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Resistance to TTKSK and TTTSK Races of *Puccinia graminis* f.sp. *tritici* in Ethiopian Tetraploid Wheat Accessions

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Abstract
Ethiopia is the leading wheat producing country in East Africa. East Africa is a known hotspot for the evolution of new rust races of wheat. A case in point could be the evolution of a new race of stem rust called Ug99 that overcame the resistance deployed in wheat breeding for stem rust for many decades. Tetraploid wheat is an excellent source of stem rust resistance. In Ethiopia recent race analysis works showed that races TTKSK and TTTSK are the two dominant races distributed in major wheat producing areas of the country such as Arsi and Bale. Taking this into account, searching for sources of stem rust resistance to races TTKSK and TTTSK is an important breeding strategy. Therefore, this study was conducted to identify sources of resistance to the two major stem rust races from tetraploid wheat accessions. A total of 40 tetraploid wheat accessions comprising *Triticcum durum*, *T. diccoccum* and *T. turgidum* were evaluated both at the seedling stage at Kulumssa and adult plant growth stages at Arsi Robe under natural epidemics. In the seedling stage, most of the accessions were resistant to both races. The infection type range from ; to 2^+. 2^+ is the maximum infection type that was displayed by few of the accessions. All the tetraploid accessions displayed low infection types to both races. Stem rust occurred at epidemic level at Arsi Robe compared to other testing locations. Almost half of the accessions were completely susceptible to stem rust infection in the field condition. Five accessions exhibited a completely immune reaction to the prevailing races. The rest displayed a trace level of moderately susceptible reaction. Those accession that combined seedling and adult plant resistance to stem rust infection could be utilized in the durum wheat breeding program of Ethiopia.
Keywords: Accessions, infection type, tetraploid, race, TTKSK, TTTSK.

1. Introduction

Wheat is one of the most important cereal crops covering an area of more than 1.6 million ha with total production of more than 34 million quintal in 2013 main season in Ethiopia (CSA, 2013). Ethiopia is one of the major wheat producing countries in the Sub-Saharan Africa. The productivity of wheat is low due to many factors among which diseases caused by fungal pathogens are the major constraints in tetraploid wheat production. Stem rust of wheat caused by *Puccinia graminis* f.sp. *tritici* is the most important wheat production bottleneck in major wheat producing areas of the country. However, grain losses have been estimated to reach up to 30-70% on susceptible varieties (Efrem *et al*., 2000) though there is variability in yield loss attributed to the variability in weather conditions. Under Kenyan condition mean grain yield loss ranging from 5.6-66.3% was recorded due to race Ug99 (Macharia and Wanyera, 2012). In 1993/94 cropping season an outbreak of stem rust occurred that attacked the previously resistant bread wheat cultivar Enkoy (Temesgen Kebede *et al*., 1995) and the yield loss due to the epidemics on this cultivar alone ranged from 65 to 100% in Bale zone of Oromia, Ethiopia (Shank, 1994). The stem rust race that was responsible for the breakdown of stem rust resistance gene deployed in Enkoy was not known at the time of the epidemics. However Enkoy is known to carry stem rust resistance gene, *Sr36* though Admassu *et al*. (2012) postulated Enkoy to have stem rust resistance gene, *Sr27*. *Pgt* population in Ethiopia is characterized by high genetic (Belayneh *et al*., 2010) and virulence diversity that makes breeding for durable stem rust resistance demanding (Belayneh and Emebet, 2005). Wild emmer wheat is excellent sources of multiple diseases resistance and has agronomic traits of breeding importance (Jaradat, 2011). Diversifying sources of resistance to stem rust of wheat with especial emphasis to race TTKSK (Ug99) and its derivatives is very crucial to avert the current situation in wheat breeding programmes throughout the world. Ethiopian tetraploid wheat including durum wheat are excellent sources of resistance to the Ug99 race group compared to bread wheat cultivars grown in the country (Worku *et al*., 2012; Worku *et al*., 2013). The major objective of the current study was to identify resistance to *Pgt* races of TTKSK and TTTSK in the seedling stage and adult plant stage to a mixture of stem rust pathotypes with different virulence combination in tetraploid wheat accessions originated from Ethiopia.

2. Materials and Methods

2.1 Seedling evaluation

All the total 40 tetraploid accessions were planted in a pot with a diameter of 6cm by 6cm. For each accession five seeds were sown. Susceptible checks Arendeto and Local Red were included for comparison purpose. Races TTKSK and TTTSK were multiplied on the universally susceptible cultivar, Morocco which is known to carry

none of the rust resistance genes (Roelfs *et al*., 1992) prior to the inoculation of the test accessions. When the seedlings were seven days old, inoculation was made using an atomizer. The inoculated seedlings were covered with moistened plastic cages and the tip of the cage was tied with a rubber band to maintain the moisture until the seedlings are taken out of the dew chamber. The same seedlings were kept in a dark room for 16 hours and taken out of the dark room and transferred to a laboratory bench and were kept in a continuous supply of light for at least 12 hours of daylight per day. After a few days the seedlings were then transferred to greenhouse where the mean temperature was 25^{o}C. 14 days post inoculation the infection type was recorded on the primary leaves based on the 0 to 4 scale (Roelfs *et al*., 1992). Infection types 0 to 2^{+} were considered as resistant and that of 3 to 4 were considered as susceptible ones.

2.2 Adult plant evaluation

All the accessions were planted in two rows of one meter length. The spacing between rows and entries was 0.2m and 0.4m respectively. Field evaluation for stem rust resistance was carried out in 2006, 2007 and 2012 main seasons. In 2006 evaluation was made at Kulumssa, Arsi Robe and Asassa. In 2007 the accessions were planted at Asassa, Bekoji and Meraro. In 2012 evaluation was made in Kulumssa and Arsi Robe. Susceptible checks Morocco and PBW343 were included every 20 entry. PBW343 is known to carry *Sr31* gene (Brama *et al*., 2003) was included to trap Ug99 and its variants. Morocco was planted to catch all *Pgt* races prevalent at Kulumssa and Arsi Robe. The accessions were bounded by spreader rows comprising Morocco to enhance stem rust epidemics. There was no artificial inoculation made at Arsi Robe. Scoring was done using the modified Cobb's scale (Peterson *et al*., 1948) and the reaction as recommended by Roelfs *et al*. (1992) two times during the cropping season and coefficient of infection (CI) was also calculated (Stubbs *et al*., 1986).

3. Result

3.1 Seedling resistance to races TTKSK and TTTSK

Of the total accessions evaluated, forty were completely resistant to stem rust race TTKSK and the maximum infection type displayed was 2^{+}. In general the infection type range from ; to 2^{+}. Only one accession (8355-1) displayed an infection type (IT=3) and it was susceptible to race TTKSK. Nine of the accessions showed an infection type (IT=2^{+}), fourteen of the total showed IT=; and twelve of them displayed an infection type of 2 (IT=2) and all categorized as resistant based on the 0-4 scoring system for stem rust of wheat. With respect to race TTTSK, the dominant infection type was ; followed by necrosis and 28 of the accessions displayed flecks (;) on the primary leaves of the seedlings. Seven of the accessions showed an IT=2 and the other two accessions showed an infection type (IT=2^{+}) and all were resistant. There was no a single accession that was susceptible to race TTTSK. However, there was only one accession that was susceptible to race TTKSK in the seedling stage.

3.2 Field evaluation in 2006

During the 2006 season, stem rust infection was very low at Asassa and Kulumssa. Only four accessions displayed a trace level of moderately resistant to moderately susceptible reaction, the remaining accessions did not show any stem rust infection at all throughout the growing season at Asassa. At Kulumssa, 32 of the accessions did not develop any stem rust symptom and the score was zero. Seven accessions displayed trace level of moderately susceptible reaction. A high level of stem rust severity of 30S and 50S was recorded on accessions codes 88229 and 226972 respectively. A severity of 5S and 10S was recorded on accession codes 8419-2 and 7348 respectively and on accession codes 7348 and 7216-1, a severity of 5MS and 10MS was recorded respectively. 37 accessions were completely free from any stem rust infection. Two accessions displayed a severity of trace MS to MR reactions. A score of 10MS was recorded on two accessions (codes 238863 and 214528). A severity of 20MS was recorded on accession code 222393. A severity of 30S-MS was recorded on two accessions, 208871 and 208872. The highest stem rust severity was recorded on accession code 236973-1. Six accessions showed a high level of stem rust infection. The reaction of the accessions to stem rust varies from location to location. Some accessions are resistant at one location and susceptible at other location. In terms of severity of stem rust infection, Arsi Robe is more important than the two locations, Asassa and Kulumssa. In general stem rust pressure was very low across the three test locations during 2006 season. This is why almost all the accessions did not show high infection level across the locations. This made necessary the evaluation of the same accessions during 2012 season at Kulumssa and Arsi Robe.

3.3. 2012 season evaluation

In 2012 main cropping season, stem rust developed at epidemic proportion at Arsi Robe station under natural infection compared to all other testing locations in Ethiopia and this allowed us to make precise phenotyping of the tetraploids for stem rust resistance. At Kulumssa stem rust pressure was very low though artificial stem rust inoculation was made three times at different growth stages. Most of the tetraploid wheat accessions showed trace to moderately susceptible reaction to stem rust of wheat at Kulumssa and hence did not develop stem rust infection that may be attributed to the un-favourable environmental conditions that prevailed at Kulumssa. 2012 main season was ideal for the development of *septoria tritici* blotch epidemics at Kulumssa. Almost half of the tetraploid accessions showed a completely susceptible reaction to stem rust epidemics that occurred in Arsi Robe

during the season. Four of the 40 tetraploids displayed a completely resistant reaction to stem rust infection and the stem rust races prevailing in Arsi Robe areas. Eleven of the accessions displayed a moderately susceptible reaction with severity of trace to 10 %. More than half of the tested accessions showed a completely susceptible reaction to stem rust of wheat under field condition at Arsi Robe. There are some accessions that depicted a moderately resistant to a moderately susceptible reaction to stem rust of wheat. The accessions consisted of *Triticcum durum*, *T. diccoccum* and *T. turgidum*.

Table. Seedling and adult plant resistance to stem rust of wheat in tetraploid wheat accessions from Ethiopia

P.No	Passport No	Accession	Species name	Altitude	Stem Rust		CI	Seedling IT to stem rust races	
					Kulumssa	Arsi Robe		TTKSK	TTTSK
1	10	6876	dicoccum	2375	0	TRS	1	2	1+-2
2	11	6920	dicoccum	2480	TMS	80S	80	2+	1-2
3	13	7216-2	dicoccum	2420	0	30S	30	2+	;-1
4	20	7348	dicoccum	2180	0	20S	30	2	;-1
5	21	7350	dicoccum	2150	TMS	50S	50	2	1-2
6	41	7615	dicoccum	2500	TMS	-	-	2+	2-2+
7	55	80-67-2	dicoccum	2590	0	20S	20	;-1	;N
8	64	8229	dicoccum	2720	0	80S	80	2	2+
9	90	8355-1	dicoccum	2150	0	10SMS	10	3	;N
10	107	8419-2	dicoccum	2070	0	-	-	-	-
11	134	208871	dicoccum	2670	0	-	-	-	-
12	135	208872	dicoccum	2560	0	-	-	1+-2	1-2
13	168	216840-1	dicoccum	2260	0	20S	20	;	;
14	170	219071	dicoccum	2560	0	20S	20	2	;
15	223	230689	dicoccum	2260	0	70S	70	1+	1-2
16	260	238863	dicoccum	2280	0	40S	40	1+-2	1+-2
17	391	209071-2	Durum	2535	0	10MS	8	;N	;N
18	392	209072	Durum	2540	TMR	10S	10	1+-2	;N
19	415	214528	Durum	2350	TR	60S	60	1	1+-2+
20	424	214587	Durum	2340	0	0	0	-	-
21	426	216078	Durum	2990	TMS	20SMS	20	1-2	;N
22	461	222554	Durum	2465	TMR	0	0	;N	;N
23	474	226233	Durum	2940	TMS	10MS	8	;N	;N
24	485	226861	Durum	2640	0	40S	40	1-2+	;N
25	486	226862	Durum	2640	0	5MS	4	;N	;N
26	489	226868-2			0	TRS	1	;N	;N
27	497	227058	Durum	2400	0	-	-	2	;N
28	500	231547	Durum	2400	TMS	TRS	1	1+-2	;N
29	501	231551	Durum	2640	0	TMS	1	;N	;N
30	502	231603-1	Durum	2420	0	0	0	;N	;N
31	503	231603-2	Durum	2420	0	TRS	1	;	;N
32	556	236973-1	Durum	2570	0	-	-	1-1	;N
33	562	236981-2	Durum	2150	0	TMS	1	;	;N
34	571	236989-1	Durum	2200	0	5S	5	;	;N
35	572	236989-2	Durum	2200	0	30S	30	2+	;N
36	574	238113-1	Durum	2410	0	20SMS	20	2+	;-1
37	580	238498	Durum	2080	0	10S	10	;N	;N
38	626	7215-2	turgidum	2450	0	40S	40	2+	;
39	627	7893-1	turgidum	2240	0	0	0	1-2	;
40	631	7958	turgidum	2600	0	60S	60	-	-
41	639	222195-1	turgidum	2440	0	10MS	8	;	;
42	641	222393	turgidum	2415	0	80S	80	1+-2+	;
43	658	226972	turgidum	2440	10S	80S	80	2	;
44	660	2315532	turgidum	2250	0	-	-	2	;
			Local Red					2+	1-2

CI=coefficient of infection, IT=infection type based on 0-4 scale (Roelfs *et al*., 1992)
S=susceptible, MS=moderately susceptible, MR=moderately resistant, R=resistant, - signifies the accession was not tested; N=necrosis, ; signifies the development of some flecks on the seedlings

4. Discussion

Admassu *et al* (2009) identified three major races including TTKSR, TTHSR and RRTTR as the dominant ones from a wheat stem rust virulence survey conducted in 2006 and 2007 main seasons from various wheat growing areas of Ethiopia. Currently, TTKSK (Ug99) is the most dominant race in major wheat producing regions of Ethiopia since 2006 onwards (Admassu *et al*., 2009; Abebe *et al*., 2012; Woldeab *et al*., 2013). Most of the tetraploid wheat accessions in the current study were resistant to the two races of stem rust in the seedling stage and this finding is agreement with the findings of Bonman *et al*. (2007) who reported that Ethiopian accessions are resistant to multiple races of stem rust in the seedling stage. Olivera *et al*. (2012a) identified sources of TTKSK resistance from emmer wheat and the infection type on the seedlings ranged from $2^{=}$ to 2^{+} and similarly in the current study the maximum seedling infection type to both races does not exceed 2^{+}. Kebede and Bekele (2009) found tetraploid wheat as a durable source of stem rust resistance to the prevalent races of stem rust in south-western part of Ethiopia where favourable climatic condition prevail. Olivera *et al*. (2012b) identified race TTKSK and others with combined virulence for stem rust resistance genes *Sr9e* and *Sr13* at Debre Zeit and also identified Ethiopian landraces of tetraploid wheat as sources of resistance to race TTKSK.

Since 2001 to 2004 cropping season, stem rust race analysis works done in Ethiopia showed that genes *Sr8b*, *Sr19* and *Sr24* were effective to all the prevailing pathotypes of stem rust (Belayneh and Emebet, 2005). Admassu *et al*., (2009) recommended the use of *Sr13*, *Sr36* and *SrTmp* in Ethiopian wheat breeding program. However, two stem rust races (TRTTF and JRCQC) prevalent in Debre Zeit are virulent to the three *Sr* genes (Olivera *et al*., 2012b).

Race TTKSK (Ug99) is the dominant stem rust race in different wheat producing regions of Ethiopia and virulence survey on wheat stem rust has detected the distribution of this particular race in various areas of Ethiopia (Admassu *et al*., 2009; Abebe *et al*., 2012). A variant of Ug99 which is virulent on *Sr24* gene is not detected in Ethiopia (Admassu *et al*., 2009; Abebe *et al*., 2012) though it was detected in neighbouring Kenya (Jin *et al*., 2008). Ethiopian bread wheat cultivar Millenium, released in 2007, carrying *Sr24* gene is still effective to the prevailing races of stem rust in stem rust hotspot areas and this is additional evidence for the effectiveness of *Sr24* gene to Ethiopian pathotypes of stem rust.

Arsi Robe station seems to have wider virulence spectrum of stem rust compared to Kulumssa during 2012 season as most of the accessions that showed resistant reaction were susceptible at Arsi Robe. This further confirms that there exists pathogenic variability of stem rust populations at Arsi Robe and Kulumssa and also the environmental condition interms of temperature and humidity was more favourable for stem rust development at Arsi Robe than at Kulumssa.

From previous studies, three of the accessions (accession codes 6870, 7348 and 7350) were resistant to stem rust race in seedling evaluations (Naod *et al*., 2007). All these accessions were resistant to the two dominant stem rust races in Ethiopia in the seedling stage in the current study and hence this finding is also in agreement with the previous report. Accession code 7350 was postulated to carry multiple stem rust resistance genes including *SrTt-3+Sr10/Sr30/Sr31/Sr9a* (Naod *et al*., 2007). This could be the probable reason was why it was resistant to both stem rust races in the seedling stage and to the prevalent races in Arsi Robe area at the adult plant stage. Accession code 8355-1 displayed a susceptible infection type (IT=3) to race TTKSK but it showed a resistant reaction to race TTTSK. It also displayed a susceptible to moderately susceptible reaction under field condition at Arsi Robe. This particular accession is valuable if used in the durum wheat breeding program as it is displaying a susceptible to moderately susceptible reaction and it may also possess minor stem rust resistance genes that are valuable to keep the variety durably resistant for a long period of time. Four of the accessions (214587, 222554, 231603-1 and 7893-1) were completely immune under field condition at Arsi Robe and the same accessions were resistant in the seedling stage to both races TTKSK and TTTSK in the seedling stage. The infection type exhibited by most was fleck followed by necrosis and in only one accession i.e. accession code 7893-1 an infection type ranging from 1-2 was recorded. These accessions have both seedling and adult plant resistance to major stem rust races prevailing in Arsi Robe area and they can be used in the durum wheat breeding program. Accessions codes including 6876, 226868-2, 231547 and 231603-2 have displayed trace level of susceptible reaction under field condition whereas accession code 231551 displayed trace level of moderately susceptible reaction at Arsi Robe. This indicates that these accessions are valuable sources of stem rust resistance in the breeding program of tetraploid wheat. The same accessions were resistant to both races in the seedling stage and the infection type ranged from ; to a maximum of 2 based on the 0-4 scale. From the current stem rust evaluation it appears that race TTKSK looks more virulent than that of race TTTSK as most of the accessions showed a little bit higher infection type compared to the low infection type in response to race TTTSK. Race TTKSK (Ug99) is the most virulent stem rust race in the world attacking most of the stem rust resistance genes in wheat excluding genes *Sr24*, *Sr36* and *SrTmp*.

5. Conclusion and Recommendation

The presence of variability in the resistance and susceptibility spectra of the accessions shows the existence of

stem rust pathogenic variability across the test locations and also depicts the variability in weather conditions favouring stem rust development. The accessions that displayed complete resistance may have major gene resistance to the prevailing race (s) of stem rust at Arsi Robe and can be valuable sources of stem rust resistance.

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