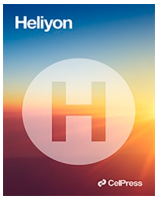
Heliyon 10 (2024) e40834

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

Unveiling the wheat-rust battleground: A transcriptomic journey

K.K. Chetan, Vaibhav Kumar Singh **, Mohammad Waris Haider, Mahender Singh Saharan, Ravinder Kumar *

Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India

ARTICLE INFO

Keywords:
Wheat
Rust
Resistance
Transcriptomics
Wheat-*puccinia* interaction

ABSTRACT

The global wheat production faces significant challenges due to major rust-causing fungi, namely *Puccinia striiformis* f. sp. *tritici*, *P. triticina*, and *P. graminis* f. sp. *tritici*, responsible for stripe, leaf, and stem rust diseases, respectively. The evolutionary relationship between wheat (host) and *Puccinia* (pathogen) renders existing wheat resistance ineffective over time. The most viable solution to this issue lies in the development of new resistant wheat varieties. However, achieving this requires a comprehensive understanding of wheat's defense mechanisms against ever-evolving pathogens. Transcriptomics emerges as a powerful tool for analyzing gene activity at the molecular level. Over the last decade, this technique has transformed our comprehension of the wheat-rust interaction. Transcriptomics has unveiled a compelling "biphasic model" of gene expression in wheat infected with rust fungi, delineating two distinct phases of defense activation. Moreover, it has illuminated the intricate signaling pathways, hormonal interactions, and diverse defense mechanisms employed by wheat. These mechanisms encompass the oxidative burst, reinforcement of cell walls, and controlled cessation of photosynthesis, all aimed at combatting the invading pathogen. However, the utility of transcriptomics extends beyond elucidating defense strategies; it enables the identification of novel genes linked to resistance or susceptibility. By unraveling the functions of these genes, researchers can uncover new avenues for breeding resistant wheat varieties, arming wheat with the molecular arsenal necessary to prevail in the ongoing battle against rust fungi. This review represents a pioneering effort in exploring transcriptomic techniques and accumulated data to present a comprehensive overview of the wheat-Puccinia interaction at the system-wide level.

1. Introduction

In wheat *Puccinia striiformis* f. sp. *tritici* (*Pst*), *Puccinia triticina* (*Pt*) and *Puccinia graminis* f. sp. *tritici* (*Pgt*) are the major rust pathogens causing stripe (yellow), leaf (brown) and stem (black) rust, respectively and responsible for frequent and substantial yield losses in wheat globally [1–3]. All the three rust fungi are obligate parasites, which can cause up to 60 percent loss in yield for leaf or stripe rust and up to 100 per cent loss for stem rust [4,5]. Wheat being a major staple food crop worldwide, consumed by 30 % of the global population [6]. It is a vital source of nutrition for humankind and world most traded crop in terms of both quantity of land required and quality of protein and calories, accounting for almost 20 % of the world total energy intake [7]. Since wheat was among the first crops to be domesticated, it has undergone over 10,000 years of selection and breeding advancements [8]. Even with a lengthy history of

* Corresponding author.
** Corresponding author.
E-mail addresses: dr.singhvaibhav@gmail.com (V.K. Singh), chauhanravinder97@gmail.com (R. Kumar).

https://doi.org/10.1016/j.heliyon.2024.e40834
Received 17 May 2024; Received in revised form 27 November 2024; Accepted 28 November 2024
Available online 29 November 2024

breeding and agronomic practices, there are still many scientific and technological obstacles in breeding rust resistant wheat cultivars in the way of meeting the need for efficient wheat production.

Plant resistance (R) genes and complementing pathogen avirulence (Avr) genes interact gene for gene to govern resistance. A hypersensitive response (localised form of programmed cell death) and systemic acquired resistance (activating defence responses in uninfected parts) are the host-resistant reactions that are triggered as a result of Avr-R interactions. Genetic resistance has been identified as the most practical, cost-effective, long-term solution for reducing wheat rust outbreaks among the available techniques. But wheat cultivars frequently lose their intrinsic resistance to rust after just three to five years of field usage due to high genetic diversity of *Puccinia* that forms multiple races with varied degrees of pathogenicity [9] and this enhanced virulence undermines already-existing plant resistance [10]. Therefore, it is crucial to comprehend the wheat defence response mechanism against the rust pathogen in order to produce innovative resistant cultivars by artificial breeding. Plants, unlike animals, lack an adaptive immune system. However, they have evolved a sophisticated defense system to combat pathogen infection. This defense system relies on a complex interplay between genes and their products. When a plant detects a pathogen, a cascade of signalling events is triggered. These signaling events lead to the upregulation of a large number of genes. The upregulated genes encode proteins that play a variety of roles in plant defense, including: pattern recognition receptors (PRRs), signal transduction proteins, pathogenesis-related (PR) proteins, transcription factors (TFs) and hormone biosynthesis genes [11].

These intricate interactions between wheat and *Puccinia* cannot be fully understood by the conventional biochemical and genetic experimental approaches. A potent method for identifying differentially expressed genes (DEGs) connected to virulence factors and regulatory processes that drive fungal disease as well as the host defence systems to repel the invasion is to monitor infection-linked transcriptome modifications [12]. One of the earliest approaches was microarray technology to quantify transcript abundance. The arrival of RNA sequencing (RNA-Seq) using next generation sequencing techniques transformed gene expression studies [13]. Over the past ten years, transcriptome approaches have been increasingly valuable in unlocking the fundamental puzzles surrounding the wheat-rust interaction. Currently, transcriptomics is experiencing a golden age as researchers use it to effectively study interactions between plants and pathogens all over the world [12]. Hence, this review is devoted to summarise the system level information that transcriptomics revealed about the wheat-*Puccinia* relationship.

2. Wheat-*Puccinia* interactions

2.1. *Puccinia* as a fungal pathogen

Puccinia infection starts when uredospore comes in contact with a film of water (Fig. 1A), absorb water, engorge (Fig. 1B) and develop germ tubes (Fig. 1C) [14]. Thigmotropic response regulates the formation of germ tubes, which grows on the leaf surface until a stoma is encountered for penetration [15]. At the stomatal aperture, protoplasm flows in the direction of the tip to create an

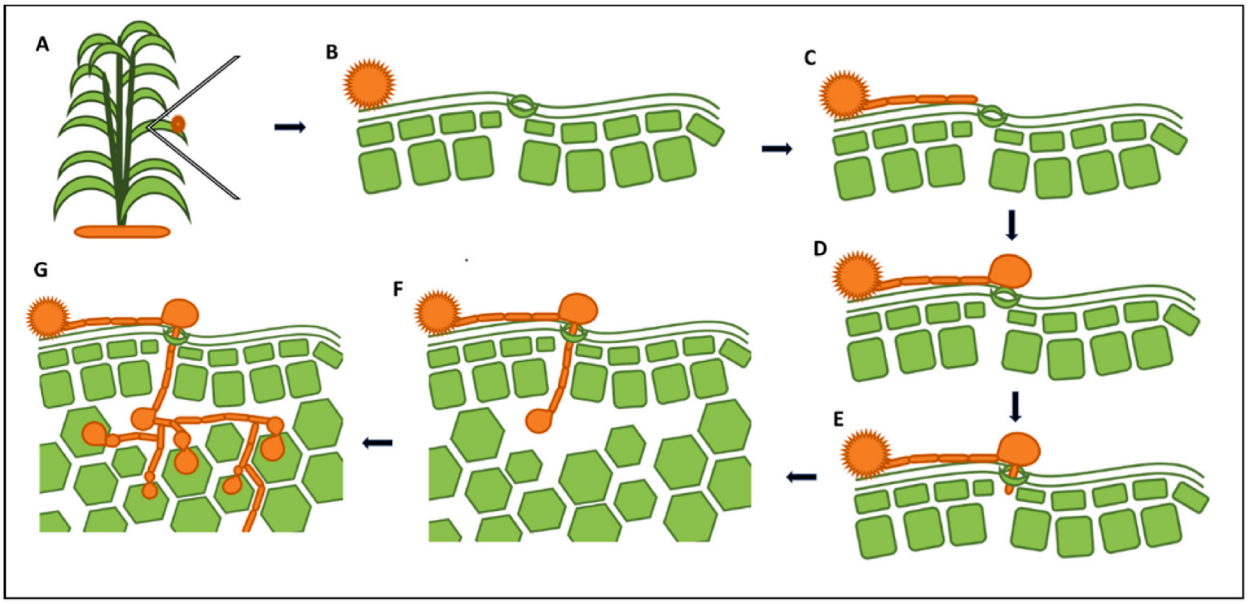


Fig. 1. Schematic representation of infection process *Puccinia* infection on wheat. A) The dikaryotic uredospore lands on the wheat leaf surface. B) The spore enlarges by absorbing dew on the leaf surface C) Later, germinates to produce a germination tube and elongates to reach stomatal aperture. D) Near stomata, the tip of the germination tube enlarges to form an appressorium. E) It enters the leaf interior by forming infection thread through the stoma F) Then, it differentiates into a substomatal vesicle. G) From there, it forms primary infection hyphae and proliferate intercellularly and forms haustorial mother cell which penetrates mesophyll cell wall to form the haustorium.

appressorium that covers the opening (Fig. 1D) [14]. A penetration peg starting from the appressorium pushes *via* stoma (Fig. 1E). Then, the fungus develops a substomatal vesicle in intercellular space and an infection hypha begins to grow inward from the substomatal vesicle towards the mesophyll cells (Fig. 1F) [16]. A septum arises behind the tip to separate the haustorial mother cell upon contact with a mesophyll cell. As seen in Fig. 1G, host cell penetration begins with the establishment of a penetration peg within the host and the haustorial mother cell. This is followed by the formation of a haustorium (fungal feeding structure) inside the host cell. Further, an extra-haustorial membrane (host plasma membrane derivative) firmly envelops the haustorium that keeps the haustoria isolated from the host cytoplasm even after the host cell wall is damaged [17]. The extra-haustorial membrane is believed to be the interface where the fungal modification of host cell metabolism to establish and maintain parasitic relationship with host [18,19].

2.2. *Wheat as a host plant*

In turn, host plants have evolved sophisticated defense mechanisms to combat pathogen invasion, including blocking the pathogen entry and activating various defense responses [20]. This defense mechanism can produce cytoplasmic, cellular, or histological structures and biochemical responses. The plant immune response consists of two arms that activate the induced defence: PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) [21]. In PTI, pathogen-associated molecular patterns (PAMPs) are recognised by cell membrane-based pattern recognition receptors (PRRs) [22]. Conversely, ETI acts within the cytoplasm, where the effector molecule is identified by the host plant cell R gene protein. This recognition sets off signalling pathways that cause the host cell to develop hypersensitive response (HR) and initiates systemic acquired resistance (SAR) [23]. Furthermore, SAR induces several signalling pathways including mitogen activated protein kinase (MAPK), calcium dependent protein kinase (CDPK), salicylic acid (SA), jasmonic acid (JA), ethylene, abscisic acid (ABA) which elicit various plant defense-related mechanisms such as oxidative burst with rapid increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) production that results in HR, cell wall fortification by lignin and callose deposition and release of various defense-related phenolics such as phytoalexin, phenolic metabolism enzymes and PR proteins [11]. The synchronistic nature between host and pathogen side by side suggests that both have been evolving together.

3. Transcriptomics for wheat crop development

Quantifying the level of gene expression under various experimental settings and identifying variations in the expression of transcripts, including mRNAs, small RNAs and non-coding RNAs are the primary goals of transcriptomics. It can be classified into three primary kinds based on the methodologies employed to identify multiple gene transcriptional changes: hybridization-based, sequencing-based, and polymerase chain reaction (PCR)-based techniques. Among the earliest approaches, differential displayed-reverse transcriptase PCR (DD-RTPCR) and cDNA amplified fragment length polymorphism (cDNA-AFLP) were used to quantify the transcript abundance. The introduction of microarray technology revolutionised the field of gene expression investigations, but it was

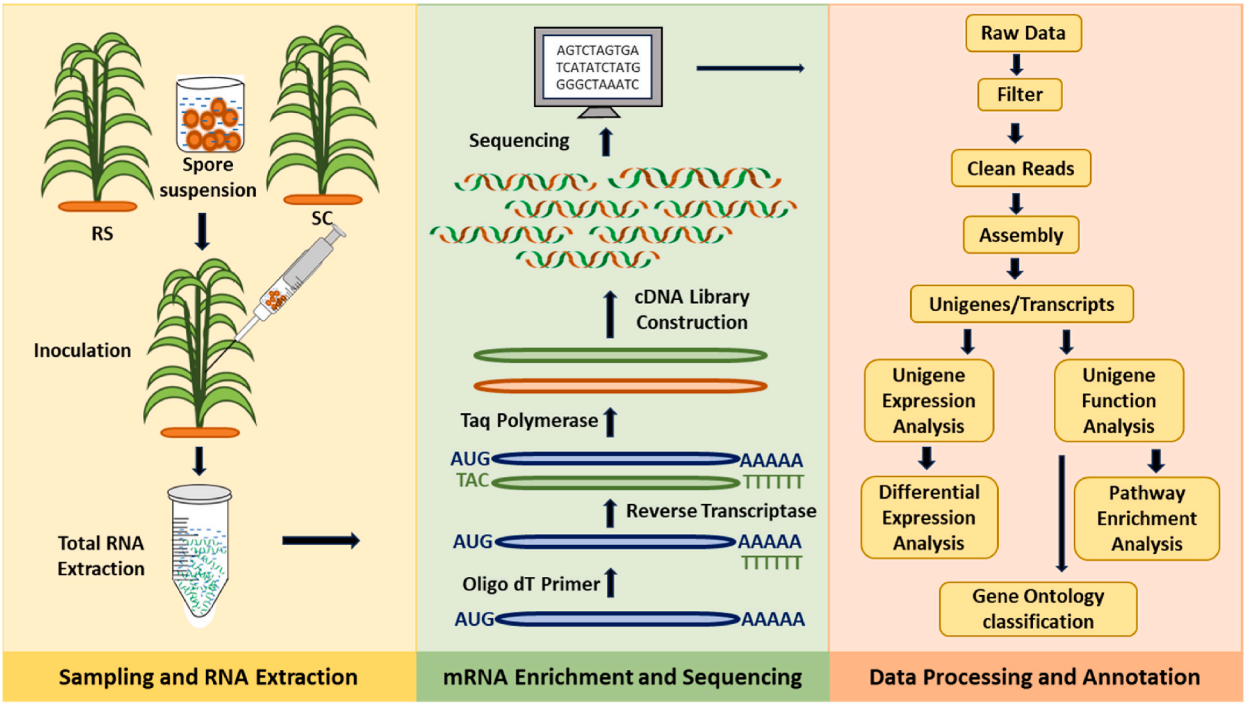


Fig. 2. Schematic representation of steps involved in transcriptomic studies by RNA Seq.

later surpassed by RNA sequencing (RNA-Seq) whose experimental methodology is shown in Fig. 2. Apart from the enhanced sensitivity, exploration of novel transcripts, non-coding RNA and alternative splicing studies made possible by high-throughput sequencing. Simultaneous investigation on host and pathogen transcriptome research is now possible because of the introduction of dual RNA-Seq. Recently, single-cell RNA-Seq has enabled sensitive and accurate transcriptional profiling at single-cell and single-nucleotide resolution, revolutionising transcriptome analysis. The advantages and disadvantages associated with these techniques were summarised in Table 1.

In the last decade, transcriptome techniques emerged and played a significant role in unlocking the fundamental secrets of wheat-rust interaction. Among these [24], conducted a gene expression profiling study using DD-RT PCR to determine the wheat resistance mechanism against yellow rust, where they cloned and sequenced 39 bands that were differently expressed out of 60 bands and quantitative real-time PCR (qRT-PCR) further validates those genes expression. Similarly, Jha [25] used DD-PCR to unravel the genetic mechanism of *Lr*28-based resistance to leaf rust in wheat. A similar kind of study by Wang et al. [26] used cDNA-AFLP technique that identified 2437 transcript-derived fragments (TDFs) with altered expression patterns in resistant wheat cultivar against *Pst*. Initially, the application of cDNA-AFLP was limited in wheat transcriptome analysis. However, with the introduction of an Affymetrix wheat gene chip, this technique is now a potent tool for analysing the wheat transcriptome. Recently, a comparative transcriptomic study using wheat gene chip in yellow rust inoculated near isogenic lines (NIL) for *Yr*5 identified 61 HR-specific transcripts, 54 basal defense transcripts and 19 biotrophic interaction specific transcripts to be induced in an incompatible interaction [27]. Similar to this, Fofana et al. [28] used cDNA microarray to study time-frame-based gene expression profiling of *Pt* infected wheat, demonstrating variations in defence pathways in *Lr*1 NILs. In case of next-generation sequencing (NGS) platforms, Illumina technology is gaining popularity for transcriptome studies due to its power of vast read depth and pair read technology. Deep sequence coverage has a significant role in gene discovery and gene expression analysis [29]. Liu et al. [30] reported a significant change in the differentially expressed genes (DEGs) in wheat inoculated with *Pst* using Illumina HiSeq platform and found to have 13892 DEGs at 24 h post-inoculation (hpi), 10195 DEGs at 48 hpi and 12268 DEGs at 72 hpi compared to 0 hpi. More recently, with a focus on the small RNA fraction from wheat plants infected with the wheat stem rust fungus, Mueth and Hulbert [31] conducted dual host-pathogen small RNA sequencing where they created small RNA libraries from infected and mock-infected plant tissue and sequenced using Ion Torrent platform.

4. Insights from transcriptomic research in wheat

Generally, infective uredospores germinate on wheat leaf surface during the invasion phase, forming a germ tube within 12 hpi [43]. After the germ tube penetrates the stoma, a substomatal vesicle is produced within 8–12 hpi, and by 16 hpi, infection hyphae and haustorial development have taken place in both compatible and incompatible interactions [44]. However, there were significant variations in fungal growth and development in susceptible and resistant cultivars after fungal penetration (16–24 hpi). The host tissues of susceptible cultivars were quickly colonized by *Puccinia* intercellularly, and a large number of haustoria developed in the neighbouring host cells. In contrast to the compatible interaction, the incompatible relationship resulted in a considerable decrease in the quantity of haustoria and the density of intercellular hyphae, as well as hypersensitive cell death in the host cells [45]. Hence, the resistance to rust in wheat is reported to be post-haustorial [26,46]. However, fungal pathogen pre-penetration induction of defense-related transcripts were also reported [47,48]. Further, the transcriptomic studies revealed that the response of wheat plant soon after *Puccinia* infection in compatible and incompatible interactions differ quantitatively but not qualitatively up to the point of penetration [49]. Accordingly, Wang et al. [26] demonstrated that in Suwon 11 (wheat cv.) challenged with either *Pst*-CYR23 (avirulent) or *Pst*-CYR31 (virulent), a significant fraction of TDFs (63 %) were shared in both interactions and were categorised as basal defense-related genes in a comparative gene expressions study. 51 genes that are often activated in both compatible and incompatible interactions between wheat and *Pst* were also identified by Ref. [27]. In case of NILs differing in the presence of resistant genes showed minor differences at early infection stages. However, in contrast to susceptible lines, the resistant lines displayed induction of many defense response-related genes [50]. Greater levels of defence readiness prior to pathogen infection are suggested by the higher basal level of transcripts, especially those implicated in pathogen detection, downstream signalling and defence mechanisms in NILs with or without resistant genes. This may be essential to "R" gene-mediated resistance. According to reports, these variations in the baseline expression of defense-related genes support the resistance mediated by *Sr*24 [50], *Yr*10 [51] and *Lr*1 [52] genes against *Pgt*, *Pst*, and *Pt* infection, respectively.

4.1. Understanding molecular signalling pathways in wheat-Puccinia interactions

4.1.1. Early signalling pathways

MAPKs are among the many protein kinases that have been connected to the signal transduction pathways that control defensive reactions upon pathogen recognition [53]. MAPKs are present in nucleus, cytoplasm and cell membranes which regulate various biological functions by phosphorylating specific molecules *viz.* transcription factors, other kinases, regulates cell proliferation, differentiation, apoptosis and immune responses. In eukaryotes they direct many of stimuli like osmotic stress, pathogen infection, heat shock, cell survival, apoptosis *etc* [54,55]. The *MKP1* gene transcripts that interacts with 20 distinct MAPKs were stimulated during the wheat-*Puccinia* interactions [26] and this cascade further modulates the expression of *WRKY* transcription factors that mediates plant immunity against rust pathogen [56]. Gene ontology of the interaction-derived transcriptomic data revealed upregulation of MAPK signalling against *Pst* [30]. Further, the higher expression levels of these genes during incompatible interactions (*Lr*28-mediated) compared to compatible interactions at early infection stage (12–24 hpi) revealed their importance in early pathogen detection and signalling to activate defense mechanisms against *Puccinia* [57], which was also supported by Dorostkar et al. [58] in *Sr*24-mediated

Table 1
Comparison of various techniques in transcriptions studies.

	Technique	Methodology	Advantages	Disadvantages	References
1	**PCR-based methods**				
1a	**Differential Displayed-Reverse Transcriptase PCR (DD-RTPCR)**	• It involves PCR amplification of cDNAs using a set of oligonucleotide primers. • One is short and arbitrary in sequence, annealing at varied places in relation to the first primer, while the other is linked to the poly-A tail of mRNAs. • Cloning, sequencing and characterization of differentially expressed bands.	• Rapidity, simplicity, and sensitivity. • Able to discern up-regulated and down-regulated genes in multiple samples. • Requires relatively minute quantities of starting material. • Prior information of the genome is not necessary.	• Rare transcripts are not identified. • False positive results during band elution. • Contamination from the adjacent bands results in overlapping expression patterns.	[32]
1b	**Quantitative Real-time PCR (qRT-PCR)**	**SYBR Green fluorescent dye:** • It attaches itself to double-stranded DNA, allowing for the measurement of the PCR amplicons that remain after each amplification cycle. • The amount of beginning template has a positive correlation with the cycle at which PCR enters log-linear amplification. **TaqmanTM system:** • In this technique, three oligonucleotides are utilized to enumerate each sequence. • Two sequences are the primers, and the third (probe) is designed to hybridize specific to the amplified sequence.	• Rapidity, simplicity, and sensitivity. • Overcoming limitations associated with conventional reverse transcriptase-PCR	• A limited number of biological samples can be examined • Requirement of the nominal amount of starting material	[33]
1c	**cDNA Amplified Fragment Length Polymorphism (cDNA-AFLP)**	• It involves digestion by two restriction enzymes followed by ligation with adaptors. • Then, a mix of short oligonucleotide primers extends restriction fragments are extended by PCR amplification. • Consequently, amplified restriction fragments will only be those whose restriction site nucleotide sequence coincides with the primer selective nucleotide sequence.	• Enables a quick search of all genome polymorphisms. • Does not need the creation of probes or previous sequence information.	• Since each primer combination results in a large number of bands with varying intensities, the analysis must choose a specific set of bands.	[34]
2	**Hybridization methods**				
2a	**Suppression Subtractive Hybridization (SSH)**	• After hybridising the driver (normal cDNA) and tester (differentially expressed transcript) cDNAs, the hybrid sequences are eliminated. • As a result, the genes represented by the remaining unhybridized cDNAs are expressed in the tester but not in the driving mRNA and are further enriched by PCR.	• Need for small amounts of sample materials. • Excellent success rate in identifying rare genes. • Greater specificity.	• Only two samples can be evaluated in one assay	[35]
2b	**Microarray analysis**	**Oligo microarray:** • Either oligonucleotides or PCR-amplified cDNA microarrays are printed robotically on a glass slide. Subsequently, the labelled cDNA populations with distinct fluoro-chromes are hybridised simultaneously. **Affymetrix gene chip microarrays:** • Gene chip microarrays are hybridised using chip with only DNA sequence. And uses in-situ oligonu-cleotide synthesis or photolithography.	• Array specificity and reproducibility. • Rapid technique, provides the information for thousands of genes at a time	• Restricted to species with genome sequence information • Relatively low throughput and costly and not used in quantitative analysis	[36]
3	**Sequencing-based methods**				

(*continued on next page*)

Table 1 (*continued*)

	Technique	Methodology	Advantages	Disadvantages	References
3a	**Large-scale cDNA sequencing/ Expressed sequence tag (EST)**	• EST is a unique, unedited, short, single sequence produced from the 5′ or 3′ end of cDNA libraries that have been randomly chosen from target cells. • These DNA sequences solely codes for gene coding region.	• No necessity of prior sequence information of the transcripts. • Consistently finds hundreds of new genes encoding proteins.	• Exorbitant for sequencing of whole cDNA libraries of an organism	[37]
3b	**Serial Analyses of Gene Expression (SAGE)**	• Here, the two linker fragments with Type IIS restriction endonuclease recognition sequence and primer binding sites were ligated to restriction-digested cDNAs • Following PCR amplification, these two sets of cDNAs are ligated to create a "ditag" with linkers on both ends, which is subsequently concatenated, cloned, and sequenced.	• Extremely sensitive for identifying new genes without known sequence information and transcripts with low abundance.	• Error may occur, when two distinct genes share the same tag or when a single gene is alternatively spliced and has distinct tags at the 3′ ends. • A fraction of mRNAs may be lost due to lack of enzyme recognition site.	[38]
3c	**Ultra-High Throughput RNA Sequencing (UHTS)**	• Transcriptomic studies using next-generation sequencing technologies like Roche 454, Illumina, and ABI SOLiD (Sequencing by Oligo Ligation and Detection) at the nucleotide level. Also known as RNA-Seq.	• Highly effective in cutting down the time needed to analyse several samples. • Identification of low-abundance genes, alternative splice transcripts, and novel genes	• High price required	[39,40] [29]
3d	Dual RNA Sequencing	• Dual RNA sequencing makes use of next generation sequencing enabled RNA-Seq techniques that simultaneously capture genome-wide transcriptional changes of infecting pathogen and host cells	• Simultaneously capture genome-wide transcriptional changes of both pathogen and host at the same point of time	• Pathogen mRNA usually constitutes a small portion of an infected cell RNA sample that results in poor sequence recovery.	[41]
3e	Single Cell RNA Sequencing	• Single-cell RNA offers a greater resolution of the interactions, enabling an examination of the makeup of the host cells and how they react to infection.	• Makes it possible to accurately identify and measure transcript isoforms. • To assess gene expression even when there is little RNA present, as in the case of single cells.	• Does not reveal the stage at which the pathogen is infecting host cells.	[42]

resistance against stem rust in wheat. However, in later stages (>72 hpi) there will be downregulation of these genes due to the excess effectors secreted by *Puccinia* at late infection stages, which interrupts MAPK signalling and was evident by the suppression of resistance response in wheat cv. FLW29 against *Pst* [59].

Apart from MAPK, plants possess an extensive array of Ca^{2+} sensors, including CDPKs, calmodulins (CMLs) and calcineurin B-like protein (CBLs), which are crucial for PTI and ETI processes [60–62]. Among them, CDPKs are thought to be vital promoters of plant innate defence responses [63]. In reaction to pathogen infection, CDPKs can detect changes in cytosolic Ca^{2+} levels and phosphorylate their target proteins. The expression of genes associated to defense is caused by these phosphorylated proteins by connecting downstream signalling pathways. Accordingly, Mir et al. [59] hypothesised the seedling stage stripe rust resistance as a result of elevated CDPK gene expression. These transcripts encode CDPK that phosphorylates the plasma membrane NADPH oxidase which controls the generation of ROS resulting in a resistant phenotype during *Pst* infection [59]. In addition to CDPK, genes encoding calcium sensor proteins, such as CMLs and CBLs, were shown to exhibit differential expression across NILs after *Puccinia* infection [59]. By raising Ca^{2+} concentrations in the cytosol, CML contributes to the wheat-*Puccinia* interaction as they regulate cellular signalling cascades, cell wall strengthening and HR [64]. Moreover, calcium influx triggers MAPK cascades, which encourage stomatal closure to prevent pathogen entrance into leaves [65]. In this context, Dorostkar et al. [58] reported the upregulation of gene encoding EF domain protein which is involved in CML pathway in resistant wheat cultivar (AT349) challenged with *Pt*. Furthermore, the previously stated DEG may be involved in the recognition of pathogen based on its relationship with the C-type lectin receptor signalling pathway. Since β-Glucan is a dominant polysaccharide found in the fungal cell wall [66], this route is responsible for initiating the defensive response. As a component of the signalling cascade, this gene has been linked to CML, which in turn may drive cell differentiation and shield it from the leaf rust infection. Additionally, the integration of this gene with the MAPK signalling pathway is accomplished by CML4, which activates MAPK and promotes the negative control of ROS buildup while preserving cell homeostasis [58].

4.1.2. Hormonal signal transduction

SA is essential for plant immunity including SAR and local defence against *Puccinia* [51,67] as evident by the greater endogenous levels of SA in resistant cultivars than susceptible ones [68]. SA specifically acts as a pre-activated defense response and the related genes like phenylalanine ammonia-lyase (PAL) encoding genes that are involved in SA biosynthesis were upregulated even at 0 hpi in

resistant cultivars [51]. Further, SA receptor NPR1 and SA-activated PR genes encoding PR proteins PR1, PR2, and PR5 [69], also exhibited a similar transcription profile to PAL in *Yr*10-mediated resistance against *Pst* [51]. Similar kind of increased PAL gene expression was reported in *Sr*24-mediated resistance against *Pgt* [50]. Additionally, in susceptible genotypes to leaf rust, DEGs implicated in SA-mediated signal transduction were dramatically knocked down, showing their significance in displaying rust resistance [58]. Interestingly, *Puccinia*-induced SA signalling showed an antagonistic action on JA signalling in wheat [51]. In contrast to SA biosynthesis genes, the genes encoding jasmonate resistant protein 1 (JAR1), fatty acid desaturase (FAD), allene oxide cyclase (AOC) and lipoxygenase (LOX) that are crucial in JA biosynthesis were neither up-regulated nor down-regulated and showed similar kind of expression in resistant and susceptible reactions in wheat-*Puccinia* interaction [51]. However, there were reports of overexpressed JA-related genes like plant defensins (PDF1.2), ethylene responsive factor (ERF), PR5 and PR10 in inoculated resistant cultivars [58,59] but, the magnitude of transcriptional peak was not great as SA-induced PR genes [51]. Hence, SA-mediated defence system is the primary signalling pathway against *Puccinia*, despite the upregulation of JA-responsive genes. Furthermore, recent investigations by Mir et al. [59] reported null expression of auxin-related genes with upregulated SA pathways at early infection stages during *Pst* infection where the auxin and SA pathways also operate in an antagonistic manner with one another during plant defense [70].

In addition, ethylene can also stimulate the production of defensive compounds and promote the activity of other defense hormones like JA and SA. In this line, Liu et al. [30] found the down-regulated DEGs in cysteine and methionine metabolism that were related to ethylene pathways and suggested that the mechanism of susceptibility of wheat cv. CY12 to *Pst* as the effect of ethylene pathway suppression as shown in Fig. 3. It was also found that the RNA helicase involved in ethylene-influenced gene expression [71] was specifically induced in resistant reaction against *Pst* [26] and *Pt* [58]. Similarly, another ethylene-responsive gene encoding EFR proteins was highly expressed at 72 hpi in resistant wheat cultivar challenged with *Pst* [72] and *Pgt* [50], indicating ethylene involvement in the defense mechanism against rust. More interestingly, ABA that found to provide a positive signal in defense responses against other pathogens, such as *P. irregular* [73] have a negative influence on resistance to rust pathogen. In this context, Wang et al. [26] reported the upregulation of gene encoding phosphatase type 2C protein that acts as a negative regulator of ABA responses in resistant genotype against *Pst*. Similarly, Ton et al. [74] also observed, ABA enhancing vulnerability to rust pathogen by undermining SA-dependent defense in wheat. Thus, an interplay between SA, JA and ET signalling pathways is a crucial element to refine the defense activation against rust pathogen.

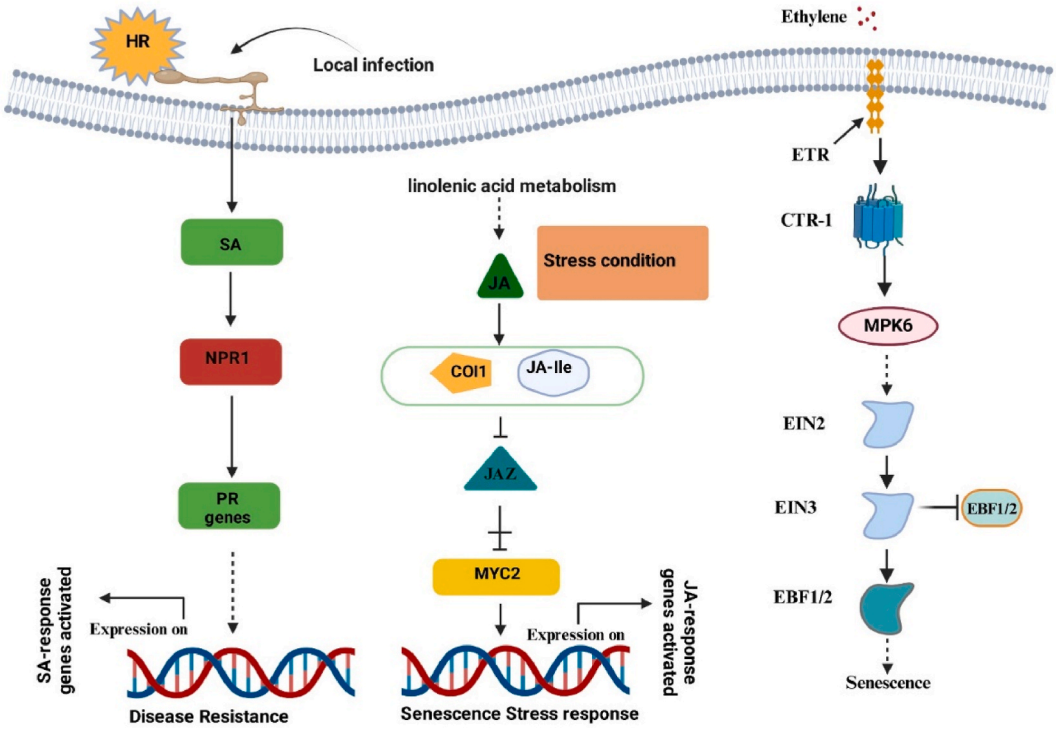


Fig. 3. The schematic diagram represents the DEGs expression enriched in SA, JA and ET signalling pathway. In SA signalling pathway, genes encoding NPR1 and PR genes were downregulated as a response to *Puccinia* infection in susceptible wheat cultivar. In JA signalling pathway, genes encoding COI1 and JAZ protein were sightly downregulated. In case of ET signalling pathway, genes encoding ETR, CTR1, EIN2, EIN3 and EBF1 were strongly downregulated that results in susceptibility to rust due to interruption of SA, JA and ET signalling pathway.

4.2. Identification of genes involved in wheat defense mechanisms

4.2.1. Positive feedback of ROS and RNS

Plant cells that participate in the HR release an enormous amount of reactive oxygen species (ROS) like hydroxyl radicals, hydrogen peroxide and superoxide anions which contribute to an oxidative burst [75]. Similarly, it has been demonstrated that reactive nitrogen species (RNS) such as nitrous oxide (N_2O) and nitric oxide (NO) also contribute to plant defence mechanisms against disease caused by biotrophic pathogens and influence HR as well as primary defence [76]. In the studies involving transcriptomic profiling showed the upregulation peroxidase and peroxisomal membrane protein encoding genes at 12 and 24 hpi, respectively, that are essential for ROS production [77,78] in a resistant wheat cv. Suwon11 against *Pst*-CY23 [26]. At the later stage of infection (48 hpi), the transcript level of plasma membrane-localized NADPH oxidases was significantly up-regulated in the *Yr*10-mediated resistance pathway, suggesting the cruciality of these enzymes in ROS generation and enhanced wheat resistance to rust [51,79,80]. Similarly, in response to *Pst*, H_2O_2 accumulation was detected in host guard cells as early as 6–8 hpi [81] and also observed in wheat mesophyll and stoma cells in the later stage of infection in resistance interaction with rust [68]. Also, Singh et al. [82] and Vishwakarma et al. [50] reported ROS production as major defense mechanism in *Lr*28 and *Sr*24-mediated defense mechanism against leaf and stem rust respectively. Regarding RNS, NO serves as a crucial signalling compound in controlling uredospore germination during wheat-rust interaction and requires NO synthase activity for endogenous NO production in resistant plants [83]. By using RT-PCR investigations, Wu et al. [51] discovered higher nitric oxide synthase 1 enzyme transcript levels in the resistant reaction against *Pst*-CYR32 at four distinct time periods (24, 48, 72, and 120 hpi). The NO works synergistically with ROS and balance of NO and ROS levels can affect wheat resistance to rust [83]. Additionally, NO can activate SA-dependent gene expression [84].

4.2.2. HR as hallmark of resistance

The perception of rust fungus by wheat will result in a programmed cell death bought by oxidative burst and other defense related compounds in order to limit and combat the *Puccinia* infection in resistant cultivars [85]. The transcriptomic analysis of relative defense gene expression in the incompatible reaction revealed rich diversity in HR-specific transcripts that are involved in both basal and R gene-mediated immunity that differentiates them from susceptible ones [27]. The induction of genes encoding peroxidase enzyme triggers subsequent NADPH oxidase-mediated ROS production [86] which contributes to the hypersensitive phenotype in the resistant reaction against *Puccinia* [27]. Further, the nucleases and chromosome condensation factors were involved in the apoptosis of neighbouring cells that were adjacent to the first dying cells in the HR by endo-nucleolytic cleavage and chromosome condensation.

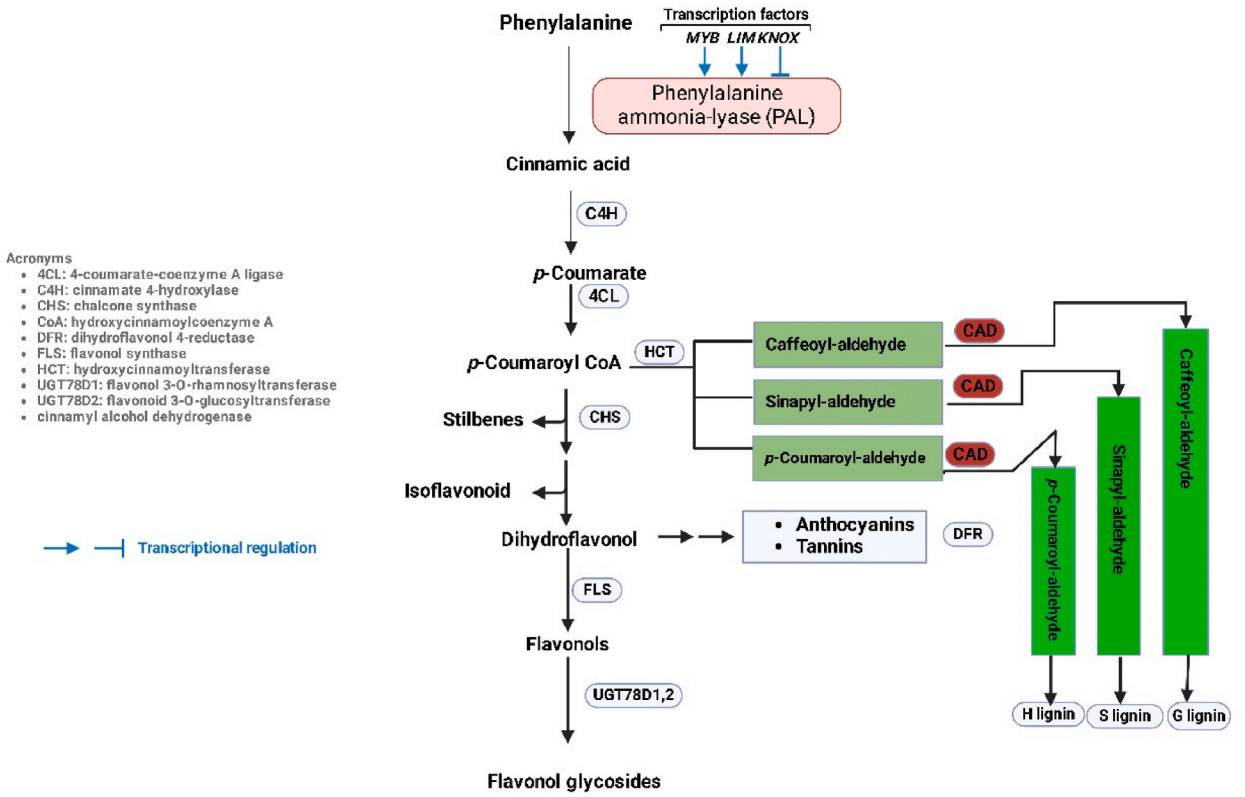


Fig. 4. Flow chart of phenylalanine pathway that leads to lignin and flavonoid production during wheat-rust interaction. The DEGs encoding the intermediary substrates and enzymes were strongly upregulated in resistant wheat cultivar after infection by *Puccinia*.

The transcripts of these genes were enhanced in *Yr*5-mediated resistance to *Pst* [27]. Additionally, during an incompatible interaction, the oxidative burst induced HR-specific structural proteins (proline-rich) of the primary cell wall were involved in cell wall strengthening [87,88] and these proteins were reported to be successful in limiting *Pst* penetration [27]. Also, Vishwakarma et al. [50] reported the upregulation of genes encoding phosphoinositide specific phospholipase and lipoxygenase which are involved in HR signalling in *Sr*24-mediated resistance against *Pgt*.

4.2.3. Cell wall reinforcement as an active resistance mechanism

In order to provide a physical barrier that limits pathogen infection, lignin tends to accumulate at the locations of pathogen invasion. This compound can strengthen cell wall and give mechanical strength [89]. Additionally, it stops the pathogen from obtaining nutrients and water from the plant, which limits the pathogen ability to proliferate and spread [90]. An essential enzyme involved in the production of lignin is caffeineyl-CoA O-methyltransferases (Fig. 4). It is particularly crucial in the formation of plant cell wall ferulic esters, which are essential for reinforcing the cell wall during the induced disease resistance response. Incompatible interactions with *Pst* [26] and *Pgt* [50] have been shown to trigger the genes encoding this enzyme at 24 hpi and peak at 48 hpi. There have also been reports of induction of other lignin biosynthesis genes, such as genes encoding dirigent-like proteins and cinnamonyl alcohol dehydrogenase (CAD) in the incompatible relationship compared to compatible interaction [27]. Further, Liu et al. [30] reported the loss of resistance and successful invasion of *Pst* in susceptible wheat cv. CY12 with down-regulated DEGs in the phenylpropanoid metabolism pathway, which led to a decrease in lignin biosynthesis. In addition to lignification, the resistant plants defend against rust fungal entry by modification of plant cell wall by the deposition of callose [91]. Callose is a polysaccharide present in the specialized cell walls. Thickenings of cell wall is seen at site of pathogen infection and forms a physical barrier to avoid pathogen invasion [92]. In one study, there found an enhanced expression of gene encoding callose synthase 12 in resistant genotype than the susceptible genotype against *Pt* [93].

4.2.4. Reduced photosynthesis as an indirect resistance mechanism

Photosynthesis supplies the raw materials and energy required for a wide variety of physiological metabolic activities and is intricately connected to plant defence response [94]. According to studies [95,96], photosynthesis could play a role in wheat defence

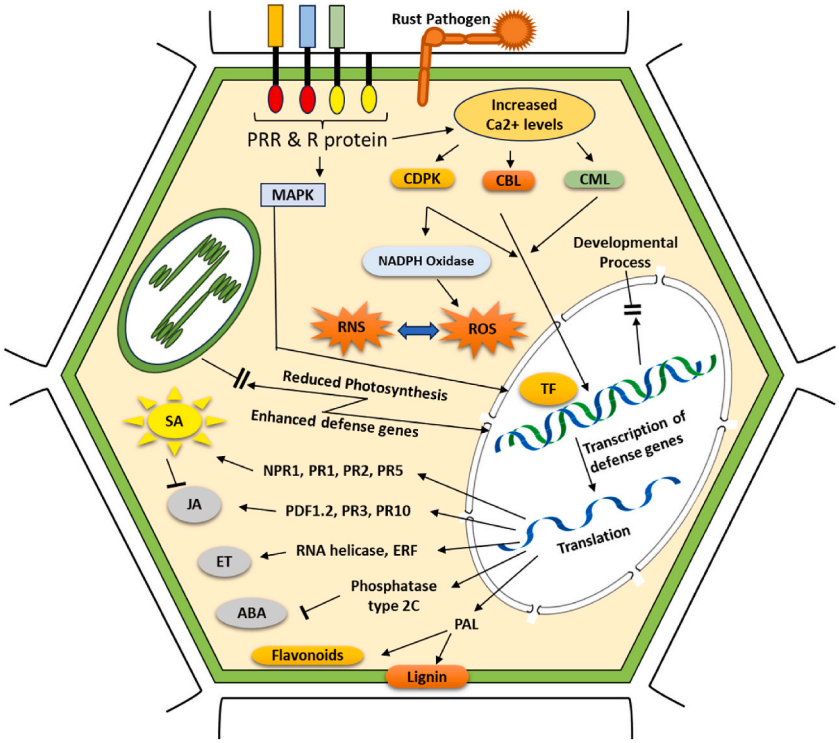


Fig. 5. Schematic representation of various signalling pathways and resistance mechanisms in wheat in response to *Puccinia* infection. During infection process, the pathogen is sensed by the pattern recognition receptors (PRR) or recognised by R proteins. It activates early signalling pathways involving mitogen associated protein kinases (MAPK) and Calcium dependent protein kinases (CDPK). MAPK enhances defense related transcription factors (TF) while the CDPKs enhances reactive oxygen (ROS) species production which in turn enhances reactive nitrogen (RNS) species production. The signal transduction results in enhancement of secondary signal molecules like salicylic acid (SA), ethylene (ET) and down regulation or no change in the genes involved in jasmonic acid (JA) and abscisic acid (ABA) production. Also, reduced photosynthesis and developmental process in response to *Puccinia* infection.

reaction against rust pathogen. The effector proteins produced by *Puccinia* have the ability to decrease photosynthesis and prevent the generation of ROS produced from chloroplasts in susceptible genotypes [97] whereas, resistant wheat genotypes can proactively adjust photosynthetic changes to minimise pathogen infection [98]. Early in the defense reaction to *Puccinia*, wheat plants seem to turn off photosynthesis locally by reducing gene expression related with photosynthesis. This reduction was brought about by a change in the host metabolism in addition to the removal of the green, photosynthetic leaf area during HR. The research by Hao et al. [99] made this conclusion quite clear when it was reported that no enrichments of photosynthesis were found at 24 or 48 hpi, but the genes encoding photosynthesis II assembly, light harvesting system and photosynthetic electron transport in photosystem I were enriched at 120 hpi. Therefore, it has been documented that photosynthesis influences the defense mechanisms elicited by *Puccinia* infection in wheat plants. Similarly, Liu et al. [30] found that the DEGs involved in photosynthesis and carbon fixation pathways were down-regulated in

Table 2
List of candidate DEGs identified through qRT-PCR in wheat-rust interaction.

Candidate genes	Peak (hpi)	Cultivar	Regulation	Pathogen	Role
UDP-glucosyltransferase	24	S	+	*Pst*	Flavonoid biosynthesis [30]
Tricetin 3′,4′,5′-O-trimethyltransferase	24	S	+	*Pst*	Methylation of tricetin [30]
Ferredoxin	24	S	+	*Pst*	Redox regulation [30]
30S Ribosomal protein S17	168	S	+	*Pst*	Protein synthesis [30]
Chalcone synthase 2-like protein	168	S	+	*Pst*	Flavonoid biosynthesis [30]
Metallothionein	0, 72	S	+	*Pst*	Ion homeostasis [30]
SRG1- like protein	24	S	+	*Pst*	Nitric oxide bioactivity [30]
GDSL esterase/lipase	72	S	+	*Pst*	Lipolytic activity [30]
Trypsin inhibitor	120	S	+	*Pst*	Inhibition of protease activity [51]
4-Hydroxyphenylpyruvate dioxygenase	120	S	+	*Pst*	Tyrosine metabolism [51]
Nitric oxide synthase 1	120	R	+	*Pst*	Oxidative burst [51]
WRKY transcription factor	72	R	+	*Pst*	Activation of defense genes [51]
Thiopurine S-methyltransferase	24, 48	R	+	*Pst*	Alters redox capacity of cells [51]
Ferritin	72	R	+	*Pst*	Ion homeostasis [51]
Leucine Rich Repeat family protein	12	R	+	*Pst*	Pathogen recognition [26]
CBL-interacting protein kinase	12	R	+	*Pst*	Ca-dependent signalling [26]
Serine/threonine Kinase	12	R	+	*Pst*	Pathogen recognition [26]
Ethylene-responsive RNA helicase	12	R	+	*Pst*	ET-dependent signalling [26]
Protein phosphatase type 2C	12	R	+	*Pst*	Negative regulator of ABA [26]
Caffeoyl-CoA O-methyltransferases	48	R	+	*Pst*	Lignin biosynthesis [26]
Peroxidase	12, 24	R	–	*Pst*	ROS scavenging [26]
Peroxisomal membrane protein	12, 24	R	–	*Pst*	ROS scavenging [26]
Wheat PR-5-like protein gene	24, 48	R	+	*Pst*	Antifungal PR protein [26]
MKP1	12	R	+	*Pst*	Signalling pathway [26]
ABC transporter C 10	36	R	+	*Pt*	Stress responsive [93]
Callose synthase 12	24	R	+	*Pt*	Cell wall strengthening [93]
Coatomer alpha subunit	16	R	+	*Pt*	Vesicular trafficking [93]
Cysteine-rich RLK 10	8, 16	R	+	*Pt*	Pathogen recognition [93]
Disease resistance protein RPM1	72	R	+	*Pt*	Effector recognition [93]
E3 ubiquitin-protein ligase UPL6	24	R	+	*Pt*	Post-transcriptional modification [93]
LRR-RLK EFR	48	R	+	*Pt*	Pathogen recognition [93]
MAP kinase	24	R	+	*Pt*	Signalling pathway [93]
Potassium transporter 18	24	R	+	*Pt*	Signalling/Cellular homeostasis [93]
Receptor-like protein kinase	24	R	+	*Pt*	Pathogen recognition [93]
Wall-associated kinase 4-like	20	R	+	*Pt*	Pathogen recognition [93]
Cell division cycle 5 like protein	16, 36	S	+	*Pt*	Pre-RNA processing factor [93]
Molybdenum cofactor sulfurase	16	S	+	*Pt*	Enhance enzymatic activity [93]
Calmodulin transcription activator	48	S	+	*Pt*	Signalling pathway [93]
Beta-fructofuranosidase	48	R	+	*Pst*	Hydrolyse β-glucans [99]
Thaumatin-like protein	24	R	+	*Pst*	Pathogenesis related protein [99]
Beta-1,3-glucanase	24	R	+	*Pst*	Hydrolyse β-glucans [99]
Cell wall-associated hydrolase	24	R	+	*Pst*	Fungal wall degradation [99]
Class I chitinase	24	R	+	*Pst*	Hydrolyse chitin [99]
Glutathione S-transferase 2	24	R	+	*Pst*	Detoxification [99]
root peroxidase	48	R	+	*Pst*	ROS production [99]
Ribonuclease 1	24, 48, 120	R	–	*Pst*	Pathogenesis related proteins [99]
3-beta-hydroxysteroid-dehydrogenase	24, 120	R	+	*Pst*	Plant growth and development [99]
lipoxygenase 1	120	R	+	*Pst*	Cell wall strengthening [99]
Thiol methyltransferase 2	24	R	–	*Pst*	Methylation of glucosinolates [99]
ATP synthase subunit alpha	24, 48, 120	R	–	*Pst*	Energy generation [99]
Protein WIR1A	48	R	+	*Pst*	Defense-related protein [99]
Pathogenesis-related 5	24	R	+	*Pst*	Pathogenesis related protein [99]
NAD(P)H oxidoreductase 1	48	R	+	*Pst*	Metabolic activity [99]
Pathogenesis-related protein 1	24	R	+	*Pst*	Pathogenesis related protein [99]
Class III peroxidase	24	R	+	*Pst*	Pathogenesis related protein [99]
Peroxidase 12	24	R	+	*Pst*	Pathogenesis related protein [99]

Where, hpi-hours post inoculation; S- susceptible; R-resistant; + upregulated; - downregulated.

wheat (CY12) after being inoculated with *Pst*-CYR34 at 18 and 48 hpi. The downregulated DEGs include genes encoding ribulose-1, 5-bisphosphate carboxylase-oxygenase, photosystem I and II proteins, photosynthetic electron transport, F-type ATPase, photosynthesis-associated protein (D1), cytochrome B6-F complex, nicotinamide adenine dinucleotide phosphate dehydrogenase and chlorophyll A-B binding proteins [30,58]. However, the specific effects of wheat rust on PS I and PS II are still unknown. Furthermore, the R-genes were also anticipated to exert their resistance effect by modifying photosynthesis which was well documented in *Yr*36, *Lr*28 and *Sr*24 mediated resistance against stripe, leaf and stem rust respectively [50,58,100]. The various signalling pathways and resistance mechanisms in wheat in response to *Puccinia* infection is summarised in Fig. 5.

5. Differential gene expression analysis

The post-infectional changes in the wheat is dynamic and differs qualitatively and quantitatively with time after infection in order to combat *Puccinia* infection. Baseline evidence to this is revealed by the infection-monitored gene expression profiling which showed the transcriptomic reprogramming (upregulated or downregulated) in wheat plants upon infection by all three kinds of rust [50,51,58, 101]. The time frame-based analysis of transcript accumulation in resistant and susceptible cultivars showed a biphasic response in wheat against *Puccinia*. In which, a transcriptional peak was observed at an early stage of 12–24 hpi [27,50] reflecting the activation of defense response against *Puccinia* at early infection stage. After this peak, there will be a sharp decline (48–72 hpi) in the transcripts that were differentially expressed earlier [26,102], followed by another increase from 72 to 168 hpi [30]. The rust fungi produce a large number of proteins from the haustoria into the extra-haustorial matrix during establishment in host, of which a portion of proteins were further carried into the host cell [103]. These proteins presumably allow pathogen to get nutrients and avoid host defense in order to infect host cells [104] and also believed that will rewire the host defense mechanisms in the plant cytoplasm which appeared as decreased host response/DEGs at 48–72 hpi [26] but sustained defense response with co-ordinated modulation of gene expression at late stages [50]. A similar kind of upward peak was observed by Liu et al. [30] at 24 hpi and 72 hpi with downward peak at 48 hpi and 160 hpi against *Pst* race CYR34. Several other studies were also shown similar pattern of transcript accumulation at early and late infection stages against *Pt* [52] and *Pgt* [50]. The list of some important candidate genes identified and validated through qRT-PCR were listed in Table 2 by different studies.

5.1. Functional analysis of the DEGs

Gene Ontology (GO) categorization was employed to classify the functions of the predicted coding sequences (CDS) based on gene product characteristics within three primary domains: biological processes, molecular functions and cellular components. Within the biological process domain, cellular processes emerged as the most prominent group in *Puccinia*-infected wheat samples. Other notable DEGs in infected samples were associated with metabolic and signaling processes. For the cellular component domain, the largest groups were identified in the cytoplasm, membrane and organelle categories [72,93,102]. Meanwhile, DEGs linked to molecular functions exhibited significant activity in ion binding, organic cyclic compound binding, heterocyclic compound binding, catalytic functions, electron carrier activity, nucleic acid-binding transcription factor activity, transporter activity, and structural molecule activity, specifically within rust-infected samples [72,93,105]. Further analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway highlighted an enrichment of genes involved in carbohydrate metabolism, signal transduction, and energy metabolism. Particularly, genes associated with the phenylpropanoid biosynthesis pathway were markedly enriched during both the early and late stages of infection [72]. Secondary metabolites, such as salicylic acid (SA), lignins, flavonoids, phytoalexins, and coumarins are products of the phenylpropanoid pathway that are essential in systemic plant resistance. Activation of this pathway has been implicated in plant defense responses against rust pathogens [106]. Additional pathways involved in plant stress responses, such as phenylalanine, thiamine, glutathione, and purine metabolism, also exhibited differential expression of enzymes like phosphatase, kinase, and adenyl pyrophosphatase. These pathways support enhanced responses in plants combating rust pathogen [72].

5.2. Upregulated genes in response to Puccinia infection

The defense system in plants involves complex interplay between genes and their products. The signalling events triggered due to pathogen detection led to the upregulation of a large number of genes. The upregulated genes encode proteins including: PRRs, signal transduction proteins, pathogenesis-related (PR) proteins, transcription factors, hormone biosynthesis genes and many other proteins intended to fight against plant pathogens were identified in wheat-*Puccinia* interaction from transcriptomic studies as described in above sections. Defense-related enzymes like protease, peptidase, endopeptidase and hydrolase were upregulated in incompatible interactions and activation of genes encoding their negative regulators causes susceptibility of plants to rust pathogen [50]. Similarly, the transcripts of wheat gene corresponding to a PR-5-like protein strongly accumulated at early infection stage (24-48hpi) in the resistant cultivars compared to the susceptible ones, indicating their broader role in wheat resistance to rust [26]. Several other PR proteins, including chitinase, thaumatin-like protein and beta-1,3-glucanase reported to be HR-specific, were also upregulated during *Puccinia* infection [27]. Secondary metabolites like flavonoids, betalins and indole alkaloids which are involved in HR, oxygen scavenging and signal transduction, respectively were involved in wheat resistance and the gene involved in their biosynthesis were upregulated in wheat [59].

Additionally, disease resistance (R) genes that encode different types of R proteins including receptor like kinases (RLK) and receptor like proteins (RLP) were upregulated in wheat in response to rust infection [59,107] and most of the upregulated R genes comprised of protein kinases and leucine rich repeats (LRR) receptors like STPK [108]. RLP genes, such as RPP (resistance to

Peronospora parasitica protein) 8 and RPP13, have been shown to express themselves more relative to resistant cultivars than susceptible cultivars after *Pst* infection [59]. In accordance with McDowell et al. [109], these RLPs offer disease resistance by indirectly interacting with avirulence protein, which in turn triggers HR. Moreover, during wheat-rust interaction, a number of R genes from the RLK family have been discovered. Among them, G-type lectin S-receptor-like protein kinases and cysteine-rich receptor-like protein kinases exhibited the highest levels of upregulation [59]. Although, RLKs are linked to cell membranes and facilitating signal transmission [110], Mir et al. [59] have shown the downregulation of wall-associated kinase genes in resistant cultivars in response to *Pst* infection. Interestingly, contrasting in wheat-*Pt* interaction [58].

5.3. Downregulated genes associated with susceptibility

Susceptibility and resistance are two sides of the same coin. S-genes, or genes coding for effector targets, are known to operate as susceptibility factors or negative defense regulators in diseases. According to Eckardt (2002), recessive resistance results from the disruption of dominant genes known as S-genes. It has been demonstrated that effectors activate the proteins encoded by S-genes, leading to effector-triggered susceptibility. Recently, Wang et al. [79,111] discovered susceptible genes encoding *TaPsIPK1* (a receptor-like cytoplasmic kinase) and *TaWRKY1*9 that found to be downregulated in resistant cultivars and knocking out this gene conferred broad-spectrum resistance to *Pst*. Furthermore, the genes encoding cell division cycle 5 like protein were speculated as S gene in wheat-rust interaction as their expression positively corelates with susceptibility to *Pt* [93] and *Pst* [112]. Additionally, reported that calmodulin binding transcription activator (CAMTA) was found to have low expression in resistant genotype and also showed that mutation of CAMTA genes leads to upregulation of 99 genes out of which 32 genes reported to have role in defense. Similar results obtained by Manjunatha [93], where CAMTA was down regulated in resistant NIL and up regulated in their corresponding susceptible NIL, hence categorised as susceptible genes that enable the *Pt* growth in the wheat during the compatible reaction. These susceptible genes will facilitate in selection for resistance by focusing on eliminating these genes harbouring or favouring pathogen in the host which supported disease susceptibility.

6. Regulatory mechanisms

6.1. Transcription factors regulating defense responses in wheat

TFs like *WRKY, GRAS*, *BHLH, FAR1, ERF, MYB* and *NAC* are reported to be crucial in plant response to pathogen infection [113]. Among them, *WRKY* TFs are a diverse collection of transcription factors peculiar to plants that include a *WRKY* DNA-binding domain. These factors are favourably controlled by MAPK signalling and aid in plant immune reaction [114]. Seven *WRKY* genes, *TaWRKY34, TaWRKY46, TaWRKY47, TaWRKY64, TaWRKY70, TaWRKY76,* and *TaWRKY146,* were shown to be elevated in a resistant wheat cultivar during the *Pst* infection [59]. As they cause SA synthesis that initiates signalling pathways and stimulates host defence mechanism, these *WRKY* TFs have been linked to wheat resistance to *Puccinia* [115]. Wang et al. [116] have observed a similar type of constructive control of *WRKY70* in rust resistance. Furthermore, it is known how important *WRKY70* is for resistance to rust because silencing it made wheat seedlings more vulnerable to *Pst* infection [117]. Further interaction of *WRKY7*0 TF with *WRKY64* and *WRKY53* enabled them to confer broad-spectrum defence response [118]. Also, the plant TF *WRKY1*8 that coordinates the expression of cysteine-rich kinase-10 were also upregulated during incompatible reaction [119]. Furthermore, TFs are reported to influence defense pathways (JA/SA) and modulates plant reaction from susceptibility to resistance in coordination with defense genes [120, 121]. In accordance with this Dorostkar et al. [58] identified *BHLH* encoding TF as a key regulator in resistant genotype against *Pt*. Similarly, *zf-RVT* TF that act as potential effector decoys [122] and *HSF* TF, involved in sulphur compound biosynthesis (phytoanticipin or phytoalexin) were upregulated against rust pathogen [58]. In addition, differential modulation of TFs including *MADS* box, *MYB2, AP2, BZIP, ERF, RAV, NAC, FAR1, NF-YB*, *MYC4* and multiple *WRKY* in response to rust pathogen has been reported by several workers [27,52,123,124].

6.2. Epigenetic regulation of gene expression during Puccinia infection

Epigenetic regulation in host-pathogen interaction involves modifications on DNA or chromatin (DNA packaging proteins) that affect gene expression without altering the DNA sequence itself. These modifications can activate or silence genes, influencing a plant response to disease. The increase of transcripts of genes expressing histone H1 linked to chromatin assembly and disassembly suggested chromatin remodelling as a useful strategy to regulate gene transcription and modulate defense reaction against *Puccinia* [58]. According to Alvarez et al. [125], histone H1 attaches to the nucleosome core, shields the linker DNA between nucleosomes which compacts the chromatin and results in secondary chromatin structures that lowers its expression. More interestingly, in the inoculated resistant cultivar, there was reduced cellular transcription linked to the Rb-EF2 complex due to the downregulation of the genes encoding retinoblastoma protein (Rb) that regulates the cell cycle [126]. Therefore, it is hypothesised that resistant genotypes choose to delay cell division and the S phase in order to preserve cell energy against the rust fungi. Additionally, the cell cycle pathway-related gene encoding Rb-associated protein A domain was also down-regulated. In particular, DEGs that control the metabolism of nitrogen compounds, RNA and nucleobase-containing compounds ultimately regulate cellular transcription in resistant cultivars upon infection [58].

7. Discussion and conclusion

Wheat researchers have identified numerous quantitative trait loci (QTLs) that influence resistance to various rust diseases caused by *Pt*, *Pgt* and *Pst*. Studies on gene expression analysis have provided insights into how these QTLs might defend against rust. However, comparing results from different studies is challenging due to variations in genotypes, pathogen strain, technical procedure involved (artificial inoculation method, time of sampling, duration of intervals between sampling and number of samples), experimental setup during expression and expression data analysis. Additionally, many QTLs with smaller effects on rust resistance have not been studied in detail, possibly due to their subtle impact or lack of suitable resources (NILs). New approaches like expressed QTL (eQTL) analysis are being developed to combine gene expression data with QTL mapping. This helps to locate chromosomal regions having genes that influence wheat resistance against rust. A recent eQTL study identified a novel rust resistance QTL (*Sr*24) on chromosome 3E of tetraploid wheat [50]. Further investigation, including isolating the genes underlying these QTLs, is necessary to understand how they function. Interestingly, durum wheat varieties tend to be more resistant to leaf rust compared to hexaploid bread wheat. While transcriptomic studies on durum wheat are limited, these wheats share many resistance-related QTLs with bread wheats. Recent success in transferring resistance genes *Sr*13 and *Sr*9 from bread wheat to durum wheat opens doors for further research using transcriptomics to understand how these QTLs function in the durum background [127].

Traditionally, most research has focused on understanding how wheat resists these pathogens. However, an alternative approach is to investigate what makes wheat susceptible to rust infection. Disabling these susceptibility genes using mutation or gene editing techniques (CRISPR/Cas9) could offer new strategies for disease resistance. However, this requires a deeper understanding of both the wheat defense mechanisms and the pathogen virulence factors. Despite being one of the most studied plant-microbe interactions, only a handful of candidate resistance genes identified through transcriptomics have been functionally validated in wheat. This is partly due to the limitations of genetic transformation techniques in many wheat research labs. Virus-induced gene silencing (VIGS) offers a promising alternative for analysing gene function [51]. Additionally, isolating wheat mutants lacking susceptibility genes could provide valuable resistant germplasm for breeding programs. However, the complex polyploid nature of the wheat genome presents challenges. Mutations in a single sub-genome (A, B, or D) might not always produce a noticeable effect [128], and most transcriptome studies cannot distinguish between expression changes in all three copies of a gene (homoeologous) present in the polyploid wheat. The large and intricate wheat genome also presents hurdles for some types of genetic studies [129].

In conclusion, our understanding of this economically significant plant–microbe interaction has been progressed because of gene expression profiling. As a result, transcriptomics is a well-developed platform for figuring out how plants and pathogens interact. Through transcriptome profiling, we are able to identify the key metabolic and signalling pathways that are altered during the interaction between wheat and rust in the wheat plant, indicating the distinction between resistance and susceptibility reactions which will be a valuable asset in wheat resistance breeding program.

8. Future directions

8.1. Utilization of identified S genes in breeding programme

Plant disease susceptibility can be better understood molecularly, and this knowledge may be employed to creating plants resistant to a wide range of diseases. The field is quickly progressing in determining the plant components that pathogen effectors target and delineating the mechanisms governing plant disease susceptibility. By utilising cutting-edge molecular technologies, research in this area will add to comprehension of the wheat-*Puccinia* relationship and the development of rust-resistant cultivars.

8.2. Integration of multi-omics approaches

Even though RNA-seq techniques show the relative abundance of various mRNAs inside a cell, but the expression level of the proteins that these mRNAs code for is not directly correlated with the degree of mRNA expression. The translation-initiation characteristics of the mRNA sequence have a significant impact on the quantity of protein molecules that can be synthesised using a particular mRNA molecule as a template. Hence, by considering expressed genes (Transcriptomics), proteins (Proteomics), metabolites (Metabolomics) *etc.* together, we gain a deeper understanding of how cells function in resistant and diseased condition.

8.3. Validation of candidate genes for breeding resistant wheat varieties

Validating candidate genes is a crucial step in developing wheat varieties resistant to rust disease. Since, candidate genes identified through initial methods like QTL mapping (Quantitative Trait Loci) or homology may not directly cause resistance. Validation ensures the genes play a functional role. Further, validated genes allow development of DNA markers for marker assisted selection, which helps breeders to select desirable traits more efficiently during breeding programs. It involves techniques like over-expression or knock-out of the candidate gene in wheat plants can directly assess its impact on resistance or by comparing the candidate gene sequence between resistant and susceptible varieties can reveal variations potentially linked to resistance.

8.4. Beyond conventional breeding

Transcriptomic data has significant potential to inform and optimize genome-editing approaches, particularly CRISPR/Cas9, to

develop rust-resistant wheat varieties with precision. Genes identified through transcriptomic studies as central to immune signaling, metabolic pathways, and structural defense responses could serve as prime candidates for CRISPR-based editing.

CRediT authorship contribution statement

K.K. Chetan: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Vaibhav Kumar Singh:** Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mohammad Waris Haider:** Validation, Methodology, Investigation, Formal analysis. **Mahender Singh Saharan:** Writing – review & editing, Supervision, Resources, Project administration. **Ravinder Kumar:** Writing – review & editing, Supervision, Software, Methodology, Funding acquisition, Data curation.

Consent to participate

Not applicable.

Ethics approval

Ethical approval is not applicable.

Consent for publication

Not applicable.

Code availability

Not applicable.

Data availability statement

This is a literature review, in which no original data were generated. For this reason, no data were deposited in a publicly repository. The information extracted from the primary studies has been summarised and also tables and figures were made based on literature.

Funding

This work was financially supported by ICAR-Indian Agricultural Research Institute, New Delhi-110012, India

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Corresponding authors is associate editor of this journal. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Author K.K. Chetan thanks DST for providing INSPIRE fellowship for his Ph.D. program. All authors would like to acknowledge the Directors of ICAR-Indian Agricultural Research Institute, New Delhi.

References

[1] M. Figueroa, K.E. Hammond-Kosack, P.S. Solomon, A review of wheat diseases—a field perspective, Mol. Plant Pathol. 19 (2018) 1523–1536, https://doi.org/10.1111/mpp.12618.
[2] K. Srinivas, V.K. Singh, K.K. Sameriya, O.P. Gangwar, S. Kumar, L. Prasad, G.P. Singh, Multi-environment phenotyping to identify broad-based, stable resistance in wheat germplasms against leaf and stripe rust diseases, Cereal Res. Commun. 51 (2023) 931–944, https://doi.org/10.1007/s42976-022-00315-9.
[3] K. Srinivas, V.K. Singh, B. Srinivas, K.K. Sameriya, U. Kumar, O.P. Gangwar, S. Kumar, L. Prasad, G.P. Singh, Documentation of multi-pathotype durable resistance in exotic wheat genotypes to deadly stripe and leaf rust diseases, Cereal Res. Commun. 52 (2024) 189–201, https://doi.org/10.1007/s42976-023-00364-8.
[4] R.F. Park, Stem rust of wheat in Australia, in: Aust J Agric Res, 2007, pp. 558–566, https://doi.org/10.1071/AR07117.
[5] A.N. Mishra, K.N. Tiwari, V.K. Singh, M. Sivasamy, D. Pal, S.C. Bhardwaj, O.P. Gangwar, T.L. Prakasha, K.S. Solanki, R.M. Phuke, S.V. Sai Prasad, C.N. Mishra, S.G. Sawashe, T.K. Narute, G.P. Singh, Insights into the rust resistance base of common wheat (Triticum aestivum L.) in India, Indian Phytopathol. 74 (2021) 537–548, https://doi.org/10.1007/s42360-021-00374-8.
[6] T. Nazarov, Y. Liu, X. Chen, D.R. See, Molecular mechanisms of the stripe rust interaction with resistant and susceptible wheat genotypes, International Journal of Molecular Sciences 25 (5) (2024) 2930, https://doi.org/10.3390/ijms25052930.
[7] B.C. Curtis, S. Rajaram, H. Macpherson, Bread Wheat: Improvement and Production, FAO Plant Production and Protection Series, Rome, Italy, 2002.

[8] A. William, B. Alain, V.G. Maarten, The World Wheat Book: A History of Wheat Breeding, 2011. Lavoisier, Paris, France.
[9] K Singh Vaibhav, M S Saharan, D Kamil, B M Bashyal, M S Gurjar, PopulationDiversity, Pathogenomics and Development of Diagnostics of Emerging Fungal PlantPathogens - A Training Manual, TB-ICN: 310/2023. ICAR-Indian Agricultural ResearchInstitute (IARI), New Delhi. pp. 171, in: (PDF) Population Diversity, Pathogenomics and Development of Diagnostics of Emerging Fungal Plant Pathogens- A Training Manual, 2023. Available from: https://www.researchgate.net/publication/377473061_Population_Diversity_Pathogenomics_and_Development_of_Diagnostics_of_Emerging_Fungal_Plant_Pathogens-_A_Training_Manual [accessed Nov 29 2024].
[10] X. Chen, T. Coram, X. Huang, M. Wang, A. Dolezal, Send Orders of Reprints at, Understanding molecular mechanisms of durable and non-durable resis-tance to stripe rust in wheat using a transcriptomics approach. r eprints@benthamscience.net, 2013. www.affymetrix.com.
[11] C. Zipfel, Early molecular events in PAMP-triggered immunity, Curr. Opin. Plant Biol. 12 (2009) 414–420, https://doi.org/10.1016/j.pbi.2009.06.003.
[12] N. Chakraborty, J. Basak, C. Nibedita, B. Jolly, Transcriptomics: a successful approach to unravel the molecular mechanism of plant-pathogen interaction in post-genomic era. https://www.researchgate.net/publication/318948718, 2017.
[13] S. Nathan, Transcriptome profiling to understand host-bacteria interactions: past, present and future, Sci. Asia 46 (2020) 503–513, https://doi.org/10.2306/SCIENCEASIA1513-1874.2020.083.
[14] L. Zhang, H. Meakin, M. Dickinson, Isolation of genes expressed during compatible interactions between leaf rust (Puccinia triticina) and wheat using cDNA-AFLP, Mol. Plant Pathol. 4 (2003) 469–477, https://doi.org/10.1046/j.1364-3703.2003.00192.x.
[15] S. Dickinson, Studies in the physiology of obligate parasitism, Phytopathology 66 (1969) 38–49.
[16] R. Allen, A cytological study of Puccinia triticina physiologic form II on Little Club wheat, J. Agric. Res. 33 (1926) 201–222.
[17] L.J. Szabo, W.R. Bushnell, Hidden robbers: the role of fungal haustoria in parasitism of plants. www.pnas.orgcgidoi10.1073pnas.151262398, 1972.
[18] M. Hahn, K. Mendgen, Signal and nutrient exchange at biotrophic plant-fungus interfaces. http://nbn-resolving.de/urn:nbn:de:bsz:352-opus-37201, 2001.
[19] K. Mendgen, C. Struck, R.T. Voegele, M. Hahn, Biotrophy and rust haustoria, Physiol. Mol. Plant Pathol. 56 (2000) 141–145, https://doi.org/10.1006/pmpp.2000.0264.
[20] H. Zhang, C. Wang, Y. Cheng, X. Chen, Q. Han, L. Huang, G. Wei, Z. Kang, Histological and cytological characterization of adult plant resistance to wheat stripe rust, Plant Cell Rep. 31 (2012) 2121–2137, https://doi.org/10.1007/s00299-012-1322-0.
[21] J. Heller, P. Tudzynski, Reactive oxygen species in phytopathogenic fungi: signaling, development, and disease, Annu. Rev. Phytopathol. 49 (2011) 369–390, https://doi.org/10.1146/annurev-phyto-072910-095355.
[22] M. Ali, Z. Cheng, H. Ahmad, S. Hayat, Reactive oxygen species (ROS) as defenses against a broad range of plant fungal infections and case study on ros employed by crops against verticillium dahlia wilts, J. Plant Interact. 13 (2018) 353–363, https://doi.org/10.1080/17429145.2018.1484188.
[23] I.P. de León, M. Montesano, Activation of defense mechanisms against pathogens in mosses and flowering plants, Int. J. Mol. Sci. 14 (2013) 3178–3200, https://doi.org/10.3390/ijms14023178.
[24] O. Bozkurt, T. Unver, M.S. Akkaya, Genes associated with resistance to wheat yellow rust disease identified by differential display analysis, Physiol. Mol. Plant Pathol. 71 (2007) 251–259, https://doi.org/10.1016/j.pmpp.2008.03.002.
[25] S.K. Jha, Expression Profiling of Leaf Rust Resistance (Lr) Gene in Wheat (Triticum aestivum), Indian Agricultural Research Institute, 2011.
[26] X. Wang, W. Liu, X. Chen, C. Tang, X. Huang, Z. Kang, Differential gene expression in incompatible interaction between wheat and stripe rust fungus revealed by cDNA-AFLP and comparison to compatible interaction, BMC Plant Biol. 10 (2010) 1–15.
[27] T.E. Coram, M. Wang, X. Chen, Transcriptome analysis of the wheat-Puccinia striiformis f. sp. tritici interaction, Mol. Plant Pathol. 9 (2008) 157–169, https://doi.org/10.1111/j.1364-3703.2007.00453.x.
[28] B. Fofana, T.W. Banks, B. McCallum, S.E. Strelkov, S. Cloutier, Temporal gene expression profiling of the wheat leaf rust pathosystem using cDNA microarray reveals differences in compatible and incompatible defence pathways, Int. J. Plant Genom. 2007 (2007), https://doi.org/10.1155/2007/17542.
[29] R.K. Varshney, S.N. Nayak, G.D. May, S.A. Jackson, Next-generation sequencing technologies and their implications for crop genetics and breeding, Trends Biotechnol. 27 (2009) 522–530, https://doi.org/10.1016/j.tibtech.2009.05.006.
[30] R. Liu, J. Lu, L. Zhang, Y. Wu, Transcriptomic insights into the molecular mechanism of wheat response to stripe rust fungus, Heliyon 8 (2022) e10951, https://doi.org/10.1016/j.heliyon.2022.e10951.
[31] N.A. Mueth, S.H. Hulbert, Dual host-pathogen small RNA sequencing during wheat stem rust infection, BMC Res. Notes 16 (2023), https://doi.org/10.1186/s13104-023-06426-8.
[32] P. Liang, A.B. Pardee, Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction, Science 257 (5072) (1992) 967–971.
[33] S. Harris, D.B. Jones, Optimization of the polymerase chain reaction, Br. J. Biomed. Sci. 54 (1997) 166–173.
[34] P. Vos, R. Hogers, M. Bleeker, M. Reijans, T. Van De Lee, M. Homes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, M. Zabeau, AFLP: a new technique for DNA fingerprinting. https://academic.oup.com/nar/article/23/21/4407/1076973, 1995.
[35] L. Diatchenko, Y.-F.C. Laut, A.P. Campbellt, A. Chenchik, F. Moqadam, B. Huang, S. Lukyanovt, K. Lukyanovt, N. Gurskayat, E.D. Sverdlovt, P.D. Siebert, Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. https://www.pnas.org, 1996.
[36] P.R.J. Birch, S. Kamoun, December 2000 New technologies for life sciences: a Trends Guide | Review Studying interaction transcriptomes: coordinated analyses of gene expression during plant-microorganism interactions. http://trends.com, 2000.
[37] S. Kamoun, P. Hraber, B. Sobral, D. Nuss, F. Govers, Initial assessment of gene diversity for the oomycete pathogen phytophthora infestans based on expressed sequences. http://www.ncgr.org/pgi, 1999.
[38] V.E. Velculescu, L. Zhang, B. Vogelstein, K.W. Kinzler, Serial analysis of gene expression, Science (1979) 270 (1995) 484–487.
[39] M. Margulies, M. Egholm, W.E. Altman, S. Attiya, J.S. Bader, L.A. Bemben, J. Berka, M.S. Braverman, Y.J. Chen, Z. Chen, S.B. Dewell, L. Du, J.M. Fierro, X. V. Gomes, B.C. Godwin, W. He, S. Helgesen, C.H. Ho, G.P. Irzyk, S.C. Jando, M.L.I. Alenquer, T.P. Jarvie, K.B. Jirage, J.B. Kim, J.R. Knight, J.R. Lanza, J. H. Leamon, S.M. Lefkowitz, M. Lei, J. Li, K.L. Lohman, H. Lu, V.B. Makhijani, K.E. McDade, M.P. McKenna, E.W. Myers, E. Nickerson, J.R. Nobile, R. Plant, B. P. Puc, M.T. Ronan, G.T. Roth, G.J. Sarkis, J.F. Simons, J.W. Simpson, M. Srinivasan, K.R. Tartaro, A. Tomasz, K.A. Vogt, G.A. Volkmer, S.H. Wang, Y. Wang, M.P. Weiner, P. Yu, R.F. Begley, J.M. Rothberg, Genome sequencing in microfabricated high-density picolitre reactors, Nature 437 (2005) 376–380, https://doi.org/10.1038/nature03959.
[40] A. Barski, S. Cuddapah, K. Cui, T.Y. Roh, D.E. Schones, Z. Wang, G. Wei, I. Chepelev, K. Zhao, High-resolution profiling of histone methylations in the human genome, Cell 129 (2007) 823–837, https://doi.org/10.1016/j.cell.2007.05.009.
[41] A.J. Westermann. Dual RNA-seq of Pathogen and Host: Duale RNA-Sequenzierung Eines Pathogens und Seines Wirts, 2014 (Doctoral dissertation, Julius-Maximilians-Universität Würzburg).
[42] N.J. Loman, A.R. Quinlan, Poretools: a tool kit for analyzing nanopore sequence data, Bioinformatics 30 (2014) 3399–3401.
[43] D.J. Mares, S. Cousen, The interaction of yellow rust (Puccinia striiformis) with winter wheat cultivars showing adult plant resistance: macroscopic and microscopic events associated with the resistant reaction, Physiol. Plant Pathol. 10 (1977), https://doi.org/10.1016/0048-4059(77)90030-3.
[44] J. Moldenhauer, B.M. Moerschbacher, A.J. Van Der Westhuizen, Histological investigation of stripe rust (Puccinia striiformis f.sp. tritici) development in resistant and susceptible wheat cultivars, Plant Pathol. 55 (2006) 469–474, https://doi.org/10.1111/j.1365-3059.2006.01385.x.
[45] Z.S. Kang, L. Huang, H. Buchenauer, Ultrastructural changes and localization of lignin and callose in compatible and incompatible interactions between wheat and Puccinia striiformis, J. Plant Dis. Prot. 109 (2002) 25–37.
[46] F.J. Kloppers, Z.A. Piietorius, Histology of the infection and development of Puccinia recondita f.sp. tritici in a wheat line with Lr37, J. Phytopathology 143 (1995) 261–267.
[47] R.A. Caldo, D. Nettleton, R.P. Wise, Interaction-dependent gene expression in Mla-specified response to barley powdery mildew, Plant Cell 16 (2004) 2514–2528, https://doi.org/10.1105/tpc.104.023382.
[48] M. Van De Mortel, J.C. Recknor, M.A. Graham, D. Nettleton, J.D. Dittman, R.T. Nelson, C. V Godoy, R. V Abdelnoor, Á.M.R. Almeida, T.J. Baum, S.A. Whitham, Distinct biphasic mRNA changes in response to asian soybean rust infection, 887 MPMI 20 (2007) 887–899, https://doi.org/10.1094/MPMI.

[49] Y. Tao, Z. Xie, W. Chen, J. Glazebrook, H.S. Chang, B. Han, T. Zhu, G. Zou, F. Katagiri, Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen Pseudomonas syringae, Plant Cell 15 (2003) 317–330, https://doi.org/10.1105/tpc.007591.

[50] G. Vishwakarma, A. Saini, S.C. Bhardwaj, S. Kumar, B.K. Das, Comparative transcriptomics of stem rust resistance in wheat NILs mediated by Sr24 rust resistance gene, PLoS One 18 (2023), https://doi.org/10.1371/journal.pone.0295202.

[51] Z. Wu, G. Zhang, R. Zhao, Q. Gao, J. Zhao, X. Zhu, F. Wang, Z. Kang, X. Wang, Transcriptomic analysis of wheat reveals possible resistance mechanism mediated by Yr10 to stripe rust, Stress Biology 3 (2023), https://doi.org/10.1007/s44154-023-00115-z.

[52] S. Kumar, Z. Wang, T.W. Banks, M.C. Jordan, B.D. McCallum, S. Cloutier, Lr1-mediated leaf rust resistance pathways of transgenic wheat lines revealed by a gene expression study using the Affymetrix GeneChip® Wheat Genome Array, Mol. Breed. 34 (2014) 127–141, https://doi.org/10.1007/s11032-014-0022-6.

[53] T. Nurnberger, D. Scheel, Signal transmission in the plant immune response, Trends Plant Sci. 6 (2001) 372–379.

[54] CJ Marshall, MAP kinase kinase kinase, MAP kinase kinase and MAP kinase, Curr Opin Genet Dev 4 (1) (1994 Feb) 82–89, https://doi.org/10.1016/0959-437x(94)90095-7.

[55] R. Seger, E.G. Krebs, The MAPK signaling cascade, The FASEB journal 9 (9) (1995) 726–735.

[56] T. Eulgem, I.E. Somssich, Networks of WRKY transcription factors in defense signaling, Curr. Opin. Plant Biol. 10 (2007) 366–371, https://doi.org/10.1016/j.pbi.2007.04.020.

[57] S. Chandra, D. Singh, J. Pathak, S. Kumari, M. Kumar, R. Poddar, H.S. Balyan, P.K. Gupta, K.V. Prabhu, K. Mukhopadhyay, De novo assembled wheat transcriptomes delineate differentially expressed host genes in response to leaf rust infection, PLoS One 11 (2016), https://doi.org/10.1371/journal.pone.0148453.

[58] S. Dorostkar, A. Dadkhodaie, E. Ebrahimie, B. Heidari, M. Ahmadi-Kordshooli, Comparative transcriptome analysis of two contrasting resistant and susceptible Aegilops tauschii accessions to wheat leaf rust (Puccinia triticina) using RNA-sequencing, Sci. Rep. 12 (2022), https://doi.org/10.1038/s41598-021-04329-x.

[59] Z.A. Mir, D. Chauhan, A.K. Pradhan, V. Srivastava, D. Sharma, N. Budhlakoti, D.C. Mishra, V. Jadon, T.K. Sahu, M. Grover, O.P. Gangwar, S. Kumar, S. C. Bhardwaj, J.C. Padaria, A.K. Singh, A. Rai, G.P. Singh, S. Kumar, Comparative transcriptome profiling of near isogenic lines PBW343 and FLW29 to unravel defense related genes and pathways contributing to stripe rust resistance in wheat, Funct. Integr. Genomics 23 (2023), https://doi.org/10.1007/s10142-023-01104-1.

[60] K. Zhang, D. Yue, W. Wei, Y. Hu, J. Feng, Z. Zou, Characterization and functional analysis of calmodulin and calmodulin-like genes in Fragaria vesca, Front. Plant Sci. 7 (2016), https://doi.org/10.3389/fpls.2016.01820.

[61] X. Gao, K.L. Cox, P. He, Functions of calcium-dependent protein kinases in plant innate immunity, Plants 3 (2014) 160–176, https://doi.org/10.3390/plants3010160.

[62] M. Boudsocq, M.R. Willmann, M. McCormack, H. Lee, L. Shan, P. He, J. Bush, S.H. Cheng, J. Sheen, Differential innate immune signalling via Ca 2+ sensor protein kinases, Nature 464 (2010) 418–422, https://doi.org/10.1038/nature08794.

[63] B. Li, X. Meng, L. Shan, P. He, Transcriptional regulation of pattern-triggered immunity in plants, Cell Host Microbe 19 (2016) 641–650, https://doi.org/10.1016/j.chom.2016.04.011.

[64] D.R. Bergey, R. Kandel, B.K. Tyree, M. Dutt, S.A. Dhekney, The Role of Calmodulin and Related Proteins in Plant Cell Function: an Ever-Thickening Plot, Springer Sci Rev, 2014, https://doi.org/10.1007/s40362-014-0025-z.

[65] D. Tang, G. Wang, J.M. Zhou, Receptor kinases in plant-pathogen interactions: more than pattern recognition, Plant Cell 29 (2017) 618–637, https://doi.org/10.1105/tpc.16.00891.

[66] P.H. Fesel, A. Zuccaro, β-glucan: crucial component of the fungal cell wall and elusive MAMP in plants, Fungal Genet. Biol. 90 (2016) 53–60, https://doi.org/10.1016/j.fgb.2015.12.004.

[67] S.H. Spoel, A. Koornneef, S.M.C. Claessens, J.P. Korzelius, J.A. Van Pelt, M.J. Mueller, A.J. Buchala, J.P. Métraux, R. Brown, K. Kazan, L.C. Van Loon, X. Dong, C.M.J. Pieterse, NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol, Plant Cell 15 (2003) 760–770, https://doi.org/10.1105/tpc.009159.

[68] X. Zhu, X. Li, Q. He, D. Guo, C. Liu, J. Cao, Z. Wu, Z. Kang, X. Wang, TaMYB29: a novel R2R3-MYB transcription factor involved in wheat defense against stripe rust, Front. Plant Sci. 12 (2021), https://doi.org/10.3389/fpls.2021.783388.

[69] X. Wang, H. Zhang, B. Nyamesorto, Y. Luo, X. Mu, F. Wang, Z. Kang, E. Lagudah, L. Huang, A new mode of NPR1 action via an NB-ARC–NPR1 fusion protein negatively regulates the defence response in wheat to stem rust pathogen, New Phytol. 228 (2020) 959–972, https://doi.org/10.1111/nph.16748.

[70] K. Kazan, J.M. Manners, Linking development to defense: auxin in plant-pathogen interactions, Trends Plant Sci. 14 (2009) 373–382, https://doi.org/10.1016/j.tplants.2009.04.005.

[71] H. Zegzouti, B. Jones, P. Frasse, C. Marty, B. Maitre, A. Latché, J.C. Pech, M. Bouzayen, Ethylene-regulated gene expression in tomato fruit: characterization of novel ethylene-responsive and ripening-related genes isolated by differential display, Plant J. 18 (1999) 589–600, https://doi.org/10.1046/j.1365-313X.1999.00483.x.

[72] P. Das, M. Grover, D. Chauhan, D.C. Mishra, S. Kumar, K.K. Chaturvedi, S.C. Bhardwaj, A. Kumar Singh, A. Rai, Comparative transcriptome analysis of wheat isogenic lines provides insights into genes and pathways associated with stripe rust resistance. https://doi.org/10.31742/ISGPB.83.1.7, 2023.

[73] B.A.T. Adie, J. Pérez-Pérez, M.M. Pérez-Pérez, M. Godoy, J.J. Sánchez-Serrano, E.A. Schmelz, R. Solano, ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis, Plant Cell 19 (2007) 1665–1681, https://doi.org/10.1105/tpc.106.048041.

[74] J. Ton, B. Mauch-Mani, β-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose, Plant J. 38 (2004) 119–130, https://doi.org/10.1111/j.1365-313X.2004.02028.x.

[75] M.C. Heath. Hypersensitive response-related death. *Programmed cell death in higher plants*, 2000, pp. 77–90.

[76] L.A.J. Mur, I.E. Santosa, L.J.J. Laarhoven, N.J. Holton, F.J.M. Harren, A.R. Smith, Laser photoacoustic detection allows in planta detection of nitric oxide in tobacco following challenge with avirulent and virulent Pseudomonas syringae pathovars, Plant Physiol. 138 (2005) 1247–1258, https://doi.org/10.1104/pp.104.055772.

[77] C.S. Bestwick, I.R. Brown, J.W. Mansfield, Localized Changes in Peroxidase Activity Accompany Hydrogen Peroxide Generation during the Development of a Nonhost Hypersensitive Reaction in Lettuce 1, n.d.

[78] HM Do, JK Hong, HW Jung, SH Kim, JH Ham, BK Hwang, Expression of peroxidase-like genes, H2O2 production, and peroxidase activity during the hypersensitive response to Xanthomonas campestris pv. vesicatoria in Capsicum annuum, Mol Plant Microbe Interact 16 (3) (2003 Mar) 196–205, https://doi.org/10.1094/MPMI.2003.16.3.196.

[79] N. Wang, X. Fan, M. He, Z. Hu, C. Tang, S. Zhang, D. Lin, P. Gan, J. Wang, X. Huang, C. Gao, Z. Kang, X. Wang, Transcriptional repression of TaNOX10 by TaWRKY19 compromises ROS generation and enhances wheat susceptibility to stripe rust, Plant Cell 34 (2022) 1784–1803, https://doi.org/10.1093/plcell/koac001.

[80] M. Yuan, B.P.M. Ngou, P. Ding, X.F. Xin, PTI-ETI crosstalk: an integrative view of plant immunity, Curr. Opin. Plant Biol. 62 (2021), https://doi.org/10.1016/j.pbi.2021.102030.

[81] C.F. Wang, L.L. Huang, H. Buchenauer, Q.M. Han, H.C. Zhang, Z.S. Kang, Histochemical studies on the accumulation of reactive oxygen species (O2- and H2O2) in the incompatible and compatible interaction of wheat-Puccinia striiformis f. sp. tritici, Physiol. Mol. Plant Pathol. 71 (2007) 230–239, https://doi.org/10.1016/j.pmpp.2008.02.006.

[82] P. Prasad, S. S Siddanna, C. Bhardwaj, P.L. Kashyap, O.P. Gangwar, H. Khan, S. Kumar, R. Kumar, VPatil, Stage-specific reprogramming of defense responsive genes during Lr24-mediated leaf rust resistance in wheat, J. Plant Pathol. 101 (2019) 283–293.

[83] S. Yin, Z. Gao, C. Wang, L. Huang, Z. Kang, H. Zhang, Nitric oxide and reactive oxygen species coordinately regulate the germination of Puccinia striiformis f. sp. tritici urediniospores, Front. Microbiol. 7 (2016), https://doi.org/10.3389/fmicb.2016.00178.

[84] L.A.J. Mur, E. Prats, S. Pierre, M.A. Hall, K.H. Hebelstrup, Integrating nitric oxide into salicylic acid and jasmonic acid/ethylene plant defense pathways, Front. Plant Sci. 4 (2013), https://doi.org/10.3389/fpls.2013.00215.

[85] M. Poretti, A.G. Sotiropoulos, J. Graf, E. Jung, S. Bourras, S.G. Krattinger, T. Wicker, Comparative transcriptome analysis of wheat lines in the field reveals multiple essential biochemical pathways suppressed by obligate pathogens, Front. Plant Sci. 12 (2021), https://doi.org/10.3389/fpls.2021.720462.

[86] L.V. Bindschedler, J. Dewdney, K.A. Blee, J.M. Stone, T. Asai, J. Plotnikov, C. Denoux, T. Hayes, C. Gerrish, D.R. Davies, F.M. Ausubel, G.P. Bolwell, Peroxidase-dependent apoplastic oxidative burst in Arabidopsis required for pathogen resistance, Plant J. 47 (2006) 851–863, https://doi.org/10.1111/j.1365-313X.2006.02837.x.

[87] T.E. Coram, E.C.K. Pang, Expression profiling of chickpea genes differentially regulated during a resistance response to Ascochyta rabiei, Plant Biotechnol. J. 4 (2006) 647–666, https://doi.org/10.1111/j.1467-7652.2006.00208.x.

[88] O. Otte, W. Barz, Characterization and oxidative in vitro cross-linking of an extensin-like protein and a proline-rich protein purified from chickpea cell walls, Phytochemistry 53 (1) (2000) 1–5.

[89] P. Bonello, J.T. Blodgett, Pinus nigra-Sphaeropsis sapinea as a model pathosystem to investigate local and systemic effects of fungal infection of pines, Physiol. Mol. Plant Pathol. 63 (2003) 249–261, https://doi.org/10.1016/j.pmpp.2004.02.002.

[90] Q. Hu, L. Min, X. Yang, S. Jin, L. Zhang, Y. Li, Y. Ma, X. Qi, D. Li, H. Liu, K. Lindsey, L. Zhu, X. Zhang, Laccase GhLac1 modulates broad-spectrum biotic stress tolerance via manipulating phenylpropanoid pathway and jasmonic acid synthesis, Plant Physiol. 176 (2018) 1808–1823, https://doi.org/10.1104/pp.17.01628.

[91] J.R. Aist, Papillae and related wound plugs of plant cells, Annual Review of Phytopathology 14 (1) (1976) 145–163.

[92] B.A. Stone, A.E. Clarke, Chemistry and Biology of (1→3) β Glucans, La Trobe University Press, Bundoora, 1992.

[93] C. Manjunatha, Development of DNA Based Detection Assay for Puccinia Triticina and Gene Expression Studies during Host Pathogen Interaction , Doctoral Thesis, ICAR-Indian Agricultural Research Institute, 2015.

[94] V. Göhre, A.M.E. Jones, J. Sklenář, S. Robatzek, A.P.M. Weber, Molecular Crosstalk between PAMP-Triggered Immunity and Photosynthesis, vol. 25, 2012, pp. 1083–1092, https://doi.org/10.1094/MPMI.

[95] Y. Lu, J. Yao, Chloroplasts at the crossroad of photosynthesis, pathogen infection and plant defense, Int. J. Mol. Sci. 19 (2018), https://doi.org/10.3390/ijms19123900.

[96] S. Kangasjärvi, M. Tikkanen, G. Durian, E.M. Aro, Photosynthetic light reactions - an adjustable hub in basic production and plant immunity signaling, Plant Physiol. Biochem. 81 (2014) 128–134, https://doi.org/10.1016/j.plaphy.2013.12.004.

[97] Q. Xu, C. Tang, X. Wang, S. Sun, J. Zhao, Z. Kang, X. Wang, An effector protein of the wheat stripe rust fungus targets chloroplasts and suppresses chloroplast function, Nat. Commun. 10 (2019), https://doi.org/10.1038/s41467-019-13487-6.

[98] M.D. Bolton, Primary Metabolism and Plant Defense-Fuel for the Fire,/487 MPMI 22 (2009) 487–497, https://doi.org/10.1094/MPMI.

[99] Y. Hao, T. Wang, K. Wang, X. Wang, Y. Fu, L. Huang, Z. Kang, Transcriptome analysis provides insights into the mechanisms underlying wheat plant resistance to stripe rust at the adult plant stage, PLoS One 11 (2016), https://doi.org/10.1371/journal.pone.0150717.

[100] S. Wang, Q.P. Li, J. Wang, Y. Yan, G.L. Zhang, H. Zhang, J. Wu, F. Chen, X. Wang, Z. Kang, J. Dubcovsky, J.Y. Gou, YR36/WKS1-Mediated phosphorylation of PsbO, an extrinsic member of photosystem II, inhibits photosynthesis and confers stripe rust resistance in wheat, Mol. Plant 12 (2019) 1639–1650, https://doi.org/10.1016/j.molp.2019.10.005.

[101] D. Chauhan, D.C. Mishra, S. Mittal, S. Rani, J. Bhati, S. Kumar, S.C. Bhardwaj, M. Grover, N. Budhlakoti, S. Khan, Identification of hub genes associated with stripe rust disease in wheat through integrative transcriptome and gene-based association study, South Afr. J. Bot. 171 (2024) 583–591, https://doi.org/10.1016/j.sajb.2024.06.038.

[102] X. Wang, C. Tang, G. Zhang, Y. Li, C. Wang, B. Liu, Z. Qu, J. Zhao, Q. Han, L. Huang, X. Chen, Z. Kang, cDNA-AFLP analysis reveals differential gene expression in compatible interaction of wheat challenged with Puccinia striiformis f. sp. tritici, BMC Genom. 10 (2009), https://doi.org/10.1186/1471-2164-10-289.

[103] A.M. Catanzariti, P.N. Dodds, J.G. Ellis, Avirulence proteins from haustoria-forming pathogens, FEMS Microbiol. Lett. 269 (2007) 181–188, https://doi.org/10.1111/j.1574-6968.2007.00684.x.

[104] R.T. Voegele, K. Mendgen, Rust haustoria: nutrient uptake and beyond, New Phytol. 159 (2003) 93–100, https://doi.org/10.1046/j.1469-8137.2003.00761.x.

[105] J. Zhao, W. Duan, Y. Xu, C. Zhang, L. Wang, J. Wang, S. Tian, G. Pei, G. Zhan, H. Zhuang, J. Zhao, Z. Kang, Distinct transcriptomic reprogramming in the wheat stripe rust fungus during the initial infection of wheat and barberry, MPMI (Mol. Plant-Microbe Interact.) 34 (2021) 198–209, https://doi.org/10.1094/MPMI-08-20-0244-R.

[106] T.E. Coram, M. Wang, X. Chen, Transcriptome analysis of the wheat-Puccinia striiformis f. sp. tritici interaction, Mol. Plant Pathol. 9 (2008) 157–169, https://doi.org/10.1111/j.1364-3703.2007.00453.x.

[107] L. Qiao, J. Luo, H. Wang, Y. Kong, T. Du, P. Qin, B. Yang, Integrated genome-wide association and transcriptomic analysis to identify receptor kinase genes to stripe rust resistance in wheat germplasm from southwestern China, BMC Plant Biol. 24 (2024) 319, https://doi.org/10.1186/s12870-024-05020-9.

[108] V. Klymiuk, E. Yaniv, L. Huang, D. Raats, A. Fatiukha, S. Chen, L. Feng, Z. Frenkel, T. Krugman, G. Lidzbarsky, W. Chang, M.J. Jääskeläinen, C. Schudoma, L. Paulin, P. Laine, H. Bariana, H. Sela, K. Saleem, C.K. Sørensen, M.S. Hovmøller, A. Distelfeld, B. Chalhoub, J. Dubcovsky, A.B. Korol, A.H. Schulman, T. Fahima, Cloning of the wheat Yr15 resistance gene sheds light on the plant tandem kinase-pseudokinase family, Nat. Commun. 9 (2018), https://doi.org/10.1038/s41467-018-06138-9.

[109] J.M. Mcdowell, M. Dhandaydham, T.A. Long, M.G.M. Aarts, S. Goff, E.B. Holub, J.L. Dangl, Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the RPP8 locus of arabidopsis. https://academic.oup.com/plcell/article/10/11/1861/5999428, 1998.

[110] Z.-H. He, I. Cheeseman, D. He, B.D. Kohorn, A cluster of five cell wall-associated receptor kinase genes, Wak1-5, are expressed in specific organs of Arabidopsis. http://nasc.nott.ac.uk/, 1999.

[111] N. Wang, C. Tang, X. Fan, M. He, P. Gan, S. Zhang, Z. Hu, X. Wang, T. Yan, W. Shu, L. Yu, J. Zhao, J. He, L. Li, J. Wang, X. Huang, L. Huang, J.M. Zhou, Z. Kang, X. Wang, Inactivation of a wheat protein kinase gene confers broad-spectrum resistance to rust fungi, Cell 185 (2022) 2961–2974.e19, https://doi.org/10.1016/j.cell.2022.06.027.

[112] P. Ling, M. Wang, X. Chen, K.G. Campbell, Construction and characterization of a full-length cDNA library for the wheat stripe rust pathogen (Puccinia striiformis f. sp. tritici), BMC Genom. 8 (2007), https://doi.org/10.1186/1471-2164-8-145.

[113] H. Zhang, Y. Yang, C. Wang, M. Liu, H. Li, Y. Fu, Y. Wang, Y. Nie, X. Liu, W. Ji, Large-scale transcriptome comparison reveals distinct gene activations in wheat responding to stripe rust and powdery mildew, BMC Genom. 15 (2014), https://doi.org/10.1186/1471-2164-15-898.

[114] Y. Chen, F. Li, L. Tian, M. Huang, R. Deng, X. Li, W. Chen, P. Wu, M. Li, H. Jiang, G. Wu, The phenylalanine ammonia lyase gene ljpal1 is involved in plant defense responses to pathogens and plays diverse roles in lotus japonicus-rhizobium symbioses, Mol. Plant Microbe Interact. 30 (2017) 739–753, https://doi.org/10.1094/MPMI-04-17-0080-R.

[115] N. Li, X. Han, D. Feng, D. Yuan, L.J. Huang, Signaling crosstalk between salicylic acid and ethylene/Jasmonate in plant defense: do we understand what they are whispering? Int. J. Mol. Sci. 20 (2019) https://doi.org/10.3390/ijms20030671.

[116] J. Wang, F. Tao, F. An, Y. Zou, W. Tian, X. Chen, X. Xu, X. Hu, Wheat transcription factor TaWRKY70 is positively involved in high-temperature seedling plant resistance to Puccinia striiformis f. sp. tritici, Mol. Plant Pathol. 18 (2017) 649–661, https://doi.org/10.1111/mpp.12425.

[117] J. Li, G. Brader, E.T. Palva, The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense, Plant Cell 16 (2004) 319–331, https://doi.org/10.1105/tpc.016980.

[118] Y. Hu, Q. Dong, D. Yu, Arabidopsis WRKY46 coordinates with WRKY70 and WRKY53 in basal resistance against pathogen Pseudomonas syringae, Plant Sci. 185–186 (2012) 288–297, https://doi.org/10.1016/j.plantsci.2011.12.003.

[119] K. Lu, S. Liang, Z. Wu, C. Bi, Y.T. Yu, X.F. Wang, D.P. Zhang, Overexpression of an Arabidopsis cysteine-rich receptor-like protein kinase, CRK5, enhances abscisic acid sensitivity and confers drought tolerance, J. Exp. Bot. 67 (2016) 5009–5027, https://doi.org/10.1093/jxb/erw266.

[120] O. Lorenzo, J.M. Chico, J.J. Sánchez-Serrano, R. Solano, JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in arabidopsis, Plant Cell 16 (2004) 1938–1950, https://doi.org/10.1105/tpc.022319.

[121] C. Yamamura, E. Mizutani, K. Okada, H. Nakagawa, S. Fukushima, A. Tanaka, S. Maeda, T. Kamakura, H. Yamane, H. Takatsuji, M. Mori, Diterpenoid phytoalexin factor, a bHLH transcription factor, plays a central role in the biosynthesis of diterpenoid phytoalexins in rice, Plant J. 84 (2015) 1100–1113, https://doi.org/10.1111/tpj.13065.
[122] P.A. Tobias, D.I. Guest, C. Külheim, R.F. Park, De novo transcriptome study identifies candidate genes involved in resistance to Austropuccinia psidii (myrtle rust) in Syzygium luehmannii (riberry), Phytopathology 108 (2018) 627–640, https://doi.org/10.1094/PHYTO-09-17-0298-R.
[123] M.D. Bolton, J.A. Kolmer, W.W. Xu, D.F. Garvin, Lr34-Mediated leaf rust resistance in wheat: transcript profiling reveals a high energetic demand supported by transient recruitment of multiple metabolic pathways, 1515 MPMI 21 (2008) 1515–1527, https://doi.org/10.1094/MPMI.
[124] B. Nyamesorto, H. Zhang, M. Rouse, M. Wang, X. Chen, L. Huang, A transcriptomic-guided strategy used in identification of a wheat rust pathogen target and modification of the target enhanced host resistance to rust pathogens, Front. Plant Sci. 13 (2022), https://doi.org/10.3389/fpls.2022.962973.
[125] M.E. Alvarez, F. Nota, D.A. Cambiagno, Epigenetic control of plant immunity, Mol. Plant Pathol. 11 (2010) 563–576, https://doi.org/10.1111/j.1364-3703.2010.00621.x.
[126] J.A. Park, J.W. Ahn, Y.K. Kim, J.K. Su, J.K. Kim, T.K. Woo, H.S. Pai, Retinoblastoma protein regulates cell proliferation, differentiation, and endoreduplication in plants, Plant J. 42 (2005) 153–163, https://doi.org/10.1111/j.1365-313X.2005.02361.x.
[127] R.P. Singh, Occurrence and Impact of a New Leaf Rust Race on Durum Wheat in Northwestern Mexico from 2001 to 2003, 2004.
[128] T.L. Fitzgerald, J.J. Powell, J. Stiller, T.L. Weese, T. Abe, G. Zhao, J. Jia, C.L. McIntyre, Z. Li, J.M. Manners, K. Kazan, An assessment of heavy ion irradiation mutagenesis for reverse genetics in wheat (Triticum aestivum L.), PLoS One 10 (2015), https://doi.org/10.1371/journal.pone.0117369.
[129] H.C. Brewer, K.E. Hammond-Kosack, Host to a stranger: arabidopsis and Fusarium ear blight, Trends Plant Sci. 20 (2015) 651–663, https://doi.org/10.1016/j.tplants.2015.06.011.