

Characterization of Advanced Hexaploid Wheat Lines Against Stripe Rust (*Puccinia striiformis* f. Sp. *tritici*) and Identification of Employed Pathogen Races

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Abstract: Wheat is one of the world's most important crops whose grain production is increasing year after year. However, its production is badly constrained by wheat rusts. Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* is an important disease of wheat resulting significant yield failure in wheat growing areas around the globe. The pathogen is one of the very important yield limiting factors in Ethiopia. The severity is worse due to emergency of virulent stripe rust races at one point of the world spread to the rest of wheat producing countries by wind and human travels. Thus, development and cultivation of hereditarily diverse and tolerant varieties is the most sustainable option to overcome these diseases. The present study was carried out with the aim to identify possible sources of stripe rust resistance among Ethiopian bread wheat breeding pipelines to enhance cultivar improvement efforts and identify physiologic races involved during screening process. A total of four mono-pustule isolates were collected from Meraro and Kulumsa, stripe rust hot spot locations. Out of these, two *P. striiformis* races; namely, PstS2 and PstS11 were identified. The former was detected at Meraro and virulent to seven of the 19-differential lines while PstS11 displayed across Meraro and Kulumsa and virulent to nine of the 19-differential lines. Twenty-eight advanced bread wheat pipelines and a universal susceptible cultivar, Morocco were evaluated for their resistance at the seedling stage against identified stripe rust races (PstS2 and PstS11) in a controlled environment. Of the 28, twenty and seventeen lines exhibited susceptible seedling reactions to PstS2 and PstS11 with infection types ranging from 7 to 9, respectively. Those groups of lines that showed susceptible reaction at seedling stage are expected to possess poly minor genes that could be used for durable stripe rust resistance breeding in wheat. However, is advised to evaluate for adult plant resistance and postulate inherent resistance genes in these lines for fruitful recommendations.

Keywords: Inherent Genes, Pathogen Races, Seedling Resistance, Slow Rusting, Yellow Rust

1. Introduction

Wheat (*Triticum aestivum* L.) one of foremost widely cultivated cereal grain crop worldwide, providing approximately 20% of global calories to humans (International Research Associates [1]). Similarly in Ethiopia, wheat is an important staple food crop, afford about 15 percent of the caloric intake for the country's over 90 million population [2]. The figures indicated production and yield of wheat crop has been increasing globally including Ethiopia [3, 4]. However, wheat production and productivity is

constrained by factors including diseases mainly rusts, insect pests, variable climatic conditions, inadequate land and socio-economic problems [5, 6].

Stripe rust (yellow rust), caused by an obligate fungal pathogen *Puccinia striiformis* Westend. f. sp. *tritici* Eriks, is one of the most damaging diseases of wheat [7]. Historically, wheat production is gravely constrained due to this disease in Ethiopia and the rest of world. Severe losses due to wheat stripe rust diseases have been reported both in the past to date. It is projected that globally 5.47 million tons of wheat are lost to the stripe rust pathogen each year, equivalent to a

loss of US\$979 million [8, 9].

As of other stripe rust vulnerable parts of the world, stripe rust of wheat is the major wheat production bottleneck in the highlands of Arsi and Bale areas, the wheat belts of Ethiopia [10, 11]. Major stripe rust epidemics were experienced in Ethiopia in 1970's, 1988s, 2010, 2017 and 2018 and resulted in significant grain yield losses of 30 to 69% [12], 58-100% [13] 96% [14] depending on the susceptibility of the cultivars and environmental conditions. For instance in 2010, when Pst pathotypes overcame the resistance conferred by Yr27 more than US\$3.2 million expended for purchasing fungicides alone, and food and nutritional security of 3.5 million Ethiopian smallholder farmers involved in wheat production were heavily threatened [15].

To reduce stripe rust-associated losses, developing and growing resistant cultivars is widely recognized as the most environmentally and economically feasible approach [16]. Ethiopian national wheat breeding and improvement program with international help, ICARDA and CIMMYT have been developed high yielding, stable, disease resistant varieties with good grain quality and adaptable to the different agro-ecologies of the country [17]. However, the promising varieties developed were susceptible to breakdown and invariably short-lived.

The major reasons for periodic outbreaks of yellow rust disease in the world and Ethiopia is the emergence of new races of rust and scarce information on the genetic variation of host-pathogen interactions and unreliability of current sources of resistance to the prevailing race population respectively [18, 19, 20]. Therefore, characterization of genotypes for diverse sources of resistances partial (slow rusting), all stage (seedling) resistance to yellow rust resistances in combination is imperative for developing and releasing durable rust resistant variety. Thus, the present

study was executed with the aim to evaluate bread wheat breeding pipelines for stripe rust seedling resistance and to identify races in *P. striiformis* populations involved in germplasm screening through Phenotyping and molecular approaches.

2. Materials and Methods

2.1. Descriptions of the Study Area

Kulumsa research center is located at 08°01'10"N, 39°09'11"E and at 2200 meters above sea level (m. a. s. l). It receives mean annual rainfall of 820 mm representing highland and high rainfall agro ecology. The monthly mean minimum and maximum temperature is 10.5 and 22.8°C, respectively. This site has dominantly fertile loam soil type [21].

Meraro substation is located at 07°24'27"N, 39°14'56"E and 2990 m. a. s. l. Its average annual rainfall is 1196 mm representing extreme highland and frost prone agro ecology. The minimum and maximum temperature is 5.7 and 18.1°C, respectively. The dominant soil type is clay soil (Nitosols) which is slightly acidic (pH = 5.0). Both locations represent major wheat-growing and yellow rust prone areas in the highlands of Arsi [21].

2.2. Experimental Materials

Twenty-eight advanced bread wheat breeding pipeline genotypes with one universal susceptible check Morocco were employed. All 28 test wheat genotypes were obtained from Kulumsa Agricultural Research Center, a center coordinating national wheat-breeding program. These test lines have been under testing for yield performance at the national testing sites.

Table 1. List of advanced bread wheat lines employed in the study.

Number	Name	Pedigree	Selection History
1	ETBW 8751	SUP152//ND643/2*WBLL1	CMSS08Y00274S-099Y-099M-099NJ-099NJ-7WGY-0B
2	ETBW 8858	SWSR22T. B./2*BLOUK #1//WBLL1*2/KURUKU	CMSS08Y01116T-099M-099Y-099M-099NJ-099NJ-14WGY-0B
3	ETBW 8870	WAXWING*2/TUKURU//KISKADEE #1/3/FRNCLN	CMSS08B00861T-099TOPY-099M-099NJ-099NJ-45WGY-0B
4	ETBW 8802	CHAM-4/SHUHA'S/6/2*SAKER/5/RBS/ANZA/3/KVZ/HYS/YMH/TOB/4/BOW'S"	ICW00-0634-6AP-0AP-0AP-23AP-0AP-0DZ/0AP-0DZ/0KUL/0SIN/0AP-0NJ/0AP-0ALK/0AP
5	ETBW 8991	SUP152//ND643/2*WBLL1	CMSS08Y00274S-099Y-099M-099NJ-099NJ-2WGY-0B
6	ETBW 8862	C80.1/3*BATAVIA//2*WBLL1/3/C80.1/3*QT4522//2*PASTOR/4/WHEAR/SOKOLL	CMSS08B00337S-099M-099NJ-099NJ-29WGY-0B
7	ETBW 8804	TURACO/CHIL/6/SERI 82/5/ALD'S/4/BB/GLL//CNO67/7C/3/KVZ/TI FALCIN/AE. SQUARROSA	ICW99-0052-2AP-0AP-0AP-11AP-0AP-0DZ/0AP-0DZ/0KUL/0SIN/0AP-0NJ/0AP-0ALK/0AP
8	ETBW 8996	(312)/3/THB/CEP7780//SHA4/LIRA/4/FRET2/5/DANPHE #1/11/CROC_1/AE. SQUARROSA (213)//PGO/10/ATTILA*2/9/KT/BAGE//FN/U/3/BZA/4/TRM/5/ALDAN/6/SERI/7/VEE#10/8/OPATA	CMSA08Y00096T-099B-050Y-040M-0NJ-8Y-0B
9	ETBW 8583	MINO/898.97/4/PFAU/SERI. 1B//AMAD/3/KRONSTAD F2004	CMSS08B00391S-099M-099Y-10M-0WGY
10	ETBW 8668	BAVIS*2/3/ATTILA/BAV92//PASTOR	CMSA08M00054T-050Y-040M-0NJ-5Y-0B
11	ETBW 8595	BAVIS*2/3/ATTILA/BAV92//PASTOR	CMSA08M00054T-050Y-040M-0NJ-5Y-0B
12	ETBW 8684	PASTOR/HXL7573/2*BAU/3/WBLL1/4/1447/PASTOR//KRICH	CMSA08M00406S-040ZTM-050Y-37ZTM-010Y-

Number	Name	Pedigree	Selection History
13	ETBW 9486	AUFF FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD	0B CMSS10B00779T-099TOPY-099M-099NJ-099NJ-5WGY-0B
14	ETBW 9547	#1//INQALAB 91*2/TUKURU MUTUS*2/AKURI//MUTUS*2/TECUE #1	CMSS11B00372S-099M-099NJ-099NJ-19WGY-0B
15	ETBW 9548	REEDLING #1//KFA/2*KACHU	CMSS11B00167S-099M-0SY-70M-0WGY
16	ETBW 9549	KFA/2*KACHU/3/KINGBIRD #1//INQALAB 91*2/TUKURU/4/KFA/2*KACHU	CMSS11B00958T-099TOPY-099M-099NJ-099NJ-8WGY-0B
17	ETBW 9550	KFA/2*KACHU*2//WAXBI	CMSS11B00961T-099TOPY-099M-099NJ-099NJ-20WGY-0B
18	ETBW 9551	KFA/2*KACHU/4/KACHU #1//PI 610750/SASIA/3/KACHU/5/KFA/2*KACHU	CMSS11B00957T-099TOPY-099M-099NJ-099NJ-8WGY-0B
19	ETBW 9552	KACHU #1/4/CROC_1/AE. SQUARROSA (205)//BORL95/3/2*MILAN/5/KACHU/6/KFA/2*KACHU	CMSS11B00214S-099M-099NJ-099NJ-22WGY-0B
20	ETBW 9553	MURGA/KRONSTAD F2004/3/KINGBIRD #1//INQALAB 91*2/TUKURU	CMSS11Y00116S-099Y-099M-0SY-4M-0WGY
21	ETBW 9554	SAUAL/MUTUS/6/CNO79//PF70354/MUS/3/PASTOR/4/BAV92 *2/5/FH6-1-7/7/CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7	CMSS11B00652T-099TOPY-099M-099NJ-099NJ-23WGY-0B
22	ETBW 9555	KFA/2*KACHU/5/WBLL1*2/4/BABAX/LR42//BABAX/3/BAB AX/LR42//BABAX/6/KFA/2*KACHU	CMSS11B00956T-099TOPY-099M-0SY-8M-0WGY
23	ETBW 9556	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/PARUS/PASTOR	PTSA08M00046S-050ZTM-050Y-50ZTM-010Y-0B-020Y-0MXI
24	ETBW 9557	SOKOLL/WBLL1/4/D67.2/PARANA 66.270//AE. SQUARROSA (320)/3/CUNNINGHAM	CMSA09M00466S-050ZTM-050Y-3WGY-0B
25	ETBW 9558	BABAX/LR42//BABAX/3/ER2000/5/ATTILA/4/WEAVER/TSC// WEAVER/3/WEAVER/6/KA/NAC//TRCH	CMSA09Y00383T-099B-050Y-050ZTM-0NJ-099NJ-11WGY-0B
26	ETBW 9559	CHIBIA//PRLII/CM65531/3/MISR 2*2/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF0 7	CMSS09Y00853T-099TOPM-099Y-099ZTM-099NJ-099NJ-24WGY-0B
27	ETBW 9560	CHWINK/GRACKLE #1//FRNCLN	CMSS09Y00753T-099TOPM-099Y-099ZTM-099NJ-099NJ-13WGY-0B
28	ETBW 9561	TRAP#1/BOW/3/VEE/PJN//2*TUI/4/BAV92/RAYON/5/KACHU #1*2/6/KINGBIRD #1	CMSS10Y00842T-099TOPM-099Y-099M-1WGY-0B
29	Morocco	Universal susceptible check/Cultivar (used in green house)	

ETBW: Ethiopian bread wheat; Source: Ethiopian national wheat research program and Ethiopian crop registration directory of 2016.

2.3. Collection of Wheat Stripe Rust Samples

A total of 6-stripe rust infected leave samples were collected from Kulumsa (3) and Meraro (3) respectively. Four to five freshly infected young leaves taken from experimental treatments and susceptible checks using sterile scissors and placed in glassine bags. Then after, infected leaf samples transported to a laboratory at KARC under dry conditions and then allowed to dry at room temperature for 24 hrs. Spores from each sample collected using motorized pump in a gelatin capsule and then stored at 4°C under freez dry conditions for a while [22].

2.4. Isolation and Multiplication of *P. Striiformis* Inoculum

The spores from each infected dried stripe rust samples were collected in a gelatin capsule using a motorized vacuum pump. Then, the spores were suspended in light mineral oil (i. esoltrol 130) and sprayed on to seven days old seedlings of susceptible bread wheat cultivar "Morroco" using motorized pump [22]. The plants were then misted with fine droplet of distilled water and incubated in a plastic dew chamber for 24 hrs at 9-10°C with close to 100% relative humidity. The seedlings were transferred to a greenhouse bench of temperature 15-18°C

and RH of 70%. Portions of spore samples were kept in a refrigerator at 4°C as a backup. Seven days after inoculation leaves containing a single fleck selected from the base of a leaf. Secondary leaves within a pot removed using sterilized scissors. A single leaf with fleck was covered with plastic bag envelop and tied up at the base with a rubber band to avoid cross contamination [23].

About 14-15 days after inoculation when the monopustule was well developed, each monopustule were collected in gelatin capsule using motorized vacuum pump. Spores harvested from a mono pustule were suspended with light weight mineral oil (soltrol 130) and inoculated on to seven days old seedlings of susceptible bread wheat cultivar "Morocco" as indicated in the previous section. About 14-15 days after inoculation, the spores from each mono pustule were collected in separate gelatin capsule as indicated above and stored at 4°C to be used for inoculation of stripe rust differential lines. These procedures repeated until sufficient amount of single spores produced for race analysis purpose. In this way, four mono pustules isolates developed for race analysis.

2.5. Growing of Seedlings of Differential Lines and Inoculation of *P. Striiformis* Isolates

Five seeds from each 19 differential lines and universal

susceptible check, Morocco were grown in $3 \times 3 \times 3$ cm diameter pots containing heat-sterilized soil, sand, and compost in 2:1:1 (v/v/v) ratio, respectively [24]. A single pustule derived spores that seem light yellow was suspended in lightweight mineral oil (soltrol 130), sprayed on to a seven days old seedlings using motorized pump, and then placed in dew chamber close to 100% RH at 9-10°C for 24 hours. Then, seedlings in each pot transferred to the greenhouse within a plastic cubicle to avoid contamination. The greenhouse temperature and humidity maintained between 15-18°C, 60-70%, respectively [22].

2.6. Disease Assessment on Differentials and Race Phenotyping

Genotypes seedling response to yellow rust scored 16-17 days after spraying using a 0-9 disease-scoring scale [25]. Infection types were grouped into low and high reaction type; where 'low' refers to resistance or incompatible (infection type 0-6) and 'high' refers to susceptible or compatibility (infection type 7-9). Accordingly, avirulence and virulence of isolates were determined by low (L) and high (H) infection types respectively. Physiological race designations and nomenclature or virulence phenotyping in this study was made based on full agreement with molecular works through single sequence repeat (SSR) [26, 27] procedure. Each lineage consisted of one or more closely related multi-locus genotypes ("strains") of a particular race (virulence phenotype), was named *P. striiformis*, followed by a forthcoming digit.

2.7. Genotyping of the Pathogens

For whole genome sequencing of *Pst* isolates, DNA was extracted for each isolate from dried single stripe lesion of infected plant urediniospores using the method as described by [28] and DNA quantity was confirmed using multiplex-based microsatellite genotyping as described by [29, 30]. While race nomenclature and designation was performed as

procedures of [26]. Molecular works were executed at Holeta biotechnology research laboratory by sending copy of the prepared monopustules with the help of CIMMYT and British field pathogenomics laboratory research team.

2.8. Seedling Evaluation of Test Wheat Lines Against Stripe Rust

All the twenty-eight bread wheat genotypes with universal susceptible check 'Morocco' were evaluated against two different *P. striiformis* races under greenhouse conditions. Similar procedures applied as indicated in sections 2.4, 2.5 and 2.6 for seedling growth, inoculation, incubation and disease scoring. Data on infection types were recorded 15 days after inoculation using standard disease score scale 0-9 [25]. Infection types 0 to 6 were considered resistant, while 7 to 9 as susceptible [22].

3. Results and Discussions

3.1. Identification of *P. Striformis* races Employed in Seedling Screening Process

Among six samples collected from both Kulumsa and Meraro nursery sites, two monopustules isolates from each site, were analyzed to race types. The number of isolates was determined based on the amount of available seeds of differential lines needed to conduct the race analysis work. Of these four isolates, two races of *P. striiformis* were identified variably with experimental sites i.e. Kulumsa and Meraro as presented along with common name, genetic lineage and virulence phenotype (Table 2). Isolates collected from Meraro experimental station yielded only race *PstS2* while Kulumsa site yielded races *PstS11* and *PstS2*. Race *PstS11* was more frequent than race *PstS2*. Both phenotypes, *PstS11* and *PstS2* were previously detected in Ethiopia and other parts of the world [31, 27, 32].

Table 2. Identified stripe rust races and their distributions across locations.

Location	Number of Isolates	Common name	Genetic Linage	Virulence Phenotype
Meraro	Isolate 1	<i>PstS2</i>	<i>PstS2</i>	-2,-,-,6,7,8,9,-,-,-,25,-,-,-, AvS,-
	Isolate 2	<i>PstS2</i>	<i>PstS2</i>	-2,-,-,6,7,8,9,-,-,-,25,-,-,-, AvS,-
Kulumsa	Isolate 1	<i>PstS2</i>	<i>PstS2</i>	-2,-,-,6,7,8,9,-,-,-,25,-,-,-, AvS,-
	Isolate 2	"AF2012"	<i>PstS11</i>	-2,-,(4)-,6,7,8,-,-,-,17,-,-,27,32,-, AvS,-

Symbols designate virulence and avirulence (-) corresponding to yellow rust resistance genes: *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr25*, *Yr27*, *Yr32*, and the resistance specificity of Spalding Prolific (Sp), Avocet S (AvS) and Ambition (Amb), respectively. *Pst*; *Puccinia striiformis tritici*.

The race *PstS11* was detected for the first time in East Africa in the autumn of 2016, after being first detected in Afghanistan in 2012 and 2013 (for the moment designated "AF2012"). The race was prevalent in epidemics in Ethiopia, where a series of varieties were severely affected by yellow rust. *PstS11* and its variants known to overcome

the resistance of *Yr2*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr17*, *Yr25*, *Yr27* and *Yr32* [31] where most of the Ethiopian cultivar wheat genotypes carry. For instance, it severely affects one of the popular cultivar "Digalu" and result heavy economic injury in wheat farming community since 2016 to present [33].

Similarly, the destructive race *PstS2* was again noticed across wide areas including Ethiopia [34, 31, 26]. In East Africa, races from the *PstS2* and the *PstS6* lineage dominated the overall population and these races are virulent on *Yr17* and *Yr2*, two extensively deployed resistance genes in the region [35].

3.2. Seedling Evaluation of Tested Hexaploid Wheat Lines Against Identified Phenotypes of Stripe Rust

Seedlings of all 28 breeding lines including susceptible check 'Morocco' were assessed for the seedling resistance against two physiologic stripe rust races namely, *PstS2* and *PstS11* identified in the present study. There were successful inoculations as shown by the susceptible infection types of the check "Morocco" IT of 9 (Table 3).

Table 3. Seedling Infection Types of Advanced Hexaploid Wheat Lines against Identified *Pst* Races in 2018.

Line	Seedling infection types	
	<i>PstS2</i>	<i>PstS11</i>
ETBW-8751	8	8
ETBW-8858	7	7
ETBW-8870	8	8
ETBW-8802	7	8
ETBW-8991	6	6
ETBW-8862	1	2
ETBW-8804	5	7
ETBW-8996	5	5
ETBW-8583	5	0
ETBW-8595	7	7
ETBW-8668	7	7
ETBW-8684	7	6
ETBW-9486	7	7
ETBW-9547	6	3
ETBW-9548	7	7
ETBW-9549	8	7
ETBW-9550	7	8
ETBW-9551	8	8
ETBW-9552	6	8
ETBW-9553	8	8
ETBW-9554	5	5
ETBW-9555	6	7
ETBW-9556	8	8
ETBW-9557	8	8
ETBW-9558	8	8
ETBW-9559	9	8
ETBW-9560	6	4
ETBW-9561	7	7
Morocco	9	9

Generally, the greenhouse experiments revealed that the bread wheat lines differed in their reaction to the stripe rust phenotypes and were categorized in to two groups as shown in Figure 1.

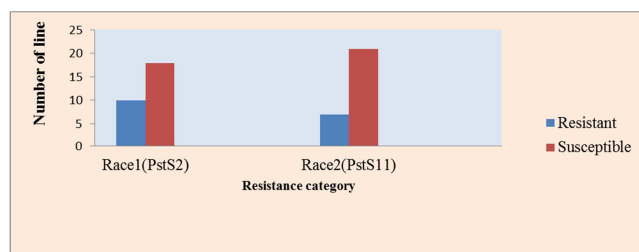


Figure 1. Frequency of tested wheat lines under resistance and susceptible reaction against two *Puccinia striiformis* f. sp. *tritici* isolates at seedling stage.

Out of 28 wheat lines tested in a greenhouse, ten lines (ETBW-8991, ETBW-8862, ETBW-8804, ETBW-8996,

ETBW-8983, ETBW-9547, ETBW-9552, ETBW-9554, ETBW-9555 and ETBW-9560); and seven lines (ETBW-8862, ETBW-8996, ETBW-8583, ETBW-8684 and ETBW-9560) showed resistance reactions (0 to 6) infection types against *PstS2* and *PstS11* races respectively. This group most probably carried major gene (s) that were effective against all the pathotypes present. However, the genotypes with race-specific resistance often become susceptible within a few years after their release. This could be due to the rapid evolution of new virulent races of the rust pathogens [36] except resistance conditioned by APR genes [37]. The lines included in this group may also contain race-nonspecific resistance genes that their effects were masked by effective race-specific resistance genes [38].

Conversely, 28.6% (eight) and 75% (twenty-one) lines had compatible reactions (infection types 7-9) against *PstS2* and *PstS11* respectively. While the susceptible check, Morocco, displayed infection type 9 to both races. The result of seedling assessment for the 28 tested lines is presented in Appendix Table 1. Wheat lines could be susceptible at seedling tests but exhibit moderate resistance to moderate susceptible reaction at adult plant stage and these lines with slow-rusting resistance parameters at adult plant stage could have durable resistance [39]. This kind of resistance can be kept for a long time even if the pathogen changes its genotype, because durable resistance, such as slow rusting and high temperature adult plant resistance (HTAP), is governed by more than one gene (at least 2–3) [40]. Thus, these lines were supposed to confer genes for varying degrees of adult plant rusting resistance that can be /used for future manipulation in wheat improvement programs after confirmatory studies.

4. Conclusion

The present study revealed, majority of the tested hexaploid wheat lines lacked seedling resistance against both races indicating that the lines probably carried many minor genes. These lines may have genes that could be effective against multiple stripe rust races. However, field based stripe rust evaluation should be conducted for adult plant resistance (durable resistance). In addition marker assisted selection is needed to confirm what resistant genes are carried in adult plant resistant lines therefore may play an important role to develop resistant wheat cultivars in Ethiopia.

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