Mining a Collection of *triticum diccoccoid* Landraces for Resistance to Races of *Puccinia graminis* f. sp. t*ritici* at Seedling Stage

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Abstract

Stem rust caused by **Puccinia graminis** *Pers. f. sp.* **tritici** *Eriks. and E. Henn. (Pgt), is one of the most destructive pathogen of wheat which causing considerable yield losses. It has become a renewed threat to global wheat production after the emergence and spread of new race known as TTKSK or Ug99 and related races from Africa. Races of the pathogen in the "Ug99 lineage" are of international concern due to their virulence for widely used stem rust resistance genes and their spread throughout Africa. Seedlings of 183 diccocciod wheat accessions which were assembled by University of Bologna, department of agricultural sciences were evaluated for their response to stem rust (***Puccinia graminis** f. sp. **tritici***) infection under greenhouse condition at University of Minnesota St. Paul campus during 2012 with an objective of identifying diccoccoid wheat accessions that could serve as sources of resistance to stem rust to enhance durable resistant variety development. Seedling infection types were evaluated using three stem rust races viz TTKSK, TRTTF and TTTTF. A high level of phenotypic variation was observed in response to races TTKSK, TRTTF and TTTTF in the test entries, allowing for selection in these germplasm as a pre-breeding work. Out of the tested accessions, 32 diccoccoid wheat accessions exhibited low infection types (0–2) response to all the three races and hence selected as a source of resistance to stem rust. These wheat germplasm which are identified as resistant along with adult plant resistant germplasm will be promising genetic stocks for accumulating resistances genes to acquire durable resistance and long lasting variety/ies.*

Key words: Landraces, *Puccinia graminis*, seedling resistance, *triticum diccocid*,

Introduction

Stem rust caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and E. Henn. (*Pgt*), is one of the most important diseases of wheat in many regions of the world (Leonard, 2001; Hodson, 2011). During severe epidemics, the disease can cause yield losses exceeding 50–70% in both hexaploid (*Triticum aestivum* L.) and tetraploid (*Triticum turgidum* ssp.) wheats. Additionally, wheat infected by stem rust can also suffer reduced end use quality and food security (Singh *et al*.,_2005). Combinations of different stem rust resistance (*Sr*) genes were successfully introgressed into wheat cultivars worldwide since the 1950s, and this gene deployment scheme has effectively controlled the disease for many years (Singh *et al*., 2011). However, the discovery of a new aggressive race (TTKSK, isolate Ug99) of *Pgt* in Uganda in 1998 (Pretorius *et al*., 2000) threatens wheat production due to its wide virulence on over 80% of wheat cultivars worldwide (Singh *et al*., 2006; Yu *et al*., 2012). Moreover, at least eight variants with different virulence patterns have been described from the "Ug99 lineage" of African stem rust races, further complicating the resistance breeding process.

Both qualitative and quantitative resistances have been reported in wheat against stem rust. Qualitative resistance is controlled by major race-specific genes ("*R*" genes) that are effective against some pathogen isolates, but not others. Oftentimes, this resistance is expressed throughout all growth and development stages of the plant. Quantitative resistance is usually based on multiple genes, each with a minor effect on slowing disease development by delaying pathogen infection, growth and/or reproduction. Since this type of resistance is phenotypically evident in adult plants only, it has been described as "adult plant resistance" (APR). To date, more than 58 stem rust resistance genes have been designated in wheat (McIntosh *et al*., 2011).

In addition, several alleles conferring unique race specificities have been identified for many of these genes, resulting in a total of 65 numerically designated resistance genes and alleles (*Yu et al*., 2014). Of this number, at least 27 are effective or partially effective against races in the Ug99 lineage (Yu *et al*., 2014). With regards to quantitative resistance, five APR genes have been designated (Yu *et al*., 2014). *Sr2*, identified in *T. turgidum*, is a quantitative resistance gene located on chromosome arm 3BS. It has been widely used in wheat breeding programs, providing durable APR for more than 50 years (Yu *et al*., 2011). However, *Sr2* only provides partial APR and is associated with pseudo black chaff, a trait that facilitates the selection of breeding lines carrying *Sr2* but may reduce yield, especially when expressed in the glumes (Mago *et al*., 2011).

Ethiopia is one of the most hot spot areas for the development of the present wheat stem rust complex (Betasilassie, 2007). The disease has become a major threat of wheat production after the epidemics of 1974 and 1993, 2010 and 2014 that drove out many Ethiopian bread wheat (*Triticum aestivum* L.) varieties, such as *Lacketch*, *Enkoy* and *Digelu* of production. Achieving more durable resistance will depend on deploying diverse combinations of race-specific qualitative resistance and/or race-nonspecific quantitative resistance genes.

To combat with stem rust, various strategies are being applied worldwide. Use of fungicide and agronomic practices has proved to be fruitful to reduce the losses to some extent. Although chemical method is in vogue all around the world, this remedial measure is not affordable by the farmers in developing countries. Most feasible method otherwise is host genetic resistance to control stem rust. Utilization of genetic resistance is economical, and carries no health and environmental hazards (Chen, 2007; Farrokhi *et al*., 2011). Furthermore, resistant varieties fight with the disease for a longer time ensuring crop sustainability.

Two types of genetic resistance, race specific and race non-specific are well-recognized. The former type of resistance works according to gene for gene model first proposed by Flor *et al*. (1942). After the evolution of new stem rust strains, race specific genes become ineffective approximately within three to five years (Line and Qayoum, 1992). Race non-specific resistance is controlled by minor genes and is long lasting. Judicious use of genetic resistance has been proposed as gene pyramiding of major (race specific) and minor (race non-specific) genes. Resulting varieties equipped with conglomeration of both type of resistances will sustain longer against the pathogen (Singh *et al.,* 2004). Therefore to manage the pathogen adequately, wheat genetic resources with diverse resistances are needed (Bux *et al*., 2011). In this regard, characterization of wheat germplasm for identification of such diverse resistances is paramount. Present study was carried out to identify wheat genetic resources with different types of resistance to enhance cultivar improvement efforts in Ethiopia.

. The wild and cultivated relatives of wheat offer a tremendous potential to be used as a source of stem rust resistance, and to broaden the genetic basis of wheat cultivars. Landraces are priority, as they may possess wide range of variation, specific adaptation to the different environmental conditions in their regions of growth, and resistance or tolerance to diseases and insect pests (Alex *et al.,* 1997).

The development of rust-resistant wheat cultivars using seedling reaction type as a predictor of adult-plant resistance has been conducted globally, with different countries placing emphasis on those rust species of economic concern to them. In the present study, a diccoccoid wheat collection was evaluated for resistance to stem rust races such as TTKSK, TRTTF and TTTTF under the controlled conditions of a greenhouse. Hence, this study was carried out with the aim of evaluating diccoccoid wheat accessions to enhance the breeding strategies in identifying the best parents to be used in the breeding program in fight against stem rust.

Materials and Methods

Plant materials

A total of 183 durum wheat accessions representing 24 countries were included in this study. Of these, 41, 27, 22, 15, 12, 12, accessions are from Ethiopia, Iran, Spain, UK, Italy and Russia, whereas the remain countries are represented by accessions ranging from 1 to 7. Details of sources of accessions, their code, and response to the three races were presented in Table 3.

Stem rust evaluation

Pathogen races and their virulence

All diccoccoid wheat accessions were evaluated for seedling resistance to three *Pgt* races: TTKSK, TRTTF and TTTTF in a greenhouse at the USDA Cereal Disease Laboratory in St. Paul, MN during 2012. The race designation is based on the letter code nomenclature system (Roelfs and Martens, 1988; Roelfs *et al.*, 1993), modified to further delineate races in the TTKS lineage (Jin *et al.*, 2008). These races were selected based on their differential virulence pattern and/or importance for durum wheat. Race TTKSK (Ug99) has a wide virulence spectrum and is rapidly evolving in East Africa. Race TTTTF is the most widely virulent race known in the United States, producing high infection types (ITs) on the majority of stem rust differential lines (Jin *et al.*, 2007). Races TRTTF present in Ethiopia, possess a virulence combination that overcomes both the resistance genes *Sr13* and *Sr9e*, two genes present at high frequency in durum wheat (Klindworth *et al.*, 2007). All isolates were derived from single pustules, increased in isolation, and stored at -80^{o}C. Information about the stem rust isolates used in the disease phenotyping test is summarized in Table 1.

Table 1. Origin and virulence properties of the *Puccinia graminis f.sp. tritici* races used to evaluate the diccocoid wheat accessions.

Race	Isolate	Origin	Virulence/avirulence formula
TTKSK (Ug99)	04KEN156/04	Kenya	*Sr5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 30, 31, 38, McN/ Sr24, 36, Tmp*
TRTTF	06YEM34-1	Yemen	*Sr5, 6, 7b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 30, 36, 38, McN/ Sr8a, 24, 31*
TTTTF	01MN84A-1-2	United States	*Sr5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 30, 36, 38, McN/ Sr24, 31*

(Source: Olivera *et al*., 2012)

Inoculation, incubation, and disease assessment

Diccoccoid wheat accessions were evaluated under controlled environment at the Cereal Disease Laboratory, St. Paul, MN using a Completely Randomized Design with two replications for each of the three races. Any accessions exhibiting variable reactions across the replicates were repeated again in a third test. Five to six seedlings per line were inoculated on the fully expanded primary leaves 8 to 9 days after planting. The experimental procedures in inoculation and disease assessment were performed as described by Jin *et al*. (2007). Wheat cultivar McNair 701 (CItr 15288) was used as susceptible control in all evaluations to monitor the virulence of the race. Wheat seedlings were evaluated for their Infection Types (ITs) 14 days post inoculation using the 0 to 4 scale according to Stakman *et al*. (1962), where ITs of 0, ;, 1, 2, or X are considered as incompatible (low ITs), where as ITs 3 or higher were considered as compatible (high ITs) (Table 2).When IT = 0 (immune reaction) occurred, the test was repeated to exclude the possibility of disease escape. Accessions giving variable reactions between experiments were repeated two to three times to confirm the most likely reactions.

Table 2. Description of infection type (ITs) and symptoms based on Stakman *et al* (1962) scale of seedling score

Infection type	Symptoms
0	No uredinia or other macroscopic sign of infection
0;	Few faint flecks
;	No uredinia but hypersensitive necrotic or chlorotic flecks are present
1	Small uredinia often surrounded by a necrosis
2	Small to medium uredinia often surrounded by chlorosis
3	Medium sized uredinia without chlorosis or necrosis
4	Large uredinia without chlorosis or necrosis

Results

Evaluation of the dicoccoides wheat collection for resistance to stem rust races

Seedling Infection Types (ITs) for each diccoccoides accessions were presented in Table 3. In all of the seedling tests, the susceptible controls of McNair 701 was heavily infected and exhibited the expected compatible ITs ranging from 3 to 4 to all the three races. The high levels of infection achieved in each experiment allowed for the reliable scoring of ITs on all accessions. A high level of variability was observed in response to stem rust races TTKSK, TRTTF and TTTTF in the diccoccoides wheat collection (Figure 1), thereby allowing for the identification of resistant germplasm in these world collection of diccoccoides gene pool of wheat accessions.

The test accessions showed significant variation in the responses to different rust races. At the seedling stage, a relatively high proportion of the accessions were resistant (converted scale values of 0 to 4) to TTTTF (78%) with almost 50% of accessions showing flecks to small uredinia often surrounded by a necrosis (converted scale 0 and 1) and about 37.7% of accessions showing resistance to the race TTKST (Ug99) with score of 0, 1 and 2 (converted scale 0, 1, and 2). For races TRTTF, a lower proportion of accessions (24.6%) showed resistance (all of these resistance accessions showed a score of 2 and with almost zero accessions having score of 0 or 1. About 22%, 62.3% and 75.4% accessions were highly susceptible (IT= 3 and 4) to races TTTTF, TTKSK and TRTTF, respectively. A total of 32 (17.5%) accessions showed resistance to all races and 34 (18.6%) accessions were susceptible to all the three stem rust races.

The frequencies of the accessions categorized as resistant and susceptible in their reaction to the three races varied markedly depending on the race. The presence of highly virulent races in the population of stem rust represents a great threat to commercial wheat production. The results of tests of the diccoccoides accessions to three races showed that the tested entries differ in their resistance to stem rust disease (Table 4). For example, seedling resistance to TTTTF, TTKSK and TRTTF was observed in 114 (77.1%), 67 (36.6%) and 45 (24.6%) accessions respectively (Table 3). The ranking values of the three races based on their frequencies of avirulence/virulence interactions considering the diccoccoides accessions as a whole (with TTTTF showing the highest degree of avirulence interactions, followed by TTKSK and TRTTF, which showed the highest frequency of virulent interactions).

Generally, the high variability in response to seedling infection to different races supports the significance of the race x accessions interaction. The current result reveals that these diccoccoides germplasm are rich source of stem rust resistance. Resistance to wheat stem rust at the seedling stage was reported in tetraploids by Olivera *et al.* (2011).

Similar result was also reported by Beteselassie *et al.* (2007) in Ethiopian emmer wheat, as 18 out of 41 accessions were resistant to a bulk of six local isolates of stem rust in Ethiopia.

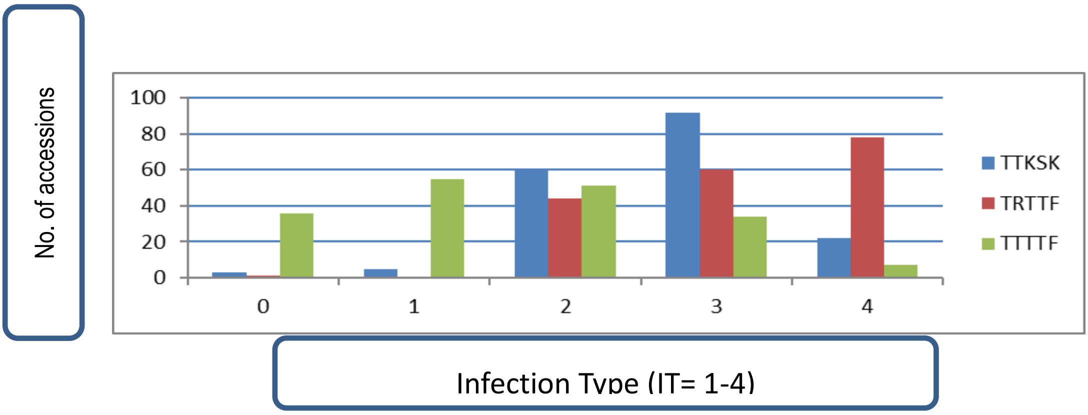

Figure 1. Frequency distribution of infection types (ITs) of 183 diccocoid accession evaluated at the seedling stage with three stem rust races at St. Paul, MN during 2012.

Geographical distribution of resistance germplasm to the three stem rust races

TTKSK: Out of 41 accessions collected from Ethiopia, 16 accessions (39%) were rated as resistant (ITs 0; 1; or 2) to race TTKSK (Table 1), Similarly, out of 27 diccoccoid accessions collected from Iran, the majority has showed resistance reaction (ITs 0, 1 or 2) to this race (18 accessions = 67%) and for those collection that made from Spain only 3 out of 22 (13.7%) were found to be resistant to race TTKSK and similarly for accession collected from UK, only 2 out of 15 (13%) were found to be resistant to race TTKSK. All accessions collected from Italy, however, were found to be susceptible to race TTKSK.

TRTTF: Most of the diccoccoides accessions collected from Ethiopia were susceptible to this race (26 out of 41= 63.4%) and similarly, for accessions collected from Iran the majority (22 out of 27 (81.5%) were susceptible to this race. Out of the three races, relatively, this race is the most aggressive race and most of the accessions collected from different countries are susceptible to this race (example, for accessions that were collected from Spain, only two accessions showed resistance to this race).

TTTTF: Except for those accessions collected from Ethiopia and UK which showed susceptible reaction to this race, most of the accessions collected from different countries showed resistance to this race. Out of 41 accessions collected from Ethiopia, 21 accessions (51.2%) and out of 15 accessions collected from UK, 8 accessions (53.3%) were susceptible to this race.

All races: out of 183 diccoccoides accessions, 32 accessions were found to be resistant to all the three stem rust races used in this study, while, 34 accessions were found to be susceptible to all the three stem rust races used in this study. Out of 32 accessions which showed resistance reaction to all the three races, 9 accessions were collection from Ethiopia and also out of 34 accessions which showed susceptible reaction to all the three races, 16 of them were collection from Ethiopia (Table 3). These identified accessions are considered in breeding program to develop wheat varieties with durable resistance. Some of them are now included in breeding program as parental lines to improve commercial cultivars of wheat.

Table 3. Code, origin maturity group and phenotypic response to three stem rust races (TTKSK, TRTTF and TTTTF) of 183 diccoccoid landraces evaluated at St. Paul MN during 2012

S.N	Diccoccoid code	source	Maturity group	TTKSK	TRTTF	TTTTF
1	IDG 114	Ethiopia	Early	3	3	3
2	IDG 392	Ethiopia	Early	2	2	3
3	IDG 2133	Ethiopia	Early	3	3	3
4	IDG 5819	Italy	Medium	4	4	2
5	I DG 8632/1	Italy	Medium	3	4	1
6	I DG 8632/2	Italy	Medium	3	4	1
7	IDG 8634	Italy	Medium	3	4	1
8	IDG 8649	Italy	Medium	3	4	2
9	IDG 8727	Italy	Medium	3	4	1
10	IDG 8784/1	Italy	Medium	3	4	2
11	IDG 8784/2	Italy	Medium	4	4	2
12	IDG 10391/1	Italy	Medium	3	4	2
13	IDG 10391/4	Italy	Late	3	4	1
14	IDG 10899	Italy	Medium	3	4	2
15	MG 3428	Kenya	Medium	0	2	2
16	MG 3429	Kenya	Late	3	4	1
17	MG 3430	Kenya	Late	2	2	0
18	MG 3431	Kenya	Medium	2	2	2
19	MG 3521	Russia	Medium	2	4	0
20	MG 3521/1	Russia	Late	3	4	3
21	MG 4356	UK	Medium	3	3	1
22	MG 4358	UK	Late	3	4	3
23	MG 4360	UK	Late	3	4	3
24	MG 4366/1	UK	Late	3	4	1
25	MG 4366/2	UK	Early	2	2	2
26	MG 4367	UK	Medium	2	4	2
27	MG 4369/1	Iran	Late	2	4	1
28	MG 4369/2	Iran	Medium	3	4	2
29	MG 4374/1	UK	Medium	3	3	1

30	MG 4374/2	UK	Late	3	4	4
31	MG 4375/1	UK	Late	4	4	3
32	MG 4375/2	UK	Medium	4	4	2
33	MG 4376	UK	Late	3	4	3
34	MG 4382	UK	Medium	3	4	3
35	MG 4384	UK	Early	3	4	3
36	MG 5268	Ukrain	Medium	1	3	0
37	MG 5269	Russia	Medium	1	3	0
38	MG 5270	Russia	Medium	2	2	0
39	MG 5273	Iran	Medium	2	3	1
40	MG 5274	Armenia	Medium	2	3	0
41	MG 5275	Iran	Medium	2	3	0
42	MG 5276	Iran	Medium	2	4	0
43	MG 5277	Iran	Medium	3	4	0
44	MG 5281/1	Ethiopia	Late	1	3	0
45	MG 5282	Ethiopia	Early	2	2	2
46	MG 5285	Morocco	Early	1	2	2
47	MG 5287/1	Iran	Medium	3	3	3
48	MG 5287/2	Iran	Medium	4	2	0
49	MG 5290	Iran	Medium	2	2	2
50	MG 5297/1	Bulgaria	Late	3	4	2
51	MG 5300/3/1	Bulgaria	Late	3	4	0
52	MG 5300/3/2	Bulgaria	Medium	4	4	1
53	MG 5302/1	Armenia	Late	3	2	0
54	MG 5303/1	Germany	Late	3	4	1
55	MG 5305	Unknown	Late	3	4	0
56	MG 5306	Ethiopia	Late	2	2	3
57	MG 5307	Russia	Late	3	3	1
58	MG 5310	Russia	Medium	3	4	0
59	MG 5312	Afghanistan	Early	3	2	3
60	MG 5313	Armenia	Late	2	3	1
61	MG 5314	Georgia	Late	0	2	0
62	MG 5315	Georgia	Late	2	2	1
63	MG 5317/1	Russia	Early	2	3	2
64	MG 5317/2	Russia	Early	3	3	1
65	MG 5320/1	Germany	Late	4	3	1
66	MG 5327	India	Early	2	2	0
67	MG 5331/1	Spain	Late	3	3	2
68	MG 5333/1	Spain	Late	3	3	1
69	MG 5334/1	Spain	Late	4	4	3
70	MG 5335/1	Spain	Late	3	4	1
71	MG 5335/2	Spain	Late	2	2	0
72	MG 5338/1	Ethiopia	Medium	3	4	1
73	MG 5339/1	Ethiopia	Late	3	3	0
74	MG 5339/2	Ethiopia	Early	3	2	2
75	MG 5340	Ethiopia	Early	4	3	3
76	MG 5343	Unknown	Early	2	2	2
77	MG 5344/2	Ethiopia	Late	4	3	1
78	MG 5344/3	Ethiopia	Late	3	3	1
79	MG 5345	Ethiopia	Early	3	3	3
80	MG 5346/1	Ethiopia	Early	2	0	3
81	MG 5346/2	Ethiopia	Early	2	2	2
82	MG 5347/1	Ethiopia	Early	3	3	3
83	MG 5347/2	Ethiopia	Early	2	2	1
84	MG 5350/2/1	Ethiopia	Early	3	3	3
85	MG 5350/2/2	Ethiopia	Early	3	3	3

86	**MG 5351**	**Ethiopia**	**Early**	2	2	2
87	**MG 5353**	**Ethiopia**	**Medium**	3	3	3
88	**MG 5354/1**	**Ethiopia**	**Early**	3	4	4
89	**MG 5354/2**	**Ethiopia**	**Early**	3	3	3
90	**MG 5357/4**	**Ethiopia**	**Early**	4	2	4
91	**MG 5359**	**Ethiopia**	**Early**	3	3	3
92	**MG 5361**	**Ethiopia**	**Early**	2	3	2
93	**MG 5362**	**Ethiopia**	**Early**	3	3	1
94	**MG 5363**	**Ethiopia**	**Early**	3	3	4
95	**MG 5366**	**Ethiopia**	**Early**	2	2	2
96	**MG 5368**	**Ethiopia**	**Early**	2	2	4
97	**MG 5377**	**Ethiopia**	**Early**	3	3	4
98	**MG5380/1/1**	**Serbia**	**Late**	4	3	3
99	**MG5380/1/2**	**Serbia**	**Late**	3	4	3
100	**MG 5381**	**Yugoslavia**	**Medium**	2	3	2
101	**MG 5383**	**Yugoslavia**	**Medium**	3	4	2
102	**MG 5384**	**Yugoslavia**	**Medium**	3	4	2
103	**MG 5385**	**Iran**	**Medium**	2	3	1
104	**MG 5386**	**Iran**	**Medium**	2	3	0
105	**MG 5388/1**	**Iran**	**Early**	2	2	2
106	**MG 5388/2**	**Iran**	**Early**	2	2	0
107	**MG 5389**	**India**	**Early**	2	2	0
108	**MG 5390**	**India**	**Early**	2	3	2
109	**MG 5394**	**Ethiopia**	**Medium**	3	3	1
110	**MG 5395/1**	**Ethiopia**	**Early**	2	2	2
111	**MG 5395/2**	**Ethiopia**	**Early**	2	2	2
112	**MG 5397/1**	**Unknown**	**Early**	2	2	2
113	**MG 5397/2**	**Unknown**	**Early**	3	3	2
114	**MG 5398**	**Germany**	**Early**	2	3	1
115	**MG 5399/3**	**Germany**	**Medium**	2	3	1
116	**MG 5401**	**Iran**	**Medium**	2	4	1
117	**MG 5402/1**	**Iran**	**Medium**	3	4	0
118	**MG 5402/2**	**Iran**	**Medium**	3	4	1
119	**MG 5405**	**Iran**	**Medium**	3	4	3
120	**MG 5407**	**Iran**	**Late**	2	4	0
121	**MG 5409**	**Iran**	**Late**	2	3	0
122	**MG 5411**	**Iran**	**Late**	2	4	0
123	**MG 5416**	**Iran**	**Medium**	3	4	1
124	**MG 5423**	**Iran**	**Medium**	2	4	0
125	**MG 5427**	**Iran**	**Medium**	2	3	0
126	**MG 5431**	**Iran**	**Medium**	2	2	1
127	**MG 5432**	**Iran**	**Medium**	2	3	0
128	**MG 5433**	**Iran**	**Late**	2	3	1
129	**MG 5437**	**Russia**	**Medium**	0	2	0
130	**MG 5442**	**USA**	**Late**	3	3	3
131	**MG 5446**	**UK**	**Medium**	3	4	0
132	**MG 5447**	**Hungary**	**Late**	4	4	1
133	**MG 5449**	**Hungary**	**Medium**	2	3	1
134	**MG 5450**	**Hungary**	**Late**	3	4	1
135	**MG 5451**	**Hungary**	**Medium**	3	4	2
136	**MG 5453/1**	**Hungary**	**Medium**	3	3	0
137	**MG 5453/2**	**Hungary**	**Medium**	3	3	0
138	**MG 5454**	**Hungary**	**Late**	3	2	3
139	**MG 5463**	**Spain**	**Early**	3	4	1
140	**MG 5465**	**Spain**	**Late**	4	4	1
141	**MG 5466**	**Spain**	**Late**	3	4	1

142	**MG 5467**	**Spain**	**Late**	3	4	2
143	**MG 5468/1**	**Spain**	**Medium**	4	4	2
144	**MG 5468/2**	**Spain**	**Early**	2	2	1
145	**MG 5469**	**Spain**	**Late**	2	4	2
146	**MG 5470**	**Spain**	**Medium**	3	4	2
147	**MG 5473**	**Spain**	**Late**	3	4	1
148	**MG 5474**	**Spain**	**Late**	3	4	1
149	**MG 5476**	**Spain**	**Medium**	3	4	0
150	**MG 5477**	**Spain**	**Late**	3	4	1
151	**MG 5478**	**Spain**	**Late**	3	4	1
152	**MG 5481**	**Spain**	**Late**	3	4	1
153	**MG 5492**	**Spain**	**Medium**	3	4	2
154	**MG 5494/1**	**Spain**	**Late**	3	3	1
155	**MG 5494/2**	**Spain**	**Medium**	3	4	2
156	**MG 5504**	**Ethiopia**	**Early**	4	3	3
157	**MG 5508**	**Ethiopia**	**Early**	2	3	1
158	**MG 5513**	**Romania**	**Medium**	3	4	3
159	**MG 5516**	**Romania**	**Medium**	4	2	2
160	**MG 5527/1**	**Austria**	**Medium**	3	4	1
161	**MG 5527/2**	**Austria**	**Early**	2	2	1
162	**MG 5529**	**UK**	**Medium**	3	3	3
163	**MG 5530**	**Ethiopia**	**Early**	3	3	2
164	**MG 5536**	**India**	**Early**	2	2	1
165	**MG 5537**	**Russia**	**Early**	2	2	0
166	**MG 5545**	**Russia**	**Medium**	3	3	1
167	**MG 5546**	**Russia**	**Early**	3	4	2
168	**MG 5549**	**Ethiopia**	**Early**	2	2	2
169	**MG 5550**	**Ethiopia**	**Early**	2	2	2
170	**MG 5556**	**Ethiopia**	**Early**	4	4	3
171	**MG 5557**	**Ethiopia**	**Early**	3	3	4
172	**MG 5563/1**	**China**	**Medium**	2	3	1
173	**MG 5563/2**	**China**	**Medium**	1	2	2
174	**MG 5567**	**USA**	**Early**	3	2	2
175	**MG 15516**	**Syria**	**Early**	2	2	1
176	**MG 15518**	**Syria**	**Medium**	3	4	2
177	**MG 15528**	**Germany**	**Medium**	4	3	3
178	**MG 15529**	**Germany**	**Medium**	4	4	3
179	**IMG 15530**	**Italy**	**Early**	4	4	0
180	**MG 28056**	**Yemen**	**Medium**	3	3	1
181	**MG 28057**	**Yemen**	**Medium**	2	4	2
182	**MG 29292**	**Yugoslavia**	**Medium**	4	4	2
183	**MG 5400/5**	**Iran**	**Medium**	3	4	2

Table 4. Numbers, frequencies of infection types (ITs) , resistant/ susceptible reactions of the 183 diccoccoid wheat collections to three races of Puccinia graminis f.sp. tritici and the combined reaction to all the three races

IT1/Reaction	TTKSK (Ug99)		TRTTF		TTTTF		All races	
	Lines	%	Lines	%	Lines	%	Lines	%
0	3	1.6	1	0.5	41	22.4	-	-
1	6	3.3	0	0.0	49	26.8	-	-
2	58	31.7	44	24.0	51	27.9	-	-
Resistant reaction	67	36.6	45	24.6	141	77.1	32	17.5
3	88	48.1	60	32.8	35	19.1	-	-
4	28	15.3	78	42.6	7.0	3.8	-	-
Susceptible reaction	116	63.4	138	75.4	42	22.9	34	18.6

NB: 1Infection types observed on seedlings at 14 days post-inoculation using a 0 to 4 scale according to Stakman et al. (1962), where infection types of; 1 or 2, are considered as a low IT and ITs of 3 or higher are considered as a high IT

Conclusions and Breeding Perspectives Recommendations

Broadening the genetic base of wheat varieties for their resistance to rust diseases needs to consider resistance sources. The current study addressed the responses of diccoccoid accessions to stem rust infection with objective to identify wheat genetic resources for stem rust resistance to enhance cultivar improvement efforts. This world collection of diccoccoid wheat accessions revealed the potential genetic variation present in this germplasm pool. Based on a set of *Pgt* isolates belonging to three races chosen to represent the most virulent, diverse, and aggressive pathotypes challenging durum wheat worldwide, that is, the TTKSK (= Ug99) race now diffused throughout Central and Northeast Africa and Iran in Asia (Singh *et al*., 2006), The North American TTTTF race (Jin *et al*., 2008) and one recently described and highly virulent Ethiopian races (TRTTF) that overcame some of the few resistance genes effective against Ug99 (Olivera *et al*., 2012). This collection of diccoccoides accession is a potential reservoir for several novel resistance genes of seedling resistance.

Identifying new sources of resistance to stem rust including Ug99 and other virulent races provides wheat breeders with an increased diversity of *Sr* genes to be combined in new cultivars. Efficient exploitation of genetic resistance to stem rust demands a detailed examination of the occurrence and distribution of seedling genes. The diccoccoides wheat accessions evaluated in the present study encompasses a large portion of the genetic variation present in the gene pools and therefore is a good resource for identifying new stem rust resistance genes.

Major gene resistance/seedling resistance can offer complete protection and significant economic benefits to farmers. Nevertheless, this kind of resistance is known to lack durability. Adult plant resistance (APR) is not complete and not limited to specific physiological races of the pathogen but unlike major gene resistance, it can be durable, hence a major concentration for wheat breeders and valuable to farmers. However, the APR genes can render the plant completely susceptible to the pathogen at seedling stage. Thus, if major genes/seedling resistances genes are identified and systematically employed through gene pyramiding (pyramiding 3-4 major genes using marker assisted selection by the aid of diagnostic markers), they provide resistance to stem rust for adequate period of times. This study provides novel information that can be exploited for pre-emptive breeding efforts to reduce the vulnerability of durum wheat to stem rust.

Accessions carrying resistant genes against the three wheat stem rust races should be tested against a collection of other different stem rust isolates in the greenhouse to determine whether they possess a broad-based resistance. The 32 selected accessions were resistant to most of the races of *Pgt* races used in the study. Out these 32 accessions, nine accessions (28%) were collections from Ethiopia. It is evident that Ethiopia has a long history of wheat rust epidemics; its native wheat germplasm harbors potentially valuable resistance loci. Moreover, the Ethiopian germplasm has been historically underutilized in breeding of modern wheat worldwide and thus the resistant alleles from the Ethiopian germplasm represent potentially novel sources of rusts resistance. Thus, the present study confirms that, most germplasms from Ethiopia has showed resistance to these three races of wheat stem rust and can serve as a source of resistance in wheat improvement program.

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