

## First report of Ug99 Wheat Stem Rust (*Puccinia graminis* f. sp. *tritici*) in South Asia

GRRC lab report and recommendations – 28/03/2024

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**Abstract:** Stem rust Ug99 has been confirmed within samples of bread wheat from two nearby sampling sites in Nov 2023, where spring wheat was grown as off-season fodder crops in an isolated mountain area at Dandunge and Kharidhunga in the Dolakha district in Nepal. Initial SSR genotyping based on incoming specimens without prior recovery in the laboratory confirmed stem rust clade I, which is a unique and characteristic group of Ug99 stem rust. Independent assays of recovered single pustule isolates from both sampling sites confirmed the presence of Ug99 by a unique race termed TTKTT based on differential responses on internationally agreed wheat lines representing a diverse set of stem rust resistance (Sr) genes. The identity of one of the recovered isolates was confirmed by additional and independent genotyping assays revealing the diagnostic pattern of Clade I. Results in this report have been presented and discussed with colleagues in Nepal, who were involved in the rust survey and sampling, and development of overall recommendation at the end of this report.

**Background:** By November 25, 2023, GRRC received samples of rust infected bread wheat from two nearby sites in the Bagmati region of Nepal, which was part of an off-season rust survey. Morphological investigation upon arrival at GRRC suggested infections by two or more wheat rust fungi, including wheat stem rust based on divergence in color, pustule shape and spore morphology (Fig. 1). Recovery of rust isolates from independent leaf specimens from both sites (separate envelopes) confirmed the presence of wheat stem rust (*Puccinia graminis* f.sp. *tritici*) at both sampling sites, whereas other rust fungi were not recovered. Note that stem rust is known to survive longer on dry or dead plant material compared with leaf- and yellow/stripe rust infecting wheat.



Fig 1: Specimens of dried wheat leaves infected by rust fungi sampled in off season wheat fodder crop in the Bagmati region of Nepal, November 2023

## Genetic and phenotypic characterization of samples

Several sample specimens from both sampling sites (envelopes) entered standard genotyping procedures without prior recovery attempts according to Patpour et al. (2022). This resulted in a unique multi-locus genotype, which is diagnostic for Ug99 stem rust in East Africa. Additional samples based on unique specimens with signs of rust infection gave no clear response, which suggests that other rust fungi in addition to stem rust was present, likely leaf rust or yellow/stripe rust.

Multiple infections were recovered from both sampling sites after standard procedures for recovery of incoming samples at GRRRC (Patpour et al. 2022). Independent assays of two recovered spore samples, a single pustule isolate from one sampling site and a bulk sample with no signs of mixed stem rust races from the other sampling site, confirmed the presence of a single race within the Ug99 group, termed TTKTT (Fig 2) based on differential responses on internationally agreed wheat lines representing a diverse set of stem rust resistance (*Sr*) genes (Jin et al. 2008). The set of wheat lines used for the assays are shown in Table 1. SSR genotyping of a recovered material confirmed Ug99.

TTKTT is the most virulent races within the Ug99 group of stem rust, with additional virulence to *SrTmp* and *Sr24* compared to the original Ug99 race termed TTKSK that was first detected in East Africa in 1998 (Patpour et al. 2015; Pretorius et al. 2000).

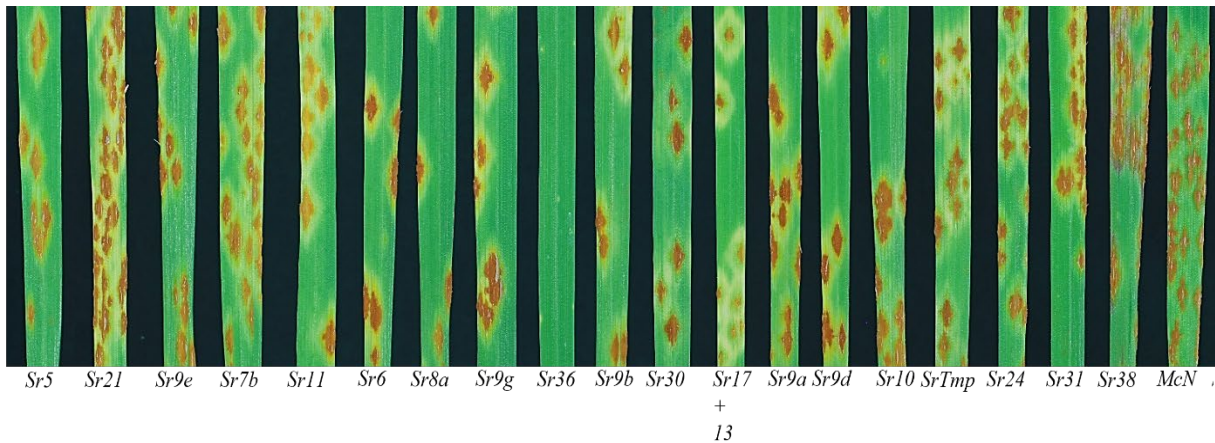


Fig. 2. Infection type responses of stem rust race TTKTT on 20 differential lines of wheat according to Table 1. In this figure, the main *Sr*-gene(s) are indicated for each line.

**Conclusion:** Stem rust Ug99 was represented in both samples submitted to GRRRC from Nepal in November 2023. Genotyping of original dried specimens of incoming samples, and identical outcome of additional tests of recovered isolates has confirmed the validity of the results.

**Recommendations and context:** Stem rust of wheat is a known disease in Nepal and South Asia, but generally less prevalent than leaf rust and yellow rust. The results document that stem rust Ug99 has been detected in Nepal, likely spread by airborne spores from epidemics in East Africa in October-November 2023, although no final conclusion on source areas and dispersal mechanism can be made on the basis of available data at present. Immediate risks of onwards spread from the actual sampling sites in Nepal, November 2023, are considered limited because the wheat (fodder) crops were removed no later than December 2023, followed by the establishment of other crops, e.g., potatoes, which do not support stem rust. However, since wheat rust fungi spreads by wind potentially across very large distances, detailed onwards rust surveillance efforts on wheat in the entire region of South Asia, where wheat is grown, and assays of crop vulnerability to stem rust Ug99 and other races, is highly recommended.

## References

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Table 1: Infection types (ITs) produced on 20 standards stem rust differentials and additional resistance genes to *P. graminis* f.sp. *tritici* isolates collected from Bagmati region of Nepal<sup>a</sup>

Variety/Line	Resistance gene	NP210a/23 (TTKTT)	NP211/23 (TTKTT)
ISr5-RaCI14159	<i>Sr5</i>	3+	3+
Tmonococcum/8*LMPG-6DK13	<i>Sr21</i>	3+	3+
VernsteinPI442914	<i>Sr9e</i>	3+	3+
ISr7b-RaCI14165	<i>Sr7b</i>	3+	3+
Lee/6*LMPG-6DK37	<i>Sr11</i>	3+	3+
ISr6-RaCI14163	<i>Sr6</i>	3+	3+
CI14167/9*LMPG-6DK04	<i>Sr8a</i>	3+	3+
ChineseSpring*7/Marquis2B	<i>Sr9g</i>	3+	3+
W2691SrTt-1CI17385	<i>Sr36</i>	0;	0;1-
Prelude*4/2/Marquis*6/Kenya117A	<i>Sr9b</i>	3	3
SelectionfromWebsterF3:F4#6	<i>Sr30</i>	3	3
Prelude/8*Marquis*2/2/Esp518/9	<i>Sr17+13</i>	2+	22+
ISr9a-RaCI14169	<i>Sr9a</i>	3	3
ISr9d-RaCI14177	<i>Sr9d</i>	3+	3+
W2691Sr10CI17388	<i>Sr10</i>	3+	3+
CsSSrTmp	<i>SrTmp</i>	3	3
LcSr24Ag	<i>Sr24</i>	3	3
Sr31(Benno)/6*LMPG-6DK42	<i>Sr31</i>	3+	3+
VPM1	<i>Sr38</i>	3+	3+
MvNair701	<i>SrMcN</i>	3+	3+

<sup>a</sup> ITs scored on seedling at 16 days post inoculation using a 0-4 scale according to Stakman et al. (1962), where ITs of 0, ;, 1, 2, or combinations were considered as a low, and IT and 3 or higher were considered as a high IT. Minus (-) and plus (+) signs indicated small and large pustules within a class, respectively (Roelfs and Martens 1988).