Send Orders for Reprints to reprints@benthamscience.net

Current Genomics, 2020, *21*, 179-193

REVIEW ARTICLE

Genomic and Molecular Perspectives of Host-pathogen Interaction and Resistance Strategies against White Rust in Oilseed Mustard

Chatterjee Anupriya[1], Nirwan Shradha[1], Bandyopadhyay Prasun[2], Agnihotri Abha[3,*], Sharma Pankaj[4], Malik Zainul Abdin[5] and Shrivastava Neeraj[1,*]

[1]Amity Institute of Microbial Technology, Amity University, Uttar Pradesh, Noida-201313, India; [2]International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India; [3]Centre for Agricultural Biotechnology, Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Noida-201313, India; [4]Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida-201313, India; [5]Department of Biotechnology, Jamia Hamdard University, New Delhi-110062, India

ARTICLE HISTORY

Received: January 15, 2020
Revised: February 25, 2020
Accepted: March 14, 2020

DOI:
10.2174/1389202921999200508075410

Abstract: Oilseed brassicas stand as the second most valuable source of vegetable oil and the third most traded one across the globe. However, the yield can be severely affected by infections caused by phytopathogens. White rust is a major oomycete disease of oilseed brassicas resulting in up to 60% yield loss globally. So far, success in the development of oomycete resistant Brassicas through conventional breeding has been limited. Hence, there is an imperative need to blend conventional and frontier biotechnological means to breed for improved crop protection and yield.

This review provides a deep insight into the white rust disease and explains the oomycete-plant molecular events with special reference to *Albugo candida* describing the role of effector molecules, *A. candida* secretome, and disease response mechanism along with nucleotide-binding leucine-rich repeat receptor (NLR) signaling. Based on these facts, we further discussed the recent progress and future scopes of genomic approaches to transfer white rust resistance in the susceptible varieties of oilseed brassicas, while elucidating the role of resistance and susceptibility genes. Novel genomic technologies have been widely used in crop sustainability by deploying resistance in the host. Enrichment of NLR repertoire, over-expression of *R* genes, silencing of avirulent and disease susceptibility genes through RNA interference and CRSPR-Cas are technologies which have been successfully applied against pathogen-resistance mechanism. The article provides new insight into Albugo and Brassica genomics which could be useful for producing high yielding and WR resistant oilseed cultivars across the globe.

Keywords: *Albugo candida*, brassica, effector molecules, resistance (*R)* genes*,* nucleotide-binding leucine-rich repeats (NLRs), white rust resistance.

1. INTRODUCTION

Oilseed crops have been the backbone of several agricultural economies from antiquity and play a prominent role in agricultural industries and trade throughout the world [1]. Indian mustard (*Brassica juncea.* (L.) Czern.), a member of the Brassicaceae family, is an economically important oilseed crop cultivated globally. It is a major oilseed crop of Indian subcontinent and the second most important source of edible oil, contributing about 30% to the total edible oilseeds production in the country. It occupies 80% of the total area of oilseed Brassica cultivation in India [2]. The yield is limited due to environmental adaptability challenges and damage caused by phytopathogens. One of the major diseases of crucifer includes white rust caused by *Albugo candida* (Pers.) Kuntze resulting in up to 60% yield loss of Indian mustard globally depending upon host genotype, planting time, plant population, nutrition and climatic conditions across the globe [3].

Disease resistance to oomycete pathogens has been a major target of plant disease management programs, and also a prime focus of genomics research to identify the major resistance genes and their molecular basis of resistance.

2. WHITE RUST: DISEASE BIOLOGY

White Rust (WR), white blister and white blister rust are the common names of the disease caused by Albugo spp. in more than 400 species of plants worldwide [4-6]. Name of the disease is derived from the appearance of white pustules, due to enzymatic digestion of epidermal cell wall, on the surface of leaves and other aerial parts of the host [7]. The

*Address correspondence to these authors at the Amity Institute of Microbial Technology, Amity University, Uttar Pradesh, Noida-201313, India; Tel/Fax: +919855658406; E-mails: neersar@gmail.com; nsrivastava1@amity.edu; agnihotri.abha@gmail.com

white pustules are mass of dehydrated sporangiospores that upon re-hydration in water droplets lead to stomatal infection [8]. It is a member of the eukaryotic oomycete order Albuginales under class oomycota, which constitutes exclusively obligate biotrophic parasites with a broad plant host range [9-13]. *A. candida* exhibits obligate biotrophic nutrition being completely reliant on host tissues. *A. candida* reproduces by asexual sporangia or zoospores and extremely resistant thick-walled sexual oospores (Fig. **1**). In all species of WR pathogens, oospores are the primary source of inoculum [3]. The oospores are responsible for long-term survival in plant debris and liberated when the host tissues decay [10-13]. The presence of oospores in crop residues and/or perennial mycelium in living host (including weed) tissue enables the pathogen to survive between host growing seasons [14]. Moisture on host surfaces is essential for the germination of sporangia and infection by the zoospores. The most likely primary infection sites are the emerging cotyledons of host plants [3]. Albugo sp. enter *via* stomata, form intercellular hyphae, penetrate the plant cell wall and invaginate the plant plasma membrane with haustoria in order to take up plant nutrients and release effector proteins into host cells [12]. When zoospores come in contact with the plant leaf surface, they settle in stomata, encyst and produce a germ tube, which extends into the sub-stomatal chamber and penetrates the host cell. A primary vesicle forms in the host cell, which enables further development of intercellular hyphae in a susceptible interaction [8]. When an Albugo infection matures, zoosporangia forcibly rupture the plant epidermis and further enzymatic digestion results in characteristic "white blister" pustules [14]. Both local and systemic types of expression characterize the disease. Local infection appears as white or creamy yellow pustules or "blisters" on leaves and stems. Systemic infection results in abnormal growth, distortion of inflorescence and sterility of flowers commonly called stag-head, which appears as a result of hypertrophy and hyperplasia. Apart from *A. candida*, which infects oil yielding Brassicas and crucifer vegetables, several other Albugo species are also well-known plant pathogens causing huge yield loss in field crops of economic importance, *e.g.*, *Albugo tragopogonis* in sunflower, *Albugo ipomoeaepandurate* in sweet potato, and *Albugo occidentalis* in spinach. *A. candida* is an obligate biotrophic homothallic oomycete pathogen of white rust [15]. As per the molecular investigations, the genus Albugo constitutes ~50 (usually) specialist pathogens such as *A. laibachii* in *Arabidopsis thaliana*, and *A. candida* in *B. juncea* [16-19]. The impact of the disease is very high in Indian sub-continent as almost all released lines grown commercially in India are susceptible to this disease.

A. candida forms 24 physiological races as reported to date which infect over 200 species of plants in 63 genera from the families of Brassicaceae, Cleomaceae and Cappraceae, each of which specializes in different host species, out of which at least 10 specialize in different Brassicaceae species [20-30]. Among the identified races, race 2 (Ac2VRR) (the 'RR' suffix was added to the pathotype name Ac2v to indicate the standard isolate chosen from the collection of the late Dr. Roger Rimmer) causes a severe annual loss of oilseed mustard (*Brassica juncea* [L.] Czern. and Coss.) in India, Canada and Australia and also infects some genotypes of other *Brassica* spp., including oilseed turnip rape (*Brassica rapa* L.) [21, 30-32]. Race 1 (Ac1) infects *Raphanus sativus*, Race 4 (Ac4) infects *Capsella bursa-pastoris*, Race 5 (Ac5) infects *Sisymbrium officinale*, and Race 6 (Ac6) infects *Rorippa islandica*. Race 7 (Ac7) is largely restricted to *B. rapa*, but has also been reported to cause disease in some cultivars of *B. napus* [33], and some genotypes of *B. juncea* [31]. The Race 9 (Ac9) infects *B. oleracea*.

3. EFFECTOR MOLECULES – MAJOR WEAPON TO ESTABLISH INFECTION

The genomes of several oomycetes, including *Phytophthora infestans, Phytophthora ramorum, Phytophthora sojae*, *Pythium ultimum*, *Albugo laibachii*, *A. candida*, and *Hyaloperonospora arabidopsidis*, have been sequenced and found to encode hundreds of effector candidate proteins [12]. They are an interesting clad of microorganisms to be studied with 18 Mb to 240 Mb genome and rich secretome [8]. *A. candida* with a 45.3 Mb genome is significantly smaller than a comparable significant biotroph such as *H. arabidopsidis*. In the early phases of the interaction, the invading oomycete needs to deal with biochemical barriers in the plant apoplast to overcome the plant immunity. Both pathogen and host secrete proteins and metabolites to control the extracellular environment.

An oomycete-plant interaction is characterized by molecular coevolution, with each side battling for control over the other. Being obligate parasites they are highly specialized in suppressing the Pattern Triggered Immunity (PTI) in plants and colonizing the host keeping it alive. *Albugo* sp. enter into the plant cell *via* stomata, invaginating the plasma membrane with haustoria to take up the plant nutrients and release the effector proteins into the host cells [13]. Effector protein further leads to infection by suppressing host plant defense responses as well as adapting the host metabolism [33]. Effectors and elicitors delivered to the apoplast include protease inhibitors, glucanase inhibitors, small cysteine-rich proteins, necrosis-like proteins (NEPs) and elicitins [34, 35].

"Effectors" are secreted protein molecules that help both the invasion and the propagation of the pathogen by suppressing host plant defense responses as well as adapting the host metabolism [36]. They are studied to alter host cell structure and function, thereby facilitating infection (virulence factors or toxins) and/or triggering defense responses (avirulence factors or elicitors). Dawkins (1999), referred to the effectors as "parasite genes having phenotypic expression in host bodies and behavior" [37]. Pathogen utilizes the effector proteins to interfere with host cellular mechanisms by suppressing the host immunity for successful infection. [38-41]. The secretion of effectors is the prime mode of infection shown by the obligate parasites. Effectors can be classified into apoplastic or extracellular and cytoplasmic or intracellular on the basis of their target sites in the host plant [40, 42]. Apoplastic effectors are secreted into the plant extracellular space (host-pathogen interface), where they interact with extracellular targets and surface receptors. Three types of apoplastic effectors from oomycetes have been studied to interfere with plant processes; inhibitors of host enzymes, RGD (Arginine–Glycine–Aspartic acid) containing proteins disrupt cell wall-plasma membrane adhesions and

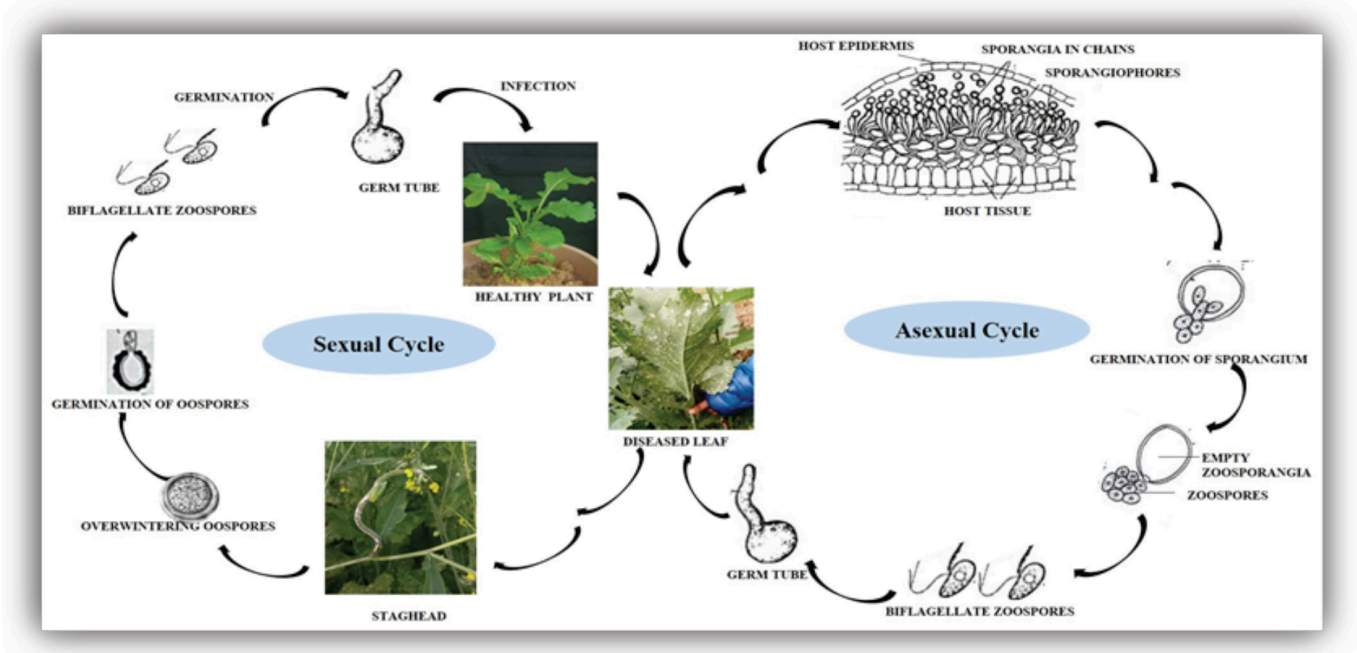

Fig. (1). *A. candida* life cycle representing the sexual and asexual cycle ([3]). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

toxins that lead to host cell death [43]. The suppression of plant defense, a consequence of PTI gets activated upon the invasion of pathogens, is key to successful infection of the host. It is evident that oomycete pathogens actively suppress innate immunity, as witnessed in *A. thaliana* by *A. candida* [44, 45]. Cytoplasmic effectors carry conserved peptide motifs at their terminals which aid them in their functioning and translocation. On the basis of the conserved motif, two important groups of translocated intracellular effectors, namely the 'RxLR-effectors' and the 'Crinklers' have been identified to be present abundantly in many plant pathogenic oomycetes. RxLR effectors (with conserved N-terminal Arg-Xaa-Leu-Arg motif) are the largest group of oomycete effector proteins [46]. Since 2005, more genome sequences of several oomycete pathogens have become available facilitating the computational analysis of the secretome in different oomycetes.

3.1. *A. Candida* Secretome

The *de novo* sequence assembly of *A. candida* revealed a small genome relative to other biotrophic oomycetes [8]. The draft genome sequence generated from the Ac2VRR, which infects the Indian mustard, reports the presence of minimal gene repertoire. Pen sequencing has helped us to assess the genome organization of *A. candida* and analyze the degree to which orthologous genes appear to have been retained relative to other oomycetes. Using BLAST, 5,975 putative orthologs to *P. ultimum*, 5,858 to *P. infestans*, 5,581 to *P. sojae*, 5,592 to *P. ramorum*, and 4,922 to *H. arabidopsidis* have been identified in *A. candida*. The genome analysis of the oomycete *H. arabidopsidis* reports a reduction in the number of genes encoding secretory pathogenicity proteins, enzymes for assimilation of inorganic nitrogen and sulphur representing a genomic signature for the evolution of obligate biotrophy [47]. Similarly, *A. candida* also lacks the assimilation enzymes such as nitrate, nitrite, and sulphite reductases, as a consequence, there is an absence of certain metabolic pathways [8]. These pathways are under investigation and not reported to date.

The Ac2VRR secretome appears to be of about 2/3 the size of the *P. infestans* secretome, a notable pathogenic oomycete, a responsible agent for Irish potato famine [48]. In contrast to the extensive family expansions observed in Phytophthora genomes, the gene families of the *A. candida* secretome have relatively few members. Sequencing of *A. candida* transcripts from infected host tissue and zoosporangia combined with genome-wide annotation revealed 15,824 predicted genes [8, 49]. Most of the predicted genes lack significant similarity with sequences from other oomycetes. There are twelve *A. candida* predicted genes that bore a similarity to other oomycetes, suggesting a shared evolutionary lineage to some of these genes amongst oomycetes [8]. A total of 929 proteins have been identified from the annotated set of *A. candida* proteins based on the criteria such as the presence of amino-terminal signal peptide, presence of a motif indicating the protein's secretory function and the absence of additional transmembrane domain [8, 50]. The *A. candida* secretome consists of proteins without transmembrane domains, reflecting its wide host range. It secretes both apoplastic and cytoplasmic types of effectors. As already discussed, *A. candida* has a much smaller repertoire of pathogenicity-related proteins as compared to Phytophthora spp. and *H. arabidopsidis* including genes that encode RXLR effector proteins, CRINKLER-like genes, and elicitins. For example, the large *A. candida* secreted protein family consists of six Crinkler-like (CRN) proteins, whereas the largest family in Phytophthora and pathogen *H. arabidopsidis* has hundreds of members [47]. Some of the gene families of *A. candida* encoded proteins, resembling those associated with the elicitation of innate defense in plants and/or compatibil-

ity in plant-microbe interactions, and the function of several proteins are yet to be discovered.

In general, the *A. candida* genome harbors fewer genes in each of the major classes of PAMP or effector-like proteins. Cellulose-binding elicitor lectin (CBEL) are elicitors, and those of oomycetes are reported to play a role in adhesion to the plant cell surface. As reported by Links *et al*. (2011) [8], the genome of *A. candida* also contains two putative *CBEL* genes: *Ac2VRR-CBEL1* and *Ac2VRR-CBEL2*. Ac2VRR-CBEL1 has a signal peptide, which may be responsible for its secretion primarily during the infection process. It also describes that Ac2VRR-CBEL2 does not contain a signal peptide indicating that it would not be secreted. Canonical structure of CBELs from Phytophthora species is a sec-dependent signal followed additionally by interleaved Cellulose Binding (CBD) and Apple Domains which mediate protein-protein or protein-carbohydrate interactions. Variation is observed amongst *CBEL* orthologs, which appears to be conserved within Oomycete genera. NEPs, capable of triggering plant cell death are not found in *A. candida,* whereas common among diverse groups of plant pathogens [51, 52].

A small group of genes in *A. candida* genome encodes secreted proteins with a variant RXLR motif (Ac-RXL). The translocation mechanism of these proteins in the plant is not fully clarified, but probably RxLR uptake may involve binding to a receptor on lipid rafts as shown for a host-targeted protein from the animal pathogenic oomycete *Saprolegnia parasitica* [53]. RxLRs and CRNs are known to defeat plant immune responses through many routes, which include reprogramming of host gene expression, altering RNA metabolism, and binding to host proteins involved in signaling [54]. Twenty-six predicted gene models with a putative sec-dependent signal peptide, non - homologous to known proteins, and had an Ac-RXL motif were estimated by string searches within the predicted proteins with amino-terminal signal peptides. Five candidate Ac-RXLs were reported to transiently induce necrosis when infiltrated into *N. benthamiana* [8]. CRNs have been proposed to be an ancient group of host-targeting proteins that evolved in the oomycete lineage, six gene models have been identified as putative CRNs. The *A. candida* CRNs form the largest secreted gene family in *A. candida*. CRN type effectors are not highly abundant in *A. candida* but are similar in number to *P. ramorum*. None of the domains present in the C- terminal as present in other oomycetes [51, 55, 56] were detected in Albugo, which supports the independent origin of Albugo effectors in the evolution of biotrophy. Systematically searched for novel host-targeting motifs amongst the proteins containing a sec-dependent signal with the program MEME [57], showed one motif namely, CHxC occurring in several proteins. Forty CHxC proteins were found, the motif showed strong conservation of two Cys residues with no detectable homolog in Phytophthora or Hyaloperonospora species. The high level of sequence divergence from other species is indicative of their specific adaptation in Albugo species. Albugo effector molecules are still not identified. The identification of Albugo effectors and their compatible plant NLR receptors will be a breakthrough, enhancing the scopes of deploying white rust resistance in the susceptible crops with economic importance, like the oilseed Brassicas.

4. PLANT RESPONSE AGAINST OOMYCETES EFFECTORS

4.1. Plant Genetic Resistance Architecture

"Genetic resistance" is a phenomenon exercised by plants upon encountering a virulent pathogen or effector molecules with the help of genetic elements to restrict the entry and establishment of pathogens. The resistance mechanism culminated by plants is a result of either expression of disease resistance genes (*R* genes) or loss or mutation of susceptibility genes (*S* genes).

Extensive studies have been executed to understand the role of the *R* gene by overexpressing them in susceptible crop varieties. The *R* genes convey plant disease resistance against pathogens by producing R proteins and have successfully proved themselves as a promising genetic source for resistance. In the 1940s, a classic "gene-for-gene" theory was established, which states that the outcome of any given plant-pathogen interaction is largely determined by a *R* gene from the host and the matching avirulence factor (avr) from the pathogen [58]. When both the *R* gene in the plant host and the cognate *avr* in the pathogen are present, the plant-pathogen interaction becomes incompatible and the host exhibits full resistance to the pathogen [59]. Guard model elucidates that the *R* gene products may not directly bind to avirulence gene products, but rather detect alterations in host proteins that are caused by the pathogen gene products [60]. The effectiveness of *R* gene-mediated resistance was first demonstrated in the early twentieth century by British scientist Rowland Biffen in wheat (Triticum sp.) breeding [61]. The majority of known R proteins are grouped into a few main classes based primarily upon their combination of a limited number of structural motifs. Most R proteins restrict only pathogen races, which express the corresponding effector protein(s) and therefore known as race-specific R proteins. Occasionally, the effective resistance is conferred against multiple races and even different pathogen species. These R proteins are called race-non-specific. The R protein structures of monocotyledonous and dicotyledonous species possess striking similarity implying that fundamental modes of pathogen recognition as well as defense signaling have been preserved through plant evolution and diversification [62]. The majority of plant *R* genes encode nucleotide-binding site leucine-rich repeat (NLR) type protein which can be further grouped into three subclasses on the basis of the NB-ARC domain. The three monophyletic classes of plant NLRs can be distinguished by their N-terminal domains; toll/interleukin1 receptor (TIR) NLRs (TNLs), Coiled-coil (CC) NLRs (CNLs) and NLRs containing an N terminal RPW8 domain (RNLs) (Fig. **2**) [63]. The N-terminal domain precedes an evolutionarily conserved domain with a Nucleotide-Binding site present in Apoptotic protease-activating factor, R proteins, and *Caenorhabditis elegans* death-4 protein (NB-ARC), followed by highly variable Leucine-rich repeats (LRR) [64]. *CNL* and *RNL* genes are found in both monocots and dicots, whereas *TNL* genes are restricted only to the dicots (Fig. **2** and Table **1**) [65]. Alike PRR, variability in the LRR enables to recognize various effector structures [66]. The NB-ARC contains several conserved motifs: P-loop/Walker-A, resistance nucleotide-binding site A (RNBS-A), Kinase-2/Walker-B, RNBS-B,

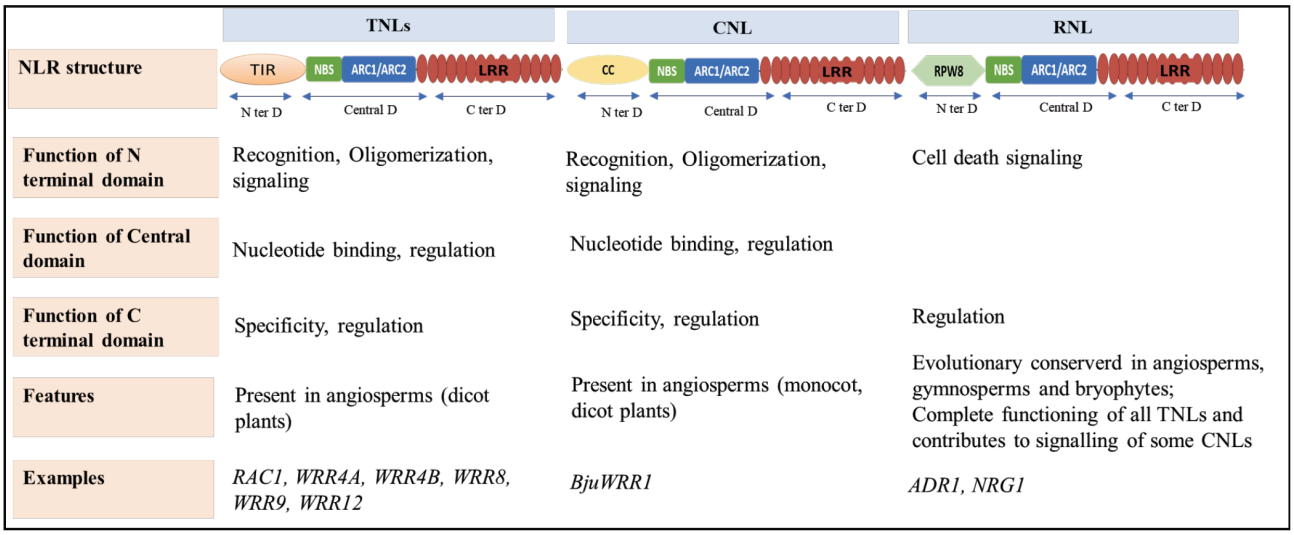

Fig. (2). Architecture of the monophyletic group of plant NLRs. The predicted and experimentally documented functions or properties of the individual domains are indicated, as well as particular features and example. TIR – Toll/interleukin-1; CC- Coiled-coil; RPW8- Resistance to powdery mildew n8; NB- Nucleotide binding; ARC1/2- Apaf-1; LRR- Leucine-rich repeat; NLR – Nucleotide-binding and leucine-rich repeat-containing proteins; TNL-TIR-NLR; CNL- CC-NLR;; RNL – RPW8-NLR. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

RNBS-C, GLPL, RNBS-D, and MHDV, GLPL and MHDV named after the conserved amino acids present. LRRs are involved in protein-protein interactions and occur in a number of proteins with diverse functions [67, 68]. Domain exchange between LRR of closely related *R* genes supports their role in pathogen recognition [59]. Variation among *R* genes occurs mainly in their LRR domain, typically in the solvent-exposed β-strand/β-turn structure within the LRR domain. A comparison of this motif among *R* gene homologs suggests that the β-strand/β- turn structure has been under diversifying selection [69]. *R* genes encoding these intracellular receptors were first identified in Arabidopsis through genetic mapping of *R* genes [70]. Several other receptor-like genes, actively engaged in different modes of defense regulation have been also identified in *A. thaliana* and Brassica species conferring resistance against *A. candida* isolates [44].

In genetic term, *S* genes can be defined as genes that are involved in disease susceptibility thus contributing positively to the infection progression. Thus, all plant genes that facilitate infection and support compatibility can be considered as *S* genes. Functional impairment of the *S* gene by mutation or deletion can alter the ability of the pathogen to cause disease. This may lead to pathogen-specific resistance if the gene is involved in the production of a component required for host penetration or in broad-spectrum resistance if the gene suppresses constitutive defenses. *S* gene was successfully identified first time in *A. thaliana*, while exploring resistance against *H. parasitica* (powdery mildew) [33]. *S* genes that have been identified as susceptibility factors for colonization by important oomycetes are listed in Table **2**. *S* genes can be categorized into three groups based on the point at which they act during the infection process. These three are those involved in early pathogen establishment, negative regulators of plant immunity, such as the *CesA3* gene, which is involved in cellulose synthesis and pathogen sustenance such as metabolite biosynthesis and sugar transport [33].

4.2. Manifestation of Oomycetes Resistance in Plants by NLR Signaling (with Reference to *A. candida*)

Plants can sense diverse extracellular oomycete-derived patterns. Oomycete patterns are derived from the pathogen's cell wall or membrane, whereas others are secreted to the extracellular environment before being detected by the plant's immune system.

As we have already discussed above, oomycete pathogens secrete effectors during invasion and propagation into the plant cell. The cytoplasmic nucleotide-binding and leucine-rich repeat receptors (NLR) present in plants detect oomycete effectors or their activity, thus triggering the immunity (ETI). Many effector–NLR interactions have also been studied in Arabidopsis [71]. Several key plant defense regulators, downstream of NLRs have been identified which includes ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1), and two NLR helpers - ADR1 and NGR1. EDS1 is a lipase-like protein that is required for the functioning of TNLs, leading to oxidative stress signaling and as a positive regulator as well as basal resistance to virulent pathogens [72]. EDS1 is well reported to be involved in the functioning of *TNL* genes such as *RPP1*, *RPP4*, *RPP5*, and *WRR4*. NLR helper proteins play a very significant role in the NLR downstream signaling. ADR1 and NGR1 are two RNL proteins, which have been recently identified as NLR helper proteins and transmit effector immunity in the host infected by *A. candida* and other notable oomycetes. ADR1 helps in the signaling of both TNLs and CNLs receptors along with the regulation of the Salicyclic acid (SA) dependent pathways. NRG1 is found to help only TNLs during the downstream signaling coupled with regulation of HR and other defense responses. Additionally, NRG1 has also shown complete resistance to *A. candida* (Fig. **3**). On the basis of the previous findings by Cevik *et al*. (2019) [30] and Caster *et al*. (2019), we understand that direct or indirect detection of the *A. candida* effector molecules by a flowering plant harboring NLRs

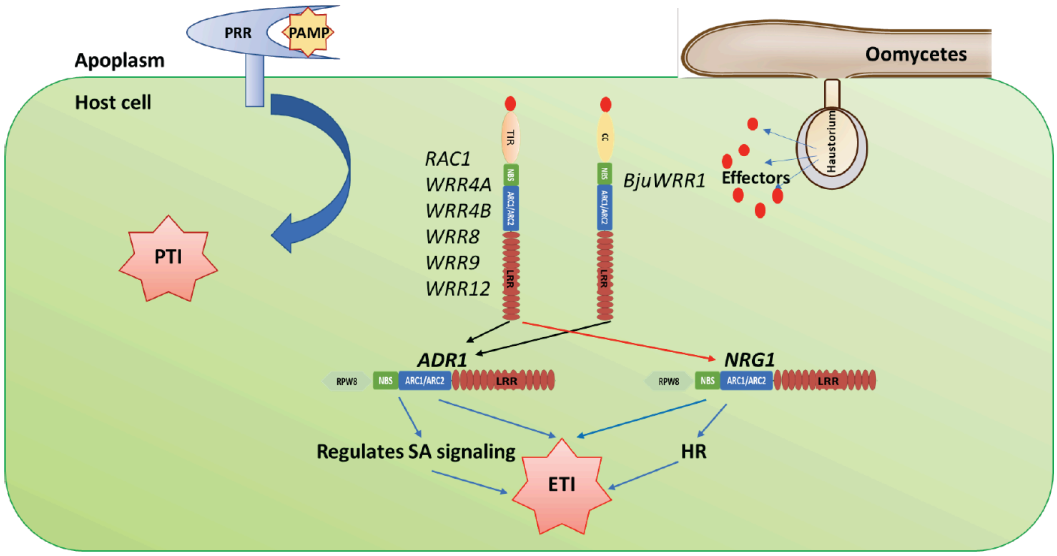

Fig. (3). Schematic representation of PTI and ETI in plants against *A. candida*. The recognition of the PAMPs of *A. candida* by PRR leads to PTI. Specific activation of TNL & CNL receptor by *A. candida* effectors triggers ETI. Few *TNL* genes are identified, for example, *RAC1, WRR4A, WRR4B, WRR8, WRR9, and WRR12* reported in Aradopsis [30]; *CNL* genes, for example, *BjuWRR1* in Donskaja IV, is an east European variety of *B. juncea* [77]. All the TNLs signal *via* NRG1 and some can also signal *via* ADR1. CNLs signal *via* ADR1 and partially *via* NRG1. ADR1 and NRG1 are conserved within both monocots and dicots suggesting this model can be applied for all the flowering plants. *WRR4A* gene has been proved to signal *via* NRG1 [80]. Rest *TNL* genes - *RAC1, WRR4B, WRR8, WRR9, WRR12* are hypothsised to signal *via* NRG1 and ARD1, whereas the *CNL* gene(s) – *BjuWRR1 is* hypothesized to signal ADR1. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

leads the NLRs to undergo a conformational change from a condensed, ADP-bound state to an open ATP-bound state with exposed N-terminal domains for the initiation of downstream signaling [66, 73-75].

The activated NLRs along with the other proteins like EDS1 reported in *B. juncea*, *B. napus*, and *A. thaliana* initiate a downstream defense-signaling pathway. Furthermore, they are transmitted by ADR1 intracellular receptor protein in the case of both TNLs and CNLs and NRG1 in the case of only TNLs (Fig. **3**). We can conclude suggesting that TNL, CNL and RNL proteins, on activation, trigger a complex network of responses, including gene induction, the production of reactive oxygen species and salicylic acid, transcriptional reprogramming and finally lead to cell death phenomenon 'Hypersensitive Response (HR)', resulting in host resistance.

5. NOVEL APPROACHES TO TRANSFER WHITE RUST RESISTANCE IN *BRASSICA JUNCEA*

The Indian gene pool lines of *B. juncea* are highly susceptible to the *A. candida* [76]. Contrary to the success in the production of oilseeds through conventional techniques, the development of oomycetes resistant brassicas has been limited. This drives an urgent need to blend the conventional, unconventional and frontier technologies for crop protection. Some accessions of *A. thaliana* and the east European mustard germplasm harbor resistance against Ac2VRR as well as other *A. candida* races [30, 76]. The non-host resistance factors present the resistant plants that can be of significant interest to introduce WRR in the susceptible commercial varieties of *B. juncea*. Studies report that the non-host resistance present in *A. thaliana* is majorly due to the presence of *R* genes [27]. Therefore, the transfer of *A. candida* effector compatible *R* genes through genetic engineering gives us high scope to deploy durable white rust resistance in the susceptible commercial varieties of *B. juncea* (Fig. **4**).

The genome of *B. juncea* variety Tumida and *B. juncea* variety Varuna has been sequenced [77]. Further analysis of the sequences will enable us to identify *S* genes responsible for WR infection in the host tissue. Genomic approaches like RNA interference and CRISPR-Cas will help us to silence or knock out the pathogen *Avr* genes and host *S* genes respectively.

Here, we have tried to brief the novel genomic approaches which can help us to develop WRR Indian mustard (Fig. **4**).

5.1. Deploying Resistance Genes for Broad-Spectrum and Durable Resistance

Plant disease resistance genes (*R* genes) play a key role in recognizing proteins expressed by specific avirulence (*Avr*) genes of pathogens encoding the effector molecules. The discovery of *R* genes (including their homologs and analogs) opened interesting possibilities for controlling plant diseases caused by several pathogens. However, due to high selection operated by pathogens along with environmental pressure, several crop plants have lost their specificity, broad-spectrum or durability of resistance. On the contrary, the advances in plant genome sequencing and identification of numerous *R* genes have provided new insights into the acquisition of resistance to pathogens in host plants through transgenesis, or gene editing. The main class of *R* genes consists of a nucleotide-binding domain (NB) and a leucine-rich

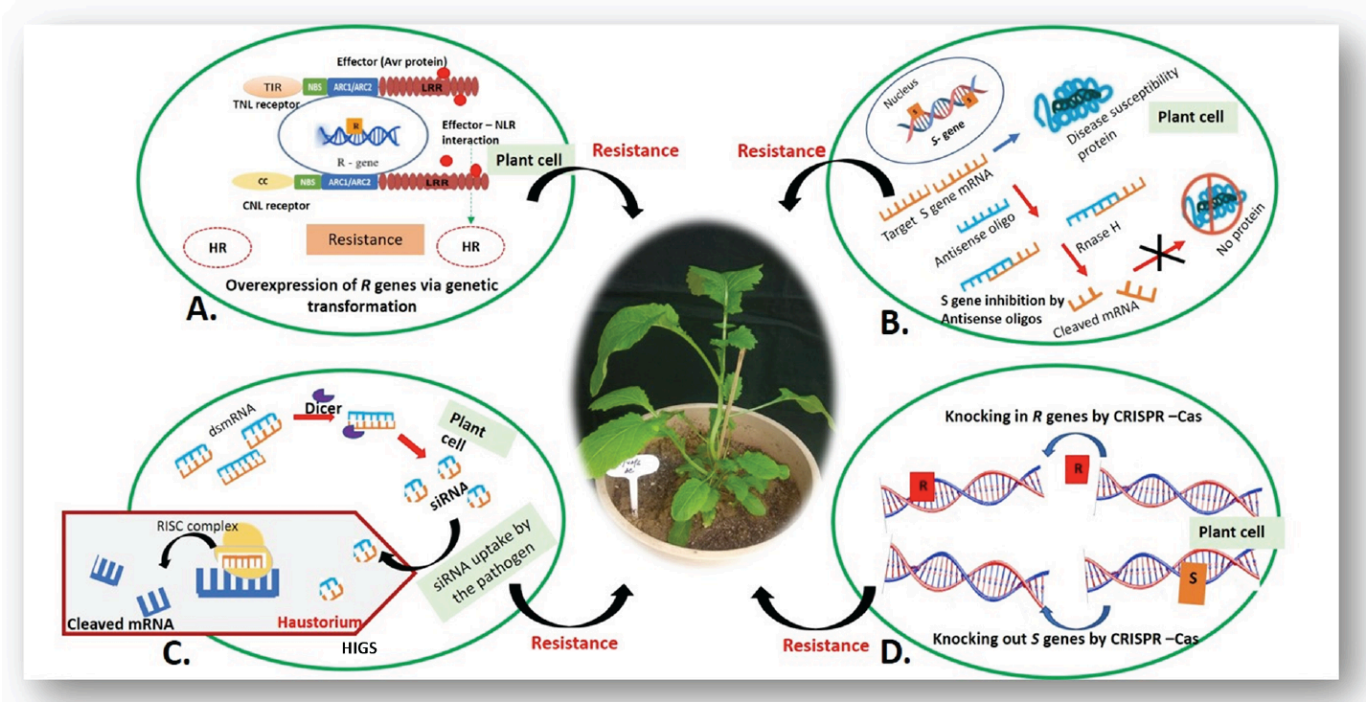

Fig. (4). Hypothetical figure for application of novel genomic approaches to transfer white rust resistance in *B. juncea.* A) Transfer of *R* genes in *B. juncea* by genetic transformation followed by overexpression leads to deployment of resistance; **B)** Inhibition of the target *S* genes in *B. juncea* by introduction of antisense oligonucleotides by genetic transformation leading to resistance; **C)** Host Induced Gene Silencing (HIGS) mediated by the siRNA in the pathogen, while invasion of the genetically modified siRNA synthesizing transgenic *B. juncea*; **D)** The targeted knocking in *R* genes and knocking out of *S* genes in the susceptible *B. juncea* varieties. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. The table represents *NLR* and *NLR*-like gene numbers corresponding to NB-ARC-LRR-encoding genes. The number for *TNLs* and *CNLs* correspond to genes encoding either full-length TNLs, CNLs or the NB-ARC-LRR-containing proteins if these can be clearly assigned to one of the NLR types based on their motif composition at the NB-ARC domain. The respective complete genome sizes are also mentioned.

Species	Common Name	Genome Size (Mbp)	*NLRs*	*TNLs*	*CNLs*	References
Arabidopsis thaliana	Thale cress	125	151	94	55	Meyers *et al.* [65]
Arabidopsis lyrata	Lyre-leaved rock-cress	230	138	103	21	Guo *et al.* [65]
Brassica rapa	Mustard	485	80	52	28	Mun *et al.* [65]
B. juncea	Indian mustard	922	NR	NR	NR	Paritosh *et al.* [99]
B. oleracea	Wild cabbage	630	NR	NR	NR	Paritosh *et al.* [99]
B. napus	Rapeseed	1130	NR	NR	NR	Paritosh *et al.* [99]
Carica papaya	Papaya	372	34	6	4	Porter *et al.* [65]
Cucumis sativus	Cucumber	367	53	11	17	Wan *et al.* [65]
Glycine max	Soybean	1115	319	116	20	Kang *et al.* [65]
Medicago truncatula	Barrel medic	186[a] (500)	270	118	152	Ameline-Torregrosa *et al.* [65]
Oryza sativa	Rice	466	458	0	274	Li *et al.* [65]
Sorghum bicolor	Sorghum	760	184	0	130	Li *et al.* [65]
Solanum tuberosum	Potato	840	371	55	316	Jupe *et al.* [65]
Vitis vinifera	Wine grape	487	459	97	215	Yang *et al.* [65]
Zea mays	Maize	2400	95	0	71	Li *et al.* [65]

Table 2. Cloned resistance (*R*) and susceptibility (*S*) genes affecting oomycete-plant interactions.

Oomycete Pathogen	Cloned *R* Gene(s)	Source of *R* Gene(s)	Cloned *S* Gene(s)	Source of *S* Gene(s)
Albugo candida	*RAC1,WRR4A, WRR4B-Col-0, WRR4B-Ws,WRR8, WRR9, WRR12* [27, 30]	Arabidopsis	NR	NR
Hyaloperonospora arabidopsidis	*RPP1* (*ATR1*), *RPP2*, *RPP4*, *RPP5*, *RPP7*, *RPP8*, *RPP13* (*ATR13*) [70]	Arabidopsis	*AGD5, IOS1*, *PUB22/23/24* (154, 55), *SON1*, *EDR2*, *SNI1,Cdd1*, *DMR1*, *RSP1/2*, *PMR4*, *DMR6 [70]*	Arabidopsis
Peronospora manshurica	*Rpm* [33]	Soybean	*MPK4* [33]	Soybean
Phytophthora cinnamomi	*TIR1* [33]	Arabidopsis	NR	NR
Phytophthora infestans	*R1*, *R2* (*AVR2*), *R3a* (*AVR3*), *R3b* (*AVR3b*) , *R4* (*AVR4*), *R 6* and *R7*, *R10* and *R11*, *RB/Rpi-Blb1* (*AVR-Blb1/IPI-O1*), *Rpi-Blb2*, *Ph-3*, *Rpi-vnt1*, *Rpi-blb3*, *Rpi-abpt* [33]	Potato	*StREM1.3* and *N. benthamiana REM1.3* orthologs [33]	*N. benthamiana*
Phytophthora palmivora	NR	NR	*RAM2*, *LATD* [33]	*Medicago*
Phytophthora sojae	*Rps1d* (*AVR1d*), *Rps1b* (*AVR1b*) [33]	Soybean	NR	NR
Plasmopara viticola	Grape *Rpv1* and *Rpv2*, *Rpv3* (*avrRpv3*), *Rpv10* [33]	Grape	NR	NR

(NR - Not Reported).

repeat (LRR) domain(s) and often referred to NLR/NB-LRR as discussed already in section 4.1. The NLR family is massively expanded in several plant species. The number of *NLR* genes in flowering plants is largely variable suggesting species-specific mechanisms in *NLR* genes expansion and/or contraction (Table **1**). This variability can be explained by three species in the Brassicaceae family; *A. thaliana, A. lyrata, and B. rapa*, which have 151, 138, and 80 full-length *NLRs*, respectively. The first *NLR* gene, *RPS2*, was cloned from Arabidopsis conferring resistance against Pseudomonas bacteria, expressing the AvrRpt2 effector molecule [70].

Scientists across the globe are working to explore *R* genes conferring WRR. Several *R* genes conferring WRR against different races of *A. candida* have been mapped in *A. thaliana, B. rapa, B. napus, B. carinata and B. juncea* [30]. Among the identified *WRR* genes, *RAC1*(Arabidopsis accession Ksk-1) and *WRR4* (Arabidopsis accession Col-0) were identified and cloned from *A. thaliana* [30]. *WRR4* gene was overexpressed into susceptible cultivar of *B. juncea* and *B. napus* by genetic transformation and was reported to confer significant levels of resistance against Ac2VRR and Ac7V [45]. *WRR4* gene has been studied to arrest the development of the pathogen in the plant epidermal cell, which shows hypersensitivity response [30]. Recently, a few more genes namely *WRR4BCol-0*, *WRR8Sf-2*, *WRR9Hi-0*, *WRR12* have been identified in Arabidopsis accessions conferring resistance to WR [30]. All these genes belong to the TNL subfamily (Tables **1** and **2**). Few candidate genes have also been mapped in two east European *B. juncea* gene pool lines Heera and Donskaja-IV, and one Chinese *B. juncea* variety Tumida conferring resistance against Indian isolate AcB1 (*Albugo candida* Bharatpur-1) [76]. Gene *BjuWRR1* from Donskaja IV, encoding a CNL domain-containing protein has been identified to confer WRR. The transgenic *B. juncea* variety Varuna constitutively expressing *BjuWRR1* conferred WRR to various isolates of *A. candida* [77].

5.2. Enriching the Known Repertoire of Immune Receptors

Plants possess immune receptors that recognize pathogens and trigger cellular defense responses [13]. Discovery of novel immune receptors recognizing major virulence factors will enrich the repertoire of known immune receptor genes that may be deployed in the crop genome for better performance in the field [78]. Once a collection of germplasm exhibiting various degrees of resistance (non-host resistance) to a particular pathogen strain is identified, a comparative genomic tool such as resistance gene enrichment sequencing (RenSeq) can be applied to identify genomic variants in *NLR* genes that are linked to disease phenotypes [79]. This promotes the cloning of new *NLR* genes and their potential deployment in crop protection through genetic engineering. RenSeq was successfully applied in the accelerated identification of *NLRs* for example, anti-*P. infestans NLR* gene *Rpi-amr3i* from *Solanum americanum*, anti-potato-late-blight *NLR gene Rpi-ver1, stem rust NLR*

genes, Sr22 and *Sr45*, which confer resistance to commercially important races of the stem rust pathogen from a mutagenized hexaploid bread wheat population [79].

Further, focusing on white rust disease, host/race specificity of *A. candida* is determined by the NLR repertoire of the host plant and the cognate effectors of the pathogen race, rather than host compatibility factors. Therefore, some of the NLRs recognizing specific races or multiple races are maintained in different Brassicaceae species. This, in turn, provides an excellent resource to identify *WRR* genes for different Brassica species.

RenSeq enabled to identify three *WRR* genes (*WRR4B*Col-0, *WRR8*Sf-2, and *WRR9*Hi-0) against *B. juncea*- infecting *A. candida* race 2 (Ac2V), and a gene, *WRR12* (*SOC3*), conferring NHR to AcBoT, in addition to the previously identified broad-spectrum resistance gene *WRR4ACol-0* (previously known as *WRR4*) [13, 30]. The distribution and sequence variation of *WRR4A*, *WRR4B, WRR8, WRR9*, and *WRR12* genes was determined while sequencing the bulk DNA of different accessions of Arabidopsis using SMRT RenSeq [30]. The sequences of the WRR alleles from each *A. thaliana* accession were identified by BLASTn against the SMRT RenSeq assemblies [80]. BLASTn hits with less than 95% identity, are not considered the alleles of the query gene [30]. Although future field experiments are required to evaluate the transfer potential of these newly identified *NLR* genes in the host. These lab studies confer that RenSeq is a powerful tool to rapidly identify novel *NLR* genes (Tables **1** and **2**).

5.3. RNA Interference Technology

RNA interference (RNAi) is a potential tool for silencing genes in a broad range of organisms. It involves the expression of a double-stranded RNA (dsRNA) or small interfering RNA (siRNAs) that trigger the degradation of target mRNA sequences by post-transcriptional gene silencing (PTGS). It is not so much successful in oomycetes pathogen alike viruses and fungus. Studies have reported that the endogenous miRNAs and siRNAs present in plants, regulate the expression of some *NLR genes* (Li *et al.*, 2012; Prasad *et al.*, 2012). This was validated by demonstrating the function of the diverse endogenous miR482 family in tomato targeting *NLR* transcripts in a sequence-specific manner and induced subsequent cleavage of the transcripts. This paves the way for the application of RNAi through the transfer of artificial miRNAs and siRNA in the plants for suppression of *S* genes and for *Avr* gene suppression in the pathogen. Genetic transformation helps to transfer the siRNA and miRNA constructs in the target plant or pathogen. There are several reports of gene silencing in plants through RNAi [79]. Polyploidy in plant challenges the targeted silencing of specific homolog(s) of a multigene family RNAi [81, 82]. Oilseed crop *B. juncea* (AABB) is an amphidiploid which is expected to have 4-6 copies of every Arabidopsis gene ortholog [82]. A recent study in polyploid *B. juncea* suggests that designing intron-spliced hairpin RNAi constructs with optimally expressed gene targets and promoters can lead to efficient silencing, especially when a specific member of a multigene family in a polyploid crop is targeted [83].

S genes render disease susceptibility in plants thus contributing positively to the infection process. On the basis of their working mechanism, *S* genes have been classified into three classes as discussed in section 4.1. Silencing *S* genes involved in pathogenesis has proven to suppress few significant bioprocesses supporting pathogenesis leading to enhanced disease resistance in plants (Fig. **4**). The bioprocess can be biosynthesis of wax, cellulose, metabolites and sugar transport [84]. This strategy has been very successfully applied to plant viruses [85]. Silencing of *S* genes through RNAi has also shown its success in Arabidopsis (against downy mildew) as well as significant crop plants like potato (late blight resistance, Botrytis blight), tomato (powdery mildew) as detailed in Table **2**. The orthologs of the *S* genes whose silencing has conferred resistance to these abovementioned oomycetes diseases can also be the target candidate genes for attaining WRR in the host.

RNAi also gives us a platform to target the host invading pathogen. The genes identified in important bioprocesses of the oomycetes pathogen, for example, *chitin synthase* gene required for chitin synthesis (an important component of cell wall), *carbohydrate myco-laminarin* gene (involved in energy storage) as well as avirulent genes are an ideal target for silencing [86]. Gene silencing has not yet been reported in *A candida* but has been applied to several Phytophthora species to generate strains deficient in particular gene products. This can be a thrust area for future studies. The transformation of *P. infestans* with sense, antisense, and promoterless constructs of the pathogen endogenous gene, for example, *inf1* gene, leads to the silencing of the *inf1* gene in as many as 20% of the transformants [86].

RNAi based Host Induced Gene Silencing (HIGS) is also a potential strategy for silencing the pathogen *Avr* genes of the crop infecting pathogen. *Avr* genes encoding the effector molecules have been proved to be ideal targets to attenuate the pathogen during the invasion of the host cell. As reported, HIGS has been successful in limiting various phytopathogens such as insects, nematodes, viruses (Virus-Induced Gene Silencing), and fungus [79]. Interestingly, effectors from oomycetes have been identified that function as suppressors of RNA silencing [79]. Gene knockouts are easier in fungus as compared to oomycetes [86]. Oomycetes are typically diploid during their asexual stages, which means for gene disruption both the copies of the gene have to be targeted. In oomycetes, most studies that involve gene silencing have been conducted in *P. infestans* [80]. Classical gene disruption approaches have not been so much successful with oomycetes, since homologous recombination occurs at very low levels. Therefore, attempts at targeted gene knockout have dominated on the gene-silencing strategies that have proven successful in plants

The advancement in *B. juncea* and *A. candida* genome sequencing will enable us to identify the *S* genes and *Avr* genes present in it providing future scopes for the attainment of WRR in Indian mustard.

5.4. Scope of CRISPR for Developing White Rust Resistance in Plant

In recent years, the bacterially derived clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated (Cas) technology has proven to be a promising approach for engineering resistance to plant viruses [87].

CRISPR-Cas site-specific nucleases evolved as components of prokaryotic immunity against viruses, and are widely deployed as tools to deploy operator-specified nucleotide sequence changes in genomes of interest. It can be used to precisely modify the genome of any organism including plants to achieve the desired trait. In the past decade, CRISPR emerged as a cheap, fast, precise and highly efficient tool as compared to other genome editing tools such as zinc finger nucleases (ZFNs) and transcriptional activator-like effector nucleases (TALENs), even at the multiplex level to edit genomes and create genetic variations quickly addressing emerging challenges in agriculture [84]. The CRISPR/Cas9 is becoming a user-friendly technique for development of non-transgenic genome edited crop plants to counteract the phytopathogen menace ensuring future food security of the increasing population in tropical countries.

In plants, it enables to knock-out the *S* genes by targeted mutagenesis and knock-in the *R* genes. Random mutagenesis can be utilized to generate polymorphism and characterize genes in a forward genetic approach (Fig. **4**). However, the large genome size and high copy number in polyploidy crops possess several challenges in site-directed mutagenesis for knocking-out of multiple genes with high homology. In 2010, Birch *et al.* [38] showed that the transgene-silencing is promotor sequence-specific, which makes it obligatory to choose diverse and proficient promoters to trim down the silencing effects [81]. Complete annotated genomes will enable us to design sgRNAs to target genes in the crop. The requirement of a large number of mutants to study the multiple forms of alleles present in polyploid is another fundamental limitation [81]. As we have discussed, *S* genes are responsible for susceptibility in plants. It has been demonstrated to be efficient for gene disruption in many plant species, including Arabidopsis, *Nicotiana benthamiana*, *N. tabacum*, rice, wheat, maize, sorghum, tomato, potato, sweet orange, poplar, and liverwort [88]. Recent studies report, several examples of CRISPR-mediated *S* gene disruption resulting in resistant plants [80]. *MLO,* a dominant *S* gene that confers broad-spectrum susceptibility to powdery mildew in diverse plants from monocots to dicots was knocked out by CRISPR. The raised *mlo2-mlo6-mlo12* triple mutant was fully resistant to an Arabidopsis-compatible powdery mildew strain [80]. CRISPR was used successfully to knock-out *MLO* genes in wheat and tomato inculcating resistance to powdery mildew. Lab study demonstrated that overexpression of SpCas9 and guide RNAs in *N. benthamiana*, Arabidopsis (*Arabidopsis thaliana*), tomato conferred resistance to the geminivirus family member Beet severe curly top virus [87]. CRISPR-Cas has also been successful in engineering resistance to RNA viruses, which comprise the most known plant viral pathogens [88]. For example, stable expression of the RNA-targeting nuclease Cas13a and the corresponding guide RNA in *N. benthamiana* conferred resistance to the RNA virus Turnip mosaic virus. Using Cas13a to target viral RNA substrates does not induce DNA breakage and thus would not introduce undesired off-target mutations to the host genome (Abudayyeh *et al.*, 2017). Similarly, FnCas9 has been used to engineer resistance against RNA viruses Cucumber mosaic virus and Tobacco mosaic virus in *N. benthamiana* and Arabidopsis [84].

In Brassica, only a few successful instances of genome editing have been reported [88]. One of these cases demonstrated the *GA4* gene knockout in a doubled-haploid genotype AG DH1012 (a broccoli-like Brassica) from the *Brassica oleracea* var. *alboglabra* (A12DHd) × *B. oleracea* var. *italica* (Green Duke GDDH33) mapping population [89]. The *BoPDS* gene, the self-incompatibility gene *BoSRK3*, the *BoMS1* gene associated with male sterility, and some of their paralogous genes were successfully knocked out using an array of sgRNA-tRNA units designed to express more than one sgRNA and showed obvious mutant phenotypes in the T0 generation. In addition, the CRISPR/Cas9 system can target multiple sites or multiple genes in a single transformation event and produce homozygous knockouts, even in the T0 generation, as reported in other species [88]. This approach enables us to develop resistant crops in a few months, with mutation undistinguishable from naturally occurring mutation.

CRISPR has helped to identify the function of two *NLR* helper genes: *NRG1* and *ADR1* in Arabidopsis and *N. benthamiana* during *A. candida* effector-triggered downstream signaling [84]. The two genes were studied to redundantly understand the full function of CC-NLR Rx2 and TIR-NLR pair RRS1/RPS4 [85]. The generation of null alleles *via* CRISPR is quick and simple nowadays, facilitating the investigation of gene function.

Reviewed literatures indicate that homology-directed recombination (HDR) mediated repair of CRISPR-induced double-strand break (DSB) can result in gene replacement or insertion in plant and animal. Gene targeting *via* HDR occurs through homologous recombination between a template and genomic DNA at very low frequency and drastically increases when DSB occurs [84]. Although CRISPR-mediated gene targeting is a routine assay in animals, it remains presently a challenge in plants. Stable gene targeting was achieved in Arabidopsis and tomato in 6% to 25% of transformed plants [90]. Theoretically, CRISPR could be employed to introgress an *R*-gene in a susceptible line quickly and with no other DNA introgression as compared to classic breeding. It is yet to be demonstrated. In a small span of five years, CRISPR has been deployed to develop significant resistance in plants *via* RNA virus targeting and *S* gene knock-out. If gene targeting is one of the current challenges, we can expect to witness much more CRISPR-derived applications in the coming years, for plant disease resistance and beyond.

However, the lack of genetic data for *B. juncea* impedes the rapid cloning of *WRR* genes from this species. The advances in *B. juncea* sequencing and optimization of CRISPR in the polyploid *B. juncea* will enable us to integrate the *WRR* genes in the susceptible varieties of *B. juncea,* deploying durable resistance to white rust disease. CRISPR-based genome editing techniques will facilitate the study of genes and proteins and will be beneficial for both basic and applied plant science (Fig. **4**).

CONCLUSION AND FUTURE DIRECTIONS

A. candida, a homothallic biotrophic oomycete, can form hybrids between genetically distinct isolates *via* co-culture recombining genetic variability in *A. candida* populations [15]. Comparative genomics has revealed an extensive genetic exchange between races of *A. candida* and this genetic

exchange could result in races with novel repertoires of effector alleles that, in turn, might enable colonization of new hosts. Therefore, understanding the underlying mechanism of NHR in different Brassica species could help us in breeding for resistance to *A. candida*. Host/race specificity of *A. candida* is determined by the NLR repertoire (encoded by *R* genes) of the host plant and the recognized effectors of the pathogen race rather than host Identifying *R* and *S* genes in *B. juncea* as well as other species of Brassica is must for the deployment of resistance to white rust. Rapid identification of more *R* genes with advanced genomic tools such as RenSeq should be practiced to enrich the NLR protein repertoire enhancing the prospects of varied pathogen recognition and the manifestation of ETI. For example, a study reported that ResSeq enabled the identification of the *R* genes namely *WRR4B* Col-0, *WRR4B* Col-0, *WRR8* Sf, *WRR9*-Hi in Arabidopsis deploying resistance to *B. juncea*-infecting race Ac2V and *WRR*12 in Arabidopsis deploying resistance to *B. oleracea*-infecting race AcBoT encoding NLR [30]. Efforts to identify *R* genes have also been assisted by the development and application of differential host-pathogen pathotype/race/isolate combinations. Further improvements can be improvised by establishing freely-available universal host differential sets for each pathogen, differential sets that constitute the host resistances, and pathogen sub-specific variation worldwide. One of the approaches that could be included is an international collaborative network building a world database for each pathogen. The initiative established at the Brassica 2016 conference to resolve the Blackleg nomenclature issue is a remarkable step for this approach [91]. Since dynamic changes can happen with either of the host cultivars and pathogens in terms of cultivar resistance breakdowns and/or pathogens losing/gaining in terms of their pathogenicity/virulence over time, every universal database set up must be regularly updated and kept current. For example, the international DivSeek database, for phenotypic data that can be linked with genotypic data (for seeds that are stored in seed bank), that was launched in 2015 could be utilized as a model to set up the databases needed. Successful phytopathogens often evade detection by host *R* genes [92]. Thus, disease resistance conferred by a single *R* gene often fails to provide durability in the field as pathogens can evolve to evade recognition by mutating the corresponding *Avr* gene. For improved durability and to broaden the resistance spectrum, multiple *WRR* genes can be introduced simultaneously, which is commonly known as stacking [93]. Three late blight resistance genes have been stacked and successfully transformed in highland potato varieties deploying durable resistance to late blight. Functional impairment of *S* genes in the host plant is also a favorable approach for sustaining disease resistance in Indian mustard as well as other economically significant crops. RNAi interference and CRISPR-Cas technology are two promising approaches which can knock out the *S* genes impairing the disease manifestation in the host plant. Future efforts in improving CRISPR-Cas for anti-oomycete resistance may also be focused on establishing an in-plant adaptive immunity by exploiting the spacer acquisition machinery in the CRISPR-Cas adaptive immune system in prokaryotes [94].

Along with the genomic approaches, the plant and seed microbiome of oilseed Brassica is an interesting aspect and should be explored to develop a resistance strategy against Albugo infection. Plant microbiome includes a microbial community that typically interacts extensively with a plant [95-97]. The plant microbiome can survive either inside or outside of plant tissues including seed and phyllosphere, performing various plant beneficial activities such as biocontrol potential against phytopathogens and promotion of plant growth. The seed microbiome of oilseed rape, is cultivar-specific [95], posing a strong influence on its interaction pattern with pathogens. Therefore considered as an interesting biomarker for breeding strategies [95]. We recommend that microbiome studies should be incorporated into breeding programs for sustainable agriculture practices. We also believe that the assessment of seed and phyllosphere microbiomes combined with network analysis may open new opportunities for the targeted selection of biocontrol strains for a given host plant at the cultivar level. These novel insights into plant and seed microbiome structure will enable the development of next-generation strategies combining both biocontrol and breeding approaches to address world agricultural challenges [98, 99].

Furthermore, the advancement in the Brassica-Albugo genomics will help us to identify more *R, S* and *A. candida* avirulent/pathogenic genes unraveling the underlying infection and non-host resistance mechanism, thus enabling us to develop white rust resistant Indian mustard.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

This study is supported by the Department of Biotechnology (DBT), Government of India under the Indo - UK project, Grant no. BT/IN/Indo-UK/CGAT/12/DP/2014-2015 dated 8th May 2015 (PI/CoPI – Dr. Abha Agnihotri and Dr. Pankaj Sharma).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Authors are thankful to the Department of Biotechnology (DBT), Government of India for grant of funds under the Indo - UK project.

REFERENCES

[1] Abrol, D.P.; Shankar, U. Integrated pest management. *Breeding Oilseed Crops for Sustainable Production*; Academic Press, **2016**, pp. 523-549. http://dx.doi.org/10.1016/B978-0-12-801309-0.00020-3

[2] Kumar, A.; Sharma, P.; Thomas, L.; Agnihotri, A.; Banga, S.S. *Proceedings of the 16th Australian Research Assembly on Brassicas,* Ballarat Victoria, **2009**.

[3] Saharan, G.S.; Verma, P.R. *White rusts: a review of economically important species*; IDRC: Ottawa, ON, CA, **1992**.

[4] Persoon, C.H. *Synopsis methodica fungorum*; Leipzig: Germany, **1801**, p. 2.

[5] Kuntze, O. *Revisio generum plantarum*; Leipzig, Germany, Vol. 2, **1891**.

[6] Biga, M.L.B. Review of the species of the genus *Albugo* based on the morphology of the conidia. *Sydowia,* **1955**, *9*, 339-358.

[7] Heller, A.; Thines, M. Evidence for the importance of enzymatic digestion of epidermal walls during subepidermal sporulation and pustule opening in white blister rusts (Albuginaceae). *Mycol. Res.,* **2009**, *113*(Pt 6-7), 657-667. http://dx.doi.org/10.1016/j.mycres.2009.01.009 PMID: 19484808

[8] Links, M.G.; Holub, E.; Jiang, R.H.; Sharpe, A.G.; Hegedus, D.; Beynon, E.; Sillito, D.; Clarke, W.E.; Uzuhashi, S.; Borhan, M.H. *De novo* sequence assembly of *Albugo candida* reveals a small genome relative to other biotrophic oomycetes. *BMC Genomics,* **2011**, *12*(1), 503. http://dx.doi.org/10.1186/1471-2164-12-503 PMID: 21995639

[9] Choi, D.; Priest, M.J. A key to the genus *Albugo*. *Mycotaxon,* **1995**, *53*, 261-272.

[10] Thines, A.; Spring, O. A revision of *Albugo* (Chromista, Peronosporomycetes). *Mycotaxon,* **2005**, *92*, 443-458.

[11] Walker, J.; Priest, M.J. A new species of *Albugo* on *Pterostylis* (Orchidaceae) from Australia: confirmation of the genus *Albugo* on a monocotyledonous host. *Australas. Plant Pathol.,* **2007**, *36*, 181-185. http://dx.doi.org/10.1071/AP07011

[12] Kamoun, S.; Furzer, O.; Jones, J.D.; Judelson, H.S.; Ali, G.S.; Dalio, R.J.; Roy, S.G.; Schena, L.; Zambounis, A.; Panabières, F.; Cahill, D.; Ruocco, M.; Figueiredo, A.; Chen, X.R.; Hulvey, J.; Stam, R.; Lamour, K.; Gijzen, M.; Tyler, B.M.; Grünwald, N.J.; Mukhtar, M.S.; Tomé, D.F.; Tör, M.; Van Den Ackerveken, G.; McDowell, J.; Daayf, F.; Fry, W.E.; Lindqvist-Kreuze, H.; Meijer, H.J.; Petre, B.; Ristaino, J.; Yoshida, K.; Birch, P.R.; Govers, F. The top 10 oomycete pathogens in molecular plant pathology. *Mol. Plant Pathol.,* **2015**, *16*(4), 413-434. http://dx.doi.org/10.1111/mpp.12190 PMID: 25178392

[13] McMullan, M.; Gardiner, A.; Bailey, K.; Kemen, E.; Ward, B.J.; Cevik, V.; Robert-Seilaniantz, A.; Schultz-Larsen, T.; Balmuth, A.; Holub, E.; van Oosterhout, C.; Jones, J.D. Evidence for suppression of immunity as a driver for genomic introgressions and host range expansion in races of *Albugo candida*, a generalist parasite. *eLife,* **2015**, *4*e, 04550. http://dx.doi.org/10.7554/eLife.04550 PMID: 25723966

[14] Beakes, G.W.; Honda, D.; Thines, M. 3 Systematics of the Stramenipila: Labyrinthulomycota, Hyphochytriomycota, and Oomycota. *Systematics and evolution*; Springer: Berlin, Heidelberg, **2014**, pp. 39-97. http://dx.doi.org/10.1007/978-3-642-55318-9_3

[15] Adhikari, T.B.; Liu, J.Q.; Mathur, S.; Wu, C.X.; Rimmer, S.R. Genetic and molecular analyses in crosses of race 2 and race 7 of *Albugo candida*. *Phytopathology,* **2003**, *93*(8), 959-965. http://dx.doi.org/10.1094/PHYTO.2003.93.8.959 PMID: 18943862

[16] Thines, M.; Voglmayr, H. An introduction to the white Blister rusts (Albuginales). *Oomycete genetics and genomics: diversity, interactions and research tools*; Lamour, K.; Kamoun, S., Eds.; John Wiley & Sons, Inc.: Hoboken, New Jersey, **2009**, pp. 77-92. http://dx.doi.org/10.1002/9780470475898.ch4

[17] Ploch, S.; Choi, Y.J.; Rost, C.; Shin, H.D.; Schilling, E.; Thines, M. Evolution of diversity in Albugo is driven by high host specificity and multiple speciation events on closely related Brassicaceae. *Mol. Phylogenet. Evol.,* **2010**, *57*(2), 812-820. http://dx.doi.org/10.1016/j.ympev.2010.06.026 PMID: 20643217

[18] Choi, Y.J.; Shin, H.D.; Ploch, S.; Thines, M. Three new phylogenetic lineages are the closest relatives of the widespread species *Albugo candida*. *Fungal Biol.,* **2011**, *115*(7), 598-607. http://dx.doi.org/10.1016/j.funbio.2011.02.006 PMID: 21724165

[19] Thines, M. Phylogeny and evolution of plant pathogenic oomycetes- a global overview. *Eur. J. Plant Pathol.,* **2014**, *138*, 431-447. http://dx.doi.org/10.1007/s10658-013-0366-5

[20] Hiura, M. Biologic forms of *A. candida* (Pers.) Kuntze on some cruciferous plants. *Shokubutsu Kenkyu Zasshi,* **1930**, *5*, 1-20.

[21] Pound, G.S.; Williams, P.H. Biological races of *Albugo candida*. *Phytopathology,* **1963**, *53*, 1146-1149.

[22] Delwiche, P.A.; Williams, P.H. 1977. Genetic studies in *Brassica nigra* (L.) Koch. *Cruciferae NewsLett.,* **1963**, *2*, 39.

[23] Hill, C.B.; Crute, I.R.; Sherriff, C.; Williams, P.H. Specificity of *Albugo candida* and *Peronospora parasitica* pathotypes toward rapid-cycling crucifers. *Cruciferae NewsLett.,* **1988**, *13*, 112.

[24] Rimmer, S.R.; Mathur, S.; Wu, C.R. Virulence of isolates of *Albugo candida* from western Canada to *Brassica* species. *Can. J. Plant Pathol.,* **2000**, *22*, 229-235. http://dx.doi.org/10.1080/07060660009500468

[25] Kaur, P.; Sivasithamparam, K.; Barbetti, M.J. Pathogenic behaviour of strains of *Albugo candida* from *Brassica juncea* (Indian mustard) and *Raphanus raphanistrum* (wild radish) in Western Australia. *Australas. Plant Pathol.,* **2008**, *37*, 353-356. http://dx.doi.org/10.1071/AP08008

[26] Saharan, G.S.V.P.; Meena, P.D.; Kumar, A. *White Rust of Crucifers: Biology, Ecology and Management*; Springer: New Delhi, **2014**.

[27] Borhan, M.H.; Holub, E.B.; Kindrachuk, C.; Omidi, M.; Bozorgmanesh-Frad, G.; Rimmer, S.R. WRR4, a broad-spectrum TIR-NB-LRR gene from *Arabidopsis thaliana* that confers white rust resistance in transgenic oilseed Brassica crops. *Mol. Plant Pathol.,* **2010**, *11*(2), 283-291. http://dx.doi.org/10.1111/j.1364-3703.2009.00599.x PMID: 20447277

[28] Meena, P.D.; Verma, P.R.; Saharan, G.S.; Borhan, M.H. Historical perspectives of white rust caused by *Albugo candida* in oilseed Brassica. *J. Oilseed Brassica,* **2014**, *5*, 1-41.

[29] Jouet, A. *Albugo candida* race diversity, ploidy and host associated microbes revealed using DNA sequence capture on diseased plants in the field. *New Phytol.,* **2019,** *221*(3), 1529-1543. http://dx.doi.org/10.1111/nph.15417 PMID: 30288750

[30] Cevik, V.; Boutrot, F.; Apel, W.; Robert-Seilaniantz, A.; Furzer, O.J.; Redkar, A.; Castel, B.; Kover, P.X.; Prince, D.C.; Holub, E.B.; Jones, J.D.G. Transgressive segregation reveals mechanisms of *Arabidopsis* immunity to *Brassica*-infecting races of white rust (*Albugo candida*). *Proc. Natl. Acad. Sci. USA,* **2019**, *116*(7), 2767-2773. http://dx.doi.org/10.1073/pnas.1812911116 PMID: 30692254

[31] Petrie, G.A. Races of *Albugo candida* (white rust and stag head) on cultivated cruciferae in Saskatchewan. *Can. J. Plant Pathol.,* **1988**, *10*, 142-150. http://dx.doi.org/10.1080/07060668809501746

[32] Pidskalny, R.S.; Rimmer, S.R. Virulence of *Albugo candida* from turnip rape (*Brassica campestris*) and mustard (*Brassica juncea*) on various crucifers. *Can. J. Plant Pathol.,* **1985**, *7*, 283-286. http://dx.doi.org/10.1080/07060668509501692

[33] Fawke, S.; Doumane, M.; Schornack, S. Oomycete interactions with plants: infection strategies and resistance principles. *Microbiol. Mol. Biol. Rev.,* **2015**, *79*(3), 263-280. http://dx.doi.org/10.1128/MMBR.00010-15 PMID: 26041933

[34] Kamoun, S. A catalogue of the effector secretome of plant pathogenic oomycetes. *Annu. Rev. Phytopathol.,* **2006**, *44*, 41-60. http://dx.doi.org/10.1146/annurev.phyto.44.070505.143436 PMID: 16448329

[35] Hein, I.; Gilroy, E.M.; Armstrong, M.R.; Birch, P.R. The zig-zag-zig in oomycete-plant interactions. *Mol. Plant Pathol.,* **2009**, *10*(4), 547-562. http://dx.doi.org/10.1111/j.1364-3703.2009.00547.x PMID: 19523107

[36] Wawra, S.; Belmonte, R.; Löbach, L.; Saraiva, M.; Willems, A.; van West, P. Secretion, delivery and function of oomycete effector proteins. *Curr. Opin. Microbiol.,* **2012a**, *15*(6), 685-691. http://dx.doi.org/10.1016/j.mib.2012.10.008 PMID: 23177095

[37] Dawkins, R. *The extended phenotype: The long reach of the gene*; Oxford University Press, **2016**.

[38] Birch, P.R.J.; Rehmany, A.P.; Pritchard, L.; Kamoun, S.; Beynon, J.L. Trafficking arms: oomycete effectors enter host plant cells. *Trends Microbiol.,* **2006**, *14*(1), 8-11. http://dx.doi.org/10.1016/j.tim.2005.11.007 PMID: 16356717

[39] Chisholm, S.T.; Coaker, G.; Day, B.; Staskawicz, B.J. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell,* **2006**, *124*(4), 803-814. http://dx.doi.org/10.1016/j.cell.2006.02.008 PMID: 16497589

[40] Vandenkoornhuyse, P.; Quaiser, A.; Duhamel, M.; Le Van, A.; Dufresne, A. The importance of the microbiome of the plant holobiont. *New Phytol.,* **2015**, *206*(4), 1196-1206. http://dx.doi.org/10.1111/nph.13312 PMID: 25655016

[41] O'Connell, R.J.; Panstruga, R. Tête à tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. *New Phytol.,* **2006**, *171*(4), 699-718. http://dx.doi.org/10.1111/j.1469-8137.2006.01829.x PMID: 16918543

[42] Kamoun, S. Groovy times: filamentous pathogen effectors revealed. *Curr. Opin. Plant Biol.,* **2007**, *10*(4), 358-365. http://dx.doi.org/10.1016/j.pbi.2007.04.017 PMID: 17611143

[43] Huang, J.; Chen, L.; Lu, X.; Peng, Q.; Zhang, Y.; Yang, J.; Zhang, B.Y.; Yang, B.; Waletich, J.R.; Yin, W.; Zheng, X.; Wang, Y.; Dong, S. Natural allelic variations provide insights into host adaptation of Phytophthora avirulence effector PsAvr3c. *New Phytol.,* **2019**, *221*(2), 1010-1022. http://dx.doi.org/10.1111/nph.15414 PMID: 30169906

[44] Cooper, A.J.; Latunde-Dada, A.O.; Woods-Tör, A.; Lynn, J.; Lucas, J.A.; Crute, I.R.; Holub, E.B. Basic compatibility of *Albugo candida* in *Arabidopsis thaliana* and *Brassica juncea* causes broad-spectrum suppression of innate immunity. *Mol. Plant Microbe Interact.,* **2008**, *21*(6), 745-756. http://dx.doi.org/10.1094/MPMI-21-6-0745 PMID: 18624639

[45] Lee, H.A.; Lee, H.Y.; Seo, E.; Lee, J.; Kim, S.B.; Oh, S.; Choi, E.; Choi, E.; Lee, S.E.; Choi, D. Current understandings of plant nonhost resistance. *Mol. Plant Microbe Interact.,* **2017**, *30*(1), 5-15. http://dx.doi.org/10.1094/MPMI-10-16-0213-CR PMID: 27925500

[46] Wawra, S.; Trusch, F.; Matena, A.; Apostolakis, K.; Linne, U.; Zhukov, I.; Stanek, J.; Koźmiński, W.; Davidson, I.; Secombes, C.J.; Bayer, P.; van West, P. The RxLR motif of the host targeting effector AVR3a of *Phytophthora infestans* is cleaved before secretion. *Plant Cell,* **2017**, *29*(6), 1184-1195. http://dx.doi.org/10.1105/tpc.16.00552 PMID: 28522546

[47] Baxter, L.; Tripathy, S.; Ishaque, N.; Boot, N.; Cabral, A.; Kemen, E.; Thines, M.; Ah-Fong, A.; Anderson, R.; Badejoko, W.; Bittner-Eddy, P.; Boore, J.L.; Chibucos, M.C.; Coates, M.; Dehal, P.; Delehaunty, K.; Dong, S.; Downton, P.; Dumas, B.; Fabro, G.; Fronick, C.; Fuerstenberg, S.I.; Fulton, L.; Gaulin, E.; Govers, F.; Hughes, L.; Humphray, S.; Jiang, R.H.Y.; Judelson, H.; Kamoun, S.; Kyung, K.; Meijer, H.; Minx, P.; Morris, P.; Nelson, J.; Phuntumart, V.; Qutob, D.; Rehmany, A.; Rougon-Cardoso, A.; Ryden, P.; Torto-Alalibo, T.; Studholme, D.; Wang, Y.; Win, J.; Wood, J.; Clifton, S.W.; Rogers, J.; Van den Ackerveken, G.; Jones, J.D.G.; McDowell, J.M.; Beynon, J.; Tyler, B.M. Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome. *Science,* **2010**, *330*(6010), 1549-1551. http://dx.doi.org/10.1126/science.1195203 PMID: 21148394

[48] Raffaele, S.; Win, J.; Cano, L.M.; Kamoun, S. Analyses of genome architecture and gene expression reveal novel candidate virulence factors in the secretome of *Phytophthora infestans*. *BMC Genomics,* **2010**, *11*, 637. http://dx.doi.org/10.1186/1471-2164-11-637 PMID: 21080964

[49] Haas, B.J.; Salzberg, S.L.; Zhu, W.; Pertea, M.; Allen, J.E.; Orvis, J.; White, O.; Buell, C.R.; Wortman, J.R. Automated eukaryotic gene structure annotation using EVidenceModeler and the Program to Assemble Spliced Alignments. *Genome Biol.,* **2008**, *9*(1), R7. http://dx.doi.org/10.1186/gb-2008-9-1-r7 PMID: 18190707

[50] Haas, B.J.; Kamoun, S.; Zody, M.C.; Jiang, R.H.; Handsaker, R.E.; Cano, L.M.; Grabherr, M.; Kodira, C.D.; Raffaele, S.; Torto-Alalibo, T.; Bozkurt, T.O.; Ah-Fong, A.M.; Alvarado, L.; Anderson, V.L.; Armstrong, M.R.; Avrova, A.; Baxter, L.; Beynon, J.; Boevink, P.C.; Bollmann, S.R.; Bos, J.I.; Bulone, V.; Cai, G.; Cakir, C.; Carrington, J.C.; Chawner, M.; Conti, L.; Costanzo, S.; Ewan, R.; Fahlgren, N.; Fischbach, M.A.; Fugelstad, J.; Gilroy, E.M.; Gnerre, S.; Green, P.J.; Grenville-Briggs, L.J.; Griffith, J.; Grünwald, N.J.; Horn, K.; Horner, N.R.; Hu, C.H.; Huitema, E.; Jeong, D.H.; Jones, A.M.; Jones, J.D.; Jones, R.W.; Karlsson, E.K.; Kunjeti, S.G.; Lamour, K.; Liu, Z.; Ma, L.; Maclean, D.; Chibucos, M.C.; McDonald, H.; McWalters, J.; Meijer, H.J.; Morgan, W.; Morris, P.F.; Munro, C.A.; O'Neill, K.; Ospina-Giraldo, M.; Pinzón, A.; Pritchard, L.; Ramsahoye, B.; Ren, Q.; Restrepo, S.; Roy, S.; Sadanandom, A.; Savidor, A.; Schornack, S.; Schwartz, D.C.; Schumann, U.D.; Schwessinger, B.; Seyer, L.; Sharpe, T.; Silvar, C.; Song, J.; Studholme, D.J.; Sykes, S.; Thines, M.; van de Vondervoort, P.J.; Phuntumart, V.; Wawra, S.; Weide, R.; Win, J.; Young, C.; Zhou, S.; Fry, W.; Meyers, B.C.; van West, P.; Ristaino, J.; Govers, F.; Birch, P.R.; Whisson, S.C.; Judelson, H.S.; Nusbaum, C. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature,* **2009**, *461*(7262), 393-398. http://dx.doi.org/10.1038/nature08358 PMID: 19741609

[51] Gijzen, M.; Nürnberger, T. Nep1-like proteins from plant pathogens: recruitment and diversification of the NPP1 domain across taxa. *Phytochemistry,* **2006**, *67*(16), 1800-1807. http://dx.doi.org/10.1016/j.phytochem.2005.12.008 PMID: 16430931

[52] Kemen, E.; Gardiner, A.; Schultz-Larsen, T.; Kemen, A.C.; Balmuth, A.L.; Robert-Seilaniantz, A.; Bailey, K.; Holub, E.; Studholme, D.J.; Maclean, D.; Jones, J.D. Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of *Arabidopsis thaliana*. *PLoS Biol.,* **2011**, *9*(7), e1001094. http://dx.doi.org/10.1371/journal.pbio.1001094 PMID: 21750662

[53] Trusch, F.; Loebach, L.; Wawra, S.; Durward, E.; Wuensch, A.; Iberahim, N.A.; de Bruijn, I.; MacKenzie, K.; Willems, A.; Toloczko, A.; Diéguez-Uribeondo, J.; Rasmussen, T.; Schrader, T.; Bayer, P.; Secombes, C.J.; van West, P. Cell entry of a host-targeting protein of oomycetes requires gp96. *Nat. Commun.,* **2018**, *9*(1), 2347. http://dx.doi.org/10.1038/s41467-018-04796-3 PMID: 29904064

[54] Wang, Y.; Xu, Y.; Sun, Y.; Wang, H.; Qi, J.; Wan, B.; Ye, W.; Lin, Y.; Shao, Y.; Dong, S.; Tyler, B.M.; Wang, Y. Leucine-rich repeat receptor-like gene screen reveals that Nicotiana RXEG1 regulates glycoside hydrolase 12 MAMP detection. *Nat. Commun.,* **2018**, *9*(1), 594. http://dx.doi.org/10.1038/s41467-018-03010-8 PMID: 29426870

[55] Situ, J.; Jiang, L.; Fan, X.; Yang, W.; Li, W.; Xi, P.; Deng, Y.; Kong, G.; Jiang, Z. An RXLR effector PlAvh142 from Peronophythora litchii triggers plant cell death and contributes to virulence. *Mol. Plant Pathol.,* **2020**, *21*(3), 415-428. http://dx.doi.org/10.1111/mpp.12905 PMID: 31912634

[56] Jiang, R.H.; Tripathy, S.; Govers, F.; Tyler, B.M. RXLR effector reservoir in two Phytophthora species is dominated by a single rapidly evolving superfamily with more than 700 members. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(12), 4874-4879. http://dx.doi.org/10.1073/pnas.0709303105 PMID: 18344324

[57] Bailey, T.L.; Elkan, C. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proc. Int. Conf. Intell. Syst. Mol. Biol.,* **1994**, *2*, 28-36. PMID: 7584402

[58] Flor, H.H. The complementary genic systems in flax and flax rust. *Advances in genetics*; Academic Press, **1956**, Vol. 8, pp. 29-54.

[59] Petit-Houdenot, Y.; Fudal, I. Complex interactions between fungal avirulence genes and their corresponding plant resistance genes and consequences for disease resistance management. *Front. Plant Sci.,* **2017**, *8*, 1072. http://dx.doi.org/10.3389/fpls.2017.01072 PMID: 28670324

[60] Glazebrook, J. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.,* **2005**, *43*, 205-227. http://dx.doi.org/10.1146/annurev.phyto.43.040204.135923 PMID: 16078883

[61] Biffen, R.H. Mendel's laws of inheritance and wheat breeding. *J. Agric. Sci.,* **1905**, *1*, 4-48. http://dx.doi.org/10.1017/S0021859600000137

[62] Dong, X. NPR1, all things considered. *Curr. Opin. Plant Biol.,* **2004**, *7*(5), 547-552. http://dx.doi.org/10.1016/j.pbi.2004.07.005 PMID: 15337097

[63] Shao, Z.Q.; Xue, J.Y.; Wu, P.; Zhang, Y.M.; Wu, Y.; Hang, Y.Y.; Wang, B.; Chen, J.Q. Large-scale analyses of angiosperm nucleotide-binding site-leucine-rich repeat genes reveal three anciently diverged classes with distinct evolutionary patterns. *Plant Physiol.,* **2016**, *170*(4), 2095-2109. http://dx.doi.org/10.1104/pp.15.01487 PMID: 26839128

[64] Takken, F.L.; Goverse, A. How to build a pathogen detector: structural basis of NB-LRR function. *Curr. Opin. Plant Biol.,* **2012**, *15*(4), 375-384. http://dx.doi.org/10.1016/j.pbi.2012.05.001 PMID: 22658703

[65] Jacob, F.; Vernaldi, S.; Maekawa, T. Evolution and conservation of plant NLR functions. *Front. Immunol.,* **2013**, *4*, 297. http://dx.doi.org/10.3389/fimmu.2013.00297 PMID: 24093022

[66] Cui, H.; Tsuda, K.; Parker, J.E. Effector-triggered immunity: from pathogen perception to robust defense. *Annu. Rev. Plant Biol.,* **2015**, *66*, 487-511. http://dx.doi.org/10.1146/annurev-arplant-050213-040012 PMID: 25494461

[67] Kobe, B.; Deisenhofer, J. The leucine-rich repeat: a versatile binding motif. *Trends Biochem. Sci.,* **1994**, *19*(10), 415-421. http://dx.doi.org/10.1016/0968-0004(94)90090-6 PMID: 7817399

[68] Kobe, B.; Deisenhofer, J. Proteins with leucine-rich repeats. *Current Opin. Struc. Biol.,* **1995**, *1, 5*(3), 409-16. http://dx.doi.org/10.1016/0959-440X(95)80105-7

[69] Borhan, M.H.; Holub, E.B.; Beynon, J.L.; Rozwadowski, K.; Rimmer, S.R. The arabidopsis TIR-NB-LRR gene RAC1 confers resistance to *Albugo candida* (white rust) and is dependent on EDS1 but not PAD4. *Mol. Plant Microbe Interact.,* **2004**, *17*(7), 711-719. http://dx.doi.org/10.1094/MPMI.2004.17.7.711 PMID: 15242165

[70] Herlihy, J.; Ludwig, N.R.; van den Ackerveken, G.; McDowell, J.M. Oomycetes used in arabidopsis research. *Arabidopsis Book, 2019*(17), pp. 1-26.

[71] Meyers, B.C.; Kozik, A.; Griego, A.; Kuang, H.; Michelmore, R.W. Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. *Plant Cell,* **2003**, *15*(4), 809-834. http://dx.doi.org/10.1105/tpc.009308 PMID: 12671079

[72] Wiermer, M.; Feys, B.J.; Parker, J.E. Plant immunity: the EDS1 regulatory node. *Curr. Opin. Plant Biol.,* **2005**, *8*(4), 383-389. http://dx.doi.org/10.1016/j.pbi.2005.05.010 PMID: 15939664

[73] Tran, D.T.N.; Chung, E-H.; Habring-Müller, A.; Demar, M.; Schwab, R.; Dangl, J.L.; Weigel, D.; Chae, E. Activation of a plant NLR complex through heteromeric association with an autoimmune risk variant of another NLR. *Curr. Biol.,* **2017**, *27*(8), 1148-1160. http://dx.doi.org/10.1016/j.cub.2017.03.018 PMID: 28416116

[74] de Araújo, A.C.; Fonseca, F.C.D.A.; Cotta, M.G.; Alves, G.S.C.; Miller, R.N.G. Plant NLR receptor proteins and their potential in the development of durable genetic resistance to biotic stresses. *Biotechnol. Res. Innov.,* In press. https://doi.org/10.1016/j.biori.2020.01.002

[75] Michelmore, R.W.; Christopoulou, M.; Caldwell, K.S. Impacts of resistance gene genetics, function, and evolution on a durable future. *Annu. Rev. Phytopathol.,* **2013**, *51*, 291-319. http://dx.doi.org/10.1146/annurev-phyto-082712-102334 PMID: 23682913

[76] Panjabi-Massand, P.; Yadava, S.K.; Sharma, P.; Kaur, A.; Kumar, A.; Arumugam, N.; Sodhi, Y.S.; Mukhopadhyay, A.; Gupta, V.; Pradhan, A.K.; Pental, D. Molecular mapping reveals two independent loci conferring resistance to *Albugo candida* in the east European germplasm of oilseed mustard *Brassica juncea. Theor. Appl. Genet.,* **2010**, *121*(1), 137-145. http://dx.doi.org/10.1007/s00122-010-1297-6 PMID: 20213517

[77] Arora, H.; Padmaja, K.L.; Paritosh, K.; Mukhi, N.; Tewari, A.K.; Mukhopadhyay, A.; Gupta, V.; Pradhan, A.K.; Pental, D. BjuWRR1, a CC-NB-LRR gene identified in *Brassica juncea*, confers resistance to white rust caused by *Albugo candida*. *Theor. Appl. Genet.,* **2019**, *132*(8), 2223-2236. http://dx.doi.org/10.1007/s00122-019-03350-z PMID: 31049632

[78] Cesari, S. Multiple strategies for pathogen perception by plant immune receptors. *New Phytol.,* **2018**, *219*(1), 17-24. http://dx.doi.org/10.1111/nph.14877 PMID: 29131341

[79] Dong, O.X.; Ronald, P.C. Genetic engineering for disease resistance in plants: recent progress and future perspectives. *Plant Physiol.,* **2019**, *180*(1), 26-38. http://dx.doi.org/10.1104/pp.18.01224 PMID: 30867331

[80] van West, P.; Kamoun, S.; van 't Klooster, J.W.; Govers, F. Internuclear gene silencing in *Phytophthora infestans*. *Mol. Cell,* **1999**, *3*(3), 339-348. http://dx.doi.org/10.1016/S1097-2765(00)80461-X PMID: 10198636

[81] Vats, S.; Kumawat, S.; Kumar, V.; Patil, G.B.; Joshi, T.; Sonah, H.; Sharma, T.R.; Deshmukh, R. Genome editing in plants: exploration of technological advancements and challenges. *Cells,* **2019**, *8*(11), 1386. http://dx.doi.org/10.3390/cells8111386 PMID: 31689989

[82] Chen, Z.J.; Ni, Z. Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. *BioEssays,* **2006**, *28*(3), 240-252. http://dx.doi.org/10.1002/bies.20374 PMID: 16479580

[83] Augustine, R.; Bisht, N.C. Targeted silencing of genes in polyploids: lessons learned from *Brassica juncea*-glucosinolate system. *Plant Cell Rep.,* **2019**, *38*(1), 51-57. http://dx.doi.org/10.1007/s00299-018-2348-8 PMID: 30306251

[84] Castel, B. Natural and CRISPR-induced genetic variation for plant immunity*,* PhD Thesis, University of East Anglia, **2019**.

[85] Wu, C-H.; Krasileva, K.V.; Banfield, M.J.; Terauchi, R.; Kamoun, S. The "sensor domains" of plant NLR proteins: more than decoys? *Front. Plant Sci.,* **2015**, *6*, 134. http://dx.doi.org/10.3389/fpls.2015.00134 PMID: 25798142

[86] Kamoun, S. Molecular genetics of pathogenic oomycetes. *Eukaryot. Cell,* **2003**, *2*(2), 191-199. http://dx.doi.org/10.1128/EC.2.2.191-199.2003 PMID: 12684368

[87] Ali, Z.; Eid, A.; Ali, S.; Mahfouz, M.M. Pea early-browning virus-mediated genome editing *via* the CRISPR/Cas9 system in *Nicotiana benthamiana* and Arabidopsis. *Virus Res.,* **2018**, *244*, 333-337. http://dx.doi.org/10.1016/j.virusres.2017.10.009 PMID: 29051052

[88] Ma, C.; Zhu, C.; Zheng, M.; Liu, M.; Zhang, D.; Liu, B.; Li, Q.; Si, J.; Ren, X.; Song, H. CRISPR/Cas9-mediated multiple gene editing in *Brassica oleracea* var. *capitata* using the endogenous tRNA-processing system. *Hortic. Res.,* **2019**, *6*, 20. http://dx.doi.org/10.1038/s41438-018-0107-1 PMID: 30729010

[89] Lawrenson, T.; Shorinola, O.; Stacey, N.; Li, C.; Østergaard, L.; Patron, N.; Uauy, C.; Harwood, W. Induction of targeted, heritable mutations in barley and *Brassica oleracea* using RNA-guided Cas9 nuclease. *Genome Biol.,* **2015**, *16*, 258-270. http://dx.doi.org/10.1186/s13059-015-0826-7 PMID: 26616834

[90] Van Vu, T.; Sivankalyani, V.; Kim, E.J.; Tran, M.T.; Kim, J.; Sung, Y.W.; Doan, D.T.H.; Kim, J.Y. Highly efficient homology-directed repair using transient CRISPR/Cpf1-geminiviral replicon in tomato. *Plant Biotechnol. J*., **2020**, 521419. https://doi.org/10.1101/521419

[91] Neik, T.X.; Barbetti, M.J.; Batley, J. Current status and challenges in identifying disease resistance genes in *Brassica napus*. *Front. Plant Sci.,* **2017**, *8*, 1788. http://dx.doi.org/10.3389/fpls.2017.01788 PMID: 29163558

[92] Wu, Z.; Li, M.; Dong, O.X.; Xia, S.; Liang, W.; Bao, Y.; Wasteneys, G.; Li, X. Differential regulation of TNL-mediated immune signaling by redundant helper CNLs. *New Phytol.,* **2019**, *222*(2), 938-953. http://dx.doi.org/10.1111/nph.15665 PMID: 30585636

[93] Cesari, S.; Bernoux, M.; Moncuquet, P.; Kroj, T.; Dodds, P.N. A novel conserved mechanism for plant NLR protein pairs: the "integrated decoy" hypothesis. *Front. Plant Sci.,* **2014**, *5*, 606. http://dx.doi.org/10.3389/fpls.2014.00606 PMID: 25506347

[94] Castel, B.; Tomlinson, L.; Locci, F.; Yang, Y.; Jones, J.D.G. Optimization of T-DNA architecture for Cas9-mediated mutagenesis in Arabidopsis. *PLoS One,* **2019**, *14*(1), e0204778. http://dx.doi.org/10.1371/journal.pone.0204778 PMID: 30625150

[95] Orozco-Mosqueda, M.D.C.; Rocha-Granados, M.D.C.; Glick, B.R.; Santoyo, G. Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms. *Microbiol. Res.,* **2018**, *208*, 25-31. http://dx.doi.org/10.1016/j.micres.2018.01.005 PMID: 29551209

[96] Bandyopadhyay, P.; Bhuyan, S.K.; Yadava, P.K.; Varma, A.; Tuteja, N. Emergence of plant and rhizospheric microbiota as stable interactomes. *Protoplasma,* **2017**, *254*(2), 617-626. http://dx.doi.org/10.1007/s00709-016-1003-x PMID: 27468993

[97] Rybakova, D.; Mancinelli, R.; Wikström, M.; Birch-Jensen, A.S.; Postma, J.; Ehlers, R.U.; Goertz, S.; Berg, G. The structure of the *Brassica napus* seed microbiome is cultivar-dependent and affects the interactions of symbionts and pathogens. *Microbiome,* **2017**, *5*(1), 104. http://dx.doi.org/10.1186/s40168-017-0310-6 PMID: 28859671

[98] Links, M.G.; Demeke, T.; Gräfenhan, T.; Hill, J.E.; Hemmingsen, S.M.; Dumonceaux, T.J. Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between micro-organisms within a shared epiphytic microbiome on Triticum and Brassica seeds. *New Phytol.,* **2014**, *202*(2), 542-553. http://dx.doi.org/10.1111/nph.12693 PMID: 24444052

[99] Kumar, P.; Yadava, S.K.; Singh, P.; Bhayana, L.; Mukhopadhyay, A.; Gupta, V. A chromosome-scale assembly of allotetraploid *Brassica juncea* (AABB) elucidates comparative architecture of the A and B genomes. *bioRxiv,* **2019**. http://dx.doi.org/10.1101/681080