

SHORT COMMUNICATION

SOURCES OF STEM RUST RESISTANCE IN ETHIOPIAN TETRAPLOID WHEAT ACCESSIONS

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ABSTRACT

Stem or black rust of wheat caused by the fungus *Puccinia graminis* f. sp. *tritici* Ericks and Henn (Pgt) is an important disease on wheat worldwide. Pgt is an obligate biotroph, heteroecious in its life cycle and heterothallic in mating type. Seedlings of 41 emmer (*Triticum dicoccum*), 56 durum (*T. durum*) wheat accessions were tested for their response to stem rust (*Puccinia graminis* f. sp. *tritici*) infection under greenhouse condition at Kulumsa Agricultural Research Center, Ethiopia. The test included screening of accessions and multipatotype testing to postulate sr genes. Vigorous screening of accessions was conducted using ten stem rust races, namely TKM/J, SKM/J, TTM/J, STM/J, TTL/K, TKR/J, TKM/J, TTM/H, SKM/J and JKM/G and, 33 stem rust differential lines. Flor's gene-for-gene theory was applied to postulate Sr genes in the tested accessions. Eighteen emmer and 6 durum accessions were found to be good sources of resistance to stem rust infection. In addition, the presence of Sr 7b, 8b, 9a, 9b, 10, 14, 24, 27, 28, 29, 30, 31, 32 and Tt-3 + 10 genes was postulated in 16 selected emmer and 5 durum wheat accessions. Hence, efforts to exploit these valuable Sr genes in Ethiopian cultivated tetraploid wheats could be rewarding to get stem rust resistant varieties and boost wheat production.

Key Words: *Puccinia graminis*, *F. sp tritica*, *T. durum*, *T. dicoccum*, Ethiopia

RÉSUMÉ

La tige ou la rouille noire du blé causée par la moisissure *Puccinia graminis f. tritici* Ericks et Henn (Pgt) est une maladie sérieuse universelle du blé. Le Pgt est une biotrophe, un hétéroecios obligatoire dans le cycle de sa vie et un hétérothallique dans l'accomplissement de son espèce. Les jeunes plantes de 41 emmer (*Triticum decoccum*), 56 durum (*T. durum*) nouvellement acquises étaient examinées en fonction de l'infection de la tige par l'infection de la rouille (*Puccinia graminis f. sp. tritici*) sous la condition de serre au Centre de Recherche Agronomique de Kalunsa en Ethiopie. L'expérience comprenait la sélection du blé nouvellement acquis et le test multipatotype pour postuler les gènes Sr. Une sélection rigoureuse de nouvelles acquisitions était entreprise en utilisant 10 races de tiges rouillées: TKM/J, SKM/J, TTM/J, STM/J, TTL/K, TKR/J, TKM/J, TTM/H, SKM/J et JKM/G et 33 tiges rouillées de traits différentiels. La théorie de Flor's gène-pour-gène était d'application pour postuler les gènes Sr pour l'examen des acquisitions nouvelles. Dix-huit emmer et six acquisitions nouvelles de durum étaient estimées comme source de résistance contre la contamination de la tige rouillée. En plus, la présence du Sr 7b, 8b, 9a, 9b, 10, 14, 24, 27, 28, 29, 30, 31, 32 et Tt-3+10 gènes étaient postulés dans 16 emmer sélectionnées et 5 durum du blé nouvellement acquis. Dès lors, des efforts pouvant permettre l'exploitation de ces Sr gènes précieux des tétraploïdes éthiopiens cultivables pouvaient être considérés comme un don en tant que variétés résistant à la rouille des tiges et à la multiplication de la production du blé.

Mots Clés: *Puccinia graminis*, *F. sp tritica*, *T. durum*, *T. dicoccum*, Ethiopie

INTRODUCTION

Stem or black rust of wheat caused by the fungus *Puccinia graminis* f. sp. *tritici* Ericks and Henn (Pgt) is an important disease on wheat worldwide. Pgt is an obligate biotroph, heteroecous in its life cycle and heterothallic in mating type (Alexopoulos *et al.*, 1996). It is known to bear many physiologic races generated mainly by mutation (Roelfs, 1985). Ethiopia is one of the hot spot areas for the development of the present wheat stem rust complex (Leppik, 1970). The disease has become a major threat to wheat production after the epidemics of 1974 and 1993 that drove out two bread wheat (*Triticum aestivum* L.) varieties, *Lacketch* and *Enkoy* out of production. The disease has been the most biotic constraint on wheat, causing yield loss ranging from 30 to 70% on a susceptible variety (Ephrem *et al.*, 2000). In Ethiopia, more than 70 wheat varieties have been released for production since 1940s. However, the national average yield of wheat is still 1.4 t ha⁻¹ (FAOSTAT, 2003). Rusts including stem, leaf and yellow or stripe are main constraints of production in different agro-ecologies.

The major cause of the ineffectiveness of wheat varieties against stem rust is the narrow genetic base on which the breeding for resistance has been founded. In areas where mono, culture of wheat production it is not uncommon to see the existing gene(s) for resistance being ineffective due to occurrence of physiologic races with the new virulence characteristics (Alex *et al.*, 1997).

Wheat in Ethiopia is represented by hexaploid (2n = 6X = 42, AABBDD) and tetraploid (2n = 4X = 28, AABB) species. Bread wheat is a widely grown hexaploid wheat, while durum (*T. durum* Desf.) and emmer wheat (*T. dicoccum* Schrank) are the two cultivated tetraploid wheats. The enormous genetic variability of the cultivated tetraploid wheats makes Ethiopia the centre of diversity for cultivated tetraploid wheats, especially durum wheat (Vavilov, 1929). Porceddu and Perrino (1973) reported the presence of six pieces of tetraploid wheats in Ethiopia namely, *T. dicoccum*, *T. Turgidum*, *T. durum*, *T. Polonocim*, *T. pyramidale* and *T. carthilcum*. Out of these, *T. durum* (durum wheat) and *T. dicoccum* (emmer

wheat) are currently under production. At present, the Institute of Biodiversity Conservation (IBC) has collected more than 12,726 accessions of *Triticum* species from various agro-ecological zones of Ethiopia. The tetraploid wheats comprise 72% of the collected accessions (Abebe and Giorgis, 1991).

The wild and cultivated relatives of wheat offer a tremendous potential to be used as a source of stem rust resistance. Landraces are priority, as they may possess a wide range of variation, specific adaptation to the different environments in their regions of growth, and resistance or tolerance to diseases and insect pests (Alex *et al.*, 1997; Mujeeb-Kazi and Rajaram, 2000). However, little work has been done to study the Ethiopian cultivated tetraploid wheats for sources of resistance to stem rust infection.

The objectives of this study were to: (i) evaluate disease reactions of tetraploid wheat accessions for use in breeding and identify tetraploid wheat accessions that could serve as sources of resistance to stem rust; and (ii) postulate the stem rust (*Sr*) genes through multipathotype testing.

MATERIALS AND METHODS

Forty one emmer and Fifty-six durum wheat accessions were used in this study. The accessions were collected by IBC/E from the major wheat producing provinces of Ethiopia, including Arsi, Bale, Shewa, Gondar, Hararghe, Gojam, Welo, Gamugofa and Tigray. The accessions were tested for resistance to stem rust infection.

The study was conducted at Kulumsa Agricultural Research Centre, Ethiopia, under greenhouse conditions during 2003. The study comprised of two parts. The first was screening accessions for stem rust resistance, while the second was multipathotype testing to postulate *Sr* genes in the selected accessions showing low infection type (IT). The ITs are the phenotypic expression of host-pathogen interaction.

Screening accessions. Five to six seeds per accessions were sown in 9-cm diameter plastic pots. The plastic pots were filled with a mixture of soil, compost and sand in 1:1:1 by volume.

Seedlings were grown in the greenhouse with a diurnal temperature and relative humidity of 18-25°C and 82-89%, respectively.

To ensure vigorous screening, a mixture of six stem rust isolates collected from severely infected emmer, drum and bread wheats varieties of the major wheat growing areas were used. The isolates mixed were SI-1a, Am-2, Ku-3, Dz-4a, Ro-4 and NA - 22. The isolates mixture was prepared by bulking equivalent amount of spores in a single test tube. When the first leaf is fully unfolded, the spore suspension that was adjusted to 4 x 10⁶ spores/ml was sprayed with an atomizer in an isolation room. Inoculated seedlings were incubated in a transparent plastic cage for 18 dark hours with a temperature ranging from 18-22°C and 98-100% relative humidity (Stubbs *et al.*, 1986).

Multipathotype testing. Ten stem rust races (Table 1) that were developed from a single pustule and with known avr/vir formula were used in this test. Each race was inoculated on 33 stem rust differential lines and the tetraploid wheat accessions. *Morocco*, a universal susceptible bread wheat variety was used as a check. Seedling raising and inoculation procedures were similar with the screening test. However, to increase the precision of the test, the inoculated seedlings were incubated in a growth chamber at 20 + 2°C and 98% relative humidity for 18 dark hours.

Disease assessment. In both parts of the study, disease assessments were done at 12 and 14 days after inoculation. A 0 to 4 (0 = Immune, ; = Nearly immune, 1 = Very resistant, 2 = Moderately resistant, 3 = Moderately susceptible and 4 = Susceptible) that was originally developed by Stakman *et al.* (1962) was used for scoring seedlings ITs.

Resistance gene postulation. Microsoft spread sheet software was used to arrange and sort the IT data in such a way that it fits to the procedure of gene postulate. Briefly, the steps were (i) races were arranged to identify columns and test lines identify rows, (ii) low infection type and high infection type were coded as 1 and 0, respectively, (iii) The last column identified the frequency of each test line frequency was obtained by simple summation of the values 0 and 1 along each row. (iv) The LIT frequency values were sorted in an ascending order. (v) Matching LIT/HIT patterns will appear in consecutive rows. Hence, steps of gene postulation described in Dubin *et al.* (1989) were applied first in groups that contain differential lines and test lines together. Further, matching to postulate additional genes was carried in the groups above it. Every matching pattern of ITs were done based on the assumptions and conditions of the gene-for-gene theory (Flor, 1946).

TABLE 1. Stem rust races used in multipathotype testing and list of Sr genes that the races are virulent on

Races*	Sr genes
TKM/J	Sr-8b, 9a, 9b, Tt-3+10, 11, 14, 18, 28, 30, 21
SKM/J	Sr-7b, 8b, 9a, 9b, Tt-3+10, 10, 11, 14, 24, 28, 30, 31, 32
TTM/J	Sr-9a, 9b, Tt-3+10, 18, 27, 28, 29, 30, 31
STM/J	Sr-7b, 8b, 9a, 9b, 9d, Tt-3+10, 14, 28, 30, 31
TTL/K	Sr-9a, 9b, Tt-3+10, 10, 14, 17, 24, 25, 30, 31
TKR/J	Sr-8b, 9a, Tt-3+10, 30, 11, 15, 28, 31
TKM/J	Sr-8b, 9a, 9b, Tt-3+10, 10, 11, 14, 18, 19, 28, 30, 31, 32
TTM/H	Sr-9a, 9b, Tt-3+10, 13, 10, 12, 14, 15, 18, 19, 24, 26+9g, 27, 29, 30, 31, 32
SKM/J	Sr-9b, 8b, 91, 9b, Tt-3+10, 11, 28, 30, 31
JKM/G	Sr-5, 7b, 8b, 9a, 9b, Tt-3+10, 10, 11, 14, 18, 19, 22, 24, 26 + 9g, 27, 28, 29, 30, 31

*Pgt-code for stem rust races were designed as described in (Roelfs and Martens, 1988) fourth letter represents additional differential lines with Sr9a, 9d, 10 and Sr28

RESULTS

Sources of resistance. Eighteen emmer and six durum wheat accessions that showed resistance response to a mixture of stem rust isolates were selected as a source of resistance to stem rust (Table 2). Ten of the selected emmer accessions were collected from Hararghe and Bale, while the others were collected from Arsi, Shewa, Gamugofa and Gojam provinces. The altitudes of these places range from 1750 to 3110 metres above sea level (masl). From the selected six durum wheat accessions, three, 7965, 7975 and 8002 were collected from Shewa, whereas one accessions was found from Arsi, Gamugofa and Gondar provinces. Durum accessions were

TABLE 2. Selected stem rust resistant accessions of emmer and durum wheat and their infection type (IT)

No.	Accession code	Province ²	Altitude observed (m.a.s.l.)	IT ³
1	6870	Bale	2470	1
2	6876	Bale	2375	1
3	7220	Shewa	1800	1
4	7347	Hararghe	2350	1
5	7348	Hararghe	2180	;
6	7350	Hararghe	2150	2
7	7622	Shewa	3100	;
8	7691	Gojam	2620	2
9	7913	Hararghe	2220	1
10	7922	Hararghe	2400	;
11	8011	Shewa	2800	1
12	8295	Arsi	3110	1
13	8296	Arsi	2800	1
14	8298	ARSI	2530	1
15	8303	Gamugofa	2590	1
16	8407	Bale	2500	2
17	8411	Bale	2410	2
18	212880	Bale	1750	2
19	7073	ARSI	2480	1
20	7449	Gonder	2240	1
21	7965	Shewa	2080	1
22	7975	Shewa	2080	1
23	8002	Shewa	2260	1
24	8299	Gamugofa	2340	1
	Morocco*	-	-	-

¹1-18T. dicoccum 19-23 T. durum * Susceptible check

²The names of location refer to the former names of Ethiopian provinces

³(Nearly immune) 1 (Moderately resistant) 2 (Moderately resistant) 4 (Susceptible) (Roelfs *et al.*, 1992)

collected from a narrow range of altitude that ranges from 2080 to 2480 masl.

Postulated Sr. genes. Among the tested accessions that showed resistance, the presence of stem rust resistance genes in sixteen emmer and five durum wheat accessions was postulated based on the IT pattern exhibited by differential lines and accessions to ten stem rust races (Table 3). The presence of *Sr8b* was postulated only in one durum accession 7984. Accessions 6867, 8010, 8312 and 206523 were shown to possess *Sr14*, *10*, *7b* and *Sr32* genes, respectively. The presence of *Sr27* and/or *Sr29* was also postulated in one emmer accession, 215419. In addition, the presence of either *Sr Tt-3 + 10*, *30*, *31* or *Sr9a* was postulated in two durum wheat accessions, 7350 and 7913 and in one durum wheat accessions 7449.

In addition to the known *Sr genes*, the presence of unidentified resistant genes, indicated as U, were also detected. These genes are referred to as unidentified because the tested differential lines may not represent them or they might not been identified yet. These genes are postulated in ten emmer and three durum wheat accessions. In seven of the accessions these Sr. genes are postulated in addition (+) to the identified genes, while in seven accessions they are postulated as the only resistance gene.

DISCUSSION

The most important and durable source of stem rust resistance in hexaploid wheat are probably those transferred from tetraploid wheats (McIntosh, 1988). The results of this study also support this fact and show that Ethiopian cultivated tetraploid wheat accessions are still good sources of stem rust resistance. Efforts made to study and exploit the merits of Ethiopian tetraploid accessions were mainly concentrated on durum wheat due to its higher commercial value than emmer wheat. Several researchers (Ephrem, 1987; Dyck and Sykes, 1995; Knott, 1996) used durum wheat accessions to transfer some resistance genes and study the inheritance of stem rust resistance genes. However, the present study showed that emmer wheat accessions have better potential than durum

TABLE 3. Postulated Sr genes in emmer and durum wheat accessions and matching IT pattern of the accession and stem rust differential lines to ten stem rust races

Differentials/ Accessions	Sr genes	Stem rust races/Isolates										Postulated genes
		T K M J A2	S K M J A9	T T M J A11	S T M J A14	T T L K A16	T K R J A17	T K M J B3	T T M H B7	S K M J B15	J K M G B21	
ER 5155	32	. ^b	1	1	1	.	.	
ISR7B-Ra	7b	.	1	.	1	1	1	
W2961sr10	10	.	1	.	.	1	.	1	1	.	1	
WRT238-5	27	.	.	1	1	.	1	
PUSA*4/ETOILE	29	.	.	1	1	.	1	
DEC*OISY BARNETA												
BENBENUTO	8b	1 ^a	1	.	1	.	1	1	.	1	1	
BiSr30Wst	30	1	1	1	1	1	1	1	1	1	1	
W2691Sr28Kt	28	1	1	1	1	.	1	1	.	1	1	
W2691Sr9b	9b	1	1	1	1	1	.	1	1	1	1	
ISR9a-Ra	9a	1	1	1	1	1	1	1	1	1	1	
St.1.25.TcLr26	31	1	1	1	1	1	1	1	1	1	1	
FED*2/SrTt-3	Tt-3+10	1	1	1	1	1	1	1	1	1	1	
LINE A SEL	14	1	1	.	1	1	.	1	1	.	1	
ACC. 6876	<i>T. dicoccum</i>	1	1	1	1	1	1	1	1	.	1	14, +c
ACC. 7347	<i>T. dicoccum</i>	1	1	1	1	.	1	1	.	1	1	28
ACC.8010	<i>T. dicoccum</i>	1	1	.	.	1	.	1	1	1	1	10, +
ACC.8011	<i>T. dicoccum</i>	1	1	.	1	1	1	1	1	1	1	U ^d
ACC.8296	<i>T. dicoccum</i>	1	1	1	1	1	1	1	.	1	1	U
ACC.8298	<i>T. dicoccum</i>	1	1	1	1	1	1	1	1	1	.	U
ACC.8312	<i>T. dicoccum</i>	1	1	.	1	.	.	1	.	1	1	7b, +
ACC. 7220	<i>T. dicoccum</i>	1	1	1	1	1	.	1	1	1	1	9B
ACC.7350	<i>T. dicoccum</i>	1	1	1	1	1	1	1	1	1	1	Tt-3+10/30/31/9a
ACC. 7913	<i>T. dicoccum</i>	1	1	1	1	1	1	1	1	1	1	Tt-3+10/30/31/9a
ACC.8295	<i>T. dicoccum</i>	1	1	1	1	.	1	1	.	1	1	28
ACC.8411	<i>T. dicoccum</i>	1	1	1	1	1	.	1	1	1	1	9b
ACC.8425	<i>T. dicoccum</i>	.	1	1	1	1	1	.	1	1	.	7b, +
ACC.212880	<i>T. dicoccum</i>	1	1	1	1	1	1	1	.	1	1	U
ACC.215419	<i>T. dicoccum</i>	.	.	1	1	.	1	1	1	.	1	29/27e, +
ACC.206523	<i>T. dicoccum</i>	.	1	.	1	.	1	1	1	.	.	32, +
ACC.7965	<i>T. durum</i>	1	1	1	1	1	1	1	1	1	.	U
ACC.7975	<i>T. durum</i>	1	1	1	1	.	1	1	1	1	1	U
ACC.8299	<i>T. durum</i>	.	1	1	1	1	1	1	1	1	1	U
ACC.7449	<i>T. durum</i>	1	1	1	1	1	1	1	1	1	1	Tt-3+10/30/31/9a
ACC.7984	<i>T. durum</i>	1	1	.	1	.	1	1	.	1	1	8b

^a1 stands for resistance (Low infection type or resistance)

^b. Stands for 0 that represents susceptibility (High infection types or susceptibility)

^c+ additional unidentified gene

^dU stands for the presence of unidentified resistant gene

^e/ either or both genes could be present

accessions as a source of resistance to stem rust diseases. The 24 selected accession were resistant to most of the races of Pgt used in the study. Hence, they can be used as alternative sources of resistance for wheat improvement programs.

In this study, the presence of Sr28, 29, 30, 31, 32, 8b, 9a, 9b and SrTt-3 genes was postulated in emmer and durum accessions. Most of these genes were reported to be effective worldwide (Roelfs and Martens, 1988). Even though virulence for Sr30 and Sr31 was reported in some countries, they are commonly found in European winter wheats and in spring wheats developed by CIMMYT (McIntosh, 1988). These genes can express themselves at seedling stage. In addition, none of them were reported to be temperature sensitive. This fact strengthens the promising future of these genes in adult plant test. These genes are also known to be effective for most of the present stem rust races in Ethiopia (Naod 2004). Multipathotype testing also showed the presence of other unidentified Sr genes in Ethiopian tetraploid accessions. These genes could be new or known genes that could not be identified by the differential lines used in this study. Hence, further refinement could be made to identify and described these genes.

The AB tetraploids generally cross readily with bread wheat. The two historical crosses 'lumillo' durum with 'Marquis' bread wheat, and 'Yaroslav' emmer with "Marquis" were typical examples for a successful effort of transferring Sr genes such as *Sr9b*, *Sr9d*, *Sr17*, *Sr18* and *Sr2* (Knott, 1989). Recently done wheat disease surveys in Ethiopia have shown that none of the cultivated bread wheat varieties are resistant to the present stem rust complex. Hence, effort to transfer valuable Sr genes from cultivated tetraploid wheats could be rewarding for Ethiopian wheat improvement efforts.

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