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Selection of Pyramided Barley Advanced Lines for Stripe Rust, Leaf Rust and Crown Rust Diseases Using Molecular Markers

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Abstract

Barley diseases are the major yield limiting factors for barley cultivation in Nepal. Stripe/Yellow rust (*P. striformis f.sp. hordei* and *P. striformis f.sp. tritici*), leaf rust (*Puccinia hordei*), and crown rust (*P. coronata*) are the major rust diseases in Nepal. Pyramiding resistance genes against all these rust diseases are possible through molecular marker assisted breeding. Sweden originated barley variety 'Bonus' is found resistant to stripe rust and having linked microsatellite markers for stripe rust and crown rust resistance. Similarly, Nepalese hull-less barley variety 'Solu Uwa' and Nepalese awn-less barley landrace NPGR Acc# 2478 have linked microsatellite markers for leaf rust resistance. Therefore, one polymorphic sequence tagged sites (STS) marker (ABG054) for stripe rust resistance, two polymorphic simple sequence repeats (SSR) markers (Bmac0144h and HVM049) for leaf rust and one polymorphic SSR marker (Bmag0006) for crown rust resistance were used to select the advanced barley lines (at F8 stage) from above parents. Field screening of stripe rust resistance was also conducted. Among 51 advanced and field disease resistance lines from Bonus/Solu Uwa cross, 10 pyramided lines for all three types of barley rust resistance were selected. Similarly, among 39 advanced and field disease resistance lines from Bonus/NPGR Acc#2478 cross, three pyramided lines were selected and advanced for further yield testing for general cultivation purpose. The chances of losing the desired gene are higher in late generation selection using molecular marker assisted selection (MAS), but the chances of getting agronomically superior varietal output is expected to increase.

Keywords: Rust, Pyramiding, Advanced lines, Barley, Marker Assisted Selection (MAS)

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Introduction

Barley diseases are the major yield limiting factors in Nepal. Among many barley diseases, rust diseases are critical from the crop production view. Strip/yellow rust (caused by *Puccinia striformis f.sp. hordei* and *P. striformis f.sp. tritici*) is prevalent rust in the Nepalese barley field. Prasad et al. [1] also observed it as a major disease causing a problem in the Nepalese barley field. However, leaf rust (caused by *P. hordei*) can be observed in some warm barley cultivating areas. Crown rust of barley (caused by *P. coronata*) can be observed very sporadically only. Any barley variety having resistance gene for all three types of rust pathogen is highly sought in Nepalese barley breeding program.

Since, Nepalese barley germplasm has a high grain yielding capacity for hill and mountain regions of the country [2], adding rust resistance characteristics to them may improve their yield and stability. Selection, identification and incorporation of rust resistance genes is the only option for the development of rust resistance barley varieties for Nepal. Therefore, pyramiding major rust resistance genes for barley will be beneficial to farmers. Molecular markers are highly preferred for gene pyramiding program like this.

The sporadic nature of the crown rust occurrence in the Nepalese barley field and overlapping of leaf rust and stripe rust in the disease screening field further pushed molecular marker assisted selection (MAS) as the most viable option for gene pyramiding for rust resistance varietal development.

Materials and Methods

Parent and Advanced lines selection

A Swedish introduced variety 'Bonus' is the two-rowed stripe rust resistance barley variety for Nepal [2] and also have linked microsatellite markers for stripe rust and crown rust resistance. The polymorphic linked microsatellites are described in 'identification of polymorphism in parents' sub-heading. Similarly, Nepalese hull-less barley variety 'Solu Uwa' and Nepalese awn-less barley landrace 'NPGR Acc# 2478' has linked microsatellite markers for leaf rust resistance. Therefore, crosses between Bonus with Solu Uwa and Acc #2478 will have a lot of chances of having pyramided lines.

Use of marker assisted selection at early stage of barley breeding such as in F2 and F3 is practically not feasible in our context due to cost, time and manpower shortage.

Table 1. List of barley advanced breeding lines used for molecular marker assisted selection.

Bonus/Solu Uwa-3	Bonus/Solu Uwa-51	Bonus/Solu Uwa-99	Bonus/Solu Uwa-153	Bonus/Acc#2478-201	Bonus/Acc#2478-231
Bonus/Solu Uwa-6	Bonus/Solu Uwa-54	Bonus/Solu Uwa-102	Bonus/Solu Uwa-156	Bonus/Acc#2478-202	Bonus/Acc#2478-235
Bonus/Solu Uwa-9	Bonus/Solu Uwa-57	Bonus/Solu Uwa-105	Bonus/Solu Uwa-159	Bonus/Acc#2478-204	Bonus/Acc#2478-238
Bonus/Solu Uwa-12	Bonus/Solu Uwa-60	Bonus/Solu Uwa-108	Bonus/Acc#2478-162	Bonus/Acc#2478-205	Bonus/Acc#2478-244
Bonus/Solu Uwa-15	Bonus/Solu Uwa-63	Bonus/Solu Uwa-111	Bonus/Acc#2478-165	Bonus/Acc#2478-206	Bonus/Acc#2478-246
Bonus/Solu Uwa-18	Bonus/Solu Uwa-66	Bonus/Solu Uwa-114	Bonus/Acc#2478-168	Bonus/Acc#2478-209	Bonus/Acc#2478-248
Bonus/Solu Uwa-21	Bonus/Solu Uwa-69	Bonus/Solu Uwa-117	Bonus/Acc#2478-171	Bonus/Acc#2478-210	Bonus/Acc#2478-254
Bonus/Solu Uwa-24	Bonus/Solu Uwa-72	Bonus/Solu Uwa-126	Bonus/Acc#2478-174	Bonus/Acc#2478-213	Bonus/Acc#2478-257
Bonus/Solu Uwa-27	Bonus/Solu Uwa-75	Bonus/Solu Uwa-129	Bonus/Acc#2478-177	Bonus/Acc#2478-216	Bonus/Acc#2478-259
Bonus/Solu Uwa-30	Bonus/Solu Uwa-78	Bonus/Solu Uwa-132	Bonus/Acc#2478-180	Bonus/Acc#2478-218	Bonus/Acc#2478-268
Bonus/Solu Uwa-33	Bonus/Solu Uwa-81	Bonus/Solu Uwa-135	Bonus/Acc#2478-183	Bonus/Acc#2478-222	Bonus/Acc#2478-278
Bonus/Solu Uwa-36	Bonus/Solu Uwa-84	Bonus/Solu Uwa-138	Bonus/Acc#2478-186	Bonus/Acc#2478-225	
Bonus/Solu Uwa-39	Bonus/Solu Uwa-87	Bonus/Solu Uwa-141	Bonus/Acc#2478-189	Bonus/Acc#2478-227	
Bonus/Solu Uwa-42	Bonus/Solu Uwa-90	Bonus/Solu Uwa-144	Bonus/Acc#2478-192	Bonus/Acc#2478-228	
Bonus/Solu Uwa-45	Bonus/Solu Uwa-93	Bonus/Solu Uwa-147	Bonus/Acc# 2478-195	Bonus/Acc#2478-229	
Bonus/Solu Uwa-48	Bonus/Solu Uwa-96	Bonus/Solu Uwa-150	Bonus/Acc#2478-198	Bonus/Acc#2478-230	

Table 2. Polymorphism observed in parental lines for different molecular markers and disease characteristics

Parent	Field Stripe Rust	ABG054 (Stripe Rust QTL)	Bmac0144h (Leaf Rust-R gene)	HVM049 (Leaf Rust-Rph19)	Bmag0006 (Crown Rust-Rpc1)
Bonus	0	1	0	0	1
Solu Uwa	10S	0	1	1	0
Acc#2478	60S	0	0	1	1

Note: Number in bracket is the linked resistance gene. Acc# = NPGR Accession Number

So, we have selected 51 advanced barley lines at F8 stage from 'Bonus' and 'Solu Uwa' crosses. Similarly, we have also selected 39 advanced barley lines at F8 stage from 'Bonus' and 'Acc#2478' crosses to detect the pyramided lines (**Table 1**). All the selected lines showed the field disease resistance. Similarly, they are forwarded to F8 based on their superior agronomic characteristics comparing to their parents.

Field Rust Evaluation

Two rows per line were sown at Khumatar, Lalitpur during normal barley growing season (November to April) in 2017. Spacing between each row was 20 cm and the length of the row was 1.5m. Resistance and susceptible parental lines were sown repeatedly after every 15 advanced lines. A susceptible landrace 'Local Jau' was used in two spreader rows around the disease screening plots. Modified Cobb scale [3] was used for rust scoring at the heading stage for all three types of rust.

Identification of polymorphism among parents

A series of microsatellite markers linked with rust resistance genes were screened to identify the polymorphic markers among the parents. Sequence Tagged Sites (STS) marker ABG54, and Simple Sequence Repeats (SSR) markers Bmac144h, HVM49 and Bmag6 were found polymorphic among the parents (**Table 2**). These markers were used for the selection of advanced barley lines.

DNA extraction and PCR reaction

Modified CTAB method as described by Sul and Korban [4] was used to extract the genomic DNA of selected barley advanced lines. PCR reaction mixture of 15 µl

Table 3. List of molecular markers showing polymorphism and used in selection process

Marker Name	Forward Primer [5' … 3']	Reverse Primer [5'… 3']	Annealing Temperature	Resistance Gene	PCR Product Size	Chromosome No.	Reference
ABG054	GTGCTTGG CGGTCGA CCAGT	GATGTCCAAC GGTGGCTTGA	55	Stripe Rust (QTL)	180bp*	4H	[5]
Bmag0006	TTAAACCC CCCCCCTC TAG	TGCAGTTACT ATCGCTGATT TAGC	58	Crown Rust (Rpc1)	174	3H	[6]
Bmac0144h	TACGTGTA CATACTCT ACGATTTG	ACTTATTCTG CATCCTGGGT	55	Leaf Rust (R-gene)	179	1H	[7]
HVM049	CTCTATAG GCACGAA AAATTCC	TTGCACATAT CTCTCTGTCA CA	55	Leaf rust (Rph19)	105	7H	[8]

Note: *=Field disease resistance data is used to identify the product size

volume was prepared using 1.5 µl (1 µM) for each primer, 7.5 µl of PCR Master Mix (Promega Corporation, USA), 2.5 µl water and 2 µl (100ng) DNA template. This PCR mixture was amplified as per the following protocol.

For Marker ABG54, Bmag6 and Bmac144h

Thirty cycles: denaturation 30 sec at 95°C, annealing 1 min with temperature as per **Table 3** and extension 2 min at 72°C.

For Marker HVM49

Touch down PCR protocol was followed as 18 cycles of 1 min denaturation at 94°C, 30 sec of touchdown protocol with decreasing 1°C per 2 cycle from 64°C until 55°C as annealing and 1 min at 72°C for the extension. This touchdown cycle was followed by another 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C and 1 min extension at 72°C. The final extension was 7 min at 72°C and the final holding is at 4°C.

The PCR products were separated in 2% agarose gel in 1XTAE buffer at 100V for one hour. Gels were stained with ethidium bromide (0.1 µg/ml) and visualized under UV rays. The presence of a particular band size (**Table 3**) was considered the presence of a particular linked gene.

Results

We have observed stripe rust in susceptible parents; however, we had not observed leaf rust and crown rust in Khumaltar conditions (**Table 2**). This suggests that the use of MAS techniques is very essential for pyramiding any resistance gene that cannot be screened in field condition at that time.

Many breeding lines showed the presence of one or more genes for the rust resistance based on the particular marker band (**Figure 1-4**).

However, the pyramided lines were very limited than our expectation for both types of crosses (**Table 4** and **Table 5**).

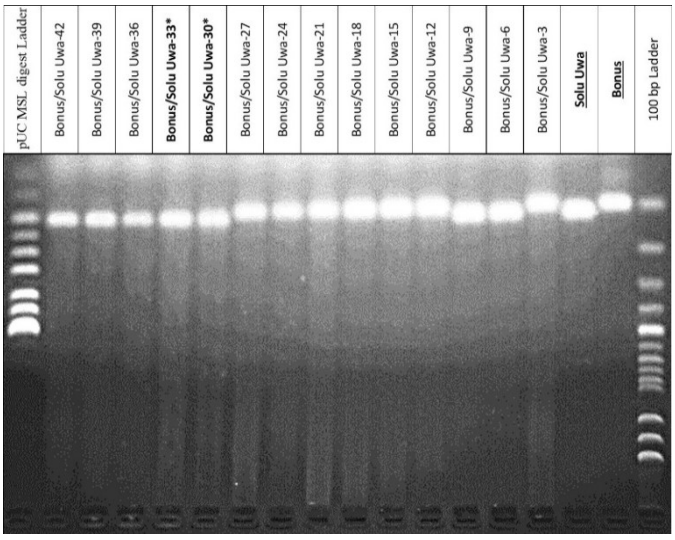

Figure 1. Amplification of SSR marker HVM049 (105bp) in barley advanced lines. (Note: Parents are underlined and bold; and selected lines are bold with asterisk mark)

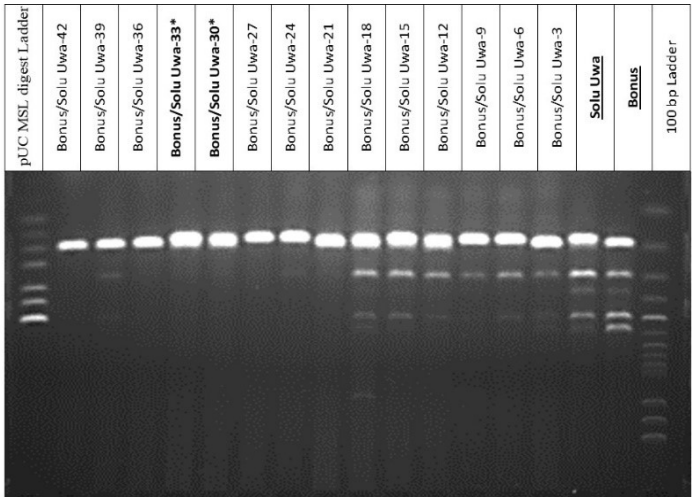

Figure 2. Amplification of SSR marker ABG054 (180bp) in barley advanced lines from Bonus/Solu Uwa. (Note: Parents are underlined and bold; and selected lines are bold with asterisk mark)

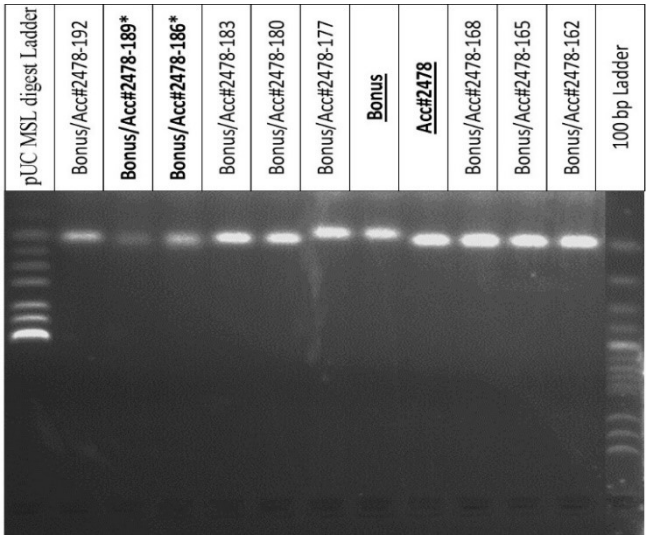

Figure 3. Amplification of SSR marker HVM049 (105bp) in barley advanced lines. (Note: Parents are underlined and bold; and selected lines are bold with asterisk mark)

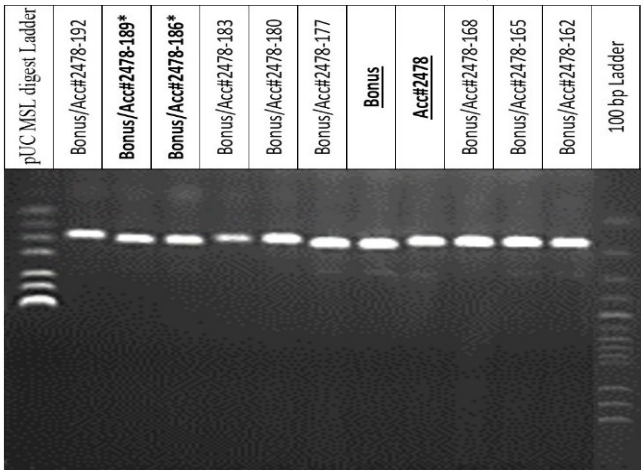

Figure 4. Amplification of SSR marker ABG054 (180bp) in barley advanced lines from Bonus/Acc#2478. (Note: Parents are underlined and bold; and selected lines are bold with asterisk mark)

Discussion

Selection on the late stage of the breeding may lead to eroding many useful lines with the important genes that showed the neutral effect in the previous season of field disease screening. The leaf rust and crown rust could not be screened in Khumaltar condition for all previous seasons, which ultimately lead us a few lines with leaf rust and crown rust resistance along with stripe rust resistance. But, the agronomic characteristics of our selected lines are superior and always safe from ending with disease resistance but poor yielding varieties.

Due to the less polymorphism between the parent Bonus and Acc#2478; we can select the lines with pyramided stripe rust and leaf rust resistance linked markers only. The linked marker for crown rust resistance found in 'Bonus' is also found in Nepalese landrace 'Acc#1478' (**Table 2**). We identified linked markers for the leaf rust resistance gene in Nepalese local variety 'Solu Uwa' and landrace 'Acc#1478' which support our observation of barley field at Khumaltar and surroundings with negligible infection from leaf rust.

Higher leaf rust resistance in Nepalese barley is also supported by the observation of Tyrshkin [9] and Henderson [10]. Tyrshkin [9] also concluded that Nepalese barley germplasm NB-3002 has one dominant gene for leaf rust resistance.

Table 5. List of selected advanced breeding lines from Bonus and Acc#2478 cross with corresponding molecular marker polymorphism.

Line	Field Stripe Rust	ABG054	HVM049
Bonus	0	1	0
Acc#2478	60S	0	1
Bonus/Acc#2478-186	0	1	1
Bonus/Acc#2478-189	0	1	1
Bonus/Acc#2478-209	0	1	1

Note: 1 = Present, 0 = Absent

Table 4. List of selected advanced breeding lines from crosses between Bonus and Solu Uwa with corresponding molecular marker polymorphism.

Line	Field Stripe Rust	ABG054	Bmac0144h	HVM049	Bmag0006
Bonus	0	1	0	0	1
Solu Uwa	10S	0	1	1	0
Bonus/Solu Uwa-30	0	1	0	1	1
Bonus/Solu Uwa-33	0	1	0	1	1
Bonus/Solu Uwa-45	0	1	0	1	1
Bonus/Solu Uwa-48	0	1	0	1	1
Bonus/Solu Uwa-60	0	1	1	1	1
Bonus/Solu Uwa-63	0	1	1	1	1
Bonus/Solu Uwa-81	0	1	1	0	1
Bonus/Solu Uwa-90	0	1	1	1	1
Bonus/Solu Uwa-135	0	1	1	1	1
Bonus/Solu Uwa-138	0	1	0	1	1

Note: 1 = Present, 0 = Absent

Similarly, we also observed that hull-less parent ('Solu Uwa') is less stripe rust susceptible than the hulled parent ('Acc#2478') (**Table 2**) as Baniya et al. [11] already concluded for covered (hulled) barley and naked (hull-less) barley collection for Nepal.

Conclusion

Selection in an early generation for marker assisted selection (MAS) program is considered a thumb rule; however, in the condition where laboratory resource is poor and costly than growing crops in the field, late generation selection for pyramided lines using molecular techniques will be still competitive. On one hand, the chances of losing the desired gene (or marker) are high; but in another hand, the chances of getting agronomical superior varietal output will also increase by late use of MAS techniques since early generation selection on MAS largely depends on particular gene/marker rather than crop performance itself.

Author's Contribution

RBA selected parents and made the crosses. SuP, AP, SS advanced the lines and maintain them. RBA, SuP did disease scoring. RBA, ShP, SS did DNA extraction, PCR and gel electrophoresis. RBA did data analysis, wrote and finalized the manuscript. All the authors read and approved the final manuscript.

Competing Interests

No competing interests were disclosed.

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Ethical Approval and Consent

Not Applicable.

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