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Identification of three new resources of resistance to Fusarium head blight in wheat

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Abstract: Fusarium head blight (FHB) mainly caused by *Fusarium* species is one of the most important diseases threatening worldwide wheat production. To develop new FHB resistance resources, disease resistances of three new wheat lines L958, L962 and L987 were evaluated over a period of several years. We employed L699 (PI672540) and L661 as the resistant and susceptible control, respectively. Moderate FHB resistance was observed in these three wheat lines, among which the genotype L958 was found a good resource for yield, quality and FHB resistance improvement. In addition, genotype L962 was found to be resistant to powdery mildew, and both L958 and L987 carried the *YrL693* gene conferring stripe rust resistance. The study provides promising results for accelerating the resistance breeding of the three wheat lines.

Keywords: agronomic trait; FHB; resistance evaluation; *Triticum aestivum*

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum* Schwabe and *F. culmorum* WG Smith, is a serious wheat (*Triticum aestivum* L.) disease worldwide (Bai & Shaner 2004; Li *et al.* 2017). In China, FHB epidemics have frequently occurred in the middle to lower valleys of the Yangtze River (Yao & Lu 2000). In recent years, FHB has spread to the southwest of China, including Sichuan Province (Zhang *et al.* 2011; Liu *et al.* 2015; Yang *et al.* 2016), where the climate was warm and rainy. Furthermore, FHB is also a fungal disease threatening food and economic security due to its contamination with mycotoxins, decreasing the wheat yield and quality (Zwart *et al.* 2008). At present, there is still no economic and effective way to control this disease. Developing resistant cultivars is an important approach to control this disease, for which the decisive step is to identify new germplasm with multi-resistance.

Some effective FHB resistance sources have been identified in common wheat (Chu *et al.* 2011), and some FHB resistance quantitative trait loci (QTLs) have also been found and mapped in wheat relatives (Cai *et al.* 2005; Chen *et al.* 2007). In recent years, some geneticists and breeders have paid much at-

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tention to intermediate wheatgrass, *Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey (2*n* = 6*x* = 42; JJJ^{s}J^{s}SS), for its easy hybridization with wheat *Triticum aestivum* (2*n* = 6*x* = 42; AABBDD) (Luo *et al*. 2009; Liu *et al*. 2013). In addition, four new FHB resistant wheat germplasms were identified and released (Liu *et al*. 2015), which demonstrated the possibility of using *Th. intermedium* to develop novel wheat germplasm with different disease resistance. However, some breeding problems still remain and new materials with effective resistance to various diseases need to be developed.

The research in this paper is aimed at screening wheat FHB resistance germplasm derived from an MY11 × YU25 cross for FHB resistance improvement.

MATERIAL AND METHODS

Plant material. The three wheat lines L958, L962, and L987 were selected from the F_7 populations derived from an MY11 × YU25 cross during 2007. MY11 is a wheat cultivar widely grown in southwestern China, while YU25 is a wheat cultivar with a foreign pedigree of *Th. intermedium* (Liu *et al*. 2015).

FHB resistance evaluation. The experiment was conducted over three years: in greenhouse at Kansas State University, Manhattan, USA during 2014; Wenjiang field at Sichuan Agricultural University, Chengdu, China during 2015 and 2017; field at Neijiang Academy of Agricultural Science in Sichuan province, China during 2017.

During autumn 2014, all wheat lines were planted in plastic trays filled with Metro-mix 360 soil mix (Hummert International, Country) in a greenhouse. We used a randomized complete block to design an experiment with three replications. Four main stem spikes were randomly chosen from each block and total of 12 spikes per genotype were inoculated with *F. graminearum* (GZ3639) at the anthesis stage (Bai *et al*. 1999) according to Cai and Bai (2014). To evaluate FHB resistance, PSS (the percent of symptomatic spikelets) of each spike was recorded at 15 days after inoculation.

During 2015 and 2017, each wheat line was sown in 5 rows with a blank row between each genotype. Each row was 1.5 m long with 30 seeds and a row spacing of 25 cm. Wheat spikes were inoculated with 10 µl mixed macroconidial suspension (200 macroconidia per µl) of the spore-derived isolate of *F. graminearum* No. 4 (provided by Professor Zhengqiang Ma at Nanjing Agricultural University, Nanjing, Jiangsu Province, P.R. China) and 30 spikes of each genotype were inoculated. The inoculated spikes were covered with plastic bags for 3 days to maintain relatively high humidity. The number of diseased spikelets was recorded at 28 days after inoculation, and the PSS was calculated.

Evaluation of powdery mildew resistance. Twenty-seven *Blumeria graminis* f.sp. *tritici* (*Bgt*) single-spore isolates (Shen *et al*. 2015) were collected from Beijing, Shandong, Hebei, Henan, and Jiangsu provinces to assess the reactions of all genotypes to the pathogen causing powdery mildew. Evaluation of seedling reactions to 27 isolates was conducted in a greenhouse using the previously reported method (Zhao *et al*. 2013). In addition, the natural powdery mildew occurrence in a greenhouse was observed and recorded.

Diagnosis of stripe rust resistance gene *YrL693*. Recently, the wheat copper-binding protein (WCBP1) encoded by a candidate stripe rust gene *YrL693* located on chromosome 1B was reported (Li *et al*. 2015) and a marker named LS36 was derived from the MY11 × YU25 cross (Huang *et al*. 2014). Therefore, the diagnostic marker LS36 was used to determine the *YrL693* gene in the three wheat lines analysed in this study. PCR amplification and product analysis were performed according to Huang *et al*. (2014).

Spike productivity comparison. In the greenhouse during 2014, four non-inoculated main stem spikes were randomly chosen from each block; both 12 non-inoculated and 12 inoculated spikes of each genotype were harvested at the mature stage. The grain number per spike (GNS) and plant height (PH) were recorded. After drying the grain to constant moisture, the grain weight per spike (GWS) and thousand grain weight (TGW) per spike were calculated. The TGW of each spike was calculated as follows;

$$\mathrm{TGW} = \frac{\mathrm{GWS}}{\mathrm{GNS}} \times 1000$$

Then the TGW of each genotype can be obtained from the average of that of each spike, the loss of GNS was calculated as below;

$$\mathrm{loss}\left(\mathrm{GNS}\right) = \left[1 - \frac{\mathrm{GNS(inoculation)}}{\mathrm{GNS(control)}}\right] \times 100\%$$

Other spike productivity indices were also calculated by the same formula.

In the field during 2015, all the inoculated spikes were harvested, GNS, GWS and TGW were investi-

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Table 1. The evaluation of Fusarium head blight resistance in five wheat lines over three years

Genotype	2014 greenhouse		2015 Wenjiang		2017 Wenjiang		2017 Neijiang	
	N	PSS	*N*	PSS	*N*	PSS	*N*	PSS
L699	12	0.210 ± 0.025^{b}	21	0.282 ± 0.068^{b}	29	0.123 ± 0.005^{c}	30	0.095 ± 0.000^{b}
L958	12	0.321 ± 0.046^{b}	28	0.285 ± 0.051^{b}	31	0.156 ± 0.021^{bc}	31	0.124 ± 0.011^{b}
L987	11	0.321 ± 0.040^{b}	25	0.487 ± 0.065^{a}	27	0.177 ± 0.026^{bc}	30	0.124 ± 0.003^{b}
L962	12	0.323 ± 0.039^{b}	24	0.586 ± 0.064^{a}	29	0.250 ± 0.046^{b}	31	0.154 ± 0.017^{b}
L661	12	0.687 ± 0.070^{a}	20	0.538 ± 0.066^{a}	29	0.364 ± 0.054^{a}	30	0.391 ± 0.051^{a}

All indices are described by mean ± standard error; *N* – number of plant spikes; PSS – the percent of symptomatic spikelets; means in a column followed by the same letter(s) are not significantly different at a 5% probability level

gated and calculated. In addition, the percentage of *Fusarium*-damaged kernels (PFDK) was calculated.

Statistical analysis. Significant differences in the means of different genotypes for the percent of symptomatic spikelets (PSS), GNS, PFDK, GWS, and TGW were determined by the multiple samples *t*-test using IBM SPSS Statistics 19 software (SPSS Inc., Chicago, IL), and the significance of differences in the same genotype indices between inoculated and non-inoculated plants was also determined by the independent samples *t*-test with the same software.

RESULTS

FHB resistance. The greenhouse evaluation performed in autumn 2014 showed that L958, L962, and L987 exhibited an obvious resistance to FHB; their PSS values at 15 days were less than 35% (Table 1). The resistant control L699 had the lowest PSS value with 21%. In contrast, the susceptible sister line L661 had the highest value of 68.7% (Table 1). The dried and dead part of the inoculated spike in L699 was obviously smaller than that in L661, while L958, L987 and L962 were intermediate (Figure 1A).

The field evaluations performed during springs 2015 and 2017 also exhibited that L699 had a very low PSS value (Table 1). The PSS value for L958 was larger than that of L699 in the greenhouse in 2014, but not significantly in 2015 and 2017, which was significantly lower than that of L987 and L962 in 2015.

Powdery mildew resistance. Only *Bgt73-3* was avirulent to all genotypes tested while L962 was resistant to 7 out of 27 isolates. L699 was resistant to all isolates and both L962 and L987 were susceptible to all isolates except *Bgt73-3*. The natural occurrence of powdery mildew in the greenhouse further confirmed the existence of powdery mildew resistance gene in L962 (Figure 1C).

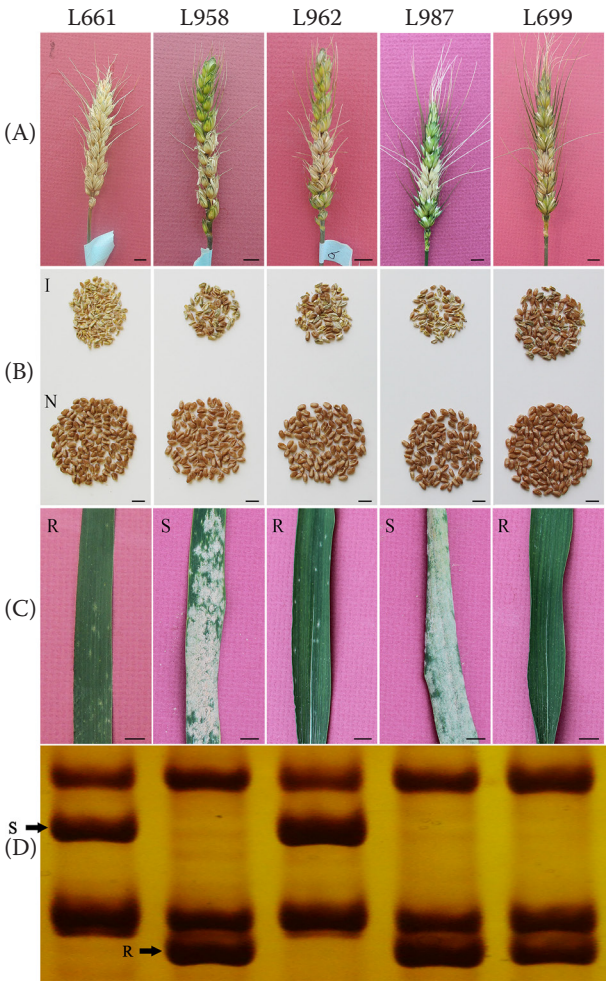

Figure 1. Phenotypes and genotypes of five analysed wheat lines: (A) phenotypes with different Fusarium head blight resistance evaluated in the greenhouse in 2014, scale bar 1.0 cm, R – resistant, S – susceptible; (B) inoculated (I) and non-inoculated (N) grains, scale bar 1.0 cm; (C) identification of powdery mildew resistance (R) and susceptibility (S), scale bar 1.0 cm; (D) resistant (R) and susceptible (S) genotypes evaluated using LS36 marker for the stripe rust resistance gene *YrL693*

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Table 2. The effects of Fusarium head blight on the grains of various wheat genotypes in the greenhouse in 2014 and in the field in 2015

Year	Genotype	*N*	GWS (g)		TGW (g)		GNS		PFDK
			mean	loss (%)	mean	loss (%)	mean	loss (%)	mean
2014	L699	12	0.65 ± 0.07^{a}	60.0**	26.15 ± 2.63^{a}	48.0**	24.5 ± 0.7^{b}	23.0**	–
	L958	12	0.21 ± 0.04^{b}	81.1**	14.79 ± 1.87^{bc}	64.9**	13.3 ± 1.2^{d}	50.3**	–
	L962	12	0.25 ± 0.08^{b}	77.0**	11.11 ± 2.49^{c}	74.5**	19.0 ± 1.9^{c}	22.7*	–
	L987	11	0.31 ± 0.05^{b}	66.7**	17.20 ± 2.33^{b}	56.1**	17.6 ± 0.8c	26.4**	–
	L661	12	0.26 ± 0.02^{b}	78.3**	8.09 ± 0.60^{c}	77.2**	31.1 ± 0.7^{a}	0.07	–
2015	L699	22	0.74 ± 0.06^{b}	–	19.55 ± 1.73^{b}	–	37.9 ± 1.8^{b}	–	0.355 ± 0.042^{a}
	L958	27	1.18 ± 0.08^{a}	–	27.45 ± 1.90^{a}	–	43.5 ± 1.2^{a}	–	0.279 ± 0.041^{b}
	L962	24	1.03 ± 0.12^{ab}	–	21.96 ± 2.23^{b}	–	45.5 ± 1.7^{a}	–	0.326 ± 0.043^{a}
	L987	25	0.81 ± 0.08^{b}	–	20.51 ± 1.76^{b}	–	40.0 ± 1.6^{b}	–	0.344 ± 0.045^{a}
	L661	19	0.89 ± 0.13^{b}	–	21.05 ± 2.46^{b}	–	40.2 ± 2.6^{b}	–	0.474 ± 0.069^{a}

All indices are described by mean ± standard error; *N* – number of plant spikes; GWS – grain weight per spike; TGW – thousand grain weight; GNS – grain number per spike; PFDK – the percent of *Fusarium*-damaged kernels; loss – the percentage of GNS, GPS, TGW decrease for the same genotype after inoculation in the greenhouse in 2014; means in a column followed by the same letter(s) are not significantly different at a 5% probability level; *, **significant difference level between inoculated and uninoculated wheat lines at $P < 0.05$ and 0.01, respectively

Diagnosis of stripe rust resistance gene *YrL693*. The results of PCR amplification of the diagnostic marker LS36 showed that L958 and L987 had the specific DNA fragment (Figure 1D), which suggested that they carry the *YrL693* gene for stripe rust resistance.

Spike productivity comparison. In the greenhouse, L699 had a significantly higher average value of GWS at 1.60 g and TGW at 50.27 g than that in other genotypes. In all genotypes, the inoculated plants had a sharp decrease of GWS, TGW and GNS except GNS of L661, compared with the corresponding control (Table 2 and Figure 1B).

However, in the field, the inoculated L958 had significantly larger values for GWS and TGW than those of the inoculated L699; the values of GWS and TGW of L958 were 1.18 g and 27.45 g, respectively; the descending order is L958, L962, L661, L987 and L699 (Table 2). Furthermore, L958 had a lower value of PFDK at 27.9% than the other lines with infection treatment.

DISCUSSION

Breeding for FHB resistance is difficult because it is controlled by multiple genes and affected by the environment. Previous studies showed that Sumai3 and Wangshuibai carried the well-known major resistance QTL *Fhb1* with the strongest FHB resistance (Cuthbert *et al*. 2006; Rawat *et al*. 2016). Since the use of Sumai3 in breeding is still unsuccessful (Chen *et al*. 2012), breeders appreciate other germplasm of resistance with some improved and important traits. Previous studies showed that L699 had a strong FHB resistance, which was similar to those of Sumai3 and Wangshuibai (Zhang *et al*. 2011; Liu *et al*. 2015; Yang *et al*. 2016). However, it does not meet the requirement for FHB resistance. The three analysed lines showed middle FHB resistance, higher spike productivity and lower PFDK in the field, especially in L958, despite their FHB resistance not being as strong as in L699. Furthermore, the three genotypes exhibited positive agronomic traits such as PH (70~75 cm) and TGW (Table 2). In addition, L962 was found to be resistant to powdery mildew (Shen *et al*. 2015) (Figure 1C) while both L958 and L987 were susceptible to powdery mildew. Regarding the stripe rust resistance, the specific PCR amplification result indicated that L958 and L987 carry the *WCBP1* gene (Figure 1D) for resistance to stripe rust. Therefore, the new germplasms L958, L962 and L987 would play an important role in wheat disease improvement in future, especially in FHB resistance improvement.

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References

Bai G., Shaner G. (2004): Management and resistance in wheat and barley to *Fusarium* head blight. Annual Review of Phytopathology, 42: 135–161.

Bai G.H., Kolb F.L., Shaner G., Domier L.L. (1999): Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology, 89: 343–348.

Cai J., Bai G.H. (2014): Quantitative trait loci for *Fusarium* head blight resistance in Huangcandou × 'Jagger' wheat population. Crop Science, 54: 2520–2528.

Cai X., Chen P.D., Xu S.S., Oliver R.E., Chen X. (2005): Utilization of alien genes to enhance Fusarium head blight resistance in wheat – A review. Euphytica, 142: 309–318.

Chen S.H., Zhang Y., Bie T.D., Gao D.R., Zhang B.Q. (2012): Damage of wheat Fusarium head blight (FHB) epidemics and genetic improvement of wheat for scab resistance in China. Jiangsu Journal of Agriculture Science, 28: 938–942.

Chen X., Faris J.D., Hu J.G., Stack R.W., Adhikari T., Elias E.M., Kianian S.F., Cai X. (2007): Saturation and comparative mapping of a major Fusarium head blight resistance QTL in tetraploid wheat. Molecular Breeding, 19: 113–124.

Chu C., Niu Z., Zhong S., Chao S., Friesen T.L., Halley S., Elias E.M., Dong Y., Faris J.D., Xu S.S. (2011): Identification and molecular mapping of two QTLs with major effects for resistance to Fusarium head blight in wheat. Theoretical and Applied Genetics, 123: 1107–1119.

Cuthbert P.A., Somers D.J., Thomas J.B., Cloutier S., Brulebabel A. (2006): Fine mapping *Fhb1*, a major gene controlling fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics, 112: 1465–1472.

Huang Q., Li X., Chen W.Q., Xiang Z.P., Zhong S.F., Chang Z.J., Zhang M., Zhang H.Y., Tan F.H., Ren Z.L., Luo P.G. (2014): Genetic mapping of a putative *Thinopyrum intermedium*-derived stripe rust resistance gene on wheat chromosome 1B. Theoretical and Applied Genetics, 127: 843–853.

Li X., Liu T.G., Chen W.Q., Zhong S.F., Zhang H.Y., Tang Z.X., Chang Z.J., Wang L., Zhang M., Li L.Q., Rao H.F., Ren Z.L., Luo P.G. (2015): Wheat *WCBP1* encodes a putative cooper-binding protein involved in stripe rust resistance and inhibition of leaf senescence. BMC Plant Biology, 15: 239.

Li X., Xiang Z.P., Chen W.Q., Huang Q.L., Liu T.G., Li Q., Zhong S.F., Zhang M., Guo J.W., Lei L., Luo P.G. (2017): Reevaluation of two quantitative trait loci for type II resistance to fusarium head blight in wheat germplasm PI 672538. Phytopathology, 107: 92–99.

Liu J., Chang Z.J., Zhang X.J., Yan Z.J., Li X., Ji J.Q., Zhang H.X., Guo H.J., Wang J.M. (2013): Putative *Thinopyrum intermedium*-derived stripe rust resistance gene *Yr50* maps on wheat chromosome arm 4BL. Theoretical and Applied Genetics, 126: 265–274.

Liu Z.H., Xu M., Xiang Z.P., Li X., Chen W.Q., Luo P.G. (2015): Registration of the novel wheat lines L658, L693, L696, and L699, which are resistance to Fusarium head blight, stripe rust, and powdery mildew. Journal of Plant Registration, 9: 121–124.

Luo P.G., Hu X.Y., Chang Z.J., Zhang M., Zhang H.Y., Ren Z.L. (2009): A new stripe rust resistance gene transferred from *Thinopyrum intermedium* to hexaploid wheat (*Triticum aestivum* L.). Phytoprotection, 90: 57–63.

Rawat N., Pumphrey M.O., Li S., Zhang X., Tivari V.K., Ando K., Trick H.N., Bockus W.W., Akhunov E., Anderson J.A., Gill B.S. (2016): Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. Nature Genetics, 48: 1576–1580.

Shen X.K., Ma L.X., Zhong S.F., Liu N., Zhang M., Chen W.Q., Zhou Y.L., Li H.J., Chang Z.J., Li X., Bai G.H., Zhang H.Y., Tan F.Q., Ren Z.L., Luo P.G. (2015): Identification and genetic mapping of the putative *Thinopyrum intermedium*-derived dominant powdery mildew resistance gene *PmL962* on wheat chromosome arm 2BS. Theoretical and Applied Genetics, 128: 517–528.

Yang S.M., Li X., Chen W.Q., Liu T.G., Zhong S.F., Ma L.X., Zhang M., Zhang H.Y., Yu D.L., Luo P.G. (2016): Wheat resistance to Fusarium head blight is associated with changes in photosynthetic parameters. Plant Disease, 100: 847–852.

Yao J.B., Lu W.Z. (2000): Progress on breeding for wheat scab resistance in China. Jiangsu Journal of Agriculture Science, 16: 242–248.

Zhang L., Chang Z.J., Li X., Zhang H.Y., Ren Z.L., Luo P.G. (2011): Screen and identification of wheat new resistant germplasm to Fusarium head blight. Acta Phytophylacica Sinica, 38: 569–570.

Zhao Z.H., Sun H.G., Song W., Lu M., Huang J., Wu L.F., Wang X.M., Li H.J. (2013): Genetic analysis and detection of the gene *MlLX99* on chromosome 2BL conferring resistance to powdery mildew in the wheat cultivar Liangxing 99. Theoretical and Applied Genetics, 126: 3081–3089.

Zwart R.S., Muylle H., Bockstaele E.V., Ruiz I.R. (2008): Evaluation of genetic diversity of Fusarium head blight resistance in European winter wheat. Theoretical and Applied Genetics, 117: 813–828.

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