Review

Emerging Trends in Allelopathy: A Genetic Perspective for Sustainable Agriculture

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Abstract: Over the past decades, a growing interest in allelopathy has been recorded due to the effective use of allelochemicals as growth regulators, bioherbicides, insecticides, and antimicrobial crop protection in the sustainable agriculture field. So far, the genetic aspects of the allelopathic effects have been poorly studied, and the identification of allelopathic genes and/or genomic regions (QTLs) has become a challenge to implement specific breeding programs. Here, we review the recent genetic and genome-based research findings in allelopathy, with a particular emphasis on weed control, which is one of the major crop yield-limiting factors. We discuss the key plant–microorganism interactions, including the cross-kingdom RNAi phenomenon and the involvement of microRNAs in allelopathy. Through this review, we wanted to lay the foundation for advancing knowledge in allelopathy and uncover the areas where research is needed.

Keywords: allelopathic genes; quantitative trait loci (QTL); weed control; microorganisms; next-generation sequencing (NGS); cross-kingdom RNAi

1. Introduction

Allelopathy is an ecological phenomenon, in which the chemicals produced by plants and microorganisms affect the growth, development, and fitness of other organisms [1]. This discipline represents a topic of growing interest due to the sustainability discussion currently in progress [2]. Over the years, several definitions have been adopted, in which "interaction" has been the key common word. Many definitions of allelopathy have been given throughout history [3–5]. More recently, the International Allelopathy Society (IAS) has further expanded the definition as follows: "any process involving secondary metabolites produced by plants, microorganisms, viruses, and fungi that influence the growth and development of agricultural and biological systems" (IAS, 1996) (Figure 1). However, although the different definitions mentioned above have tried to include all the possible physiological responses due to allelopathic interactions induced by secondary metabolites among organisms, to date the positive or negative effects of allelopathy are not well defined [5].

Indeed, the study of plant responses to allelochemicals is markedly influenced by the used methods, the considered biological traits, and the evolutionary history of the organisms [5]. In addition, the allelopathic phenomenon increases with the genetic distance between the species, and this concept opens a new scenario in which kin recognition takes place among species [6] and where root exudates play a pivotal role [7]. In this context, Crepy and Casal [8] described for the first time the molecular mechanisms of recognition responses in the shoot in which phytochrome B and cryptochrome 1 genes were involved. Moreover, plants can be passive organisms, responding only to environmental fluctuations, or active, transmitting, receiving, and reacting directly with other plants and microorganisms to chemical signals, regardless of environmental variations (Figure 1) [9].

These responses, which determine a fundamental role in the acquisition of resources, are the key to how a plant community is organized and how species-specific mechanisms, such as coevolution, are modulated [10–12].
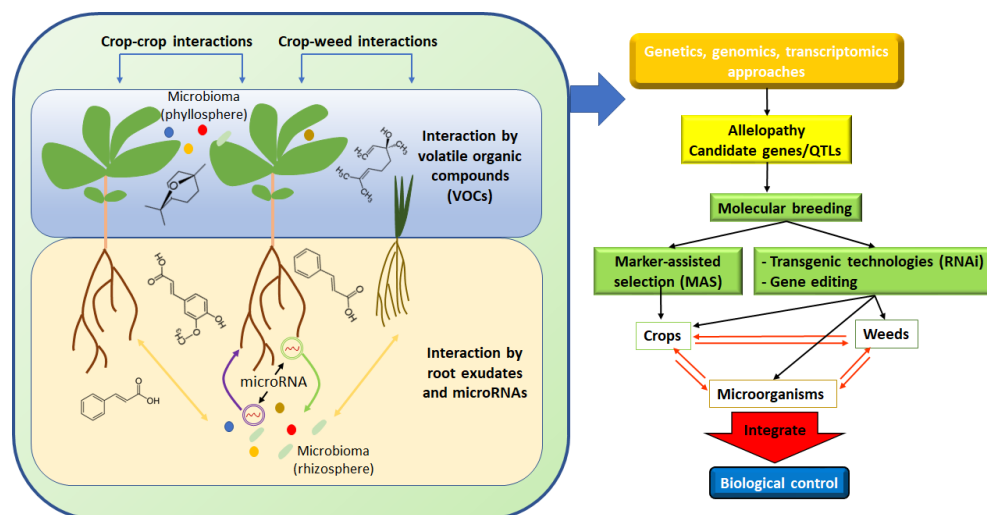

Figure 1. Schematic diagram highlighting the different allelopathic interactions. Aboveground interactions are mediated by volatile organic compounds (VOCs), whereas root exudates represent the main factor mediating allelopathic interaction in the soil. Two strategies (transgenic organisms or MAS) can be pursued to create new varieties with higher allelopathic potential. Microbiota modulates, reduces, or enhances, allelopathic interactions even through cross-kingdom microRNA exchanges.

In field conditions, the allelopathic phenomenon can also be easily understood from the spatial distribution of species [13–17], taking into account the architecture of the root system as well [18]. Moreover, for many years, allelopathy was considered an aspect of the plant competition phenomenon, but today, the distinction between the two phenomena is very clear [19,20].

Allelochemicals are secondary metabolites from different classes, such as phenolic, terpenoid, and alkaloid compounds [21]. As the main allelochemicals within plants, in terms of proportion, the phenolic compounds were extensively studied to identify their allelopathic mechanism of action in model species, such as *Arabidopsis thaliana* and *Lactuca sativa*, although no field applications have been performed [21]. Several studies reported the allelopathic effect of simple phenolic compounds on the morphophysiological processes in many crops, such as root morphology in maize plants [22], membrane permeability [23], nutrient uptake [22], cell division and elongation [24], photosynthesis and respiration [25], and hormones synthesis and balance [26].

On the other hand, more complex metabolites were also studied in allelopathic interaction such as terpenoids, including mono-, di-, triterpenoids, and sterols [21], which are involved in seed germination and oxidative damage [26], plant communication with other organisms [27], and plant defense as well [28].

Moreover, in agroecosystems, the allelopathic effects take on considerable importance in weed management [29]. Indeed, among crop pests, agricultural weeds represent the major limitation to agricultural production [30], and chemical weed control currently represents the most adopted strategy, leading to environment and human health cues [31]. A strategy to overcome these problems could be the use of allelochemicals, which possess a high potential as bioherbicide or/and herbicide bioinspired, exploiting new mechanisms of action, thereby overcoming specific resistances [12], thus representing an alternative to weed control in terms of sustainability.

In this context, genetic and genomic studies (see Figure 1) are necessary to understand the impact of allelochemicals on the plant and their biosynthesis mechanisms to switch from chemical to allelopathic weed control [32]. Despite this necessity, the genetic approach

to allelopathy studies is still in its infancy, as highlighted by the low number of published studies in the most common databases. However, several interesting genomic, evolutionary findings and genetic approaches (Figure 1) have emerged in recent years, revealing the high complexity of these genetic mechanisms. Some of these studies focused on the clustering of nonhomologous biosynthesis genes in chromosomes [33], but more recently, new generation technologies related to genomic studies applied to plant biology identified genes and/or genomic regions involved in plant allelopathic responses [34–36].

Thus, although the emerging number of genome-based investigations on allelopathic phenomena record a positive trend, the use of these technologies in specific breeding programs for allelopathy still represents a largely unexplored field [12].

In this regard, we briefly review how genetic and genomic approaches in allelopathy could provide valid support to understanding the mechanisms underlying the action mode of allelochemicals. In addition, we discuss the interplay between plants and microorganisms, the last one playing a key role in plant physiology through the production, transformation, and degradation of secondary metabolites. Finally, in this review, we emphasize how allelopathic potential is genetically controlled, and the identification and transfer of allelopathic traits from specific genotypes into elite cultivars offers the potential to improve sustainably cropping systems.

2. Genomic Approaches in Allelopathy

In recent years, genetic technologies have developed rapidly, and whole-genome sequencing has become an increasingly routine technique in many areas, such as medicine, biotechnology, and agriculture. Although global expression responses of plant genomes in allelopathy using DNA microarrays were reported [37], the new technologies could detect novel transcripts, and predict the gene regulatory networks of a biological response. Indeed, to analyze gene and/or regulatory chromosome regions, a complete sequence assembly is necessary, and, at the same time, the biological processes associated with likely phenotypic traits can be determined. To date, these sequencing technologies, such as whole-genome sequencing and RNA sequencing (RNA-seq), can be performed at different biological levels: from plant tissue to single cell [38]. However, in allelopathy, the application of these approaches is markedly limited to a few experiments (Table 1), including either gene expression or RNA sequencing technologies, as well as the identification of quantitative trait loci (QTL) useful for plant breeding.

Table 1. Representative articles of the genetic and genomic approaches in allelopathy.

Plant Material	Methods	Targets	References
Common reed	RNA-seq	Phytohormones	He et al. [39]
Rice/barnyard grass	Microarray	Phytohormones	Chi et al. [40]
Tomato	RNA-seq	Antioxidants and Hormones	Cheng et al. [34]
Rice/barnyard grass	RNA-seq	Shikimic acid and acetic acid pathways	Zhang et al. [41]
Rice/barnyard grass	RNA-seq	Diterpenoid and flavonoid biosynthesis pathway	Li et al. [42]
Rehmannia glutinosa	Cloning, qRT-PCR	Phenolic biosynthesis: *C3H* gene	Yang et al. [43]
Soybeans	RNA-seq	Oxidative stress and jasmonic acid signaling (*PIF3*)	Horvath et al. [44]
Rice	SNPs genotyping	QTL regions	Chung et al. [45]
Rice	qRT-PCR	Biosynthesis of phenolic acids	Zhang et al. [46]
Wheat/ryegrass	AFLP, RFLP and SSR genotyping	QTL regions	Wu et al. [47]
Lettuce/rice	RFLP genotyping	QTL regions	Zeng et al. [48]
Lettuce/*Triticum Speltoides*	RAPD genotyping	Genetic diversity in allelopathic potential	Quader et al. [49]
Rice	RNA interference	PAL gene expression	Fang et al. [50]
Rice	T-DNA insertion	*OsCPS4, OsKSL4*	Xu et al. [51]
Rice/barnyard grass	qRT-PCR	*PAL, C4H, F5H,* and *COMT* genes	Zhang et al. [52]

Table 1. *Cont.*

Plant Material	Methods	Targets	References
Sorghum	SSR genotyping	QTL regions	Shehzad et al. [53]
Rice/barnyard grass	qRT-PCR, ChIP-seq, ChIP-qPCR	MYB transcription factor	Fang et al. [35]
Rice	qRT-PCR, RNA-seq	Biosynthetic gene clusters	Sultana et al. [54]
Arabidopsis	RNA-seq	Signal transduction, nutrient transporter, detoxification genes	Zhang et al. [36]
Rice	RNA-seq	Chlorophyll and nitrogen metabolisms	Li et al. [55]
Rice	Genome sequencing	Detoxification-related genes (CYP450, GST) DIMBOA gene cluster.	Guo et al. [56]

2.1. From Metabolite to Gene in Plants

Despite the considerable number of manuscripts dealing with crop allelopathy, the main works aiming to understand the molecular networks involved in allelopathic traits and their potential involvement in breeding programs for field application were performed on cereals (Table 1). In particular, the main metabolites considered to be involved in this phenomenon belong to the classes of indoles (benzoxazinoids and their derivatives, produced by wheat, maize, rice, etc.), phenylpropanoids (i.e., cinnamic acids derivatives), and terpenoids (momilactones a and b, produced by rice).

Among the molecular approaches used to clarify some aspects related to the allelopathic phenomenon, the expression analyses of plant secondary metabolism pathway-related genes turned out to be an informative technique for the regulation of metabolite biosynthesis. The first correlation between metabolite produced and gene expression was demonstrated in cereals [50,57]. In particular, some studies focused on the biosynthesis of benzoxazinoide compounds, the cyclic hydroxamic acids 2,4-dihydroxy-1,4-benzoxazin-3-one and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIBOA and DIMBOA, respectively), identifying five key responsible genes in maize [58]. In this regard, a mutation in the Bx1 gene in maize demonstrated a clear correlation between gene (Bx1) and metabolite (DIMBOA). Then, a deeper study of the same biosynthetic pathway led to the identification of other genes (named Bx2 through Bx5) encoding cytochrome P450-dependent monooxygenases [58]. Similarly, Song et al. [59] investigated in hydroponic experiments the weed suppressive ability of several rice accessions and banyardgrass (*Echinochloa crus-galli*) exposed to different nitrogen supplies, identifying two contrasting lines (accession PI312777, highly allelopathic, and accession Lemont, with low suppressive activity). After a subtractive hybridization suppression, to construct a forward SSH-cDNA library of PI312777, the authors sequenced and annotated 35 clones, identifying genes related to allelochemicals. In particular, they reported that in the accession PI312777, the phenylalanine ammonia lyase (PAL) and cytochrome P450 genes strongly increased their transcript abundance at a low N level, suggesting that the higher ability of PI312777 to suppress banyardgrass might be connected to the stronger activation of genes involved in de novo synthesis of allelochemicals [59]. Using the previously described rice accessions treated with SA as elicitor, Fang et al. [60] observed an increase in the allelopathic potential of the accession PI312777, together with an upregulation of genes involved in phenylpropanoid metabolism and antioxidant-related proteins. On the contrary, the cocultured plants of barnyardgrass highlighted a reduced activity of cell-protective enzymes (SOD, POD, and CAT). The authors concluded that allelopathic PI312777 accession was characterized by an active chemical defense and auto-detoxifying enzyme system associated with xenobiotic detoxification [60].

Finally, in 2019, exposing the accession PI312777 to barnyardgrass root exudates, a transcript upregulation of allelochemical-related biosynthesis pathways was observed, such as the acetic and shikimic acid, suggesting an accumulation of metabolites (phenolic

acids, fatty acids, and flavonoids) belonging to the downstream shikimic and acetic acids pathway in response to root exudates of barnyardgrass [41].

Besides gene expression and transcriptomic analysis, the involvement of PAL-related genes in rice allelopathy was further elucidated using proteomics and bioinformatics approaches. A significant correlation between inhibitory effects of allelopathic rice on weeds and a higher expression of *PAL* in the phenylpropanoid metabolism was demonstrated using the RNA interference (RNAi) approach to silence this gene in rice, highlighting also a quali-quantitative modulation of microorganisms in the rhizosphere [39,51]. Recently, the key role of *PAL* on weed suppression was further emphasized through the genetic transformation of rice (silencing or overexpressing), which also showed a direct impact on the microbial community in the rhizosphere, thereby changing root exudated secretion and consequently the capacity of weed suppression [61].

In a recent paper, Zhang et al. [46] confirmed in field experiments that the allelochemical effects in rice are highly dependent on specific genes related to phenolic biosynthesis by comparing the two previously mentioned contrasting rice cultivars (Lemont and PI312777) for allelopathy potential. *PAL*, cinnamate-4-hydroxylase (*C4H*), ferulic acid 5-hydroxylase (*F5H*), caffeic acid O-methyltrans-ferases (*COMT*), cinnamoyl-CoA reductase (*CCR*), and cinnamoyl alcohol dehydrogenases (*CAD*) genes were found upregulated in both PI312777 root and shoot [62,63], thereby indicating that the enhancement of the phenolic compound metabolic processes increase the allelopathic potential [46].

Reverse genetic approaches were also employed, such as knockout lines to overcome the limits imposed by previous studies in which the allelopathic effect was based exclusively on the ability to modify any physiological process and/or reduce any morphological parameter by a mix of metabolites present in root exudate [51,64], dependent on a plethora of genes, and therefore not assigning a specific role to certain genes.

Another important class of allelopathic metabolites produced by rice is terpenic-derived compounds. In particular, momilactone a and b are among the most important in allelopathic interactions. These metabolites were first isolated in rice husks [65,66] and then in root exudates, as reported in the extensive review written by Kato-Noguchi and Peters [67] and Serra Serra et al. [68]. These molecules, constantly produced by rice during its life cycle, are highly active at low concentrations (ED_{50} between 20 μM and 40 μM) against two of the most noxious weeds affecting rice crops, i.e., barnyardgrass and Jungle rice (*Echinochloa colonum)*. In contrast, no autotoxicity phenomenon was observed on rice at natural concentrations [67]. Their biosynthesis is mediated by the enzymes copalyl diphos-phatesynthase 4 OsCPS4 and kaurene synthase-like 4 OsKSL4 [69,70], which are required to produce syn-pimaradiene, the committed intermediate in momilactone biosynthesis. Through reverse genetics, it was possible to obtain insertion gene knockouts for *OsCPS4* and *OsKSL4* [71,72], allowing for defining the role of these specialized metabolites. In fact, in silenced plants, a significant reduction of its allelopathic activity was observed, confirming the role of these terpenoids on rice weed suppressive ability.

These results emphasize once again how the genetic approach related to the biosynthesis of metabolites with allelopathic potential could be useful for plant breeding programs, in which allelopathic crops could represent a potential alternative to agrochemicals and pharmaceuticals, thereby preventing many problems derived from the massive use of synthetic herbicides.

However, both conventional breeding methods and new technologies (genome editing) cannot neglect the evaluation of productivity, and many studies in this field have focused only on the vegetative phase.

However, the analysis of a single gene expression does not provide a clear view of its regulation and function. For a detailed analysis of the molecular aspects underlying the allelopathic mechanisms, other factors at different levels must be considered, such as transcription factors (TFs), as well as post-transcriptional and post-translational regulations. Furthermore, in a complex biological system such as the plant, there are complex molecular

networks that interact to regulate the phenotypic expression of the organisms, and new technologies could lead to an easier understanding of these interactions.

2.2. From Genome to Gene

The first technique to sequence a gene or genome dates to 1977 by Sanger and Coulson [73], who revolutionized research in biology by contributing to a new viewpoint in molecular biology. This method, among others [74], dominated genetic research up to 2005 [75,76], in which a new method based on automated capillary electrophoresis was developed to improve data knowledge of genes and genome sequences [77], generating pivotal information related to genetics, epigenetics, and transcriptomics. This new technology, named next-generation sequencing (NGS), has opened new scenarios in health, environment, and agriculture-related studies due to the highly accessible low-cost and fast high-throughput sequencing technique [78]. In addition, the capacity to obtain large genomic data sets (Giga base), the scalability, the de novo sequencing and resequencing, the discovery of genomic variants, and molecular markers in crops are other features that distinguish NGS from the older technologies. Moreover, NGS technologies can be applied for TF binding site identification and chromatin alteration studies [74]. Despite all the mentioned benefits related to NGS technologies, their applications in the allelopathic field are limited. The literature can be divided into two aspects inherent to the allelopathic phenomenon: on the one hand, there are transcriptomic analyses on weeds to identify which molecular mechanisms are induced when in cocultivation with crops, and on the other hand, the transcriptomic approaches applied on crops to understand the molecular aspects of the allelochemical's action mode useful in breeding strategies.

Concerning the first aspect (from weed to crops), the study by Guo et al. [56] is an important milestone in the elucidation of the allelopathic mechanisms implemented in barnyardgrass. Since barnyardgrass is among the most widespread weeds in the world, the authors shed light on the molecular mechanisms underlying its high allelopathic potential. In particular, using genome assembly and annotation (RNA-seq), the authors identified two gene clusters, involved in DIMBOA and phytoalexin momilactone A biosynthesis, which were activated in response to cocultivation with rice, and detoxification seemed to be the main mechanism conferring an extreme adaptation to the weed [56].

However, the limits of this work could be traced back to the polyploid nature of the species used and the technologies adopted as they do not allow to associate cause–effect in a statistically significant way.

Regarding the second aspect, an important work that highlights the importance of molecular approaches in the understanding of the crop's allelopathy, as well as its responses to allelochemicals is that of Zhang et al. [44]. Indeed, in a previous comparative analysis [79], the authors tested the effects of barnyardgrass root exudates on two contrasting rice varieties for the allelopathic trait (PI312777 and Lemont), discerning induced allelopathy (IA) from genetic allelopathy (GA). Although no differences between rice varieties were detected in IA, the GA exhibited higher activity in PI312777 than Lemont. In addition, the authors stated the pivotal role of the *PAL* gene in PI312777 variety during exposure to barnyardgrass root exudates [79]. These results lead to the hypothesis of the existence of probable induction systems in regulating the expression of genes involved in the allelopathic response, in which a simple gene expression is insufficient to individuate target genes. Then, in the subsequent article [44], the authors carried out a transcriptomic analysis on a similar experimental design, and the approach allowed the identification of some TFs, such as TGA related to salicylic acid, and biosynthetic pathways, such as shikimic acid pathway, involved in rice allelopathic response. To elucidate the molecular nature of the allelopathic signal in the rice–barnyardgrass interaction, Li et al. [42] revealed that in response to barnyardgrass root exudates, the elicitation of rice allelochemicals momilactons B and tricin biosynthesis matched with an upregulation of the key genes involved in the diterpenoid and flavonoid biosynthesis pathways (*CPS4*, *KSL4*, *CYP99A2*, *CYP99A3*, and *MAS*). To complement these results, a weighted gene coexpression network analysis (WGCNA)

revealed the presence of a coexpression module significantly and positively correlated to the expression of four out of the five above-cited genes and allowed the identification of seven TFs among the hub genes within the regulatory network, such as C2H2 and bHLH, supporting their role in the allelopathic interaction between rice and barnyardgrass [54]. Furthermore, an in-depth study of this interaction highlighted the pivotal role played by the TF *OsMYB57*, which regulates L-phenylalanine content interacting with *OsMAPK11* (Figure 2) [35].
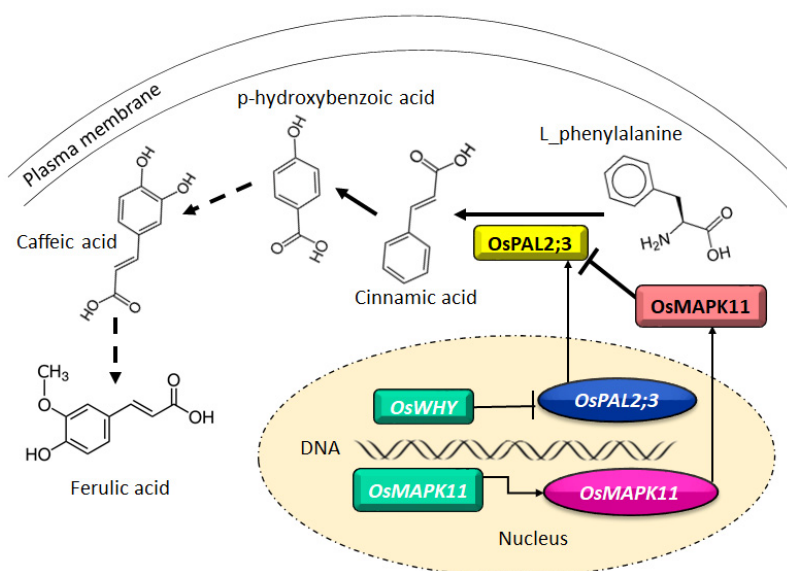

Figure 2. Schematic summary proposed by Fang et al. [35] describing OsMYB57 as a positive regulator of *OsPAL2;3* gene expression, since it is involved in the transcriptional regulation of *OsMAPK11* gene expression. OsMAPK11 protein then interacts with OsPAL2;3 to regulate its activity in the phenylpropanoid pathway. *OsWHY* transcription factor represses the transcription of *OsPAL2;3*.

Studying the allelopathic effect of *Artemisia argyi* on rice transcriptome, Li et al. [55] discovered the molecular mechanisms by which allelochemicals significantly reduce rice germination rate and growth. Photosynthesis and chlorophyll metabolism inhibition was the key action mode of *A. argyi* extracts, targeting a series of chlorophyll synthesis-related genes, such as *HEMA, HEML, CHLH, CRD*, and *CHLD*, as well as genes involved in the photosynthesis pathway, such as *PetC, PsbY*, and *LHCII*, the transcripts of which resulted significantly reduced in *A. argyi* extract-treated rice with respect to the control [55].

Moreover, transcriptome results revealed that the caffeic acid present in *A. argyi* extract inhibited *Setaria viridis* weed growth by downregulating multiple genes involved in gibberellin (GA), phytoalexin biosynthesis, and mitogen-activated protein kinase (MAPK) signaling pathways [80].

Although many allelochemical pathways and gene targets have been identified, few studies described the cellular signaling machinery in response to allelochemicals. Recently, Zhang et al. [36] highlighted the involvement of signal molecules such as PK, receptor-like kinases (*RLKs*), calcium-dependent protein (*CIPKs*), and calmodulin-related genes in *Arabidopsis thaliana* roots exposed to aqueous extracts of the invasive weed *Conyza canadensis* (Figure 3).
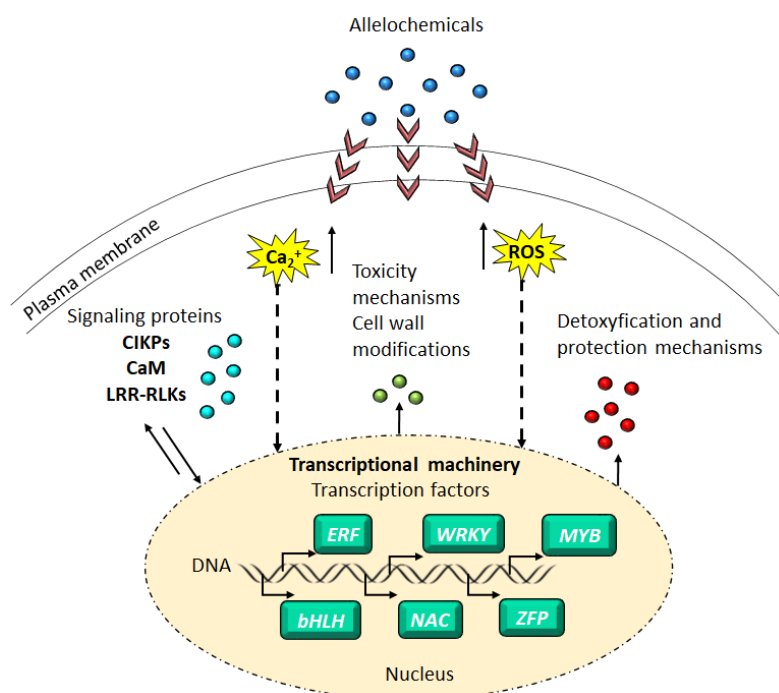
Figure 3. Molecular model of *Conyza canadensis* allelopathic effect proposed by Zhang et al. [36], illustrating the signaling machinery, the transcriptional regulation, and the cellular processes involved in stress response in *Arabidopsis thaliana* roots.

Moreover, plenty of phytohormones, such as ABA, auxin, and ethylene-related genes, were also involved in tomato and Arabidopsis signal transduction pathways [34,36]. Finally, even microRNAs (miRNAs) are involved in the rice–barnyardgrass molecular allelopathic interaction, as described by Fang et al. [61]. The authors emphasized the enhancement of miRNAs transcript levels relevant to plant hormone signal transduction, nucleotide excision repair, and the peroxisome proliferator-activated receptor pathways in barnyardgrass cocultured with the allelopathic rice cultivar PI312777 and showed that the expression levels of these miRNAs in barnyardgrass plants were positively correlated with allelopathic potential of the cocultured rice varieties.

Despite the appreciable efforts done to elucidate the signaling mechanisms of allelopathic compounds, both through simple gene expression and transcriptome analysis, more in-depth research is required to identify some genomic regions (QTLs), which could be used in plant breeding programs.

2.3. Plant Breeding in Allelopathy

The main goal of plant breeding is to maintain the quality of life on earth. Improving crops for allelopathy falls within sustainable agriculture, and different strategies, based on genetic variability or transformation, can be adopted to reach this goal. In the first case, natural genetic variability is used to obtain multiple genotypic variants with small phenotypic effects, whereas the genetic transformation forms variants with a significant effect on phenotype. Genetic variability among and within species provides a genetic pool on which to select crop with high allelopathic ability [47], highlighting how improving allelopathy in crops depends on the understanding of the genetic control of these traits. However, as demonstrated by different authors, allelopathic traits follow a normal distribution, thereby outlining the quantitative nature of the traits and their polygenic control [48,81]. Thus, the approaches used to study and understand multiple traits in plants are quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS). QTL mapping is based on statistical analysis that links phenotypes, and in this context, it is represented by allelopathic traits, with genotypes (chromosome regions) [81]. In recent years, genetic studies have

been performed only on crops with considerable economic importance, such as wheat and rice (Table 1). A full-bodied study was performed by Olofsdotter et al. [82], who report some examples in rice. However, it is important not to overlook the environment's effect on these quantitative traits. Indeed, for these studies, fixed segregant populations are needed for QTL analysis, in which near isogenic lines (NILs), recombinant inbred lines (RILs), and doubled haploid lines (DHLs) represent the most used plant material. Initially, with the lack of current technology, segregation ratios and first-generation molecular markers were taken into consideration, as well as euploid, aneuploid, and substitution lines, to investigate the loci of genes controlling the accumulation of DIMBOA on chromosomes [83]. Yet, QTLs associated with allelopathic traits in rice were also identified using restriction fragment length polymorphism (RFLP) markers in the F2 population from a cross between two contrasting varieties for this trait [84], while Wu et al. [47] identified such QTLs studying the allelopathic effect of wheat on ryegrass using the "equal-compartment agar method". In this work, the authors used a double-haploid (DH) population and different molecular markers, such as RFLP, amplified fragment length polymorphism (AFLP), and microsatellite (SSRs). They identified two QTLs in chromosome 2B associated with allelopathic traits [47]. As in wheat, a DH population was also used in rice (123 genotypes), and its parents were employed to elucidate the genetic mechanisms of rice allelopathy [48]. The authors detected four QTLs related to allelopathy traits and located them on chromosomes 3, 9, 10, and 12, confirming some QTLs previously localized [84].

High-throughput SNP genotyping was recently adopted to identify QTLs associated with the allelopathic traits in rice [45]. For this purpose, 98 F8 RILs were produced by single-seed descent by crossing a cultivar with high allelopathic potential (Sathi) with a nonallelopathic cultivar (Non-an). On chromosome 8, two QTLs, qlTL-8 and qlSL-8, were detected and were responsible for shoot and root length inhibition, explaining 20 and 15% of the phenotypic variation, respectively. Interestingly, between these QTLs, 31 genes were located [45]. Recently, these technologies have been applied to identify QTLs associated with allelopathic traits in several crops. In sorghum, 107 accessions were employed to perform linkage disequilibrium (LD)-based association mapping [53], and F2:3 lines derived from a cross between Japanese and African landraces were used in linkage mapping. In both cases, different QTLs were detected, which could be implicated in breeding programs. Moreover, the results demonstrated that there are many other allelopathic metabolites in addition to sorgoleone, and that *SOR1*, a gene related to sorgoleone biosynthesis, may not be the only gene conferring allelopathy to sorghum [53]. Finally, in another study, rice's morphological and molecular traits were associated with weed competitive ability to develop new varieties with high grain yield under competition [85]. In this investigation, a BC1F2:3 population between *Oriza glaberrima* (IRGC105187) and *O. sativa* cultivar IR64 was employed to assay allelopathic traits. Fifty-nine out of seventy-two detected QTLs were found to be major QTLs. Moreover, many alleles from the *O. glaberrima* parent contributed to the phenotype, thereby demonstrating that *O. glaberrima* species may be a potential source for improving weed competitive traits in rice [85]. Nevertheless, further studies are needed to identify genes included in the QTLs detected, also uncovering the upstream TFs regulating gene clusters and genes or pathways responsible for allelopathy traits. Moreover, there are many factors that influence the detection of QTLs segregating in a population such as environmental effects, population size, traits considered, and experimental error. Thus, the validation of the QTLs detected in different environments and also with an increase in the size of the populations assumes considerable importance.

2.4. Microorganism in Allelopathy

Microorganisms colonizing the rhizosphere, mycorrhizosphere, and phyllosphere play a pivotal role in plant health and performance through different mechanisms, including allelopathy [86,87]. There is continuous allelopathic crosstalk between plants and the complex microbial communities in which plant roots secrete a variety of molecules able to shape the rhizosphere microbiota, which in turn produce feedback on the plant [88–90].

The role of root-exuded coumarins in shaping the root microbiome clearing the rhizosphere from competing microorganisms to give coumarin-resistant microorganisms a competitive advantage has been recently highlighted and represents a good example of how plants affect soil microorganism communities [91]. Subsequently, Stinglis et al. [92] described the molecular basis of the *Arabidopsis thaliana–Pseudomonas simiae* WCS417 beneficial model system, in which the plant and the probiotic rhizobacteria closely collaborate to induce the root-specific *MYB72* TF and the MYB72-controlled β-glucosidase *BGLU42* scopoletin-dependent biosynthesis. The excretion of this metabolite selectively inhibits the soil-borne fungal pathogens *Fusarium oxysporum* and *Verticillium dahliae* and promotes the growth of rhizobacteria *P. simiae* WCS417 and *Pseudomonas capeferrum* WCS358 responsible for the rhizobacteria-induced systemic resistance (ISR). This molecular collaboration led to plant protection and growth enhancement and improved the niche establishment of the microbial partner as well.

In the same context, another step toward crop improvement is the identification of plant genes and QTLs obviously responsible for the modulation of rhizosphere microbiota composition and allelopathic interactions. In their recent paper, Li et al. [93] revealed that allelopathic rice could affect the metabolism of phenolic acid allelochemicals by regulating the key gene *OsPAL2-1*. The phenolic acids secreted by allelopathic rice into soil induced the gathering of myxobacteria in the rhizosphere. The latter is responsible for the production of a large number of secondary metabolites with allelopathic activity, among which quercetin, a potential allelochemical deriving from the ferulic acid-induced *Myxococcus xanthus* cultured medium and playing a role in weed germination and growth suppression. In addition, Escudero-Martinez et al. [94] identified the *QRMC-3HS* genomic region as the major determinant of the composition of barley rhizosphere microbiota communities. Then, performing a root comparative RNA-seq profiling on the barley lines with contrasting alleles at *QRMC-3HS*, they identified a nucleotide-binding leucine-rich repeat (NLR) gene among the primary candidate genes.

In their turn, microorganisms produce allelochemicals such as phytohormones (e.g., ABA, auxins, ethylene), volatile organic compounds (e.g., ketones, alcohols, alkanes, terpenoids), quorum sensing molecules (e.g., N-acylhomoserine-lactones, AHL), and antibiotics [95], that can promote plant growth [96], resistance to stress [96,97], induce resistance to diseases, antagonize phytopathogens [98], and control weeds [42,99].

Among rhizosphere microbiota, plant growth-promoting rhizobacteria (PGPR) is a group of beneficial microorganisms (fungi and bacteria) that can promote plant growth by regulating phytohormones synthesis/transport and inducing plant systemic resistance and tolerance through VOCs production. Among the most recent papers on the topic, the study conducted by Lee et al. [100] revealed that *Arabidopsis* plants exposed to 1-decene (a VOC identified from the biocontrol fungus *Trichoderma*) exhibited the greatest increase in plant fresh shoot weight (38.9%) and chlorophyll content (67.8%) respect to other VOCs treatments. The RNA-seq profiling revealed that VOC treatment affected the expression of 123 genes, among which cell wall modification, auxin induction, stress, and defense response-related genes, with a notable downregulation of several stress-related genes. Furthermore, a transcriptome analysis of the growth-promoting effect of VOCs produced by *Microbacterium aurantiacum* GX14001 on tobacco (*Nicotiana benthamiana*) revealed that most of the upregulated genes in response to the bacterium VOCs were involved in plant hormone signal transduction, phenylpropyl biosynthesis, plant–pathogen interaction, and flavonoid biosynthesis pathways. The authors suggested that plant hormone signal regulation was the way by which GX14001's VOCs promoted tobacco growth, a suggestion that was validated by further *Arabidopsis* mutant experiments [101].

Moreover, Berendsen et al. [102] defined the rhizosphere microorganisms as the "plant secondary genome" reporting the impact of the microbe-derived compounds on plant performances. In addition, Rout et al. [103] exposed an interesting perception of the plant microbial genomes as integrated components of the plant genome and highlighted the importance of considering the plant microbiota (all microorganisms) as a plant microbiome

(all microbial genomes) that constantly dialogues with the host genes [104]. In addition to the allelochemical substances, a more elaborated communication process, a kind of "molecular allelopathy", was recently discovered. The "cross-kingdom RNAi" phenomenon is described as a bi-directional communication channel organized by the plant and its associated rhizospheric microorganisms through extracellular vesicles (EVs) carrying miRNAs to induce gene silencing (Figure 1) [105].

In their interesting opinion paper, Middleton et al. [106] proposed the implication of miRNAs in shaping the abundance, function, and composition of the rhizospheric microbiota, based on experimental evidence of the connection between plant miRNAs and pathogens [107,108]. In particular, the cross-kingdom trafficking between *Arabidopsis* roots and the fungus *Botritis cinerea* has been well described [109]; authors showed that *Arabidopsis* cells secrete exosome-like EVs hosting miRNAs that are delivered into fungal cells at the infection sites, inducing fungal virulence-related genes silencing. This phenomenon has been further studied revealing the involvement of RNA-binding proteins in *Arabidopsis*, such as Argonaute 1 (AGO1), RNA helicases (RHs), and annexins (ANNs), in the selective loading of miRNAs into EVs [110].

Considering the strict association between plants and microorganisms, it is clear how plant breeding programs should be based on a holistic approach without overlooking microbiota. The genomic approaches are useful for understanding the plant-microbe interactions even if the need for more specific tools for deep knowledge is required. NGS technologies have stimulated research in meta-genomics and meta-transcriptomic fields [111], and the integration of these approaches allows gaining knowledge of microbial community (taxa composition, community structure), plant (genotype, metabolism), and environmental characteristics [112] to improve plant performances. Such approaches would significantly impact global food supplies by improving plant performance in a sustainable manner by reducing chemical supply, although this complex interaction requires modeling for improving predictable outcomes, which are still missing.

3. Conclusions

Although genetic technologies have undergone exponential progress in recent years, allowing the reduction of costs and time, these are limited in the allelopathic field, with respect to others, and the reasons for this could be the complexity of the phenomenon. Because allelopathy is a quantitative trait, any approach should consider the effect of the environment on this trait. The findings reported in this review can represent a good starting point to consolidate breeding strategies, considering, however, that much remains to be done to develop competitive crop varieties, thereby maintaining sustainable and environment-friendly agriculture. In this context, the continuous improvements in advanced technologies will make possible the identification of ever more precise genomic regions and specific genes that could have greater solidity in reproducibility. Furthermore, with the advent of new holistic metabolomic techniques, which will allow the identification of a broad spectrum of specialized metabolites, their intermediates, and the metabolic pathways involved in their biosynthesis, it will be possible to integrate these data with the genetic and phenotypic ones within the QTL analysis, leading to the elucidation of genes and pathways responsible for allelopathy activity and enabling the development of novel alleles to expand breeding programs for weed interference. Indeed, the cross-kingdom RNAi discovery open the door to developing plenty of effective breeding strategies, which will allow the control of plant rhizosphere microbiota in various crops and for many purposes, such as microbes allelochemicals production for weed control or pathogen immunity as described above. Finally, although the CRISPR/Cas9 was applied to plant genome editing for improving or studying many aspects, allelopathy has still not been the target of this technology, which could elucidate the precise mechanism for specific responses and/or molecular regulation, thereby opening new scenarios in this field.

References

1.　Einhellig, F.A. Mechanism of action of allelochemicals in allelopathy. In *Allelopathy: Organisms, Processes, and Applications*; Inderjit, K.M., Dakshini, M., Einhellig, F.A., Eds.; ACS Symposium Series 582; American Chemical Society: Washington, DC, USA, 1995; pp. 96–116.

2.　Latif, S.; Chiapusio, G.; Weston, L.A. Allelopathy and the role of allelochemicals in plant defence. *Adv. Bot. Res.* **2017**, *82*, 19–54.

3.　Molisch, H. *Der Einfluss einer Pflanze auf die Andere-Allelopathie*; Gustav Fisher Verlag: Jena, Germany, 1937.

4.　Rice, E.L. *Allelopathy*, 2nd ed.; Academic Press, Inc.: Orlando, FL, USA, 1984.

5.　Zhang, Z.; Liu, Y.; Yuan, L.; Weber, E.; van Kleunen, M. Effect of allelopathy on plant performance: A meta-analysis. *Ecol. Lett.* **2021**, *24*, 348–362. [CrossRef] [PubMed]

6.　Bais, H.P. We are family: Kin recognition in crop plants. *New Phytol.* **2018**, *220*, 357–359. [CrossRef] [PubMed]

7.　Biedrzycki, M.L.; Jalany, T.A.; Dudley, S.A.; Bais, H.P. Root exudates mediate kin recognition in plants. *Commun. Integr. Biol.* **2010**, *3*, 28–35. [CrossRef] [PubMed]

8.　Crepy, M.A.; Casal, J.J. Photoreceptor-mediated kin recognition in plants. *New Phytol.* **2015**, *205*, 329–338. [CrossRef] [PubMed]

9.　Durret, R.; Levin, S. Allelopathy in Spatially Distributed Populations. *J. Theor. Biol.* **1997**, *185*, 165–171. [CrossRef]

10.　Inderjit, K.; Callaway, R.M. Experimental designs for the study of allelopathy. *Plant Soil* **2003**, *256*, 1–11.

11.　Inoye, B.; Stinchcombe, J.R. Relationships between ecological interaction modifications and diffuse coevolution: Similarities, differences, and causal links. *Oikos* **2001**, *95*, 353–360. [CrossRef]

12.　Guo, L.; Qiu, J.; Li, L.-F.; Lu, B.; Olsen, K.; Fan, L. Genomic clues for crop-weed interactions and evolution. *Trends Plant Sci.* **2018**, *23*, 1102–1115.

13.　Del Moral, R.; Willis, R.J.; Ashton, D.H. Suppression of coastal heath vegetation by *Eucalyptus baxteri*. *Aust. J. Bot.* **1978**, *26*, 203–219. [CrossRef]

14.　Fischer, N.H.; Williamson, G.B.; Weidenhamer, J.D.; Richardson, D.R. In search of allelopathy in the Florida scrub: The role of terpenoids. *J. Chem. Ecol.* **1994**, *20*, 1355–1380. [PubMed]

15.　Katz, D.A.; Sneh, B.; Friedman, J. The allelopathic potential of *Coridothymus capitatus* L. (Labiatae). Preliminary studies on the roles of the shrub in the inhibition of annuals germination and/or to promote allelopathically active actinomycetes. *Plant Soil* **1987**, *98*, 53–66.

16.　Weidenhamer, J.D.; Romeo, J.T. Allelopathic properties of *Polygonella myriphylla*: Field evidence and bioassay. *J. Chem. Ecol.* **1989**, *15*, 1957–1970.

17.　Bais, H.P.; Vepachedu, R.; Gilroy, S.; Callaway, R.M.; Vivanco, J.M. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science* **2003**, *301*, 1377–1380. [PubMed]

18.　Schenk, H.J.; Callaway, R.M.; Mahall, B.E. Spatial Root Segregation: Are Plants Territorial? *Adv. Ecol. Res.* **1999**, *28*, 145–180.

19.　Rizvi, S.G.H.; Rizvi, V. *Allelopathy: Basic and Applied Aspects*; Chapman and Hall: London, UK, 1992.

20.　Bais, H.P.; Walker, T.S.; Stermitz, F.R.; Hufbauer, R.A.; Vivanco, J.M. Enantiomeric-dependent phytotoxic and antimicrobial activity of (±)-catechin. A rhizosecreted racemic mixture from spotted knapweed. *Plant Physiol.* **2002**, *128*, 1173–1179.

21.　Macías, F.A.; Mejías, F.J.R.; Molinillo, J.M.G. Recent advances in allelopathy for weed control: From knowledge to applications. *Pest. Manag. Sci.* **2019**, *75*, 2413–2436.

22.　Lupini, A.; Sorgonà, A.; Princi, M.P.; Sunseri, F.; Abenavoli, M.R. Morphological and physiological effects of trans-cinnamic acid and its hydroxylated derivatives on maize root types. *Plant Growth Regul.* **2016**, *78*, 263–273.

23.　Politycka, B. Free and glucosylated phenolics, phenol-beta-glucosyltransferase activity and membrane permeability in cucumber roots affected by derivatives of cinnamic and benzoic acid. *Acta Physiol. Plantarum* **1997**, *19*, 311–317.

24.　Cruz, O.R.; Anaya, A.L.; Hernandez-Bautista, B.E. Effects of allelochemical stress produced by sicyosdeppei on seedling root ultrastructure of *Phaseolous vulgaris* and *Cucubita ficifolia*. *J. Chem. Ecol.* **1998**, *24*, 2039–2057.

25.　Patterson, D.T. Effects of allelopathic chemicals on growth and physiological response of soybean (*Glycine max*). *Weed Sci.* **1981**, *29*, 53–58.

26.　Araniti, F.; Bruno, L.; Sunseri, F.; Pacenza, M.; Forgione, I.; Bitonti, M.B.; Abenavoli, M.R. The allelochemical farnesene affects Arabidopsis thaliana root meristem altering auxin distribution. *Plant Physiol. Bioch.* **2017**, *121*, 14–20.

27.　Yazaki, K.; Arimura, G.; Ohnishi, T. "Hidden" terpenoids in plants: Their biosynthesis, localization and ecological roles. *Plant Cell Physiol.* **2017**, *58*, 1615–1621.

28.　Gershenzon, J.; Dudareva, N. The function of terpene natural products in the natural world. *Nat. Chem. Biol.* **2007**, *3*, 408–414.

29.　Chou, C.C. Introduction to allelopathy. In *Allelopathy: A Physiological Process with Ecological Implications*; Reigosa, M.J., Pedrol, N., González, L., Eds.; Springer: Dordrecht, The Netherlands, 2006; pp. 1–9.

30.　Zimdahl, R.L. *Weed–Crop Competition: A Review*, 2nd ed.; Blackwell: Hoboken, NJ, USA, 2004.

31. MacLaren, C.; Storkey, J.; Menagat, A.; Metcalfe, H.; Dehnen-Schmutz, K. An ecological future for weed science to sustain crop production and the environment. A review. *Agron. Sustain. Dev.* **2020**, *40*, 24.

32. Wink, M. Biochemistry, physiology and ecological functions of secondary metabolites. In *Biochemistry of Plant Secondary Metabolism*, 2nd ed.; Wink, M., Ed.; Wiley-Blackwell: West Sussex, UK, 2010; pp. 1–19.

33. Boycheva, S.; Daviet, L.; Wolfender, J.; Fitzpatrick, T.B. The rise of operon-like gene clusters in plants. *Trends Plant Sci.* **2014**, *19*, 447–459.

34. Cheng, F.; Cheng, Z.H.; Meng, H.W. Transcriptomic insights into the allelopathic effects of the garlic allelochemical diallyl disulfide on tomato roots. *Sci. Rep.* **2016**, *6*, 38902.

35. Fang, C.; Yang, L.; Chen, W.; Li, L.; Zhang, P.; Li, Y.; He, H.; Lin, W. MYB57 transcriptionally regulates MAPK11 to interact with PAL2;3 and modulate rice allelopathy. *J. Exp. Bot.* **2020**, *71*, 2127–2142.

36. Zhang, H.; Rutherford, S.; Qi, S.; Huang, P.; Dai, Z. Transcriptome profiling of Arabidopsis thaliana roots in response to allelopathic effects of Conyza canadensis. *Ecotoxicology* **2022**, *31*, 53–63.

37. Duke, S.O.; Baerson, S.R.; Pan, Z.; Kagan, I.A.; Sanchez-Moreiras, A.; Reigosa, M.J.; Pedrol, N.; Schulz, M. Genomic Approaches to Understanding Allelochemical Effects on Plants. In *Allelopathy in Sustainable Agriculture and Forestry*; Zeng, R.S., Mallik, A.U., Luo, S.M., Eds.; Springer: New York, NY, USA, 2008.

38. Yuan, Y.; Lee, H.T.; Hu, H.; Scheben, A.; Edwards, D. Single-Cell genomics analysis in plants. *Genes* **2018**, *9*, 50.

39. He, H.Q.; Lin, W.X.; Liang, Y.Y.; Song, B.Q.; Ke, Y.Q.; Guo, Y.C.; Liang, K.J. Analyzing the molecular mechanism of crop allelopathy by using differential proteomics. *Acta Ecol. Sin.* **2005**, *25*, 3141–3146.

40. Chi, W.-C.; Chen, Y.-A.; Hsiung, Y.-C.; Fu, S.-F.; Chou, C.-H.; Trinh, N.N.; Chen, Y.-C.; Huang, H.-J. Autotoxicity mechanism of Oryza sativa: Transcriptome response in rice roots exposed to ferulic acid. *BMC Genom.* **2013**, *14*, 351.

41. Zhang, Q.; Zheng, X.-Y.; Lin, S.-X.; Gu, C.-Z.; Li, L.; Li, J.-Y.; Fang, C.-X.; He, H.-B. Transcriptome analysis reveals that barnyard grass exudates increase the allelopathic potential of allelopathic and non-allelopathic rice (*Oryza sativa*) accessions. *Rice* **2019**, *12*, 30. [CrossRef]

42. Li, L.-L.; Zhao, H.-H.; Kong, C.-H. (-)-Loliolide, the most ubiquitous lactone, is involved in barnyard grass-induced rice allelopathy. *J. Exp. Bot.* **2020**, *71*, 1540–1550.

43. Yang, Y.; Zhang, Z.; Li, R.; Yi, Y.; Yang, H.; Wang, C.; Wang, Z.; Liu, Y. RgC3H involves in the biosynthesis of allelopathic phenolic acids and alters their release amount in *Rehmannia glutinosa* roots. *Plants* **2020**, *9*, 567. [CrossRef] [PubMed]

44. Horvath, D.P.; Hansen, S.A.; Moriles-Miller, J.P.; Pierik, R.; Yan, C.; Clay, D.E.; Scheffler, B.; Clay, S.A. RNAseq reveals weed-induced PIF3-like as candidate target to manipulate weed stress response in soybean. *New Phytol.* **2015**, *207*, 196–210. [CrossRef] [PubMed]

45. Chung, I.-M.; Ham, T.-H.; Cho, G.-W.; Kwon, S.-W.; Lee, Y.; Seo, J.; An, Y.-J.; Kim, S.-Y.; Lee, J. Study of quantitative trait loci (QTLs) associated with allelopathic trait in rice. *Genes* **2020**, *11*, 479. [CrossRef]

46. Zhang, Q.; Zhang, Q.; Lin, S.; Wang, P.; Li, J.; Wang, H.; He, H. Dynamic analysis on weed inhibition and phenolic acids of allelopathic rice in field test. *Arch. Agron. Soil Sci.* **2020**, *67*, 1809–1821. [CrossRef]

47. Wu, H.; Pratley, J.; Ma, W.; Haig, T. Quantitative trait loci and molecular markers associated with wheat allelopathy. *Theor. Appl. Genet.* **2003**, *107*, 1477–1481. [CrossRef]

48. Zeng, D.; Qian, Q.; Teng, S.; Dong, G.; Fujimoto, H.; Yasufumi, K.; Zhu, L. Genetic analysis of rice allelopathy. *Chin. Sci. Bull.* **2003**, *48*, 265–268. [CrossRef]

49. Quader, M.; Daggard, G.; Barrow, R.; Walker, S.; Sutherland, M.W. Allelopathy, Dimboa production and genetic variability in accessions of Triticum speltoides. *J. Chem. Ecol.* **2001**, *27*, 747–760. [CrossRef] [PubMed]

50. Fang, C.; Zhuang, Y.; Xu, T.; Li, Y.; Li, Y.; Lin, W. Changes in rice allelopathy and rhizosphere microflora by inhibiting rice phenylalanine ammonialyase gene expression. *J. Chem. Ecol.* **2013**, *39*, 204–212. [CrossRef] [PubMed]

51. Xu, M.; Galhano, R.; Wiemann, P.; Bueno, E.; Tiernan, M.; Wu, W.; Peters, R.J. Genetic evidence for natural product-mediated plant-plant allelopathy in rice (*Oryza sativa*). *New Phytol.* **2012**, *193*, 570–575. [CrossRef] [PubMed]

52. Zhang, Q.; Lin, S.; Zhang, Q.; Wang, P.; Wang, H.; He, H. Comparative analysis on metabolites and gene expression difference of various allelopathic potential rice under weed stress. *Weed Res.* **2022**, *in press*. [CrossRef]

53. Shehzad, T.; Okuno, K. Genetic analysis of QTLs controlling allelopathic characteristics in sorghum. *PLoS ONE* **2020**, *15*, e0235896. [CrossRef]

54. Sultana, M.H.; Liu, F.; Alamin, M.; Mao, L.; Jia, L.; Chen, H.; Wu, D.; Wang, Y.; Fu, F.; Wu, S.; et al. Gene modules co-regulated with biosynthetic gene custers for allelopathy between rice and bernyargrass. *Int. J. Mol. Sci.* **2019**, *20*, 3846. [CrossRef]

55. Li, J.; Chen, L.; Chen, Q.; Miao, Y.; Peng, Z.; Huang, B.; Guo, L.; Liu, D.; Du, H. Allelopathic effect of *Artemisia argyi* on the germination and growth of various weeds. *Sci. Rep.* **2021**, *11*, 4303. [CrossRef]

56. Guo, L.; Qiu, J.; Ye, C.; Jin, G.; Mao, L.; Zhang, H.; Yang, X.; Peng, Q.; Wang, Y.; Jia, L.; et al. *Echinochloa crus-galli* genome analysis provides insight into its adaptation and invasiveness as a weed. *Nat. Commun.* **2017**, *8*, 1031. [CrossRef]

57. Gierl, A.; Frey, M. Evolution of benzoxazinone biosynthesis and indole production in maize. *Planta* **2001**, *213*, 493–498. [CrossRef]

58. Frey, M.; Chomet, P.; Glawischnig, E.; Stettner, C.; Grün, S.; Winklmair, A.; Eisenreich, W.; Bacher, A.; Meerley, R.B.; Briggs, S.P.; et al. Analysis of a chemical plant defense mechanism in grasses. *Science* **1997**, *277*, 696–699. [CrossRef]

59. Song, B.; Xiong, J.; Fang, C.; Qiu, L.; Lin, R.; Liang, Y.; Lin, W. Allelopathic enhancement and differential gene expression in rice under low nitrogen treatment. *J. Chem. Ecol.* **2008**, *34*, 688–695. [CrossRef] [PubMed]

60. Fang, C.X.; Xiong, J.; Qiu, L.; Wang, H.B.; Song, B.Q.; He, H.B.; Lin, R.Y.; Lin, W.X. Analysis of gene expressions associated with increased allelopathy in rice (*Oryza sativa* L.) induced by exogenous salicylic acid. *Plant Growth Regul.* **2009**, *57*, 163–172. [CrossRef]

61. Fang, C.; Li, Y.; Li, C.; Li, B.; Ren, Y.; Zheng, H.; Zeng, X.; Shen, L.; Lin, W. Identification and comparative analysis of microRNAs in barnyard grass (*Echinochloa crus-galli*) in response to rice allelopathy. *Plant Cell Environ.* **2015**, *38*, 1368–1381. [CrossRef]

62. Dixon, R.A.; Paiva, N.L. Stress-induced phenylpropanoid metabolism. *Plant Cell* **1995**, *7*, 1085–1097. [CrossRef] [PubMed]

63. He, H.B.; Wang, H.B.; Fang, C.X.; Wu, H.; Guo, X.K.; Lin, Z.H.; Lin, W.X. Barnyard grass stress up regulates the biosynthesis of phenolic compounds in allelopathic rice. *J. Plant Physiol.* **2012**, *169*, 1747–1753. [CrossRef] [PubMed]

64. Field, B.; Jordan, F.; Osbourn, A. First encounters—Deployment of defence related natural products by plants. *New Phytol.* **2006**, *172*, 193–207. [CrossRef]

65. Kato, T.; Kabuto, C.; Sasaki, N.; Tsunagawa, M.; Aizawa, H.; Fujita, K.; Kato, Y.; Kitahara, Y.; Takahashi, N. Momilactones, growth inhibitors from rice, *Oryza sativa* L. *Tetrahedron Lett.* **1973**, *14*, 3861–3864. [CrossRef]

66. Takahashi, N.; Kato, T.; Tsunagawa, M.; Sasaki, N.; Kitahara, Y. Mechanisms of dormancy in rice seeds: II. New growth inhibitors, momilactone-A and -B isolated from the hulls of rice seeds. *Jpn. J. Breed.* **1976**, *26*, 91–98. [CrossRef]

67. Kato-Noguchi, H.; Peters, R.J. The role of momilactones in rice allelopathy. *J. Chem. Ecol.* **2013**, *39*, 175–185. [CrossRef]

68. Serra Serra, N.; Shanmuganathan, R.; Becker, C. Allelopathy in rice: A story of momilactones, kin recognition, and weed management. *J. Exp. Bot.* **2021**, *72*, 4022–4037. [CrossRef]

69. Peters, R.J. Uncovering the complex metabolic network underlying diterpenoid phytoalexin biosynthesis in rice and other cereal crop plants. *Phytochemistry* **2006**, *67*, 2307–2317. [CrossRef] [PubMed]

70. Toyomasu, T. Recent advances regarding diterpene cyclase genes in higher plants and fungi. *Biosci. Biotechnol. Biochem.* **2008**, *72*, 1168–1175. [CrossRef] [PubMed]

71. Zhang, J.; Li, C.; Wu, C.; Xiong, L.; Chen, G.; Zhang, Q.; Wang, S. RMD: A rice mutant database for functional analysis of the rice genome. *Nucl. Acids Res.* **2006**, *34*, D745–D748. [CrossRef] [PubMed]

72. Jeon, J.S.; Lee, S.; Jung, K.H.; Jun, S.H.; Jeong, D.H.; Lee, J.; Kim, C.; Jang, S.; Yang, K.; Nam, J.; et al. T-DNA insertional mutagenesis for functional genomics in rice. *Plant J.* **2000**, *22*, 561–570. [CrossRef]

73. Sanger, F.; Nicklen, S.; Coulson, A.R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5463–5467. [CrossRef] [PubMed]

74. Afzal, F.; Gul, A.; Kazi, A.M. Next-generation sequencing technologies and plant improvement. In *Plant Omics: Trends and Applications*, 1st ed.; Hakeem, K.R., Tombuloğlu, H., Tombuloğlu, G., Eds.; Springer International Publishing: Cham, Switzerland, 2016; pp. 271–294.

75. Maxam, A.M.; Gilbert, W. A new method for sequencing DNA. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 560–564. [CrossRef]

76. Morozova, O.; Marra, M.A. Applications of next-generation sequencing technologies in functional genomics. *Genomics* **2008**, *92*, 255–264. [CrossRef]

77. Schuster, S.C. Next-generation sequencing transforms today's biology. *Nat. Methods* **2008**, *5*, 16–18. [CrossRef]

78. Margulies, M.; Egholm, M.; Altman, W.E.; Attiya, S.; Bader, J.S.; Bemben, L.A.; Berka, J.; Braverman, M.S.; Chen, Y.-J.; Chen, Z.; et al. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **2006**, *437*, 376–380. [CrossRef]

79. Zhang, Q.; Li, L.; Li, J.; Wang, H.; Fang, C.; Yang, X.; He, H. Increasing rice allelopathy by induction of barnyard grass (Echinochloa crus-galli) root exudates. *J. Plant Growth Regul.* **2018**, *37*, 745–754. [CrossRef]

80. Chen, L.; Li, J.; Zhu, Y.; Guo, L.; Ji, R.; Miao, Y.; Guo, L.; Du, H.; Liu, D. Caffeic Acid, an Allelochemical in *Artemisia argyi*, Inhibits Weed Growth via Suppression of Mitogen-Activated Protein Kinase Signaling Pathway and the Biosynthesis of Gibberellin and Phytoalexin. *Front. Plant Sci.* **2021**, *12*, 802198. [CrossRef] [PubMed]

81. Asins, M.J.; Bernet, G.P.; Villalta, I.; Carbonell, E.A. QTL Analysis in Plant Breeding. In *Molecular Techniques in Crop Improvement*; Jain, S., Brar, D., Eds.; Springer: Dordrecht, The Netherlands, 2010.

82. Olofsdotter, M.; Jensen, L.B.; Courtois, B. Improving crop competitive ability using allelopathy—An example from rice. *Plant Breed.* **2002**, *121*, 1–9. [CrossRef]

83. Niemeyer, H.M.; Jerez, J.M. Chromosomal location of genes for hydroxamic acid accumulation in wheat using wheat aneuploids and wheat substitution lines. *Heredity* **1997**, *79*, 10–14. [CrossRef]

84. Ebana, K.; Yan, W.; Dilday, R.H.; Namai, H.; Okuno, K. Analysis of QTL Associated with the Allelopathic Effect of Rice Using Water-soluble Extracts. *Breed. Sci.* **2001**, *51*, 47–51. [CrossRef]

85. Bharamappanavara, M.; Siddaiah, A.M.; Ponnuvel, S.; Ramappa, L.; Patil, B.; Appaiah, M.; Maganti, S.M.; Sundaram, R.M.; Kadadanamari, S.; Tuti, M.D.; et al. Mapping QTL hotspots associated with weed competitive traits in backcross population derived from *Oryza sativa* L. *and O. glaberrima* Steud. *Sci. Rep.* **2020**, *10*, 22103. [CrossRef]

86. Fierer, N. Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* **2017**, *15*, 579–590. [CrossRef] [PubMed]

87. Müller, H.; Berg, C.; Landa, B.B.; Auerbach, A.; Moissl-Eichinger, C.; Berg, G. Plant genotype-specific archaeal and bacterial endophytes but similar Bacillus antagonists colonize mediterranean olive trees. *Front. Microbiol.* **2015**, *6*, 138. [CrossRef] [PubMed]

88. Frąc, M.; Hannula, S.E.; Bełka, M.; Jędryczka, M. Fungal Biodiversity and Their Role in Soil Health. *Front. Microbiol.* **2018**, *9*, 707. [CrossRef]

89. Barrera, S.E.; Sarango-Flóres, S.W.; Montenegro-Gómez, S.P. The phyllosphere microbiome and its potential application in horticultural crops. A review. *Rev. Colomb. Cienc. Hortic.* **2019**, *13*, 384–396. [CrossRef]

90. Ding, L.-J.; Cui, H.-L.; Nie, S.-A.; Long, X.-E.; Duan, G.-L.; Zhu, Y.-G. Microbiomes inhabiting rice roots and rhizosphere. *FEMS Microbiol. Ecol.* **2019**, *95*, fiz040. [CrossRef]

91. Lundberg, D.S.; Teixeira, P.J. Root-exuded coumarin shapes the root microbiome. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 5629–5631. [CrossRef] [PubMed]

92. Stringlis, I.A.; Yu, K.; Feussner, K.; de Jonge, R.; Van Bentum, S.; Van Verk, M.C.; Berendsen, R.; Bakker, P.A.H.M.; Feussner, I.; Pieterse, C.M.J. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E5213–E5222. [CrossRef] [PubMed]

93. Li, Y.; Jian, X.; Li, Y.; Zeng, X.; Xu, L.; Khan, M.U.; Lin, W. OsPAL2-1 Mediates Allelopathic Interactions Between Rice and Specific Microorganisms in the Rhizosphere Ecosystem. *Front. Microbiol.* **2020**, *23*, 1411. [CrossRef]

94. Escudero-Martinez, C.; Coulter, M.; Alegria Terrazas, R.; Foito, A.; Kapadia, R.; Pietrangelo, L.; Maver, M.; Sharma, R.; Aprile, A.; Morris, J.; et al. Identifying plant genes shaping microbiota composition in the barley rhizosphere. *Nat. Commun.* **2022**, *13*, 3443. [CrossRef] [PubMed]

95. Müller, C.A.; Obermeier, M.M.; Berg, G. Bioprospecting plant-associated microbiomes. *J. Biotechnol.* **2016**, *235*, 171–180. [CrossRef] [PubMed]

96. Egamberdieva, D.; Wirth, S.; Alqarawi, A.A.; Elsayed, F.A.A.; Hashem, A. Phytohormones and Beneficial Microbes: Essential Components for Plants to Balance Stress and Fitness. *Front. Microbiol.* **2017**, *8*, 2104. [CrossRef]

97. Ho, N.-G.; Mathew, D.C.; Huang, C.-C. Plant-Microbe Ecology: Interactions of Plants and Symbiotic Microbial Communities. In *Plant Ecology-Traditional Approaches to Recent Trends*; IntechOpen: London, UK, 2017.

98. Yi, H.-S.; Yang, J.W.; Ryu, C.-M. ISR meets SAR outside: Additive action of the endophyte Bacillus pumilus INR7 and the chemical inducer, benzothiadiazole, on induced resistance against bacterial spot in field-grown pepper. *Front. Plant Sci.* **2013**, *4*, 122. [CrossRef]

99. Abbas, T.; Zahir, Z.A.; Naveed, M.; Abbas, S.; Alwahibi, M.S.; Elshikh, M.S.; Adnan, M. Large Scale Screening of Rhizospheric Allelopathic Bacteria and Their Potential for the Biocontrol of Wheat-Associated Weeds. *Agronomy* **2020**, *10*, 1469. [CrossRef]

100. Lee, S.; Behringer, G.; Hung, R.; Bennett, J. Effects of fungal volatile organic compounds on *Arabidopsis thaliana* growth and gene expression. *Fungal Ecol.* **2019**, *37*, 1–9. [CrossRef]

101. Gao, Y.; Feng, J.; Wu, J.; Wang, K.; Wu, S.; Liu, H.; Jiang, M. Transcriptome analysis of the growth-promoting effect of volatile organic compounds produced by *Microbacterium aurantiacum* GX14001 on tobacco (*Nicotiana benthamiana*). *BMC Plant Biol.* **2022**, *22*, 208. [CrossRef]

102. Berendsen, R.L.; Pieterse, C.M.J.; Bakker, P.A.H.M. The rhizosphere microbiome and plant health. *Trends Plant Sci.* **2012**, *17*, 478–486. [CrossRef] [PubMed]

103. Rout, M.E.; Southworth, D. The root microbiome influences scales from molecules to ecosystems: The unseen majority. *Am. J. Bot.* **2013**, *100*, 1689–1691. [CrossRef]

104. Bever, J.D. Feedback between plants and their soil communities in an old field community. *Ecology* **1994**, *75*, 1965–1977. [CrossRef]

105. Baldrich, P.; Rutter, B.D.; Karimi, H.Z.; Podicheti, R.; Meyers, B.C.; Innes, R.W. Plant extracellular vesicles contain diverse small RNA species and are enriched in 10- to 17-nucleotide "tiny" RNAs. *Plant Cell* **2019**, *31*, 315–324. [CrossRef] [PubMed]

106. Middleton, H.; Yergeau, E.; Monard, C.; Combier, J.P.; El Amrani, A. Rhizospheric plant-microbe interactions: miRNAs as a key mediator. *Trends Plant Sci.* **2021**, *26*, 132–141. [CrossRef]

107. Zhang, T.; Zhao, Y.-L.; Zhao, J.-H.; Wang, S.; Jin, Y.; Chen, Z.-Q.; Fang, Y.-Y.; Hua, C.-L.; Ding, S.-W.; Guo, H.-S. Cotton plants export microRNAs to inhibit virulence gene expression in a fungal pathogen. *Nat. Plants* **2016**, *2*, 16153. [CrossRef]

108. Wang, M.; Weiberg, A.; Dellota, E., Jr.; Yamane, D.; Jin, H. Botrytis small RNA Bc -siR37 suppresses plant defense genes by cross-kingdom RNAi. *RNA Biol.* **2017**, *14*, 421–428. [CrossRef] [PubMed]

109. Cai, Q.; Qiao, L.; Wang, M.; He, B.; Lin, F.-M.; Palmquist, J.; Huang, S.-D.; Jin, H. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Science* **2018**, *360*, 1126–1129. [CrossRef]

110. He, B.; Cai, Q.; Qiao, L.; Huang, C.-Y.; Wang, S.; Miao, W.; Ha, T.; Wang, Y.; Jin, H. RNA-binding proteins contribute to small RNA loading in plant extracellular vesicles. *Nat. Plants* **2021**, *7*, 342–352. [CrossRef]

111. Knief, C. Analysis of plant microbe interactions in the era of next generation sequencing technologies. *Front. Plant Sci.* **2014**, *5*, 216. [CrossRef]

112. Bringel, F.; Couée, I. Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Front. Microbiol.* **2015**, *6*, 486. [CrossRef] [PubMed]