




Evaluation of leaf rust resistance and characteristics of Korean wheats

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Abstract Leaf rust is the most widespread and destructive fungal disease, and outbreaks have always caused considerable losses in wheat yields. Thus, worldwide increases in wheat production depend on the development of leaf rust-resistant wheat varieties. In this study, we evaluated the resistance of forty Korean wheat cultivars to leaf rust at the seedling stage. Only two Korean wheats, Ol and Jonong, were resistant to leaf rust, whereas the remaining thirty-eight Korean wheats were susceptible to leaf rust. The Ol and Jonong varieties presented larger dry seed weights and higher antioxidant activity in response to leaf rust than the susceptible wheat varieties. No differences in β -1,3-glucanase activity or chlorophyll content between resistant and susceptible wheat varieties were observed. Overall, these results are important for the development of wheat varieties that are highly resistant to leaf rust and to understand the underlying mechanisms that confer leaf rust resistance.

Keywords Antioxidant capacity · β -1,3-glucanase · Dry seed weight · Korean wheat · Leaf rust resistance · *Puccinia triticina*

Introduction

Wheat accounts for 19% of the total production of major cereal crops worldwide and its consumption is on the rise [1]. To ensure

a sustainable future for humanity, wheat production yields must thus be increased. Wheat fungal diseases result in significant wheat yield losses. In particular, wheat leaf rust (*Puccinia triticina*) constitutes a major impediment to wheat production. Leaf rust disseminates rapidly and may produce catastrophic effects under optimal conditions [2]. It can be blown by winds and travel long distances, will end their crops within weeks of infection if they are not treated on time. There are various strategies to control wheat leaf rust, including the use of chemicals, biological controls, molecular markers, or the promotion of host resistance. The establishment of genetic resistance to leaf rust is the most studied and preferred method to prevent wheat yield loss. Currently, more than eighty leaf rust resistance (*Lr*) genes have been identified from diverse wheat varieties [3]. However, most *Lr* genes are not effective in conferring resistance to current races of leaf rust. Moreover, leaf rust has variable virulence and readily overcomes resistance through mutation [4]. Accordingly, new sources of resistance to leaf rust must be continually created and deployed. In this study, we evaluated forty Korean wheat cultivars for leaf rust resistance and characterized their distinct degrees of resistance and susceptibility. This study will help secure leaf rust-resistant wheat resources while expanding the foundation for studying the mechanisms of leaf rust resistance.

Materials and Methods

Plant Materials

Forty Korean wheat cultivars (*Triticum aestivum* L.) were used in this study [5]. Leaf rust resistance was evaluated using the first leaves of 14-day-old seedlings. Cultivar seeds were sterilized with 5% NaOCl for 7 min and washed five times with distilled water. The seeds were then stored at 4 °C for germination. After 3 days incubation, the seeds were sown in autoclaved soil. The seedlings were installed in a greenhouse and grown at 25 °C for 14 days under daylight conditions. The average of 50-dry seed weight of selected wheat cultivars was determined with three replicates randomly.

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Leaf rust inoculation

The *P. triticina* strain was provided by the Korea Research Institute of Chemical Technology. The inoculum was prepared by diluting the uredospores of *P. triticina* in distilled water containing 120 μ L of Tween 20 (DAEJUNG, Siheung, Korea) [6]. The 14-day-old seedling leaves were sprayed evenly with inoculum using an airbrush and incubated under dark conditions at 100% relative humidity for 24 h at 20 °C. The phenotypic infection type and seedling response (0-4 scale) were evaluated 10 days post-inoculation. The phenotypic performance of the cultivars varied from 0 to 4: where, 0 indicated no visible symptoms; 0; indicated hypersensitive flecks; 1 indicated minute uredinia surrounded by mostly necrotic tissue; 2 indicated small- to medium-sized uredinia surrounded by chlorotic and/or necrotic tissue; 3 indicated large uredinia, without surrounding chlorosis; and 4 indicated large uredinia, without chlorosis or necrosis.

Chlorophyll Assay

Based on the evaluation of leaf rust resistance, four kinds of wheat which showed the most resistant (Keumkang, Jokyung) and susceptible (Ol, Jonong) to leaf rust were used in the future characteristics experiments. A total of 50 mg of leaf material was collected from the first leaves of the 14-day-old seedlings. The leaf material was ground with a mortar and pestle in liquid nitrogen. Acetone (80%) was used to extract the chlorophyll from the samples. Chlorophyll extraction was performed overnight under dark conditions at 4 °C. The concentrations of chlorophyll a, chlorophyll b, and total chlorophyll were measured using a SpectraMax M2 spectrophotometer at 663 and 645 nm (Molecular devices, San Jose, CA, USA) [7,8] and calculated using the following equations.

$$\text{Chlorophyll a} = (12.7 A_{663} - 2.69 A_{645}) V / 1000 W$$

$$\text{Chlorophyll b} = (12.7 A_{663} - 2.69 A_{645}) V / 1000 W$$

$$\text{Total Chlorophyll (a+b)} = (12.7 A_{663} - 2.69 A_{645}) V / 1000 W$$

In the equations, V is the extract volume (mL) and W is fresh leaf weight (g). The blank consisted of 80 % acetone.

β -1,3-Glucanase Assay

The first leaves from 14-day-old selected cultivars were sampled at 0, 12, and 24 h after leaf rust inoculation. A protein crude extraction was performed by grinding the first leaves in Pro-prep protein extraction solution (iNtRON, KOREA) using 700 μ L of buffer per 500 mg leaf. The activity of β -1,3-glucanase was measured using 1% laminarin (Sigma-Aldrich, USA) as a substrate. The reaction mixture, which contained 40 μ L 1% (w/v) laminarin (in 0.2 M phosphate buffer, pH 6.5) and 20 μ L of crude enzyme solution, was incubated for 1 h at 37 °C. Subsequently, 40 μ L 1% NaOH solution was added and the mixture was boiled for 5 min to terminate the reaction. One percent DNS (3,5-dinitrosalicylic acid) reagent and supernatant mixed in a 1:1 ratio were boiled for 5 min. After cooling, the color that developed was

measured using a SpectraMax M2 spectrophotometer at 540 nm (Molecular devices, San Jose, CA, USA) [9-11]. The activity of β -1,3-glucanase was calculated from the standard curve using glucose.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

Antioxidants with weak A-H bonding will react with stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich), leading to discoloration of the molecule [12-14]. The 14-day-old seedlings were exposed to leaf rust stress for 0, 12, and 24 h under dark conditions. Leaf samples (0.05 g) were collected from three seedlings. Three replicates were included for each treatment. The samples were ground in liquid nitrogen and incubated using 400 μ L of MeOH (1% HCl) overnight under dark conditions at 4 °C. The supernatant was collected by adding 400 μ L of distilled water and 400 μ L of chloroform at a 1:1 ratio before centrifuging at 3000 rpm for 2 min. A total of 300 μ L supernatant was dried overnight in a vacuum evaporator at 20 °C. The pellet was resuspended in 600 μ L of MeOH and centrifuged at 3000 rpm for 2 min. The 400 μ L supernatant was collected by adding equal amounts of 500 μ M DPPH. The reaction was completed in the dark at 25 °C for 30 min. The absorbance of DPPH was measured using a SpectraMax M2 spectrophotometer at 517 nm (Molecular devices, San Jose, CA, USA).

Results and Discussion

Leaf rust resistance of forty Korean wheat cultivars

Most of the Korean wheat cultivars were susceptible to leaf rust. Thirty-eight wheat cultivars were classified as having a phenotypic performance of either 2 or 3 against leaf rust infection. Only two cultivars, Ol and Jonong, were classified as having a phenotypic performance of 1 against leaf rust infection (Table 1 and Fig. 1). Most of the Korean wheat cultivars were relatively sensitive to leaf rust infection, and, consequently, it is important to develop a domestic wheat variety resistant to leaf rust.

Dry seed weight and chlorophyll content of resistant and susceptible wheat cultivars

Based on phenotypic evaluation of leaf rust resistance, ten Korean wheat cultivars, including Ol and Jonong, were examined for dry seed weight. The weight of fifty dry seeds from each of the ten Korean wheat cultivars were averaged and compared. The average seed weight of the two resistant wheat cultivars, Ol and Jonong, was much larger than the average seed weight of the susceptible wheat cultivars except for Jokyung wheat (Fig. 2). This suggests that seed weight might be associated with leaf rust resistance.

We compared the chlorophyll a, chlorophyll b, and total chlorophyll content of the leaves from 14-day-old seedlings of two susceptible wheat cultivars, Keumkang and Jokyung, and the

Table 1. Phenotypic performance evaluation of the forty Korean wheat cultivars at the seedling stage to leaf rust infection

Accession name	Phenotypic reaction*	Wheat type	Accession name	Phenotypic reaction*	Wheat type
OI	1	Red wheat	Jonong	1	Red wheat
Geuru	3	Red wheat	Jokyung	3	Hard white wheat
Dahong	3	Red wheat	Younbaeck	2	White wheat
Chung-kye	3	Red wheat	Shinmichal No.1	2	Red waxy wheat
Eunpa	2	Red wheat	Dabun	3	Red wheat
Tapdong	2	Red wheat	Baekjoong	2	Soft white wheat
Namhae	3	Red wheat	Jeokjoong	3	Red wheat
Uri	2	Soft red wheat	Sukang	3	Red wheat
Olgeru	2	Soft red wheat	Hanbaek	3	White wheat
Alchan	3	Soft red wheat	Suan	3	Red wheat
Gobun	3	Soft red wheat	Dajung	3	Red wheat
KeumKang	3	Hard white wheat	Goso	3	Soft red wheat
Seodun	2	Soft Red wheat	Joa	3	Red wheat
Saeol	3	Red wheat	HoJoong	2	Red wheat
Jinpum	3	Red wheat	Baekchal	3	White wheat
Milseoung	2	Hard red wheat	Jojoong	3	Red wheat
Jo-eun	2	Red wheat	Baek-kang	2	White wheat
Anbaek	3	Red wheat	SaeKeumKang	3	Red wheat
Jopum	3	Hard red wheat	Taejoong	2	Red wheat
Shinmichal	3	Red wheat	Johan	2	Red wheat

*; Phenotypic performance after leaf rust infection: 0 = no visible symptoms; 0; = hypersensitive flecks; 1 = minute uredinia surrounded by mainly necrotic tissue; 2 = small- to medium sized uredinia surrounded by chlorotic and/or necrotic tissue; 3 = large uredinia, without surrounding chlorosis; and 4 = large uredinia, without chlorosis or necrosis

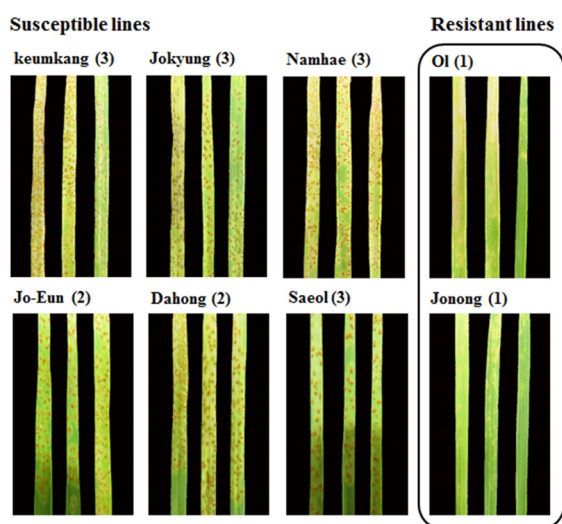


Fig. 1 Leaf rust-susceptible and leaf rust-resistant Korean wheat varieties. The type of leaf rust infection is shown according to the phenotypic performance scale for susceptible and resistant Korean wheat varieties. The phenotypic performance of the cultivars varied from 0 to 4 were showed in the images

two resistant wheat cultivars, OI and Jonong. No difference in chlorophyll content between the susceptible and the resistant cultivars was observed (Fig. 3). Overall, the leaf rust-resistant

Korean wheat cultivars presented relatively large seed weights compared to the leaf rust-susceptible wheat cultivars. However, chlorophyll content was similar between leaf rust-susceptible and leaf rust-resistant Korean wheat cultivars. Therefore, it is important for future studies to evaluate the underlying mechanisms responsible for seed weight variation, which may be associated with leaf rust resistance.

Increase of β -1,3-Glucanase activity after leaf rust inoculation

Give that β -1,3-glucanase breaks down the β -1,3-glucan that forms in the cell walls of fungal pathogens and thus contributes to the protection of plants from fungal infection [15,16], the specific activity of β -1,3-glucanase was measured in this study. As expected, the specific activity of β -1,3-glucanase in all wheat cultivars tended to increase from 0 to 24 h after leaf rust inoculation (Fig. 4). However, no difference in β -1,3-glucanase activity between leaf rust-susceptible and leaf rust-resistant wheat cultivars was found. This suggest that that although β -1,3-glucanase activity increased after leaf rust inoculation, it is not a determining factor for leaf rust resistance in Korean wheat cultivars.

DPPH Assay for Antioxidant Activity Analysis

Leaf rust induced the host oxidative stress in the resistance to invasive fungal disease. The change of antioxidant activity after

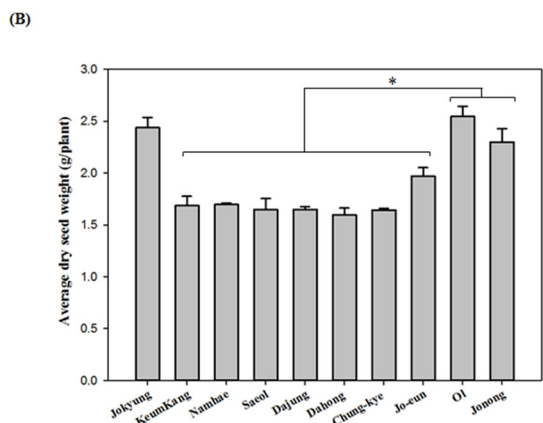
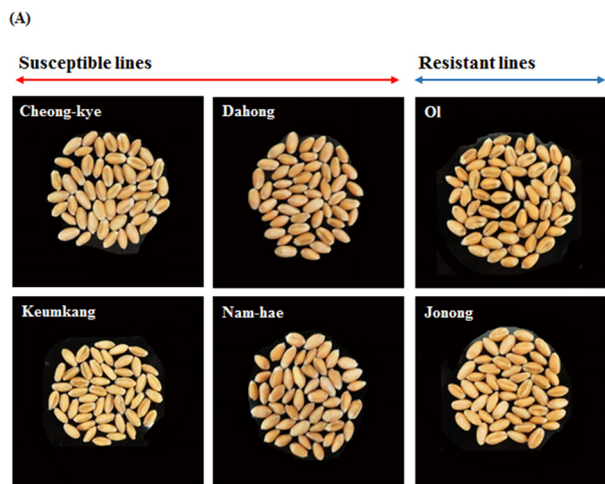


Fig. 2 Seed size and dry weight of Korean wheats. Comparison of (A) seed size and (B) average dry seed weight of susceptible and resistant Korean wheat varieties. For average dry seed weight, a total 50 seeds from each wheat cultivar were used (**p* < 0.05, Student's *t*-test)

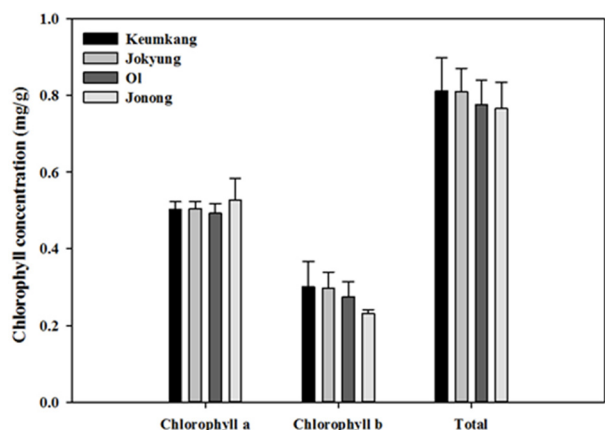


Fig. 3 Chlorophyll content of Korean wheat varieties. Comparison of chlorophyll content in two susceptible (Keumkang, Jokyung) and two resistant (OI, Jonong) Korean wheat varieties. Chlorophyll a, chlorophyll b, and total chlorophyll content were analyzed (**p* < 0.05, ***p* < 0.01, Student's *t*-test).

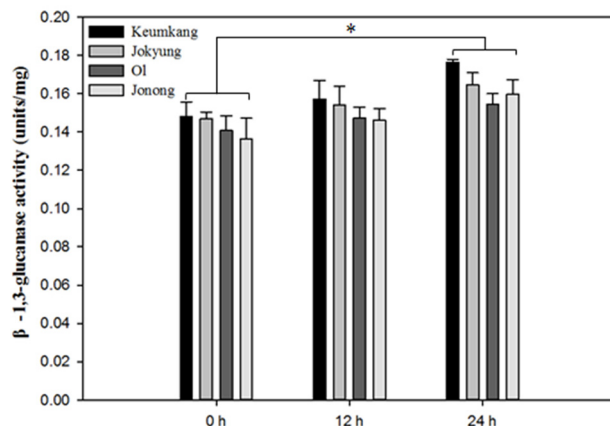


Fig. 4 β -1,3-Glucanase activity of Korean wheat varieties in response to leaf rust infection. Extracts of the first leaves of two susceptible (Keumkang, Jokyung) and two resistant (OI, Jonong) Korean wheat varieties were sampled at 0, 12, and 24 h after leaf rust inoculation and then analyzed for β -1,3 glucanase activity (**p* < 0.05, Student's *t*-test)

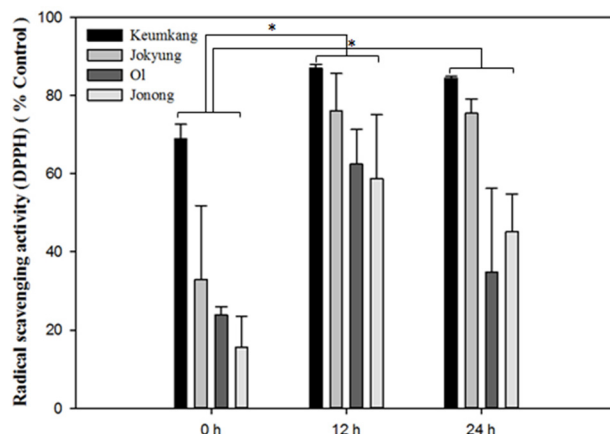


Fig. 5 Radical scavenging activity of Korean wheat varieties in response to leaf rust infection. Extracts of the first leaves of two susceptible (Keumkang, Jokyung) and two resistant (OI, Jonong) Korean wheats were sampled at 0, 12, and 24 h after leaf rust inoculation and then analyzed for radical scavenging activity using DPPH (**p* < 0.05, ***p* < 0.01, Student's *t*-test)

leaf rust inoculation was examined for two susceptible and two resistant wheat cultivars. Antioxidant activity was measured by using DPPH, which quantifies radical scavenging activity [17]. The radical scavenging activity of all wheat cultivars increased at 12 and 24 h after inoculation. In particular, radical scavenging activity was maximal at 12 h. Interestingly, the radical scavenging activity of OI and Jonong wheat cultivars increased rapidly at 12 h to 61 and 73%, respectively. In contrast, the radical scavenging activity of Kumkang and Jokyung wheat cultivars at 12 h increased to 20 and 56%, respectively. These results indicate that resistant wheat cultivars might have a higher antioxidant capacity against leaf rust infection than susceptible wheat cultivars.

Declarations The authors declare that they have no competing interests.

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