Development of the panel of SSR markers to estimate the level of genetic diversity among the Siberian stem rust population

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Abstract: *Puccinia graminis* f. sp. *tritici* is the causal agent of stem rust disease in wheat. The rust fungus has caused devastating epidemics of disease throughout history and is currently a potential threat to wheat production in some regions of Western Siberia. The samples were analyzed using 14 polymorphic simple sequence repeat (SSR) markers. The panels of specific SSR markers were suggested to distinguish the samples from different regions and single pustule isolates.

Key words: SSR markers; Stem Rust; Western Siberia.

1. Introduction

The genetic diversity of *Puccinia graminis* is extremely high. Within the species, there are several special forms (f. sp) virulent to different cereals, and there is a differentiation of *P. graminis* f. sp. *tritici* (Pgt) determined by the resistance of wheat varieties. Single sequence repeat (SSR) markers are developed to target highly unstable parts within an organism's genome. They present high levels of polymorphism due to high mutation rates e. g. caused by slippage during DNA replication. The advantage of using SSR markers in a dikaryotic organism like *P. graminis* is that they are co-dominant and alleles from both nuclei can be detected. By using SSR markers, many samples can be processed and differences within one species or closely related species may be detected. Tri-nucleotide repeat SSR markers have been developed for Pgt and have been shown to be useful in distinguishing between race groups (Zhong et al., 2009). The objective of this study was to develop a panel of SSR markers to estimate the level of genetic diversity among the Siberian stem rust population.

2. Materials and methods

Sample collection and storage. Infected stems of the susceptible wheat cv. 'Chernyava 13' were collected in the Novosibirsk region (test fields of ICG SB RAS and test fields of SibRIPPB – Branch of ICG SB RAS) and in the Omsk region (Omsk State Agrarian University) in 2018. Single pustule isolates of Pgt from heterogeneous samples were multiplied and stored in a freezer at –80 °C at the ICG SB RAS.

SSR marker analysis. Fourteen of the SSR markers used in international rust laboratories (Zhong 2009; Berlin 2012, 2017) were validated for Russian Pgt isolates. Genomic DNA was extracted from roughly 40 mg of tissue from germinated or ungerminated urediniospores, teliospores and aeciospores of stem rust fungus. Spore material was pulverized by using liquid nitrogen. DNA isolation directly from urediniospores was carried out according to the method described by Justesen et al. (2002), and DNA extraction from infected plant material, according to the method described by Plaschke et al. (2002).

The PCR reaction was performed in a volume of 13 µl. The reaction mixture contained of 30 ng DNA, 1.3 nmol of the M13-tailed forward and 13 nmol of the reverse primers, 0.54 mM of each dNTP, 1 U/µl of HP-Taq DNA polymerase and buffer (67 mM Tris-HCl pH 8.8; 18 mM; 1.7 mM; 0.01 % Tween 20), 30 pmol of each the M13-tailed primer labeled with FAM.

We used touchdown PCR to increase specificity and reduce background amplification, which is essential to correct allele calling and critical when pooling markers. PCR conditions were as follows: initial denaturation at 95 °C followed by 12 cycles (ramp annealing temperature 0.5 gr/cycle) of denaturation for 30 s at 95 °C, annealing for 1 min 30 s at 63 °C and extension for 30 s at 72 °C; and initial denaturation at 95 °C, followed by 23 cycles of denaturation for 30 s at 95 °C, annealing for 1 min 30 s at 57 °C and extension for 30 s at 72 °C; and a final extension for 10 min of 72 °C. The length of the amplicons was determined using an ABI 3130XL Genetic Analyzer (SB RAS Genomics Core Facility, Novosibirsk, Russia) and was scored using the Peak Scanner Software v1.0 (Applied Biosystems).

3. Results and discussion

To develop the panel of SSR markers effective for use on the Siberian population of stem rust, it was important to find out about the marker resolution.

The SSR-based profiles of Pgt samples from the same wheat cv. 'Chernyava 13', but collected in Omsk and Novosibirsk regions, showed difference when the following five markers were used: 109AGGF/R, 227AAGR/F, 293F/R, PgCAA8F1/R1, CAA49F1/R1 (SSR panel#1). These results indicate that (1) two distant Pgt populations exist in these regions of Western Siberia; (2) the SSR markers listed as panel#1 are able to differentiate the geographical samples of the stem rust population.

Four SSR markers (SSR panel#2: 109AGGF/R, CAA98F1/R1, CAA53F1/R1, CAA49F1/R1) were enough to differentiate single-pustule isolates (sp isolates) from the Novosibirsk population. By using SSR markers, stable marker fragments were obtained. By using 109AGGF/R, the following four

Table 1
Primer sequences and characteristics of the 14 SSR makers used among Siberian stem rust

Locus	Primer name	Forward primer (5′–3′)	Revers primer (5′–3′)	Size range, bp	Motif	Reference
Pgestssr021	21AAGF/R	GTTTGCCTGATGATGGATGA	CCGAATGCAGATTACCCTTG	260–271	AAG	Stoxen S., 2012
Pgestrre024	24R/F	TCATCGACCAAGAGCATCAG	TTCGGGAGTGAGTCTCTGCT	166–178	CAT	Stoxen S., 2012
Pgestssr109	109AGGF/R	TCATCGACCAAGAGCATCAG	TTCGGGAGTGAGTCTCTGCT	187–193	CCT	Zhong et al., 2009
Pgestssr173	173R/F-ATC	TCCCTTGACCTTTCTCAACG	TCCATTGAGTTCCATCGTGA	195–213	ATC	Zhong et al., 2009
Pgestssr227	227AAGR/F	CACACGTCTCGAGGAACAGA	CTCGTGGGATGAAGTCCATT	210–231	AAG	Zhong et al., 2009
Pgestssr293	293F/R	GAACCTTGGCCTGAGTGCTA	GCAGCCTACAGCAAGAATCC	258–270	GGT	Zhong et al., 2009
Pgestssr341	341AACR/F	GATGTCGCACTCGGTTTCTT	GGCCTTGGTACCCAATTTCT	232–235	TGG	Zhong et al., 2009
Pgestssr353	353F/R	TCGAATCCCAAGGAACAGAG	ACGTCTTGGGTTTCTGTGGA	253–259	AGG	Zhong et al., 2009
PgtCAA49	CAA49F1/R1	TCGTCTGATCGTGAGAAACG	GACGATTGCTGAGGATTGCT	128–164	CAA	Stoxen S., 2012
PgtCAA53	CAA53F1/R1	AGGCTCAACACCACCCATAC	AGGAGGAGGTGAAGGGGATA	204–240	CAA	Jin et al., 2009
PgtCAA93	CAA93F1/R1	CACTCTCGCCAAACCTCATT	CGCCTGTGATGGTTGTATTG	176–262	CAA	Jin et al., 2009
PgtCAA98	CAA98F1/R1	ATTCGGATGGTCCGTTACTG	CCATCCCACTCAAATCATCC	183–198	CAA	Jin et al., 2009
PgtGAA8	PgCAA8F1/R	GGATGATCGGTCAGTTGGTT	TGTCTGCCTGTCTGTCGAAC	198–228	CAA	Stoxen S., 2012
PgtCAT4.2	PgCAT4F2/R1	CCGTGTCGATCCCAATAATC	AGCAAGGTGAGAATCGGAAA	141–150	CAT	Stoxen S., 2012

marker fragments were obtained: 181, 187, 190, and 193 bp. One of them (190 bp) was polymorphic between single-pustule isolates. By using CAA98F1/R1, the following five marker fragments were obtained: 183, 189, 192, 195, and 198 bp. Two of them (189 and 195 bp) were polymorphic between single-pustule isolates. By using CAA53F1/R1, the following five marker fragments were obtained: 229, 231, 234, 237, and 246 bp. One of them (246 bp) was polymorphic between single-pustule isolates. By using CAA49F1/R1, the following six marker fragments were obtained: 128, 131, 134, 146, 149, and 158 bp. Four of them (128, 146, 149, and 158 bp) were polymorphic between single-pustule isolates.

4. Conclusions

Validation of 14 SSR markers on the Siberian stem rust population allowed us to propose two SSR panels efficient for differentiation:

1) SSR panel#1: the geographical samples of the stem rust population (from Omsk and Novosibirsk regions);
2) SSR panel#2: single-pustule isolates of Pgt.

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Conflict of interest. The authors declare no conflict of interest.