



# Virulence Phenotypes of Siberian Wheat Stem Rust Population in 2017–2018

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Management of wheat stem rust in Western Siberia has gained importance since the first outbreaks in 2007–2010 and 2016. The race composition and virulence patterns were investigated for the enlarged *Puccinia graminis* f. sp. *tritici* (*Pgt*) samples collected in three neighboring regions Omsk, Novosibirsk, and Altai during 2017–2018. Most of *Pgt* isolates were identified as virulent to wheat lines with genes Sr5, Sr9a, Sr10, Sr38, SrMcN, and avirulent to Sr24, Sr31. Differentiation ability of genes Sr6, Sr7b, Sr8a, Sr9b, Sr9d, Sr9g, Sr9e, Sr11, Sr17, Sr21, Sr30, Sr36, and SrTmp to distinguish between the regional populations was established. A total of 33 virulence phenotypes or races were detected among 115 *Pgt* isolates tested. Based on virulence phenotypes, two different *Pgt* subpopulations were identified in the Altai and Omsk regions likely originating from asexual and sexual cycles, respectively. The Novosibirsk pathogen population seems to be a mixture of isolates originated from both neighboring regions with virulence phenotypes that developed in the west, Omsk (TKRPF, QHHSF, and MLLTF), and in the south, Altai (NFMSF, LKCSF, LKMSF, and PKCSF), of Western Siberia.

**Keywords:** black rust, race typing, *Puccinia graminis* f. sp. *tritici*, Sr genes, Western Siberia

## INTRODUCTION

Western Siberia is an extremely important wheat production area in Russia with ~7 million ha of spring wheat, along with increasing areas of winter wheat and some durum wheat. The genetic basis of resistance to stem rust in Siberian germplasm is narrow and limited to several *Sr* genes, including *Sr25*, *Sr31*, *Sr36*, *Sr6Ai*, *Sr6Ai#2*, and some unknown major genes (Shamanin et al., 2016). The devastating disease caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) was not considered economically important in Western Siberia until the first reports of stem rust outbreak in 2007–2010 (Shamanin et al., 2015; Sochalova and Lihenko, 2015). In the Omsk region, southwest of Western Siberia, wheat production was affected by heavy epidemic in 2015; yield loss was estimated at more than 2 million tons. Understanding population structure, virulence diversity, and distribution of *Pgt* in the Omsk and neighboring regions has been essential for effective disease management based on resistance genes. Integrated analysis of pathogen virulence in Western Siberia has just been launched. In 2017, field trip for assessing the stem rust situation was conducted, and the samples of stem rust were collected for determining virulence phenotypes at the Global Rust Reference Center (GRRC) (GRRC Report: Samples of Stem Rust Infected Wheat From Russia, 2017). Significant race diversity coupled with high virulence of local pathogen was revealed in the samples from Omsk and Altai (Shamanin et al., 2020). Further study of virulence dynamics in the vast

territory of Western Siberia was highlighted as the main result of international screening (Hodson et al., 2017). Moreover, the global tracking of aggressive races Ug99 and its variants spreading across the continents makes the race surveys highly actual at every location of wheat crops (Pretorius et al., 2000; Olivera et al., 2015; Bhattacharya, 2017). The objective of our study was to investigate the race composition and virulence patterns of the enlarged *Pgt* samples collected in three neighboring regions Omsk, Novosibirsk, and Altai during 2017–2018.

## MATERIALS AND METHODS

In 2017–2018, 77 infected stems of susceptible cultivars of bread wheat (cvs. Chernyava 13, Khakasskaya, Duet) with urediniospores of *Pgt* were sampled in the neighboring regions Omsk, Novosibirsk, and Altai. Additionally, 30 samples of infected leaves with aeciospores of *Pgt*, were collected from barberry, the alternative host of stem rust, in Omsk 2018 (Figure 1). Bulk samples were stored in the automatic desiccator

at dry cold conditions. After recovering on susceptible wheat cultivars Khakasskaya and Morocco, a total of 115 single pustule *Pgt* isolates were obtained from 107 bulk field samples. In 2017, 51 single pustule isolates were obtained from 39 samples and tested at the GRRC in Denmark. The rest 64 single pustule isolates were obtained from 68 samples and analyzed at the Institute of Cytology and Genetics.

Single pustule isolates were used for determining virulence phenotypes based on a set of 20 North American wheat differential lines, containing *Sr* genes: *Sr5* (ISr5-Ra), *Sr21* (CnS\_ *Triticum monoc.* Deriv.), *Sr9e* (Vernstein), *Sr7b* (ISr7b-Ra), *Sr11* (ISr11-Ra), *Sr6* (ISr6a-Ra), *Sr8a* (ISr8a-Ra), *Sr9g* (CnSr9g), *Sr36* (W2691SrTt-1), *Sr9b* (W2691Sr9b), *Sr30* (BtSr30Wst), *Sr17+13* (Combination VII), *Sr9a* (ISr9a-Ra), *Sr9d* (ISr9d-Ra), *Sr10* (W2691Sr10), *SrTmp* (CnsSrTmp), *Sr24* (LcSr24Ag), *Sr31* (Benno Sr31/6\*LMPG), *Sr38* (VPM-1), and *SrMcN* (McNair 701). According to the international protocols of virulence analysis, seedling plants were used (Roelfs et al., 1992). Each of 115 single pustule isolates was tested at least twice on the set of differentials with the susceptible checks



(cultivars Khakasskaya and Morocco). The spores were dissolved in Novec 7100 oil and applied with an airbrush on seedlings planted 5 to 10 per pot. Inoculated plants were placed in a dark dew chamber at  $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 24 h and then transferred to a growing room at  $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$  with a photoperiod of 16 h. The entire set of tested plants was covered with a cellophane box to avoid contamination (**Figure 2**). Infection types as described

by Stakman et al. (1962) were assessed 14 to 16 days after inoculation; infection types 0 to 2 and 3 to 4 were interpreted as low (avirulent) and high (virulent), respectively. Races were designated following the North American (hexadecimal) *Pgt* coding system (Roelfs and Martens, 1988; Jin et al., 2008). A total of 33 virulence phenotypes or races were detected among 115 *Pgt* isolates tested.



**FIGURE 2** | Procedures for laboratory testing of spore material, facility of the Institute of Cytology and Genetics (IC&G). **(A)** Spore recovering; **(B)** spore multiplication on susceptible cultivar; **(C,D)** spore collection with the vacuum pump; **(E)** spore inoculation with the airbrush; **(F)** infected wheat seedlings of the differential set covered with cellophane isolating box.

## Data Analysis

Virulence frequencies were calculated for all regional populations for each separate year and for the pool of 2017 and 2018. The relationships of the *P. graminis* f. sp. *tritici* populations were analyzed using the assignment-based approaches. The corresponding Kosman dispersion (*KW*) within and distance (*KB*) between populations with regard to the simple mismatch dissimilarity between virulence patterns were calculated (Kosman, 1996, 2014; Kosman and Leonard, 2007). Differentiation among populations was estimated with the permutation test (1,000 random partitions) for differentiation statistic  $dif_{KW}$  (Equation 1 in Gulyaeva et al., 2020) similar to those of Jost (2008; Equations 9, 11), and in accordance with explanations in Kosman (2014, p. 565). The corresponding calculations were performed with Virulence Analysis Tool (VAT) software (Kosman et al., 2008; Schachtel et al., 2012) and its extension. The structural diversity of a population (effective number of equally distant isolates) was measured by the Hill number of order 1,  ${}^1D(T)$ , based on the matrix of simple mismatch dissimilarities between isolates (Equation 4 in Scheiner et al., 2017). Then the structural variation within a given population (effective number of different isolates) is a function of both the dispersion *KW* and structural diversity  ${}^1D(T)$ , where *k* is the number of isolates in the population:

$${}^1D(T, KW) = 1 + \frac{k-1}{k} \times {}^1D(T) \times KW \quad (1)$$

This estimate is similar to  ${}^qD(TM)$  from Equation 5 for  $q = 1$  and dispersion *M* in Scheiner et al. (2017). The structural variation  ${}^1D(T, KW)$  has a range [1, *k*], whereas its normalized version (corrected Equation 5 in Kosman et al., 2019) ranges between 0 and 1 and thus is more suitable for comparison of populations with different numbers of isolates:

$${}^1nD(T, KW) = \frac{{}^1D(T, KW) - 1}{k - 1} \quad (2)$$

This indicator expresses an extent of variability within the pathogen populations.

NMDS plots with regard to the Kosman distance (*KB*) between the regional collections of *P. graminis* f. sp. *tritici* in 2017 and 2018 were derived using the Mdscale program of the NTSYSpc package, version 2.2 (Exeter Software, Setauket, NY, USA). A UPGMA dendrogram based on the simple mismatch dissimilarity between virulence phenotypes detected in each separate population was derived using the SAHN program of the NTSYSPC package, v. 2.1 (Exeter Software).

## RESULTS

Wheat crop occupies the territory of more than 1.4 million ha in the Omsk region, more than 1 million ha in Novosibirsk region, and ~2.1 million ha in the Altai. Stem rust was observed as a late infection. When sampled, wheat crop was close to maturity or mature. Both years, 2017 and 2018, were non-epidemic for stem rust in Western Siberia. Among the inspected field plots and commercial fields, the disease incidence ranged between 0

and 45% with severity up to 60S. The Novosibirsk region is the central part of Western Siberia, bordered by the Omsk region in the west and the Altai region in the south.

## Virulence Frequencies

Out of 115 *Pgt* isolates tested, 46 isolates originated from Omsk samples, 35 from Novosibirsk samples, and 34 from Altai. The virulence phenotypes of *Pgt* isolates are listed in **Table 1**. During the survey of 2017–2018, no avirulent isolates to *Sr5*, *Sr9a*, *Sr10*, *Sr38*, *SrMcN* resistant genes were revealed in West Siberian population of stem rust. All tested *Pgt* isolates were avirulent to *Sr31*, which was consistent with the field screening of wheat lines *Sr31*(Benno)/6\*LMPG-6 DK42, *Seri 82*, *PBW343*, *Cham 10*, *Bacanora* carrying the rye translocation (Shamanin et al., 2010; Skolotneva et al., 2018). An alarming occurrence of virulence to *Sr24* was recorded among Omsk samples in 2017 (four *Pgt* isolates, **Table 1**) because other samples consisted of 100% avirulent to *Sr24* pathotypes.

The virulence of *Pgt* isolates to *Sr6*, *Sr8a*, *Sr9b*, *Sr9d*, *Sr9g*, *Sr17*, and *Sr36* were high in each geographical sample, with frequencies of 52.0 to 95.7%. Virulence frequency of 100% was detected toward *Sr9d*, *Sr9g*, *Sr17* among the Altai samples (**Table 2**). The virulence of *Pgt* isolates to *Sr9e*, *Sr11*, *Sr30* were low, with maximum frequencies of 44.1, 34.2, and 14.7% in Omsk, respectively. For *Sr30*, no virulent isolates were identified in the Altai samples. Geographic samples varied greatly in virulence for *Sr7b*, *Sr21*, and *SrTmp* (**Figure 3**). While the Omsk samples were the most virulent with frequencies of 91.1, 67.7, and 85.3%, respectively, the Altai samples were low virulent (23.5% of *Pgt* isolates infected differential line *Sr7b*) or even avirulent (toward differential lines *Sr21*, *SrTmp*).

## Virulence Phenotypes

A total of 33 virulence phenotypes or races were detected among 115 *Pgt* isolates tested. No races were identified as common for all three geographical samples. But Novosibirsk samples shared races TKRPF, QHHSE, and MLLTF with Omsk samples and races NFMSE, LKCSF, LKMSE, and PKCSF with the Altai. Among the Omsk and Novosibirsk samples, the most frequently identified race was TKRPF (virulent to *Sr5*, *Sr21*, *Sr9e*, *Sr7b*, *Sr6*, *Sr8a*, *Sr9g*, *Sr36*, *Sr9b*, *Sr17*, *Sr9a*, *Sr10*, *SrTmp*, *Sr38*, and *SrMcN*), which comprised up to 36% of *Pgt* collection obtained during 2017–2018 (**Table 1**). Among the Altai and Novosibirsk samples, races LKCSF and PKCSF were predominant with frequencies of 16 and 11%. Rare phenotypes were more frequent in collections from Omsk region (RRGTE, RKRSP, RFRSE, RFRTF, RCRTF, QFRCE, QHHSE, and SHHSF). In general, virulence to *Sr21* differentiated the races on geographical origin: Q, R, and T races shared by the Omsk and Novosibirsk samples, but L and P races shared by the Altai and Novosibirsk samples. The interesting group of M races (MPLTF, MTNTE, MTLTE, MLNTE, MQNTE, MLLTE, and MQLTF) was identified in the bulk sample from Omsk barberry, which was included in the survey in 2018. They differ in virulence of up to 3 resistance genes (*Sr6*, *Sr8a*, *Sr9g*, *Sr21*, *Sr30*) and look like a family of virulence phenotypes resulting from the sexual process. Race MLLTF was also identified among samples from Novosibirsk wheat in 2018.

**TABLE 1** | Virulent phenotypes of *Puccinia graminis* f. sp. *tritici* isolates identified in Western Siberia 2017–2018.

Location and year of sample (number of isolates)	Race (number of isolates)	Virulent to Sr genes	Avirulent to Sr genes
Novosibirsk, 2017 (31)	LCCSF (1)	5, 9a, 9d, 9g, 10, 17, 38, McN	6, 7b, 8a, 9b, 9e, 11, 21, 24, 30, 31, 36, Tmp
	LCHSF (1)	5, 9a, 9b, 9d, 9g, 10, 17, 38, McN	6, 7b, 8a, 9e, 11, 21, 24, 30, 31, 36, Tmp
	LCRSF (2)	5, 9a, 9b, 9d, 9g, 10, 17, 36, 38, McN	6, 7b, 8a, 9e, 11, 21, 24, 30, 31, Tmp
	LHRSF (2)	5, 6, 9a, 9b, 9d, 9g, 10, 17, 36, 38, McN	7b, 8a, 9e, 11, 21, 24, 30, 31, Tmp
	QCHSF (3)	5, 9a, 9b, 9d, 9g, 10, 17, 21, 38, McN	6, 7b, 8a, 9e, 11, 24, 30, 31, 36, Tmp
	QCRSF (6)	5, 9a, 9b, 9d, 9g, 10, 17, 21, 36, 38, McN	6, 7b, 8a, 9e, 11, 24, 30, 31, Tmp
	LKCSF (2)	5, 6, 8a, 9a, 9d, 9g, 10, 17, 38, McN	7b, 9b, 9e, 11, 21, 24, 30, 31, 36, Tmp
	MCMSF (1)	5, 7b, 9a, 9d, 9g, 10, 17, 36, 38, McN	6, 8a, 9b, 9e, 11, 21, 24, 30, 31, Tmp
	NFMSF (2)	5, 8a, 9a, 9d, 9e, 9g, 10, 17, 36, 38, McN	6, 7b, 9b, 11, 21, 24, 30, 31, Tmp
	QHHSF (1)	5, 6, 9a, 9b, 9d, 9g, 10, 17, 21, 38, McN	7b, 8a, 9e, 11, 24, 30, 31, 36, Tmp
	RFPTF (2)	5, 7b, 8a, 9a, 9d, 9g, 10, 17, 21, 30, 36, 38, McN, Tmp	6, 9b, 9e, 11, 24, 31
TKRPF (8)	5, 6, 7b, 8a, 9a, 9b, 9e, 9g, 10, 17, 21, 36, 38, McN, Tmp	9d, 11, 24, 30, 31	
Omsk, 2017 (18)	RRGTF (1)	5, 6, 7b, 9a, 9b, 9d, 9g, 10, 11, 21, 38, McN, Tmp	8a, 9e, 17, 24, 30, 31, 36
	TKRPF (7)	5, 6, 7b, 8a, 9a, 9b, 9e, 9g, 10, 17, 21, 36, 38, McN, Tmp	9d, 11, 24, 30, 31
	RKRSP (1)	5, 6, 7b, 8a, 9a, 9b, 9d, 9g, 10, 17, 21, 24, 36, 38, McN	9e, 11, 30, 31, Tmp
	RFRSF (1)	5, 7b, 8a, 9a, 9b, 9d, 9g, 10, 17, 21, 36, 38, McN	6, 9e, 11, 24, 30, 31, Tmp
	THRTP (2)	5, 6, 7b, 9a, 9b, 9d, 9e, 9g, 10, 17, 21, 24, 36, 38, McN, Tmp	8a, 11, 30, 31
	QHHSF (1)	5, 6, 9a, 9b, 9d, 9g, 10, 17, 21, 38, McN	7b, 8a, 9e, 11, 24, 30, 31, 36, Tmp
	RCRTF (1)	5, 7b, 9a, 9b, 9d, 9g, 10, 17, 21, 36, 38, McN, Tmp	6, 8a, 9e, 11, 24, 30, 31
	SHHSF (1)	5, 6, 9a, 9b, 9d, 9e, 9g, 10, 17, 21, 38, McN	7b, 8a, 11, 24, 30, 31, 36, Tmp
	RCRTP (1)	5, 7b, 9a, 9b, 9d, 9g, 10, 17, 21, 24, 36, 38, McN, Tmp	6, 8a, 9e, 11, 30, 31
	QFRCF (1)	5, 8a, 9a, 9b, 9d, 9g, 10, 17, 21, 36, 38, McN	6, 7b, 9e, 11, 24, 30, 31, Tmp
	RFRTF (1)	5, 7b, 8a, 9a, 9b, 9d, 9g, 10, 17, 21, 36, 38, McN, Tmp	6, 9e, 11, 24, 30, 31
Altai, 2017 (20)	PKCSF (5)	5, 6, 7b, 8a, 9a, 9d, 9e, 9g, 10, 17, 38, McN	9b, 11, 21, 24, 30, 31, 36, Tmp
	LKCSF (3)	5, 6, 8a, 9a, 9d, 9g, 10, 17, 38, McN	7b, 9b, 9e, 11, 21, 24, 30, 31, 36, Tmp
	LKMSF (4)	5, 6, 8a, 9a, 9d, 9g, 10, 17, 36, 38, McN	7b, 9b, 9e, 21, 24, 11, 30, 31, Tmp
	LTMSF (1)	5, 6, 8a, 9a, 9d, 9g, 10, 11, 17, 36, 38, McN	7b, 9b, 9e, 21, 24, 30, 31, Tmp
	NFMSF (4)	5, 8a, 9a, 9d, 9e, 9g, 10, 17, 36, 38, McN	6, 7b, 9b, 11, 21, 24, 30, 31, Tmp
	LHCSF (1)	5, 6, 9a, 9d, 9g, 10, 17, 38, McN	7b, 8a, 9b, 9e, 11, 21, 24, 30, 31, 36, Tmp
	LHMSF (2)	5, 6, 9a, 9d, 9g, 10, 17, 36, 38, McN	7b, 8a, 9b, 9e, 11, 21, 24, 30, 31, Tmp
Novosibirsk, 2018 (16)	TKRPF (7)	5, 6, 7b, 8a, 9a, 9b, 9e, 9g, 10, 17, 21, 36, 38, McN, Tmp	9d, 11, 24, 30, 31
	MLLTF (2)	5, 7b, 9a, 9b, 10, 11, 36, 38, McN, Tmp	6, 8a, 9b, 9e, 9g, 17, 21, 24, 30, 31
	LKCSF (4)	5, 6, 8a, 9a, 9d, 9g, 10, 17, 38, McN	7b, 9b, 9e, 11, 21, 24, 30, 31, 36, Tmp
	LKMSF (2)	5, 6, 8a, 9a, 9d, 9g, 10, 17, 36, 38, McN	7b, 9b, 9e, 11, 21, 24, 30, 31, Tmp
	PKCSF (1)	5, 6, 7b, 8a, 9a, 9d, 9e, 9g, 10, 17, 38, McN	9b, 11, 21, 24, 30, 31, 36, Tmp
Omsk, 2018 (16)	MPLTF (2)	5, 7b, 8a, 9a, 9d, 9g, 10, 11, 36, 38, McN, Tmp	6, 9b, 9e, 17, 21, 24, 30, 31
	MTNTF (1)	5, 6, 7b, 8a, 9a, 9d, 9g, 10, 11, 30, 36, 38, McN, Tmp	9b, 9e, 17, 21, 24, 31
	MTLTF (1)	5, 6, 7b, 8a, 9a, 9d, 9g, 10, 11, 36, 38, McN, Tmp	9b, 9e, 17, 21, 24, 30, 31
	TKRPF (7)	5, 6, 7b, 8a, 9a, 9b, 9e, 9g, 10, 17, 21, 36, 38, McN, Tmp	9d, 11, 24, 30, 31

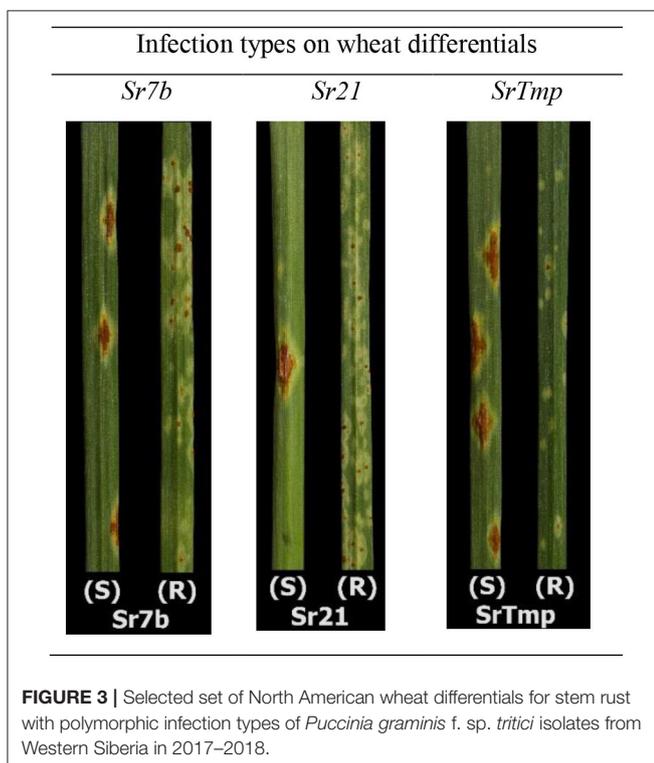
(Continued)

TABLE 1 | Continued

Location and year of sample (number of isolates)	Race (number of isolates)	Virulent to Sr genes	Avirulent to Sr genes
	MLNTF (2)	5, 7b, 9a, 9d, 10, 11, 30, 36, 38, McN, Tmp	6, 8a, 9b, 9e, 9g, 17, 21, 24, 31
	MQNTF (1)	5, 6, 7b, 9a, 9d, 10, 11, 24, 36, 38, McN, Tmp	8a, 9b, 9e, 9g, 17, 21, 24, 31
	MLLTF (1)	5, 7b, 9a, 9d, 10, 11, 36, 38, McN, Tmp	6, 8a, 9b, 9e, 9g, 17, 21, 24, 30, 31
	MQLTF (1)	5, 6, 7b, 9a, 9d, 10, 11, 36, 38, McN, Tmp	8a, 9b, 9e, 9g, 17, 21, 24, 30, 31
Altai, 2018 (14)	LKMSF (5)	5, 6, 8a, 9a, 9d, 9g, 10, 17, 36, 38, McN	7b, 9b, 9e, 11, 21, 24, 30, 31, Tmp
	LKCSF (4)	5, 6, 8a, 9a, 9d, 9g, 10, 17, 38, McN	7b, 9b, 9e, 11, 21, 24, 30, 31, 36, Tmp
	PKCSF (3)	5, 6, 7b, 8a, 9a, 9d, 9e, 9g, 10, 17, 38, McN	9b, 11, 21, 24, 30, 31, 36, Tmp
	LKRSF (2)	5, 6, 8a, 9a, 9b, 9d, 9g, 10, 17, 31, 38, McN	7b, 9e, 11, 21, 24, 30, 31, Tmp
<b>Total: 115 isolates</b>	<b>33 races</b>		

TABLE 2 | Virulence frequency of *Puccinia graminis* f. sp. *tritici* on a selected set of NA differentials with polymorphic reaction type in the regions of Western Siberia in 2017–2018, %.

Regions	Sr21	Sr9e	Sr7b	Sr11	Sr6	Sr8a	Sr9g	Sr36	Sr9b	Sr30	Sr17	Sr9d	SrTmp	Sr24
Novosibirsk	57.4	38.3	44.7	4.3	57.5	59.6	95.7	72.3	57.4	4.3	95.7	68.1	40.4	0
Omsk	67.7	44.1	91.1	34.2	67.7	58.8	79.4	91.1	67.7	14.7	64.7	64.7	85.3	11.7
Altai	0	35.3	23.5	2.9	88.2	91.2	100	52.9	5.9	0	1	1	0	0



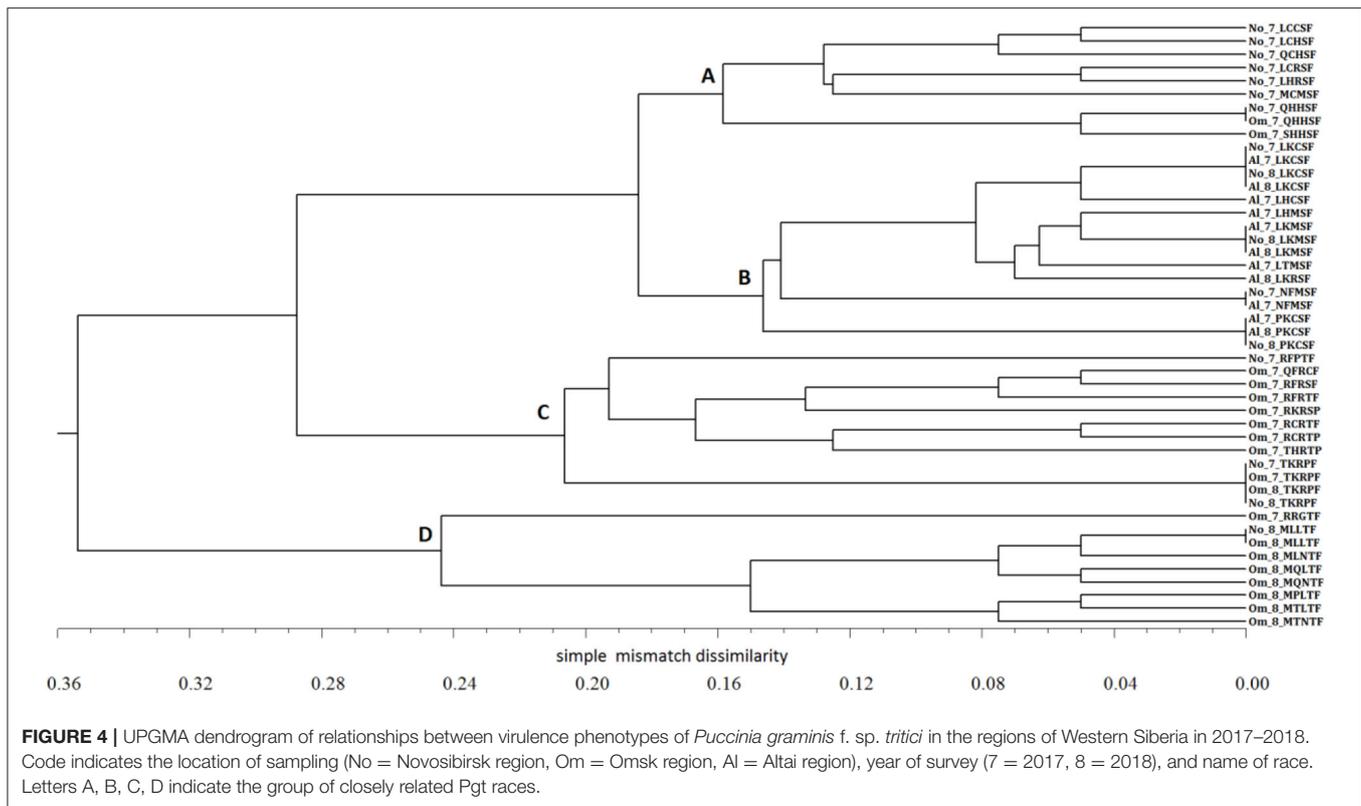
The relationships between virulence phenotypes are shown on the UPGMA dendrogram (Figure 4). Virulence phenotypes were separated into four clusters. Two clusters contain phenotypes shared by samples from Novosibirsk and Omsk only in 2017

(group A) and in both years surveyed (group C). All the Altai phenotypes along with the races NFMSF, LKCSF, LKMSF, and PKCSF from Novosibirsk belong to cluster B. Closely related M-phenotypes detected mainly in Omsk in 2018 are grouped within the distinct cluster D.

### Variability Within and Among Samples

Variability within the geographical samples of *Pgt* changed differently from 2017 to 2018 according to the *KW* dispersion and the normalized number of effectively different isolates  ${}^1nD(T, KW)$  (Table 3). According to both the estimates, in 2018 variability was approximately 40% larger within the Omsk samples, 40% smaller within the Altai samples, and nearly the same in Novosibirsk. Differences in variability were remarkable between the regions with highest values of *KW* dispersion in Novosibirsk (0.316 in 2017 and 0.356 in 2018). In the Altai samples, variability was almost two times smaller than in other regions (0.155 in 2017 and 0.107 in 2018). Similar results were established with the normalized effective number of different isolates  ${}^1nD(T, KW)$ .

Relationships between the regional samples were estimated with the *KB* distances for the virulence profiles of *Pgt* isolates and only for phenotypes without their abundances; it is represented by the corresponding NMDS plots (Figure 5). Clear separation between the Altai and Omsk samples was established, whereas the Novosibirsk samples were in between, but more similar to the Altai ones. These findings were also confirmed by the permutation test for differentiation statistics  $dif_{KW}$ . Significant differentiation was ascertained between the Omsk and Novosibirsk samples (at  $p \geq 0.95$ ) and the Omsk and Altai samples (at  $p \geq 0.99$ ) in both years. Hypothesis of differentiation between the Novosibirsk and Altai samples was rejected.



**TABLE 3** | Variation within samples of *Puccinia graminis* f. sp. *tritici* in the regions of Western Siberia in 2017–2018.

	Altai 2017	Altai 2018	Novosibirsk 2017	Novosibirsk 2018	Omsk 2017	Omsk 2018
No. of isolates	20	14	31	16	18	16
$KW^a$	0.155	0.107	0.316	0.356	0.232	0.344
$^1D(T, KW)^b$	3.645	2.254	9.779	5.482	4.721	5.416
$^1nD(T, KW)^c$	0.139	0.096	0.293	0.299	0.219	0.294

<sup>a</sup>Dispersion of isolates within samples (Kosman and Leonard, 2007; Kosman, 2014).

<sup>b</sup>Effective number of different isolates (Equation 1).

<sup>c</sup>Normalized effective number of different isolates (Equation 2).

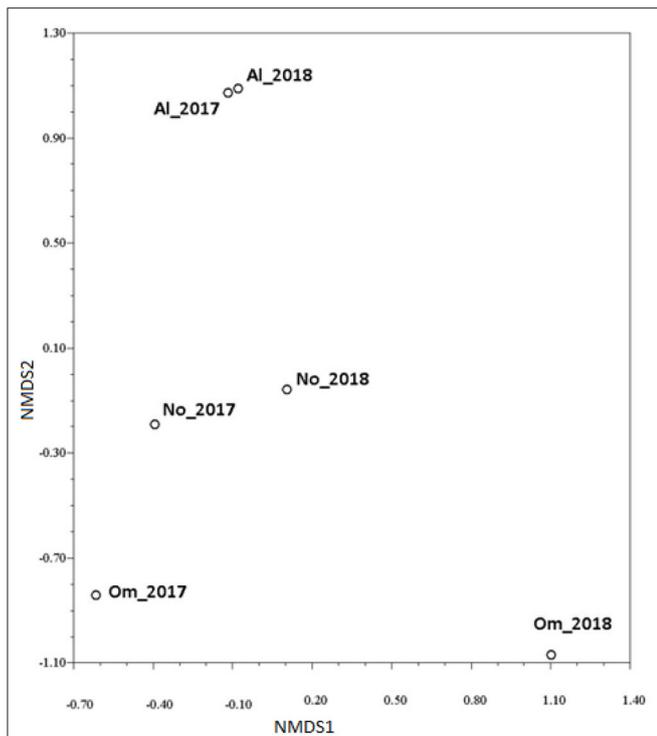
## DISCUSSION

Management of wheat stem rust can be more effective if an origin of initial infection is known. To ascertain whether inoculum of *Pgt* in a specific region is endemic or wind disseminated from neighboring areas, monitoring of the pathogen in Western Siberia was performed in 2017–2018. Based on virulence phenotypes, two absolutely different *Pgt* subpopulations were discovered in the Altai and Omsk regions. The Novosibirsk pathogen population seems to be a mixture of isolates originated from both the neighboring regions virulence phenotypes that arose in the west (TKRPF, QHHSF, and MLLTF) or in the south (NFMSF, LKCSF, LKMSF, and PKCSF) of Western Siberia.

It appears that sexual reproduction to large extent shapes the structure of the *Pgt* population in Omsk region. Indeed, barberry plants are common in this area, and they are susceptible to stem rust (Shamanin et al., 2015). High variability is indirect evidence of existing sexual stage in the pathogen development.

Race composition was characterized by single predominant TKRPF race along with significant number of rare races not detected in recent years elsewhere in Asia and Africa. The group of isolates with similar M- races was sampled from barberry and wheat in Omsk in 2018. Because new races may appear after sexual recombination on alternate host (Jin, 2011), the virulence composition and phenotype structure of *Pgt* population are highly dynamic. In North America, stem rust races 56, 15B, and QCC originated from barberry were responsible for the severe epidemics in the mid-1930s, mid-1950s, and between 1989 and 1993 (Stakman and Rodenhiser, 1958; Martens et al., 1989). Races of *Pgt* with a rich virulence spectrum have been isolated from barberry plants in the central region of Russia from 2000 to 2009 (Skolotneva et al., 2013).

Susceptible barberry plants have not been found in the Altai region yet. Variability within the Altai *Pgt* samples was much lower than in the Omsk region (e.g., 0.107–0.155 vs.



**FIGURE 5 |** Non-metric multidimensional scaling (NMDS) plot of relationships among the regional collections of isolates of *Puccinia graminis* f. sp. *tritici* in Western Siberia in 2017–2018. The collections are encoded according to the sampling location (No = Novosibirsk region, Om = Omsk region, Al = Altai region) and year of survey. The plot was generated based on KB distance between collections with regard to the simple mismatch dissimilarity between isolates (STRESS1 = 0.001).

0.232–0.344 for the *KW* dispersion, **Table 3**) and similar to *P. triticina* asexual population with *KW* values ranging between 0.09 and 0.11 (Gulyaeva et al., 2020). The Altai samples consisted of several closely related rare virulence phenotypes. Therefore, the clonal structure of *Pgt* population in the Altai region can be assumed with a high confidence. Because of the mild short winter, the urediniospores are able to survive on winter wheat, whose area has increased over the past decade in the region. The new lineages in asexual fungal populations could be rarely generated by somatic hybridization, which has been widely discussed as a source of low genetic diversity (Johnson and Newton, 1946; Watson and Luig, 1958; Cotter and Roberts, 1963; Luig and Watson, 1972). Emergence through somatic hybridization was proven for sadly famous Ug99 lineage of races (Li et al., 2019). For the Altai *Pgt* subpopulation, low virulence diversity might be provided by somatic hybridization.

Aecia observed on barberry in the Novosibirsk region explained that the rye special form, *P. graminis* f. sp. *secalis*, segregated predominantly among the sexual offspring (Peresyphkin, 1979; Skolotneva and Salina, 2016). Field and laboratory virulence tests have shown that barberry is not a source of infection for wheat crops in the Novosibirsk

region (Kelbin et al., 2019). Thus, wheat stem rust in the Novosibirsk region is not endemic but carried by wind from both neighboring regions. This makes the virulence pattern of local samples most variable (e.g., 0.316–0.356 for *KW*, **Table 3**) and dependent on breeding events in the neighboring regions. Although Novosibirsk *Pgt* population seems to be a mixture of the Omsk and Altai subpopulations, it was much more similar to the Altai one, which primarily reproduced asexually in the period of our survey.

Our results clearly demonstrate significant differentiation among the regional *Pgt* populations in Western Siberia. The main driving force of the population diversity and geographic differentiation seems to be sexual reproduction of the pathogen fungi in the Omsk region (northwest of the area), whereas only clonal propagation with no evidence of sex is assumed in the Altai region (southeast of the area). These two well-distinguished poles, both geographically and by reproductive ability of *Pgt*, predetermined extreme boundaries of composition of the pathogen populations with a gradual mixing of *Pgt* races along the gradient from southeast to northwest of Western Siberia.

## DATA AVAILABILITY STATEMENT

The data will be made available by request to the corresponding author.

## AUTHOR CONTRIBUTIONS

ESS conceived the research idea and was responsible for sampling the *Pgt* collections in Novosibirsk and Altai, and acquisition and interpretation of virulence data. EK performed data analysis and interpretation of results. MP performed the virulence analysis of Siberian samples in GRRC. VK contributed to acquisition of virulence data. AM was responsible for reviewing the current state of stem rust worldwide and organized the international collaboration between IC&G and GRRC. VS sampled the *Pgt* collections in Omsk. EAS was responsible for general design of the work. ESS and EK prepared the first draft, while all authors contributed to shaping the final version of the manuscript.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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