International Scholars Journals

Advances in Agriculture and Agricultural Sciences ISSN 2756-326X Vol. 10 (3), pp. 001-007, March, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Assessment of wheat varieties for resistance to leaf rust in Ethiopian conditions

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Received 5 August, 2023; Accepted 20 September, 2023

Leaf rust (*Puccinia triticina* Eriks) is the most common rust disease of wheat in wheat-producing areas of Ethiopia. The use of cultivars with durable resistance is the most economical way of controlling the disease. Field experiments were conducted at Ambo Plant Protection Research Center, Ethiopia during 2013 to 2014 main cropping seasons to reveal variability for field based slow rusting resistance to leaf rust among 18 improved wheat cultivars grown in Ethiopia. Parameters used as criteria to identify slow rusting included final rust severity (FRS), coefficient of infection (CI), relative area under disease progress curve (rAUDPC) and infection rate (Inf-rate). Among these parameters, FRS, CI and rAUDPC were found to be reliable to assess slow rusting in the cultivars. The results revealed that wheat cultivars Pavon 76, Africa Mayo, Bonny, Galili, Qulqulu, Hawi and Senqegna had low disease severities (< 30%) with moderately susceptible reactions, lower rAUDPC values (>30%) and CI (< 20) and were identified to have good level of slow rusting resistance. Cultivars Kubsa, Galama and PBW 343 had moderate values for slow rusting parameters and were identified as possessing moderate level of slow rusting. The slow rusting cultivars identified from the current study can be used for further manipulation in wheat improvement programs.

Key words: Leaf rust, *Puccinia triticina*, resistance, slow rusting, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the major food crops in the world. It is used by more than one-third of its population as a staple food (Kumar et al., 2011). Ethiopia is the largest wheat producer in sub-Saharan Africa (FAOSTAT, 2014). The current total area devoted to wheat production in Ethiopia is estimated to be over 1.6 million hectare (CSA, 2015). Despite the large area under wheat, average yield in Ethiopia is estimated around 2.54 t ha^{-1} which is far less than potential yields of 8 to 10 t ha^{-1} (CSA, 2015). The low productivity is partially attributed to the prevalence of wheat rust diseases and lack of durable resistant variety. Leaf rust caused by the pathogen *Puccinia triticina* Eriks has been an important disease of wheat in most wheat growing areas of Ethiopia

Table 1. Description of the wheat cultivars used for evaluation of slow rusting resistance.

S/N	Cultivar	Year of release	Source center
1	Africa Mayo	1960	Kenya
2	Bonny	1967	Kenya
3	Pavon-76	1982	KARC /EIAR
4	Kubsa	1995	KARC/EIAR
5	Galama	1995	KARC/EIAR
6	PBW 343	1995	CVRC/India
7	Medawalabu	1999	SARC\OARI
8	Hawi	1999	KARC/EIAR
9	Senkegna	2005	ADARC/ARARI
10	Mellenium	2007	KARC\EIAR
11	Qulqulu	2009	HU
12	Galil	2010	Hazera Genetics Ltd
13	Kekeba	2010	KARC\EIAR
14	Danda'a	2010	KARC/EIAR
15	Shorima	2011	KARC/EIAR
16	Hoggana	2011	KARC/EIAR
17	Jefferson	2012	Fedis/OARI
18	Huluka	2012	KARC/EIAR
19	Morocco(Sucpt.ck)		

(Badebo et al., 2008). It is the most prevalent type of rust, which causes yield losses up to 70% on susceptible cultivars (Draz et al., 2015). The best alternative to reduce loss from such a disease would be to use resistant cultivars.

To date, more than 70 leaf rust resistance genes are identified in wheat however most of the genes are race-specific that confer resistance in a gene-for-gene manner (McIntosh et al., 2012; Park et al., 2014). Wheat varieties relying on race-specific resistance often lose effectiveness within a few years by imposing selection for virulent leaf rust races (Bolton et al., 2008; Draz et al., 2015). Due to non-durability of resistance in cultivars that contain only specific major genes for resistance, recent breeding programs have focused on developing cultivars with adult plant resistance or slow rusting.

Slow rusting resistance is a type of resistance that is both race non-specific and durable (Sawhney, 1995; Priyamvada et al., 2011). It is polygenic and effective against a broad range of leaf rust races (Parlevliet, 1985; McIntosh et al., 1995; Herrera-Foessel et al., 2007). Slow rusting resistance is characterized by a slow epidemic build up despite a high infection type indicating a compatible host-pathogen relationship (Parlevliet and van Ommeren, 1975; Priyamvada et al., 2011). In wheat only a small group of leaf rust resistance genes are known as slow rusting genes such as *Lr67* (Dyck and Samborski, 1977), *Lr34* (Singh and Gupta, 1992), *Lr46* (Singh et al., 1998) and *Lr68* (Herrera-Foessel et al., 2012).

Although several studies have been carried out to assess leaf rust resistance in different wheat genotypes in Ethiopia, many of them were based on race specific resistance. The present study was thus designed to assess the levels of slow rusting resistance in some commercial bread wheat cultivars to leaf rust under field conditions.

MATERIALS AND METHODS

To evaluate 18 released bread wheat cultivars (Table 1) for their slow rusting resistance to leaf rust field experiments were conducted during 2013 and 2014 main cropping seasons (June to October) at Ambo Plant Protection Research Center (Ambo PPRC). Ambo PPRC is found at an altitude of 2147 m above sea level. The annual average temperature and rain fall is 27.5°C and 1077.68 mm, respectively. Wheat cultivar Morocco which is considered to lack resistance genes to the leaf rust pathogen was used as a comparative control in the experiments.

The experiments were laid out in randomized complete block design (RCBD) with three replications. Each plot consisted of 6 rows with a size of 1 m × 1.5 m and a spacing of 1 m between blocks and 0.5 m between plots. The inter row spacing was 0.3 m. To ensure uniform spread of inoculum and for sufficient disease development during the trial periods susceptible wheat cultivar Morocco was planted a week earlier around the experimental areas. Artificial inoculation was carried out by spraying spreader rows with mixture of isolates prevalent in the area using an ultralow volume sprayer after sunset. This took place twice when most plants were at the stem elongation. The recommended fertilizer rates (41/46 kg N/P_2O_5 ha^{-1}) and seed rates 150 kg ha^{-1} was used.

Disease assessment

Slow rusting of the wheat genotypes was assessed through final rust severity (FRS), coefficient of infection (CI), area under disease

Table 2. Final rust severities and coefficient of infections of leaf rust on the cultivars tested.

Varieties	2013 cropping season		2014 cropping season	
	FRS	CI	FRS	CI
Pavon 76	5MS	4	10 MS	8
Kekeba	2R	0.8	3.5 MR	1.4
Dendea	5R-MR	1.5	5 MR	2
Shorima	2R-MR	0.6	5 R-MR	1.5
Huluka	0R	0	0 R	0
Hoggana	0R	0	0 R	0
Kubsa	30MS	24	40 MS	32
Galama	28MS	22.4	35 MS	28
Madawalabu	10MR	4	10 MR	4
Africa Mayo	10MS	8	22.5 MS	18
Millenium	5R-MR	1.5	10 MR	4
PBW 343	35MS	28	40 MS	32
Bonny	10MS	8	22.5 MS	18
Galil	5MS	4	10 MS	8
Qulqulu	5MS	4	5 MS	4
Jefferson	5R-MR	1.5	10 MR	4
Hawi	10MS	8	22.5 MS	18
Senkegna	5MS	4	10 MS	8
Morocco	60S	60	70S	70

FRS = Final rust severity; CI = Coefficient of infection; R = Resistant; R-MR = Resistant to moderately resistant; MR = Moderately resistant; MS = Moderately susceptible; S = Susceptible.

progress curve (AUDPC) and infection rate (inf-rate).

Disease severity was assessed by estimating the approximate percentage of leaf area affected using modified Cobb scale (Peterson et al., 1948) on all tillers of 10 randomly selected and pre-tagged plants of the central four rows of each plot and the mean of the ten plants was considered as the value for a plot. Disease severity was taken three times at twenty days interval starting when leaf rust levels on Morocco reached 50% severity. The host plant response to infection was scored according to Roelfs et al. (1992).

Average coefficient of infection (CI) was calculated by multiplying the percentage severity and the constant value assigned to each reaction type (Saari and Wilcoxson, 1974). The constant values were considered as R=0.2, R-MR = 0.3, MR = 0.4, MS = 0.8 and S = 1.

Area under disease progress curve (AUDPC) was calculated by using the formula suggested by Wilcoxson et al. (1975).

$$\text{AUDPC} = \sum_{i=1}^{n} [0.5\,(x_i + x_{i+1})]\,[t_{i+1} - t_i].$$

Where, x_i = the average coefficient of infection of i^{th} record, X_{i+1} = the average coefficient of infection of $i+1^{th}$ record and $t_{i+1} - t_i$ = Number of days between the i^{th} record and $i+1^{th}$ record, and n = number of observations.

Apparent infection rate (Inf-rate) as a function of time was also calculated from the three disease severity observations as a severity of leaf rust infection at the time of rust pustules appearance and every twenty days thereafter. It was estimated using the following formula adopted by Van der Plank (1963).

$$\text{Inf-rate} = 1/t\ (\ln x/1-x)$$

Where x = the percent of severity divided by 100; t = time measured in days. The apparent infection rate is the regression coefficient of ln x/1-x on t.

Data analysis

Relative forms of the epidemiological parameters were generated by comparing the respective values of each entry with the susceptible variety Morocco. Coefficient of correlation was done using SPSS software (SPSS, 2005) to determine the relationship between disease parameters.

RESULTS AND DISCUSSION

Final rust severity

There was wide variation in the leaf rust severities ranging from 0 to 60% during the 2013 cropping season at the Ambo PPRC. Diverse field reactions ranging from resistance (R) to susceptible (S) responses were observed at the trial. The final rust severities of the cultivars and their infection types are presented in Table 2.

Final rust severity represents the cumulative result of all resistance factors during the progress of epidemics (Parlevliet and van Omeren, 1975). Based on final rust severity, the tested wheat cultivars were grouped into two groups of slow rusting resistance, that is, high and moderate levels of partial resistance having 1-30 and 31-

50% FRS, respectively. During the 2013 cropping season seventeen wheat cultivars displayed disease severities of up to 30%. Of these eight had resistant to moderately resistant (R-MR) field reactions while nine showed moderately susceptible (MS) responses. On the other hand, cultivar PBW 343 was included in the second group with 35% final rust severity and MS field response. Despite the heavy leaf rust disease pressure during 2014 cropping season, 7 wheat cultivars, including Pavon 76, Africa Mayo, Bonny, Galili, Qulqulu, Hawi and Senkegna remained in the first group, exhibiting final rust severities ranging from 1 to 30%, with compatible (MS) responses and are of great importance to achieving effective breeding for durable resistance to leaf rust (Parlevliet, 1988; Nzuve et al., 2012). According to Nzuve et al. (2012), the available resistance genes in these materials overcame the leaf rust virulence in the field and led to statistically low disease severities despite the compatible host-pathogen reactions. Previously, Ali et al. (2007), Li et al. (2010), Tabassum (2011) and Safavi (2012) also used final rust severity to assess slow rusting behaviour of wheat lines. On the other hand Kubsa, Galama and PBW 343 showed final rust severities between 31 and 50% in 2014 cropping season and were regarded as possessing moderate levels of slow rusting resistance.

Cultivars, Huluka and Hoggana showed immune responses in both seasons. The immune response on these cultivars could be as a result of hypersensitive responses; resistance often breaks down due to the development of new races of the pathogen. A suitable breeding strategy like the use of inter-specific and remote crosses or even the direct transfer of these resistances through backcrosses could be used to improve the adopted but highly susceptible wheat varieties being grown in Ethiopia (Bartos et al., 2002). On the other hand, the susceptible check, Morocco, displayed the highest disease severities of 60 and 70% with completely susceptible (S) responses during 2013 and 2014 cropping seasons, respectively, indicating that an acceptable epidemic pressure was established over the seasons for field experiments.

Coefficient of infection

The data on disease severity and host reaction were combined to calculate CI (Table 2). According to Ali et al. (2009), lines with CI values of 0-20, 21-40, 41-60 were regarded as possessing high, moderate and low levels of slow rusting resistance, respectively. In the present study, all the test genotypes except Kubsa, Galama and PBW-343 showed CI values between 0 and 20 in both seasons and were designated as having a high level of slow rusting. It was, therefore, concluded that these cultivars had a great potential to be used as a resistance sources against leaf rust. Cultivars Kubsa, Galama and PBW-343 had CI values of 21 to 40, designated as having moderate levels of slow rusting resistance. In the seasons, only the susceptible check had a CI value of more than 40. Many earlier researchers such as Patil et al. (2005); Pathan and Park (2006) and Draz et al. (2015) also appraised slow rusting resistance to wheat leaf rust using coefficient of infection and reported the presence of different partial resistance conferring genes in wheat lines.

Area under disease progress curve

Disease progress curve is a better indicator of disease expression over time (Van der Plank, 1963). Therefore, selection of cultivars having lower AUDPC values is acceptable for practical purposes. The tested wheat cultivars were categorized into two distinct groups for slow rusting resistance, based on the AUDPC values. Wheat cultivars exhibiting AUDPC values up to 30% of the check were grouped as having high level of partial resistance, consisted of 15 and 16 wheat cultivars during 2013 and 2014 cropping seasons, respectively; while those having AUDPC values to 70% of the check were grouped as moderately resistant cultivars, included Kubsa, Galama and PBW 343 in 2013 and Kubsa and Galama during 2014 cropping season (Table 3).

Of the wheat cultivars under group one, cultivars Pavon-76, Africa Mayo, Bonny, Galil, Qulqulu, Hawi and Senqegna showed MS types of infection in the field. According to Parlevliet (1988), Brown et al. (2001), Singh et al. (2005), and Kaur and Bariana (2010) the cultivars which had MS infection type may be carrying durable resistance genes, such as slow rusting resistance. These wheat cultivars first shown rust infection and sporulation but the final host reaction was characterized as chlorotic and necrotic lesions. Subsequently, the disease progression remained slower and highly retarded among these cultivars. Such partially resistant lines could highly delay evolution of new virulent races of the pathogen because multiple point mutations are extremely rare in normal circumstances (Schafer and Roelfs, 1985; Ali et al., 2008; Tsilo et al., 2010). Likewise, despite the MS infection type exhibited on moderately slow rusting cultivars, leaf rust developed slowly as indicated by their AUDPC values. None of the tested cultivars was marked as having susceptible field response. Other researchers have also reported variation among different wheat lines for slow rusting resistance to leaf rust using AUDPC (Patil et al., 2005; Draz et al., 2015).

Infection rate

The maximum mean disease progress rate (Inf-rate = 0.12) was observed on the cultivar Hawi in 2013 cropping season, while the maximum infection rate of 0.170 was observed on the cultivar Galama in 2014 (Table 3).

Table 3. AUDPC and Infection rates of leaf rust on the cultivars tested.

Varieties	2013 cropping season			2014 cropping season		
	AUDPC	rAUDPC	Inf-rate	AUDPC	rAUDPC	Inf-rate
Pavon 76	40	6.67	0.082	88	12.57	0.088
Kekeba	0	0.00	0.019	7	1.00	0.046
Dendea	20	3.33	0.081	10	1.43	0.032
Shorima	8	1.33	0.044	20	2.86	0.073
Huluka	0	0.00	0.000	0	0.00	0.000
Hoggana	0	0.00	0.000	0	0.00	0.000
Kubsa	320	53.33	0.064	360	51.43	0.169
Galama	300	50.00	0.053	340	48.57	0.170
Madawalabu	44	7.33	0.082	40	5.71	0.083
Africa Mayo	100	16.67	0.037	190	27.14	0.089
Millenium	20	3.33	0.081	28	4.00	0.157
PBW 343	400	66.67	0.052	210	30.00	0.084
Bonny	88	14.67	0.084	130	18.57	0.058
Galil	40	6.67	0.082	66	9.43	0.078
Qulqulu	40	6.67	0.082	36	5.14	0.091
Jefferson	16	2.67	0.081	48	6.86	0.058
Hawi	84	14.00	0.120	178	25.43	0.091
Senkegna	40	6.67	0.082	58	8.29	0.066
Morocco	600	100.00	0.119	700	100.00	0.130

AUDPC = Area under disease progress curve; rAUDPC = Relative area under disease progress curve; Inf-rate = Infection rate.

Table 4. Correlation coefficients (r) for disease parameters of leaf rust on wheat cultivars at Ambo, 2013 cropping season.

Parameter	2013 cropping season			2014 cropping season		
	FRS	CI	AUDPC	FRS	CI	AUDPC
FRS	1			1		
CI	0.990**	1		0.989**	1	
AUDPC	0.993**	0.983**	1	0.972**	0.982**	1
Inf-rate	0.311	0.305	0.237	0.579**	0.520*	0.570**

**Significance level at P ≤ 0.01; *significance level at P ≤ 0.05.

Cultivars Huluka and Hoggana showed a constant disease severity, thus showing no increase per unit time with an Inf-rate value of 0 in both seasons. The disease progress rate of certain lines was more than the susceptible cultivar, Morocco in the seasons due to the fact that disease scoring was initiated when disease severity was already 50% on the susceptible check. Hence, the actual infection rate for Morocco may even be more. Besides, infection rate in the present study did not distinguish cultivars with different level of slow rusting with regard to other parameters. Similarly, the more variation in infection rate among the tested cultivars than the other slow rusting parameters is partly because infection rate is a regression coefficient with larger error variance. Therefore infection rate in the present study seemed to produce unreliable estimates of slow rusting resistance when compared with FRS, CI and AUDPC. Similar results were found for rusts of wheat (Rees et al., 1979; Broers, 1989; Ali et al., 2008; Safavi et al., 2013).

Correlation between slow rusting parameters of wheat leaf rust

A positive and highly significant correlation of FRS with CI (r = 0.990) and AUDPC (r = 0.993) was found during 2013 cropping season (Table 4). Strong correlation coefficients of 0.989 and 0.972 were also observed between FRS with CI and AUDPC during the 2014 cropping season, respectively. The high correlation coefficient was also observed between AUDPC and CI in both seasons; r = 0.983 during the 2013 main season and r = 0.982 during the 2014 cropping season. These strong correlations agreed with the results of Qamar et al. (2007); Ali et al. (2008); Safavi et al. (2010) and Shah et al. (2010). Although positive correlations were observed between infection rate and other disease parameters, the relationship between the variables was weak in the season. Similarly, relatively low correlations were observed between infection rate and the other disease parameters in 2014 cropping season. This indicates that although severity or the area under the disease progress curve was increasing, the rate of infection reduced as epidemic progressed because less healthy plant tissue was available for additional infections (Freedman and Mackenzie, 1992).

Since, FRS, CI and AUDPC had strong positive correlations in the present study; selection of lines having final disease score less than 30%, CI between 0 to 20 and rAUDPC less than 30% with MS responses is normally accepted for practical purposes. Feasibility of measuring slow rusting resistance under field condition preferably by low final ratings and CI have been reported previously by Safavi et al. (2013) and Hei et al. (2014). Singh et al. (2007) also reported that field selection of the slow rusting trait preferably by low rAUDPC and terminal ratings along with CI, is feasible where greenhouse facilities are inadequate. Accordingly, wheat cultivars Pavon 76, Africa Mayo, Bonny, Galili, Qulqulu, Hawi and Senqegna with highly slow rusting resistance characteristics: FRS 0-30% with MS field responses, CI 0-20 and rAUDPC less than 30% were identified for resistance breeding. Of these cultivars Pavon 76 and Hawi were postulated to have combinations of major gene resistance genes *Lr1*, *Lr10* and *Lr 13,* and *Lr2c*, *Lr23*, *Lr27+31*, respectively (Mebrate et al., 2008). The presence of both major and minor genes in these cultivars is of paramount importance since the combined effects of several genes give the cultivar a wider base of disease resistance (Roelfs et al., 1992). Cultivars Kubsa, Galama and PBW 343 had FRS 31 to 50% with MS field responses, CI value ranging from 21 to 40 and rAUDPC between 31 and 70% and were regarded as moderately slow rusting (Table 2). Cultivar Kubsa was postulated to have major gene resistance gene *Lr44* while Galama was postulated to have a combination of major gene resistance genes *Lr23* and *Lr37* (Mebrate et al., 2008). The highly slow rusting and moderately slow rusting wheat cultivars identified in the present study were supposed to be having genes for varying degrees of slow rusting and may be used for further genetic manipulation in wheat improvement programs. Singh et al. (2004) have also reported that genotypes in both group 1 and 2 could have durable resistance controlled by more than one gene which can serve as good parents for breeding.

Conclusion

The wheat cultivars showed variation in resistance reaction, ranging from immunity to slow rusting resistance. Most of the evaluated cultivars exhibited better performance under high disease pressure shown by susceptible check. Cultivars Pavon 76, Africa Mayo, Bonny, Galili, Qulqulu, Hawi and Senqegna exhibited lower levels of FRS (< 30% with MS responses), coefficient of infection (< 20) and rAUDPC less than 30% indicating a high level of slow rusting resistance. Three wheat cultivars Kubsa, Galama and PBW 343 had moderate level of slow rusting resistance in the seasons. The correlations among the field based slow rusting parameters were highly significant. The slow rusting cultivars identified from this study with better levels of slow rusting resistance may be exploited for durable resistance in Ethiopian wheat breeding program. However, further testing for stability over years and locations for leaf rust along with other desirable characters must be made before approval.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The Alliance for a Green Revolution in Africa (AGRA) is sincerely thanked for financial support of the study. The Ethiopian Institute of Agricultural Research (EIAR) is acknowledged for hosting the field research. The all-round support provided by the wheat rust research team of Ambo Plant Protection Research Center of Ethiopia is highly appreciated.

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