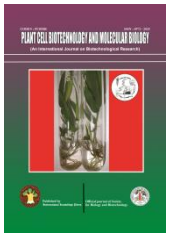

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Genomic and Molecular Outlook of Host-pathogen Interaction and Resistance Strategies against White Rust in Mustard (*Brassica juncea* L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

White rust, caused by the oomycete pathogen *Albugo candida*, poses a significant threat to mustard cultivation, leading to reduced yields and compromised oilseed quality. This study explores the genomic and molecular mechanisms underlying the host-pathogen interaction in *B. juncea* and outlines resistance strategies to counter white rust. While it is the predominant oilseed Brassica species in India, it is cultivated on a smaller scale in China and Australia. Due to its drought-hardy nature, *B. juncea* is being bred for canola-quality traits to expand its cultivation into lower rainfall regions. Research has demonstrated that resistance to white rust in *B. juncea* is regulated by a single dominant gene, and numerous lines, such as 'Bio-YSR', 'BEC-144', and 'JM-1', exhibit this trait. Comparative proteomic studies identified 19 proteins with significant expression changes

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between resistant and susceptible varieties. One key protein, PR-5, a thaumatin-like protein, has been linked to the resistance mechanism. Recent advances in genomic research, including the use of AFLPs, intron polymorphic markers, and SSRs, have enabled breeders to track and validate resistance traits. 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. These findings pave the way for genetic-based approaches to develop white rust-resistant *B. juncea* cultivars, providing a sustainable strategy to protect oilseed production in diverse climatic conditions. This study's insights contribute to a deeper understanding of host-pathogen interactions and offer promising solutions to mitigate the impact of white rust on mustard crops.

Keywords: AFLPs; CRISPR-Cas; dominant gene; host-pathogen; polymorphic markers; SSRs.

1. INTRODUCTION

Indian mustard (*Brassica juncea* L. Czern & Coss) is a significant *Rabi* oilseed crop and a member of the Brassica family. Brassica species, encompassing a variety of vegetables and oilseed crops, are integral to agricultural production worldwide [1]. Among these species, *Brassica juncea*, commonly known as Indian mustard, holds particular significance due to its amphidiploid nature, with a chromosome count of 2n = 36 (AABB) [2, 3]. This amphidiploidy arises from the combination of genomes from two diploid species: *Brassica rapa* (AA) with 2n = 20 chromosomes and *Brassica nigra* (BB) with 2n = 16 chromosomes [4, 5]. It plays a crucial role in oilseed production, especially in countries like India, China, and Pakistan. Its common names in India are rai, raya, or sarson. This versatile crop can be found in various forms such as seeds, powders, and oil. Although it is mainly grown in temperate regions during the *rabi* season, it requires relatively cool temperatures to thrive. According to the USDA National Nutrient Database (2023), mustard seeds offer a notable nutritional profile, providing 28.09 grams of carbohydrates, 6.89 grams of sugar, 12.2 grams of dietary fiber, 36.24 grams of fat, and 26.08 grams of protein per 100 grams [6]. Mustard seed is primarily crushed to produce edible oil, which is a staple in many daily diets due to its affordability. Globally, Indian mustard constitutes 10% of the total planted land under oilseeds and contributes 20.8% to India's total agricultural output. It has a global production of 72.4 million tonnes, with an annual productivity of 19.8% [7]. India accounts for 28.3% of the world's total acreage and output of Indian mustard. The country's production is approximately 8.3 million tonnes, with China and the European Union also contributing significantly, at about 11-12 million tonnes and 10-13 million tonnes [8].

In India, the primary producers of Indian mustard are the states of Rajasthan, Madhya Pradesh, Uttar Pradesh, and Haryana, which together account for over 74% of the nation's total production. Rajasthan leads with 44.9% of the production, followed by Madhya Pradesh with 11.3%, Uttar Pradesh with 10.6%, and Haryana with 8% [9]. In contrast, southern Indian states like Karnataka, Tamil Nadu, and Andhra Pradesh have less favourable conditions for mustard production due to soil salinization. However, mustard can grow in various agro-climatic conditions, from the Northeast and North-West to Central and Southern India. Indian mustard can tolerate a wide range of environmental conditions, including annual precipitation between 500 and 4200 mm, temperatures ranging from 6 to 27 degrees Celsius, and soil pH between 4.3 and 8.3 [10]. It follows the C3 pathway for carbon assimilation, demonstrating optimal photosynthetic response at temperatures between 15 and 20 degrees Celsius. Beyond this temperature range, its CO_2 exchange rate begins to decrease. Mustard grows best in well-drained sandy loam soil and is an excellent choice for rainfed farming systems due to its relatively low water requirement of 240–400 mm. The adaptability and resilience of Indian mustard contribute to its significant role in global oilseed production.

Despite its importance, *B. juncea* faces several challenges from biotic and abiotic stresses that affect its productivity. Among these, white rust, a fungal disease caused by *Albugo candida*, poses a significant threat. This fungal pathogen, which is among the top ten oomycete pathogens, is an obligate bio trophic parasite, affecting both the vegetative and reproductive phases of the plant [11]. White rust manifests as zoosporangial pustules that are white to cream-colored and can be found on various aerial parts of the plant, including cotyledons, leaves, stems, and

inflorescences [12]. The disease can lead to severe deformities such as stag head formation, resulting in greatly reduced seed production. Under late sown conditions, losses in seed yield due to white rust can be as high as 50%. To address this problem, disease resistance is a critical factor in crop production, ensuring quality, environmental safety, and optimal yield. A single gene (often referred to as an R-gene) or multiple genes with smaller effects can govern disease resistance in crops like *B. juncea* [13]. The Brassica species exhibit a wide range of genotypic variability in terms of resistance to white rust. While the Indian germplasm of *B. juncea* tends to be highly susceptible to white rust, the East European germplasm shows varying degrees of resistance, ranging from moderate to high [14].

Developing *B. juncea* varieties with inherent resistance or tolerance to white rust is an economically viable, environmentally friendly, and sustainable approach to meeting agricultural demands. Some cultivars, such as Heera and Donskaja IV, have been identified as resistant sources for white rust and are widely used in breeding programs [15]. Heera, with its resistant locus AcB1-A4.1, demonstrates moderate to strong resistance to common strains of *Albugo candida* [16]. However, Donskaja IV's locus AcB1-A5.1 provides complete resistance to the pathogen. Given the need for effective disease resistance, the current study focuses on evaluating Indian and East European gene pools of *B. juncea* for their resistance to white rust under conditions specific to the Jammu region. This research aims to identify genotypes that display strong resistance to the disease, providing a foundation for breeding efforts and sustainable crop production.

2. WHITE RUST: ITS DISEASE BIOLOGY

White rust (WR), also known as white blister or white blister rust, is a disease caused by *Albugo* species that affects over 400 species of plants worldwide. The name "white rust" comes from the distinctive white pustules that form on the surfaces of leaves and other aerial parts of the host due to enzymatic digestion of the epidermal cell wall [17]. These pustules are clusters of dehydrated sporangiospores, which, when rehydrated by moisture, can lead to infection through the stomata. Albugo spp. are part of the eukaryotic oomycete order *Albuginales* within the class Oomycota [18]. They are obligate biotrophic parasites with a wide host range, completely relying on host tissues for nutrition. *Albugo candida*, the pathogen responsible for white rust, reproduces both asexually through sporangia or zoospores, and sexually through thick-walled oospores [19], which are highly resistant and play a significant role in long-term survival (Fig 1). The presence of oospores in decaying crop debris and perennial mycelium in living host tissues allows the pathogen to persist between growing seasons.

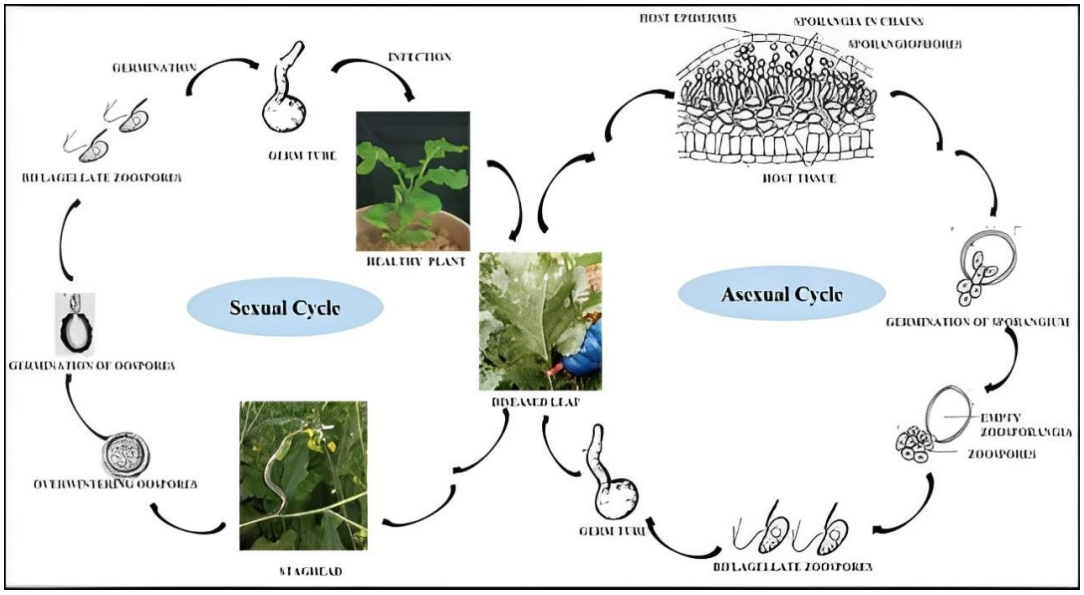

Fig. 1. Life cycle of representing the sexual and asexual cycle of *A. candida*

Moisture on plant surfaces is crucial for the germination of sporangia and infection by zoospores, with emerging cotyledons serving as likely primary infection sites. Once inside the plant, the pathogen enters via stomata and forms intercellular hyphae. It then penetrates the plant cell wall and creates a structure called a haustorium, allowing it to take up nutrients from the host while releasing effector proteins. When zoospores contact the plant leaf surface, they settle in the stomata, encyst, and produce a germ tube that extends into the sub-stomatal chamber and penetrates the host cell [17, 20]. This process leads to the characteristic "white blister" pustules when the infection matures, causing the sporangia to forcibly rupture the plant epidermis. The disease can manifest as local or systemic infection. Local infection appears as white or creamy-yellow pustules or "blisters" on leaves and stems, while systemic infection can result in abnormal growth, distortion of inflorescences, and sterility of flowers, known as staghead. White rust has a significant impact on oilseed crops, particularly in the Indian subcontinent, where most commercially grown lines of *Brassica juncea* are susceptible to the disease [21].

A. candida is known to infect over 200 plant species across 63 genera from the families *Brassicaceae, Cleomaceae*, and *Capparaceae*. There are 24 identified physiological races, with Race 2 (Ac2VRR) causing substantial annual losses in oilseed mustard in India, Canada, and Australia. Other races of *A. candida* specialize in different hosts, such as Race 1 (Ac1) that infects *Raphanus sativus,* Race 4 (Ac4) that affects *Capsella bursa-pastoris*, Race 5 (Ac5) that infects *Sisymbrium officinale*, and Race 6 (Ac6) that infects *Rorippa islandica* [22]. Race 7 (Ac7) is largely restricted to *B. rapa* but has been reported to affect some cultivars of *B. napus* and some genotypes of *B. juncea*. Race 9 (Ac9) infects *B. oleracea* [23]. Understanding the complex interactions between *A. candida* and its diverse hosts is crucial for developing effective disease management strategies and breeding resistant varieties of crops like Brassica juncea. The goal is to mitigate the significant yield losses and other adverse effects caused by this pervasive plant pathogen.

The flow chart (Fig 2) illustrates a progressive scale of rust infection on leaves, ranging from no visible symptoms to severe defoliation. At the beginning of the scale, there are no leaf symptoms, indicating that the plant is free from rust or at least visually uninfected. The next stage shows the emergence of minuscule dispersed pustules covering 5% or less of the leaf surface, suggesting early signs of rust. If the infection is not managed at this point, it can progress to the third stage, where up to 10% of the leaf surface is affected by rust pustules [24].

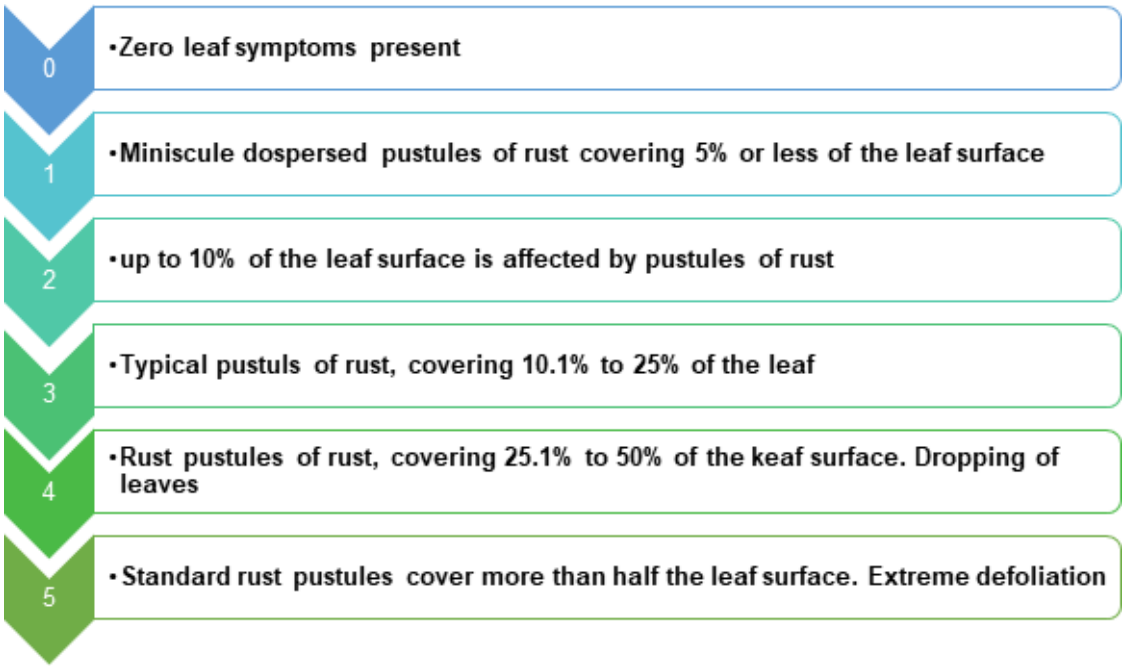

Fig. 2. Range of disease severity in white rust

Continuing along the scale, typical rust pustules develop, affecting between 10.1% and 25% of the leaf surface. This is a crucial stage where intervention may still prevent further spread. If unchecked, the infection can escalate to the fifth stage, where rust pustules cover between 25.1% and 50% of the leaf surface, leading to leaf drop and weakening of the plant. In the final stage, rust pustules cover more than half of the leaf surface, resulting in extreme defoliation. This level of infection severely impacts plant health and yield. The flow chart provides a visual guide for assessing rust severity and underscores the importance of early detection and timely management to prevent the disease from reaching the later, more destructive stages.

3. RESISTANCE SOURCES FOR WHITE RUST

To manage white rust disease in *Brassica juncea* (Indian mustard), it is critical to understand its origins and genetic resistance mechanisms. While *B. juncea* is a popular oilseed crop in India, it is less commonly grown in China and Australia, where *B. napus* is the predominant species. Despite this, *B. juncea* is cultivated in smaller regions in China, covering approximately 3,000 hectares. In regions like Canada, India, and Western China, *B. juncea* is often preferred over *B. napus* because it is more drought-resistant [25]. Given this adaptability, there is growing interest in expanding *B. juncea* cultivation, especially in areas with lower rainfall, such as parts of Australia. Australian farmers have been breeding *B. juncea* for canola-quality traits to match its resilience with the commercial standards of canola. This effort led to the release of new commercial *B. juncea* cultivars in 2006 with qualities comparable to canola [26]. The most effective and economical method for protecting mustard plants from white rust is through genetic resistance.

Research has shown that *B. juncea* has inherent resistance to *Albugo candida*, the pathogen causing white rust, including isolates from other host plants and the prominent race 2V. A study investigating the proteomic differences between resistant and susceptible B. juncea varieties identified 19 proteins with significant expression changes [27]. One of these proteins was a thaumatin-like protein, PR-5, suggesting a potential role in resistance mechanisms [28]. Further studies on *B. juncea* 'Bio-YSR' revealed that a single dominant gene regulates resistance to white rust. Monogenic dominance for resistance was confirmed through experiments using segregating populations derived from crosses with various resistant lines, such as Bio-YSR, BEC-144, and JM-1 [29]. Additionally, resistance to A. candida race 2V was successfully introduced into *B. juncea* from *B. napus*.

The data Table 1 presents a list of resistant donor plants along with the corresponding sources of their resistance to white rust. The plant "Donskaja-4" has resistance derived from the AcB1-A5.1 locus, indicating a specific genetic region associated with resistance [30]. Similarly, "Heera" sources its resistance from the AcB1-A4 locus, showing that different loci can contribute to resistance traits. "BIO YSR" draws its resistance from the BjuWRR1 gene, suggesting that a specific gene confers this resistance [31]. Lastly, "Tumida" demonstrates resistance originating from the LG A6 locus. These sources indicate the diversity of resistance mechanisms in various resistant donor plants, providing valuable genetic resources for breeding programs aimed at enhancing resistance against white rust.

Molecular mapping studies have identified specific resistance loci associated with white rust resistance in B. juncea. For example, two distinct resistance loci, AcB1-A4.1 and AcB1-A5.1, were identified in different linkage groups from East European lines. Furthermore, a single CC-NB-LRR protein expressing the R gene in *B. juncea* 'Donskaja-IV' conferred complete resistance to various [35] *A. candida* isolates, with this gene being named BjuWRR1. Molecular markers such as AFLPs, intron polymorphic markers, and SSRs have been employed in breeding programs to track and confirm resistance traits in Brassica species [36]. These markers enable breeders to select for resistant genotypes and accelerate

Table 1. List of resistant donor plants along with the sources of their resistance to white rust

Resistant donor	Resistant source	Review of literature
Donskaja-4	AcB1-A5.1 locus	Saharan et al. [32]
Heera	AcB1-A4 locus	Sharma et al. [33]
BIO YSR	BjuWRR1 gene	Devi et al. [30]
Tumida	LG A6 locus	Bhayana et al. [34]

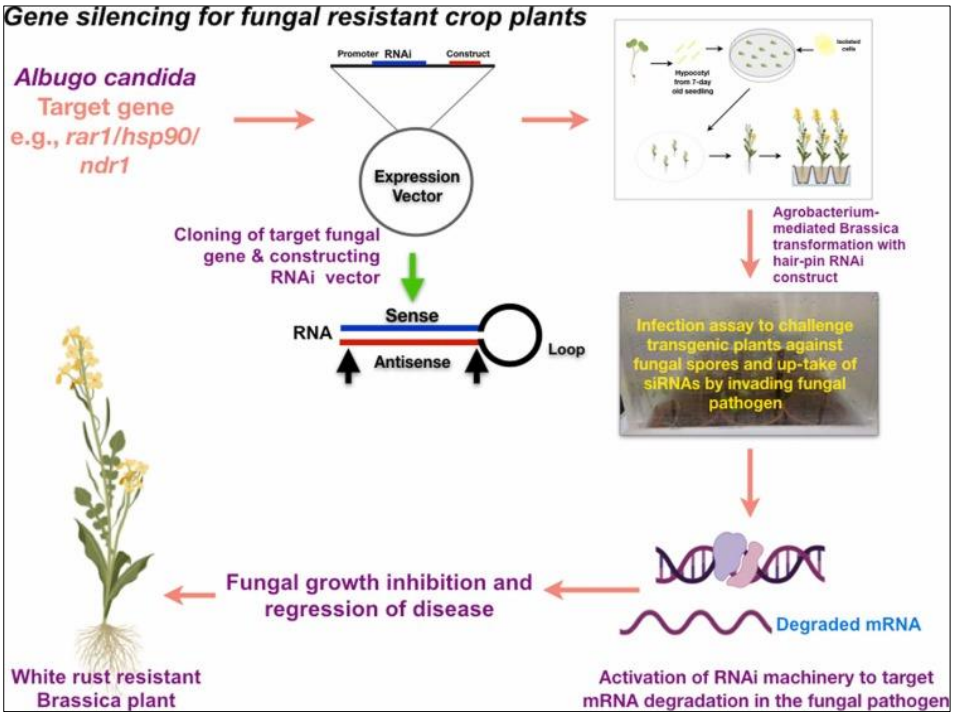

Fig. 3. Resistance strategies for defence against *AIbugo candida* causing white rust disease [22]

the development of white rust-resistant cultivars (Fig 3). These findings collectively point towards a genetic-based approach to control white rust disease in *B. juncea*. By harnessing the resistance genes and integrating them into susceptible cultivars, breeders can create more resilient crops, ensuring sustainable production of oilseed Brassica in various climates and regions [37].

4. CRISPR FOR DEVELOPING WHITE RUST RESISTANCE IN PLANT

CRISPR-Cas technology has revolutionized the field of genome editing, offering a highly precise, flexible, and efficient tool to modify the genomes of various organisms, including plants. Originating as a defense mechanism in prokaryotic cells against viruses, CRISPR-Cas has been adapted for targeted genetic changes in crops, providing a powerful method to address emerging agricultural challenges. CRISPR-Cas9 is particularly popular for its cost-effectiveness and ease of use compared to earlier genome editing methods like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) [37]. It has enabled precise gene editing, allowing researchers to knock out specific genes responsible for susceptibility to diseases or knock in resistance genes (R genes). CRISPR technology has been successfully applied to a variety of plant species, including *Arabidopsis*, rice, wheat, maize, tomato, and many others [38]. It has proven effective in creating resistant plant variants by targeting susceptibility genes (S genes) and modifying them to increase resistance to various plant pathogens.

For example, the MLO gene, which is associated with susceptibility to powdery mildew in many plants, was knocked out using CRISPR, resulting in complete resistance to the disease [39]. CRISPR technology has also been applied to combat plant viruses, such as the Beet severe curly top virus, Cucumber mosaic virus, and Tobacco mosaic virus, by targeting the viral RNA, thus providing resistance without inducing off-target mutations. In the Brassica family, a few successful applications of CRISPR genome editing have been reported, though the technique is still being optimized for polyploid species like *Brassica juncea*. One example involved knocking out the GA4 gene in a doubled-haploid genotype derived from *Brassica oleracea*. Additionally, CRISPR was used to knock out the self-incompatibility gene BoSRK3 and the BoMS1 gene, associated with male sterility, demonstrating the versatility and effectiveness of the technique [40-41].

However, there are challenges to overcome when applying CRISPR to plants, particularly in large genome sizes and high copy numbers in polyploid crops like *Brassica juncea*. Homology-directed recombination (HDR), a method used to achieve gene replacement or insertion, has a low frequency in plants, making stable gene targeting difficult. Despite these challenges, CRISPR-based genome editing holds significant promise for developing crop resistance to various diseases, including white rust in *Brassica juncea* [42]. Advances in sequencing and optimization of CRISPR for polyploid plants are expected to further enhance our ability to deploy durable resistance traits in susceptible plant varieties. Overall, CRISPR technology has the potential to transform plant breeding and agricultural practices by enabling rapid development of disease-resistant crops, reducing reliance on chemical pesticides, and increasing food security in the face of a growing global population [43]. With continued research and advancements in genetic data for *Brassica juncea*, CRISPR-based genome editing is poised to play a crucial role in sustainable agriculture and plant disease resistance [43-44].

5. STAGES OF INFECTION OF WHITE RUST IN

White rust, a disease caused by the fungus Albugo candida, can lead to significant yield losses in mustard (*Brassica juncea* L.). It appears in two main forms: as localized infections on leaves and as systemic infections affecting the inflorescence, leading to stag head formation (Table 2). The initial stage of infection involves the appearance of small white blisters or pustules on leaves. These can eventually cover large portions of the leaf surface, impacting photosynthesis and leading to reduced vigour. Stag head is a systemic infection resulting from the growth of the fungus in the plant's inflorescence, leading to deformities and a significant reduction in yield [45]. The extent of yield loss in India due to white rust ranges from 23% to 54.5%, depending on the severity and timing of infection. The blisters start as tiny creamy spots at the leaf tips, then expand and coalesce, eventually causing the leaves to wither and die.

Several factors contribute to the severity and rapid spread of white rust. Soil moisture, relative humidity, and temperature play a role in the development of white rust symptoms, with conditions of high humidity and moderate temperatures promoting fungal growth. The rapid spread of the disease can be attributed to the pathogen's aggressive nature and the dispersal of sporangia by wind and water. Stag head infections are particularly damaging, as they not only cause structural deformities in the plant but also severely reduce the quality and quantity of seeds [46]. Efforts to combat white rust focus on early detection, genetic resistance, and improved agronomic practices. Developing resistant cultivars is a priority, as it provides the most cost-effective means to reduce the impact of white rust and secure crop yield.

Table 2. Stages of infection of white rust in Indian mustard

Stage	Infection
Initial Infection	The illness begins when the *Albugo candida* fungus spores come into interaction with the host plant, which in the present instance is *Brassica juncea.* Spores can travel via wind, water, or contaminated plant debris.
Appressorium Formation	Once fungal spores have landed on the surface of a host organism, they germinate and create structures called appressoria. The fungus uses appressoria to secure its hold on the plant's surface.
Penetration and Hyphae Formation	Once the fungus has attached, it employs enzymes to eat through the plant's cuticle and cell wall, gaining access to the plant's inner tissues. Next, hyphae, which resemble threads, are developed by the fungus and used to colonize and multiply within the plant's cells.
Hypertrophy and Hyperplasia	The proliferation of the hyphae causes the host cells to swell (hypertrophy) and proliferate (hyperplasia). White rust is characterized by the appearance of pustules or swellings on the surface of infected plants.
Sporulation	There, the fungus reproduces and produces spores within the pustules. This spore is white and powdery and can be dispersed by wind, water, or people.
Secondary Infections	To further complicate matters, the pustules' spores can travel to neighbouring plants or other areas of the same plant, causing secondary infections. As long as the right conditions are met, this loop of infection, hyphal development, and sporulation will keep repeating itself.

6. FUTURE PERSPECTIVES

As the agricultural industry continues to seek sustainable solutions to combat crop diseases, the future of white rust control in mustard appears promising. Advanced genomic techniques, including CRISPR-Cas genome editing, offer new pathways for developing resistant cultivars [47-48]. By targeting specific genes responsible for susceptibility or inserting resistance genes from other Brassica species, breeders can create mustard varieties with enhanced resistance to *Albugo candida*, the causative agent of white rust [49]. This precise genetic approach not only accelerates breeding programs but also minimizes the need for chemical interventions, aligning with environmentally sustainable practices.

Proteomic and transcriptomic analyses will likely play a pivotal role in uncovering the molecular mechanisms underlying host-pathogen interactions in *B. juncea*. Understanding these interactions at a cellular level can help identify key proteins and signalling pathways involved in resistance. Future research could focus on expanding the catalogue of resistance-associated proteins, providing more targets for genetic engineering. Additionally, exploring gene regulatory networks and epigenetic factors might offer insights into how resistance traits can be stabilized across generations. Another crucial aspect for future development is the integration of traditional breeding with modern genomic tools. Marker-assisted selection (MAS) allows breeders to track resistance loci with high precision, facilitating the rapid development of resistant lines. Combining MAS with genomic selection could further streamline the breeding process, reducing the time and resources needed to produce commercially viable resistant cultivars [50]. This approach is especially relevant in polyploid crops like *B. juncea*, where the complexity of the genome poses additional challenges.

The climate resilience of *B. juncea* is another area of interest. As global weather patterns shift, mustard cultivars must adapt to varying conditions, including drought and heat stress [51]. Breeding programs that incorporate resistance to white rust while enhancing drought tolerance and other agronomic traits will be crucial for maintaining and expanding oilseed production in new regions. Furthermore, collaboration among researchers, breeders, and farmers will be essential to ensure that the development of resistant cultivars aligns with real-world agricultural needs. Overall, the future of controlling white rust in mustard relies on a combination of innovative genomic technologies, comprehensive molecular studies, and collaborative breeding efforts. By leveraging these tools, the agricultural industry can secure a more sustainable and resilient future for oilseed Brassica crops, ultimately contributing to global food security and environmental stewardship [52-53].

7. CONCLUSION

White rust, poses a significant threat to mustard crops, particularly in regions where it is a crucial oilseed. The development of effective resistance strategies is essential to ensure sustainable production and meet the growing global demand for oilseed crops. The intersection of traditional breeding and modern genomic technologies offers a promising pathway to overcome this challenge. Genomic approaches, such as CRISPR-Cas genome editing, are revolutionizing our ability to create resistant cultivars. These tools allow for precise modifications, enabling researchers to target specific genes involved in susceptibility or introduce resistance genes from related Brassica species. This precision not only accelerates the development of resistant varieties but also reduces reliance on chemical controls, promoting environmentally sustainable agriculture. Proteomic and transcriptomic studies further enhance our understanding of the host-pathogen interaction, revealing key molecular players in the resistance mechanism. By identifying and characterizing these proteins, researchers can develop new targets for genetic engineering, potentially leading to broader and more durable resistance across different strains of Albugo candida. Traditional breeding methods, combined with modern genomic tools like marker-assisted selection, play a critical role in delivering resistant cultivars to farmers. This hybrid approach is particularly effective for polyploid crops like *B. juncea*, where the complexity of the genome requires precise and reliable breeding techniques. Additionally, the resilience of mustard to drought and other climate stresses can be harnessed to develop cultivars that thrive in diverse environments. Ultimately, the success of resistance strategies against white rust in mustard depends on collaborative efforts among researchers, breeders, and farmers. By leveraging advanced genomic technologies, comprehensive molecular studies, and robust breeding programs, we can ensure a sustainable and productive future for oilseed Brassica crops. This approach not only

safeguards food security but also contributes to a more resilient agricultural system in the face of global challenges.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar R, Pandey MK, Chand P. Correlation and Path-coefficient Analysis for Oil and Its Fatty Acids Traits in Indian Mustard [*Brassica juncea* (L.) Czern & Coss.]. Research Square. 2023;01-09. Available:https://doi.org/10.21203/rs.3.rs-3278206/v1
2. Riaz U, Anum W, Murtaza G, Jamil M, Samreen T, Sohail I, Iqbal R, Ameen M. Genomics of Mustard Crops. Oil Crop Genomics. 2021:271-90.
3. Kumar R, Pandey MK, Priyanka N, Kumar V. Character Association and Path Analysis Studies in Indian Mustard [*Brassica juncea* L.]. Research Square. 2023;01-09. Available:https://doi.org/10.21203/rs.3.rs-3252020/v1
4. Gupta M, Banga SS. Heteroploidy in Brassica juncea: Basics and Applications. In The Brassica juncea Genome Cham: Springer International Publishing.2022; 115-145.
5. Singh S, Ram M, Gupta D, Meena MK, Nayak PK, Choudhary K, Kumar R, Chouhan S. Assessing genetic variability in taramira (*Eruca sativa* Mill.) germplasm for enhanced breeding strategies. International Journal of Economic Plants. 2024;11:018-25.
6. Youssef MK, El-Newihi AM, Omar SM, Ahmed ZS. Assessment of proximate chemical composition, nutritional status, fatty acid composition and antioxidants of curcumin (*Zingiberaceae*) and mustard seeds powders (*Brassicaceae*). Food and Public Health 2014;4(6):286-292.
7. Jat RS, Choudhary RL, Singh HV, Meena MK, Singh VV, Rai PK. Sustainability, productivity, profitability and soil health with conservation agriculture based sustainable intensification of oilseed brassica production system. Scientific Reports. 2021;11(1):13366.
8. Bhanu AN, Srivastava K, Singh RK. Advances in agronomic management in Indian mustard for Eastern Uttar Pradesh. Acta Sci. Agric. 2019;3:70-9.
9. Chand S, Patidar OP, Chaudhary R, Saroj R, Chandra K, Meena VK, Limbalkar OM, Patel MK, Pardeshi PP, Vasisth P. Rapeseed-mustard breeding in India: Scenario, achievements and research needs. Brassica Breeding and Biotechnology. 2021:174.
10. Malik MM. *Genetic Divergence among Local and Exotic Brassica juncea Germplasm as Revealed by Using Simple Sequence Repeats (SSRs)* (Doctoral dissertation, Capital University; 2015.
11. Kamoun S, Furzer O, Jones JD, Judelson HS, Ali GS, Dalio RJ, Roy SG, Schena L, Zambounis A, Panabières F, Cahill D. The Top 10 oomycete pathogens in molecular plant pathology. Molecular plant pathology. 2015;16(4):413-34.
12. Joy J, Rathod RR, Das P, Suryawanshi AP. Reactions of mustard varieties/germplasms/advanced breeding lines against white rust in field under Konkan conditions. Journal of Pharmacognosy and Phytochemistry. 2019;8(6):2012-4.
13. Singh KP, Kumari P, Rai PK. Current status of the disease-resistant gene (s)/QTLs, and strategies for improvement in Brassica juncea. Frontiers in Plant Science. 2021; 12:617405.
14. Kaur G, Singh VV, Singh KH, Priyamedha, Rialch I, Gupta M, Banga SS. Classical genetics and traditional breeding in Brassica juncea. InThe *Brassica juncea* Genome 2022 Mar 9 Cham: Springer International Publishing. 2022;85-113.
15. Arora H, Padmaja KL, Paritosh K, Mukhi N, Tewari AK, Mukhopadhyay A, Gupta V, Pradhan AK, Pental D. BjuWRR1, a CC-NB-LRR gene identified in *Brassica juncea*, confers resistance to white rust caused by Albugo candida. Theoretical and Applied Genetics. 2019;132:2223-36.
16. Arora H, Padmaja KL, Paritosh K, Mukhi N, Tewari AK, Mukhopadhyay A, Gupta V, Pradhan AK, Pental D. BjuWRR1, a CC-NB-LRR gene identified in Brassica juncea, confers resistance to white rust caused by Albugo candida. Theoretical and Applied Genetics. 2019;132:2223-36.
17. Anupriya C, Shradha N, Prasun B, Abha A, Pankaj S, Abdin MZ, Neeraj S. Genomic and molecular perspectives of host-pathogen interaction and resistance strategies against white rust in oilseed mustard. Current Genomics. 2020;21(3): 179-93.

18. Crowther W. Advancing genomics to enable anticipatory breeding of resistance in Brassica crops to diseases of global importance (Doctoral dissertation, University of Warwick); 2014.
19. Saharan GS, Verma PR, Meena PD, Kumar A, Saharan GS, Verma PR, Meena PD, Kumar A. The Pathogen. White Rust of Crucifers: Biology, Ecology and Management. 2014:55-90.
20. Haber S, Harder DE. Oat diseases and pathologic techniques. Oat science and technology. 1992;33:307-425.
21. Inturrisi FC, Barbetti MJ, Tirnaz S, Patel DA, Edwards D, Batley J. Molecular characterization of disease resistance in *Brassica juncea*–The current status and the way forward. Plant Pathology. 2021; 70(1):13-34.
22. Nirwan S, Chatterjee A, Cevik V, Holub EB, Jones JD, Tewari AK, Shrivastava N, Agnihotri A, Sharma P. Genetic manipulation ofF Indian mustard genotypes with WRR-gene (s) confers resistance against *Albugo candida*. Molecular Biology Reports. 2024;51(1): 199.
23. Kaur P. Agronomic challenges from novel pathotypes of Albugo candida to the emerging Brassica juncea industry in Western Australia. Phytopathologia Mediterranea. 2013:418-33.
24. Angelotti F, Scapin CR, Tessmann DJ, Vida JB, Canteri MG. The effect of temperature, leaf wetness and light on development of grapevine rust. Australasian Plant Pathology. 2014;43:9-13.
25. Song F, Meng Z, Luo T, Xin J, Xian M, Rao N, Chen Q, Wang Y, Khan MN, Hu L. Cytological identification of new-type Brassica napus materials and their physiological response to drought. Crop and Pasture Science. 2019;70(10):876-89.
26. Barve YY, Gupta RK, Bhadauria SS, Thakre RP, Pawar SE. Induced mutations for development of B. juncea canola quality varieties suitable for Indian agro-climatic conditions. Induced plant mutations in genomics era. Food and Agriculture Organization of the United States. 2009: 373-5.
27. Kaur P, Jost R, Sivasithamparam K, Barbetti MJ. Proteome analysis of the Albugo candida–Brassica juncea pathosystem reveals that the timing of the expression of defence-related genes is a crucial determinant of pathogenesis. Journal of Experimental Botany. 2011;62 (3):1285-98.
28. Chand S, Singh N, Prasad L, Nanjundan J, Meena VK, Chaudhary R, Patel MK, Taak Y, Saini N, Vasudev S, Yadava DK. Inheritance and allelic relationship among gene (s) for white rust resistance in Indian mustard [*Brassica juncea* (L.) Czern & Coss]. Sustainability. 2022;14(18):11620.
29. Sharma R, Sharma M, Salgotra RK. Biochemical profiling of Brassica juncea Germplasm. Primus. 2022;41(16.29):50-13.
30. Devi YS, Devi TR, Thakur AK, Ngangkham U, Devi HN, Kh P, Sinha B, Senjam P, Singh NB, Mishra LK. Evaluation of Indian mustard genotypes for white rust resistance using BjuWRR1 gene and their phenotypic performance. Agronomy. 2022; 12(12):3122.
31. Arora H, Padmaja KL, Paritosh K, Mukhi N, Tewari AK, Mukhopadhyay A, Gupta V, Pradhan AK, Pental D. BjuWRR1, a CC-NB-LRR gene identified in Brassica juncea, confers resistance to white rust caused by *Albugo candida*. Theoretical and Applied Genetics. 2019;132:2223-36.
32. Singh Saharan G, Mehta NK, Meena PD. Genomics of Crucifer's Host-Pathosystem: Prologue. In Genomics of Crucifer's Host-Pathosystem. Singapore: Springer Nature Singapore. 2023;1-126
33. Sharma R, Sharma M, Salgotra RK. Evaluation of *Brassica juncea* germplasm for white rust resistance. The Pharma Innovation Journal. 2022;11(10):1883-1886.
34. Bhayana L, Paritosh K, Arora H, Yadava SK, Singh P, Nandan D, Mukhopadhyay A, Gupta V, Pradhan AK, Pental D. A mapped locus on LG A6 of Brassica juncea line Tumida conferring resistance to white rust contains a CNL type R gene. Frontiers in Plant Science. 2020;10:496858.
35. Arora H, Padmaja KL, Paritosh K, Mukhi N, Tewari AK, Mukhopadhyay A, Gupta V, Pradhan AK, Pental D. BjuWRR1, a CC-NB-LRR gene identified in Brassica juncea, confers resistance to white rust caused by Albugo candida. Theoretical and applied genetics. 2019;132:2223-36.
36. Kumar M, Choi JY, Kumari N, Pareek A, Kim SR. Molecular breeding in Brassica for salt tolerance: importance of microsatellite (SSR) markers for molecular breeding in *Brassica*. Frontiers in Plant Science. 2015;6:688.

37. Elouafi I, Shahid MA, Begmuratov A, Hirich A. The contribution of alternative crops to food security in marginal environments. Emerging Research in Alternative Crops. 2020:1-23.
38. Singh M, Singh VV, Singh N, Monika. Drought Tolerance in Rapeseed-Mustard: Conventional and Molecular Approaches. InGenomic Designing for Abiotic Stress Resistant Oilseed Crops. Cham: Springer International Publishing.2022;199-218
39. Ton LB, Neik TX, Batley J. The use of genetic and gene technologies in shaping modern rapeseed cultivars (*Brassica napus* L.). Genes. 2020;11(10):1161.
40. Gupta D, Bhattacharjee O, Mandal D, Sen MK, Dey D, Dasgupta A, Kazi TA, Gupta R, Sinharoy S, Acharya K, Chattopadhyay D. CRISPR-Cas9 system: A new-fangled dawn in gene editing. Life sciences. 2019; 232:116636.
41. Tyagi S, Kumar R, Kumar V, Won SY, Shukla P. Engineering disease resistant plants through CRISPR-Cas9 technology. GM Crops & Food. 2021;12(1):125-44.
42. Santillán Martínez MI, Bracuto V, Koseoglou E, Appiano M, Jacobsen E, Visser RG, Wolters AM, Bai Y. CRISPR/Cas9-targeted mutagenesis of the tomato susceptibility gene PMR4 for resistance against powdery mildew. BMC Plant Biology. 2020;20:1-3.
43. Dai C, Tian X, Ma C. A Twentieth-Century Challenge. InGenome editing for precision crop breeding. Burleigh Dodds Science Publishing. 2021;403-419
44. 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. Horticulture Research. 2019;6.
45. Moon TT, Maliha IJ, Khan AA, Chakraborty M, Uddin MS, Amin MR, Islam T. CRISPR-Cas genome editing for insect pest stress management in crop plants. Stresses. 2022;2(4):493-514.
46. Rialch I, Dhaliwal I, Rana K, Kaur J, Kaur G. Genomic Designing for Biotic Stress Resistance in Rapeseed. InGenomic Designing for Biotic Stress Resistant Oilseed Crops. Cham: Springer International Publishing. 2022;55-84
47. Raza A, Tabassum J, Fakhar AZ, Sharif R, Chen H, Zhang C, Ju L, Fotopoulos V, Siddique KH, Singh RK, Zhuang W. Smart reprograming of plants against salinity stress using modern biotechnological tools. Critical Reviews in Biotechnology. 2023;43 (7):1035-62.
48. Saharan GS, Mehta N, Meena PD, Saharan GS, Mehta N, Meena PD. Association or Mixed Infection of Downy Mildew and White Rust Disease Complex. Downy Mildew Disease of Crucifers: Biology, Ecology and Disease Management. 2017:199-213.
49. Latif MZ, Haq IU, Ijaz S, Faraz A. Rapeseed and Mustard: Diseases, Etiology, and Management. InSustainable Winter Fodder. CRC Press.2021;199-222.
50. Wang Y, Zafar N, Ali Q, Manghwar H, Wang G, Yu L, Ding X, Ding F, Hong N, Wang G, Jin S. CRISPR/Cas genome editing technologies for plant improvement against biotic and abiotic stresses: advances, limitations, and future perspectives. Cells. 2022;11(23):3928.
51. Langner T, Kamoun S, Belhaj K. CRISPR crops: plant genome editing toward disease resistance. Annual Review of Phytopathology. 2018;56:479-512.
52. Mushtaq M, Ahmad Dar A, Skalicky M, Tyagi A, Bhagat N, Basu U, Bhat BA, Zaid A, Ali S, Dar TU, Rai GK. CRISPR-based genome editing tools: Insights into technological breakthroughs and future challenges. Genes. 2021;12(6):797.
53. Ragimekula N, Varadarajula NN, Mallapuram SP, Gangimeni G, Reddy RK, Kondreddy HR. Marker assisted selection in disease resistance breeding. Journal of Plant Breeding and Genetics. 2013;1(2): 90-109.

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