Increasing Disease-Resistance in Cereals

Subjects: Plant Sciences

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Nowadays, biotechnology represents our best resource both for protecting crop yield and for a science-based increased sustainability in agriculture. Over the last decades, agricultural biotechnologies have made important progress based on the diffusion of new, fast and efficient technologies, offering a broad spectrum of options for understanding plant molecular mechanisms and breeding. This knowledge is accelerating the identification of key resistance traits to be rapidly and efficiently transferred and applied in crop breeding programs.

Keywords: crop disease resistance ; plant-microbe interaction ; molecular mechanisms in plant immunity ; sustainable agriculture ; Plant biotechnology

1. Introduction

Food availability and security challenge may be overcome by boosting crop yield, particularly that of cereals, and/or by reducing crop yield losses (20–40%) to pests and diseases, therefore diminishing further consequences for livelihoods, public health and the environment [1]. Moreover, effectiveness of long-term use of pesticides is impeded by different levels of resistance developed by phytopathogens [2]. Crop rotation, aiming to prevent the pathogen accumulation by alternating an incompatible host, together with the introduction of plant disease resistance genes (R genes) through specific breeding programs, represents alternative methods to combat yield losses to pests.

In this scenario, it would be very difficult, if not impossible, to succeed with conventional breeding, and the role of plant sciences and biotechnology becomes crucial for the future of humankind. Therefore, to find harmless control strategies for crop disease management, we need to exploit the plant innate immunity that, if timely activated, can efficiently contrast and restrict plant infection by microorganisms. In fact, although in nature plants face many types of biotic stresses caused by various organisms including fungi, viruses, bacteria, nematodes and insects, they generally resist most pathogens, and plant infection is usually the exception, not the rule [3].

Plants possess an innate ability to sense and recognize potential invading microorganisms and to mount successful defenses. Only pathogens with an evolved ability to evade recognition or suppress host defense mechanisms, or both, are successful. These biotic stress agents cause different kinds of diseases, infections, and damage to cultivated plants and significantly impact crop productivity [4].

2. Plant Biotechnology: From Random to Directed, Precise and Safe Mutagenesis

Over thousands years since 10,000 BP, humans have domesticated plants in an unconscious manner, selecting phenotypes with traits essential either for wide adaptation to different environments or improved agronomic performance. At the turn of 19th century, the introduction of Mendelian laws led to a scientific approach in crop breeding, thus representing the first revolution in the field of plant science (Figure 1).

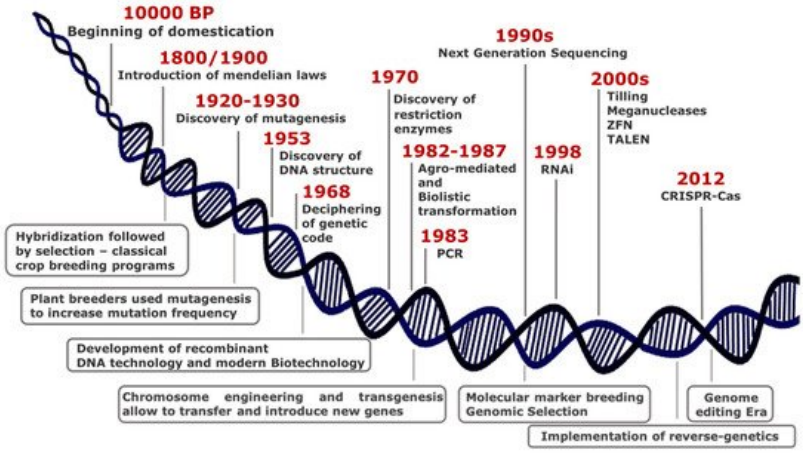

Figure 1. Agricultural biotechnology timeline. A timeline showing how biotechnology in agriculture has evolved, changing the ability to develop new crops.

Although the most common way of generating genetic variability is to mate (cross) two or more parents that have contrasting genotypes, the selection of best resulting phenotypes fostered the development of monotypic crop fields, with consequent loss of biodiversity.
Genetic variability is the basis to discover new beneficial traits and results from mutations that have occurred in genomes, either naturally or induced.

Plant breeders have used mutagenesis intensively since 1950, and to date, the FAO/IAEA Mutant Varieties Database includes more than 3300 varieties that have been released worldwide for commercial use.

Some important achievements in plant sciences characterized the second half of the last century, among which the genetic engineering technology including chromosome engineering and transgenesis for gene transfer between species distantly related.

Genetic manipulation quickly proved to have a great potential in functional genomics contributing to unravel essential in plant physiology mechanisms. In few years, transgenesis
was widely adopted in plant breeding programs since it renders possible introgression of genes or any DNA sequence from other species and enables targeted editing of plant genome to increase genetic variability.

In the last decades, new breeding techniques (NBTs) are rapidly emerging from advances in genomic research and for application in crop traits improvement. They enable precise, targeted, and reliable changes in the genome and do not create multiple, unknown, unintended mutations, unlike chemical or radiation-induced mutagenesis.

Genome-editing methods produce defined mutants, thus becoming a potent tool in functional genomics and crop breeding. Zinc Finger Nucleases (ZFN) and Transcription Activator-Like Effector Nucleases (TALENs) were the dominant genome editing tools until the rise of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and Crispr associated protein (Cas).

For the first time ever, researchers and breeders can select and target any location in the genome by the use of a short synthetic guide RNA (sgRNA) along with an endonuclease enzyme (Cas9) [[5]]. The discovery of new Cas9 orthologs (Cpf1, Cas13) and the introduction of prime editing by fusing Cas9 to reverse transcriptase [6] enable to extend genome editing applications [7][8][9][10]. Such technology is applied in a wide range of applications spanning from gene silencing and gene insertions to base, RNA, and epigenome editing

For several genome-editing techniques, the resultant plants are free from foreign genes and would be indistinguishable both from plants generated by conventional breeding techniques and from naturally mutated plants.

Indeed, a recently published study of the European Commission regarding the status of new genomic techniques (NGT) under Union law identified limitations to the capacity of the legislation to keep pace with scientific developments, causing implementation challenges and legal uncertainties. It concluded that the applicable legislation is not fit for the purpose of some NGTs and their products and that it needs to be adapted to scientific and technological progress.

3. Increasing Disease-Resistance in Cereals by Implementing Plant Immunity Through Transgenesis

During evolutionary warfare with pathogens, plants have evolved sophisticated detection and inducible defense systems to properly defend themselves (Figure 2). Therefore, plants deploy hundreds of pattern recognition receptors (PRRs) in the cell plasma membrane, conceptually analogous to Toll-like receptors in animal cells [11], that can identify both non-self-molecules, referred to as pathogen-associated molecular patterns (PAMPs), and altered self-molecules or damage-associated molecular patterns (DAMPs) [12][13]. Ligand binding by its cognate receptor, belonging to the Receptor-Like Kinases (RLKs) or Receptor-Like Proteins (RLPs) classes, triggers the socalled PAMP/DAMP-triggered immunity (P/DTI).

A second level of the plant immune system involves plant resistance proteins able to recognize pathogen specific effectors (Avr proteins) and triggers plant defense mechanisms in a more robust way [14]. This kind of resistance is called effector-triggered immunity (ETI). Most resistance genes (R genes) encode proteins with unique domains that contain a conserved Nucleotide Binding Site called NBS. LRR (Leucin-Rich Repeat) is the second most important domain. NB-LRR receptors may recognize pathogen effectors delivered inside the cell to favor plant colonization [15].

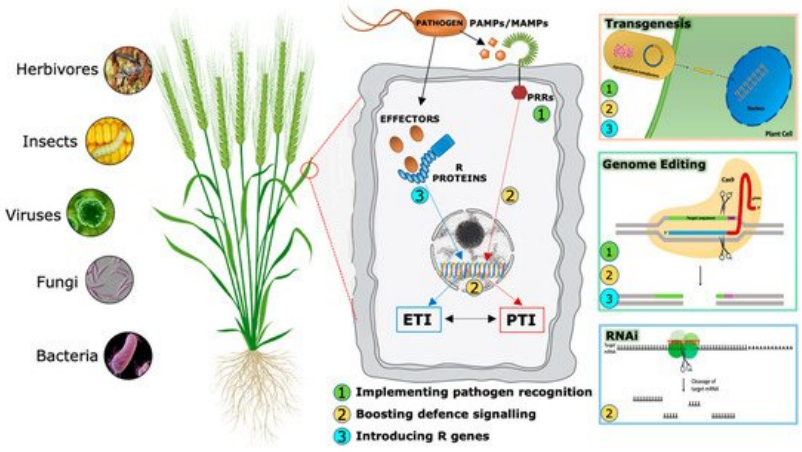

Figure 2. Biotechnological approaches and their possible involvement to enhance cereal resistance to pathogens.

Traditionally, PTI and ETI have been considered to act sequentially but independently. However, recent accumulating evidence shows that the distinction between PAMPs and effectors, PRRs and R proteins, therefore between PTI and ETI, cannot strictly be maintained [16][17], suggesting an alternative model in which the two systems interact and share common elements but in which the cellular responses they evoke appear to be distinct. Analyses of specific mutants concluded that the activation of PTI is essential for ETI to function, while ETI can boost the efficiency of PTI and prolong the immune response duration.

Plant hormones, or phytohormones, are naturally occurring signaling compounds with diverse chemical properties. The activity of a given hormone depends on its biosynthesis, conjugation, transport, and degradation as well as hormone activation and inactivation [18][19]. Although all hormones regulate several processes independently, inducible defense responses are fine-tuned by very complex crosstalk among hormone signaling outputs [20][21][22]. Such a complex and multilayered plant immune system offers different levels on which researchers could act through biotechnological approaches in order to enhance or implement plant resistance (Table 1).

Table 1. Biotechnological interventions to increase disease resistance in cereals.

Immunity Level of Intervention	Biotechnological Intervention	Gene	Species	Enhanced Resistance to	References
Pathogen sensing	**Interspecies/interfamily transfer of known PRRs**	***AtEFR***	**Wheat**	***Pseudomonas syringae* pv. *oryzae***	[23]
		AtEFR	**Rice**	***Xanthomonas oryzae* pv. *oryzae*-derived *elf18***	[24]
		AtEFR	**Rice**	***Acidovorax avenae* subsp. *avenae***	[25]
		OsXa21	**Rice**	***Xanthomonas oryzae* pv. *oryzae***	[26]

Immunity Level of Intervention	Biotechnological Intervention	Gene	Species	Enhanced Resistance to	References
Effector detection		***TaRLK1* and *TaRLK2***	**Wheat**	***Blumeria graminis* f. sp. *tritici***	[27]
		HvLEMK1	**Barely, Wheat**	***Blumeria graminis f.sp. hordei; Blumeria graminis* f. sp. *tritici***	[28]
		HvLecRK-V	**Wheat**	***Blumeria graminis* f. sp. *tritici***	[29]
	Production of chimeric receptor kinases and *R* genes	***AtEFR-OsXa21***	**Rice**	***Pseudomonas syringae pv. tomato; Agrobacterium tumefaciens; Xanthomonas oryzae pv. oryzae***	[30][31]
		OsXa21-OsCEPiP	**Rice**	***Magnaporthe oryzae***	[32]
	Deletion of effector binding sites	***Os11N3/OsSWEET14***	**Rice**	***Xanthomonas oryzae pv. oryzae***	[33]
	Addition of effector binding sites	**OsXa27**	**Rice**	***Xanthomonas oryzae pv. oryzae***	[34]
Immune signaling	**Altered expression of signaling components**	***AtNPR1***	**Rice**	**Broad-spectrum of pathogens**	[35]
	Altered expression of transcription factors	***TaPIMP1***	**Wheat**	***Bipolaris sorokiniana***	[36]
		OsIPA1/OsSPL14	**Rice**	***Magnaporthe oryzae***	[37]
R genes	**Transfer of APR alleles**	***TaLr34***	**Barely, Rice, Sorghum Maize, Durum wheat**	**Multiple biotrophic pathogens**	[38][39][40][41][42]
		TaLr67	**Barely**	**Multiple rusts and powdery mildew**	[43]

3.1. Pathogen Detection

Knowledge of the plant immune system offers the opportunity to develop new strategies of intervention at the pathogen perception level (Table 1). Increased or new recognition ability may be generated in different ways, for example by intra- and interspecies introduction of PRRs from other plants with novel recognition specificity [11][27][28][44][45][46]. In a recent study, the *Arabidopsis thaliana* EF-Tu (elongation factor thermo unstable) receptor, abbreviated as EFR, was transferred to monocot rice to confer resistance to two *Xanthomonas oryzae* pv. *Oryzae* isolates. Rice plants expressing such receptors were able to sense the bacterial ligand of EFR and to elicit an immune response. *AtEFR* was also expressed in wheat [23] driven by the rice actin promoter, and the plants showed enhanced induction of defense-related genes, callose deposition, and resistance against the cereal bacterial pathogen *P. syringae* pv. *Oryzae.* In another study, a lectin receptor-like kinase gene (LecRK) of *Haynaldia villosa*, a diploid wheat relative, has been transferred to wheat variety Yangmai158, which is powdery mildew susceptible [37]. Transgenic wheat plants showed a significant increase in powdery mildew resistance.

A different original approach is represented by engineering novel recombinant PRRs by producing chimeric receptors. Modular assemblies between Arabidopsis EFR and rice Xa21 [47] have shown that it is reliable to engineer PRRs to induce signaling and quantitative immunity against the bacterium Pseudomonas syringae pv. Tomatoe and Agrobacterium tumefaciens in Arabidopsis.

For bacterial pathogens expressing transcription activator-like (TAL) effectors that activate the expression of susceptibility genes in the host, resistance can be engineered introducing deletions in the TAL DNA binding sites on the promoter of those genes [48][49]. Another approach to engineer resistance to these bacterial pathogens is to add TAL effector binding sites to a cell-death-promoting (“executor”) gene that is triggered by TAL effectors present in common pathotypes [50][51].

3.2. Boosting the Immune Signaling

P/DTI and ETI lead to the activation of the membrane-localized ion channels and an increase in the amount of cytoplasmic calcium. Other early response events include the activation of mitogen-activated protein kinases (MAPKs) [52]. Three hormones are principally involved in downstream signaling pathways caused by P/DTI and ETI: SA, jasmonic acid (JA), and ethylene (ET). Even though SA pathway stimulates resistance to biotrophic and hemibiotrophic pathogens, JA and ET pathways are typically induced upon sensing necrotrophic pathogens and chewing insects [53]. JA and SA have important roles in the activation of transcription factors controlling biotic stress responses, the interplay between different defense signaling pathways, and chemical priming to improve plant resistance through systemic acquired resistance (SAR).

Activated defense programs require cellular rearrangements at different levels, including machinery involved in transcription, translation, and protein secretion as well as metabolism prioritization of carbon and nitrogen towards production of defense compounds, such as pathogenesis-related (PR) proteins.

Therefore, the overexpression of specific transcription factors is a potential strategy to engineer resistance, with minimized or no effects on yield. One interesting study concern the rice gene *Ideal Plant Architecture 1 (IPA1)*, known as *OsSPL14*, in which a naturally occurred allelic variant increased yield and resistance to rice blast (Table 1).

3.3. R Gene Transfer

Adult plant resistance (APR) or “slow rusting” wheat genes represent a class of potential transferable *R* genes [54]. Different APR genes are known, but only two, *Lr34* and *Lr67* (Table 1), have been cloned [55][56]. Transgenic wheat lines expressing *Lr34* gene displayed enhanced resistance to multiple biotrophic pathogens including the leaf rust pathogen and powdery mildew both at seedling and adult stages [38][57]. The mechanism by which resistance is triggered by Lr34 and Lr67 is poorly understood, although it is likely that it provides the activation of biotic or abiotic stress responses allowing the host to limit pathogen development and growth.

Wheat resistance to Fusarium species has been greatly improved by expressing either a barley uridine diphosphate-dependent glucosyltransferases (UGT), HvUGT13248, involved in mycotoxin detoxification [58], or pyramided inhibitors of cell wall-degrading enzymes secreted by the fungi, such as the bean polygalacturonase inhibiting protein (PvPGIP2) and TAXI-III, a xylanase inhibitor [59].

4. Increasing Disease-Resistance in Cereals by Using Gene Expression or Editing Techniques

4.1. RNA Interference (RNAi)

RNA interference (RNAi) was first discovered in plants as a molecular mechanism involved in the recognition and degradation of non-self-nucleic acids, principally directed against virus-derived sequences. In addition to its defensive role, RNAi is essential for endogenous gene expression regulation [60]. RNAi-based resistance can be engineered against many viruses by expressing “hairpin” structures, double-stranded RNA molecules that contain viral sequences, or simply by overexpressing dysfunctional viral genes [61]. Moreover, a single double-stranded RNA molecule can be processed into a variety of small interfering (si)RNAs and thereby effectively target several virus sequences using a single hairpin construct.

Over the last two decades, RNAi has emerged as a powerful genetic tool for scientific research. In addition to basic studies on the determination of gene function, RNA-silencing technology has been used to develop plants with increased resistance to biotic stresses (Figure 2), (Table 2) [62][63].

In short, RNAi appears to be a promising additional control strategy in the arsenal of plant breeders against at least some pathogens.

Table 2. Examples of gene expression or editing techniques to increase disease resistance in cereals.

Molecular Technique	Biotechnological Intervention	Gene	Species	Enhanced Resistance to	References
RNAi	**Viral gene silencing**	**Wheat streak mosaic virus genes**	**Wheat**	**Wheat streak mosaic virus (WSMV)**	**[64]**

Molecular Technique	Biotechnological Intervention	Gene	Species	Enhanced Resistance to	References
CRISPR/Cas9		Wheat dwarf virus genes	Barely	Wheat dwarf virus (WDV)	[65]
	Host-induced gene silencing	*FgCYP51A*, *FgCYP51B* and *FgCYP51C*	Barely	*Fusarium graminearum*	[66]
		FgCh3b	Wheat	*Fusarium graminearum*	[67]
		PtMAPK1, *PtCYC1*, *PtCNB*	Wheat	*Puccinia triticina*, *P. graminis* and *P. striiformis*	[68][69]
		FcGls	Wheat	*Fusarium culmorum*	[70]
	Silencing of host genes	*TaMlo-A1*	Wheat	*Blumeria graminis* f. sp. *tritici*	[71]
		OsSWEET13	Rice	*Xanthomonas oryzae pv. oryzae*	[72]
		OsERF922	Rice	*Magnaporthe oryzae*	[73]
		TaEDR1	Wheat	*Blumeria graminis f. sp. tritici*	[74]
		OsSEC3A	Rice	*Magnaporthe oryzae*	[75]
		TaLpx-1	Wheat	*Fusarium graminearum*	[46]
		TaHRC	Wheat	*Fusarium graminearum*	[76]

4.2. CRISPR/Cas9 Mediated Genome Editing

In plant research, NBTs are attracting a lot of attention. NBTs appear to be suitable for many different fields in plant science, such as developmental processes and adaptation/resistance to (a)biotic stresses [77]. NBTs include the most recent and powerful molecular approaches for precise genetic modifications of single or multiple gene targets. They employ site-directed nucleases to introduce double-strand breaks at predetermined sites in DNA.

The rapid increase in scientific publications documenting the use of CRISPR/Cas highlights how this technique has a greater success rate in gene modification compared to the other available nucleases. Actually, the application of CRISPR/Cas technologies to edit plant genomes is proving to be a powerful tool for future enhancement of agronomic traits in crops, qualitative and health parameters, tolerance to abiotic stress [78], and also for the improvement of biotic stress resistance (Table 2) [79].

MLO loci have been targeted by RNA-guided Cas9 endonuclease inbread wheat [80]. It had previously been reported that MLO were susceptibility genes and that homozygous loss-of-function mutants had significantly increased resistance to powdery mildew in barley, Arabidopsis, and tomato [81][82][83]. Bread wheat plants mutated by CRISPR/Cas9 in one (TaMLO-A1) of the three MLO homeoalleles showed improved resistance to *Blumeria graminis* f. sp. *tritici* infection. Another example of CRISPR/Cas9-derived resistance against the same disease is the knockout of TaEDR1 [84], conferring resistance to powdery mildew in wheat.

Plants resistant to rice blast disease were generated through CRISPR/Cas9-mediated disruption of *OsERF922* and *OsSEC3A* genes in rice.

Relatively few studies have been published on the application of the CRISPR/Cas systems to counteract crop bacterial diseases. CRISPR/Cas9 editing of *OsSWEET13* has been performed in rice to achieve resistance to bacterial blight disease caused by bacterium *Xanthomonas oryzae* pv. *oryzae* [72]. X. oryzae produces an effector protein, PthXo2, which induces *OsSWEET13* expression in the host and the consequent condition of susceptibility. Zhou et al. [85] obtained a null mutation in *OsSWEET13* in order to better explore PthXo2-dependent disease susceptibility, and resultant mutants were resistant to bacterial blight.

Further genome editing strategies for multiplexed recessive resistance using a combination of the major effectors and other R genes will be the next step toward achieving bacterial blight resistance.

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