Yanzhan No. 4110" wheats revealed 377 genes that overlapped putative drought-responsive genes, enriched in signaling transduction and MAP (mitogen-activated protein) kinase activity. (Ali et al., 2013; Ali et al., 2016; Lv et al., 2018). Since RNA editing sites were found in both genotypes, wheat's reaction to drought may involve RNA editing as a mechanism (Lv et al., 2018). RNA editing has been demonstrated to be a stress reaction and occurs during transcription, allowing for post-transcriptional change of genes (Licht and Jantsch, 2016). Targets were found in untranslated sections and coding sequences with single nucleotide editing potential, introducing amino acid alterations with the most common mutation in codons being C to T (Shabalina et al., 2013). The study of genetic diversity in landraces of Prunus salicina Lindl in Argentina's Paraná River Delta, which has a very harsh agro-ecosystem, particularly concerning water stress, demonstrated the effectiveness of the inexpensive and simple sequence repeat (SSR) producers based on PCR.

It was discovered that these neutral markers were sufficient for cultivar identification and population genetic research (Brown, 1996). To find potential genes linked to stress tolerance or other noteworthy agronomic features, they also evaluated the 25 kb SSR bordering the genome in silico (Wenzl et al., 2006). Interestingly, at least 26 of the 118 genes were connected to stress tolerance, plant development, and fruit quality (Dolferus, 2014). This work implies that a valuable method for locating putative breeding-value genes is the molecular characterization of particular landraces of Japanese plum that have acclimated to harsh agroecosystems (Auxcilia and Shabha, 2017). In summary, exploring the broad field of molecular genetics, genomics, and biotechnology in crop plant breeding has demonstrated the noteworthy advancements achieved over the past forty years (Kole et al., 2015). From the first days of DNA sequencing to the present day of sophisticated marker techniques, the field has developed and now offers priceless resources for improving crop resilience, quality, and yield (Ali et al., 2010a; Ali et al., 2011; Saini et al., 2020). The several ways that molecular markers, including CRISPR/Cas9 site-directed mutagenesis, ddRAD sequencing, and genomic selection, might be used in conjunction with conventional breeding techniques have been highlighted in the Special Issue on "Molecular Genetics, Genomics, and Biotechnology in Crop Plant Breeding". Major agricultural plants can now be improved by integrating genotyping and phenotyping, emphasising tailoring technology to various crops and trees (Costa et al., 2019).

Innovative solutions are needed to meet the difficulties provided by a changing climate, and this Special Issue's papers discuss the significance of genetic tools in the development of climate-smart crops (Aaliya et al., 2016; Ali et al., 2014; Ali and Malik, 2021; Ali et al., 2010b; Taranto et al., 2018). The variety of applications and difficulties encountered in crop breeding is exemplified by the discovery of important genes controlling characteristics such as pre-harvest sprouting resistance in wheat and the investigation of genetic diversity in Prunus salicina under challenging agro-ecosystems. The papers also highlight the important role that genetic modifications, like mutations produced by CRISPR/Cas9, play in accomplishing particular breeding objectives (Ahmar et al., 2023). The research demonstrated the accuracy and effectiveness of various methods in obtaining desired characteristics, from increasing the amount of seed oil in rapeseed to increasing the weight of rice grains (Hu et al., 2017). The issues of feeding a growing global population will largely be handled by integrating molecular tools with traditional breeding approaches as we move forward (Ahmar et al., 2020). The success stories in this collection provide hope for the future of agriculture, showing how molecular genetics and biotechnology may be used to improve crop plants sustainably while also creating a resilient and diverse agricultural environment (Saini et al., 2020).

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Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

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Not applicable

Conflict of Interest

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