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Research Article

Diversity of Ethiopian Durum Wheat Landraces for Resistance to Stem Rust Seedling Resistance Genes

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Stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is one of the most important diseases of wheat worldwide. Breeding for resistance to diseases is the most important approach for mitigation of yield losses. This study was conducted to estimate the diversity of all stage stem rust resistance (ASR) genes on the 142 durum wheat landrace accessions at seedling stage. The study was conducted in greenhouse at Ambo Plant Protection Research Center on the 142 durum wheat landrace accessions using 20 differential lines, one susceptible line (McNair), and eight *Pgt* races. The result depicted the presence of *Sr7b, Sr8a, Sr9b, Sr10, Sr11, Sr13, Sr17, Sr30, Sr31, Sr36, and SrTmp* in the Ethiopian durum wheat accessions. Among the 142 durum wheat accessions, 83 accessions were identified for possessing single ASR genes, and four accessions including the universal susceptible line (McNair) did not have effective resistance genes to the pathogen races tested in this study. The remaining 55 accessions had either a combination of two resistance genes, unknown number and kind of genes, or unidentified genes displaying resistance across all the pathogen races. This study demonstrated the prevalence of significant genetic diversity for stem rust ASR genes in the Ethiopian durum wheat landraces.

1. Introduction

In sub-Saharan Africa (SSA), Ethiopia is the largest producer of wheat with approximately 1.7 million ha of land under cultivation [1]. Wheat is fourth in area coverage and third in amount of grain production following maize and teff in the country [2]. In Ethiopia, more than 90 bread and 36 durum wheat varieties have been released for production since 1950s. However, the national average yield is 2.42 t/ha, which is far less than the world average of 3.43 t/ha [1]. The low productivity is attributed to lack of varieties resistant to the prevalent wheat rusts, namely, the stem rust (*Puccinia graminis* f. sp. *tritici* Eriks. and E. Henn), leaf rust (*P. triticina* Eriks.), and stripe rust (*P. striiformis* Westend. f. sp. *tritici* Eriks.), which are the major important diseases.

Stem rusts are managed by cultural control, chemical applications, and use of resistant varieties, in which case the third option is the best strategy [3,4]. Wheat producers in Ethiopia require disease resistant varieties which were farmers-friendly, environmentally safe, and cost effective. It is important to identify sources of resistance genes in order to develop disease resistant wheat cultivars. One of the rich sources of stress resistance germplasm is landraces or farmers' varieties, which are also known to be reservoirs of genetic resources like resistance genes for several plant diseases including wheat rust [5–7].

Use of crop diversity is a key approach to improve productivity and achieve food security [8]. Ethiopian durum wheat landraces are diverse and possess high variation for economically important agronomic traits including resistance/tolerance to both biotic and abiotic stresses but are not exploited enough [9–11]. The durum accessions contributed to the world wheat varietal improvement; for instance, the Ethiopian durum wheat landrace ST464 was one of the major sources of *Sr13* [12].

Most commonly three of the stem rust resistance genes (*Sr9d*, *Sr9e*, and *Sr13)* are present in many *T. durum* genotypes alone or in various combinations [13]. However, the adequacy of the resistance genes believed to be present in those cultivars may not be effective in providing full protection against the pathogen [14]. Therefore, it is important to expand our knowledge on the response of the Ethiopian durum wheat accessions to the current pathogen populations. Hence, we need to search new sources of stem rust resistance genes, particularly host plants possessing durable resistance genes/nonrace specific resistance genes [5, 6].

The source of seeds used by majority of durum wheat growing farmers in Ethiopia is landraces consisting of large numbers of different genetic backgrounds [9]. For identification of resistant sources of genes, germplasms were assessed from known sources and screened for triple resistance to wheat rust diseases [15, 16]. Colomba and Gregorini [17] reported that there are six types of *Triticum* species grown in Ethiopia, namely, *T. dicoccum, T. turgidum, T. durum, T. polonocim, T. pyramidale*, and *T. carthilcum*. Among those, *T. durum* (durum wheat) and *Triticum aestivum* are the most dominantly grown species. For future use in research and maintenance of the available germplasm, the Institute of Biodiversity Conservation (IBC) has collected more than 12,726 accessions of *Triticum* species from various agroecological zones of Ethiopia and, out of those, tetraploid wheat species accounted for 72% of the germplasm collection [18]. Therefore, durum wheat accessions collected from different agroecologies and locations are considered to vary for resistance to diseases and pests, grain yield, and adaptation to specific environmental situations and are generally considered initial ground for durum wheat improvement program [18]. Hence, this study was conducted with the general objective of evaluating the genetic diversity of durum wheat accessions grown in Ethiopia for resistance to stem rust pathogen (*Puccinia graminis* f. sp. *tritici*).

2. Materials and Methods

2.1. Description of Study Areas. Greenhouse study was conducted at Ambo Plant Protection Research Center (APPRC), Jibat Woreda, during 2020 crop growing season. The center has national mandate for stem rust race analysis and gene postulation tests. It is located at geographic coordinates of 08°57′58″N and 37°51′33″E latitude and longitude, respectively. The study site is also situated at an altitude of 2175 m.a.s.l.

2.2. Experimental Materials

2.2.1. Plant Materials. 142 durum wheat accessions were obtained from the Ethiopian Biodiversity Institute, and 20 stem rust near isogenic differential lines were used in combination with the durum wheat accessions. In addition, a universal susceptible cultivar (McNair 701) was included as a control (Tables 1–4).

2.2.2. Pathogen Materials. Eight *Pgt* races (TTKSK (Ug99), TTTTF, TTRTF, JRCQC, TKTTF, TRTTF, TTKTT, and TKKTF) were used for gene postulation from Ambo plant protection laboratory.

2.3. Green House Experiment

2.3.1. Experimental Design and Treatments. Pot experiment for evaluating seedlings in the greenhouse was conducted to infer resistance genes in the 142 wheat accessions including one control susceptible cultivar (McNair) and 20 differential lines following the method described by Roelfs and Marten [19]. 25 seeds from 142 durum wheat accessions and 15 seeds of each differential line were pregerminated on moist filter paper in 90 cm Petri dish. After two days, five sprouting seeds were transplanted into a 5 cm diameter plastic pot filled with sterilized soil, sand, and compost at the ratio of 2 : 1 : 1. Each pot was replicated twice and placed in seedling growth chamber room until two primary leaves were emerged for inoculation. In the same manner, the differential accessions were planted in pots and were arranged in four sets of five groups according to the following orders (Table 5): Group i: *Sr5*, *Sr2l*, *Sr9e, and Sr7b*; Group ii: *Sr11, Sr6, Sr8a, and Sr9g*; Group iii: *Sr36, Sr9b, Sr30, and Sr17*; Group iv: *Sr9a, Sr9d, Sr10, and SrTmp*; Group v: *Sr24, Sr31, and Sr38,* including *SrMcN*, the susceptible accession McNair without *Sr* gene, used as control [19].

2.3.2. Inoculum Preparation and Inoculation. For the eight predominant stem rust races (TTKSK (Ug99), TKTTF, JRCQC, TRTTF, TTRTF, TTKTT, TKKTF, and TTTTF), their virulence spectra on the stem rust differentials are described in Table 6. Techniques for inoculum production, collection, storage, and inoculation followed the standard guideline produced by Roelfs et al. [21]. Increasing of urediospore bulk sample was conducted on susceptible cultivar (McNair). The method used for deriving single isolate, characterization, and nomenclature was described by Fetch and Dunsmore [22]. One gelatin capsule of freshly harvested urediospores prepared by suspending 14 mg in 0.75 ml lightweight mineral oil, Soltrol 170 (Chevron Phillips Chemical Company, The Woodlands, Texas, United States) was used to inoculate on 48 accessions (240 seedlings per tray). Inoculation was done using atomized inoculator by spraying when the seedlings have fully expanded primary leaves and the second leaves begin to grow after seven days at seedling stage [23]. Inoculated seedlings were moistened with fine droplets of distilled water produced with an atomizer and placed in a dew chamber in darkness for 18 hours at 18 to 22°C temperature and 98 to 100% relative humidity. Upon removal from dark chamber, plants were exposed to 4 hours of fluorescent light to provide condition for infection and allowed to dry dew for about 2 hours. Inoculated plants were then transferred to greenhouse benches where conditions were regulated at 12 hours' photoperiod, with temperature range of 18 to 25°C and relative humidity (RH) of 60 to 70% [24].

Table 1: List of durum wheat accessions postulated to carry only a single *Sr* gene.

	Pgt races								
Accessions	TTKSK	TKTTF	JRCQC	TRTTF	TTRTF	TTKTT	TKKTF	TTTTF	Postulated genes
222469	3^-	3^-	2^-	3^-	3^-	3^-	3^-	3^-	*Sr7b*
204453	3^-	3^-	2^-	3^-	3^-	3^-	3^-	3^-	*Sr7b*
238127	3^-	3^-	2^-	3^-	3^-	3^-	3^-	3^-	*Sr7b*
ISr7b-Ra	3^-	3	2^-	3^-	3^-	3	3^-	3	
238129	3^-	3^-	;1^+	2^+	3^-	3^-	3^-	3^-	*Sr8a*
222582	3^-	3^-	2^+	2^+	3	3^-	3^-	3^-	*Sr8a*
222553	3^-	3^-	;1^+	2^+	3^-	3^-	3^-	3^-	*Sr8a*
222426	3^-	3^-	2^+	2^+	3^-	3^-	3^-	3^-	*Sr8a*
ISr8a-Ra	3^-	3^-	;1^+	2^+	3^-	3^-	3^-	3^-	
222388	3^-	3^-	2	3	3^-	3	3^-	3^-	*Sr9b*
216069	3^-	3	2	3^-	3^-	3^-	3^-	3^-	*Sr9b*
226889	3^-	3^-	2	3^-	3^-	3^-	3^-	3^-	*Sr9b*
214605	3^-	3^-	2	3^-	3^-	3^-	3^-	3^-	*Sr9b*
208200	3^-	3^-	2	3^-	3^-	3^-	3^-	3^-	*Sr9b*
226876	3^-	3^-	2	3^-	3^-	3^-	3^-	3^-	*Sr9b*
226880	3^-	3^-	2	3^-	3^-	3^-	3^-	3^-	*Sr9b*
208188	3^-	3^-	2	3^-	3^-	3^-	3^-	3^-	*Sr9b*
W2691Sr9b	3^-	3^-	2	3^-	3	3	3	3^-	
213036	3^-	3^-	2^+	3^-	3^-	3^-	3^-	3	*Sr10*
222432	3	3^-	2^+	3	3	3^-	3^-	3^-	*Sr10*
208183	3^-	3^-	2^+	3^-	3	3^-	3^-	3^-	*Sr10*
214312	3^-	3^-	2^+	3^-	3^-	3^-	3	3^-	*Sr10*
208128	3^-	3^-	2^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
221740	3	3^-	2^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
212648	3^-	3^-	2^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
238113	3^-	3^-	2^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
222560	3^-	3^-	2^+	3^-	3^-	3	3^-	3	*Sr10*
222474	3^-	3	2^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
222488	3^-	3^{--}	2^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
214527	3^-	3^-	2^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
204410	3^-	3^-	2^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
208201	3^-	3^-	2^+	3^-	3^-	3	3^-	3^-	*Sr10*
226858	3^-	3^-	2^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
204454	3^-	3^-	2^+	3	3^-	3^-	3^-	3^-	*Sr10*
208189	3^-	3^-	2^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
222439	3^-	3^-	1^+	3^-	3^-	3^-	3^-	3^-	*Sr10*
W2691Sr10	3^-	3^-	2^+	3^-	3^-	3	3	3^-	
226886	3^-	2^+	3^-	3^-	3	3^-	2^+	3^-	*Sr11*
222464	3^-	2^+	3^-	3^-	3^-	3^-	2^-	3^-	*Sr11*
226882	3^-	2	3^-	3^-	3^-	3^-	2	3^-	*Sr11*
238124	3^-	2	3^-	3^-	3^-	3^-	2^+	3	*Sr11*
204409	3^-	2^+	3^-	3^-	3^-	3	2^-	3^-	*Sr11*
238115	3^-	2	3^-	3^-	3^-	3^-	;1^+	3^-	*Sr11*
204432	3^-	2^-	3^-	3^-	3^-	3^-	2^-	3^-	*Sr11*
214495	3^-	2^+	3^-	3^-	3^-	3^-	;1	3^-	*Sr11*
222705	3^-	2^+	3^-	3^-	3^-	3	2^+	3^-	*Sr11*
222381	3^-	2^+	3^-	3^-	3^-	3	2	3	*Sr11*
238125	3^-	2^+	3^-	3^-	3^-	3^-	2^+	3^-	*Sr11*
238114	3^-	2^+	3^-	3^-	3^-	3^-	1^+	3^-	*Sr11*
204560	3^-	2^+	3^-	3^-	3^-	3^-	;2	3^-	*Sr11*
238132	3^-	2^+	3^-	3^-	3^-	3^-	1^+	3^-	*Sr11*
216098	3^-	2^+	3^-	3^-	3^-	3^-	2^+	3^-	*Sr11*
204545	3^-	2^+	3^-	3^-	3^-	3^-	;1^+	3^-	*Sr11*
204521	3^-	2	3^-	3^-	3^-	3^-	;1^+	3^-	*Sr11*
214589	3^-	2^+	3^-	3^-	3^-	3^-	1^+	3^-	*Sr11*
222520	3^-	2^+	3^-	3^-	3^-	3^-	2^-	3^-	*Sr11*
238123	3^-	2^+	3^-	3^-	3^-	3^-	2^-	3^-	*Sr11*
222435	3^-	2^-	3^-	3^-	3^-	3^-	2^-	3^-	*Sr11*
222815	3^-	2^+	3^-	3^-	3^-	3^-	2^-	3^-	*Sr11*

Table 1: Continued.

	Pgt races								
Accessions	TTKSK	TKTTF	JRCQC	TRTTF	TTRTF	TTKTT	TKKTF	TTTTF	Postulated genes
206627	3^-	$;1^+$	3^-	3^-	3^-	3^-	;1	3^-	*Sr11*
222552	3^-	2^-	3^-	3^-	3^-	3^-	$;1^+$	3^-	*Sr11*
226977	3^-	2^+	3^-	3^-	3^-	3^-	1^+	3^-	*Sr11*
236987	3^-	2^-	3^-	3	3^-	3^-	2^-	3^-	*Sr11*
ISr11-Ra	3^-	2^+	3^-	3^-	3^-	3^-	$;1^+$	3^-	
208934	3^-	3^-	2^+	3^-	2^-	3^-	3	3^-	*Sr30*
222505	3^-	3^-	2^+	3^-	2^+	3^-	3^-	3^-	*Sr30*
BtSr30Wwst	3^-	3^-	$;1^+$	3^-	$;1^+$	3	3^-	3^-	
208785	3^-	2^-	2^+	2^+	2^-	3^-	2	$;1^+$	*Sr31*
226898	3^-	2^+	;	2	2^-	3^-	;1	;1	*Sr31*
5250	3^-	2^+	2^+	2^-	1^+	3^-	2^+	2^+	*Sr31*
236988	3^-	;1	;	2	2^-	3^-	$;1^+$	2^-	*Sr31*
204391	3^-	2	2^+	2^+	2^+	3^-	$;1^+$	2^-	*Sr31*
Sr31/6*LMPG	3^-	;1	2^+	$;1^+$	2	3^-	1^+	$;1^+$	
204562	2^+	3^-	2^-	3^-	3^-	2^+	2	3^-	*Sr36*
204506	2^+	3^-	;	3^-	3^-	2^+	;	3^-	*Sr36*
211488	2^+	3^-	2^-	3^-	3^-	2^+	2^+	3^-	*Sr36*
222680	2^+	3^-	2^-	3^-	3^-	2^-	2^+	3^-	*Sr36*
208476	2^-	3^-	2	3^-	3^-	2^+	2^+	3^-	*Sr36*
226857	2^-	3^-	2^+	3^-	3^-	2^+	2^-	3^-	*Sr36*
226859	2^+	3^-	;2	3^-	3^-	2^+	$;1^+$	3^-	*Sr36*
238126	2^-	3^-	2^-	3^-	3^-	2^+	;1	3^-	*Sr36*
238121	2	3^-	;1	3^-	3^-	2^+	;2	3^-	*Sr36*
8063	2^+	3^-	2	3^-	3^-	2^+	;2	3^-	*Sr36*
222428	2^+	3^-	;	3^-	3^-	2^+	;2	3^-	*Sr36*
204522	2^+	3^-	;	3^-	3^-	2^+	;	3^-	*Sr36*
204542	2^+	3^-	2^-	3^-	3^-	2	2^-	3^-	*Sr36*
W2691SrTt-1	2^+	3^-	;	3^-	3	2^-	;	3^-	
238128	2^+	3^-	2^-	3^-	3^-	3^-	3^-	3^-	*SrTmp*
238120	2	3^-	2^+	3^-	3^-	3^-	3^-	3^-	*SrTmp*
226869	2^+	3^-	2^-	3^-	3^-	3^-	3^-	3^-	*SrTmp*
204444	2^+	3^-	2^+	3^-	3^-	3^-	3^-	3^-	*SrTmp*
CnsSrTmp	2^+	3^-	$;1^+$	3^-	3^-	3	3	3	

2.4. Data Collection

2.4.1. Disease Parameters. In the greenhouse, data recording for seedling infection types began after 14 days of inoculation using the 0–4 scoring scale developed by Stakman et al. [25], where 0 indicates immune or fleck; 1 indicates small uredia with necrosis; 2 indicates small-to-medium uredia with chlorosis or necrosis; 3 indicates medium-size uredia with/without chlorosis; and 4 indicates large uredia without chlorosis or necrosis. The scales up to the rate of 2 were considered to be incompatible, while the rates above 3 were regarded as compatible reactions. The infection types were defined by modifying characters as follows: –, uredinia somewhat smaller than normal; +, uredinia somewhat larger than normal for the infection type.

2.5. Data Analysis and Interpretation. Seedling resistance genes were postulated based on the gene-for-gene specificity hypothesis between the host resistance genes and the avirulence genes in the pathogen by correlating the response of the differential sets of each pathogen with the response of the host genotypes. To interpret the results from the multipathotype tests, a differential response key for a given gene based on responses of the target differential genotype against an array of pathotypes was generated (Table 5). This key was used to postulate the resistance genes which occurred singly in various entries. Postulation of more than one resistance gene per entry was performed when deviation from the usual infection type was shown by a particular resistance gene, as evidenced by the specific infection types.

3. Results and Discussion

The gene postulation result depicted the presence of diverse types and numbers of stem rust resistance genes in the Ethiopian durum wheat accessions (Tables 1–4). Among the 142 durum wheat accessions evaluated for the presence of stem rust resistance genes, 83 were found to possess nine different kinds of (*Sr7b, Sr8a, Sr9b, Sr10, Sr11, Sr30, Sr31, Sr36,* and *SrTmp*) singly postulated stem rust resistance genes. Four accessions (5180, 204463, 222433, and 203968) including the universal susceptible line (McNair) did not have effective resistance genes to the pathogen races tested in this study. The remaining 55 accessions had either a combination of two resistance genes (like *Sr13* and *Sr17*

Table 2: List of durum wheat accessions postulated to carry two *Sr* genes.

Accessions	TTKSK	TKTTF	JRCQC	TRTTF	TTRTF	TTKTT	TKKTF	TTTTF	Postulated genes
208191	;1^{+}	2^{-}	;1	2^{-}	2^{+}	3^{-}	;1	2^{+}	*Sr13* + *Sr17*
226893	2	2	2^{+}	2^{+}	2^{+}	3^{-}	2^{+}	2	*Sr13* + *Sr17*
214608	2	2^{-}	2	;1	;1	3^{-}	2^{-}	;1	*Sr13* + *Sr17*
214418	2^{-}	2^{+}	2^{-}	2	2^{+}	3^{-}	2^{+}	2^{+}	*Sr13* + *Sr17*
214348	2^{+}	2^{+}	2^{-}	2^{+}	2^{+}	3^{-}	1^{+}	2	*Sr13* + *Sr17*
226860	;1	;1^{+}	;1^{+}	;1^{+}	2^{+}	3^{-}	2	;1^{+}	*Sr13* + *Sr17*
222764	2^{+}	2^{+}	2^{+}	2^{+}	2^{+}	3^{-}	2^{-}	2^{+}	*Sr13* + *Sr17*
222449	2^{+}	2	2^{+}	2	;1^{+}	3^{-}	2^{-}	2^{+}	*Sr13* + *Sr17*
226884	;1	2^{+}	2^{+}	2^{+}	2	3^{-}	2^{-}	2^{+}	*Sr13* + *Sr17*
212650	2	2	2^{+}	2^{+}	2^{+}	3^{-}	2^{+}	2	*Sr13* + *Sr17*
208331	2	2	2	2^{+}	2^{-}	3^{-}	2^{+}	2^{+}	*Sr13* + *Sr17*
222494	2^{+}	2^{+}	2	2^{+}	2	3^{-}	2	2^{+}	*Sr13* + *Sr17*
222405	2^{-}	2^{+}	2^{+}	2^{+}	;1	3^{-}	2^{+}	2	*Sr13* + *Sr17*
226965	2^{+}	2^{-}	;1	2	;1^{+}	3^{-}	2^{-}	2^{+}	*Sr13* + *Sr17*
222437	;1^{+}	;1^{+}	;1^{+}	2^{-}	2^{+}	3^{-}	;1	2^{+}	*Sr13* + *Sr17*
226885	;1^{+}	2^{-}	2^{+}	2^{+}	2	3^{-}	2^{+}	2^{+}	*Sr13* + *Sr17*
222454	2^{-}	2^{-}	2^{+}	2^{+}	2	3^{-}	2	2	*Sr13* + *Sr17*
222450	;1	;2	;1	2^{+}	2^{+}	3^{-}	;	;1	*Sr13* + *Sr17*
226883	;1^{+}	;	;1	;1^{+}	2	3^{-}	;1	;1	*Sr13* + *Sr17*
204011	2	;1	2^{+}	2^{-}	1^{+}	3^{-}	;	;1	*Sr13* + *Sr17*
204476	2	2^{+}	2^{-}	2^{+}	2	3	2	2	*Sr13* + *Sr17*
204555	2	;1^{+}	2^{-}	2^{+}	2^{-}	3	2^{-}	2^{-}	*Sr13* + *Sr17*
204589	;1	;1	;1	2^{+}	2^{+}	3^{-}	;1	2^{+}	*Sr13* + *Sr17*
Combination V	2^{+}	2^{+}	2^{+}	2^{+}	2^{+}	3^{-}	2^{+}	2^{+}	

Table 3: List of durum wheat accessions postulated to carry more than two stem rust resistance genes.

	Pgt races								
Accessions	TTKSK	TKTTF	JRCQC	TRTTF	TTRTF	TTKTT	TKKTF	TTTTF	Postulated genes
226867	2^{-}	;1	2^{-}	3^{-}	3^{-}	2^{-}	2^{+}	2^{+}	Sr11^{+} Sr36^{+*}
222422	;1^{+}	;1^{+}	2^{+}	3^{-}	3^{-}	2^{-}	2	;1^{+}	Sr11^{+} Sr36^{+*}
208206	2^{-}	2^{+}	2^{+}	3^{-}	3^{-}	2^{-}	2^{-}	2^{-}	Sr11^{+} Sr36^{+*}
ISr11-Ra	3^{-}	2^{+}	3^{-}	3^{-}	3^{-}	3^{-}	;1^{+}	3^{-}	
W2691SrTt-1	2^{+}	3^{-}	;	3^{-}	3	2^{-}	;	3^{-}	

*Unknown stem rust resistance gene(s).

postulated on 23 of the accessions), unknown number and kind of genes (where the ITs displayed could not be correlated/matched with the ITs of the tested 20 differential lines), or unidentified genes displaying resistance across all the pathogen races (Table 4).

3.1. Group 1: Durum Wheat Accessions Postulated for Possessing Single Resistance Genes. The largest portions (58.45%) of the durum wheat accessions were found to possess only one (a single) stem rust resistance gene. Among the singly postulated stem rust resistance genes, the most frequent resistance gene was *Sr11* (18.31%), followed by *Sr10* (12.67%), *Sr36* (9.15%), and *Sr9b* (5.6%). On the other hand, *Sr31* (3.5%), *Sr8a* (2.8%), *SrTmp* (2.1%), *Sr7b* (1.4%), and *Sr30* (0.7%) were the least postulated genes. The durum wheat accessions and their phenotypic expressions (disease reaction) against each of the eight *Pgt* races with the corresponding disease reaction of the differential cultivars are presented in Table 1. The above nine kinds of *Sr* resistance genes were postulated by comparing the IT patterns of the eight different *Pgt* races on the 83 durum wheat accessions with those of the differential lines possessing the known resistance genes as displayed.

3.2. Group 2: Durum Wheat Accessions with Two All Stage Resistance (ASR) Genes. Accessions that showed low ITs similar to a combination of two resistance genes with compensating pathotypic specificities were postulated to carry the corresponding two *Sr* genes. In this case, 23 durum wheat accessions exhibited exactly identical ITs displayed by the differential cultivar "Combination V" that is known to carry *Sr13* + *Sr17* together (Table 2).

3.3. Group 3: Durum Wheat Accessions Postulated to Carry More than Two Genes. The three durum wheat accessions 226867, 222422, and 208206 were postulated to carry three or more (*Sr11, Sr36*, and other unknown) resistance genes in combination (Table 3). These accessions displayed low infection types to six of the *Pgt* races (TTKSK, TKTTF, JRCQC, TTKTT, TKKTF, and TTTTF) and high ITs for the remaining two *Pgt* races (TRTTF and TTRTF). The *Sr36*

Table 4: Durum wheat accessions postulated for carrying uncharacterized seedling resistance (USR) gene(s) against the eight *Pgt* races.

Ser. no.	Accessions	TTKSK	TKTTF	JRCQC	TRTTF	TTRTF	TTKTT	TKKTF	TTTTF	Postulated *Sr* gene(s)
1	213037	2$^+$	2	2$^-$	2$^+$	;1	2$^+$	;1	2$^-$	USR
2	203992	;1	;1	;1$^+$	;1	;1	2$^-$	;1	;1$^+$	USR
3	236986	2	;1$^+$	2$^-$	2$^-$	2$^-$	;1$^+$	2	;1	USR
4	238131	2$^+$	2$^-$	1$^+$	2$^+$	;1$^+$	;1	;	;1	USR
5	204566	;1	;1	;1$^+$	2$^+$	2$^-$	;1$^+$	2$^-$	2$^+$	USR
6	222389	;1	;1	2$^-$	2$^-$	2$^-$	2$^-$	;1	2$^+$	USR
7	214264	;1	2$^-$	;1$^+$	2	2$^+$	2$^+$	2	2$^+$	USR
8	232119	;1	;1$^+$	2	2$^-$	2$^-$	;1$^+$	;1$^+$	;1$^+$	USR
9	204509	;1	;1$^+$	;1	;1	;1$^+$	;1	;	2$^-$	USR
10	214606	2$^-$	;1$^+$	2$^+$	2$^+$	;1$^+$	2$^-$	;1	;1$^+$	USR
11	226866	2$^-$	;1	2$^+$	2$^+$	;1	;1$^+$	;1	;1	USR
12	214467	;1	;1	2	;1	;1	2	;1	;1$^+$	USR
13	222451	2	;1$^+$	;2	;1$^+$	2$^-$	2$^-$	;	2	USR
14	226978	;1	;1$^+$	;1$^+$	2	2$^-$	;1$^+$	;1	;	USR
15	226821	;1$^+$	2$^-$	2	;1	2$^-$	2$^+$	;1	;1	USR
16	222482	2	2$^-$	3	3$^-$	3$^-$	2$^+$	2$^-$	2$^+$	USR
17	208197	2$^+$	2$^+$	3$^-$	3$^-$	3$^-$	2$^-$	2$^-$	2$^+$	USR
18	222550	2$^+$	2$^+$	3$^-$	3$^-$	3$^-$	2$^-$	;	;1$^+$	USR
19	222559	2$^+$	;1	3$^-$	3$^-$	3$^-$	2$^+$	2	2$^-$	USR
20	5204	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	2$^-$	2$^+$	3$^-$	USR
21	226973	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	2$^+$	2$^+$	3$^-$	USR
22	204363	3$^-$	3$^-$	3$^-$	3$^-$	3	3$^-$	2	2	USR
23	7974	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	2$^-$	2	USR
24	204428	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	2$^-$	2$^+$	USR
25	226971	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	2$^-$	2$^-$	USR
26	204543	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	2$^+$	2$^+$	USR
27	5071	3$^-$	2$^-$	3$^-$	2$^-$	3$^-$	3$^-$	3$^-$	2$^+$	USR
28	204586	3$^-$	2$^+$	3	2$^+$	3$^-$	3$^-$	3$^-$	2$^-$	USR
29	222556	3$^-$	2$^+$	3$^-$	3$^-$	3$^-$	2$^+$	;1	3$^-$	USR

Table 5: Seedling infection types produced on eight *Pgt* races of *Sr* genes.

Differential lines	Sr genes	TTKSK	TKTTF	JRCQC	TRTTF	TTRTF	TTKTT	TKKTF	TTTTF
ISe5-Ra	*5*	3$^-$	3$^-$	;	3$^-$	3$^-$	3$^-$	3	3$^-$
CnS-T-mono	*21*	3$^-$	3$^-$	3	3$^-$	3$^-$	3	3$^-$	3$^-$
Vernstine	*9e*	3$^-$	3$^-$	3	3$^-$	3$^-$	3	3$^-$	3$^-$
ISr7b-Ra	*7b*	3$^-$	3	2–	3$^-$	3$^-$	3	3$^-$	3
ISr11-Ra	*11*	3$^-$	2$^+$	3$^-$	3$^-$	3$^-$	3	;1$^+$	3$^-$
ISr6-Ra	*6*	3$^-$	3$^-$	3$^-$	3$^-$	3$^-$	3	3	3$^-$
ISr8a-Ra	*8a*	3$^-$	3$^-$	;1$^+$	2$^+$	3$^-$	3$^-$	3$^-$	3
CnSr9g	*9g*	3$^-$	3$^-$	3	3$^-$	3$^-$	3$^-$	3$^-$	3
W2691SrTt-1	*36*	2$^+$	3$^-$	;	3$^-$	3	2–	;	3$^-$
W2691Sr9b	*9b*	3$^-$	3$^-$	2	3$^-$	3	3	3	3$^-$
BtSr30Wwst	*30*	3$^-$	3$^-$	;1$^+$	3$^-$	;1$^+$	3	3$^-$	3$^-$
Combination V	*17*	2$^+$	2$^+$	2$^+$	2$^+$	2$^+$	3-	2$^+$	2$^+$
ISr9a-Ra	*9a*	3$^-$	3$^-$	3$^-$	3	3$^-$	3$^-$	3	3$^-$
ISr9d-Ra	*9d*	3$^-$	3$^-$	3	3$^-$	3$^-$	3	3$^-$	3
W2691Sr10	*10*	3$^-$	3$^-$	2$^+$	3$^-$	3$^-$	3	3	3$^-$
CnsSrTmp	*Tmp*	2$^+$	3$^-$	;1$^+$	3$^-$	3$^-$	3	3	3
LeSr24ag	*24*	;1	;1	;1	2–	;1	3	;	;1
Sr31/6*LMPG	*31*	3$^-$	;1	2$^+$	;1$^+$	2	3$^-$	1$^+$	;1$^+$
VPMI	*38*	3$^-$	3$^-$	2	3$^-$	3$^-$	3	3	3$^-$
McNair 701	*McN*	3$^-$	3$^-$	3$^-$	3$^-$	3	3	3$^-$	3

Table 6: The origin and virulence/avirulence formulae of the Ethiopian *Pgt* races used in the study.

Race	Origin	Avirulence	Virulence
TTKSK	Uganda	*Sr24, 36, Tmp*	*Sr5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38, McN*
TKTTF	Ethiopia	*Sr11, 24, 31*	*Sr5, 21, 9e, 7b, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 38, McN*
TRTTF	Yemen	*Sr8a, 24, 31*	*Sr5, 21, 9e, 7b, 11, 6, 9g, 9b, 30, 17, 9a, 9d, 10, 38, McN*
JRCQC	Ethiopia	*Sr5, 7b, 8a, 9b, 10, 24, 30, 31, 36, Tmp*	*Sr21, 9e, 11, 6, 9g, 9a, 9d,*
TTRTF	Georgia	*Sr24, Sr30, 31*	*Sr5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, 38, McN*
TTKTT	Kenya	*Sr36*	*Sr5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*
TKKTF	Kenya	*Sr11,24,31,36*	*Sr5, 21, 9e, 7b, 6, 8a, 9 g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*
TTTTF	USA	*Sr24, 31*	*Sr5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*

Source: [20].

carrying differential line W2691SrTt-1 had low ITs to four of the *Pgt* races (TTKSK, JRCQC, TTKTT, and TKKTF), whereas it showed high ITs to the remaining four *Pgt* races. Meanwhile *Sr11* carrying differential line ISr11-Ra displayed low ITs to only two *Pgt* races (TKTTF and TKKTF) and high ITs to the remaining six *Pgt* races. The three durum wheat accessions' IT patterns matched with the combinations of the IT patterns displayed by *Sr11* and *Sr36* except the low IT displayed for the *Pgt* race TTTTF that prompted the inclusion of another (other) unknown *Sr* gene(s).

3.4. Group 4: Durum Wheat Accessions Carrying Unknown/Unidentified Sr Genes. In this group, 29 durum wheat accessions were included, which were further divided into two subgroups. The first subgroup contained 15 accessions, which exhibited low infection types for all the tested races (Table 4). We could not postulate a known *Sr* gene(s) to this subgroup because the differential lines were susceptible to at least one of the *Pgt* races that did not match with the infection patterns produced by a specific or a combination of two or greater number of genes. The second subgroup consisted of 14 durum wheat accessions that displayed unique high and low IT patterns that we could not find a match to either a specific known gene or combinations of two or more genes with the available data of the seedling reaction of differential lines with the eight *Pgt* races. The differential lines carrying *Sr* genes *Sr21, Sr9e, Sr6, Sr9g, Sr9a, and Sr9d* did show high infection types for all the eight tested *Pgt* races; hence, they were excluded from this study.

4. Discussion

Stem rust is economically one of the most important diseases of wheat that could lead to 100% yield loss if susceptible cultivar is used. Germplasm development and enhancement activities should be given more attention for mining novel stem rust resistance genes by screening diverse sources of germplasm collections in order to combat the devastating effects of stem rust disease. Ethiopia is highly endowed with durum wheat genetic diversity owing to the presence of large number of collections of tetraploid wheat germplasms maintained in the Institute of Biodiversity Conservation. Additionally, cultivation of landraces of durum wheat by millions of smallholder farmers in the central highlands ensures the continuation of genetic conservation of the crop for future use. Landraces are known to be reservoirs of genetic diversity for several economically important genes that can be easily deployed to modern cultivars through conventional and modern breeding methodologies.

Gene postulation studies (multipathotype tests) conducted at seedling stages in the greenhouse facilitate identification of resistance genes controlled by major genes in the host genotypes (durum wheat plant in this case). In this study, 142 Ethiopian durum wheat accessions obtained from IBC, Ethiopia, were screened for resistance to stem rust pathogens in the greenhouse during the 2019 cropping season. The greenhouse study depicted 11 stem rust resistance genes (*Sr7b, Sr8a, Sr9b, Sr10, Sr11, Sr17, Sr24, Sr30, Sr31, Sr36, and SrTmp*). In agreement with the current finding, Belayneh et al. [26] postulated 11 stem rust resistance genes (*Sr5, Sr7a, Sr7b, Sr8a, Sr9e, Sr11, Sr21, Sr27, Sr29, Sr30,* and *Sr37*) from a set of 60 wheat genotypes constituted from 30 durum wheat and 30 bread wheat genotypes using 40 differential cultivars and 10 *Pgt* races isolated from Ethiopian *Pgt* population obtained from annual *Pgt* pathotype surveys. Similarly, Randhawa et al. [27] postulated seven kinds of stem rust resistance genes (*Sr7b, Sr8a, Sr12, Sr15, Sr17, Sr23,* and *Sr30*) from a set of 87 Nordic spring wheat cultivars using eight Australian *Pgt* races. In general, the multipathotype test indicated the presence of relatively appreciable diversity (11 *Sr* genes including *Sr13*+*Sr17* postulated in combination) of stem rust resistance genes in the Ethiopian durum wheat landrace accessions; in particular, the set of germplasm accessions that displayed unknown and or uncharacterized *Sr* genes could add to additional Ug99 effective resistance genes in combinations or novel genes which are effective against all the pathotypes used in this study. Similar views were endorsed by Belayneh et al [26], Randhawa et al. [28], and Dakouri et al. [29] who identified uncharacterized/unknown genes effective against all the *Pgt* races used in their respective seedling tests in the greenhouse.

Regarding the number and type of stem rust resistance genes, Beteselassie et al. [16] postulated *Sr7b, Sr8b, Sr9a, Sr9b, Sr10, Sr14, Sr24, Sr27, Sr28, Sr29, Sr30, Sr31, Sr32, and SrTt-3 + Sr10* on 16 emmer and 5 durum wheat landrace accessions (obtained from IBC, Ethiopia) using 10 *Pgt* races and 33 differential accessions. These authors reported more diverse types of *Sr* genes compared to the current study that might be due to the use of 33 differential lines and 10 types of *Pgt* races and evaluation of two different tetraploid wheat species (emmer and durum wheat). In the same manner,

Belayneh et al. [26] used 40 differential cultivars and lines representing 40 different *Sr* genes and 10 *Pgt* races. Those authors were able to postulate *Sr9e, Sr14, Sr21, Sr27, Sr28, Sr29, Sr32,* and *Sr37*, whereas this study lacks the differential cultivars that help to detect these genes in the durum wheat germplasm. More findings similar to the results of this study have been reported by Dyck and Sykes [30] who carried out genetic analysis of stem rust resistance in Ethiopian durum wheat germplasm that showed the presence of *Sr6, Sr8a, Sr9a, Sr9d, Sr9e, Sr11, Sr13, Sr30,* and *Sr36*.

The presence of *Sr13* + *Sr17* in the Ethiopian durum wheat germplasm has been reported by Banchigize-Getie [31] who evaluated 45 Ethiopian durum wheat landraces along with 35 differential cultivars and nine *Pgt* races for seedling resistance using multipathotype test conducted at the University of Sydney, Australia. Klindworth et al. [12] and Periyannan et al. [32] reported that durum wheat is the major source of *Sr13*, which is in agreement with this study. The *Pgt* race JRCQC in Ethiopia and other races of stem rust in several countries were reported to show virulence to *Sr13*; however, this gene was found to be effective against the *Pgt* race Ug99 (TTKSK) and its derivatives that gave it major importance worldwide to be deployed in new cultivars in order to combat this aggressive *Pgt* race [32]. Hence, these 23 durum wheat accessions could be good sources of stem rust resistance to be incorporated in breeding lines to develop wheat cultivars.

Several researchers postulated combination of three or more rust resistance genes in a single genotype [16, 26, 27, 31]. The durum accessions carrying a greater number of resistance genes could be excellent candidates (sources of germplasm) for gene pyramiding in transgressive breeding.

In order to solve the mysteries of the USR genes presented in Table 4, carrying out of additional such experiments with other additional differential lines and foreign *Pgt* races might help to identify the genes conferring resistance to stem rust in some or more of the genotypes [26]. The other option is to conduct molecular marker analysis using diagnostic DNA markers for the genes frequently found in durum wheat germplasm [33]. The last option could be to conduct genetic analysis for each of the landraces and study the stem rust inheritance to determine the type and number of gene(s) conferring resistance to these genotypes [34, 35]. Discovery of USR genes in such kinds of (multipathotype tests) experiments conducted for Ethiopian durum wheat landraces had been reported by Naod-Beteselassie et al. [16], Belayneh et al. [26], and Getie [31] who have endorsed similar views indicated above.

Generally, this study depicted 11 stem rust resistance genes either singly or in combinations of two, three, or greater number of genes. Most of these ASR genes (60% or 83 accessions) are not effective when used singly to protect the host from *Pgt* in the field. Hence, they should be used in combination with other effective ASR and/or APR genes; particularly the ASR genes should be selected based on their compensating race specificities. For instance, 23 of the 142 durum wheat accessions possessed *Sr13* + *Sr17* in combination; also 3 of them possessed *Sr11* + *Sr36* + USR genes. These genes showed compensating race specificities; due to this, the *Sr11* + *Sr36* + USR possessing accessions were resistant to six of the eight races, whereas the *Sr13* + *Sr17* possessing accessions were resistant to all races of the pathogens tested. The remaining 29 accessions possessed unidentified stem rust resistance genes. Such findings are not uncommon in such kinds of experiments. These types of genetic materials could be potential sources of novel genes for rust resistance genes. Further studies such as genetic analysis (inheritance studies) and molecular marker analysis would lead to identification and characterization of the USR genes.

Data Availability

All the data related to this manuscript are included in the manuscript. If any additional information is needed and is available, the authors can provide it upon request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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