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Response of wheat lines to yellow rust (*Puccinia striiformis* f. sp. Tritici) in central and south eastern parts of Ethiopia

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Abstract: **The present investigation was carried out to detect the prevailing virulent races of yellow rust at field, to identify resistance wheat lines and effective genes for yellow rust. The experiments were carried out in non-replicated design having about 100 entries and differentials both at Sinana and Kulumsa research stations. YR5, YR8, YR15, YR17, YR27, Lassik (-Yr5) and Lassik (+Yr5) resistance genes have immune field reaction at Kulumsa and also Yr15 was the only differential lines resistant to the existing isolates at Sinana. The result depicted that 7, 17 and 76 wheat lines were resistant, moderately resistant and susceptible reaction at seedling stage to Kubsa isolate, respectively and 12, 27 and 61 wheat lines exhibited resistant, moderately resistant and susceptible reactions, respectively, to the Digelu isolate. At field condition 62,7,31 wheat lines were found to be resistant, moderately resistant and susceptible respectively at Sinana. At Kulumsa 97 and 3 wheat lines were found to be resistant and susceptible respectively. Wheat lines Av Yr 15, WT6/12, Sr 33/Cs # 4, Sr 33/Cs #5, Sr 33+Sr 2/Cs #14, Sr 33+Sr 2/Cs #15, Sr 45/Cs #20 and Sr 45+Sr 2/Cs #29 were immune at field condition of all locations. However, Westonia, Thatcher, Avocet, CANTHACHK, CT4-NS1, Pavon Sr 26+ SR 31, Westonia Sr 24+ Sr 31, Pavon Sr 24+ Sr 31 and CTH- Ns 2 exhibited maximum field severity and coefficient of infection (CoF) and hence the aforementioned resistant wheat lines may serve as better parent in resistant wheat breeding program.**

Keywords: **Immune, susceptible, effective genes, resistant, virulence, isolates.**

1. INTRODUCTION

The great majority of Ethiopian -population (85%) lives in rural areas, where agriculture is the major stake of livelihood activities. The ever increasing population needs to have increased food source but the only way of increasing productivity is through intensification because the land couldn't have an infinite space for extension. In order to feed the ever increasing population we have to increase wheat production at the rate 1.6% which can be achieved by developing high yielding varieties having a good tolerance level for biotic and a-biotic stresses (Khan et al., 2013).

Wheat is one of the most widely grown cereal crops globally. In 2010, world wheat production was 651 million tonnes, making it the third most produced cereal after maize and rice. The most common species grown are *Triticum aestivum* L. (common wheat) and *Triticum turgidum var. Durum* L. (durum wheat). Common wheat accounts for 95% of the total wheat consumed worldwide (Randhawa et al., 2013). It is also major crop in Ethiopia that is central to achieving development in agriculture. Most wheat production in Ethiopia comes from small holder farmers. Wheat is mainly grown in the central and south-eastern highlands during the main (Meher) rainy season (June to September) and harvested in October-November. It is the third most important cereal crop after teff (*Eragrostis tef*) and maize (*Zea mays*) in area

coverage and production in Ethiopia. On average, from 2004-2009, wheat production covered 1.51 million hectares of land, and yielded 2.29 million metric tons of grain yield per year(Abebe *et al*., 2013). Ethiopia produces 4.8 million tons of wheat grain from 1.7 million ha in Meher season (CSA, 2018/19). Ethiopia is the second largest wheat producer in sub Saharan Africa, unfortunately, the productivity is remained low, 2.7t/ha as compared to the world average,3t/ha. Arsi and Bale highlands in south eastern Ethiopia are the major common wheat producing provinces of Ethiopia and are considered the wheat belts of East Africa. The highlands of Bale alone contribute about 11% of the country's wheat production. However, high yield and quality losses due to diseases are confronting farmers (Dereje and Chemeda, 2009).

Among the most important diseases of wheat that significantly reduce wheat production are the rusts (yellow, stem and leaf rusts). The rusts of wheat are among the most widely spread pathogens that can be found in most areas of the world where wheat is grown. The disease causing wheat rust fungi are spread in the form of clonally produced dikaryotic urediospores, which can be dispersed by wind for thousands of kilometers from initial infection sites across different areas from continent to oceans. Epidemics of wheat rusts can occur on a continental scale due to the widespread dispersal of urediospores (Khan *et al*., 2013). Wheat rust fungi are highly specific obligate parasites. Rust populations can be characterized by distribution of races and the frequencies of virulence against specific rust resistance genes on a defined set of wheat differential hosts (Khan *et al*., 2013).

Yellow rust prevalence changes from year to year and from place to place, depending on climatic conditions and variety grown (Abebe T. Et al., 2013). Major stripe rust (*Puccinia striiformis* f. Sp. *Tritici*) epidemics occurred in Ethiopia in 1970's, 1988, 2010(Nazari, 2011). In the Bale highlands, wheat is produced twice a year favoring the continuous perpetuation of the three rusts pathogen year round. Yellow rust monitoring conducted over 1996 to 2000, and surveys indicated that the disease is more important, endemic and severe in the main season (August to December) than in short season (March to July). The development of yellow rust epidemics, in addition to favorable weather factors, depends on the level of cultivar susceptibility, which affects the disease occurrence and its progress, cropping systems and management practices (Dereje and Chemeda, 2009).

Yellow rust, caused by *Puccinia striiformis* f. Sp. Tritici, is an important foliar wheat disease in Ethiopia. It has been reported to be prevailing at higher altitudes and cool and temperate regions where wheat is grown (Johnson, 1992; mcintosh *et al*., 1995; Boyd, 2005). The disease was first described by Gaddin Europe in 1777 and it has been reported in more than 60 countries on all continents except Antarctica (Chen, 2005). Stripe rust prevalence changes from year to year and from place to place, depending on climatic conditions and variety grown (Abebe et al., 2013). Major stripe rust epidemics occurred in Ethiopia in 1970's, 1988 and 2010 (Nazari, 2011). However, the development of yellow rust epidemics, in addition to favorable weather factors, depends on the level of cultivar susceptibility, which affects the disease occurrence and its progress, cropping systems and management practices (Hailu and Fininsa, 2009). Justesen et al (2014) used clustering methods and approximate Bayesian computation (ABC) to compare different competing scenarios describing ancestral relationship among ancestral populations and more recently founded populations. Their analyses confirmed that the Middle East-East Africa as the most likely source of newly spreading, high- temperature-adapted strains; Europe as the source of South American, North American and Australian populations; and Mediterranean-Central Asian populations as the origin of South African populations (Justesen et al., 2014).

The existence of a high genotypic diversity, a high sexual ability as well as the independent maintenance of strongly differentiated populations in the Himalayan region pinpoint this region as the possible centre of origin of *Puccinia striiformis* f. Sp. Tritici (Sajid, 2013).

In Ethiopia yellow rust yield loss was assessed on superimposed trials of wheat and the result from the assessment indicated that grain yield losses of 47.2% and 32% were observed in the worst scenario in north Shewa and in east Gojam zones, respectively (Wendale et al., 2016). Grain yield loss was lowest (28%) for south Gondar zone (Wendale et al., 2016). Therefore, the present investigation was carried out to detect the prevailing virulent races of yellow rust at field, to identify resistance wheat lines and effective genes for yellow rust in hot spot areas of south eastern and central Ethiopia.

2. METHODOLOGY

The study comprises the phenotypic evaluation activity both at green house and field conditions. It contains 93- spring bread wheat genotypes from Australia. The aforementioned spring bread wheat genotypes was originated from Dr.Evans Lagudah of CSIRO, were multiplied first by Dr.Wuletaw Tadesse, at Terbol station of ICARDA in Lebanone. In 2015,

these materials were planted at Sinana and Kulumsa stations in Ethiopia for yellow rust evaluation. Additionally, seven checks and 17 yellow rust differential lines were used.

2.1 DESCRIPTION OF THE STUDY AREA

The study was conducted at two locations in Central and Southeast of Ethiopia, namely Sinana and Kulumsa. The selected places have different agro ecological zones which favor the development of yellow rust at various degrees. Sinana is located at 7° 7'N, 40°10'E and at 2450masl. It receives mean annual rainfall of 808 mm. The monthly mean minimum and maximum temperatures are 9.3 and 20.9 °C, respectively. The dominant soil type is pellic vertisol which is slightly acidic. Kulumsa is located at $39^{0}09$'East $08^{0}01$' North and at 2200m above sea level. It receives 820mm of rainfall annually. The monthly mean minimum and maximum temperatures are 10.5°C and 22.8°C, respectively. The dominant soil type is Clay soil (Luvisols). Sinana is well known hot spot area for yellow rust while Kulumsa is hot spot for the three rusts.

2.2 FIELD TEST

2.2.1 PLANTING AND MANAGEMENT

Wheat lines were planted in June, 2015 at Kulumsa and in August, 2015 at Sinana using noo-replicated design, along with various differentials and checks. The wheat lines were planted in four blocks with two rows keeping the distance of 0.2m apart and 2m long following seed rate of 100 kg/ha. Fertilizers such as Urea and DAP were applied at the rate of 50kg/ha and 100kg/ha, respectively. Weed managment and intercultivation was carried out according to the recommendation in each location.

2.2.2 INOCULATION

Rust spores collected from different fields during previous years were multiplied in the greenhouse using susceptible plants grown in pots during June-August, 2015 were used for inoculating wheat lines at tillering. Spreader rows were planted in mixtures of the most susceptible bread wheat cultivars, Morocco and Kubsa in a row around the experimental field. The inoculation was carried out by spraying method at tillering. Spraying of urediospores suspension which was made by diluting 0.275g of spore in one litre of mineral oil (soltrol 70) were done in spreader row with the help of pumping machine (ULV) during after noon.

2.2.3 DATA COLLECTION AND ANALYSIS

Disease severity as a percentage of leaf area covered with the disease was assessed following a modified Cobb's scale considering the perecentages of the rust severity and reaction (Peterson *et al*., 1948). Field response was recorded three times at 10 days interval from tillering to early flowering. The data on disease severity and host reaction was combined to calculate the coefficient of infection (CI) by multiplying the severity value by constant values of 0, 0.2, 0.4, 0.6, 0.8, or 1.0. After the last disease score when the disease progress ceased, according to Stubbs *et al*. (1986); Pathan and Park (2006), the field severity data was converted to Coefficient of Infection (CI) by multiplying with constant values of response. Wheat lines with coefficient of infections ranging from 0 to 20 were considered as resistant while 20 to 30, 30 to 40, 40 to 60 and 60 to 100 were moderately resistant, moderately susceptible, moderately susceptible to susceptible, and susceptible, respectively.

2.3 GREEN HOUSE TEST

2.3.1 COLLECTION AND MULTIPLICATION OF YELLOW RUST ISOLATES IN GREENHOUSE

Yellow rust isolates were collected from 2015 survey in Arsi and West Arsi. Kubsa isolates of yellow rust were collected from the area where Kubsa was dominantly produced and the isolate was widely distributed in Kubsa Kebele, Gedeb Assasa woreda,West Arsi, Oromia. Digelu isolates were collected from the area where Digelu variety was mostly produced (Lemmuna Bilbilo). Fresh and sufficient inoculum was prepared on susceptible hosts. A maleic hydrazide (MH) solution was prepared at a rate of 0.3 g/l. At emergence, seedlings of hosts were treated with a solution of MH to retard plant development and encourage sporulation. Two days after applying MH, seedlings were fertilized with solution as described above. One week old seedlings were infected by spraying with urediniospores of yellow rust in separate greenhouse compartments. A fresh spore of stripe rust was used from growing room for inoculating the selected hosts. For

inoculations, urediospores were suspended in light mineral oil (Soltrol 70). The upper surfaces of primary leaves were uniformly inoculated with a pressurized sprayer by putting pots in an inoculation booth that was automatically rotating to allow uniform spraying at spore concentration of 0.275g/1l urediospores/ L mineral oil. The booth was thoroughly cleaned using alchol after spraying and different isolates were handled separately to prevent contamination. Inoculated seedlings were allowed to dry for about 2 hours before they were incubated by placing in a moist chamber (96% RH) and incubated for 48 hours at 6°C. Seedlings were taken from the moist chambers and allowed to dry slowly for another 2 hours and moved to glasshouse until sufficient spores were harvested for inoculating test plants.

2.3.2 PROCEDURE AND ANALYSIS OF SEEDLING RESISTANCE TEST FOR YELLOW RUST

This experiment was conducted under greenhouse condition at the Ethiopian Institute of Agricultural Research, Kulumsa Agricultural Research Center. Seedlings were inoculated by spraying the bulk of isolates urediospores suspended in light mineral oil (Soltrol 70) using an automizer when the first leaves were fully expanded (8 days after sowing). Inoculated plants were allowed to dry for 5 minutes and were fine-misted with water and placed in a wet plastic cage with a small amount of water at the bottom.

The inoculated seedlings were incubated at 10ºc for 24 hours in a dew chamber with relative humidity close to 100%. Seedlings were transferred to a greenhouse with mean temperature of about 18ºc. A week after inoculation, 2 grams of nitrogen fertilizer per 100 ml was added as liquid fertilizer to each pot.

At seedling, disease assessment was carried out from two replicated experiments to avoid diseases escape: on the 14th days after inoculation using 0–9 scale as indicated below (Table 4, macneal *et al*.1971). Infection types 0-6 were classified as low or resistant while 7-9 scores were considered as high or susceptible infection types (Stubbs, 1985).

Table 1: Infection types representing 0-9 scale according to McNeal *et al.* (1971).

Infection type	Host response	Symptoms Immune
0	Immune	No visible uredia
1	Very resistance	Necrotic flecks
2	Resistance	Necrotic areas without sporulation
3-4	Resistance to moderately resistance	Necrotic and chlorotic areas with restricted sporulation
5-6	Moderately resistance	Moderate sporulation with necrosis and chlorosis
7-8	Moderately susceptible	Sporulation with chlorosis
9	Susceptible	Abundant sporulation without chlorosis

3. RESULTS

3.1 FIELD RESPONSE OF WHEAT LINES TO YELLOW RUST

Control lines carrying the genes *YR5*, *YR8*, *YR15*, *YR17*, *YR27*, Lassik (-*Yr5*) and Lassik (+Yr5) resistance genes have immune field reaction against yellow rust with the severity values of zero at Kulumsa Agricultural Research station (Table 2), suggested that the lack of the aforementioned races in the study area. However, at Sinana only Control lines carrying the genes *Yr 15* was immune to yellow rust. The current results also demonstrated that absence of virulence race to *Yr15* both location i.e Kulumsa and Sinana. The yellow rust Control lines during 2015 was exhibited in but, the next season the yellow rust infection at field condition of Kulumsa was higher on most of control lines. During 2016 cropping season the yellow rust virulence was not detected only for the control lines which have yellow rust resistant genes Yr15 and Yr10 and the rest control lines showed variable field reaction to yellow rust and it could be good indication for the detection of complex yellow rust races at the field conditions of Kulumsa or there would be aggressive races for most of control lines except for the aforementioned resistant yellow rust genes.

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Table 2: Response of yellow rust differentials both at Kulumsa and Sinana, 2015.

No	Host differentials	Field response			
		Yr-genes	Sinana	Kulumsa 2015	Kulumsa 2016
1	YR1/6*Avocet S	1	50S	TrMR	30S
2	YR5/6*AOC CX86.6.1.20	5	30S	0	40S
3	YR6/6*AOC CX94.2.2.25	6	60S	TrSMS	80S
4	YR7/6*Avocet S	7	70S	30S	80S
5	YR8/6*Avocet S	8	60S	0	80S
6	YR9/6*Avocet S	9	60S	TrS	80S
7	YR10/6*Avocet S	10	20S	TrMS	0
8	YR15/6*Avocet S	15	0	0	0
9	YR17/3*AOC CX94.8.1.25	17	80S	0	50S
10	YR18/3*AOC CX94.10.1.7	18	60S	TrMR	60S
11	YR26/3*AOC CX96.17.1.	26	20S	TrMR	-
12	YRSP/6*AOC CX94.14.1.15	Sp	5MS	TrMS	30S
13	YR27/3*AOC CX94.19.1.1	27	40S	0	50S
14	AVOCET R	R	30S	TrMR	70S
15	AVOCET S	S	80S	5Mr	5MR
16	Lassik (-Yr5)	-Yr5	TrMS	0	TMR
17	Lassik (+Yr5)	+Yr5	TrMS	0	10S

S = Susceptible MS = Moderately Susceptible; MSS = Moderately susceptible and susceptible; MR = Moderately resistance SMS = Susceptible and moderately susceptible; MSMR = moderately susceptible moderately resistance; - = not germinated.

62 lines of the tested wheat lines in the current experiment were found to be resistant to yellow rust (Figure 1). The coefficient of infection of the wheat lines varied from 0 (immune) to 80 (susceptible) suggesting wide variations among the lines in resistant to yellow rust. Seven wheat lines were moderately resistant to yellow rust at Sinana and also the rest 31 wheat lines were exhibited susceptible reaction with coefficient of infection of above 31 at hot spot area, Sinana during 2015/16 cropping season. As indicated below in (figure 1) 97 of wheat lines were resistance at field condition of Kulumsa with infection types below 30 and only 3 wheat lines were susceptible at Kulumsa field condition and thus the result revealed that low yellow rust infection at Kulumsa were recorded compared as to Sinana area.

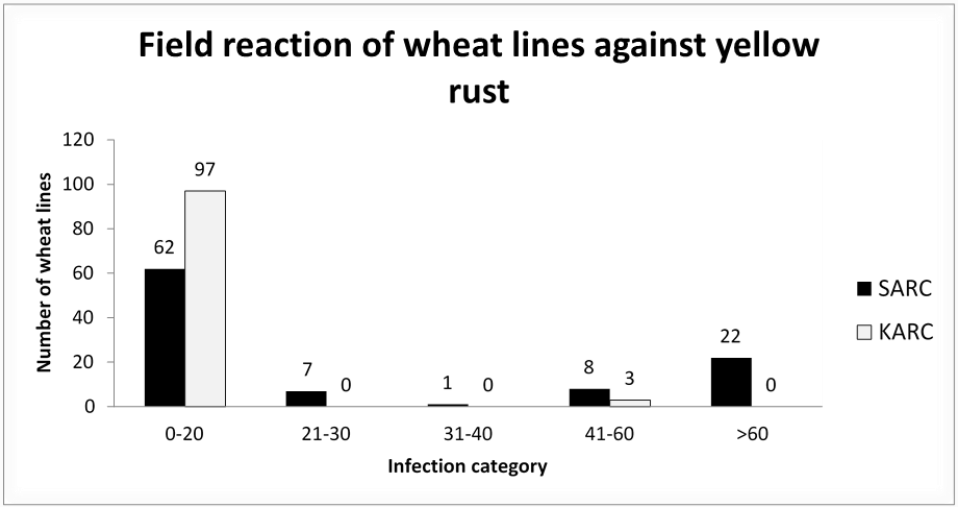

Figure 1: Response of wheat lines to yellow rust in Central and South eastern Ethiopia.

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3.2 FREQUENCY DISTRIBUTION OF SEEDLING REACTION OF WHEAT LINES TO YELLOW

Screening of wheat lines at seedling stage was done at Kulumsa Agricultural Research Center under greenhouse by using two selected yellow rust isolates collected from Arsi zone.

Results revealed that 7, 17 and 76 wheat lines were resistant (0-4 IT), moderately resistant (5-6 IT) and susceptible (7-9 IT) reaction at seedling stage to Kubsa isolate, respectively, as shown in (Figure 2). Besides, 12, 27 and 61 wheat lines exhibited resistant (0-4IT), moderately resistant (5-6IT) and susceptible (7-9 IT) reactions, respectively, to the second isolate (Digalu).

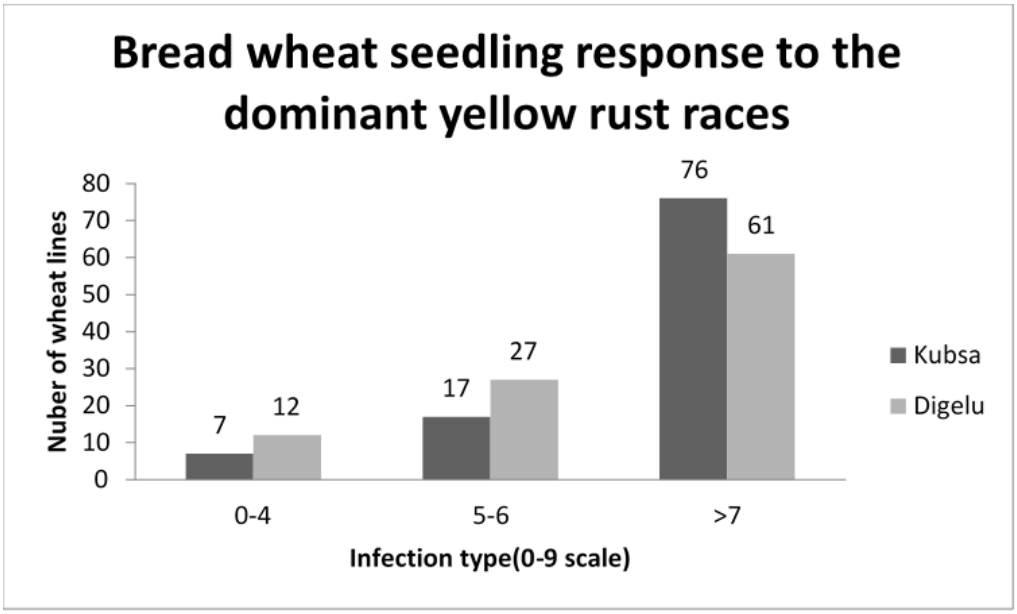

Figure 2: Frequency distribution of seedling reaction of wheat lines against two selected yellow rust islates.

3.3 YELLOW RUST FIELD REACTION

During the course of this experiment, severe yellow rust was prevalent at Sinana Agricultural Research station as it is evident on the susceptible wheat cultivars Thatcher and westonia (Figure 3 below). This high disease pressure led to total failure of the aforementioned varieties (data not shown).

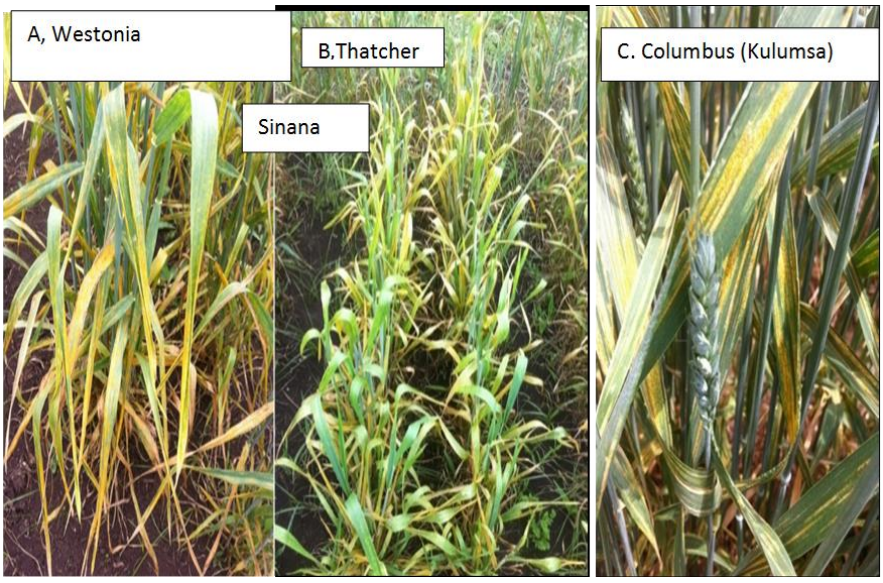

Figure 3: Yellow rust of wheat in Sinana (A and B), and Kulumsa (C).

Wheat lines Av Yr 15, WT6/12, Sr 33/Cs # 4, Sr 33/Cs #5, Sr 33+Sr 2/Cs #14, Sr 33+Sr 2/Cs #15, Sr 45/Cs #20 and Sr 45+Sr 2/Cs #29 were immune at field condition at both the locations. However, Westonia, Thatcher, Avocet, CANTHACHK, CT4-NS1, Pavon Sr 26+ SR 31, Westonia Sr 24+ Sr 31, Pavon Sr 24+ Sr 31 and CTH- Ns 2 exhibited maximum field severity and higher values of coefficient of infection both field conditions of Kulumsa and Sinana.

Among the tested check varieties, all except one were good against yellow rust in both locations, having minimum coefficient of infection. The susceptible check variety Avocet, on the other hand, had the maximum field severity at both locations.

When tested under greenhouse condition for seedling resistance against Kubsa and Digalu isolates, wheat lines Av Yr 15, avyr 5+18, Sr 33+Sr 2/Cs # 11, Pavon Sr 24+ SR 26+Sr 31, Angas Sr 32 and Sr 50+Sr 45 # 28 were found to have lower level of infection hence considered as relatively resistant to the disease. Wheat lines Pavon Sr 24+ Sr 31+Sr 50, Gato Sr 50, Co 1 NS 765, Columbus, ND Sr 1, Westonia, Thatcher, CANTHACHK, CT4-NS1, Avocet, Pavon Sr 26+ SR 31, Westonia Sr 24+ Sr 31, Pavon Sr 24+ Sr 31 and CTH- Ns 2 had higher disease level as it was evident from the infection types.

In a similar manner wheat line Av Yr 15, avyr 5+18, Sr 45/Cs #23, Sr 33+Sr 2/Cs # 11, Sr 33/Cs #1, Angas Sr 32 and Sujata wheat lines were found resistant to only one of the two isolates, Digelu isolate. But the Check variety Ogolcho was the only variety which had low infection type to both Kubsa and Digelu isolates. Shorima and Hidasse exhibited resistance or low infection types to Digelu isolate but both of them were susceptible to Kubsa isolate. The rest of check varieties had highest infection types at seedling stage to both yellow rust isolates. The susceptible avocet check variety also exhibited highest infection type in green house.

4. DISCUSSION

In Ethiopia, several wheat cultivars have been released since the inception of wheat breeding in the 1950s. However, most of those cultivars were abandoned from production due to their susceptibility to diseases especially yellow rust (Badebo, 2002). Screening wheat lines against triple rusts at both field and greenhouse, and invention of the resistance genes in the current wheat cultivars and searching for new sources of resistance are amongst the major objectives of successful wheat improvement program. Currently, most of produced wheat varieties in wheat belt areas of the country are whipped out due to rust especially yellow rust within a short period of time. Such a problem has occurred due to the development of virulent races in the Ethiopian highlands.

The most destructive rust type is yellow rust, which threaten wheat production in the country and cause considerable yield losses, sometimes even crop failures. Screening or developing wheat lines against specific (single) rust race does not guarantee better yield or diseases resistance in any cropping season as wheat yellow rust races often occur in combination than in isolation. As a result screening and evaluating wheat lines to different rust races should be given due attention to minimize the loss of wheat yields and feed the ever increasing population of the world. In view of the above facts, field and greenhouse experiments were conducted in two yellow rust hot beds, namely: Kulumsa and Sinana.

Yellow rust differentials were also planted at Sinana and Kulumsa and also the field response of differential lines revealed that only virulence race to *Yr15* was not detected at both locations. Additionally, virulence races to *YR5*, *YR8*, *YR15*, *YR17*, *YR27*, Lassik (-*Yr5*) and Lassik (+Yr5) differentials were also not detected at Kulumsa field condition. In contrary yellow rust races virulent to *Yr1*, *Yr6*, *Yr7*, *Yr9* and *Yr18* was detected at Kulumsa field condition and this result agreed with the finding of Denbel (2014). *Yr15*, *Yr5* yellow rust resistance genes were found to be effective both at field and green house conditions and the current results are in agreement with (Sharma-Poudyal *et al*.,2013; Denbel, 2014). In the same manner, wheat line with seedling resistance gene *yrsuj* also showed resistance to stripe/yellow rust both at field and greenhouse condition. This confirmed the findings of Lan *et al*. (2015).

5. CONCLUSION

Wheat rusts are major devastating fungal diseases worldwide and in Ethiopia. Yellow rust cause grain yield losses of 100 percent in susceptible wheat cultivars during diseases epidemics. Despite frequent occurrence of yellow rust, there has been not enough study concerning yellow rust alternative host and the current pathotypes existing in wheat producing areas of the country. In the present study resistant wheat lines and effective genes were evaluated wheat breeding

Programme as a new source of resistance lines to overcome the yellow rust problem in Ethiopia. 93 wheat lines, 7 check varieties and 17 differential lines were evaluated for the effectiveness against yellow rust at hot spot areas of Ethiopia. Wheat lines Av Yr 15, WT6/12, Sr 33/Cs # 4, Sr 33/Cs #5, Sr 33+Sr 2/Cs #14, Sr 33+Sr 2/Cs #15, Sr 45/Cs #20 and Sr 45+Sr 2/Cs #29 were identified as resistant to yellow rust which will serve as source of resistance lines if the research institutions and researcher will consider the above materials for crossing program based on resistance genes those materials confer towards yellow rust to increase the production and productivity of wheat in the country. Among tested differential lines, differential lines with Yr15 resistance gene was effective at both tested locations and the effective gene Yr15 needs to be incorporated into breeding program of the country to boost wheat production.

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