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Seedling and Adult Plant Resistance to Stem Rust (*Puccinia graminis* f.sp. *tritici*) in Selected Ethiopian Durum Wheat Landraces, Cultivars, and CIMMYT Advanced Lines

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Abstract: Breeding wheat for stem rust resistance caused by *Puccinia graminis f.sp. tritici* (Pgt) is a priority work worldwide including Ethiopia. Most of the major stem rust resistance genes deployed in commercial durum wheat cultivars and breeding lines succumb to emerging races in Ethiopia. In this study, 156 durum wheat accessions consisted of cultivars, landraces and advanced lines were exposed to the three e stem rust races (TTTTF, TTRTF, and JRCQC) at the seedling and adult plant growth stages. The seedling test was conducted in the greenhouse while the field tests in a single race nursery at Debre Zeit at Debre Zeit research center during 2019 and 2020 seasons. An augmented design with three replicated checks in every 20 entries was used in the field experiments while the seedling test was carried out based on the standard procedures. Of the 156 entries; only 22 (14%) and 25 (16%) exhibited resistance at seedling and adult plant growth stages. Of the tested CIMMYT advanced lines, 89% of the lines were susceptible to the race TTTTF, while 11% of the lines were resistant to this race at seedling stage. Of the total tested entries, 16 and 11% were resistant to all the three races at seedling and field condition, respectively. Six accessions exhibited overall resistance (at seedling and adult plant growth stages), while seven entries (two cultivars, two landraces, and three CIMMYT advanced lines) showed susceptible reaction (high infection types) at seedling stage and low severity (resistance) under field conditions to the three races; these accessions possibly have adult plant resistance to stem rust. Further pre-breeding (and genotyping) research is recommended to identify and characterize the stem rust resistance genes in those wheat germplasm associated to overall and adult plant resistance.

Keywords: Durum Wheat, Virulent Races, Adult Plant Resistance, Seedling Resistance, *Puccinia graminis* f.sp. *tritici*

1. Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum*, 2n = 4x = 28; AABB) is among the top 10 most important crops. It is the only tetraploid species of commercial wheat commonly cultivated worldwide with an annual production average of 40 million tonnes in 2016/17 cropping season on 16 million hectares of land [1, 2]. Durum wheat accounts about 8% of the world's total wheat production [18] and it is the primary source of semolina, often used in food products such as pasta, couscous, bulgur, various bread, and other local products [8]. Ethiopia is considered as secondary center of origin for tetraploid wheat and the largest durum wheat producer in sub-Saharan Africa, with approximately 0.6 million hectares of land [15, 29]. However, the future wheat production must increase due to an expected 50% increase in the global demand for agricultural products as the world population is estimated to exceed nine billion by 2050 [10]. Supplying enough and ensuring sustainable wheat production for a rapid growing world population faces several challenges. Among those, wheat stem rust caused by *Puccinia graminis* f. sp.

tritici (*Pgt*) Ericks and Henn remains the most problematic to the world's wheat production [22]. The continuous emergence of virulent races of *Pgt* poses a threat to food security and continues to affect production through overcoming the effectiveness of resistance genes. The sudden changes appeared in the stem rust virulence patterns remind us the vulnerability of commercial varieties and continuously threaten global wheat production [3, 5, 23].

The highland of Ethiopia is considered as a hot spot for the development of stem rust diversity [31]. Repeated epidemics have been recorded in different parts of the country in the recent history that have caused great losses [23, 20]. Apart from the rapid rate of change in the genetic make-up of stem rust populations induced by mutation and selection pressure, the current resistance breeding strategies and the increasingly narrow deployment of resistance in the field result in easily breakdown of resistance by the pathogen. Most Ethiopian cultivars widely grown and advanced breeding lines are known to possess the *Sr24* resistance gene [13]. To date, 13 races have been identified within the Ug99 race lineage in Eastern Africa and the Middle East, and it is projected to further increase, threatening the major wheat growing regions in the world [24].

Nowadays, new races that are not members of the Ug99 race group, have caused disease epidemics on durum wheat in East Africa and worldwide [23]. Races out of the Ug99 race group with relevant virulence on durum wheat such as TTTTF, JRCQC, and TTRTF have been reported. Race TTTTF was detected in Iran from samples collected during 2010-2014 [26, 9]. This race devastated several thousands of hectares of durum wheat on the Italian Islands of Sicily in 2016, causing the largest stem rust outbreak that Europe has seen in decades [9]. In Ethiopia, TTTTF was first detected from samples collected in 2009 in Eastern Shoa zone of central Ethiopia at trace level and had been spread to most of the wheat growing areas of the country [16, 12].

TTRTF is the other virulent race on durum wheat that caused a severe epidemic on durum wheat in Sicily, Italy, epidemic in 2016 [4, 25]. This race was observed in Georgia in 2014 and carries virulence to several resistance genes in durum and common wheat. TTRTF has virulence on resistance genes including *Sr9e*, *Sr13b*, *Sr35*, *Sr36*, *Sr37*, *Sr38*, *Sr45*, and *SrTmp* [21]. Race JRCQC was identified in Ethiopia in 2009 and has a combined virulence on alleles of commonly deployed resistance genes in durum wheat (*Sr9*e and *Sr13b)* [19, 35]. Resistance of durum wheat cultivars to the Ug99 lineage is mainly conferred by Sr13 and Sr9e [34, 28]. The appearance *Pgt* races TTTTF, JRCQC, and TTRTF with combined virulence to *Sr13b* and *Sr9e* has increased the susceptibility of durum wheat to stem rust [19]. Moreover, released varieties and breeding lines having single race specific resistance genes have been resulted in boom-and-bust cycles in which case breeding programs should ensure multiple gene combinations to enhance the durability of resistance [6, 5]. The failure of all-stage resistance genes because of virulence diversity in the *P. graminis* f. sp. *tritici* population threatens wheat production worldwide and has led researchers to select for adult plant resistance [31]. Sounding up the alarm on finding resistant breeding lines, landraces or varieties is the most economical and environmentally feasible approach for proper management of the pathogen. Field resistant and seedling susceptible phenotype of existing varieties, landraces and advanced breeding lines against these three races may facilitate the identification of adult plant resistance (APR) germplasms. Therefore, this study was designed to evaluate durum wheat cultivars, landraces and advanced breeding lines for adult plant resistance (field resistance) and seedling susceptible to races TTTTF, TTRTF, and JRCQC.

2. Materials and Methods

2.1. Description of the Experimental Site

The experiment was conducted at Debre Zeit Agricultural Research Center on black soil (vertisols) and light soil for two consecutive (2019–2020) cropping seasons under rain-fed condition. Debre Zeit Agricultural Research Center is located at 8°44′ N and 38°58′ E, and with a distance of 47 km from Addis Ababa.

The altitude is 1,998 m above sea level, while most the trial fields are heavy soils (Vertisols) with few pockets of light soils (Alfisols/ Mollisols) Total rain fall, mean maximum temperature, minimum temperature, relative humidity during the cropping seasons were 775.6 (64.6 mean) mm, 26.5°C, 7.8°C, 55.5%, respectively (Figure 1).

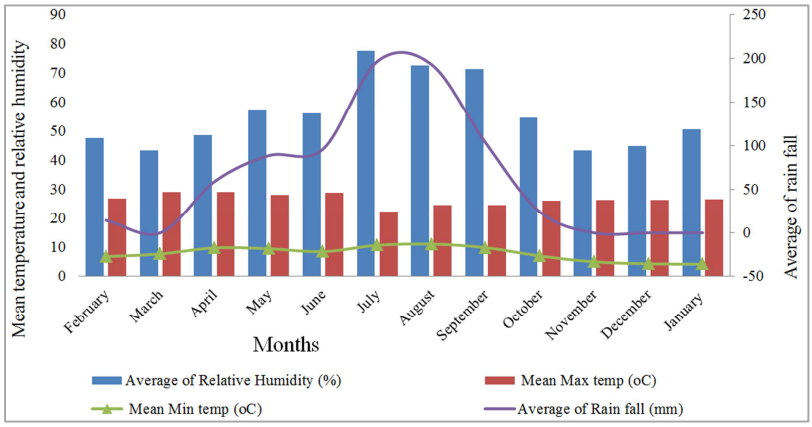

Figure 1. *Mean monthly minimum, maximum temperature, relative humidity, and rain fall during 2019 and 2020.*

2.2. Plant Materials

A total of 156 germplasm consisting of 38 durum wheat cultivars, 28 durum wheat land races, and 90 CIMMYT advanced durum wheat breeding lines and the checks (Table 1).

Table 1. *Durum wheat accessions and their sources.*

Accessions	Number	Source
Commercial cultivars	38	DZARC
Landraces	28	DZARC
Advanced lines	90	CIMMYT

2.3. Stem Rust Isolates (Pgt Races)

The three *Pgt* races (TTTTF, JRCQC, and TTRTF) and their virulence/avirulence on the twenty stem rust differential lines with known genes are shown in Table 2 below. The isolates were obtained from Ambo Agricultural Research Center and individually multiplied on susceptible variety (McNair) in the greenhouse at Debre Zeit Agricultural Research Center. These races were selected based on their virulence on standard differential lines and global deployed durum wheat varieties.

Table 2. *Virulence and Avirulence of selected Pgt races on standard differential lines with known genes at seedling stage.*

Pgt races	Virulence	Avirulence	% virulence
JRCQC	*21, 9e, 11, 6, 9g, 17, 9a, 9d, McN*	5, 7b, 8a, 36, 9b, 30, Tmp, 10, 24, 31, 38	45
TTRTF	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, 38, McN	30, 24, 31	85
TTTTF	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*	24, 31	90

2.4. Seedling Test Procedures

Five seeds of each accession were planted in plastic pots (6.7 cm width x 6.7 cm length x 9 cm height) filled with soil: compost: sand combination in 2:1:1 (v/v/v) ratio and replicated twice for each isolate. After planting, trays were incubated in a greenhouse maintained at 19 to 22°C with a photoperiod of 16 h manipulated by supplemental inflorescent lighting. The seedling plants were inoculated when the primary leaf had fully emerged and the second leaf starting to grow. Urediniospores were heat shocked by submergence of plastic bags containing the isolates within a water bath set at 45°C for 15 min and suspended in lightweight mineral oil (Soltrol 70) in preparation for inoculation. A total of 0.75 ml of oil was added to individual gelatin capsules each including 14 mg of urediniospores. One gelatin capsule was used to inoculate 48 wheat accessions (5 seeds x 48 lines =240 seedling plants total/capsule) using a vacuum pump. Immediately after inoculation, the oil was allowed to evaporate for 15 minutes, and inoculated plants were subsequently placed into dew chambers at 22°C where the chamber was misted for 2 min every 15 min for ~16 h without light. After exposing the inoculated seedlings to fluorescent light for three hours, the inoculated seedlings were moved to greenhouse benches with 70% RH, day/night and 25°C day/night temperatures with supplemental light using florescent lamps to develop infection. Fourteen days after inoculation, the seedlings were scored based on 0-4 scale. Accordingly, infection types (ITs) ";", "0", "1–", "1", "1+", "2–", "2", and "2+" were considered resistant whereas "3–", " 3", " 3+", and "4" were considered susceptible. This scale was linearized to 0-to-9 scale as ';' and '0' = 0, '1–' = 1, '1' = 2, '1+' = 3, '2–' = 4, '2' = 5, '2+' = 6, '3–' = 7, '3' = 8, '3+' = 9, '4' = 9 for statistical analysis. Accessions with linearized scale ≤6 (IT ≤ 2+) and >6 (IT > 2+) were considered seedling resistant, and susceptible, respectively.

2.5. Field Evaluation Procedures

The same durum wheat accessions (n=156) which were used in the seedling test were exposed to the three stem rust races (TTTTF, TTRTF, and JRCQC) in a single race nursery at Debre Zeit Agricultural Research Center during the 2019 and 2020 main cropping season. The durum wheat accessions were sown in two rows of 1 m length x 20 cm wide plot size. Two susceptible (Local red and Arendeto) and a moderately resistant cultivar (Mangudo*)* were used. An augmented design was used and checks were replicated every 20 entries. Spreader rows were sown perpendicular to the plots in the middle of 1-m alleys Spreader rows were composed of mixtures of susceptible cultivars, namely Arendato, Local red, Leeds (Sr13/Sr13b) and Malefia in equal proportion of each cultivar. Each nursery was surrounded by 2 m of border planted with two rows of Oat (*Avena sativa* L.) which is a non-host to *Pgt* and used as a physical barrier to filter stray spores (contaminants). The borders and spreaders were planted 1 to 2 weeks before the entries were planted.

2.5.1. Multiplication and Inoculation

Each race (TTTTF, TTRTF, and JRCQC) maintained and increased on two susceptible wheat cultivars (McNair 701 (CItr 15288) and local red). Twenty seedlings were raised in 7x7 diameters plastic pots field with sterilized soil, sand and compost in 2:1:1 (v/v/v) ratio. After 4 days of sowing the emerging seedlings were treated with 25 ml of maleic hydrazide solution per pot (0.33 g maleic hydrazide per 1 liter water) to limit excessive growth and stay green Urediniospores were collected from the sporulating pustules of inoculated plants starting 14 days after inoculation using a vacuum pump. The spores allowed to dry for 24 hrs. at room temperature and then be used for inoculation or stored in a refrigerator at 4°C for few Both syringe and spray methods used. About 2mg/ml spore was suspended in distilled water and then a drop of tween 20 is applied per 10 ml of suspension and inoculated using syringe (disposable syringes

are available in pharmacy/drug shop). Each spreader rows surrounded the nursery was inoculated 2-3 times every week at stem elongation (Zadok's growth stage = 31). Four random tillers per meter were inoculated. In addition, the spreader rows were inoculated with a suspension of 0.5 g urediniospores, 500 ml water, and 1 drop of Tween 20 surfactant using knapsack sprayer from the booting to flowering stages.

2.5.2. Data Collection

The disease severity was scored according to the modified Cobb's scale by estimating the proportion of the stem area (0-100%) covered by rust pustules [27]. Host responses were scored based on the size of pustules and amount of chlorosis and necrosis on the stem and the constant value for each response(s) (Table 3). At seven days interval the disease data was recorded four times.

Table 3. *Response classes of genotypes, description of each response and constant value.*

Response classes	Description of each response	Constant value
0	No visible infection	0
R	Resistant	0.2
MR	Moderately resistant	0.4
MR-MS/MS-MR	Moderately Resistance to Moderately susceptible or moderately susceptible to susceptible	0.6
MS	Moderately Susceptible	0.8
MSS	Moderately Susceptible to Susceptible	0.9
S	Susceptible	1

The mean of scale responses was used to calculate coefficient of infection (CI) in the case of combination of infection responses for a given germplasm. Then the coefficient of infection (CI), area under disease progress curve (AUDPC) and final stem rust severity were used for further statistical analysis and the final stem rust scoring was considered to calculate the coefficient of infection for each single-race nursery.

The AUDPC is calculated using the midpoint rule method [7] as follows.

$$AUDPC = \frac{\sum_{i=1}^{n-1}(\mathrm{Yi}+Y_{i+1})}{2} x\ (ti + 1 - ti)$$

Where "y" is the percentage of affected tissue at each reading,

"t" is time in days of each reading and "n" is the number of readings.

2.6. Data Analysis

Analysis of variance was conducted on area under the disease progress curve (AUDPC), coefficient of infection (CI) and final rust severity (disease parameters) to determine resistance differences among the durum wheat accessions. The analysis was conducted using the PROC MIXED in SAS (Version 9.00; SAS Institute, Inc.) following mixed model procedures for an augmented design, where blocks and checks considered as fixed effects and the effect of tested genotypes were considered as random.

Correlation coefficient was used to estimate the relationship between the different disease parameters (AUDPC (%days), CI and final rust severity) for each single race nurseries both at seedling and adult plant growth stage. R statistical software version 4.0.5 was used to analyze correlation between the final rust severity (FS), coefficient of infection (CI) and area under disease progress curve (AUDPC) field response against the three races.

3. Results and Discussion

3.1. Seedling Reaction

All the tested cultivars showed susceptible reaction to race TTTTF at the seedling stage whereas 60.5% and 39.5% of the cultivars exhibited resistance and susceptible reaction, respectively to the race TTRTF (Table 4). Of the tested CIMMYT advanced lines, 89% were susceptible to the race TTTTF, while 11% were resistant to this race at seedling stage. Of the tested durum wheat landraces, 43 and 82% were resistant to races TTTTF and TTRTF, respectively whereas 57 and 18% exhibited susceptible reaction to races TTTTF and TTRTF, respectively. Overall focusing on the seedling susceptible lines were the most important criteria to identify adult plant resistance lines, cultivars, and landraces.

Table 4. *The response of tested entries at seedling against the Pgt races.*

Tested entries and Reaction	Stem Rust Races		
Cultivars (n=38)	**TTTTF**	**TTRTF**	**JRCQC**
Resistant	0	23	28
Susceptible	38	15	10
Land Races (n=28)			
Resistant	12	23	17
Susceptible	16	5	11
CIMMYT Advanced Lines			
Resistant	10	48	48
Susceptible	80	42	42

The frequency of resistance in durum wheat accessions varied according to the stem rust races (Table 5). Of the total accessions, 30.8 and 69.2% exhibited susceptible and resistance reaction to race TTTTF at seedling, respectively while 75.6 and 82.1% showed resistance to JRCQC and TTRTF races, respectively. Race TTTTF was the most virulent on the tested entries followed by the race TTRTF at seedling stage (Table 5). Of the total tested entries, 16% were resistant to all the three races at seedling stage while 17% was susceptible to the three races at seedling stage (Table 5).

Of the total evaluated entries, 52% showed seedling resistant to the races combination TTRTF and JRCQC. Similar results reported by Megerssa et al. [17] who states that the lowest resistant panels evaluated against TTRTF and JRCQC 53.4% races. Relatively the lowest percentage of lines resistant to races TTTTF (69.2%) were recorded as compared with races JRCQC (75.6%) and TTRTF (82.1%) is expected because of the virulence of this race on durum wheat [19, 21]. The seedling resistances observed in the germplasms ranged from single-to multiple-race resistance indicating the effectiveness of the same resistance source against multiple races (Table 5).

Table 5. *Germplasms Resistant and susceptible against single race and combination of races at seedling stage.*

Single Race	Resistant (n=156)	Susceptible (n=156)	Races combined	Resistant (n=156)	Susceptible (n=156)
JRCQC	118	38	TTTTF+TTRTF+JRCQC	25	26
TTRTF	128	28	TTTTF+TTRTF	29	41
TTTTF	108.0	48	TTTTF+JRCQC	30	49
			TTRTF+JQCQC	81	26

The seedling correlation coefficient between TTRTF and TTTTTF was very weak (r=0.16) and weak correlation between JRCQC and TTRTF (r=0.22), JRCQC and TTTTF (r= 0.24) (Figure 1) and this implies that the virulence level between these races is different. According to Megerssa et al. [17] high correlation coefficient (r= 0.64) between the seedling response of races JRCQC and TTRTF (r=0.64) calculated. However, this study showed weak correlation coefficient between the seedling response of races JRCQC and TTRTF with the value of (r= 0.22).

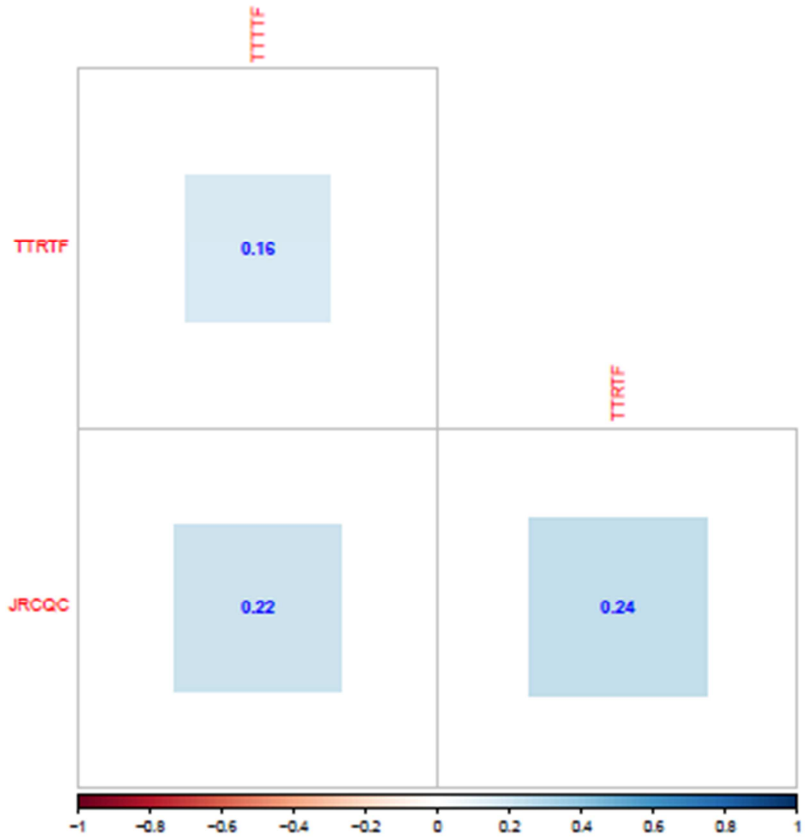

Figure 2. *Correlation between durum wheat germplasms against the three races at seedling stage.*

3.2. Adult Plant Response to the Three Races

The germplasm were evaluated for field response to the three races. Namely: TTTTF, TTRTF and JRCQC at Debre Zeit Agricultural research Center. In the race nurseries, 84% and 80.1% of the accessions were susceptible to race TTTTF and TTRTF, respectively (Table 6). The resistant germplasm varied from 26.9% against race JRCQC to 19.9% against race TTRTF from the field result of each race. From the field single race nursery result 84 and 16% of germplasms were susceptible and resistant to race TTTTF at field, respectively (Table 6).

In general, from phenotypic results; race TTTTF was the most virulent as compared to other races. Of the germplasm evaluated, 11.5% were resistant to all the three races while 66.7% were susceptible to the three races at adult plant growth stage (Table 5). Of the tested germplasm, 14.1 and 69.2% showed resistant and susceptible against the race

combination of TTTTF and JRCQC at adult plant growth stage, respectively. 4% of the germplasm showed vertical resistant or contain major gene, and 4.5% showed horizontal or minor genes resistance to the races combination of TTTTF+TTRTF+JRCQC (Table 7). Of the tested germplasms at seedling and field growth stages, 59% susceptible to the race TTTTF (Figure 2). Similar report was done by Yesuf NS et al. [33] who states that 90% of the differential lines were ineffective against race TTTTF. The phenotypic correlation coefficient between disease parameters (AUDPC, COI and final rust severity) were from moderate (r=0.44) to very strong (r=0.99) (Figure 4).

Table 6. *Germplasms Resistant and susceptible against single race and combination of races at adult plant growth stage.*

Races	Resistant (n=156)	Susceptible (n=156)	Combined races	Resistant	Susceptible
TTTTF	25	131	TTTTF+TTRTF+JRCQC	18	104
TTRTF	31	125	TTTTF+TTRTF	19	123
JRCQC	42	114	TTTTF+JRCQC	22	108
			TTRTF+JRCQC	18	100

Table 7. *Genotypes Resistant at seedling and field, susceptible at seedling and field, seedling susceptible and field against single race and combination of races at field and seedling stages.*

Races	Resistance (Seedling + Field) (n=156)	Susceptible (seedling+ Field) (n=156)	Seedling susceptible + Field Resistance (n=156)
TTTTF	9	97	8
TTRTF	21	38	9
JRCQC	27	39	17
TTTTF +TTRTF	7	34	9
TTTTF+JRCQC	6	32	9
TTRTF+JRCQC	14	24	8
TTTTF+TTRTF+JRCQC	6	19	7

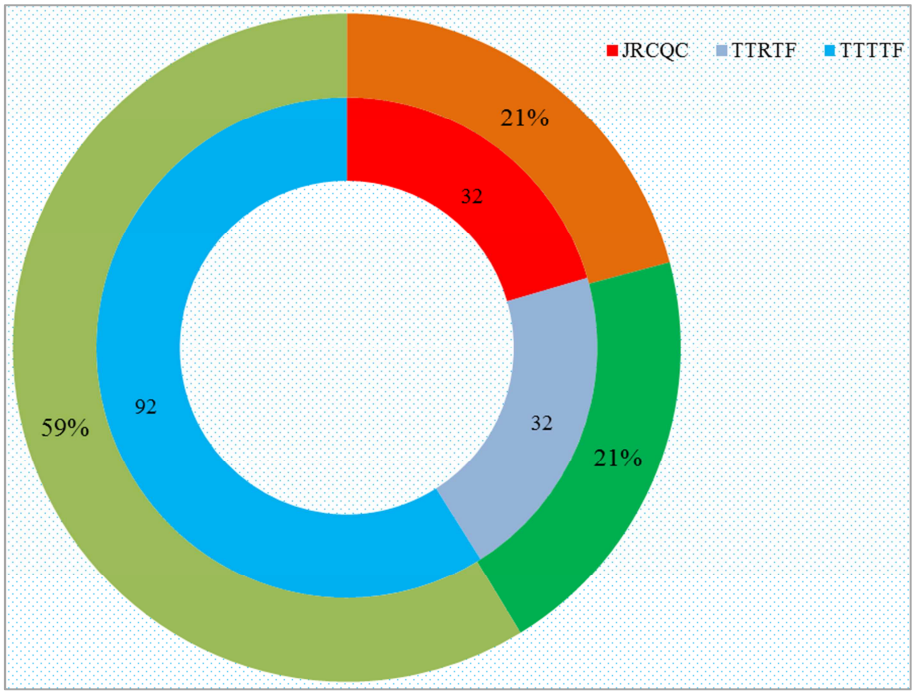

Figure 3. *Total number of entries susceptible both at seedling and field, and percentage to each race.*

Table 8. *Genotypes that showed the lowest severity for each race at field and susceptible at seedling stage (APR-gene).*

Entries	TTTTF		TTRTF		JRCQC		Seedling response		
	FRS	Response	FRS	Response	FRS	Response	TTTTF	TTRTF	JRCQC
Foka (cultivar)	20	MSMR	10	MR	20	MS	3	3	4
Kilinto (cultivar)	20	MSMR	20	MS	15	MS	3	3-	3
MCD5-13	25	MS	20	MSMR	25	MSMR	3	3	3-
MCD1-12	20	MSMR	5	MR	15	MSMR	3	4	3-
Advanced line	25	MS	25	MS	15	MS	3	3	4
Advanced line	20	MS	20	MS	15	MS	3	3	3
Advanced line	25	MS	20	MS	10	MS	3	3-	3

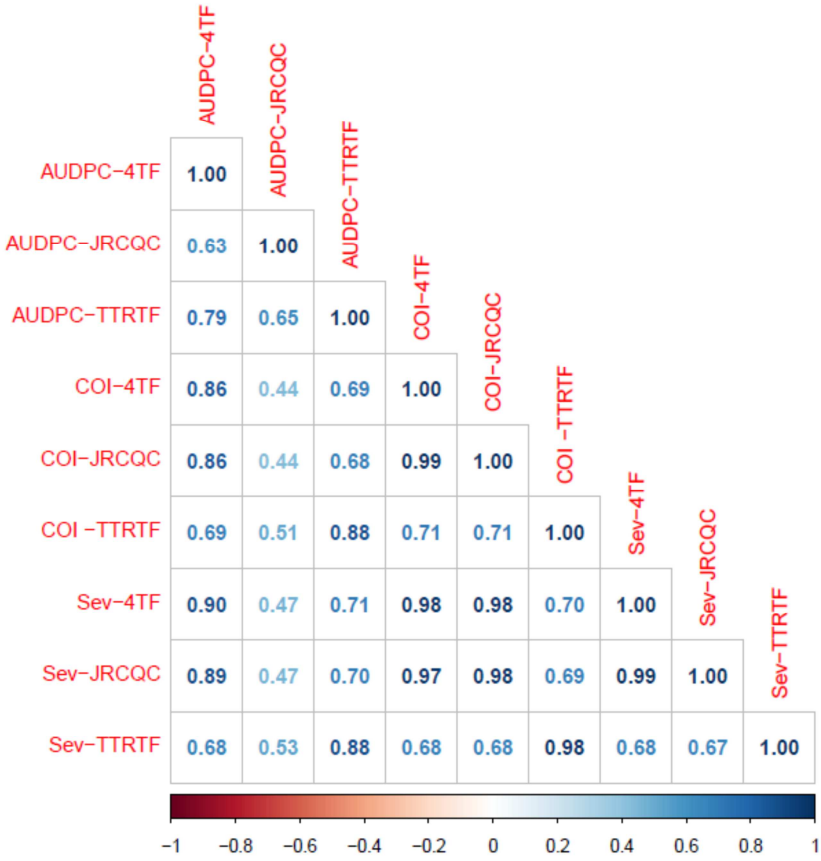

Figure 4. *Correlation coefficients among final rust severity (Sev.), coefficient of infection (COI), and area under the disease progress curve (AUDPC) for the average values for each of the three single race nurseries during 2020 and 2021.*

4. Conclusion and Recommendation

This study shown that the durum wheat released varieties, durum land races, and CIMMYT advanced durum wheat lines harbor race specific and multiple race resistance to the virulent *Pgt* races at the seedling and adult plant growth stages. The germplasms consistently resistant in the seedling assay and in the field are being used as sources of resistance in the durum wheat improvement program. Therefore, identification of sources of resistance to race TTTTF is paramount as this race is more virulent than races TTRTF and JRCQC. This study also confirmed the importance of testing durum wheat germplasm to multiple *Pgt races* both at seedling and adult plant growth stages. Further pre-breeding (genotyping) research is recommended to identify and characterize the stem rust resistance genes in those wheat germplasm associated to overall and adult plant resistance. Identification of sources of adult plant resistance is also very important in future resistance breeding of durum wheat against stem rust.

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