

# Physiologic specialization, host resistance and epidemiology of white rust and downy mildew disease complex in rapeseed and mustard – The research scenario in Haryana

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## ABSTRACT

Rapeseed and mustard group dominates amongst oilseed crops in area and production all over Haryana. More than twenty diseases are known to affect the rapeseed mustard group of crops in Haryana, but diseases like white rust and downy mildew caused by *Albugo candida* and *Peronospora parasitica* are of major consequences because of wide spread and destructive nature thereby causing heavy yield losses annually through out Haryana. Although association of these two diseases was reported long back but their importance has been realized only recently. Sources of resistance has been reported but their utility and effectiveness in breeding for disease resistance cultivars is limited due to lack of information on the occurrence and distribution of pathotypes and perfect genotypes screening technique. Different researchers in Haryana conducted studies on the topic to generate more information for improvement of rapeseed and mustard. Under Hisar conditions average temperature of more than 15°C, average RH>65 per cent and intermittent rains on susceptible cultivars proved most conducive for white rust development whereas in case of downy mildew, 15-20°C temperature and Relative humidity above 70 per cent was the best for infection and development of downy mildew. To standardize host differentials by considering the homogeneity and purity of species and varieties is demand of the day.

**Key words:** Downy mildew - Rapeseed Mustard - White rust

## INTRODUCTION

India is one of the leading oilseeds producing country in the world accounting for nearly 18 per cent of the world's rapeseed-mustard production. In Haryana during 2006-07, rapeseed and mustard was cultivated in an area of 0.60 million ha which is 8.84% of total area of India, with a production of 0.80 million tones which is 10.75% of total Indian production with average yield of 1343 kg/ha. More than twenty diseases are known to affect the rapeseed mustard group of crops in India, but diseases like White rust and Downy mildew are of major consequences because of their global distribution and heavy yield losses. The disease affects number of *Brassica* plants of economic importance but its incidence and damage is more in mustard (*B. juncea*) and rapeseed. Staghead formation (systemic infection) due to white rust and / or mixed infection of white rust and downy mildew causes losses in yield from 23 to 60 per cent (Saharan et al., 1984; Saharan and Verma. 1992). Although association of these two diseases was reported long back but their importance has been realized only recently with the assessment of losses in mustard crop from 17-54% due to mixed infection mainly on inflorescence resulting into stagheads. Sources of resistance has been reported but their utility and effectiveness in breeding for disease resistance cultivars is limited due to lack of information on the occurrence and distribution of pathotypes and perfect genotype screening techniques. The mixed infection of white rust and downy mildew is conspicuous at floral stage of crop growth. In the family cruciferae about 50 genera and more than 100 different species are susceptible to infection by downy mildew pathogen. Yield losses due to downy mildew infection alone are very difficult to estimate, since in most cases it is always associated with white rust. The information gathered on the occurrence of pathotypes and resistance will be helpful in identification of new genes for resistance affective against existing virulence from the germplasm collection. Screening of varieties against specific virulence will also give clue about quality of resistance genes (strong or weak in different combination which in turn can be utilized for breeding resistant cultivars.

## OBJECTIVES

The main objective of the above study is standardization of host differentials and Identification of pathotypes in *Albugo* and *Peronospora* causing infection as disease complex in rapeseed mustard and to investigate inheritance of rapeseed mustard resistance with known genes of resistance, virulence and epidemiology of the disease complex, perfection of screening technique against white rust and downy mildew diseases for evaluating rapeseed mustard genotypes under laboratory and field conditions, to find strong correlation between increase in rust pustules and environmental factors, to find out differential reaction between isolates of *Albugo candida* and *Peronospora parasitica* on host differentials.

## RESEARCH SCENERIO

### Physiologic Specialization

This is well established in *A. candida*. whereas isolates of *P. parasitica* differ in the range of cruciferous host that they can infect. A historical account given by Saharan and Verma (1992) indicated that it was Eberhart who recognized the specialized groupings of *Albugo*, one attacking *Capsella*, *Lepidium* and *Arabis*, and the other attacking *Brassica*, *Sinapis* and *Diplotaxis*. From India, 9 races were identified from four host species, viz., *B. juncea*, Lakra and Saharan (1988a) identified five races of *A. candida* on the basis of their reaction on a set of sixteen host differentials. They have identified two distinct races from *B. juncea*, which are different from previous records. One (race 2) attacking *B. nigra*, *B. juncea* and *B. campestris* var. brown sarson and the other (race 3), infecting only *B. juncea* and *B. campestris* var. *toria*. Gupta and Saharan (2002) identified four new pathotypes viz., AC-14, AC-15, AC-16 and AC-17 of *A. candida*.

Strains of *P. parasitica* are known to be both homothallic as well as heterothallic. Specialization of parasitism may be exhibited at the generic and lower taxonomic levels of the host. Different pathogenic varieties or formae specialis of the fungus has been recognized in species of *Brassica* (*P. parasitica* var / f. sp. *Brassicaceae*), *Raphanus* (var. f. sp. *raphani*) and *Capsella* (var./f. sp. *capsellae*). Physiologic specialization at the generic level of the host is widely reported on *Brassica* and *Sinapis*. Mehta and Saharan (1994) tested nine isolates of *P. parasitica* collected from the leaves and stagheads of six host species on seventeen host differentials. Isolates from *Brassica* oilseeds infected all species except *B. alba*, whereas isolates from cauliflower leaves do not infect *B. carinata*, *B. alba*, *B. nigra*, *B. chinensis*, *B. pekinensis* and *B. napus*. There was no significant difference among the conidial size of the isolates collected from leaves and stagheads but significant differences were observed among these groups. The isolates were classified into two distinct pathotypes, one from cauliflower and other from oilseeds *Brassicaceae*. There was no significant difference between the isolates in percentages of spore germination. Saharan et al. (1995) reported that out of 17 host differentials, seven host differentials viz., *Brassica alba*, *Brassica campestris* var. *parkland*, *B. carinata*, *B. napus*, *B. napus* var. *wester*, *Eruca sativa* and *Sinapis alba* gave resistant reaction to white rust at foliar stage and inflorescence stage. *Brassica juncea* showed susceptible reaction at inflorescence stage. Similarly there was variability in pathogen (*Peronospora parasitica*) isolated from cauliflower, turnip, sprouting broccoli (*Brassica oleracea* var. *gemmifera*) & bathu (*Chenopodium album*) and it is clear that the pathogen is host specific.

### Host Resistance

*Albugo candida* infects a large number of host plants in aizoaceae, capparidaceae, coleomaceae, cruciferae and amaranthaceae family. All accessions in *B. napus* and most cultivars in *B. oleracea* are resistant to *A. candida*. The resistance in *B. napus* cv. Regent is governed by three genes, AC-7-1, AC-7-2 and AC-7-3. In India, cvs. Tower 1, 2, 3, 4, Gullivar, Midas, Norin, Regent, H -715 and HNS-I of *B. napus* are resistant to *A. candida*. The procedure of disease scoring scale of white rust was modified / refined and was given a range of 0-5 and /or 0-9 scale at different places by different workers (Saharan, 1992). Lakra and Saharan (1988 b) developed a descriptive scale of 0-5 for assessing both phases of white rust development on mustard individually and/or combined. Subsequently Dang et al. (2000), reported that *B. alba*, *B. campestris* (varieties, BSH-I, Chamba, Gulivar, Sangam, SSK-I, TH-68), *B. carinata* (HC-I), *B. juncea* (varieties DIR-1507, DIR-1522, ZEM-I), *B. napus* (varieties Tower, GS-7027, HNS-4, HNS-IO, Midas, Norin), *B. pekinensis*, *Eruca sativa* and

*Raphanus sativus* have been found resistant to white rust in India. Similarly they also observed that varieties such as *B. alba*, HC-I, H-IIOA, PHR-I, DIR-1507, DIR-1522, TMS-50, ZEM-I, GS-7027, HNS-4, HNS- 10, Midas and Norin resistant to downy mildew. Saharan and Krishnia (2001) have reported multiple disease resistance (white rust, Alternaria blight, powdery mildew) in HC-I and PCC-I of *B. carinata* and GSL-1501 of *B. napus*. Gupta et al. (2002) have reported that genotypes EC-129126-1 and Shiva were free from white rust on leaves as well as inflorescences even under late sown conditions. The results of interspecific hybridization between *B. juncea* and *B. carinata* envisage easy transfer of white rust resistance into high seed yield background through pedigree selection from an unadapted species *B. carinata* to the adapted species *B. juncea*. The resistance which is monogenic with complete dominance in *B. carinata* could be partially introgressed into *B. juncea* cultivars by selecting disease-free plants in advance segregating generation grown under heavy disease pressure and their repeated back crossing to *B. juncea* cultivars. In a study involving inheritance of resistance to *A. candida* race 2 in mustard. Saharan et al. (1995) reported that the germplasm lines viz., GSL-1501, DOMO, B<sub>10</sub>YSR, Wester, GC 7027, RH 8539, PR 8805, DWRR 15, HNS 4, RN 248, JMM-W-7, EC-129126-1, GSL-1, HNS-3, DIR-1002, Midas, PC-3, Norin-14 & HC-1 were found resistant to white rust & downy mildew on the first observation. However, out of the above mentioned germplasm lines the DOMO, B<sub>10</sub>YSR, Wester, RH-8539, DWRR-15, JMM-W-7, DIR-1002 & Norin-14 tend to show the susceptible reaction on last observation.

### **Epidemiology**

The germination of the sporangia is closely dependent upon the prevailing temperature and relative humidity. The optimum temperature for their germination is about 10°C at which the rate of germination and the number of zoospores formed are maximum. Sporangia do not germinate if the temperature is above 25°C. For the germination of oospores 10-20°C temperature is most congenial. Date of sowing has great bearing on floral infection of white rust. When mustard sowing is delayed from 1<sup>st</sup> week of October to 3<sup>rd</sup> week of November, the disease intensity increases from 4.6 to 68.5 per cent. Epidemic development of the disease under field conditions occurred when temperature around 12°C, relative humidity > 70 per cent (mostly between 60-80%), wind velocity from 2.7-3.4 km/h and winter rains have been found as most congenial. Under Hisar conditions average temperature of more than 15°C, average RH>65 per cent and intermittent rains on susceptible cultivars proved most conducive. Early planting from 15<sup>th</sup> September to October avoids stag-head formation and gives higher yield. Hypertrophy in the plant is directly correlated with the amount of oospore formation. Sporangia germinate between 6-22°C. Maximum sporangial germination takes place between 12-14°C, dark conditions and 7 h of incubation period. Saharan et al. (1995) studied progression of white rust on different genotypes of rapeseed mustard in relation to prevailing environmental conditions in the field and developed regression equation for three germplasm Culture-1 ( $-16.65+0.72 x_1-0.32 x_2+0.09 x_3+0.01 x_4-0.38 x_5-0.05 x_6$ ), CSR-721 ( $-20.44+0.96 x_1-0.58 x_2+0.12 x_3-0.005 x_4-0.64 x_5-0.07 x_6$ ) and LWR-7 as  $-12.41+1.04 x_1-0.46 x_2+0.015 x_3 -0.006 x_4-1.07 x_5-0.23 x_6$ ) for cumulative progression of white rust pustules size on rapeseed mustard. Where  $x_1$  is maximum temperature,  $x_2$  is minimum temperature,  $x_3$  is RH (Morning),  $x_4$  is RH (Evening) and  $x_5$  is sunshine hours and  $x_6$  is average rainfall. Similarly developed regression equation for white rust pustules number and periodical progression of white rust pustules size and number in relation to environmental conditions.

Peronospora produces both sexual (oospores) and asexual (conidial/sporangia) spores, which are helpful in survival and dissemination of the pathogen. Floral infection increases in the late sown crops. The disease is favoured by damp and cool environmental conditions. More infections occur at low temperature (8-16°C), moist weather and low light intensity. Penetration of the host occurs most rapidly at 16°C. Haustoria development is optimum at 20-25°C. Downy mildew develops very fast at 24°C. Sporangia exposed to air are infectious for six weeks but direct sun light may kill them within 5-6 h. Relative humidity >70 per cent helps in rapid development of the disease. In a subsequent study, 15-20°C was the best temperature for infection and development of downy mildew. At this temperature regime infection occurs with in 24 h of inoculation. The infection frequency is reduced at 25°C while no infection is observed at 30°C. Leaf wetness duration of 4-6 h at 20°C and for 6-8 h at 15°C is essential for severe

infection and disease development on mustard. The infection frequency and disease development increases significantly with the increase in duration of leaf wetness (Mehta et al., 1995b). In India, infection of mustard foliage starts by the end of October (cotyledon stage) and progresses up to November. The crop planted after mid-November may not contract downy mildew. However, downy mildew growth as a mixed infection with white rust on floral parts can be seen upto March (Mehta and Saharan, 1998).

#### **Downy mildew and White Rust disease complex**

The associations of downy mildew and white rust infection on oilseed Brassicas have long been observed. In artificially inoculated leaves of mustard, the stimulatory effect of *A. candida* infection is more intense when *P. parasitica* is inoculated 7 days after *A. candida*. When *Peronospora* and / or *Albugo* are inoculated alone or in different combinations, the downy mildew infection takes 7 days, while white rust appears within 5-6 days of inoculations. When both the pathogens are inoculated simultaneous in a 50:50 sporangial concentration then, there is delay in the expression of infections by *Peronospora* for 2-3 days (Mehta et al., 1995a). Histopathological studies carried out by Mehta et al. (1995a) indicated that conidia of *Peronospora* and sporangia of *Albugo* inoculated on mustard leaves germinate 24 h after inoculation. Two days after inoculation infection is normally confined to the host epidermis. Six days after inoculation, the pathogens progress deeper into the tissues. When *P. parasitica* is inoculated prior or after *A. candida*, mycelium can be seen in the intercellular spaces with globose to knob-like haustoria in the mesophyll cells. When *A. candida* is inoculated alone or in combination with *P. parasitica*, the pathogen emerges from the lower epidermis and forms pustules. However, on its own *Peronospora* causes necrosis in the mesophyll cells. When both the pathogens are inoculated together, the infection is confined to the upper layer of the mesophyll with limited colonization of the cells and few haustoria or mycelium in the intercellular spaces. Nine days after inoculation, characteristic disease symptoms are visible. The white rust pustules show hyaline sporangiophore bearing globose to oval shaped sporangia in chains. The *Peronospora* mycelium is intercellular with lobe-shaped haustoria in the distorted tissue of leaves. When both pathogens are inoculated together, infection is extended to mesophyll cells and there is development of pustule below the epidermis. Twelve days after inoculation, complete colonization of the host tissue by the pathogen is evident from the development of necrotic zone by *P. parasitica* and bursting of pustules releasing sporangia in case of *A. candida*. In the inflorescence, the mycelium passes through the epidermis, hypodermis, cortex and finally reaches to the pith region. The mycelium is in abundance in the cortex and produces conidiophores bearing conidia above the epidermis layer. For *A. candida*, numerous sporangiophores bearing sporangia are observed below the epidermis layer in the form of pustules and knob-like haustoria in the tissues. In the colonized tissues both pathogens cannot be distinguished based on somatic morphology.

#### **DISCUSSION**

The purpose of presentation is to compile the research information gathered on the occurrence of pathotypes and resistance which will be helpful in identification of new genes for resistance effective against existing virulence from the germplasm collection. Screening of varieties / germplasm against specific virulence will also give us clue about quality of resistance genes (strong or weak in different combination) which in turn can be utilized for breeding resistant cultivars, analysis of components of resistance / tolerance and will also be helpful in determining nature of tolerance / resistance in varieties. The importance of resistance study will form a firm base in developing disease resistant cultivars. The information of research scenario of Haryana will be helpful for the other workers to conduct research with the futuristic approach.

#### **FUTURISTIC APPROACH OF RESEARCH**

The techniques utilized for research and development / testing of resistant plants needs standardization and more improvement. More research is required to standardize host differentials to observe the homogeneity and purity of species and varieties. More study on pathogenic variability of pathogen is required so as to allow appropriate selection but not

so severe that plants with some resistance are graded as susceptible.

In fact, more research on the nature of inoculum, its concentration and distribution on the host plants, the age of host to be tested and the environmental conditions of testing place are of paramount importance. They should be standardized based on global research and use of Information Technology in oilseed improvement programme as very little information is available regarding the resistance of the wild species to different diseases. The relationship between pathogenicity on wild hosts and crop plants needs further study since these sources of inoculum may act as a theatre for increased genetic variation in the pathogen resulting in to wider virulence.

Information needs to be generated regarding marking of specific chromosome segments involved in qualitative and quantitative traits by using ELISA. RAPDS may in fact be useful sources of RFLP probes and once the sequence of the amplified region has been characterized, can serve as genetic anchors for physical mapping of the genome. RAPD fingerprinting may distinguish between species, pathotypes or even separate clonal population.

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