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## Screening of chrysanthemum (*Dendranthema grandiflora*) genotypes for resistance to white rust (*Puccinia horiana* Henn.)

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**Abstract**

*Puccinia horiana* is the causal agent of chrysanthemum white rust. This microcyclic autoecious rust has a quarantine status and cause major damage in the commercial production of *Dendranthema grandiflora*. The experiment was carried out to identify genetic resources of chrysanthemum that are resistant/susceptible to white rust disease. A total 20 chrysanthemum genotypes were screened for resistance to *P. horiana* isolates collected from ICAR-IIHR, Bengaluru. By combing the observation methods without and with optical microscope, we established the identification standards for chrysanthemum white rust. The results showed that out of 20 genotypes screened for white rust, eleven genotypes/lines viz., Sweta Singar, Punjab Gold, Garden Beauty, Red Stone, Punjab Anuradha, Lal Pari, White Dolly, White Andaman, IIHR6-32, IIHR9-3 and IIHR6-41 were determined to be resistant. The use of white rust resistant genotypes will be useful for breeding programs in developing varieties with novel flower quality coupled with resistant to white rust for safe cultivation of chrysanthemum.

**Keywords:** Chrysanthemum, white rust, *Puccinia horiana*, screening, artificial inoculations, resistant

**Introduction**

Chrysanthemum (*Dendranthema grandiflora*) is one of the commercial flower crops grown throughout the world mainly for cut flower, potted plant, loose flower and bedding purpose. Chrysanthemum white rust (*Puccinia horiana* Henn.) was first discovered in Japan in 1895 and was confined to China and Japan until the 1960s. It is established in Europe, Africa, Australia, Central America, South America, and the Far East. Chrysanthemum white rust is one of the disastrous diseases in southern parts of India which caused severe damage to chrysanthemum production. It was first noticed in Udagamandalam district of Tamil Nadu since 2012, with a renewed occurrence there in 2014. Later, the disease was noticed in field grown chrysanthemum in and around Bengaluru, Karnataka in 2013 and 2014. The severity of occurrence of this disease was post-monsoon season followed by the winter spell (October to December) when temperature range between 15° to 26 °C and relative humidity 90 to 95% [1]. Due to intermittent rains in monsoon, the temperature falls down and become favorable for white rust initiation [2].

White rust spread quickly in greenhouse and nursery environments causing severe crop losses. The symptoms of this disease are very distinct; light green to yellow spots up to 5 mm in diameter appear on the upper surface of the leaf. These spots become brown and necrotic with age. Raised beige to pink pustules form on the underside of leaves beneath the spots. Pustules become white with age. Pustules are most common on young leaves and flower bracts but may form on any green tissue or the petals. Symptoms usually occur during cool, wet weather. The disease mainly affects the leaves, but under severe infestation, spread to stems, bracts, or even the flowers. Under severe infection, the pustules coalesced leading to complete drying and death of the plant. The optimal conditions for development of the pathogen are a high relative humidity and cool temperatures (17°-20°C) [3]. Under these conditions, symptoms appear 7–10 days post infection as chlorotic spots that develop teliospores after 14–18 days. Leaf wetness and a high relative humidity are essential for basidiospore formation, survival, and infection [3, 4]. To prevent damages and economic losses, fungicides are regularly sprayed on chrysanthemum before the plants show symptoms of the disease [5, 6]. Although white rust can be chemically controlled [5, 4], the use of fungicides is associated with both environmental hazards and an increased production cost [7]; over dependence on their use has already led to the appearance of tolerant strains [8, 9].

The more sustainable strategy of exploiting genetically determined resistance requires the identification of sources of resistance, as first attempted [10, 11]. Resistance is most commonly under monogenic control [12]. Several chrysanthemum spp. have been reported as resistant to white rust [10, 13]. An intergeneric hybrid between chrysanthemum and *Artemisia sieversiana* has been shown to be more resistant than the chrysanthemum parent [14]. Hybridization between commercial chrysanthemum and various *Ajania* and *Chrysanthum* spp. has been repeatedly achieved [15, 16], therefore, distant crossing could have considerable potential for white rust resistance breeding.

Four types of reactions of the chrysanthemum cultivars to *P. horiana* have been described [12]. They suggested that a single dominant gene controls resistance in hexaploid chrysanthemum based on the segregations for resistance. Variation for resistance to white rust in chrysanthemum species have been reported [17]. Despite the yearly introduction of newly bred cultivars, there has been no report regarding the evaluation of commercial cultivars resistant to white rust [18]. Moreover, in view of the interaction between hosts and parasites, several studies have demonstrated the existence of differential interactions between *P. horiana* isolates and Chrysanthemum species, and the highly complex race structure in this pathosystem [19, 20]. In spite of the economic importance of chrysanthemum in India, however, there is little information available on the genetic resources resistant to white rust. Therefore, the present study was carried out to identify chrysanthemum genotypes/lines resistance to *Puccinia horiana* Henn.

## 2. Materials and Methods

### 2.1 Planting materials

Twenty chrysanthemum genotypes viz., Sweta Singar, Kalpana, IIHR6-26, Ajay, Local White, Punjab Gold, IIHR9-12, Garden Beauty, IIHR6-29, Red Stone, Arka Chandrika,

Arka Kirti, Punjab Anuradha, IIHR6-32, IIHR9-3, IIHR6-41, Lal Pari, White Dolly, IIHR2-7, and White Andaman were used for the study. Screening was carried out under net house (75% shade net) condition during January to March 2017. Uniform rooted suckers of 6-7 cm length were taken from the mother plants of 20 genotypes/lines and kept in coco-peat filled media in 12" pots for 10 days to rooting for better establishment. Eighteen uniform rooted suckers of each genotypes/lines were planted in plastic pots (12") with two plants each pot, under net house with 3 replications using the completely randomized design.

### 2.2 Inoculations

After 12 days of planting, the cuttings were inoculated with teliospores collected from diseased cultivars Swapna and Marigold. To minimize disease escape, plants were inoculated by spraying spore suspension (semi-quantitative inoculation) method derived [4]. The rust pustules were removed from chrysanthemum leaf surface with the help of scalpel blade. These rust pustules were kept for germination in sterile distilled water under 17-19°C. Frequently observed for germ tube splitting and spore germination. Germ tube formation started after 5-6 hrs of incubation. Splitting of germ tube and basidiospores were released after 7 hrs of incubation. The concentration of the pathogenic spore suspension was then adjusted using a hemacytometer slide to a concentration of  $1.16 \times 10^6$  basiosporangia/ml with deionized water containing one drop of Tween 20 before application to the plants until runoff using a hand held sprayer. The plants were kept under net house condition during January-February with naturally maintained high relative humidity (80%), sufficient light (14-15 hour's photoperiod) and temperature (15 to 25°C) in southern Karnataka [21]. Different stages of infected leaves, aggregated and individual teliospores and post-inoculation symptoms on plants are shown in Figure 1.



**Fig 1:** A- overview of experiment, B- Different stages of infected leaves with white rust, C- Aggregated and individual teliospores, D- Post - inoculation symptoms on plants

### 2.3 Disease monitoring and classification

After artificial post inoculation, the important parameters such as latent period, infection type, disease incidence and disease severity were recorded at the time when teliospore containing pustules were well developed. Monitoring was continued until 50 days post-inoculation (dpi). The latent period was defined as the number of days elapsed between inoculation and the first appearance of symptoms [22]. Infection type was represented by a 0 to 5 scale, which was observed from the majority (15 or greater) of individual data, adapted [23], in which '0' indicated no visible symptoms, '1' indicated rare visible yellowish hypersensitive flecks are discernible, '2' indicated a few small yellowish flecks and very little telia on the back, '3' indicated more small or few large yellowish necrosis and clear telia on the back, '4' indicated large and

continuous yellowish necrosis and clear telia on the back, and '5' indicated massive large and continuous yellowish necrosis and diffusible telia on the back and some leaves even roll or rot [24]. Rating scale for disease index [25], used in the present study, where, 0 = no infection, 1 = fewer than five pustules per plant, 2 = five to 100 pustules per plant, 3 = more than 100 pustules per plant, 4 = more than 100 pustules per plant and two or more leaves with coalesced over at least 75% of the leaf area. The susceptibility/resistant of the genotypes/lines was established [17] as depicted in Table 1. The Average disease index was calculated by multiplication of disease score  $\times$  number of plants in that score and divided by the total number of plant was established [18] as depicted in Table 1.

**Table 1:** Average disease index for chrysanthemum white rust (*Puccinia horiana*) resistance evaluation [18].

Resistance evaluation	Average disease index (DI)	Symptoms
Resistant	0.0	No visible symptom
Moderately resistant	0.1–1.0	Few pustules developed slowly and sporulated limitedly
Susceptible	1.1–3.0	Many pustules developed
Highly susceptible	3.1–4.0	Many pustules developed quickly and coalesced over major portion of the leaf and sporulated abundantly

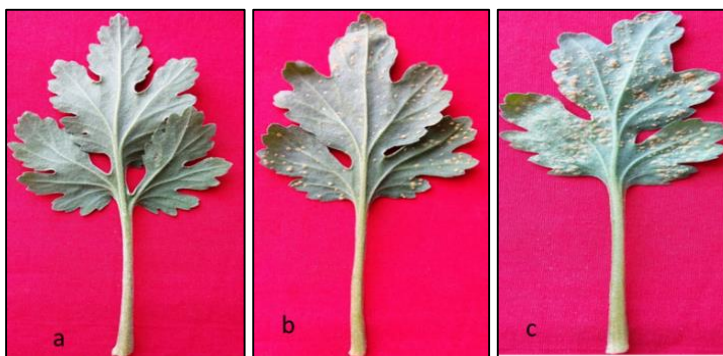
### 2.4 Statistical analysis

The WASP (Web Agri Stat Package), a web based agricultural statistics software package developed by ICAR-Central Coastal Agricultural Research Institute, Goa was used for statistical analysis to assess whether genotypes differed significantly from one another for their reaction to infection.

### 3. Results

A total of 20 genotypes/lines were evaluated for their level of resistance to infection. The latent period ranged from 7 to

greater than 50 days post inoculation (dpi). The earliest genotypes/lines to shown disease symptoms were Kalpana and Arka Chandrika, followed 1 day later (IIHR6-29), 2 days later (IIHR2-7 and Local White), 3 days later (IIHR6-26 and Local White), 4 days later (IIHR9-12), 8 days later (Ajay). Maximum latent period was recorded in Arka Kirti (25.33 days). Fewer symptoms were recorded after 25 days of post inoculation. Development of white rust pustules on abaxial surface of cv. Local White leaf at different dpi is shown in Figure 2.



**Fig 2:** Development of white rust pustules on abaxial surface of a cv. Local White leaf at a) 8 day post inoculation (dpi), b) 18 dpi, c) 28 dpi.

With respect to infection type, among the 20 genotypes/lines, five genotypes/lines namely Kalpana, IIHR6-26, Local White, IIHR9-12 and IIHR6-29 showed high levels of infection. A number of chlorotic lesions and pustules developed at faster rate on the leaves.

On the other hand, eleven genotypes/lines viz., Sweta Singar, Punjab Gold, Garden Beauty, Red Stone, Punjab Anuradha, Lal Pari, White Dolly, White Andaman, IIHR6-32, IIHR9-3 and IIHR6-41 were determined to be resistant (Table 2).



**Table 2:** Latent period, infection types, disease index, disease response of 20 genotypes/lines of chrysanthemum screened against white rust (*Puccinia horiana*)

Sl. No.	Genotypes/lines	Average latent periods (days)	Average infection types	Disease index	Disease response
1.	Sweta Singar	50.00	0.00	0.0	Resistant
2.	Kalpana	7.33	5.00	3.1-4.0	Highly susceptible
3.	IIHR6-26	10.00	4.78	3.1-4.0	Highly susceptible
4.	Ajay	14.67	3.33	1.1-3.0	Susceptible
5.	Local White	9.67	4.89	3.1-4.0	Highly susceptible
6.	Punjab Gold	50.00	0.00	0.0	Resistant
7.	IIHR9-12	11.00	4.78	3.1-4.0	Highly susceptible
8.	Garden Beauty	50.00	0.00	0.0	Resistant
9.	IIHR6-29	8.33	4.33	3.1-4.0	Highly susceptible
10.	Red Stone	50.00	0.00	0.0	Resistant
11.	Arka Chandrika	7.67	3.44	3.1-4.0	Highly susceptible
12.	Arka Kirti	25.33	1.56	1.1-3.0	Susceptible
13.	Punjab Anuradha	50.00	0.00	0.0	Resistant
14.	IIHR6-32	50.00	0.00	0.0	Resistant
15.	IIHR9-3	50.00	0.00	0.0	Resistant
16.	IIHR6-41	50.00	0.00	0.0	Resistant
17.	Lal Pari	50.00	0.00	0.0	Resistant
18.	White Dolly	50.00	0.00	0.0	Resistant
19.	IIHR2-7	9.67	3.56	1.1-3.0	Susceptible
20.	White Andaman	50.00	0.00	0.0	Resistant
	SE(m)	0.42	0.13		
	C.D. at 5%	0.77	0.37		
	C.V.	1.43	12.54		

The genotypes Ajay, Arka Kirti and IIHR2-7 showed many pustules developed on the leaves and sporulated abundantly. These three genotypes/lines were, thus, considered as susceptible. But the other six genotypes/lines including Kalpana, IIHR6-29, Local White, IIHR9-12, IIHR6-29 and Arka Chandrika were considered as highly susceptible because they developed infected leaves quickly and covered with numerous pustules. Pustules formed even on stem, bracts, and flower buds.

Eight chrysanthemum genotypes viz., Pusa Aditya, Marigold, Vijay Kiran, Ratlam Selection, Anmol, Shyamal, Vasanthika and Sunil were highly susceptible while five genotypes viz., Flirt, CO.1, Fitonia, Rekha and Arka Ravikiran were moderately resistant. However, genotypes Arka Indira, Arka Kirti, Arka Pankaj, Arka Yellow Gold and Arka Red Gold were found resistant in both *in vitro* as well as in pot culture screening [26].

#### 4. Discussion

*Puccinia horiana* is one of the most destructive fungal disease in Chrysanthemum causing serious production losses. Greenhouse cultivation of Chrysanthemum during summer is threatened by the spores build up on the greenhouse curtain wall used for the short day treatment. Variation for resistance to white rust in commercial chrysanthemum genotypes/lines and the differential interactions between *P. horiana* isolates and chrysanthemum genotypes/lines have been investigated. However, there is meager literature available on the genetic resources resistant to white rust. In this study, white rust resistance of chrysanthemum genotypes/ lines against local isolates was studied. A total of 20 genotypes/ lines were evaluated in net house conditions against white rust. White rust infection type and disease index were established. Based on previous related research literature and experimental studies, identification standards for chrysanthemum white rust resistance was established, which were able to scientifically identify and check the resistance/susceptibility to chrysanthemum white rust. According to the screening result (Table 2), we have combine latent period, infection type, and disease index together. We found that latent period is a

reliable and simpler component for screening for resistance to *P. horiana*. Infection type and Disease index (DI) have been widely used in disease resistance screens [27, 28]. This screening could be useful for the screening for *P. horiana* incidence of chrysanthemum. Among the 20 chrysanthemum cultivars screened, 11 were resistant (Sweta Singar, Punjab Gold, Garden Beauty, Red Stone, Punjab Anuradha, Lal Pari, White Dolly, White Andaman, IIHR6-32, IIHR9-3 and IIHR 6-41). The high frequency of resistance to white rust indicated that the resistance, reported to be controlled by a single dominant gene, might have been stably inherited by the next generation [12]. Therefore, these genetic resources are expected to be helpful for cross breeding programs to breed new cultivars resistant to white rust disease in chrysanthemum. However, differential interactions between *P. horiana* isolates and chrysanthemum cultivars, which have been proposed recently, indicate that more than two types of white rust resistance gene might exist in chrysanthemums [19, 20]. Likewise, these data are valuable to identify resistance genes appropriate for a particular pathotype, and pyramid those genes for improving white rust resistance in chrysanthemum.

#### 5. Conclusion

A total of 20 genotypes/lines were screened for their level of resistance to white rust (*Puccinia horiana* Henn.) following latent periods, infection types and disease index. The eleven genotypes viz., Sweta Singar, Punjab Gold, Garden Beauty, Red Stone, Punjab Anuradha, Lal Pari, White Dolly, White Andaman, IIHR6-32, IIHR9-3 and IIHR 6-41 were found to be resistant to white rust (*Puccinia horiana* Henn.). The genotypes/lines Ajay, Arka Kirti and IIHR2-7 considered as susceptible, while, genotypes/lines Kalpana, IIHR6-29, Local White, IIHR9-12, IIHR6-29 and Arka Chandrika as highly susceptible.

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