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# Variation for Resistance to White Rust (*Puccinia horiana*) among *Ajania* and *Chrysanthemum* Species

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*Abstract*. **White rust (causative pathogen *Puccinia horiana*) is a destructive disease of commercial chrysanthemum crops. A panel of 19 accessions of commercial chrysanthemum near-relatives (four *Ajania* species, 11 *Chrysanthemum* species including five accessions of *Chrysanthemum indicum*) were screened for their reaction to white rust infection in separate greenhouse trials carried out at two independent sites in eastern China, one in 2010 and the other in 2012. The reaction of the accessions to artificial inoculation ranged from immune to highly susceptible. Accessions of *Chrysanthemum indicum*, *C. yoshinaganthum*, *C. makinoi* var. *wakasaense*, *C. nankingense*, *C. vestitum*, *C. lavandulifolium*, *C. crassum,* and *Ajania tripinnatisecta* were immune, and strong resistance was present in *C. japonense*, *C.* × *shimotomaii,* and *A. przewalskii*. Most of the accessions behaved similarly in the two trials, but two of the *C. indicum* accessions produced inconsistent results, each being highly resistant in one trial but susceptible in the other. Because wide crosses are relatively easy to achieve in the chrysanthemum complex, these immune and highly resistant accessions represent promising germplasm for white rust resistance breeding.**

Chrysanthemum white rust (causative pathogen *Puccinia horiana* Hennings) is a notifiable disease, which infects chrysanthemum plants grown for the cut flower and pot plant trade, largely because they are routinely cultivated under glass or in plastic tunnels where the microclimate favors the growth of the pathogen. The global trade in chrysanthemum has dispersed the disease from the site of its first documentation in Japan in 1895 to China, South Africa, and Europe (Baker, 1967; Firman and Martin, 1968; Whipps, 1993) and by now has become endemic to most chrysanthemum-growing areas (Cook, 2001; Göre, 2008; O'Keefe and Davis, 2012). The pathogen is an obligate biotroph, which colonizes young leaves and flower buds (Yamada, 1956). Under conditions of high humidity and mild temperature, its teliospores, which develop within a pustule on the abaxial leaf surface within 14 to 18 d post-infection (Zandvoort et al., 1968b), germinate to form a promycelium. Each promycelium bears a mean of two infective propagules (basidiospores) (Kapooria and Zadoks, 1973), which can be dispersed by wind over a distance of up to 700 m (Zandvoort, 1968b).

Although white rust can be chemically controlled (Dickens, 1990; Zadoks et al., 1969; Zandvoort et al., 1968a), the use of fungicides is associated with both environmental hazards and an increased production cost (Waard et al., 1993); overdependence on their use has already led to the appearance of tolerant strains (Abiko et al., 1977; Cook, 2001). The more sustainable strategy of exploiting genetically determined resistance requires the identification of sources of resistance, as first attempted by Dickens (1968) and Martin and Firman (1970). DeJong and Rademaker (1986) have suggested that resistance is most commonly under monogenic control.

Outside of the primary gene pool, a number of related species have been shown to harbor variation of relevance to aphid tolerance (Sun et al., 2012), to *Alternaria* leaf spot resistance (Xu et al., 2011), and to tolerance of various abiotic stresses (Guan et al., 2010; Yin et al., 2009). Several *Chrysanthemum* spp. have been reported as resistant to white rust (Dickens, 1968; Hiratsuka, 1957) and an intergeneric hybrid between chrysanthemum and *Artemisia sieversiana* has been shown to be more resistant than the chrysanthemum parent (Furuta et al., 2004). Hybridization between commercial chrysanthemum and various *Ajania* and *Chrysanthmum* spp. has been repeatedly achieved (Cheng et al., 2011; Deng et al., 2011), so wide crossing could have considerable potential for white rust resistance breeding. Here, we report the evaluation for the white rust resistance shown by a panel of 19 *Ajania* and *Chrysanthemum* spp. involving two independent trials. To our knowledge, this represents the first such survey of the potential of *Ajania* and *Chrysanthemum* spp. to provide a source of genetic resistance to white rust.

## Materials and Methods

*Plant materials and inoculation.* The set of 19 accessions including species from *Ajania* (four species) and *Chrysanthemum* (10 species with five accessions of *Chrysanthemum indicum*) (Table 1) was obtained from the Chrysanthemum Germplasm Resource Preserving Center, Nanjing Agricultural University, Nanjing, China. The commercial cultivar Jinba served as a white rust-susceptible control to indicate inoculations were succeeded or not. Screening was carried out on plants grown in two different plastic greenhouses in eastern China, one in 2010 ("Trial 1") and the other in 2012 ("Trial 2"). For Trial 1, morphologically uniform 3-week-old rooted cuttings (eight to 10 leaf stage) were grown at a site located at lat. 34.06° N, long. 118.28° E. The plastic house was initially treated with carbendazim and mancozeb, after which a minimum of 30 cuttings per accession were planted in a single 10-m row with an interplant spacing of 20 to 30 cm and an interrow spacing of 0.5 m. The experiment was arranged as a randomized complete block with three replications (McIntosh, 1983). Overhead irrigation was provided with a sprinkler. No fertilizer or fungicide was applied during the whole experimental process (1 May to 30 June). Seven days after planting, the cuttings were inoculated with teliospores collected from diseased cv. Jinba plants. To minimize disease escape, they were inoculated first using the fraction method elaborated by Zhu et al. (2011), then a day later using a spray method derived from Zandvoort (1968a); briefly, the infected leaves containing white rust pustules were cut into small pieces and were dispersed in deionized water and filtered through medical gauze to remove any plant debris. The concentration of the pathogenic spore suspension was then adjusted using a hemacytometer slide to a concentration of $1 \times 10^6$ zoosporangia/mL with deionized water containing one drop of Tween 20 before application to the plants until runoff using a handheld sprayer. The plants were covered with a black polythene sheet for the first 48 h post-inoculation both to maintain a high relative humidity and to exclude light (Yamada, 1956). Thereafter the temperature was kept within the range 15 to 25 °C, and supplementary light was given to provide a 16-h photoperiod. The relative humidity was kept above 80% by the use of 360° rotating sprinklers for overhead sprinkling, in which water pressure

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Table 1. Latent period, infection type, disease index, and host response of 19 accessions of *Ajania* (four species) and *Chrysanthemum* (eleven species) tested for reaction to chrysanthemum white rust inoculation in two independent trials.

| | | Latent period (dpi) | | Infection type | | Disease index | | Host response | |
|---|---|---|---|---|---|---|---|---|---|
| Materials | Origin | 2010 | 2012 | 2010 | 2012 | 2010 | 2012 | 2010 | 2012 |
| *Chrysanthemum indicum* | Hangzhou, Zhejiang province, China | >30 | >30 | 0 | 0 | 0 f[z] | 0 f | I | I |
| *Ajania tripinnatisecta* | Hongyuan, Sichuan province, China | >30 | >30 | 0 | 0 | 0 f | 0 f | I | I |
| *Ajania przewalskii* | Jingyuan, Shannxi province, China | >30 | 27 | 0 | 1 | 0 f | 0.01 ± 0.01 e | I | HR |
| *Chrysanthemum indicum* | Hiroshima, Japan | >30 | >30 | 0 | 0 | 0 f | 0 f | I | I |
| *Chrysanthemum indicum* | Aichi, Japan | >30 | >30 | 0 | 0 | 0 f | 0 f | I | I |
| *Chrysanthemum yoshinaganthum* | Ibaraki, Japan | >30 | >30 | 0 | 0 | 0 f | 0 f | I | I |
| *Chrysanthemum indicum* | Nanjing, Jiangsu province, China | >30 | 11 | 0 | 3 | 0 f | 21.97 ± 2.07 c | I | MS |
| *Chrysanthemum makinoi* var. *wakasaense* | Ibaraki, Japan | >30 | >30 | 0 | 0 | 0 f | 0 f | I | I |
| *Chrysanthemum nankingense* | Nanjing, Jiangsu province, China | >30 | >30 | 0 | 0 | 0 f | 0 f | I | I |
| *Chrysanthemum vestitum* | Huangshan, Anhui province, China | >30 | >30 | 0 | 0 | 0 f | 0 f | I | I |
| *Chrysanthemum lavandulifolium* | Beijing, China | >30 | >30 | 0 | 0 | 0 f | 0 f | I | I |
| *Chrysanthemum crassum* | Ishikawa, Japan | >30 | >30 | 0 | 0 | 0 f | 0 f | I | I |
| *Chrysanthemum japonense* | Tokyo, Japan | 23 | 26 | 1 | 2 | 3.74 ± 1.40 e | 0.01 ± 0.001 e | HR | MR |
| *Chrysanthemum × shimotomaii* | Hiroshima, Japan | 22 | 13 | 1 | 2 | 0.25 ± 0.05 e | 0.63 ± 0.02 e | HR | MR |
| *Chrysanthemum japonense* var. *ashizuriense* | Hiroshima, Japan | 25 | >30 | 2 | 0 | 13.48 ± 0.78 d | 0 f | MR | I |
| *Chrysanthemum ornatum* | Hiroshima, Japan | 26 | 15 | 2 | 2 | 0.93 ± 0.10 e | 10.14 ± 0.28 d | MR | MR |
| *Chrysanthemum indicum* | Huangshan, Anhui province, China | 15 | 29 | 3 | 1 | 30.26 ± 1.40 b | 0.004 ± 0.001 e | MS | HR |
| *Ajania pacifica* | Ibaraki, Japan | 12 | 10 | 4 | 4 | 28.84 ± 0.87 c | 31.44 ± 2.02 b | S | S |
| *Ajania shiwogiku* var. *kinokuniense* | Ibaraki, Japan | 9 | 9 | 5 | 5 | 41.63 ± 1.84 a | 44.75 ± 2.84 a | HS | HS |

[z] Disease indices represented by means in the same column followed by the same letter are not significantly different at $P = 0.01$.

dpi = days post-inoculation; I = immune; HR = highly resistant; MR = moderate resistant; MS = moderate susceptible; S = susceptible; HS = highly susceptible.

was 200 Kpa, flow was 27.8 $L\cdot h^{-1}$, and maximum range was 3.5 m (Lemiao Irrigation Equipment Factory, Zhejiang, China) every 2 h during the day. The Trial 2 plastic house was sited at lat. 34.32° N, long. 118.12° E. Planting period, plant layout, inoculation procedure, and field management were as for Trial 1. The fungal inoculum used in this trial was collected from diseased cv. Iwanohakusen.

*Disease monitoring and classification.* The latent period, infection type, disease severity, and disease incidence were recorded at the time when teliospore-containing pustules had become well developed. Monitoring was continued until 52 d post-inoculation (dpi). The latent period was defined as the number of days elapsed between inoculation and the first appearance of symptoms (Browne and Cooke, 2004). Infection type was represented by a 0 to 5 scale, which was observed from the majority (15 or greater) of individual data, adapted from Pathan and Park (2006) and Zhu et al. (2011), 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. Disease incidence (I) was given by the ratio between the average number of diseased and nondiseased leaves on a plant from 30 individuals per accession. A mean disease severity measure ($\hat{S}$, given by the mean proportion of leaf area infected) was calculated from ≈30 infected leaves per accession; both the area of diseased leaf surface and the overall leaf area were obtained from scanned images of the leaves following Igathinathane et al. (2006). A disease index (DI) was then derived from the expression $I \times \hat{S} \times 100$ based on rule for resistance evaluation of wheat to leaf rust (*Puccinia triticina*) (Ministry of Agriculture of the People's Republic of China, 2007).

*Statistical analysis.* SPSS 17.0 software (SPSS Inc., Chicago, IL) was used for all statistical calculations. A one-way analysis of variance, in conjunction with the Duncan multiple range test, was used to assess whether accessions differed significantly from one another for their reaction to infection.

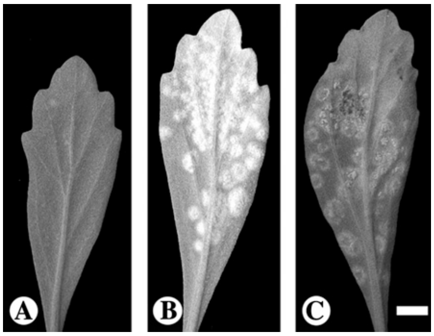

Fig. 1. The development of white rust pustules on the abaxial surface of an *A. pacifica* leaf at (**A**) 10 d post-inoculation (dpi), (**B**) 20 dpi, (**C**) 30 dpi. Bar = 5 mm.

## Results

*Trial 1.* The latent period ranged from 9 to greater than 30 dpi (Table 1). The earliest accession to display disease symptoms was *A. shiwogiku* var. *kinokuniense* followed 3 d later by *A. pacifica*. The disease symptoms started as pale green to yellow flecks of diameter up to 5 mm (Fig. 1A). Over time, the flecks turned necrotic, and buff- to pink-colored pustules formed on the leaf's surface (Fig. 1B). The pustules lost their color as they matured. In some cases, both surfaces of the leaf became postulated, leading to leaf collapse (Fig. 1C). With respect to infection type, 12 of the 19 accessions (*A. tripinnatisecta*, *A. przewalskii,* four of the *C. indicum* accessions, *C. yoshinaganthum*, *C. makinoi* var. *wakasaense*, *C. nankingense*, *C. vestitum*, *C. lavandulifolium*, *C. crassum*) were symptomless (type "0," see Fig. 2) and therefore were classed as immune. *C. japonense* and *C. × shimotomaii* had developed a small number of yellowish flecks by the end of the monitoring period (type "1," see Fig. 2) and were classed as highly resistant. *C. japonense* var. *ashizuriense* and *C. ornatum* leaves carried rather more yellowish flecks and developed small pustules on their abaxial surface (type "2," see Fig. 2) and were classed as moderately resistant. Finally, *C. indicum* from Huangshan, *A. pacifica,* and *A. shiwogiku* var. *kinokuniense* were classed as, respectively, moderately susceptible, susceptible, and highly susceptible (types "3" to "5," see Fig. 2). The DI classification exposed significant differences ($P < 0.01$) between the accessions, ranging from 0 (the 12 immune accessions) to 41.6. The DIs of the two highly resistant accessions were 0.2 and 3.7, that of the moderately resistant *C. japonense* var. *ashizuriense* was 13.5, and *C. ornatum* was 0.9, which classified as highly resistant, whereas those of susceptible accessions *A. pacifica*, *C. indicum* (Huangshan), and *A. shiwogiku* var. *kinokuniese* ranged from 28.8 to 41.6 (Table 1).

*Trial 2.* The latent period was generally slightly shorter than in Trial 1, although it was the same (9 dpi) for the earliest accession to show disease symptoms (*A. shiwogiku* var. *kinokuniense*). The order of symptom displayed thereafter was *A. pacifica* (10 dpi), *C. indicum* (Nanjing) (11 dpi), *C.* × *shimotomaii* (13 dpi), *C. ornatum* (15 dpi), *C. japonense* (26 dpi), *A. przewalskii* (27 dpi), and *C. indicum* (Huangshan) (29 dpi). The other accessions remained symptomless (Table 1). Some of the recorded infection types differed from what had been seen in the earlier trial. Thus, the classification of *A. przewalskii* changed from "0" to "1" (immune to highly resistant) and that of *C. japonense* var. *ashizuriense* from "2" to "0" (its DI fell from 13.5 to 0) and so was classified as immune. *C. indicum* (Huangshan) was reclassified as being highly resistant (previously moderately susceptible) and *C. indicum* (Nanjing) as moderately susceptible (previously immune) (Table 1). To determine if these host responses were consistent across two different field locations, Spearman rank correlation tests were performed. Significant and positive correlations between Trial 1 and Trial 2 ($r = 0.652$; $P < 0.01$) were found.

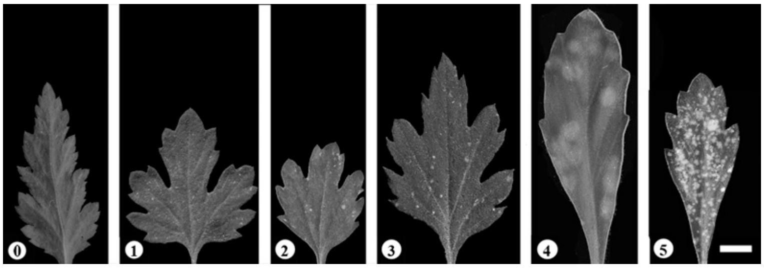

Fig. 2. Infection type of accessions inoculated with white rust, measured at 30 d post-inoculation (dpi) in Trial 1. Bar = 10 mm. 0 = no visible symptoms, as exemplified by *C. nankingense*; 1 = a very small numbers of yellowish flecks, as exemplified by *C.* × *shimotomaii;* 2 = several yellowish flecks and a few pustules, as exemplified by *C. ornatum*; 3 = the development of chlorotic and necrotic areas, and the formation of a few pustules on the abaxial leaf surface, as exemplified by *C. indicum* (Huangshan); 4 = the development of extensive areas of chlorosis and necrosis, and the formation of many pustules on the abaxial leaf surface, as exemplified by *A. pacifica;* 5 = merged pustules on the abaxial leaf surface and the development of leaf distortion and collapse, as exemplified by *A. shiwogiku* var. *kinokuniense*.

Table 2. Latent period, infection type, and disease index for chrysanthemum white rust (*Puccnia horiana*) resistance evaluation.

| Resistance evaluation | Latent period [LP, days post-inoculation (dpi)] | Infection type | Disease index (DI) |
|---|---|---|---|
| Immune | $LP > 30$ | 0 | $DI = 0$ |
| Highly resistant | $20 < LP \leq 30$ | 1 | $0 < DI \leq 10$ |
| Moderately resistant | $20 < LP \leq 30$ | 2 | $10 < DI \leq 20$ |
| Moderately susceptible | $10 < LP \leq 20$ | 3 | $20 < DI \leq 30$ |
| Susceptible | $10 < LP \leq 20$ | 4 | $30 < DI \leq 40$ |
| Highly susceptible | $LP \leq 10$ | 5 | $DI > 40$ |

## Discussion

Reliable sources of genetically determined resistance are the sine qua non of a disease resistance breeding program. Here, the white rust infection type and disease index of a set of near relatives of commercial chrysanthemum were established. The panel of 19 accessions was evaluated in two independent trials separated from one another with respect to both time and place, and the outcomes were highly (but not entirely) consistent with one another. Notable among the discrepancies were the changed classification in infection type accorded to *C. indicum* (Huangshan) and *C. indicum* (Nanjing). Such variation may because of the change of a combination of factors such as spatial and temporal variations in inoculum levels, environmental conditions, age of plants, and soil conditions, etc. (Akhtar et al., 2013). The most likely basis for this phenomenon, as seen in many host/fungal pathogen interactions, lies in present in the inoculum (Lindeberg, 2012; Zhou et al., 2007). Races of white rust were first discovered and supposed by Dickens (1968), and there may exist four races of white rust, which was reported in Japan by Kudo and Okamura (1998). A differential response in the chrysanthemum/white rust system has been recorded by Yamaguchi (1981), and this finding was later confirmed by De Backer et al.'s (2011) study of the response of 36 cultivars to 22 different white rust strains, where differential patterns of resistance were displayed. Over half of the accessions proved to be immune or at least highly resistant, despite the large inoculum load imposed on them, and most of these resistant types belonged to the genus *Chrysanthemum*, rather than to the more distant genus *Ajania*.

According to the evaluation result (Table 1), we can combine latent period, infection type, and disease index together (Table 2). These evaluation systems could be useful for the evaluation for *P. horiana* tolerance of chrysanthemum. In this study, we found that latent period is a reliable and simpler component for evaluation for resistance to *P. horiana*. Infection type and DI have been widely used in disease resistance screens (Farias Neto et al., 2008; Kolmer, 1996). The former has the advantage of speed and simplicity, because it is based on primary data; its disadvantage is that it is somewhat subjective. The DI combines disease incidence and disease severity, thereby producing a measure, which is much more objective; however, its acquisition is more laborious and requires the means to measure the area of diseased and non-diseased leaf surface.

The leaves of *A. pacifica* and *A. shiwogiku* var. *kinokuniense* typically develop a denser mat of leaf trichomes (especially on their abaxial surface) than do those *Chrysanthemum* spp. In *Arabidopsis thaliana* at least, it has been suggested that the presence of trichomes aids the adhesion of fungal spores to the leaf surface (Calo et al., 2006). A dense system of leaf trichomes can be expected to produce a microclimate where the level of relative humidity remains high, and this would certainly favor the germination of the white rust spores, so it may be that greater susceptibility shown by the *Ajania* spp. is a pleotropic effect of trichome density.

The relative ease of making wide hybrids between commercial chrysanthemum and related species from the genera *Chrysanthemum* and *Ajania* opens the way to introgressing white rust resistance into the crop species. Primary hybrids tend to form poor-quality inflorescences, but this feature should be correctable by building in a sufficient level of backcrossing (Zhao et al., 2012). Apart from the white rust resistance, the related species in question provide a reservoir of genes, which could be exploited to improve the commercial chrysanthemum's level of tolerance to a number of abiotic stresses, of resistance to a range of other diseases and pests, and possibly also to manipulate flowering time.

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