J. Hortic. Sci.
Vol. 20(2), 2025

https://doi.org/10.24154/jhs.v20i2.3065

Original Research Paper

Identification of novel sources of resistance to white rust (*Puccinia horiana*) in Chrysanthemum (*Chrysanthemum morifolium*)

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ABSTRACT

Chrysanthemum is a premier commercial floriculture crop valued for its versatility as a loose flower for garlands, a cut flower for arrangements, and an ornamental pot or bedding plant. However, white rust, caused by *Puccinia horiana* Henn., remains a major pathological constraint resulting in significant economic losses. This study evaluated the resistance of 175 chrysanthemum genotypes under natural field conditions, with a subset of 20 genotypes further validated via *in vitro* detached leaf assays. Results indicated that 18 genotypes were highly susceptible, recording the highest per cent disease index (PDI > 61%). Conversely, 27 genotypes were categorized as resistant (PDI 11–20%), while, the remaining genotypes exhibited no symptoms (PDI 1–10%) and were classified as highly resistant. Notably, genotypes such as Mayur, NBRI Little Kusum, and Arka Yellow Gold demonstrated consistent high resistance, while, genotype Marigold, Sunil, and Punjab Gold were susceptible across both screening environments. These identified resistant genotypes serve as valuable genetic resource for commercial cultivation and future breeding programs aimed at disease resistance.

Key words: Chrysanthemum, per cent disease index, resistance, screening, white rust

INTRODUCTION

Chrysanthemum, family Asteraceae, is native to the Northern Hemisphere, primarily Europe and Asia, is a major commercial flower crop for cut flower, potted plant, and herbaceous perennial markets. Chrysanthemum white rust (*Puccinia horiana* Henn.) is one of the destructive diseases globally. This microcyclic autoecious rust has a quarantine status, produces basidiospores and teliospores and completes its life cycle on a single host species and can cause major damage in the commercial cultivation of chrysanthemum.

In India, chrysanthemum white rust is an emerging disease. This rust disease was noticed in and around Bengaluru, Karnataka and Udagamandalam, Tamil Nadu during 2012, with a renewed occurrence in 2014. In certain germplasm accessions, the disease incidence was 100%, whereas in others, it ranged from 20% to 60% (Sriram et al., 2015). Given the international and often trans-continental production of planting material and cut flowers of chrysanthemum and the decreasing availability of registered fungicides in specific regions, prophylactic fungicide spray is essential to prevent damage and economic losses. However, the adoption of resistant genotypes in quality chrysanthemum production is environment friendly and long-term alternative to excessive chemical application. Therefore, the present study was carried out to screen 175 chrysanthemum genotypes for disease reaction against white rust and to identify white rust resistant genotypes.

MATERIALS AND METHODS

Screening under natural condition

The experiment was conducted at the research block of Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, India during 2020 to 2021. The experimental site was located at 13° 7′ N latitude and 77° 29′ E longitudes with an altitude of 890 meters above MSL. The experimental material consisted of 175 genotypes (Supplementary Table 1) evaluated in randomized block design with three replications under natural condition. The disease scoring was carried out at weekly intervals for 14 weeks starting from 1st week

of December 2020 to 2nd week of March 2021. Variance analysis was performed using genotype as the single factor across three replicates. To meet the assumptions of normality, per cent disease index (PDI) values were arc-sine transformed. All statistical processing was conducted using custom code in SAS (Statistical Analysis System) at the ICAR-IIHR Statistical Laboratory, Bengaluru, India.

Artificial screening using detached leaf assay

A total of 20 genotypes including 3 highly susceptible genotypes were selected based on natural screening to confirm the resistance reaction using detached leaf method assay by the method of artificial inoculation. The selected genotypes *viz.*, Mayur, Doddabyale-kere-1, Pachai Local, NBRI Little Kusum, Statesman, Autumn Joy, Basanti, Pink Cloud, Aparajita, Rekha, Arka Pink Star, Arka Yellow Gold, Co-1, White Dolley, Texas Gold, Himanshu, Yellow Charm, Punjab Gold, Sunil and Marigold were screened by detached leaves assay method in the laboratory with three replications. The leaves of highly susceptible chrysanthemum genotype Sunil infected with white rust were collected. The pustules were excised with a sterile blade, and the inoculum was collected in Eppendorf tubes and stored at 4°C in the refrigerator. Pustules were gently crushed in sterile water using a pestle and mortar; the suspension was then held in Eppendorf tubes at 17°C for a duration of 6 to 7 hours. After approximately 3 hours, teliospore germination began, followed by the release of basidiospores 6 to 7 hours later. The teliospore count per mL of *P. horiana* spore suspension was 1.29×10^6. Basidiospore suspension was sprayed on leaves of 20 chrysanthemum genotypes in petri plates and kept in the incubator at optimum temperature 17°C for initiation and development of the white rust pustules.

Disease assessment

Disease severity in the field was recorded based on evaluation of the intensity of the disease symptoms of the plant using 0-4 scale (Bonde et al., 1995). Five plants in each genotype were scored by using the disease scale for *P. horiana*. A disease rating scale was used where, 0: no visible infection, 1: fewer than 5 pustules per plant, 2: 5 to 100 pustules per plant, 3: more than 100 pustules per plant, and 4: more than 100 pustules per plant and two or more leaves with coalesced lesions covering at least 75% of the leaf area.

The PDI was calculated using the given formula and the genotypes were categorized into five groups namely highly resistant (1-10%), resistant (11-20%), moderately susceptible (21-40%), susceptible (41-60%) and highly susceptible (>61%).

$$\text{PDI} = \frac{\text{Sum of all the rating}}{\text{Total no. of rating} \times \text{Maximum disease grade}} \times 100$$

Area under disease progress curve (AUDPC)

AUDPC is another criterion which indicates the pathogen's speed of progression in plant tissue and is used to distinguish between resistant and susceptible genotypes. Disease incidence data recorded at a weekly interval was used to calculate the area under disease curve progression (AUDPC) as a measure of quantitative disease resistance involving repeated disease assessments. The AUDPC was computed based on the disease scores using the following formula (Jeger & Rollinson, 2001).

$$\text{AUDPC} = \sum_{i=1}^{n=1} \left[\left\{ \frac{X_{i+1} + X_{i+1}}{2} \right\} x \{ t_{i+1} - t_i \} \right]$$

where i is the proportion of disease on the i^{th} observation ti is the time (days) of observation expressed as days after transplanting (DAT) and N is the total number of disease severity readings (PDI) taken throughout the experimental period.

RESULTS AND DISCUSSION

Screening under natural condition

Evaluation of 175 chrysanthemum genotypes under natural field conditions revealed significant variation in their response to *Puccinia horiana*. Based on the per cent disease index (PDI), the germplasm was categorized into distinct resistance levels (Table 1; Supplementary Table 1). Of the 175 genotypes screened, 76 remained symptom-free and were classified as highly resistant. The remaining genotypes were distributed across the resistance spectrum: 27 were resistant, 35 were moderately susceptible, 19 were susceptible, and 18 were highly susceptible.

Disease severity increased due to climatic conditions in January and February. In January, there was 12.1 mm of rainfall, a mean relative humidity of 78.09% (range: 66–89%), and average temperatures ranging from 16.93°C (min) to 29.24°C (max). February recorded 15.20 mm of rainfall, 60.50% mean

Table 1 : Classification of the chrysanthemum genotypes based on disease index against white rust caused by *P. horiana*

Disease response	Disease index	Genotype	No. of genotypes
Highly resistant	1-10	NBRI Little Kusum, Yellow Delight, Mayur, Doddabyalekere 1, Statesman, Autumn Joy, Kajal, Cherabu, Kargil, Basanti, Pink Cloud, Winter Queen, Aparajita, Gulmohar, Co-1, Texas Gold, Yellow Charm, Corcom Small, Mallika Yellow, White Dolley, White Andamon, Anmol, Fitonia, Rekha, IIHR 1-1, IIHR 2-3, IIHR 2-8, IIHR 2-14, IIHR 2-24, IIHR 2-25, IIHR 2-31, IIHR 2-34, IIHR 2-40, IIHR 2-46, IIHR 3-8, IIHR 3-21, IIHR 3-22, IIHR 4-1, IIHR 4-4, IIHR 4-11, IIHR 4-17, IIHR 4-21, IIHR 4-23, IIHR 4-24, IIHR 4-29, IIHR 4-30, IIHR 4-32, IIHR 4-33, IIHR 4-34, IIHR 4-39, IIHR 5-7, IIHR 5-15, IIHR 5-31, IIHR 6-4, IIHR 6-9, IIHR 6-12, IIHR 6-18, IIHR 6-24, E 85, LYSD, Selection-2, DFR-2, IARI-1, Rajamundri collection, Pusa Sona, Maghi Yellow, Hosur 1, Yellow Spoon, Appu, IIHR 5-6, Arka Usha Kiran, Arka Chandrakant, Arka Yellow Gold, Arka Kirti, Arka Pink Star, Red Stone	76
Resistant	11-20	Karnool Yellow, Doddabyalekere 2, White Local, Shukla, Jublee, Flirt, Little Darling, Jyotsia, Haldighati, IIHR 2-5, IIHR 2-11, IIHR 2-17, IIHR 2-20, IIHR 2-28, IIHR 2-30, IIHR 2-32, IIHR 2-45, IIHR 3-3, IIHR 3-9, IIHR 3-10, IIHR 3-16, IIHR 4-7, IIHR 5-10, Roopanjali, LYD, DFR-3, Selection 5	27
Moderately Susceptible	21-40	A1 Collection, A2 collection, Heritage, Coffee, White Prolific, Dundi, Star Pink, Maghi Orange, Dolley Orange, NBRI Little Hemant, Pusa Aditya, Karnool Pink, Shaymal, IIHR 1-2, IIHR 2-1, IIHR 2-7, IIHR 2-15, IIHR 2-16, IIHR 2-27, IIHR 2-36, IIHR 3-12, IIHR 3-13, IIHR 5-13, IIHR 9-9, IIHR 6-13, IIHR 6-16, IIHR 6-23, TP-1, TP-4, Agnipath, Raja, Bhima, PAU Anmol, Chandini, Arka Chandrika	35
Susceptible	41-60	Ratlam Selection, Sunil, Gouri, Vijay Kiran, IIHR 2-18, IIHR 2-29, IIHR 2-38, IIHR 3-4, IIHR 3-6, IIHR 5-28, IIHR 5-14, IIHR 5-29, IIHR 6-5, IIHR 6-26, IIHR 6-27, HCC-3, Agnishika, HYDC-14, NBRI Little Pink	19
Highly susceptible	> 61	Marigold, Punjab Gold, Pusa Kesar, Golden Yellow, IIHR 2-21, IIHR 2-41, IIHR 3-7, IIHR 3-11, IIHR 5-28, IIHR 6-20, TP-2, TP-3, Rajat, Farr, Swapna, Bidhan Mum, Kalpana, IIHR 5-8	18

humidity (range: 38–82%), and mean temperatures between 13.27°C and 29.66°C. As temperature increased, there was sudden reduction in disease severity as recorded in several genotypes.

There was no rainfall during March and the mean minimum and maximum temperature recorded was 12.33°C and 32.38°C, respectively and mean relative humidity (46.36%) (ranged 28-67%) (Fig. 1). With the increase in temperature, the pustules started drying and the disease severity in different genotypes declined subsequently. As a result, no further progression of the disease took place. There was presence of few pustules in highly infected genotypes and there was no symptom of disease infection in other chrysanthemum genotypes.

Several studies have evaluated chrysanthemum germplasm for white rust resistance with varying results. Backer et al. (2011) found only eight resistant cultivars out of 36, Park et al. (2014) identified resistance in 41 spray cultivars and three wild species, noting that 37 others were moderately resistant. In targeted genotype screenings, Thakur et al. (2019) verified the resistance of five Arka-series genotypes in multiple environments. These findings were expanded by Kumar et al. (2021), who reported resistance in eleven specific genotypes, such as Punjab Gold, Red Stone, and Lal Pari.

The inheritance of resistance of *P. horiana* in chrysanthemum is described as complete resistance

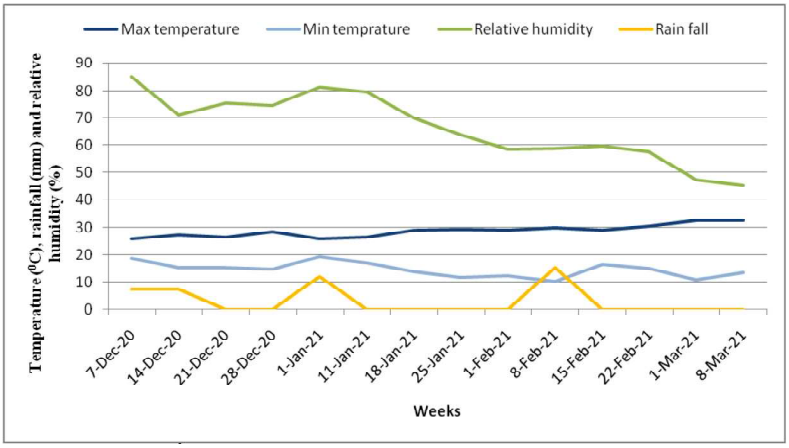

Fig. 1 : Temperature (°C), rainfall (mm) and relative humidity (%) during investigation

when no symptoms can be detected visually and it is controlled by a single dominant gene (Jong & Rademaker, 1986). Because chrysanthemum is a hexaploid, the susceptible response may occur if all copies have the recessive gene 'r'. The plant may exhibit total resistance if one or more copies possess the dominant allele 'R'. This form of host-pathogen interaction was found in 104 (76- highly resistant and 27- resistant) chrysanthemum genotypes and classified as resistant. Incomplete resistance with few pustules that develop slowly and sporulate sparsely was the second kind of host-pathogen interaction documented in this study, as observed in 35 genotypes. This could be due to the mycelium's difficulties in penetrating the leaf, or because the pustules were small and required longer to grow (Thakur et al., 2019). This needs a high spore-pressure as well as favourable fungus-growth circumstances.

This type of interaction was also described by Jong & Rademaker (1986) in the crosses of these incomplete resistance genotypes with susceptible ones. They found no obvious monogenic ratio, as well as variances in pustule size and latent time, and concluded that incomplete resistance is regulated by many genes. The third form of host-pathogen interaction reported in this study was vulnerable in remaining chrysanthemum genotypes with numerous rust pustules that develop rapidly and sporulate abundantly. In both symptomatic and asymptomatic infected host tissues, Keefe & Davis (2015) documented direct penetration of the host epidermis by the basidiospore germ tube and colonisation with intercellular mycelium and intracellular M-haustoria. Their findings revealed that the cuticle has no significant impact on resistance. According to Lone et al. (2016) another possible reason for the inhibition of fungal development in resistant chrysanthemum genotypes is the array of defense responses evoked by the pathogen, which leads to an increase in phenolic acids and flavonoids and the creation of phytoalexin.

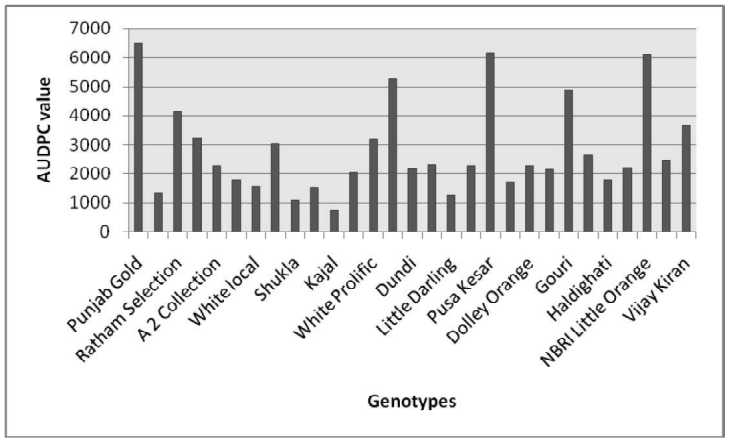

Fig. 2 : AUDPC for different genotypes of chrysanthemum screened under natural condition for white rust disease

Table 2 : Per cent disease index of chrysanthemum genotypes under *in vitro* screening

Genotype	0 dpi	2 dpi	4 dpi	6 dpi	8 dpi	10 dpi	12 dpi	14 dpi
Mayur	0	0	0	0	0	0	0	0
Doddabyalekere-1	0	0	0	0	0	0	0	0
Pachai Local	0	0	0	0	0	0	0	0
NBRI Little Kusum	0	0	0	0	0	0	0	0
Statesman	0	0	0	0	0	0	0	0
Autumn Joy	0	0	0	0	0	0	0	0
Basanti	0	0	0	0	0	0	0	0
Pink Cloud	0	0	0	0	0	0	0	0
Aparajita	0	0	0	0	0	0	0	0
Rekha	0	0	0	0	0	0	0	0
Arka Pink Star	0	0	0	0	0	0	0	0
Arka Yellow Gold	0	0	0	0	0	0	0	0
Co-1	0	0	0	0	0	0	0	0
White Dolley	0	0	0	0	0	0	0	0
Texas Gold	0	0	0	0	0	0	0	0
Himanshu	0	0	0	0	0	0	0	0
Yellow Charm	0	0	0	0	0	0	0	0
Punjab Gold	0	0	0	0	12.5	25	25	25
Sunil	0	0	0	12.5	25	25	50	37.5
Marigold	0	0	12.5	25	25	25	25	37.5

The AUDPC indicated the progress of the disease in a given crop growth period (Fig. 2). The resistant genotype Kajal showed less AUDPC value of 770, compared to other genotypes screened for resistance. The highest AUDPC value was recorded in Punjab Gold (6510) with average PDI of 69.28.

Screening under *in vitro* condition

Of the twenty chrysanthemum genotypes screened *in vitro*, three were categorized as moderately susceptible: Punjab Gold, Sunil, and Marigold, with PDI scores of 25, 37.5, and 37.5, respectively. The remaining genotypes remained asymptomatic throughout the study period (Table 2).

CONCLUSION

Puccinia horiana Henn., the causal agent of chrysanthemum white rust, is one of the most destructive diseases affecting chrysanthemum production worldwide. The increasing prevalence of fungicide-resistant strains has made chemical control less reliable, emphasizing the need for host-plant resistance. Because susceptible genotypes compromise the commercial value of the crop, this investigation focused on screening genotypes under both natural and controlled conditions. The resistant genotypes identified through this research offer a sustainable solution for commercial cultivation and serve as essential parental material for breeding programs.

ACKNOWLEDGEMENT

The senior author is thankful to the Department of Science and Technology, Govt. of India for providing DST-INSPIRE Fellowship for successful completion of Ph.D. at ICAR-Indian Institute of Horticultural Research, Bengaluru, India.

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(***Received : 18.6.2024; Revised : 3.7.2025; Accepted : 8.7.2025)***