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SHORT COMMUNICATION

Wheat germplasm development by gene pyramiding for resistance to race TTKSK of stem rust

Xiaojie Jin, Dawn Chi, Danielle Wolfe, Colin Hiebert, Tom Fetch, Wenguang Cao, Allen Xue, Gavin Humphreys, and George Fedak

Abstract: To develop wheat germplasm with resistance to race TTKSK of stem rust, five stem rust resistance genes were used to produce pyramids with up to 4 *Sr* genes of various combinations by marker-assisted selection (MAS) and doubled haploid (DH) technology. Thirteen desired pyramids with 2 to 4 of the *Sr* genes were recovered, and wheat cultivar Hoffman was improved by stacking *Sr35* and *Sr36*. TTKSK resistance test showed that pyramided lines with three or four genes plus improved Hoffman with *Sr35*+*Sr36* were all immune to TTKSK. This type of germplasm will be a useful tool for the production of cultivars with long-term sustainability.

Key words: Ug99, gene pyramiding, doubled haploids, MAS.

Résumé : Pour obtenir un plasma germinal résistant à la race TTKSK de la rouille de la tige, les auteurs ont appliqué la technique de sélection assistée par marqueur et celle de la double haploïdie à cinq gènes de résistance. L'idée était de créer des pyramides comprenant jusqu'à quatre gènes Sr combinés de diverses façons. Treize pyramides comportant deux à quatre gènes Sr ont été récupérées et le cultivar de blé Hoffman a été amélioré par empilement des gènes Sr35 et Sr36. L'épreuve de résistance à la race TTKSK indique que les lignées pyramidales à trois ou quatre gènes améliorent la variété Hoffman et que les cultivars portant les gènes Sr35+Sr36 résistent tous à cette race. Un tel plasma germinal s'avérera utile pour la production de cultivars très pérennes. [Traduit par la Rédaction]

Mots-clés : Ug99, pyramide génétique, double haploïdie, sélection assistée par marqueur.

Introduction

Stem rust of wheat (*Triticum aestivum* L.), caused by the fungus *Puccinia graminis* Pers.: Pers. f. sp. *tritici* Eriks. & E. Henn (*Pgt*) has historically been one of the most destructive diseases of wheat worldwide. Over the past several decades, stem rust has been under effective control worldwide by widespread use of resistant cultivars; however, recently most of the commonly used stem rust resistance genes were rendered ineffective against a new *Pgt* race, TTKSK, and its variants (Singh et al. 2011).

TTKSK (informally known as Ug99) with virulence to *Sr31* was first detected in Uganda in 1998 (Pretorius et al. 2000). Since then, the Ug99 race group has been evolving rapidly to overcome other resistance genes. To date, a total 13 races belonging to the Ug99 lineage have been identified and spread to 13 countries of East Africa and the Middle East. Thus, stem rust has returned as a major threat to global wheat production (Bhavani et al. 2019).

At present, most Ug99 resistant genes are originated from wild relatives of wheat (Singh et al. 2011). *Sr35* transferred into hexaploid wheat from *Triticum monococcum* L., is effective against the TTKSK race and its variants (Zhang et al. 2010; Singh et al. 2011). *Sr36* was derived from *Triticum timopheevi* (Zhuk.) Zhuk. and has been deployed in wheat cultivars globally (Tsilo et al. 2008; Jin et al. 2009). It is not only effective against many prevalent *Pgt* pathotypes but is also effective against all

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races of the Ug99 race group except TTTSK (Jin et al. 2009; Chemayek et al. 2017). Therefore, it is one of the best available sources of resistance to Ug99 and could be used effectively as a component for Ug99 resistance breeding. *Sr33* and *Sr43* derived from *Aegilops tauschii* Coss. and *Thinopyrum ponticum* (Podp.) Z.-W. Liu & R.R.-C. Wang, respectively, are among the genes effective against Ug99 races (Sambasivam et al. 2008; Niu et al. 2014). The *SrCad* gene present in the Canadian cultivar AC Cadillac confers moderate resistance to Ug99 and a high level of resistance when combined with *Lr34* (Hiebert et al. 2011).

Pyramiding of multiple effective *Sr* genes in one wheat background is an effective strategy to enhance resistance durability to stem rust. Tools such as molecular markers and doubled haploid (DH) are now readily available to facilitate the pyramiding process (Mago et al. 2011). In the present study, DH technology plus linked markers were used to manipulate five stem rust resistance genes to produce pyramids of up to four *Sr* genes of various combinations.

Materials and Methods

Plant material and DH plant production

Five wheat cultivars/lines, AC Cadillac (*SrCad*), Lang (*Sr36*), RL5405 (*Sr33*), RL6099 (*Sr35*), and RWG34 (*Sr43*) were used as resistant parents to produce two complex crosses: (AC Cadillac/Lang)//(RWG34/RL5405) and RWG34/RL5405//RL6099. Another backcross population, (Hoffman*2/RL6099)//(Hoffman*2/Lang), was produced to improve Hoffman's stem rust resistance.

The PCR-selected F_1 hybrid plants of the above three combinations were used for producing DH populations according to established protocols (Fedak et al. 1997).

DNA extraction and PCR screen

Genomic DNA was extracted from young leaves using the CTAB method. Previously reported PCR markers for these stem rust resistance genes were used for analysis. Markers cfd15, cfa2040 and FSD_RSA were used for *Sr33*, *Sr43*, and *SrCad*, respectively (Sambasivam et al. 2008; Hiebert et al. 2011; Niu et al. 2014). Markers barc51, cfa2076, cfa2193, BF485004, BE405552 were used for *Sr35* (Zhang et al. 2010). Markers stm773–2 and gwm319 were used for *Sr36* (Tsilo et al. 2008). PCR conditions for amplification were used as described in previous references and PCR products were separated by 2% agarose gel electrophoresis.

Stem rust assay

The DH lines with two/three/four gene combinations were selected and evaluated for seedling resistance to stem rust. Ten plants of each DH line, parents, and susceptible checks were tested with stem rust race TTKSK (Ug99, isolate SA31) inside a biocontainment level 3 lab at the Morden Research and Development Center, Agriculture and Agri-Food Canada. Seedlings were rated for their infection types 14 d post inoculation. Infection types were recorded using a 0–4 scale where plants with an infection type 0–2^+ were considered resistant and those with 3–4 were considered susceptible (Stakman 1962).

Results

Improvement of Canadian wheat cultivar Hoffman

To produce gene combinations (*Sr35*+*Sr36*) in the Hoffman background, PCR-selected BC_1F_1 plants from the cross Hoffman*2/ RL6099 (*Sr35*) and Hoffman*2/ Lang (*Sr36*) were crossed. Then F_1 plants from the cross combination (Hoffman*2/ RL6099)//(Hoffman*2/ Lang) were screened by PCR for the *Sr35*+*Sr36* genotype. Fifteen F_1 plants with *Sr35*+*Sr36* genotype were used to produce the DH lines that could generate four genotypes, *Sr35*+*Sr36*, *Sr35*, *Sr36*, and null. Finally, 16 of 62 DH lines with genotypes *Sr35*+*Sr36* were obtained (Table 1).

Production of plants with different gene combinations

The F_1 plants from the crosses (AC Cadillac/Lang)//(RWG34/RL5405) and RWG34/RL5405//RL6099 were screened by PCR to confirm the presence of the respective gene combinations. Seven F_1 plants carrying *Sr33*+*Sr36*+*SrCad*+*Sr43* and six F_1 plants carrying *Sr33*+*Sr35*+*Sr43* were used to make DH lines by the wheat × maize pollination method. At the end, 46 DH lines with 14 genotypes and 81 DH lines with 8 genotypes were developed, respectively, from the two crosses (Table 1). In total, 13 desired gene combinations were obtained: *SrCad* + *Sr36* + *Sr43* + *Sr33*; *SrCad* + *Sr36* + *Sr43*; *SrCad* + *Sr36* + *Sr33*; *SrCad* + *Sr43* + *Sr33*; *Sr36* + *Sr43* + *Sr33*; *SrCad* + *Sr36*; *SrCad* + *Sr43*; *Sr36* + *Sr43*; *Sr36* + *Sr33*; *Sr43* + *Sr33*; *Sr35* + *Sr33* + *Sr43*; *Sr35* + *Sr33*; *Sr35* + *Sr43*.

TTKSK resistance

DH lines with the above 14 desired gene combinations were evaluated for TTKSK resistance and the results were summarised as Table 2. The pyramided lines with two *Sr* genes confer a high-level of resistance to TTKSK. All the DH lines with pyramids of three or four *Sr* genes (*SrCad*, *Sr35*, *Sr36*, *Sr43*, and *Sr33*) conferred immunity to TTKSK. In addition, the improved Hoffman with pyramids of *Sr35* and *Sr36* also conferred immunity to TTKSK.

Discussion

Stem rust race TTKSK (Ug99) has unique virulence which renders most current wheat varieties susceptible worldwide. It has virulence to most *Sr* genes of common wheat origin and some *Sr* genes from alien relatives. Development of new germplasm with effective *Sr* genes is one effective way to control Ug99 spread, especially breeding cultivars with multiple gene combinations through marker-assisted selection (MAS) to enhance the resistance durability (Bhavani et al. 2019). Stem rust resistance genes *Sr33*, *Sr35*, *Sr36*, and *Sr43* are all alien *Sr* genes that provide resistance to TTKSK (Singh et al. 2011). *SrCad* present in Canadian cultivar AC Cadillac,

Table 1. Frequencies of gene combinations in douple haploid (DH) lines derived from three hybrid combinations in this study.

Order	Gene combinations	Frequencies in DH lines from cross (AC Cadillac/Lang)//(RWG34/RL5405)	RWG34/RL5405//RL6099	(Hoffman *2/Rl6099)//(Hoffman *2/Lang)
1	*SrCad+Sr36+Sr43+Sr33*	6	—	—
2	*SrCad+Sr36+Sr43*	2	—	—
3	*SrCad+Sr36+Sr33*	4	—	—
4	*SrCad+Sr43+Sr33*	2	—	—
5	*Sr36+Sr43+Sr33*	3	—	—
6	*SrCad+Sr36*	3	—	—
7	*SrCad+Sr43*	1	—	—
8	*SrCad+Sr33*	0	—	—
9	*Sr36+Sr43*	3	—	—
10	*Sr36+Sr33*	3	—	—
11	*Sr43+Sr33*	1	9	—
12	*Sr35+Sr33+Sr43*	—	11	—
13	*Sr35+Sr33*	—	3	—
14	*Sr35+Sr43*	—	8	—
15	*Sr35+Sr36*	—	—	16
16	*Sr33*	7	17	—
17	*Sr35*	—	10	16
18	*Sr36*	7	—	16
19	*SrCad*	2	—	—
20	*Sr43*	0	8	—
21	—	2	15	14
Totals	—	46	81	62

Table 2. TTKSK infection type of different gene combinations.

Order	Gene combinations	TTKSK infection type[a]
1	*SrCad+Sr36+Sr43+Sr33*	0
2	*SrCad+Sr36+Sr43*	0
3	*SrCad+Sr36+Sr33*	0;
4	*SrCad+Sr43+Sr33*	0
5	*Sr36+Sr43+Sr33*	0
6	*SrCad+Sr36*	0;
7	*SrCad+Sr43*	1–1
8	*Sr36+Sr43*	;
9	*Sr36+Sr33*	0;
10	*Sr43+Sr33*	1+
11	*Sr35+Sr33+Sr43*	0
12	*Sr35+Sr33*	0;
13	*Sr35+Sr43*	1–;
14	*Sr35+Sr36*	0
RL5405	*Sr33*	12
Lang	*Sr36*	0
Ac Cadillac	*SrCad*	1–
RWG34	*Sr43*	12+
RL6099	*Sr35*	0;
Hoffman	—	4
McNair 701	—	33+
Little Club	—	34

[a] According to Stakman 1962 scale.

confers the seedling resistance to Ug99 in Canadian wheat cultivars (Hiebert et al. 2011). In the present study, the above five stem rust resistance genes were used to produce pyramids of up to four genes of various combinations by using MAS and DH technology. And DH lines with pyramids of *SrCad* + *Sr36* + *Sr43* + *Sr33*, *SrCad* + *Sr36* + *Sr43*, *SrCad* + *Sr36* + *Sr33*, *SrCad* + *Sr43* + *Sr33*, *Sr36* + *Sr43* + *Sr33*, and *Sr35* +*Sr33* + *Sr43* were extremely effective against TTKSK (Table 2). These results suggest that pyramiding multiple resistance genes into a single line is an effective way to produce wheat germplasm with high resistance.

Hoffman is a Canadian commercial wheat cultivar with many good traits, especially high yield; however, it is susceptible to TTKSK and related races. To enhance its stem rust resistance, we have used a MAS-based backcross breeding strategy to stack two *Sr* genes (*Sr35* and *Sr36*) in Hoffman. Although *Sr36* is ineffective against one race of Ug99 lineage TTTSK, it is still effective against Ug99 and its other variants (Jin et al. 2009; Bhavani et al. 2019). Moreover, *Sr36* is effective against other prevailing *Pgt* pathotypes (Chemayek et al. 2017). Finally, three improved wheat lines Hoffman (*Sr35* +*Sr36*), Hoffman (*Sr35*) and Hoffman (*Sr36*) were obtained (Table 1). The TTKSK resistance test result showed that pyramided lines with genotype *Sr35*+*Sr36* had immunity to TTKSK, which indicated enhanced resistance compared with the parent 'Hoffman' (Table 2). Therefore, disease resistance of wheat cultivars can be improved by combining multiple resistance genes through a MAS-based backcross breeding strategy.

It is virtually impossible to produce a large stack of disease resistance genes by phenotyping alone. This study has shown, as have others, that it is feasible to produce pyramids with a combination of up to four *Sr* genes by selecting out of reasonable-sized DH populations (Mago et al. 2011). The pyramids produced in this study all proved to be highly resistant following inoculation with TTKSK and can be used as the donors of multiple *Sr* genes (Liu et al. 2020). As a result, the pyramided lines with three or four *Sr* genes and improved Hoffman with *Sr35* and *Sr36* can be used for future wheat breeding. New molecular tools have been developed for the more efficient detection, identification and mapping of unique alien disease resistance genes. This increasing supply of disease resistance genes will increase the scope of gene pyramiding by increasing the potential for producing gene pyramids of increasing complexity.

Conflict of Interest

The authors declare no conflict of interest.

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References

Bhavani, S., Hodson, D. P., Huerta-Espino, J., Randhawa, M. S., and Singh, R. P. 2019. Progress in breeding for resistance to Ug99 and other races of the stem rust fungus in CIMMYT wheat germplasm. Front. Agric. Sci. Eng. **6**: 210–224. doi:10.15302/J-FASE-2019268.

Chemayek, B., Bansal, U. K., Qureshi, N., Zhang, P., Wagoire, W. W., and Bariana, H. S. 2017. Tight repulsion linkage between *Sr36* and *Sr39* was revealed by genetic, cytogenetic and molecular analyses. Theor. Appl. Genet. **130**: 587–595. doi:10.1007/s00122-016-2837-5.

Fedak, G., Burvill, M., and Voldeng, H. 1997. A comparison of anther culture and maize pollination for haploid production in wheat. J. Appl. Genet. **38**: 407–414.

Hiebert, C. W., Fetch, T. G., Zegeye, T., Thomas, J. B., Somers, D. J., Humphreys, D. G., et al. 2011. Genetics and mapping of seedling resistance to Ug99 stem rust in Canadian wheat cultivars 'Peace' and 'AC Cadillac'. Theor. Appl. Genet. **122**: 143–149. doi:10.1007/s00122-010-1430-6.

Jin, Y., Szabo, L. J., Rouse, M. N., Fetch, T., Pretorius, Z. A., Wanyera, R., and Njau, P. 2009. Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. Plant Disease, **93**: 367–370. doi:10.1094/PDIS-93-4-0367. PMID:30764215.

Liu, T., Fedak, G., Zhang, L., Zhou, R., Chi, D., Fetch, T., et al. 2020. Molecular marker based design for breeding wheat lines with multiple resistance and superior quality. Plant Disease, **104**: 2658–2664. doi:10.1094/PDIS-02-20-0420-RE. PMID:32749944.

Mago, R., Lawrence, G. J., and Ellis, J. G. 2011. The application DNA marker and doubled-haploid technology for stacking multiple stem rust resistance genes in wheat. Mol. Breed. **27**: 329–335. doi:10.1007/s11032-010-9434-0.

Niu, Z., Klindworth, D. L., Yu, G., Friesen, T. L., Chao, S., Jin, Y., et al. 2014. Development and characterization of wheat lines carrying stem rust resistance gene *Sr43* derived from *Thinopyrum ponticum*. Theor. Appl. Genet. **127**: 969–980. doi:10.1007/s00122-014-2272-4.

Pretorius, Z. A., Singh, R. P., Wagoire, W. W., and Payne, T.S. 2000. Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Disease **84**: 203. doi:10.1094/PDIS.2000.84.2.203B. PMID:30841334.

Sambasivam, P. K., Bansal, U. K., Hayden, M. J., Dvorak, J., Lagudah, E. S., and Bariana, H. S. 2008. Identification of markers linked with stem rust resistance genes *Sr33* and *Sr45*. pages 351–353 in R. Appels, R. Eastwood, E. Lagudah, P. Langridge, M. Mackay, L. McIntye, P. Sharp, eds. Proc. 11th International Wheat Genetics Symposium. Sydney: Sydney University Press.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., et al. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu. Rev. Phytopathol. **49**: 465–481. doi:10.1146/annurev-phyto-072910-095423.

Stakman, E. C. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. Page 53 in E.C. Stakman, D.M. Stewart, W.Q. Loegering, eds. USDA Agricultural Research Service E-617. United States Government Printing Office, Washington.

Tsilo, T. J., Jin, Y., and Anderson, J. A. 2008. Diagnostic microsatellite markers for the detection of stem rust resistance gene *Sr36* in diverse genetic backgrounds of wheat. Crop Sci. **48**: 253–261. doi:10.2135/cropsci2007.04.0204.

Zhang, W., Olson, E., Saintenac, C., Rouse, M., Abate, Z., Jin, Y., et al. 2010. Genetic maps of stem rust resistance gene *Sr35* in diploid and hexaploid wheat. Crop Sci. **50**: 2464–2474. doi:10.2135/cropsci2010.04.0202.