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Evaluation of Local, Improved and Elite Bread Wheat Genotypes for seedling Resistance to Stem Rust

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Abstract: **Stem rust is one of the main diseases of wheat in Ethiopia, causing up to 100% of yield losses in susceptible cultivars. Stem rust resistant wheat genotypes provide one of the best means for controlling the disease and are also effective means of reducing yield losses caused by the disease. However, breeding genotypes for disease resistance is a continuous process and plant breeders need to add new effective sources to their breeding materials. Evaluation of the existing wheat genotypes for their resistance to stem rust is one of the alternatives to find sources of resistance genes against the disease. The present study was conducted to evaluate Local, Improved and Elite bread wheat genotypes to stem rust isolate. Thirty six bread wheat genotypes together with susceptible checks were tested using complete randomized design (CRD) with three replications in a greenhouse.**

Eighty percent of the Local bread wheat genotypes showed susceptible reaction to the tester isolate while 20% of them showed resistant reaction to the isolate. 95.2% of the Improved bread wheat genotypes showed susceptible reaction while 4.8% of them showed resistant reaction. Hundred percent of the Elite genotypes showed susceptible reaction to the tester isolate. In general, 91.7% of the wheat genotypes displayed susceptible reaction whereas 8.3% of them showed resistant reaction. The wheat genotypes with resistant reaction were variety Enkoy, K6295-4A and Millennium. Thus, result of the present study showed that the urgent need of replacement of stem rust susceptible wheat genotypes by resistant wheat genotypes through breeding for stem rust resistant genotypes and those wheat genotypes that showed resistant reaction to the tester isolate can be used as sources of stem rust resistant genes in the development of resistant wheat genotypes in breeding programs for stem rust disease resistance.

Keywords: **Seedling resistance, wheat, stem rust.**

1. INTRODUCTION

Wheat is one of the most important crops in the world in terms of nutrition and production and is used by more than one-third of world's population as a staple food (Kumar *et al.*, 2011). Twenty percent of the food calories consumed by the world population comes from wheat (Hawkesford*et al*., 2013). Moreover, it is one of the worlds' most productive crops in the 21st century. There is increased consumption and demand for grain, for fuel as well as food (Curtis and Halford, 2014). Wheat yields must be increased which is seen as an important strategy to prevent food shortages (Curtis and Halford, 2014). It is one of the key staple crops for global food security, providing more than 35% of the cereal calorie intake in the developing world, 74 % in the developed world and 41 % globally from direct consumption (Shiferaw*et al*., 2013).

In Ethiopia, wheat is one of the most important cereal crops and is widely grown in a wide range of altitudes and agro-ecological zones (Hailu*et al.*, 1991; Bekele*et al.*, 1999; Belay and Taner, 1999). It requires optimum amount of rainfall

with a fair distribution during the growth period and the most suitable area for its growth falls between 1900-2700m a.s.l. (Hailu*et al.*, 1991). Wheat ranks second in volume of production next to maize and third in area of cultivation next to teff and maize in descending order with the total hectarage of 1.6 million hectares and volume production of 4.5 million tons (CSA, 2017). The national average yield of wheat in Ethiopia is about 2.67t/ha (CSA, 2017). This is by far below the world's average yield/ha which is about 3.09t/ha (FAO, 2017). This low yield is attributed to multifaceted biotic and aboitic factors such as cultivation of unimproved low yielding varieties, low and uneven distribution of rainfall, poor

agronomic practices, insect pests and serious diseases like rusts (Hailu*et al.*, 1991; Solomon, 1993; Dereje and Yaynu, 2001). Rusts are considered the most destructive diseases of wheat worldwide (Roelfs*et al.*, 1992; Singh *et al.*, 2002; Agrios, 2005). They have been a scourge on human kind since the beginning of historical time (CIMMYT, 2005). Many epidemics have been recorded over the past 150 years, in the near and far east, Europe and the Americans. (Sharma, 2001; Singh *et al.*, 2002; Singh, 2005). Several devastating rust epidemics have resulted in major famines in Asia and grain losses at a massive scale in North America in 1903, and 1905 and 1950-54 (Roelfs*et al.*, 1992; Singh *et al* 2002; Agrios, 2005). In most wheat-growing regions of the world, existing environmental conditions would favor stem rust infection, which could lead to epidemic buildup (Singh *et al.*, 2011).The stem rust is the most devastating of the rust diseases and can cause losses of 50% in one month when conditions for its development are favorable. Losses of 100% can occur with susceptible cultivars (FAO, 2002). An estimated 80-90% of all global wheat cultivars growing in farmer's fields are now susceptible to Ug99 or variants (Ug99 factsheet, 2010). Ug99 is the only known race of wheat stem rust that has virulence for an extremely important resistance gene - Sr31. In addition, Ug99 has virulence against most of the resistance genes of wheat origin and other resistance genes from related species (Ug99 factsheet**,** 2010**)**.The stem rust resistance gene Sr31 derived from rye has been used as an important source of stem rust resistance in many wheat cultivars worldwide. However, isolates of stem rust with virulence to Sr31 were identified from Uganda in 1999.

In Ethiopia, stem rust is an important and widely spread disease occurring in most parts of the country where wheat is cultivated (Masresha, 1996; Temesgen*et al.*, 1996; Belayneh*et al* 2008). There was a major stem rust epidemic in 1974 and 1992/93 that drawn out Lakech and Enkoy varieties out of production, respectively (Betesilase*et al* 2007). In 2001, the cultivar Shinna, released in 1999 for north western Ethiopia, became highly susceptible to stem rust in southeastern Ethiopia (Arsi and Bale highlands). A wheat disease survey conducted in 2004 showed that 56 percent of small-scale farmers grew the two major wheat cultivars, Kubsa and Galama that are currently susceptible to stem rust (CIMMTY, 2005). Besides the widespread use of susceptible varieties, wheat cultivation throughout the year has contributed to the survival and build-up of the stem rust pathogen that in turn favors epidemics of the disease. Yield trial conducted from 2000 to 2004 in different areas showed increase of stem rust from year to year (CIMMYT, 2005). This has resulted in lower yields than that of the expected ones even at experimental level. It has been predicted that severity of stem rust in Ethiopia will increase and cause major yield loss in the future until effective and durable resistant varieties are developed against Ug99 in East Africa (CIMMYT, 2005). Recent virulence study conducted in major wheat growing areas of Ethiopia indicated that most of the pathotypes identified were virulent for most of the wheat differentials (Belayneh and Emebet, 2005; Belayneh*et al* 2008). Another report showed that Ethiopia is one of the East African countries where most of its wheat varieties have become susceptible to the previously known prevalent virulence of wheat stem rust and the newly evolved pathotype Ug99 (Singh *et al*., 2006). Therefore, replacement of the currently popular susceptible varieties with high yielding resistant varieties is very important and should be the best strategy to protect wheat production in the country. Moreover, host resistance is the most effective, economical, and environmental friendly method of disease control. An effective deployment of resistance genes for the management of stem rust in wheat requires knowledge about the resistance status and the diversity of resistance genes in varieties under consideration (Belayneh*et al.*, 2008). Thus, knowledge on the current status of varieties under cultivation against the prevailing wheat stem races is crucial; as the pathogen is known to evolve its virulence frequently (Jin *et al*., 2009). Therefore, achievement of durable resistance against wheat stem rust requires constant characterization of the pathogen, and identification and deployment of resistance genes that overcome the prevailing virulent races (Belayneh*et al.*, 2008). Thus, evaluation of the current status of available genotypes for their seedling stage and adult plant stage resistance is imperative. Hence, this study was initiated with the objective to evaluate Local, Improved and Elite wheat genotypes for their seedling resistance to highly virulent stem rust isolate.

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2. MATERIALS AND METHODS

2.1. Description of the Study Area

Evaluation of seedlings in a greenhousewas conducted at Kulumsa Agricultural Research Center in Ethiopia. Kulumsa Agricultural Research Center is located at 169 Km South-east of Addis Ababa at 080 01'10''N latitude and 390 09'11'' E longitudes and at elevation of 2200m.a.s.l. The average annual rainfall of the area is 809mm and the maximum and minimum annual mean temperatures are 23.80C and 9.890C, respectively. Kulumsa Agricultural Research Center is a center of excellence for wheat production research in the country.

2.2. Experimental Design

The experiment was carried out in a complete randomized design (CRD) with three replications.

2.3. Experimental Materials and their Sources

In the green house study; 10 Local, 21 Improved and five Elitewheat genotypes as well as two checks were used as plant host materials. Wheat cultivar "Morocco"was used a susceptible checkwhereaswheat cultivar PBW343 was used as a standard check. Both the seeds of wheat genotypes including the checks and wheat stem rust isolate were obtained from Kulumsa Agricultural Research Centre.

2.4. Spores Multiplication

Five to six seeds of susceptible wheat cultivar, "Morocco", were planted in 7 cm x 7 cm x 6 cm dimension plastic pots containing sterilized mixture of soil, sand and compost in a ratio of 2:1:1, respectively. These growth medium was sterilized using soil sterilizer at a temperature of 180^0C for an hour to avoid soil borne seedling diseases. The seedlings were grown in rust free room to protect them from contamination. When seedlings reach the age of seven days(when the first leaves were fully expanded, and the second leaves were just emerged to grow), wheat stem rust urediospores were suspended in sterile distilled water. Tween 20 was used as wetting agent to maintain the urediospores in suspension (Roelfs *et al.,* 1992)so that to disperse the spores more or less uniformly in the suspension. Before the inoculation, leaves of the seven days-old seedlings were rubbed gently between clean moistened fingers to remove the waxy layer from the surface which hinders the penetration of the pathogen into the host tissues. After rubbing, the leaves were sprayed with sterile distilled water using hand sprayer. Following this, the prepared urediospores suspensions were gently sprayed on seedlings using atomizer to produce pustules on the leaves of inoculated wheat seedlings. Soon after inoculation, seedlings were again gently sprayed with sterile distilled water to create artificial dew.

Thereafter, the inoculated seedlings were incubatedin a small cage, made of metal frame and tray, covered by clear moisture proof polyethylene bag with small layer of water at the bottom to maintain saturated condition for maximum spore germination and penetration of the host tissues(Roelfs *et al*., 1992). Additionally, the inner walls of the plastic cover of the cage were sprayed with sterile distilled water prior to incubation, to maximize the amount of humidity during incubation period of the inoculated seedlings. In this way, seedlings were incubated for 24 hours at 18-22^0C in a dark condition.The dark condition was created by placing the dew-chamber under tables and covering the cages with moistened (soaked) Sacs.After 24 hours of dark-dew condition, the dew-chamber was half open to remove the dew gradually to avoid a sudden drying effect of the dewed seedlings. After the dew was lightly removed slowly, the cages were top-covered by fine mesh cloth, tightly held in position by rubber bands, before they were transferred to greenhouse benches. After this procedure, seedlings were transferred to greenhouse benches and supplementary fluorescent light was provided them for 3-4 hours in controlled condition. The supplementary light was used to finish infection, because many penetration pegs fail to develop from the appersorium unless stimulated by supplementary light for a three to four hours period while the seedlings were slowly dried after the dew period (Roelfs *et al.,* 1992). In this way, the inoculated seedlings were kept on the greenhouse bench until urediospores developed. While the inoculated seedlings were on the greenhouse bench, they were closely inspected for appearance of symptoms.Fourteen days after inoculation, urediospores were collected. Such urediospores collection was carried out continually every two days until the uredia provideenough spores toinoculate the seedlings of the wheat genotypes. The urediospores were collected by tapping rusted leaves on a piece of glyssine sheet and were sieved using piece of sieve cloth to separate them from plant debris or dead tissues. Then,

the collected urediospores were transferred to petri-dishes and placed over a dried Silica gel desiccant in a desiccator after which sealed with Vaseline. Finally, urediospores were stored in a refrigerator at 4^{o}C until used.

2.5. Seedling Raising and Seedling Inoculation of the Wheat Genotypes

For this study, 10 Local, 21 Improved and five Elite wheat genotypes together with two susceptible checks were used (Table 1) to determine their reaction to a highly virulentstem rust isolate. Seedling sraising and seedling inoculation of the wheat genotypes were carried out following the procedures described in section 2.3 of this paper.

Table 1: List of tested wheat genotypes with their year of release and pedigrees

No	**Variety**	Year of release	Cross/Pedgree
	Local wheat genotypes		
1	Enkoy	1974	HEBRARD/SEL (W`S245 X SUP51X
2	Dereselgn	1974	C18154/2*FR
3	K6290 Bulk	1977	AF.MAYO X ROMANY2
4	K6295-4A	1980	ROMANY X GB-GAMENYA
5	ET 13-A2	1981	UQ105 SEL X ENKOY
6	Pavon-76	1982	VCM//CNO ' S ' 7C/3/KAL/BB
7	Bondie		
8	Israel		
9	Dashen		VEE 17=KVZ/BUHO"S"//KAL/BB
10	Laketch		PJ "S " /GB55
Improved wheat genotypes			
11	Mitke	1993	BOW 28 X RBC
12	Wabe	1994	MAL" S" BUC "S"
13	Galama	1995	4777(2)//FKN/GB/3/PVN "S"
14	Kubsa	1995	ND VG9/44//KAL/BB/3/YACO 'S' /4//VEE # 5 "S"=ATILLA
15	Tusie	1997	COOK/VEE "S"//DOVE "S"/SER/
16	Abola	1997	BOW 'S'/BUC "S"
17	Ketar	1999	COOK/VEE "S"//DOVE "S"/SERI/3/BJY 'S'/COC
18	Shina	1999	GOV9/A2/MUS "S" /3/R37/GHL121/KAL/BB/4/ANI "S"
19	Medewelabu	1999	TL/3/FN/TH/NAR59*2/4/BOL "S"
20	Sofumer	1999	LIRA "S"/TAN "S"
21	Tura	1999	ARO YR SEL 60/80
22	Watera	2000	MON "S" /VEE "S"//SARA
23	Bobicho	2000	BURRION
24	Hawi	2000	CHIL/PRL
25	Simba	2000	PRINIA
26	Durie	2001	BOW "S"/YO"S"//22"S"
27	Sirbo	2001	MILLAN
28	Meraro	2005	M/4/HAR1709/3/M//24/E
29	Dodota	2005	BJY/COC//PRL/BOW
30	Digelu	2005	SHA7/KAU2
31	Millennium	2007	ALD/CEP75630//CEP 75234/PT7219/3/BUC/BIY/4/
Elite wheat genotypes			
32	DAPHE #1		KIRITATI//2BABAX*2/3/VIVITS/
33	QUAIU #2		BABAX/LR42//BABAX*2/3/VIVITS/
34	PICAFLOR#1		KIRITATI//SERI/RAYON
35	CHONTE #1		SERI.B*2/3/KAUZ*2/BOW//KAUZ/4//PBW343*2/KUKUNA
36	MUNAL #1		WAXWING*2/KIRITATI
37	Morocco		Check
38	PBW343		Standard check

Source: Kulumsa Agricultural Research Center (KARC)

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2.6. Disease assessment and data collection.

Stem rust infection types were scored 14 days after inoculation using the 0-4 scale of Stakman *et al*.(1962) in the following way: 0=No uredinia or macroscopic signs of infection (Immune), ;= No uredinia, but hypersensitive necrotic or chlorotic flecks present (Nearly immune), 1= Small uredinia surrounded by necrosis (very resistant), 2=small to medium uredinia often surrounded by chlorosis or necrosis; green islands may be surrounded by chlorotic or necrotic border (Moderately resistant), 3= medium–sized uredinia that may be associated with chlorosis (moderately susceptible) and 4= large uredinia without chlorosis (susceptible). Infection types were classified into low infection types (0-2) and high infection types (3-4).

2.7. Data Analysis

The recorded infection types were used to analyze the data and to determine the reactions of each genotype tested. For this, the infection types were classified into two groups (low/high) following the method described by Roelfs *et al*. (1992). Based on the infection types observed on each genotype, in response to the causal pathogen, the genotypes were grouped into either resistant genotypes or susceptible genotypes to the tester stem rust isolate.

3. RESULT AND DISCUSSION

3.1. Determination of Reactions of Local, Improved and Elite Wheat Genotypes

In this study, a total of 36 bread wheat genotypes were used. These wheat genotypes were tested against a highly virulent stem rust isolate. The highly virulent isolate used to determine the reactions of these wheat genotypes obtained from Kulumsa Agricultural Research Center collections. Out of the 36 wheat genotypes, 10 were Local, 21 were Improved and five were Elite wheat genotypes. Out of the total 36 wheat genotypes, 26 (72.2%) of them showed highly susceptible reactions (IT=4) and seven (19.4%) of them displayed moderately susceptible reaction with ITs of 3 while three (8.3%) of them showed resistant reactions. Eighty percent of the Local genotypes showed susceptible reaction while 20% of them showed resistant reaction. Susceptible reaction was observed on 95.2% of the Improved genotypes tested while resistant reaction was observed on 4.8% of them. Hundred percent of the Elite genotypes showed susceptible reaction to the tester isolate. In general, 91.7% of the total tested wheat genotypes displayed susceptible reaction whereas 8.3% of them showed resistant reaction (Table 2). The wheat genotypes with resistant reaction were variety Enkoy, K6295-4A and Millennium.

Table 2: Response of wheat genotypes to the stem rust isolate

No	**Variety**	Year of release	Cross/Pedgree	Stem rust score
	Local wheat genotypes			
1	Enkoy	1974	HEBRARD/SEL (W`S245 X SUP51X	2
2	Dereselgn	1974	C18154/2*FR	3
3	K6290 Bulk	1977	AF.MAYO X ROMANY2	3
4	K6295-4A	1980	ROMANY X GB-GAMENYA	2
5	ET 13-A2	1981	UQ105 SEL X ENKOY	3
6	Pavon-76	1982	VCM//CNO ' S ' 7C/3/KAL/BB	4
7	Bondie			4
8	Israel			3
9	Dashen		VEE 17=KVZ/BUHO"S"//KAL/BB	4
10	Laketch		PJ "S " /GB55	4
	Improved wheat genotypes			
11	Mitke	1993	BOW 28 X RBC	4
12	Wabe	1994	MAL" S" BUC "S"	4
13	Galama	1995	4777(2)//FKN/GB/3/PVN "S"	4
14	Kubsa	1995	ND VG9/44//KAL/BB/3/YACO 'S' /4//VEE # 5 "S"=ATILLA	4

15	Tusie	1997	COOK/VEE "S"//DOVE "S"/SER/	4
16	Abola	1997	BOW 'S'/BUC "S"	4
17	Ketar	1999	COOK/VEE "S"//DOVE "S"/SERI/3/BJY 'S'/COC	4
18	Shina	1999	GOV9/A2/MUS"S"/3/R37/GHL121/KAL/BB/4/ANI "S"	4
19	Medewelabu	1999	TL/3/FN/TH/NAR59*2/4/BOL "S"	4
20	Sofumer	1999	LIRA "S"/TAN "S"	4
21	Tura	1999	ARO YR SEL 60/80	4
22	Watera	2000	MON "S" /VEE "S"//SARA	4
23	Bobicho	2000	BURRION	3
24	Hawi	2000	CHIL/PRL	4
25	Simba	2000	PRINIA	4
26	Durie	2001	BOW "S"/YO"S"//22"S"	4
27	Sirbo	2001	MILLAN	4
28	Meraro	2005	M/4/HAR1709/3/M//24/E	4
29	Dodota	2005	BJY/COC//PRL/BOW	4
30	Digelu	2005	SHA7/KAU2	3
31	Millennium	2007	ALD/CEP75630//CEP 75234/PT7219/3/BUC/BIY/4/	2
	Elite wheat genotypes			
32	DAPHE #1		KIRITATI//2BABAX*2/3/VIVITS/	4
33	QUAIU #2		BABAX/LR42//BABAX*2/3/VIVITS/	3
34	PICAFLOR#1		KIRITATI//SERI/RAYON	4
35	CHONTE #1		SERI.B*2/3/KAUZ*2/BOW//KAUZ/4//PBW343*2/KUKUNA	4
36	MUNAL #1		WAXWING*2/KIRITATI	4
37	Morocco		Check	4
38	PBW343		Standard check	4

3.2. Conclusion and Recommendation

In the present study, a total of 36 bread wheat genotypes together with two susceptible checks were used. Out of the 36 wheat genotypes tested, 10 were Local, 21 were Improved and five were Elite wheat genotypes.

Eighty percent of the Local bread wheat genotypes showed susceptible reaction to the tester isolate while 20% of them showed resistant reaction to the tester isolate. 95.2% of the Improved bread wheat genotypes showed susceptible reaction while 4.8% of them showed resistant reaction. Hundred percent of the Elite genotypes showed susceptible reaction to the tester isolate. In general, 91.7% of the wheat genotypes displayed susceptible reaction whereas 8.3% of them showed resistant reaction. The wheat genotypes with resistant reaction were variety Enkoy, K6295-4A and Millennium. Thus, result of the present study showed that the urgent need of replacement of stem rust susceptible wheat genotypes by resistant wheat genotypes through breeding for stem rust resistant genotypes and those wheat genotypes that showed resistant reaction to the tester isolate can be used as sources of stem rust resistant genes in the development of stem rust resistant wheat genotypes in breeding programs for stem rust disease resistance.

Therefore, wheat breeders and pathologists should work aggressively to develop stem rust resistant wheat genotypes to protect poor farmers from stem rust epidemics in wheat growing areas of the country.

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