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Assessment of sources of adult plant resistance genes to stem rust in Ethiopian durum wheat genotypes

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ABSTRACT:

Stem rust is a devastating disease of bread wheat and durum wheat in the major wheat-growing regions of the world. Races belonging to the Ug99 (TTKSK) lineage of the wheat stem rust fungus, carrying complex virulence combinations, and their migration to countries in Africa, Middle East and Asia continue to pose a significant threat to global wheat production. Therefore, the present study was conducted in the greenhouse and field to assess sources of durable resistance to stem rust. Fifteen durum wheat genotypes and a susceptible cultivar 'Morocco' were evaluated in the greenhouse and field at Debre Zeit Agricultural Research Center, Ethiopia in order to detect the presence of effective stem rust resistance genes. A mixture of three dominant races of Puccinia graminis f. sp. tritici (TKTTF, TTKSK and JRCQC) was used for inoculation. The field experiment was conducted using RCB design with three replications at two different locations. Phenotyping of the genotypes at seedling stage in the greenhouse showed four genotypes (Ginchi, Quami, DW-#3 and DW-#11) that carried effective ASR genes; however, the rest eleven genotypes showed susceptible reaction. On the other hand, the field assessment of the genotypes for stem rust resistance showed presence of varied levels of field resistance. The combined results from both seedling reaction test and field experiments indicated that the eleven genotypes might possess one or more adult plant resistance (APR) genes to stem rust of wheat. Molecular marker analysis for detection of the known APR genes (Sr2, Sr55, Sr56, Sr57, and Sr58) should be conducted along with multi-pathotype tests for further determination of the specific genes(s) that conferred resistance to stem rust pathogen races including Ug99 and its derivatives for each genotype. The eleven genotypes that possessed APR genes can be good sources of durable stem rust resistance genes to be incorporated in the Ethiopian durum wheat improvement program.

Keywords: Adult plant resistance, all stage resistance, durum wheat, stems rust, physiological races

INTRODUCTION

Wheat (Triticum spp.) is considered as the earliest domesticated cereal crop and currently the most important agricultural product of the world. It is one of the most important cereal crops in the world in terms of cultivation area and amount of produce. According to FAOSTAT (2014), 729 million metric tons of wheat produced in the world. The major producing countries in the world are European Union, China, India, USA and France in that order. In 2015/16 cropping season during main and minor cropping seasons the average yield of wheat in Ethiopia was 2608kg/ha (CSA, 2016).

There are many known wild and cultivated species of wheat in the genus Triticum. However, the principal wheats of commercial importance are T. aestivum L. and T. durum Desf. (Hanson et al., 1982). Durum wheat (T. durum Desf.) is the predominant tetraploid species that constitutes nearly 10% of wheat production in the world and approximately 30% in Ethiopia (Hanson et al., 1982). Durum wheat is popular for its wide range of food products, such as breads, pastas, cookies, etc. (Pena, 2002). In addition, it has high nutritive value (>10% protein, 2.4% lipid, and 79% carbohydrates) and accounts for about 20% of the caloric intake of the human diet (Khanna, 1991; Gooding and Davies, 1997).

In Ethiopia, the most suitable altitudes for wheat production are between 1900- 2700 meter above sea level (Hailu et al., 1991). Despite the large area of cultivation under wheat, average yield in Ethiopia is below the world average (FAO, 2016). The low yield of wheat in Ethiopia is attributed to a number of factors which encompass soil fertility, weeds, moisture stress and pests of which disease is rapidly spreading fungal diseases causing epidemics that require urgent actions (Hovmoller et al 2010). Stem rust is a potentially devastating fungal disease that can kill wheat plants and small grain cereals but more typically reduces foliage, root growth, and grain yields (Sawhney, 1995). Epidemics of stem rust could cause a loss of up to 100% (Chen, 2005; Pardey et al., 2013). Temesgen et al. (1995) reported that an outbreak of stem rust epidemics which occurred in Arsi and Bale regions caused 67-100% loss on commercial durum wheat cultivars. The main reason for such a disaster was the continuous release of cultivars with major gene (race-specific) resistance (Ayele, 2002). Since race-specific resistance usually overcome through emergence of new races of virulence in the pathogen population, durable resistance is of great interest to wheat breeders (Suenaga et al., 2001; Lalahamed et al., 2004).

Stem rust of wheat caused by the pathogen Puccinia graminis f. sp. tritici (Pgt) has become an important disease of wheat in the major wheat producing regions of Ethiopia. Hence, use of resistant cultivars particularly of adult plant resistance (APR) genes is the most effective, sustainable and environment-friendly way of managing rust diseases of wheat due to its durable nature of resistance (Bhavani, et.al., 2019). Presence of a single or couple of APR genes in a cultivar may not provide sufficient resistance levels in a high disease pressure area. However, cultivars with high levels of resistance were developed by pyramiding three to five APR genes (Bariana and Mclntosh 1995; Lillemo et al., 2005; Singh et al., 2010; Singh et al., 2011; Bansal et al., 2014). In Ethiopia wheat varieties are becoming vulnerable to stem rust epidemics largely due to the use of varieties with race specific major gene resistance developed materials. A case in point could be the emergence of the new race Ug99 in Uganda in 1999 (Pretorius, et al. 2000) which later appeared in Kenya and Ethiopia in 2005 that broke the resistance of the most widely deployed seedling resistance gene Sr31 after decades of control of the pathogen (Singh, et al., 2011). Ug99 has brought a major anxiety in the world wheat production as majority (>90%) of the world's commercial wheat varieties became susceptible to it (McIntosh and Pretorius, 2011). Hence, this necessitates the need to identify sources of race-nonspecific adult plant resistance germplasm to be incorporated in the wheat breeding scheme. Thus, this study was initiated to assess sources of resistance to stem rust in the durum wheat genotypes.

Materials and methods

Description of Experimental Site

Field experiments were conducted during 2017 cropping season at Debrezeit Agricultural Research Center in two different testing sites. The sites are located within the range of approximate geographical coordinates of 8o 44" N latitude and 38o 57" E longitude with altitude range of 1900-1950 meter above sea level. The average annual rainfall of the area is 851 mm and the soil type of the site is eutric vertisol (87.74%) and haplic andosols and vetric andosols constituting 5.94% each respectively. The average minimum and maximum annual temperatures of the study sites are 11.23CO and 25.19CO respectively (WRB, 2006).

Plant materials used in the study

Fifteen durum wheat genotypes and four bread wheat genotypes including 'Morocco' were used as sources of plant materials in this study. The four bread wheat genotypes were used as spreaders or susceptible cultivars to facilitate infections and also as standard for susceptibility in scoring at greenhouse and field studies. Details of the plant materials are described in table 1.

No.	Genotype	Altitude	Pedigree	Year of release	Source
1	Alem tena	1500-1800	Icasyr-1/3/Gen//Sn/Mrb3	2016	DZARC
2	Tesfaye	1800-2800	ARMENT//SRN-3/NIGRIS-4/3/CANELD-9.1/4/TOSKA-26/RASCON-37//SNITSN/5/PLAYERO	2016	DZARC
3	Mangudo	1800-2600	MRF_1STJ2/3/1718BT24//KARIM	2012	DZARC
4	Utuba	188-2600	Durum ICARDA/Ethiopia IDON-MD 53	2015	DZARC
5	Denbi	1800-2650	(AJAIA/BUASHEN)	2009	DZARC
6	Ude	1800-2400	CHEN/ALTAR 84//JO 69	2002	DZARC
7	Boohai	-	-COO "S" / CANDEAL II CD 3062- BS OGR	-	DZARC
8	Mukiye	1800-2600	STJ3//BCR/LK54/3/TER-3	2012	DZARC
9	Asasa	1680-2400	(DZ 2085)	1997	DZARC
10	Quamy	1600-2200	CD-75533-A	1996	DZARC
11	Ginchi	2000-2300	DZ-1050	2000	DZARC
12	Yeter	2000-2200	chen/Tez/3/Guil/cII CD 94026-4y-040m-030y-pAp-0y	2002	DZARC
13	DW-NVT-OHMA-16/17-set-I-#3	-	-	-	DZARC
14	Hitosa	1800-2650	CHEN/ALTAR-84	2009	DZARC
15	DW-NVT-OHMA-16/17-set-I #11	-	-	-	DZARC
16	PBW343	-	-	-	DZARC
17	Digelu	-	SHA 7/ KAUZ or HAR 3116	-	DZARC
18	Arendato	-	-	-	DZARC

Pathogen materials

An equal proportion of the mixture of currently dominant stem rust races in the field (TKTTF, TTKSK and JRC-QC) was used as source of inoculums to evaluate the durum wheat genotypes both in the greenhouse and field.

Greenhouse seedling evaluation for stem rust resistance

Phenotyping of the 15 durum wheat genotypes and one susceptible check (Morocco) was conducted to detect the presence of effective seedling resistance genes to stem rust. Ten seeds of each wheat variety and a susceptible check were planted in plastic pots containing soil, compost and sand in the ratio of 2:1:1, respectively using RCBD (Randomized Complete Blok Design) with three replications. After seven days of planting the seedlings were inoculated with mixture of the currently dominant stem rust races in the field (TKTTF, TTKSK and JRCQC) using standard procedure for inoculation of seedlings as described in McIntosh, et al (1995). To create artificial dew and facilitate spore germination, water was sprayed using sprayer. Twenty minutes after inoculation they were placed in dew chamber in dark room (incubation room) covered with polythene plastic sheets for 24 hours at 18-22oC. Upon removal from chamber, seedlings were exposed to 3 hours of fluorescent light to dry dew on the leaves. Following this, the seedlings were transferred to the greenhouse microclimate rooms where conditions were regulated at 12 hours photoperiod, at temperature range of ±25oC and RH of 60 -70%.

Scoring of the infection types (IT) commenced two weeks after inoculations (12-15 days) using 0-4 scale as described in Stakman, et al. (1962). Where, '0' = immune, ';' (flack) = practically immune, '1' = very resistant, '2' = moderately resistant to resistant, '3' = moderately susceptible, and '4' = completely susceptible.

Field evaluation for adult plant resistance to stem rust

The fifteen durum wheat genotypes and a composite of three susceptible check cultivars; PBW343, Digelu and Arendato (used as a rust infector plants) was planted using randomized complete block design with three replications in two different experimental sites. The field plot size was 1.2 x 2.5 m where each experimental plot consisted of six rows. The spacing between blocks was 1m and the spacing between plots and spreader row and within blocks was 0.5 m each respectively and seeds were drilled in rows at spacing of 20 cm with seed rate of 150 kg/ha.

Experimental plots were fertilized with DAP fertilizer at rate of 100 kg/ha at planting as source of P (phosphorus). Urea was used as source of N (nitrogen) at rate of 150 kg/ha and applied in splits where the first half at planting and the remaining half a month after planting. All crop management practices such as cultivation, weeding etc., carried out as desired. Two rows of infector plants (susceptible varieties) were planted across the borders and between the replications. After 30 days of planting the infector plants were inoculated with the spore mixtures of the stem rust races.

Disease severity recording in the field commenced after establishment of the rust in the infector rows. Recording of rust severity was made using the modified Cobb's scale (Peterson et al., 1949; Roelfs, 1992; IPO and CIMMYT, 1999) where 0% = immune and 100% = completely susceptible. The field assessment of stem rust data recording done six times from each experimental plot randomly starting from booting stage until the crop attains its phys-

iological maturity. The average coefficient of infection (ACI) was calculated by multiplying the severity data obtained for each genotype and the constant value assigned for host response as described in (CIMMYT, 1999). The area under disease progress curve was computed using the formula developed by Campbell and Maddon (1990) as described below.

$$\mathbf{AUDPC} = \sum_{\mathbf{i=1}}^{\mathbf{n-i}} [\mathbf{1/2(X_{i+1} + X_i)(t_{i+1} - t_i)}]$$

here, Xi the average coefficient of infection of the ith observation

Xi+1= the average coefficient of infection of the i+1th observation

t i+1-t i=the number of days between the ith observation and the i+1 th observation

Data Analysis

Analysis of variance

The data for disease parameters at the two sites were subjected to analysis of variance using GenStat 16th edition statistical software package (VSN International Ltd, London, UK following the procedures described in Gomez and Gomez (1984). The differences between treatment means was compared using least significant difference (LSD) test at 5% level of significance when the ANOVA showed the presence of significant difference between genotypes.

RESULTS

Results from the experiments entailing assessment of slow rusting resistance genes for stem rust studies between durum wheat genotypes were conducted both in greenhouse and field conditions. The greenhouse seedling test and field assessment data for stem rust resistance has been subjected to analysis of variance (ANOVA) and demonstrated highly significant difference between genotypes for both (ASR & APR) types of genetic resistances.

Seedling Reaction Test

Results from the greenhouse experiment showed that the durum wheat genotypes varied in their reaction. To confirm the results of the seedling tests, the greenhouse experiment was repeated three times following the same procedure and the result was similar. The seedling test data ranged between infection types '1' and '4'. Summary of the greenhouse experiment data is presented in table 2.

Genotypes possessing only adult plant resistance character (genes) showed intermediate (3) or fully susceptible (4) reaction in the seedling tests (Parlevliet, 1988; Sawhney, 1995; Bansal, et al, 2014; Bariana et al., 2016). Based on the 0 - 4 scoring scale, only two cultivars (Ginchi and Quami) showed resistance reactions IT 1 consistently, the two promising genotypes (DW-NVT-OHMA-16/17-set-1-#11,DW-NVT-OHMA-16/17-set-1-#3) showed resistance to moderately resistance reaction IT 2, five genotypes (Boohai, Denbi, Hitosa, Tesfaye, and Yerer) showed moderately susceptible reaction IT 3 ; the rest six genotypes (Alemtena, Asasa, Mangudo, Mekuye, Ude and Utuba) showed susceptible reaction IT 4 to stem rust that is comparable to the standard susceptible check cultivar 'Morocco' (Table 2).

Field Experiment

The field assessment data for stem rust resistance has been subjected to analysis of variance (ANOVA) and demonstrated highly significant difference between genotypes in both locations. The analysis of variance (ANOVA) for AUDPC showed highly significant ($p \leq 0.01$) differences among genotypes and the analysis of variance (ANOVA) for all average coefficient of infection (ACI) showed highly significant ($p \leq 0.01$) differences and the disease severity data showed significant difference at both locations (Appendix 1). This indicated the presence of sufficient genetic variability for the level of resistance/susceptibility among the genotypes investigated. Similar views were endorsed by Aida (2005) who evaluated twenty three bread wheat genotypes for durable resistance to stem rust. Adult plant resistant genotypes were identified on the bases of their area under stem rust progress curve (AUDPC), disease severity and average coefficient of infection at the two locations in addition to their susceptible disease

reaction at seedling stage (IT 3+ to 4) and relatively resistance reactions observed at adult stage in the field (Table 2 and Appendix 2 and 3).

Area under stem rust progress curve and average coefficient of infection

Area under disease progress curve (AUDPC) is the true measures of disease parameter, it directly related with the yield loss (Strange, 1993; Campbell, 1998). At Black soil the disease severity for the durum wheat genotypes showed moderately resistance disease reaction except two genotypes (Tesfaye and Ginchi) that showed moderately resistance to moderately susceptible (MR-MS) reaction (appendix 3).Whereas, the area under disease progress curve (AUDPC) for all fifteen genotypes showed moderately resistance reaction (Table 2).

At Light soil, three genotypes (Asasa, Tesfaye and DW-NVT-OHMA-16/17-set-1-#11) showed MR-MS reaction while the remaining genotypes demonstrated resistance to moderately resistance reaction (appendix 4). On the other hand for disease severity, four of the genotypes (Boohai, Yerer, Tesfaye and DW-NVT-OHMA-16/17-set-1-#3) showed MR-MS reaction while majority of the genotypes showed resistance to moderately resistance reaction (Table 2). Average coefficient of infection showed moderately resistance reaction for all fifteen genotypes at both locations (appendix 3 and 4).

The highest values of both AUDPC (83 in Black soil and 69 in Light soil) and disease severity (100 in both Black soil and 70 in Light soil) was recorded on the spreader plots that were constituted from susceptible genotypes 'Digelu', 'PBW343' and 'Arendato' (Table 2 and appendix 3 and 4). Whereas, disease severity of the experimental treatments (the fifteen durum wheat genotypes) was less compared with the susceptible genotypes since the highest corresponding values of both AUDPC (33 in Black soil and 38 in Light soil) and disease severity (38 in Black soil and 45 in Light soil) were much less than half of the values observed for the susceptible genotypes (Table 2 and appendix 3 and 4).

Genotype	Area under disease progress curve		Seedling result
	Black soil	Light soil	
Alemtena	26	27	4
Asasa	23	38	4
Boohai	26	32	3
Denbi	29	29	3
DW-NVT-OHMA-16/17-set-1-#11	24	35	2
DW-NVT-OHMA-16/17-set-1-#3	27	33	2

DISCUSSION

Durable resistance is a type of resistance that has remained effective in a cultivar during its widespread cultivation for a long sequence of generations or period of time in an environment favorable to a disease (Bariana , 2003). Durable resistance to rusts can be achieved through a combination of both APR and ASR genes deployed to a single commercial cultivar (Singh et al, 2011; Bansal et al, 2014). Since durable resistance is mostly associated with APR or slow rusting genes characterized by susceptible response to seedling tests, it is therefore, important to have seedling reaction test to identify adult plant resistance character. The present study was conducted to assess durable sources of adult plant resistance of durum wheat genotypes. The results from seedling reaction test revealed that four genotypes; Ginchi, Quami, DW-NVT-OHMA-16/17-set-I-#3, and DW-NVT-OHMA-16/17-set-I #11 carried effective ASR genes to Ug99 and its variants with ITs 1, 1+, 2 and 2, respectively. Five genotypes (Boohai, Denbi, Hitosa, Tesfaye, and Yerer) showed moderately susceptible reaction IT 3; the rest six genotypes (Alemtena, Asasa, Mangudo, Mekuye, Ude and Utuba) showed susceptible reaction IT 4 to stem rust. The greenhouse and field evaluation data together showed that eleven genotypes (Alemtena, Yerer, Asasa, Denbi, Hitosa, Mangudo, Mekuye, Ude, Boohai,Tesfaye and Utuba) had source of only adult plant resistance character to stem rust since they showed intermediate to susceptible seedling reactions to stem rust races and comparably low AUDPC values. Debebe (2003) and Aida (2005) reported that selection of genotypes having low AUDPC values with terminal disease score of less than 20S is normally accepted for practical purposes where the aim is to utilize slow rusting resistance as one of the durable resistance strategies. Therefore, these result indicated that all these fifteen durum wheat genotypes carried resistance genes to stem rust effective under field conditions (Table 2).

Several studies showed that genotypes carrying only slow rusting resistance genes or APR genes are usually susceptible at the seedling stage (devoid of effective seedling resistance genes) but become resistant as the plant matures (Mallard et al., 2005; Ellis et al.,2014; Singh et al., 2010). Therefore, these eleven genotypes (Alemtena,Yerer, Asasa, Denbi, Hitosa, Mangudo, Mekuye, Ude, Boohai, Tesfaye and Utuba) may possess three to five adult plant resistance genes since their field assessment results confirmed their adult plant resistance character

(Table 2 and Appendices 3 & 4). Significant number of findings (Bariana and Mclntosh, 1995; Lillemo et al., 2005; Singh et al. ,2010; Singh et al.,2011; Bansal et al.,2014) indicated that presence of a single or couple of APR genes in a cultivar may not provide sufficient resistance levels in high disease pressure areas; however, they mentioned that cultivars with high levels of resistance were developed by pyramiding three to five APR genes.

In general, it is possible to surmise that those genotypes that exhibited field resistance to stem rust at both locations but with seedling reactions ranged from 3 to 4 lacks effective ASR genes, hence, they can be good sources of APR genes. Therefore, these genotypes have to be selected as donor parent for incorporating durable resistance in durum wheat improvement program. For effective and precise breeding outcome knowledge of identity of the APR genes present in these genotypes is essential; hence genotyping/screening of these eleven genotypes with the already known molecular markers of the APR genes; Sr2, Sr55, Sr56, Sr57 and Sr58 is imperative. The outcome of these studies could be used as a preliminary source of information to develop high yielding stem rust resistant durum wheat cultivars for future breeding program particularly for durable resistance wheat breeding through gene pyramiding approaches using molecular marker assisted selection.

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Conflict of interest

The authors declare that there is no conflict of interest regarding publication of this manuscript

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