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ARTICLE

Identification and characterization of leaf rust (*Puccinia triticina*) and Fusarium head blight (*Fusarium graminearum*) related genes in domesticated wheat varieties in Mongolia

Odgerel Bold*[1], *Otgon-Erdene Deriilaa*[2] *and P.S. Baenziger*[3]

[1] *Department Economy, state reserve, emergency buck-up supply, University of Internal Affairs, Ulaanbaatar, Mongolia*
[2] *School of Agroecology, Mongolian University of Life Sciences, Ulaanbaatar, Mongolia*
[3] *Department of Agronomy and Horticulture, University of Nebraska Lincoln, Lincoln, USA*

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Abstract: Wheat is recognized as the major crop among all cereals. For better quality and disease-free production, the current study was designed to evaluate the prevalence of genetic leaf rust resistance and fusarium head blight in nineteen genotypes of wheat, which are commonly grown in Mongolia. For example Khalkhgol-1, Darkhan-131, Darkhan-160, Darkhan-144, Orkhon, Darkhan-34, Darkhan-74, Darkhan-193, Altaiskaya-100, Darkhan-181, Darkhan-141, Buryatskaya osistiya, Darkhan-166, Buryatskaya-79, Buryatskaya-34, Selenge, Altaiskaya-530, Altaiskaya-325 and Altaiskaya jinitsa genotypes. The presence of *Lr34* and *Fhbt1* genes were evaluated for leaf rust and fusarium head blight respectively. *Lr34* gene was reported in the Darkhan-160 and Darkhan-181 genotypes, while fusarium head blight was not reported in any of the genotype.

Keywords: *Wheat (Triticum aestivum L.); leaf rust; fusarium head blight; molecular markers;*

INTRODUCTION

Wheat is one of the most important cereal crops for human nutrition. It is grown in more than 200 million hectares worldwide and wheat provides one fifth of the calorific and protein needs of the global population [1, 2]. Wheat crop suffer from many destructive diseases. Rust diseases are the most destructive because of their range, which is also increasing. Leaf rust is an epidemic disease caused by Puccinia triticina, which affects the flag leaf and causes major losses in quality and yield of wheat crop [3]. It is a global disease. For example, it is found in Mexico, USA, Asia and Australia [4, 5].

The fusarium head blight (FHB, caused by Fusarium graminearum,) is also known as a very destructive fungal disease. Fusarium head blight spreads due to climate change, crop rotation, and humid atmosphere [6]. FHB affects the heads of wheat causing premature bleaching of spikelet, which has seriously negative implication on the economic gains [7]. FHB was known to have negative effects on grain and crop yield, for example, on poor quality grains and yield loses [8-9]. FHB prevalence can be identified under warm and wet environment [10].

***corresponding author: pstephen.baenziger@gmail.com** **https://orcid.org/0000-0003-0256-7695**

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In addition, FHB also causes grain contamination by myco-toxins, which are harmful for human and animal health [11-12]. The present study was conducted to identify the presence of Lr34 and Fhb1 genes for leaf rust and FHB in wheat cultivars commonly grown in Mongolia.

MATERIALS AND METHODS

Plant material

The seeds of the Mongolian domestic wheat varieties, such as, Khalkhgol-1, Darkhan-131, Darkhan-160, Darkhan-144, Orkhon, Darkhan-34, Darkhan-74, Darkhan-193, Altaiskaya-100, Darkhan-166, Darkhan-181, Darkhan-141, Selenge, and Russian wheat varieties Buryatskaya osistiya, Buryatskaya-79, Buryatskaya-34, Altaiskaya-530, Altaiskaya-325 and Altaiskaya jinitsa were obtained from the Plant Science and Agricultural Training Research Institute of Darkhan-Uul aimag, Mongolia. 4 genotype checks, including two for leaf rust and two for Fusarium head blight, were provided by the Small Grain Lab, University of Nebraska-Lincoln. The genotypes and checks were given the following names as in Tables 1 and 2.

Marker gene

Identification and characterization of leaf rust resistance (R) genes Lr34 and FHB resistance gene (R) gene Fhb1 in 19 Mongolian local varieties of wheat were done using different molecular marker systems. In addition, wheat leaf rust resistant line LCH14-077 for positive control, wheat leaf rust susceptible line LCH14-089 were used as negative control for the Lr34 gene. Fusarium head blight resistant line Overland_Fhb1 was used as the positive control, FHB susceptible line NE14696 was used as negative control for the Fhb1 gene in this experiment.

Table 1. Name of Wheat Accessions

Sr.	Genotypes	Origin	Growth habit	Pedigree
1	Khalkhgol-1	Mongolia	Spring	Bezostaya winter variety to spring variety
2	Darkhan-131	Mongolia	Spring	Bezostya x Scala
3	Darkhan-160	Mongolia	Spring	Odessa 51 x Calyansona
4	Darkhan-144	Mongolia	Spring	CT-416 x Grekum 114
5	Orkhon	Mongolia	Spring	USA Washington variety selection
6	Darkhan-34	Mongolia	Spring	Darkhan 74 x Darkhan 77
7	Darkhan-74	Mongolia	Spring	Poland RAH-506 variety
8	Darkhan-193	Mongolia	Spring	Darkhan 74 x Darkhan 77
9	Altaiskaya-100	Mongolia	Spring	Botanicheskaya 2 x Jnicha
10	Darkhan-181	Mongolia	Spring	USA Vasington variety selection
11	Darkhan-141	Mongolia	Spring	Poland RAH-506 variety
12	Buryatskaya osistiya	Mongolia	Spring	Mironovskaya 808 x Onohoiskaya
13	Darkhan-166	Mongolia	Spring	Orkhon x Calyansona
14	Buryatskaya-79	Mongolia	Spring	Mironovskaya 808 x Onohoiskaya
15	Byryatskaya-34	Mongolia	Spring	Bezostaya 1 x Yarovya 9009
16	Selenge	Mongolia	Spring	Buryatskaya 79 x Buryatskaya 34
17	Altaiskaya-530	Mongolia	Spring	Lutestens 281 x Lutestens 281
18	Altaiskaya-325	Mongolia	Spring	Lutestens 328 x Jigulavskaya
19	Altaiskaya jinitsa	Mongolia	Spring	Komsomolskaya x Lutestens 281

Table 2. Name of Checks

Checks for Lr34 gene		
Sr.#	Name	Control type
1	LCH14-077	Positive
2	LCH14-089	Negative
Checks for FHB		
Sr.#	Name	Control type
1	Overland_Fhb1	Positive
2	NE14696	Negative

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DNA Extraction

The 19 lines and four checks were planted in the greenhouse. After one week, fresh leaf samples were collected from each genotype separately. DNA was isolated by Qiagen kit by following (Biosprint) DNA extraction protocol. DNA quantification was done by Invitrogen Life fluorometer (Qubit 3.0). https://dx.doi.org/10.17504/protocols.io.bi8dkhs6

Markers

The identification and characterization of leaf rust (Lr34) and fusarium head blight (Fhb1) resistance gene will be done in all the genotypes. For the purpose of fine mapping, four markers were tested (Table 3). The markers sequence information was used from the online USDA website. The extracted DNA was used for PCR reactions.

Table 3. KASP assays and primer sequences for Lr34 and Fhb1 genes

Category	Gene	Primer Name	FAM Primer
Rust	Lr34	Lr34_TCCIND	GGTATGCCATTTAACATAATCATGAA
Rust	Lr34	Lr34jagger	TGTAATGTATCGTGAGAGATTTGCAG
Fusarium	Fhb1	snp3BS-8	CACATGCATTTGCAAGGTTGTTATCC
Fusarium	Fhb1	UMN10_SNP	GAATTACTCATTTTTAGATTTGTCTACATACA
Category	Gene	Primer Name	HEX Primer
Rust	Lr34	Lr34_TCCIND	GGTATGCCATTTAACATAATCATGAT
Rust	Lr34	Lr34jagger	ATTGTAATGTATCGTGAGAGATTTGCAT
Fusarium	Fhb1	snp3BS-8	CACATGCATTTGCAAGGTTGTTATCG
Fusarium	Fhb1	UMN10_SNP	GAATTACTCATTTTTAGATTTGTCTACATACG
Category	Gene	Primer Name	Commom Primer
Rust	Lr34	Lr34_TCCIND	TACTATATGGGAGCATTATTTTTTTCC
Rust	Lr34	Lr34jagger	GATCATTATCTGACCTGTGCGAATGAATA
Fusarium	Fhb1	snp3BS-8	CAAAGCAGCCTTAGGTCAATAGTTTGAAA
Fusarium	Fhb1	UMN10_SNP	GAAGTTCATGCCACGCATATGCTAGTA

PCR

PCR reaction mixture reagents used were KASP Master Mix (2x concentration) 5µl, the master mix contained FAMTM and HEXTM specific FRET cassette, ROX (passive reference dye) and Taq polymerase in an optimized buffer solution, 0.14 µl 72X SNP specific KASP Assay mix (Primers); the 72X KASP assay mix contains two allele-specific forward primers and one common primer, 5 µl of genomic DNA (50 ng/µl). Polymerase chain reaction was performed using the Eppendorf Mastercycler. The PCR programme was done in several stages: Stage 1 (Hot-start Taq activation) 94°C for 15 minutes followed as 1 cycle; Stage 2 (Touchdown) of initial denaturation at 94°C for 20 seconds, annealing at 65-57°C dropping 0.8°C per cycle (to achieve a final annealing/extension temperature of 55°C) 1 minute followed as 10 cycles; Stage 3, amplication at 94°C for 20 seconds and 55°C for 1 minute followed as 26 cycles, and, Stage 4 (end) 4°C for 1 minute followed by 1 cycle. The PCR product was analysed using three software programs in KASP analysis-Omega (to analyze the PCR products), Kluster Caller (to read the measurements from Omega), and SNP viewer.

RESULTS AND DISCUSSION

Genotyping for Lr34 gene in wheat

Lr34 is a well-known leaf rust resistance gene, which provides quantitative durable resistance against leaf rust. Presence of *Lr34* allele was tested with 2 primer combinations, such as *Lr34_TCCIND* and *Lr34 jagger*. *Lr34* jagger primer combination did not produce the desired results. The marker of *Lr34_TCCIND* was used for further characterization of the *Lr34* region. In fragment pattern, red colour represents the presence of *Lr34* gene in *LCH14-077* (experimental check) followed by Darkhan-160 and Darkhan-181 genotypes (Figure 1). Blue colour represents the absence of *Lr34* gene in *LCH14-089* (check for negative control) followed by Khalkhgol-1, Darkhan-131, Darkhan-144, Orkhon, Darkhan-34, Darkhan-74, Darkhan-193, Altaiskaya-100,

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Darkhan-141, Buryatskayaosistiya, Darkhan-166, Buryatskaya-79, Buryatskaya-34, Selenge, Altaiskaya-530, Altaiskaya-325 and Altaiskaya jinitsa.

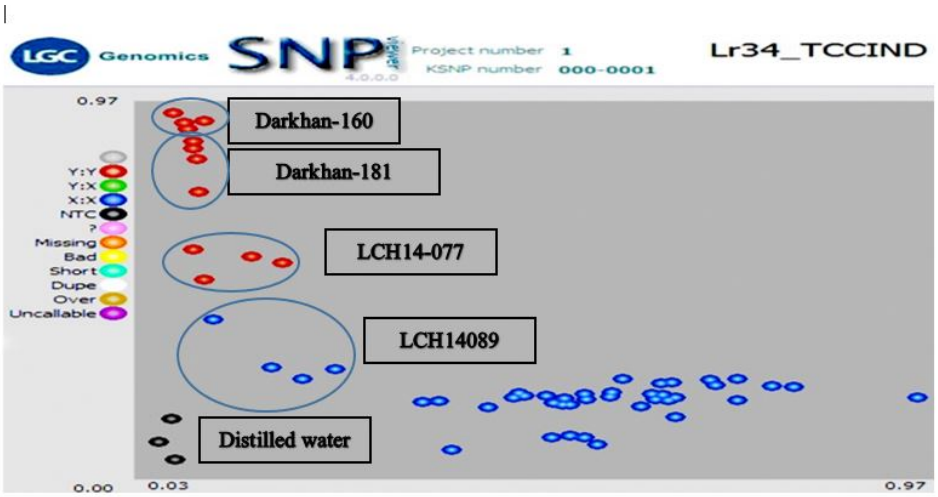

Figure 1. KASP marker Lr34_TCCIND Tested on a set of wheat lines: *Red is positive control (LCH14-077), followed by Darkhan-160 and Darkhan-181, Blue color is negative control (LCH14-089) followed by Khalkhgol-1, Darkhan-131, Darkhan-144, Orkhon, Darkhan-34, Darkhan-74, Darkhan-193, Altaiskaya-100, Darkhan-141, Buryatskaya osistiya, Darkhan-166, Buryatskaya-79, Buryatskaya-34, Selenge, Altaiskaya-530, Altaiskaya-325 and Altaiskaya jinitsa*

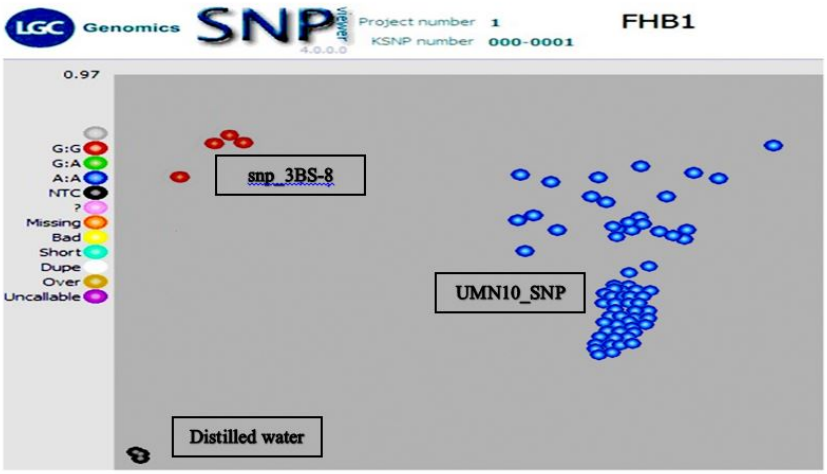

Figure 2. CASP marker UMN10_SNP (Fhb1 resistant gene) tested on a set of wheat lines: *Red is positive control (Overland_Fhb1), Blue color is negative control (NE14696) followed by Khalkhgol-1, Darkhan-131, Darkhan-144, Orkhon, Darkhan-34, Darkhan-74, Darkhan-193, Darkhan-160, Darkhan-181, Altaiskaya-100, Darkhan-141, Buryatskaya osistiya, Darkhan-166, Buryatskaya-79, Buryatskaya-34, Selenge, Altaiskaya-530, Altaiskaya-325 and Altaiskaya jinitsa*

The *UMN10_SNP* marker was used to test the *Fhbt1* resistant gene in the population. The red color in results showed the presence of *Fhbt1* gene in the Overland (positive check), while the whole population showed negative results for *Fhbt1* gene.

Rust is a major disease in wheat that causes yield losses. The high spells of humidity leading to free standing moisture on the leaves during the growing season promotes leaf rust infection. Due to the gravity of this disease and the importance of *Lr34* for disease resistance, knowledge of genetics of leaf rust resistance in wheat cultivars can be helpful in accelerating efficient gene exploitation mechanism to develop resistance lines in wheat and to make better recommendations to wheat growers. Genetic resistance for rust is the most effective and economical way to reduce yield loses [13] due to leaf rust disease.

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The role of Lr resistance genes for durable resistance against leaf rust was found to be very important [14 & 15]. The *Lr34* gene has positive association with the leaf rust resistance in the wheat [16]. Fusarium head blight (FHB) is a very devastating disease in wheat [17]. FHB has been reported to cause great economic losses in the production of wheat crop [18] and grain quality contaminated with myco-toxins. The selection of genotypes for leaf rust resistance on the basis of presence of *Lr-34* gene and for FHB resistance on the basis of the presence of *Fhb1* are useful and time-saving techniques to screen a large population in a short time.

CONCLUSIONS

- From the 19 wheat varieties surveyed, the Darkhan-160 and Darkhan-181 had the same color as the LCH14-077 variety with positive control of the Lr34 gene in the KASP marker analysis and were believed to contain the Lr34 gene for leaf rust disease resistance.

- It is possible to select Darkhan-160 and Darkhan-181 wheat varieties as parent plants to create new varieties that are resistant to leaf rust.

- Through the KASP marker analysis of 19 local and promising varieties of wheat, all varieties showed same result as UMN10_SNP negative control. Hence, all lines did not contain Fhb1 gene encoding Fusarium resistance. All lines are most likely vulnerable to FHB. However, molecular markers could be used to track Fhb1 in crosses and future released cultivars.

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