



Genetic resistance of barley genotypes to Stripe rust (*Puccinia striiformis*) in the western Midhills of Nepal

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ABSTRACT

Stripe rust, caused by *Puccinia striiformis* f. sp. *hordei*, is economically an important disease of barley (*Hordeum vulgare* L.) and causes a significant yield loss in major barley-growing countries, including Nepal, where the growing season is relatively cool and wet. A worldwide collection of 340 barley genotypes was evaluated over three years for stripe rust resistance at the research field of the Agricultural Research Station, Dailekh, western Nepal. In addition, resistant and susceptible checks for stripe rust, 'Bonus' and 'Solu Uwa', were repeated in an augmented block design. When comparing disease severity over three years, significant variation among barley genotypes was observed in disease resistance. Among 342 genotypes, 99 (29%), 120 (35%), and 130 (38%) were resistant to stripe rust in 2016, 2017, and 2018, respectively. A total of 49 genotypes were found to be resistant in all three years. These identified resistant barley genotypes can serve as a source of stripe rust resistance in barley breeding programs in Nepal and other stripe rust-prone regions in South Asia and similar environments.

Keywords: Barley, *Hordeum*, *Puccinia*, Resistance, Stripe rust.

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the major cereal crops worldwide (FAO 2025). The crop is widely used for food, feed, and malting purposes. Barley can be grown under cold, rainfed, saline, alkaline, low-input, and other limiting conditions (Kumar et al 2020). Barley's adaptability to harsh environments allows growers to cultivate it as a major cereal crop in higher hills under rainfed and marginal growing conditions as a staple food. In addition, the crop is cultivated for feed in the mid-hills and plains of Nepal (Pokharel et al 2016). Barley is the 5th most important cereal crop in Nepal. This is an important crop in the high-hill areas of Nepal, such as Karnali, Mustang, Manang, Solukhumbu, etc. The area under barley cultivation and its production have declined annually in the Terai region, while remaining relatively stagnant in the hills and mountains (Kandel et al 2019). The productivity of barley is low (1.39 t ha⁻¹) in Nepal (MoAFE 2026), mainly due to limited barley cultivars, poor-quality seeds, inadequate crop management, and abiotic and biotic stresses (Pokharel et al 2016). Among the biotic stresses, barley rusts and other diseases such as spot blotch, net

blotch, powdery mildew, and smuts reduce the grain yield and quality of barley (Kuldeep et al 2008, Wan et al 2012, Gyawali et al 2017, Gangwar et al 2018, Gurung et al 2018, Verma et al 2018).

Stripe rust disease caused by *Puccinia striiformis* Westend. f. sp. *hordei* Erikss.) (PSH) is one of the devastating diseases of barley, especially in mountain regions and the adjoining plains of Nepal (Gurung et al 2018, Baniya et al 1997, Baniya et al 1992). Stripe rust is a wind-borne disease of cooler climates (2-15°C) and is favored by prolonged leaf wetness (8-10 h), which provides optimal conditions for infection and disease epidemics (Gangwar et al 2018). In Nepal, higher altitudes in the hills (1000-2500 masl) provide cold, moist conditions conducive to infection and disease development (Manandhar et al 2016). A single infection by the fungus can produce millions of wind-borne urediniospores that can disperse over long distances. These urediniospores are capable of multiple infections and consequently cause disease epidemics under favorable environmental conditions (Wan and Chen 2012). Yield losses of up to 66% have been reported in the hilly regions of India (Vaish et al 2011). Under experimental conditions, a 90% yield reduction was recorded for a local barley variety in the Kathmandu Valley (Manandhar et al 2016). Therefore, stripe rust epidemics that cause significant yield losses in hilly regions are expected in Nepal.

The Himalayan and near-Himalayan region is considered a center of diversity for *Puccinia striiformis* f. sp. *tritici*, wheat stripe rust pathogen, due to high recombinant population structure and diversity (Khan et al 2019, Hovmoller et al 2015). Since *Puccinia striiformis* f. sp. *hordai* has a biology like that of *Puccinia striiformis* f. sp. *tritici*, PSH diversity may also be high in the region. The characterization of rust isolates and resistance screening of barley genotypes is significant for an effective breeding program. In the absence of characterized virulent isolates and artificial inoculation facilities for seedling resistance testing in Nepal, germplasm screening across multiple disease hotspots is a major strategy for identifying rust-resistant genotypes (Kumar et al 2016, Gyawali et al 2017). This study aimed to determine whether any barley genotypes collected from the International Center for Agricultural Research in the Dry Areas (ICARDA) are effective against PSH at Dailekh and a similar environment in Nepal.

2. MATERIALS AND METHODS

2.1 Barley genotypes

A total of 342 barley genotypes, comprising 340 diverse germplasm obtained from ICARDA, and two local checks (Bonus and Solu Uwa) from the Nepalese barley breeding program, were evaluated for stripe rust resistance. The Nepalese barley cultivars 'Bonus' (two-rowed, hulled type, and resistant to stripe rust) and 'Solu Uwa' (six-rowed, hull-less type, and susceptible to stripe rust) were developed and released for general cultivation in 1974 and 1990, respectively (NARC 2020). Among the six recommended or improved barley cultivars, only three are currently grown in Nepal (NARC, 2020). In addition, the collection consisted of 280 hulled and 60 hull-less genotypes, and 138 two-row and 202 six-row types, based on row patterns (Amezrou et al 2017).

2.2 Field experiments and phenotyping for stripe rust resistance

All 342 barley genotypes were tested in the research field of the Agriculture Research Station, Dailekh (ARS Dailekh), Western Nepal, a site known for testing rust in wheat and barley. Dailekh (83°58' E, 28°13' N, and 1350 masl) is in the mid-hills of Nepal. The field experiments were conducted during the three consecutive barley growing seasons: 2016,

2017, and 2018. Seeding was done on 7 November 2016, 16 November 2017, and 10 November 2018. The weather data was collected for all three years.

The response of the genotypes to stripe rust was evaluated in a field plot with two rows, each 150 cm long, spaced 25 cm apart. A continuous row of the susceptible spreader, Solu-Uwa, was planted perpendicular to the experimental block on both sides. Each experimental block and the whole experimental area were bordered by the susceptible check/spreader. A total of 17 blocks were made, consisting of 20 genotypes in each block. The checks Bonus and Solu-Uwa were repeated in each block at an interval of 20 test genotypes. Every year, the susceptible check Solu Uwa was planted 15 days before planting the test genotypes in the borders to create natural inoculum pressure and facilitate the spread of the PSH inoculum onto the experimental plots. NPK fertilizers were applied at 80, 30, and 20 kg ha⁻¹. Full doses of P and K were applied as a basal dose, while the seed was shown, and the remaining 40 kg N were applied twice: 20 kg after the first weeding and the remaining 20 kg at the booting stage of the crop. The field was frequently flood-irrigated to keep it moist for optimal crops and rust development. Stripe rust severity on test entries was assessed in the field plots when the susceptible check, Solu Uwa, began developing rust pustules, indicating a susceptible reaction. After that, the test genotypes were scored for stripe rust four times at 10-day intervals each year, and the final recording coincided with the full development of rust in the spreader variety.

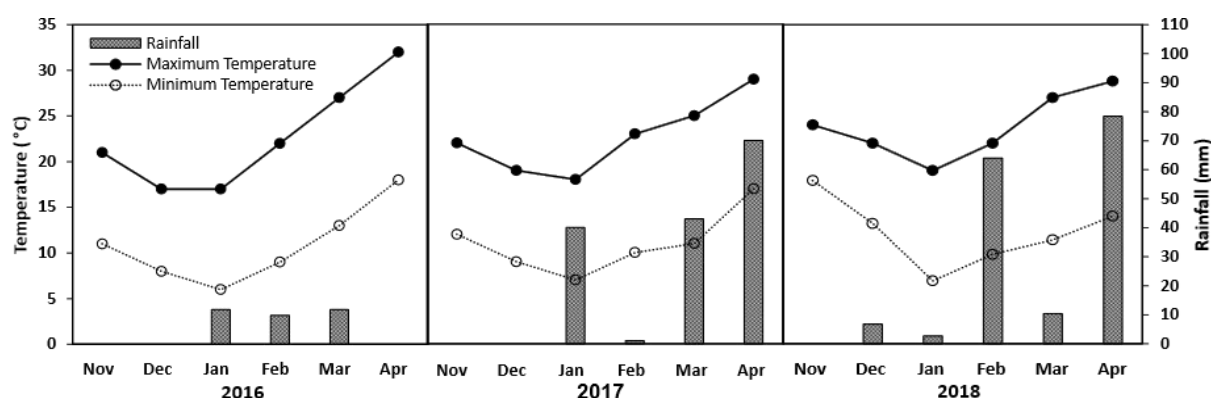


Figure 1. Weather data of the experimental site (ARS Dailekh) during the barley growing seasons of 2016, 2017, and 2018.

Rust development is related to the host plant's development and growth stage (GS), the best time for rust scoring is when the susceptible cultivar expresses a fully susceptible reaction and incidence ([Manandhar et al 2016](#)). Therefore, the ideal time for rust screening is from GS 41 (Booting) to GS 77 (Milky-ripe stage), as described by ([Zadoks et al. 1974](#)). The stripe rust score was determined by the severity (percentage of leaf rust infection) and the disease reaction (resistant, intermediate, or susceptible). Severity was recorded in percentage based on visual observations according to the modified Cobb scale ([Peterson et al 1948](#)) and reactions based on Roelfs et al. (1992) where plant responses were recorded as immune (0) = No visible infection on plants, Resistance (R) = visible chlorosis or necrosis but no uredia present, Moderately Resistance (MR) = small uredia present and surrounded by either chlorotic or necrotic areas, Moderately Susceptible (MS) = MS medium size uredia present and possibly surrounded by chlorotic areas, and Susceptible (S) = large uredia present with ample sporulation, generally with little or no chlorosis and no necrosis, uredia generally coalesced to form lesions. When calculating the resistant or susceptible reaction, the response of each genotype to stripe rust each year and the overall score were determined as follows: Immune (0) = genotype with no disease symptom and clean leaf every year, Resistant (R) =

genotype with no MR, MS, or S reactions but at least R type of disease reaction in any year or every year, Moderately Resistant (MR) = genotype with no MS or S reactions but at least MR type of disease reaction in any year or every year, Moderately Susceptible (MS) = genotype with no S reaction but at least MS type of disease reaction in any year or every year, and Susceptible (S) = at least S type of disease reaction in any year or every year. The area under the disease progress curve (AUDPC) was calculated using the disease severity values described above. The host response data were combined into a single-value coefficient of infection (CI), as described by (Afzal et al 2009). As described by (Manandhar et al 2016), the CI was calculated by multiplying the severity. Constant values 0.0, 0.2, 0.4, 0.8, and 1.0 were used for immune (I), resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) types of disease reactions, respectively. The AUDPC value was calculated using the following formula (Milus and Line 1986).

$$\text{AUDPC} = \sum_{i=1}^n [(CI_{i+1} + CI_i)/2][(t_{i+1} - t_i)]$$

Where, CI_i = Coefficient of infection as defined above on i^{th} days, t_i = time in days at i^{th} observation, and n = total number of observations.

2.3 Statistical analysis

The AUDPC value was used to analyze variance (ANOVA) using ACBD-R - Augmented Complete Block Design in R, Version 2.0 (Rodriguez et al 2016).

RESULTS

Infection types and disease severity varied among barley genotypes. The combined analysis of variance (ANOVA) over three years revealed significant differences among the genotypes ($P < 0.001$), years ($P < 0.001$), and their interactions ($P < 0.001$) for disease severity (Table 1). Similarly, significant differences in AUDPC among genotypes were observed for each of the crop years 2016, 2017, and 2018 (data not shown). In 2016, 2017, and 2018, the mean AUDPC values at ARS Dailekh for Solu Uwa were 836, 924, and 801, respectively, with an overall mean of 854. Overall, 60 genotypes at Dailekh showed higher stripe rust severity than Solu Uwa in all the years.

Table 1. Combined analyses of variance (ANOVA) for stripe rust severity of 342 barley genotypes at ARS Dailekh in the three consecutive growing seasons of 2016, 2017, and 2018.

Source	df	Mean Square	P-value
Genotype (G)	341	749399	<0.001
Year (Y)	2	7996294	<0.001
G×Y	682	99433	<0.001

df = degree of freedom, P-value = probability

When comparing disease severity over three years, significant variation among barley genotypes was observed in disease resistance. Among 342 genotypes, 99 (29%), 120 (35%), and 130 (38%) were resistant to stripe rust in 2016, 2017, and 2018, respectively. A total of 49 genotypes were found to be resistant in all three years. The summary of the disease reaction of barley genotypes is presented in Figure 2, Figure 3, and Table 2.

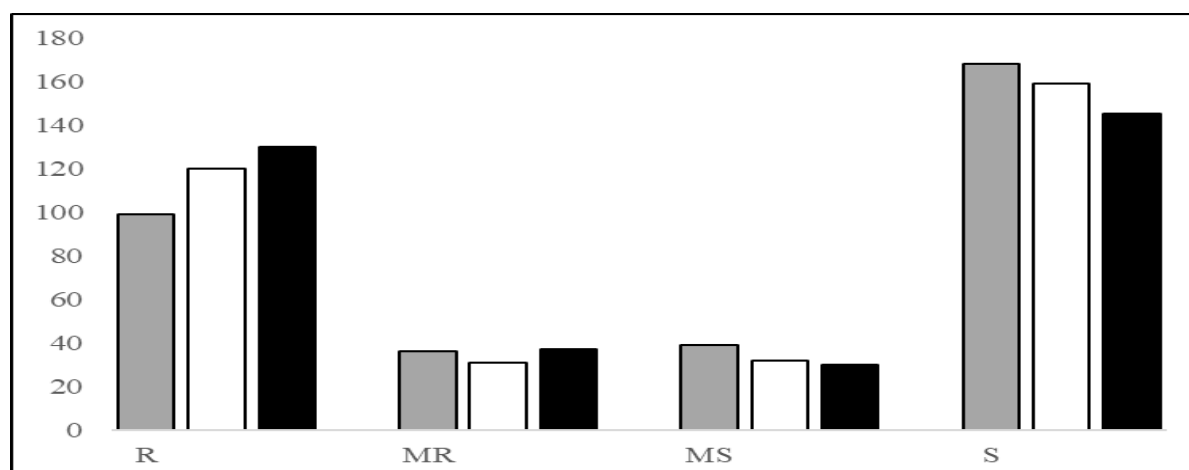


Figure 2. Reaction to stripe rust of 342 barley genotypes at ARS Dailekh, Nepal, during the three consecutive winters of 2016, 2017, and 2018. R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, and S = Susceptible. Likewise, the light gray, white, and black bars indicate the results for 2016, 2017, and 2018, respectively.

The resistant check Bonus was immune (0) and remained disease-free over the three years. Susceptible check Solu Uwa consistently exhibited susceptibility to infection and higher disease severity (Table 2). Disease reaction based on infection types revealed 99 (28.9%) genotypes as R, 36 (10.5%) as MR, 39 (11.4%) genotypes as MS, and 168 (49.1%) genotypes as S in 2016. In 2017, the numbers of genotypes showing R, MR, MS, and S disease reactions were 120 (35%), 31 (9%), 32 (9.3%), and 159 (46.4%), respectively. In 2018, 130 (38%), 37 (10.8%), 30 (8.7%), and 145 (42.4%) genotypes had R-, MR-, MS-, and S-type disease reactions, respectively (Figure 2 and Table 2).

Table 2. Genotypes showing resistance to stripe rust at ARS Dailekh, combined over three years (2016, 2017, and 2018).

Location		Genotypes showing resistance (R) type of disease reaction in each of 2016, 2017, and 2018
ARS (Combined years)	Dailekh over three years)	AM-15, AM-28, AM-30, AM-36, AM-40, AM-56, AM-63, AM-85, AM-90, AM-93, AM-94, AM-99, AM-117, AM-123, AM-126, AM-132, AM-146, AM-180, AM-181, AM-182, AM-186, AM-192, AM-193, AM-194, AM-195, AM-200, AM-202, AM-203, AM-225, AM-226, AM-242, AM-243, AM-248, AM-250, AM-263, AM-264, AM-265, AM-271, AM-273, AM-274, AM-278, AM-279, AM-285, AM-288, AM-297, AM-304, AM-305, AM-311 and Bonus.
		The susceptible check 'Solu Uwa' had a susceptible reaction type with >60S in all years.

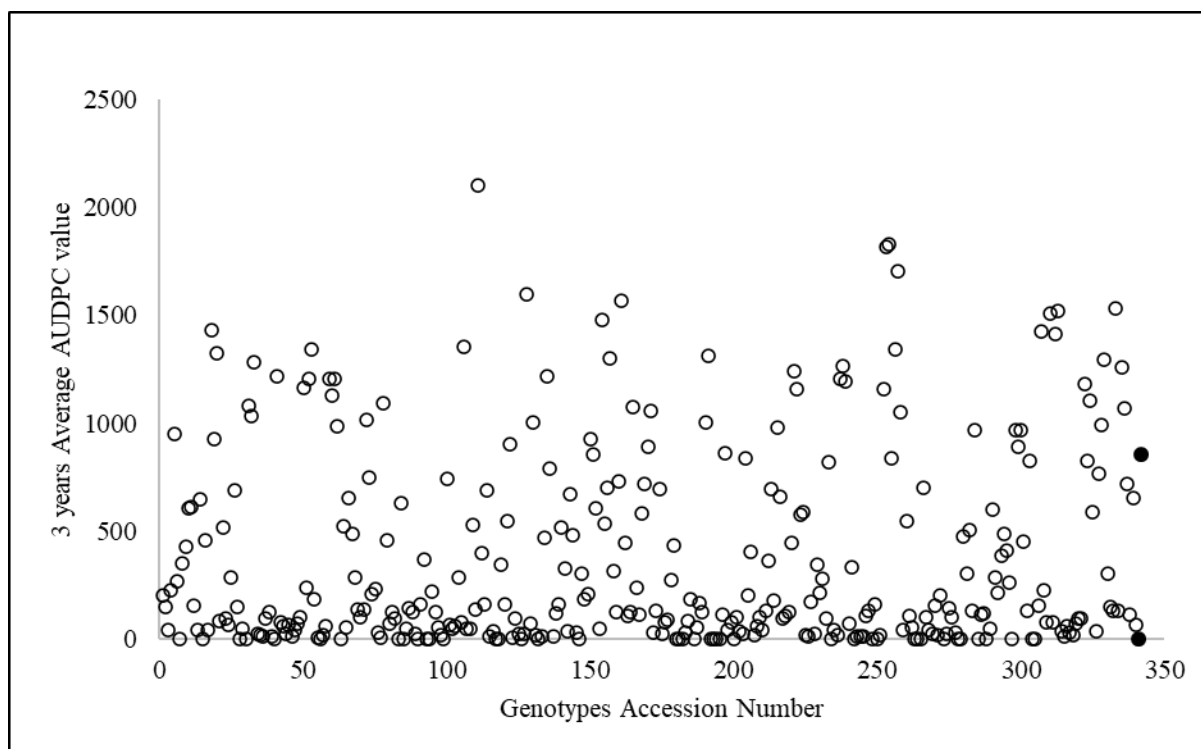


Figure 3. Ranking of the genotypes based on combined disease severity data from three consecutive crop growing seasons. The lower dark point indicates the AUDPC value of the Bonus (the resistance check), and the upper dark point indicates the AUDPC value of Solu Uwa (the susceptible check).

The genotypes shown in Figure 3 are ranked by average performance and stability. The resistant check Bonus and the other stripe rust-resistant genotypes for three years are located at the baseline (i.e., 0 value). Likewise, genotypes that are moderately resistant, moderately susceptible, or susceptible are located far from the baseline (0). All the genotypes shown in Table two are located at the baseline (0 value).

DISCUSSION

The reactions of barley genotypes of diverse origins to stripe rust varied in disease severity and infection types at Dailekh over three consecutive barley-growing seasons (2016-2018). Dailekh is known as a hotspot for stripe rust in Nepal's Himalayan region and is considered a favorable hill site for PSH resistance screening. NARC is continuously testing its barley and wheat varietal trials in Dailekh to develop a stripe rust-resistant variety in Nepal. The natural inoculum and local weather conditions favor stripe rust infection, rust development, and the secondary spread of stripe rust uredospores from the spreader rows to test genotypes. Weather variables recorded over the three consecutive barley-growing seasons at ARS Dailekh further support the site's climatic suitability for stripe rust development (Figure 1). Stripe rust development on the susceptible check, Solu Uwa, was always > 60S at both locations from 2016 to 2018.

In 2016, 2017, and 2018, the susceptible check Solu Uwa had a higher AUDPC. The rust started to appear at ARS Dailekh as usual and persisted there as a disease. The average weather data for three years (November to April) indicated that the climatic conditions were conducive to the disease's occurrence (Figure 1). The weather data further support that this location was favorable for barley stripe rust research. There was ample moisture and favorable temperatures for stripe rust development (Figure 1). In this study, the conducive

environment for stripe rust at the test location and the consistent susceptible disease reactions of the susceptible check, Solu Uwa, indicated the location's usefulness for disease screening under natural epiphytotic conditions.

High stripe rust severity was observed on Solu Uwa in this study in all three barley growing seasons, indicating that stripe rust remains a major production constraint for barley in the Himalayan regions of Nepal. (Upreti 2005) reported that Solu-Uwa was susceptible to stripe rust, resulting in a 30% yield loss in Nepal. The economic importance of barley stripe rust is also documented in similar hilly regions of the Himalayas across India, Pakistan, and Afghanistan (Vaish et al 2011, Gyawali et al 2017, Verma et al 2018). Among foliar diseases, stripe rust had a prevalence of >45% in India (Vaish et al 2011). In this study, 60 genotypes were more susceptible to stripe rust than Solu Uwa (AUDPC = 854), with the highest AUDPC, 2100, observed in AM-111 over a 3-year average.

The genotypes showing immune (no visible infection on plants) and resistance (visible chlorosis or necrosis but no uredia on leaf surfaces) reactions were not screened against specific stripe rust races because the Nepal Agricultural Research Council (NARC) lacks such facilities. Therefore, identifying sources of resistance at hot spots is necessary for the national barley breeding program (Gurung et al 2018, Sharma et al 1994). The Nepalese barley breeding program lacks facilities to screen for PSH-specific race resistance. Therefore, evaluations of barley genotypes in hotspot locations over multiple years are increasingly important for identifying broader resistance to potential PSH races prevalent in the region. The resistant genotypes identified in this study, including Bonus, exhibited resistance under field conditions across all three crop-growing seasons, providing important germplasm to improve stripe rust resistance in Nepal. We argue that these genotypes will likely resist the PSH races prevalent in the country's hotspots and other barley-growing regions, based on disease reactions observed in all seasons. Furthermore, more than 50% of the barley genotypes were classified as MR to MS in this study, suggesting that PSH resistance in these genotypes may be quantitative.

In conclusion, we identified barley genotypes that are PSH-resistant and can be used to breed barley cultivars resistant to stripe rust in favorable environments, such as the Dailekh environment in Nepal and other similar barley-growing regions. Because these barley genotypes were identified through field screening over three years, the resistance to stripe rust they exhibit is expected to be sustainable. Hence, these genotypes can be used for barley breeding programs as additional genetic resources to develop stripe rust-resistant barley cultivars in Nepal and elsewhere in the Himalayan regions.

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Authors' contribution

Bhanu Bhakta Pokharel: Germplasm import, research proposal preparation, carried out field experiments, data collection, data analysis, and manuscript preparation, **Suk Bahadur**

Gurung, Laxman Aryal, and Khem Raj Pant: assisted in field experiments and disease scoring.

Conflict of Interest

The authors declare no conflicts of interest.

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