ORIGINAL RESEARCH ARTICLE

Characterization of Nepalese Barley Gene Pool for Leaf Rust Resistance

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Abstract

Barley (*Hordeum vulagare* L) is the major crop for the people living in the high hills and mountainous region of Nepal. Leaf rust (caused by *Puccinia hordei*) is one of the major production threats for barley cultivation. A lot of variation can be observed on Nepalese barley accessions with respect to leaf rust resistance characteristics. Two hundred and forty one barley accessions were screened for leaf rust resistance characteristics on heading stage at Khumaltar, Lalitpur, Nepal. Among them, one hundred and nine Nepalese barley accessions showing promising for disease resistance were screened using six SSR markers linked to leaf rust resistance genes. Bonus and Local Jau was used as the resistant and susceptible check respectively. Leaf rust resistance genes *Rph1, Rph2, Rph3, Rph7, QBLR-P* and *QTL* on chromosome 5HS were detected on Nepalese barley accessions using respective SSR markers. Eight Nepalese barley accessions showed presence of three and more leaf rust resistant genes. The poor relationship between the field disease resistance and molecular markers linked with specific leaf rust resistance gene proved that Nepalese barley gene pool contains other leaf resistance genes.

Keywords: leaf rust, *Puccinia hordei*, resistant gene, Nepalese barley, Simple Sequence Repeats (SSR)

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Introduction

Barley (*Hordeum vulgare* L.) occupies total area of 29598 hectare and total production 33782 metric ton with average productivity of 1.141 metric ton/ha in Nepal [1]. The maximum area of the crop lies in the mid-western development region. Out of the total barley area, more than 50 % is in the hill region, while 40% is in the mountain region [2]. However, its production and productivity is declining due to diseases and unpredictable climatic condition of the mountain area [3]. Leaf rust (caused by *Puccinia hordei*) is one of the major problematic diseases for barley production in Nepal [4]. Therefore, rust resistant barley varieties are another need of mountain farmers.

Nepal harbours hundreds of the barley landraces. High level of genetic variation can be observed in Nepalese barley [5]. Similarly, a lot of variation was observed among the Jumla collection of Nepalese barley for many yield attributing characters [6]. Many of these landraces possess one or more characteristics for abiotic and biotic stress tolerance [2,3]. Variation on rust resistance characteristics is very important for rust resistance breeding program.

Therefore, use of these germplasm for rust resistance gene pyramiding is highly beneficial to Nepalese farmers. Identification of particular rust resistance gene and its incorporation is the only option for the development of barley varieties for leaf rust rust resistance. Use of the molecular marker for this purpose is highly sought for this.

Material and Methods

Germplasm Collection

One hundred and fifty five Nepalese barley accessions (NPGR No.s) collected from different parts of Nepal were obtained from National Plant Genetic Resources Centre (NPGRC); and forty seven Jumla collection (JC# series), two local collection (Acc# series) and thirty six barley breeding lines (NB, B, GR and Xveola series) were collected from Hill Crops Research Program (HCRP), Dolakha of Nepal Agricultural Research Council (NARC) (**Table 1**). Similarly, one hundred and nine Nepalese barley accessions were selected based on their disease resistance data (**Table 2**) for

Table 1: Nepalese barley accessions showing variation on leaf rust resistance characteristics.

Genotype	Leaf Rust	Genotype	Leaf Rust	Genotype	Leaf Rust	Genotype	Leaf Rust	Genotype	Leaf Rust
1522		2060		2496		7436		JC#14	
1535		2062		2505		7441		JC#15	
1537		2064		2506		8252		JC#16	
1538		2065		2507		9436		JC#17	
1540		2066		2508		9963		JC#18	
1543		2069		2511		12069		JC#19	80s
1544		2072		2512		12538		JC#21	40s
1545		2073		2513	40s	20774		JC#22	
1546		2074		2514		22463		JC#23	
1547		2075		2515		112-14		JC#24	
1550		2078		2518		Acc#1545		JC#25	
1574		2079		2520		Acc#1574		JC#26	60s
1575		2080		2523		Arupos/oy-B-oy		JC#27	
1576		2081		2525		B86019-1-0		JC#28	
1589		2082		2527		B86019-1K2		JC#29	
1664		2083		2530		B86023-1K	ts	JC#31	
1999		2084		2532		B86065-1-4		JC#32	80s
2000		2086		2533		B86099-1K		JC#33	80s
2001		2087		2534	tms	B86099-2-1		JC#34	20s
2002		2088		2539		B86099-2K		JC#35	
2003		2089		2542		B86115		JC#36	60s
2004		2090		5177		B86122-1-5		JC#37	90s
2005		2181		5617		B86122-1-5-0K3		JC#38	ts
2008		2244		6035		B86122-1K		JC#39	
2009		2447		6036		B86152-2		JC#41	
2010		2453		6038		B86152-2-2-0-0K		JC#42	60s
2011		2454		6041		B86157-1-1-5-0-0K3		JC#43	20s
2013		2456		6044		B86615-1-4		JC#44	40ms
2014		2457		6045		B90K-007-1		JC#45	40s
2016		2458		6055		B90K-01-2K		JC#48	40s
2018		2459		6063	80s	B90K-014-1	ts	JC#49	
2023		2461		6235		B90K-024-1		JC#50	
2024		2464		6289		B90K-038		JC#51	60s
2027		2465		6292		B90K-090		Jumla coll	
2029		2467		6293		Bonus		LG-51/Xve	
2033		2468		6304		COQ/KI/Pescii		Local Jau	
2035		2469		6309		GR-25-85		Maticos	
2037		2470		6310		JC#01		NB-1003-37	
2040		2471		6311		JC#02		NB-1003-37/1034	
2042		2472		6315		JC#03		NB-1003-37/1038	
2043	90ms	2473		6316		JC#04		NB-1003-37/1214	
2046		2482		6319		JC#05		NB-1207/CI	
2048		2483		6320		JC#06	20s	Xveola-12	
2049		2485		6326		JC#07	60s	XVeola-13	
2050		2486		6327		JC#08	10s	XVeola-28	
2051		2487		6334		JC#09	30s	Xveola-38	
2056		2488		6342		JC#11	20s	Xveola-43	
2057		2491		6447		JC#12			
2058		2494	40s	6557		JC#13			

Note: r-resistance, mr-moderately resistance, tms-trace moderately susceptible, tmr-trace moderately resistance, ts-trace susceptible, s-susceptible; blank=no disease i.e. 0 score

Table 2: Nepalese barley accessions used for screening leaf rust resistance using simple sequence repeats marker

1535	2002	2046	2081	2458	2483	6036	6327	8242	JC#18
1537	2003	2051	2082	2459	2489	6293	6340	8244	JC#46
1538	2004	2062	2084	2461	2494	6304	6342	8245	JC#49
1540	2008	2064	2086	2464	2496	6309	6343	8246	Local Jau
1544	2009	2069	2087	2467	2505	6310	6344	8252	Maticos
1545	2010	2072	2088	2468	2506	6314	6350	9436	NB-1003-37
1572	2014	2073	2089	2470	2508	6315	6557	9963	NB-1003-37/1038
1574	2023	2074	2090	2471	2515	6316	7436	Arupos/oy-B-oy	Solu Uwa
1575	2026	2075	2441	2478	2537	6319	7441	B86122-1-5-0K3	Xveola-12
1594	2033	2078	2456	2480	2541	6320	8239	Bonus	XVeola-13
1601	2043	2080	2457	2482	2542	6326	8240	GR-25-85	Xveola-43
2000									

Note: Name as numbers without any alphabet denote the Nepalese Plant Genetic Resource (NPGR) number.

Table 3: SSR markers used to identify presence of leaf rust resistance gene in Nepalese barley gene pool

Marker Name	Sequence-F [5'… … 3']	Sequence-R [5'… … 3']	Annealing Temperature	Stripe rust resistance gene	PCR product size	Chromosome No.	Ref
AY642926-CA11	CCAAAAAC AATTGAGAA AACCCTA	CCTCCC TGAGAG ACCTCCTAT T	58	*Rph7*	183	3H	[12]
Bmac096	GCTATGGCG TACTATGTA TGGTTG	TCACGATGA GGTATGATCA AAGA	58	*Rph2*	173	5HS	[13]
Bmag0225	AACACA CA AAATATTAC AT CA	CGAGTAGTTC CC ATG TGA C	58	*Rph7*	162	3H	[14]
Bmag337	ACAAAGAG GGAGTAGTA CGC	GACCCATGAT ATATGAAGA TCA	55	*QTL*	129-150	5HS	[13]
EBmac0755	AGCCTTGTG TATCAGGAC A	CTGCTGGTGT TCTCTAAAAG T	55	*Rhp3 Rhp19*	144-155	7HL	[15]
Ris44	ACGGATCTA CTTTAGCTA GCA	AAACAACCC CACACAATC	58	*QBLR-P*	105-110*	7HL	[16]

Note: *=product size determined based on field data for disease resistance

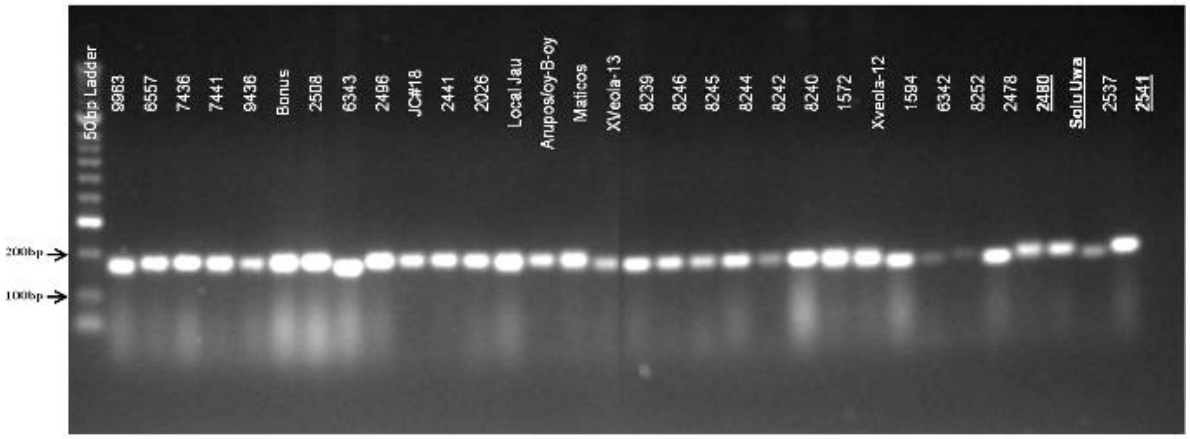

Figure 1. Amplification of SSR markers AY642926-CA11showing presence of Rph7 (183bp) (underlined accessions) in Nepalese barley

Table 4: Nepalese barley germplasm with different leaf rust resistant gene identified using different molecular markers

***Rph7* (AY642926-CA11)**	***Rph2* (Bmac096)**	***Rph3* & *Rph1* (EBmac0755)**	***Rph7* (Bmag0225)**	***QTL* on 5HS (Bmag0337)**	***QBLR-P* (Ris44)**
2480	GR-25-85	1538	GR-25-85	1540	GR-25-85
2062	2470	2008	1545	1601	1544
2483	2471	2033	1574	2010	1545
2541	2515	2062	2010	2023	1574
Solu Uwa	6036	2478	2080	2033	1575
	1537	2480	2505	2043	1601
	2074	2483	2508	2051	2002
	2082	2489	6304	2478	2003
	2506	2515	6310	2480	2004
	NB-1003-37	2541	6315	2482	2072
	NB-1003-37/1038	6342	6343	2489	2073
	Xveola-43	8252	6350	2494	2075
		9963	2000	2505	2078
		1594	2026	2515	2080
		2506	6309	2537	2081
		2537	Arupos/oy-B-oy	2541	2084
		6557	Bonus	6036	2086
		JC#46	JC#49	6340	2088
		JC#49	Local Jau	6342	2089
		NB-1003-37/1038	Maticos	6344	2090
		Solu Uwa	NB-1003-37	8246	2456
		Xveola-43	XVeola-13	8252	2457
				9963	2459
				1537	2461
				2000	2464
				2014	2467
				2046	2468
				2074	2470
				2082	6304
				2506	6310
				2542	6314
				6557	6315
				7436	6316
				8239	6319
				8245	6326
				B86122-1-5-0K3	6327
				JC#49	1535
				Local Jau	2009
				NB-1003-37	2064
				NB-1003-37/1038	2069
				Solu Uwa	2458
				Xveola-43	2471
					6293
					6309
					6320
					Bonus
					NB-1003-37

Note: combination on parenthesis is respective SSR markers used to detect particular gene.

Table 5: Nepalese barley germplasm having three and more leaf rust resistance gene detected by molecular markers

Genotype	*Rph3* & *Rph1*	QTL on 5HS	*Rph2*	*QBLR-P*	*Rph7*
GR-25-85	0	0	1	1	1
NB-1003-37	0	1	1	1	1
NPGR No. 2506	1	1	1	0	0
Xveola-43	1	1	1	0	0
NB-1003-37/1038	1	1	1	0	0
NPGR No. 2515	1	1	1	0	0
JC#49	1	1	0	0	1
Solu Uwa	1	1	0	0	1

Note: 1=present, 0=absent

molecular marker screening. Bonus and Local Jau were used as resistant and susceptible check respectively.

Leaf Rust Evaluation at Field

Barley lines were screened for leaf rust at heading stage at Khumaltar, Lalitpur, Nepal during normal barley growing season. Bonus (origin Sweden) and Local Jau (Nepalese landrace) was used as resistance and susceptible check respectively. Two rows (spacing 20cm) of 1.5m long per accession were sown. The resistance and susceptible checks were repeated after every 15 test lines. Two spreader rows of Local Jau were sown around the disease screening plots. Disease scoring was conducted according to the modified Cobb scale [7].

Molecular Marker

Six SSR markers were selected for screening leaf rust resistance gene (**Table 3**). Molecular markers are selected based on their linkage with particular leaf rust resistance gene.

DNA extraction, PCR reaction and data analysis

Genomic DNA of barley accessions was prepared using modified CTAB method as described by Sul and Korban [8]. Each PCR reaction was conducted with100ng of genomic DNA, 1 µM of each primer and 7.5 µl of 2x GoTaqGreen PCR Master Mix (Promega Corporation, Madison, WI, USA). PCR mixture was amplified in MJ Research PTC-100TM Programmable Thermal Controller (MJ Research, Inc, Watertown, MA, USA) with the following temperature regimes: initial denaturation for 2 min at 95°C followed by 30 cycles of 95°C for 30 sec, annealing as per primer for 1 min, extension at 72°C for 2 min and final extension at 72°C for 7 min followed by holding at 4°C as described on **Table 3** and Scottish Crop Research Institute [9].

Amplified PCR products were separated in 2% analytical grade agarose gel at 100V for 1H. Gels were stained with 0.1 µg/ml ethidium bromide (Promega Corporation, Madison, WI, USA) and then visualized under UV trans illuminator gel documentation system (Wilber Lourmat, Marne-La-Valleen, France) using 1 µg guide size DNA ladder (Genetix, Biotech Asia Pvt. Ltd.). The presence and absence of particular band size was scored for screening disease resistance genes.

Results and Discussion

A lot of variation was observed in Nepalese barley germplasm for leaf rust resistance characteristics (**Table 1**). Leaf rust resistance gene *Rph1, Rph2, Rph3, Rph7, QBLR-P* and *QTL* on 5HS was detected on Nepalese barley accessions using respective SSR markers. Twelve landraces showed presence of Rph2, 22 accessions showed presence of Rph1 and Rph3, 27 accessions showed presence of Rph7, forty two accessions showed presence of *QTL* on chromosome 5HS and 47 accessions possessed leaf rust resistant *QTL QBLR-P* (Table 4). Similarly, eight Nepalese barley accessions showed presence of three and more leaf rust resistant gene (Table 5).

Nepalese barley germplasm showed good resistance with leaf rust which may be due to the presence of leaf rust resistance major genes and quantitative trait loci (QTLs) as detected by different SSR markers. Tyryshkin [10] and Henderson [11] also concluded that Nepalese barley has good resistance against the leaf rust. The released hulless barley variety "Solu Uwa" show good resistance with leaf rust and have

QTLs (Table 4) and major genes including *Rph7* (Figure 1). Similarly, adult plant resistance for leaf rust was also observed by Tyryshkin [10] for Nepalese barley NB-3002 while screening world collection of barley for leaf rust and proved to have one dominant gene. This gene may be Rph7. Similarly, the poor relationship between the field disease resistance and molecular markers linked with specific leaf rust resistance gene proved that Nepalese barley gene pool contains new leaf resistance genes that cannot be defined by the tested molecular markers.

Conclusion

Many Nepalese barley landraces showed field resistance with leaf rust, however, some promising lines still lacks any major resistant genes as defined by molecular markers and need to be incorporated from other lines to address future unwanted leaf rust spread. Barley genotypes with more than three resistant genes could be the choice of donor parents for leaf rust resistant breeding through molecular marker assisted selection in Nepal.

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