Development of a Rapid Approach for Detecting Sharp Eyespot Resistance in Seedling-Stage Wheat and Its Application in Chinese Wheat Cultivars

Yan Ren,^{1,2} Peng-Bo Yu,^{1,2} Yue Wang,^{1,2} Wei-Xiu Hou,^{1,2} Xia Yang,^{1,2} Jia-Lin Fan,³ Xiao-Hui Wu,^{1,2} Xia-Lei Lv,^{1,2} Ning Zhang,^{1,2} Lei Zhao,^{1,2} Zhong-Dong Dong,^{1,2} and Feng Chen^{1,2,†}

¹ National Key Laboratory of Wheat and Maize Crop Science, Henan Agricultural University, Zhengzhou, China

² Agronomy College, Henan Agricultural University, Zhengzhou, China

³ Henan Key Laboratory of Nuclear Agricultural Sciences/Isotope Institute Co., Ltd., Henan Academy of Sciences, Zhengzhou, China

Abstract

Sharp eyespot, caused by *Rhizoctonia cerealis*, has become one of the most severe diseases affecting global wheat production in recent decades. Quick and efficient screening methods are required to accelerate the development of cultivars for sharp eyespot resistance in wheat breeding. Here, a two-step colonized wheat kernels (TSCWK) method for the inoculation and classification of sharp eyespot resistance in seedlings was established in a greenhouse. After preliminary verification of the reliability of the method in two replicates, 196 wheat cultivars were assessed for sharp eyespot resistance, and significant correlations were identified among the four replicates ($r = 0.78$ to 0.84 ; $P < 0.01$). Furthermore, the 196 cultivars were scored for sharp eyespot resistance at the milk-ripe stage using traditional toothpick inoculation in the field. Correlation and linear regression analysis showed

that the application of this approach at the seedling stage showed good consistency with the traditional field method. Moreover, the scoring of 442 cultivars using the TSCWK method indicated that most cultivars from the Huanghuai valley were susceptible to *R. cerealis*, suggesting an urgent need to improve sharp eyespot resistance in this region. Additionally, the relative resistance index of sharp eyespot decreased in the surveyed cultivars of the region with time. This study offers a rapid and effective approach for the identification of wheat sharp eyespot resistance and provides valuable germplasm for improving sharp eyespot resistance in wheat breeding.

Keywords: common wheat, sharp eyespot, colonized wheat kernels method, seedling inoculation

Wheat (*Triticum aestivum* L.) is an essential global crop. In 2018, 759.9 million metric tons of wheat was produced (FAO 2019). Soil-borne pathogens can cause extensive damage to wheat by limiting water and nutrient uptake or by reducing the quantity and quality of the grain (Hamada et al. 2011). Sharp eyespot, as one of the most serious soil-borne diseases, is mainly caused by *Rhizoctonia cerealis* (Li et al. 2014). Infection symptoms in wheat include dark-bordered lesions on the stem bases of young and mature plants, and even peduncle rot, leading to lodging at the internode and premature spike senescence or ripening (white heads) (Lemańczyk and Kwaśna 2013; Lovell et al. 2003). Since the 1980s, sharp eyespot has become a threat to wheat production in many wheat-growing regions of the world, including China (Chen et al. 2008, 2013), New Zealand (Cromey et al. 2002), the United Kingdom (Clarkson and Cook 1983), and Egypt (Hammouda 2003). In China, sharp eyespot has affected more than 8.1 million hectares of wheat, resulting in a yield loss of 530,000 t in 2005 (McBeath and McBeath 2010). Additionally, the incidence of sharp eyespot in Europe is increasing yearly due to

earlier sowing, the planting of highly susceptible cultivars, and more favorable weather for infection (Lemańczyk and Kwaśna 2013). Although sharp eyespot can be controlled through the application of fungicides, the increased use of fungicides has decreased microbial antagonism to favor *R. cerealis* (Prew and McIntosh 1975; Van der Hoeven and Bollen 1980). Therefore, developing and breeding resistant wheat cultivars to sharp eyespot is a more effective and environmentally safe approach for controlling the disease.

Developing a simple and accurate method for detecting sharp eyespot resistance is an important precondition for wheat resistance breeding. At present, the main methods for detecting sharp eyespot resistance include identification in a disease nursery (Li et al. 2013; Zhang and Ren 1999), seed inoculation (Liu et al. 2015; Ren et al. 2010; Zhang et al. 2005), colonized wheat kernels on the soil surface (Chen et al. 2013; Wu et al. 2017), and toothpick inoculation (Cai et al. 2006). The first two inoculation methods (disease nursery and seed inoculation) are influenced greatly by the field environment, resulting in them being unrepeatable. The other methods of inoculation (spreading of colonized wheat kernels on the soil surface and embedding colonized toothpicks in the plant) greatly increase the repeatability but are time-consuming and labor-intensive and thus unsuitable for the large-scale resistance screening of cultivars (Cai et al. 2006; Chen et al. 2013). Seedling trials in a greenhouse have a short cycle, have high repeatability, and are characterized by little influence from the environment. However, there is no suitable method for testing wheat sharp eyespot resistance at the seedling stage, because the correlation between seedlings in the greenhouse and adult plants in the field is low (Chen et al. 2002; Zhou et al. 2017). Genetic studies on wheat sharp eyespot resistance and breeding have progressed slowly due to the lack of an effective and reliable method. It is thus urgent that a rapid and effective approach for the identification of sharp eyespot resistance in common wheat is established.

The Huanghuai valley is the largest and most important wheat production zone in China, contributing 60 to 70% of the total production and cultivation area (Yang et al. 2019). With the incorporation of straw residues in recent years, crop rotation, and the heavy use of fertilizers, wheat sharp eyespot has become one of the major diseases in

†Corresponding author: F. Chen; chf0088@163.com

Y. Ren and P. B. Yu contributed equally to this paper.

Funding: The research was supported by National Natural Science Foundation of China (U1904109 and 3181101544), National Key Research and Development Program (2016YFD0100502), Ten Thousand Talents Plan (Z04295) of China, and Henan Science and Technology Foundation (202102110016).

*The e-Xtra logo stands for “electronic extra” and indicates that supplementary tables are published online.

The author(s) declare no conflict of interest.

Accepted for publication 27 January 2020.

the Huanghuai valley (Li et al. 2017). In this study, we developed an effective and reliable approach for the identification of wheat seedling sharp eyespot resistance and identified some sharp eyespot resistant germplasm from 442 wheat accessions that could be used as resistance donors in wheat breeding.

Materials and Methods

Plant materials. A total of 442 wheat accessions (431 historical and advanced lines from China, and 11 cultivars from foreign countries) were used to develop evaluation method for wheat sharp eyespot resistance and to screen germplasm resistant to sharp eyespot. Most (399) of the Chinese wheat accessions had been developed or released in the Huanghuai valley, including Henan, Hebei, Shaanxi, Shanxi, Shandong, Jiangsu, and Anhui provinces. Four accessions (resistant line CI12633, moderately resistant cultivar Xifeng, and moderately susceptible cultivars Yangmai15 and Yangmai158) showing different resistance levels were used as controls during the inoculating and scoring of wheat sharp eyespot. The highly susceptible cultivar Yumai49 was planted as the susceptible control.

***R. cerealis* pathogen materials.** Wheat plants in the greenhouse and field were inoculated using a Chinese *R. cerealis* isolate, R0301, kindly provided by Prof. Huaigu Chen from the Jiangsu Academy of Agricultural Sciences. R0301 is a highly virulent strain isolated from wheat samples symptomatic for sharp eyespot in Jiangsu, China, in 2003 (Li et al. 2014). An aliquot of R0301 stock, maintained on potato dextrose agar (PDA) at 4°C, was activated on fresh PDA plates under artificial incubation at 23°C in the darkness. Agar disks (10 mm in diameter) were then cut from the margin of actively growing colonies and transferred into 1.5-ml centrifuge tubes containing PDA and then stored in the incubator for inoculum preparation. Another 10-mm-diameter agar disk was transferred to a 2-ml centrifuge tube filled with sterile mineral oil and stored at -80°C.

Sharp eyespot inoculum preparation. *Wheat kernel inoculum.* After the wheat kernels had been soaked in water for 12 h, they were fully boiled for about 1 h before cracking. The boiled kernels were then moved onto clean gauze on a test bed to dry completely for 12 to 24 h and were further transferred into Erlenmeyer flasks for autoclaving at 121°C for 45 min. After cooling, five pieces of

activated medium with R0301 mycelia were placed into each Erlenmeyer flask and mixed thoroughly before being placed in the incubator to grow under the same conditions mentioned above. The flasks were fully shaken every 2 to 3 days to promote uniform colonization. After 10 days, the kernels full of white mycelia were used for seedling inoculation.

Toothpick inoculum. Ordinary wooden toothpicks were cut into approximately 0.5-cm-long pieces and then immersed into water for 48 h. After the wooden toothpicks had been autoclaved at 121°C for 20 min, about 30 sterilized toothpick segments were evenly placed onto each PDA plate under a strict asepsis procedure when the PDA had not solidified. Four pieces of active medium with R0301 mycelia were symmetrically added to each PDA plate with toothpick segments, and then the plates were placed in an incubator at 23°C in the darkness. After 7 to 10 days, the colonized toothpick segments were ready for inoculation in the field.

Evaluation of resistance in the greenhouse. A schematic diagram of the two-step colonized wheat kernels (TSCWK) method for inoculation and investigation of seedling sharp eyespot is shown in Figure 1. To improve the intensity and effectiveness of the inoculation, the sterile soil was mixed with colonized kernels before sowing, and then about 0.3 g (2‰ of sterilized soil) of colonized kernels was scattered into each plastic pot at the two-leaf stage. The TSCWK method was involved in the two-step inoculation at two different seedling stages instead of one-step inoculation at the three-leaf stage (Chen et al. 2009), which improved the distribution of the hyphae by *R. cerealis* and sharply improved inoculation efficiency and phenotyping repeatability. Below is the detailed procedure.

Wheat seeds were sterilized with 95% ethyl alcohol for 3 min and then rinsed in sterile water three times. Soil for planting was autoclaved at 121°C for 1 h. Sterilized soil (150 g) containing 0.3 g colonized wheat kernels was placed into a 7 × 7 × 7 cm plastic pot, into which 16 sterilized seeds/pot were placed and covered with 20 g of sterilized soil. Two to four pots as replicates were used for each cultivar. Twenty-four plastic pots were placed onto one tray, including one susceptible cultivar Yumai49 as the control. Inoculated seeds were watered from the bottom of the trays once a day. Furthermore, another 0.3 g of colonized wheat kernels was scattered into the four

Fig. 1. Schematic diagram of seedling inoculation identification for sharp eyespot using the two-step colonized wheat kernels method (A) and phenotypic classification (B).

corners of each plastic pot with the secondary inoculation when the second leaf was fully expanded. The conditions of the growth chamber were kept at 16 h light/8 h darkness at 18 to 25°C with 70% relative humidity.

The severity of the sharp eyespot was scored about 25 days after the secondary inoculation based on a six-point scale standard as follows, including 0 (no obvious symptoms), 1 (the outer leaf sheath turns brown or slight sharp eyespot appears), 3 (the outer leaf sheath has obvious sharp eyespot, but the inner sheath is asymptomatic), 5 (the inner sheath turns brown or has slight sharp eyespot), 7 (the inner leaf sheath has obvious sharp eyespot), and 9 (complete necrotic damage) (Fig. 1B). After scoring, the disease index (DI) was calculated for each replicate based on the formula $DI (\%) = \sum(f_i y_i) \times 100 / (\sum f_i \times 9)$, where y_i is the scale value of each plant and f_i is the number of each scale value assessed in one replicate.

Evaluation of sharp eyespot resistance in the field. To verify the inoculation approach, we used the traditional method in adult plants. A total of 200 wheat accessions including four controls were planted in the field for evaluation of sharp eyespot severity at the Zhengzhou Scientific Research and Education Center of Henan Agricultural University, Henan province, during the 2017 to 2018 cropping season. The field trial was performed using randomized complete blocks with two replicates. Four cultivars (CI12633, Xifeng, Yangmai15, and Yangmai158) with known sharp eyespot resistance were included as controls in each replicate. Approximately 40 seeds of each cultivar were sown in a row of 1.5 m length and 23 cm between rows. The highly susceptible control, Yumai49, was planted adjacently at every 20 rows. Inoculations were carried out using the method of Cai et al. (2006) with slight modifications. Briefly, the colonized toothpick segments were gently embedded between the leaf sheath and stem on 4 and 5 April 2018 during the jointing stage. Twenty stems of each cultivar were inoculated in every replicate. Sterile toothpicks were also used for the control. To enhance humidity, the plants were sprinkled with water twice a day, each time for 30 min, on the 10th day after inoculation. Disease severity was assessed at the milk-ripe stage based on a scale of 0 to 4 according to the method of Shi et al. (2000). DI was calculated using the above-mentioned formula.

Statistical analysis. Analysis of variance (ANOVA) and correlation coefficients were calculated with Windows Excel 2013. In order to better estimate the sharp eyespot resistance of the cultivars, the relative resistance index (RRI) for each cultivar was calculated using the following formula (Liu et al. 2015):

$$RRI = 1 - (DI_i / DI_{\max})$$

where DI_i is the disease index of the i^{th} cultivar or line and $DI_{\max}$ is the disease index of the most severe cultivar or line. The RRI was categorized qualitatively from immune to highly susceptible.

Results

The development and validation of the TSCWK method for evaluating sharp eyespot resistance in wheat seedlings. Four wheat cultivars with known different sharp eyespot resistances were evaluated as controls in the seedling and adult stages. As shown in Table 1, these four cultivars showed relatively consistent DI and RRI between the seedlings by TSCWK and the adult plant results in the field, and they also showed identical resistance classification

with a previous phenotype (Ren et al. 2009; Wu et al. 2017). This suggested that the new method we established in this study could preliminarily evaluate the sharp eyespot resistance of wheat cultivars at the seedling stage.

In order to verify the repeatability of this method at large sample scales, the resistance of 196 cultivars to *R. cerealis* was further investigated using TSCWK at the seedling stage. The results showed that significant correlations (averaged $r = 0.82$; Table 2) for sharp eyespot DI were obtained among the four replicates. Ren et al. (2009) and Zhou et al. (2017) previously reported methods for the evaluation of wheat seedling sharp eyespot resistance, but only a small number of cultivars were used, and the correlation coefficients among replicates were relatively low (averaged $r = 0.74$). In addition, the ANOVA showed that the resistance to sharp eyespot at the seedling stage differed significantly among the wheat cultivars surveyed (Supplementary Table S1), suggesting that screening cultivars with sharp eyespot resistance was feasible using TSCWK.

The application of TSCWK. Before we developed TSCWK in the seedlings, the inoculation of sharp eyespot in the field had been performed in our group for several years. Compared with spreading wheat kernels close to the root of the plant, the method of toothpick inoculation showed high accuracy and good repeatability. Therefore, to evaluate the application value of TSCWK, the 196 cultivars that were scored using TSCWK in the seedling stage were further identified for sharp eyespot resistance in the field using toothpick inoculation. The correlative coefficient for the averaged DI was significant ($r = 0.64$; $P < 0.01$) between the seedling and adult-plant results, suggesting that the sharp eyespot resistance of the seedlings could preliminarily predict the resistance of adult plants (Fig. 2). Further analysis showed that most of the resistant cultivars screened in the seedlings also showed intermediate to high sharp eyespot resistance in the field (Table 3), indicating that TSCWK could be used to preliminarily predict resistance in adult plants to sharp eyespot in common wheat. In summary, the TSCWK for detecting wheat seedling resistance to sharp eyespot demonstrated good repeatability and reliability and could be applied to rapidly detect sharp eyespot resistance for a large number of wheat accessions.

Distribution of sharp eyespot resistance in wheat cultivars from the Huanghuai valley. A total of 442 wheat cultivars with four replicates were used to identify sharp eyespot resistance using TSCWK, and the averaged seedling DI of all cultivars surveyed was 53.31 and ranged from 15.66 to 77.78. There were 3, 15, 54, 83, 136, 136, and 15 accessions showing averaged DI ranges of <20, 20.01 to 30, 30.01 to 40, 40.01 to 50, 50.01 to 60, 60.01 to 70, and 70.01 to 80, respectively (Fig. 3A). No accession was immune or highly resistant, but 17 showed an averaged RRI of more than 0.6 ($DI < 29$, Fig. 3, Table 3), exhibiting their potential use in wheat resistance breeding. The phenotypic results indicated that most wheat cultivars (78.05%) showed intermediate to high susceptibility to sharp eyespot, indicating an urgent need for improving sharp eyespot resistance in the Huanghuai valley of China.

Moreover, the assessment of adult plant resistance to sharp eyespot in 196 wheat cultivars showed a DI of 12.15 to 88.29 (Supplementary Table S2). The percentage of susceptible cultivars containing moderate and high susceptibility was 66.84% in the field, which was slightly higher than that in the seedling (64.80%). In addition, the averaged DI of these 196 cultivars was 47.50 in the seedlings and 57.90

Table 1. Comparison of the resistance levels of four controls at the seedling and adult-plant stages^a

Varieties	Seedling stage					Adult-plant stage		
	DI-1	DI-2	DI-Avg	RRI-Avg	Type of resistance	DI-Avg	RRI-Avg	Type of resistance
CI12633	12.12	19.19	15.66	0.78	R	16.02	0.82	HR
Xifeng	47.47	45.45	46.46	0.35	MS	45.30	0.49	MR
Yangmai15	63.09	63.64	62.54	0.12	HS	57.27	0.35	MS
Yangmai158	65.66	59.60	62.63	0.13	HS	65.68	0.26	MS

^a DI = disease index; DI-Avg = the average DI of two repeats; RRI-Avg = the average relative resistance index of two repeats; HR = highly resistant; R = resistant; MR = moderately resistant; MS = moderately susceptible; and HS = highly susceptible.

in the field, indicating that wheat sharp eyespot occurred more seriously in the field than in the seedlings. This was probably caused by the superposition of multiple pathogens in the field, such as *Fusarium* species and *Microdochium nivale*. However, there were 14

accessions showing an averaged DI of less than 35 (RRI > 0.6) (Table 3).

According to the released or developed year, the 399 accessions from the Huanghuai valley could be divided into four periods: before

Table 2. Information for sharp eyespot disease index and correlation coefficients among different environments

Environment ^a	Minimum	Maximum	CV (%)	Average	<i>r</i>				
					Seedling.2	Seedling.3	Seedling.4	Adult.1	Adult.2
Seedling.1	12.12	72	31.61	44.76	0.832**	0.779**	0.770**	0.532**	0.481**
Seedling.2	16.16	72.73	27.09	47.38		0.801**	0.831**	0.573**	0.562**
Seedling.3	12.12	82	23.58	53.83			0.847**	0.599**	0.508**
Seedling.4	16.16	82	23.43	53.95				0.547**	0.528**
Adult.1	18.59	88	26.29	55.89					0.753**
Adult.2	16.92	94	24.28	60.24					

^a Seedling.1 = repeat 1 in the greenhouse; Seedling.2 = repeat 2 in the greenhouse; Seedling.3 = repeat 3 in the greenhouse; Seedling.4 = repeat 4 in the greenhouse; Adult.1 = repeat 1 in field; and Adult.2 = repeat 2 in field. Two asterisks (**) indicate significance at $P < 0.01$.

Fig. 2. Linear regression analysis of disease index (A) and relative resistance index (B) of 196 wheat varieties in the seedling and adult stage.

Table 3. Representative cultivars with different resistance levels

Resistance level	Relative resistance index	Cultivars or lines	
		Seedling test	Adult-plant test
Immune	1	...	...
Highly resistant	0.80–0.99	...	Shaanhe6, CI12633
Resistant	0.60–0.79	CI12633, Banmangmai, Banjiemang, Ibis, Hongyouzi, Shaanhe6, Chinese Spring, Hongxingmai, Pingyuan50, Linfen139, Chuanyu12, Yongfengnong2, Yunong202, Xinmai68, Huabei187, Jinmai50, Neixiang184	Banmangmai, Chinese Spring, Huomai, Banjiemang, Pingyuan50, Pingyang181, Yumai8, Qingfeng1, Hongyouzi, Hongxingmai, Libellula, Zhengmai8998
Moderately resistant	0.40–0.59	Ningchun10, Pingyang181, Jishen5099, Yanfeng712, Yumai47, Yuami51, Jimai30, Xiaoyan22, Jimai32, Jimai210, Huomai, Yumai8, Jiamai6, Bainong1309, Fengdecun20, Changnong339-5-1, Zhengmai8998, etc.	Changle5, Changnong339-5-1, Jinmai50, Jing411, Ningchun10, Xinyin13-2, Chuanyu10, Shaan229, Xiaoyan22, Jishen5099, Xinmai18, Zhongnong28, Beijing841, Jinmai54, Xiaoyan6, Longmai157, Jingshuang2, Han94-5316, Nanda2419, etc.
Moderately susceptible	0.20–0.39	Yumai13, Yufeng2, Changle5, Zhongnong28, Yumai53, Yunong9901, Bainong160, Jinmai109, Jinchengmai12, etc.	Neixiang184, Shannong413863, Yumai47, Shuangji2, Yangmai4, Wansu9908, Xinmai13, Gaocheng8901, etc.
Highly susceptible	<0.20	Zhoumai22, Minfeng296, Han97-5297, Luomai166, Kaimai26, Pumai27, Xiza5, Yumai4, Zhongying012, etc.	Stramppli, Guinong16, Shaannong69, Xingmai1, Ji923235, Zhoumai22, Sumai3, Zhenghua0840-3, Bainong160, etc.

1980, 1980 to 2000, 2001 to 2014, and after 2014. As shown in Figure 4, the mean DI to sharp eyespot in the seedlings of all cultivars surveyed was 48.76 (before 1980), 49.06 (1980 to 2000), 49.46 (2001 to 2014), and 58 (after 2014), indicating a dramatic increase in DI after 2014. Moreover, the percentage of susceptible cultivars (including moderately susceptible and highly susceptible) rose rapidly after 2014, and the proportion of resistant cultivars (including highly resistant, resistant, and moderately resistant) decreased from 33.33% (2000 to 2014) to 11.16% (after 2014) (Fig. 4), indicating that sharp eyespot resistance has not been focused on by wheat breeders in recent years. Of the 39 wheat cultivars from other countries or regions (including seven landraces, nine from Beijing, 12 from Sichuan, one from Hubei, one from Ningxia, and nine from abroad), the averaged seedling DI was 42.11, and 10 of them (Banmangmai, Banjiemang, Ibis, Hongyouzi, Chinese Spring, Hongxingmai, Pingyuan50, Chuanyu12, and Huabei187) showed middle resistance ($RRI > 0.6$) to sharp eyespot and could be potentially used as sharp eyespot resistance germplasm in Chinese wheat breeding.

Discussion

In recent decades, wheat sharp eyespot has become increasingly globally aggravated by increased straw residue incorporation. The present study showed that the percentage of susceptible cultivars has increased sharply after 2014 (88.84% of which are susceptible). Therefore, this has possibly become a serious threat to wheat production owing to continual climatic changes and the large number of susceptible wheat cultivars planted. To avoid the potential losses of wheat yield caused by sharp eyespot, it is urgent to strengthen the selection and utilization of cultivars with improved resistance to sharp eyespot.

Sharp eyespot pathogen can infect wheat root tissues at any time during wheat development, and plants at the seedling and jointing-booting stages are easily infected. After the heading stage, growth of the pathogen is reduced, and little change is observed in disease severity. Compared with field trials, seedling sharp eyespot tests are quick, reproducible, and relatively free from environmental interference. However, few studies exist on seedling inoculation for wheat sharp eyespot. Chen et al. (2002) evaluated Yangmai158 for resistance in the seedling and adult stages by embedding matchstick inocula, which showed a significantly different DI (54.67 in the seedling stage and 16.70 in the adult stage) between the seedling and adult stages due to inappropriate grading standards. Ren et al. (2009) improved the embedding inoculation method for detecting sharp eyespot by changing the inoculation conditions and grading standards.

However, the embedding inoculation method was complex and could not be used on thin and narrow plants; thus, it was not suitable for large-scale resistance identification. Zhou et al. (2017) used soaking wheat seedlings in *R. cerealis* fungal spore suspension for the assessment of sharp eyespot resistance in the greenhouse, but testing results of 16 wheat accessions were not consistent with field scoring for sharp eyespot resistance.

Dodman and Wildermuth (1987) reported that soil-borne disease resistance is through adding inoculum to the soil rather than directly inoculating into plants. Therefore, we developed a TSCWK method for seedling inoculation identification for wheat sharp eyespot resistance, and the results showed that the TSCWK method at the seedling stage was sufficient to appropriately evaluate plants for resistance to *R. cerealis*. Compared with one-step inoculation at the three-leaf stage (Chen et al. 2009), TSCWK showed an earlier inoculation time, faster resistance identification cycle, and more stable inoculation efficiency. Erginbas-Orakci et al. (2016) showed that the colonized grains method was the most appropriate method for crown rot

Fig. 4. Distribution of mean disease index (DI), relative resistance index (RRI), and percentages of susceptible and resistant cultivars at four different periods.

Fig. 3. Disease index (DI) (A) and relative resistance index (RRI) (B) at seedling and adult-plant stages in Chinese wheat cultivars.

resistance screening, which is consistent with our results, indicating that similar inoculation methods could be used for detecting resistance among *R. cerealis*, *Fusarium culmorum*, and *F. pseudograminearum*.

In our study, no immune or highly resistant cultivars were identified, and most of the wheat cultivars (80.65%) from the Huanghuai valley showed intermediate to high susceptibility to sharp eyespot, which is similar to the results of Li et al. (1998). Therefore, there is an urgent need to improve sharp eyespot resistance in wheat breeding in China. Although few resources resistant to sharp eyespot exist, we categorized the cultivars or lines into five groups based on their sharp eyespot resistance, which will better utilize these cultivars (Table 3). In the 242 current cultivars or advanced lines, only Yongfengnong2 showed moderate resistance ($RRI > 0.60$) to sharp eyespot and could be considered for application in sharp eyespot resistance breeding. Furthermore, some landraces and historical cultivars (e.g., Banmangmai, Banjiemang, Chinese Spring, Hongyouzi, Pingyuan50, and Neixiang184) showed middle resistance to sharp eyespot and could be used in wheat resistance breeding. However, some cultivars (e.g., Xiaoyan4, Xinong979, Yanyou361, Yumai50, Yunong209, Yumai47, Zhou8425B, Zhoumai22, Zhoumai23, and Zhengmai9023) showing high susceptibility ($RRI < 0.2$) to sharp eyespot should be avoided in endemic areas of sharp eyespot.

Literature Cited

- Cai, S. B., Ren, L. J., Yan, W., Wu, J. Z., Chen, H. G., Wu, X. Y., and Zhang, X. Y. 2006. Germplasm development and mapping of resistance to sharp eyespot (*Rhizoctonia cerealis*) in wheat. *Sci. Agric. Sin.* 39:928-934.
- Chen, H. D., Wang, Z. M., and Ni, G. H. 2002. The research of embedding inoculation method to sharp eyespot using embedding method. *Jiangsu J. Agric. Sci.* 5:31-32.
- Chen, J., Li, G. H., Du, Z. Y., Quan, W., Zhang, H. Y., Chen, M. Z., Wang, Z., and Zhang, Z. J. 2013. Mapping of QTL conferring resistance to sharp eyespot (*Rhizoctonia cerealis*) in bread wheat at the adult plant growth stage. *Theor. Appl. Genet.* 126:2865-2878.
- Chen, L., Zhang, Z. Y., Liang, H. X., Liu, H. X., Du, L. P., Xu, H. J., and Xin, Z. Y. 2008. Overexpression of *TIERF1* enhances resistance to sharp eyespot in transgenic wheat. *J. Exp. Bot.* 59:4195-4204.
- Chen, Y., Li, W., Zhang, X. X., Zhang, B. Q., Yu, H. S., and Chen, H. G. 2009. Composition and virulence of pathogen of wheat sharp eyespot in North latitude 33° of China. *J. Triticeae Crops* 29:1110-1114.
- Clarkson, J. D. S., and Cook, R. J. 1983. Effect of sharp eyespot (*Rhizoctonia cerealis*) on yield loss in winter wheat. *Plant Pathol.* 32:421-428.
- Cromey, M. G., Butler, R. C., Boddington, H. J., and Moorhead, A. R. 2002. Effects of sharp eyespot on yield of wheat (*Triticum aestivum*). *N. Z. J. Crop Hortic. Sci.* 30:9-17.
- Dodman, R. L., and Wildermuth, G. B. 1987. Inoculation methods for assessing resistance in wheat to crown rot caused by *Fusarium graminearum* group 1. *Aust. J. Agric. Res.* 38:473-486.
- Erginbas-Orakci, G., Poole, G., Nicol Julie, M., Paulitz, T., Dababat Abdelfattah, A., and Campbell, K. 2016. Assessment of inoculation methods to identify resistance to Fusarium crown rot in wheat. *J. Plant Dis. Prot.* 123:19-27.
- FAO. 2019. FAOSTAT Database. <http://www.fao.org/faostat/en/#home>. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Hamada, M. S., Yin, Y. N., Chen, H. G., and Ma, Z. H. 2011. The escalating threat of *Rhizoctonia cerealis*, the causal agent of sharp eyespot in wheat. *Pest Manag. Sci.* 67:1411-1419.
- Hammouda, A. M. 2003. First report of sharp eyespot of wheat in Egypt. *Plant Dis.* 87:598.
- Lemańczyk, G., and Kwaśna, H. 2013. Effects of sharp eyespot (*Rhizoctonia cerealis*) on yield and grain quality of winter wheat. *Eur. J. Plant Pathol.* 135:187-200.
- Li, H. J., Wang, X. M., Chen, H. G., Li, W., Liu, D. T., and Zhang, H. Y. 2013. Reaction of wheat-*Thinopyrum* progenies and wheat germplasm to sharp eyespot. *Acta Agron. Sin.* 39:999-1012.
- Li, H. J., Yuan, H. X., Diao, X. G., Li, S. P., Hu, Y. X., and Wang, S. Z. 1998. Evaluation on the resistance of major wheat varieties in Henan province to sharp eyespot. *J. Henan Agric. Univ.* 32:107-111.
- Li, W., Guo, Y. P., Zhang, A. X., and Chen, H. G. 2017. Genetic structure populations of the wheat sharp eyespot pathogen *Rhizoctonia cerealis* anastomosis group D subgroup I in China. *Phytopathology* 107:224-230.
- Li, W., Sun, H. Y., Deng, Y. Y., Zhang, A. X., and Chen, H. G. 2014. The heterogeneity of rDNA-ITS sequence and its phylogeny in *Rhizoctonia cerealis*, the cause of sharp eyespot in wheat. *Curr. Genet.* 60:1-9.
- Liu, Y., Zhang, Q. F., Fu, B. S., Cai, S. B., Jiang, Y. J., Zhang, Z. L., Deng, Y. Y., Wu, J. Z., and Dai, T. B. 2015. Genetic diversity of wheat germplasm resistant to sharp eyespot and genotyping of resistance loci using SSR markers. *Acta Agron. Sin.* 41:1671-1681 (In Chinese with English abstract).
- Lovell, D. J., Parker, S. R., Baldwin, S., and Fraaije, B. A. 2003. Peduncle rot of dwarf caused by *Rhizoctonia cerealis*. *Plant Health Prog.* 4.
- McBeath, J. H., and McBeath, J. 2010. Plant diseases, pests and food security. Pages 117-156 in: *Environmental Change and Food Security in China*. Springer, Dordrecht, the Netherlands.
- Prew, R. D., and McIntosh, A. H. 1975. Effects of benomyl and other fungicides on take-all, eyespot and sharp eyespot diseases of winter wheat. *Plant Pathol.* 24:67-71.
- Ren, L. J., Chen, P. D., Chen, H. G., and Ma, H. X. 2010. Screening of resistance to sharp eyespot in wheat. *J. Plant Genet. Resour.* 11:108-111.
- Ren, L. J., Yao, J. B., Chen, P., and Ma, H. X. 2009. A new method for the seedling resistance evaluation of wheat sharp eyespot. *Jiangsu J. Agric. Sci.* 5:131-133.
- Shi, J. R., Wang, Y. Z., Chen, H. G., and Shen, S. W. 2000. Screening techniques and evaluation of wheat resistance to sharp eyespot caused by *Rhizoctonia cerealis*. *Acta Phytophylac. Sin.* 27:107-112 (In Chinese with English abstract).
- Van der Hoeven, E. P., and Bollen, G. J. 1980. Effect of benomyl on soil fungi associated with rye. 1. Effect on the incidence of sharp eyespot caused by *Rhizoctonia cerealis*. *Neth. J. Plant Pathol.* 86:163-180.
- Wu, X. J., Cheng, K., Zhao, R. H., Zang, S. J., Bie, T. D., Jiang, Z. N., Wu, R. L., Gao, D. R., and Zhang, B. Q. 2017. Quantitative trait loci responsible for sharp eyespot resistance in common wheat CI12633. *Sci. Rep.* 7:11799.
- Yang, X., Pan, Y. B., Singh, P. K., He, X. Y., Ren, Y., Zhao, L., Zhang, N., Cheng, S. H., and Chen, F. 2019. Investigation and genome-wide association study for Fusarium crown rot resistance in Chinese common wheat. *BMC Plant Biol.* 19:153.
- Zhang, H. Q., and Ren, Z. L. 1999. A preliminary study on resistance to sharp eyespot in wheat (*Triticum aestivum* L.). *Acta Phytopathologica Sin.* 3:199-202.
- Zhang, X. C., Li, S. S., Zhao, X. H., Fan, Y. X., and Li, R. J. 2005. QTL and molecular markers for resistance gene of wheat sharp eyespot. *J. Plant Genet. Resour.* 6:276-279.
- Zhou, M. P., Yao, J. B., Zhang, P., Yang, X. M., Yu, G. H., and Ma, H. X. 2017. New method for the resistance evaluation of wheat sharp eyespot in seedling. *Jiangsu J. Agric. Sci.* 33:61-66.