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Evaluation of Advanced Kenyan Barley (*Hordeum vulgare* L.) Genotypes for Resistance to Stem Rust (*Puccinia graminis* Pers:.Pers. f.sp *tritici* Eriks. & E. Henn.) Race Ttksk and Its Variants

Kaisha Victor Ambula [a], Owuoche James [a] and Miriam Karwitha Charimbu [a*]

[a] Department of Crops, Horticulture and Soils, Egerton University, Nakuru, Kenya.

Authors' contributions

This work was carried out in collaboration among all authors. Author KVA designed the study, performed the statistical analysis, wrote the protocol, managed the analyses of the study and wrote the first draft of the manuscript. Authors OJ and MKC reviewed the literature. All authors read and approved the final manuscript.

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ABSTRACT

Stem rust caused by *Puccinia graminis* Pers:.Pers. f.sp *tritici* affects all the aerial parts of wheat and barley plants leading to yield losses. The objective of this study was to determine the level of resistance in advanced Kenyan barley lines to stem rust race TTKSK and its variants. The greenhouse and field experiments were laid in complete block design and randomized complete block design respectively with all experiments replicated three times. The experiments were conducted at Kenya Agricultural and Livestock Research Organization- Njoro. In the greenhouse experiment, forty genotypes were sown in perforated plastic pots and when the seedlings attained two-leaf stage, stem rust isolate collected from wheat variety, *KS Mwamba* was used to inoculate the genotypes. In the field, forty genotypes were sown in a one meter twin rows of each genotype were planted and all agronomic practices implement except management of stem rust disease. Infection types (IT) on the seedlings were observed and data collected following a scale described by Stakman et al. 1962. Disease severity data was collected according to modified Cobbs scale and then subjected to Area Under Disease Progress Curve (AUDPC). All genotypes showed immunity to moderate resistance at seedling level ranging from 0 to 2+. Adult plant reactions to

**Corresponding author: E-mail: miriam.charimbu@egerton.ac.ke;*

race TTKSK and its variants ranged from an overlap of moderately resistant (MR) to susceptible (S). In all genotypes AUDPC ranged from 225.83 to 887.92 for under this study and the Coefficient of Infection ranged from 13 to 47. High levels of slow rusting were observed in only 12.5% of the genotypes tested while 10% of the genotypes had low levels. 77.5% of the genotypes had moderate levels of slow rusting. The genotypes with high levels of slow rusting can be advanced for release as varieties or be included in barley breeding.

Keywords: Barley; disease severity; genotypes; resistance; stem rust.

1. INTRODUCTION

Rusts are the greatest biotic threats for the production of barley (*Hordeum vulgare* L) and wheat (*Triticum aestivum* L) especially after the emergence of race *Ug99* of stem rust and rapid development of variants that are more virulent [1]. In the USA where *Ug99* is not present, yield loses in barley range from trace to 15% (simply managed by gene *Rpg1*) while in Kenya losses of up to 100% have been experienced [2]. Stem rust race Ug99 has brought a new dimension in research due to its devastating nature in barley and wheat [3]. Kenya has different agro-ecological regions with their growing period differing from each other. This ensures that there is a green crop of barley and wheat at any given point in time throughout the year forming what is referred to as "green bridges" [4]. This phenomenon facilitates the availability of pools of stem rust inocula throughout the year [3]. Most barley and wheat production regions in Kenya and the world have conditions that favour the development of rust diseases in small cereals [5]. Growing of resistant cultivars provides the most sufficient and positive effect on management strategy of reducing yield losses due to stem rust [6]. Due to the diversity in the virulence of the races available, there is need for identification of new sources of resistance and incorporation to the adapted barley genotypes [7].

Apart from crop resistance, the use of fungicide in control for stem rust has been explored and found to be successful [8]. However, the need for multiple applications of the fungicides is not economically feasible more so for small scale farmers [8]. Large scale farmers may afford to purchase fungicides but it becomes an extra cost in cereal production [9]. Wanyera et al., [10] found that application of fungicides at tillering and flowering stages had the highest effect in reducing yield loss due to stem rust, implying that the growth stage at which fungicides are applied is important for the maximizing the effect of a fungicide. Concerns of environmental pollution and health effects on consumers of farm produce is also limitation in the use of fungicides [2].

Plant resistances vary widely and act by limiting the effectiveness and spread of pathogens [11]. Seven stem rust resistance genes *Rpg1, Rpg2, Rpg3, rpg4 Rpg5, Rpg6* and *rpgBH* have been identified and catalogued [3]. Seedling resistance genes to stem rust are monogenic and race specific but the pathogen usually evolves and overcome these genes leading to a "boom and bust" cycle [12]. This race specific kind of resistance follows the gene-for-gene phenomenon between host plant resistance and the corresponding gene for avirulence in the pathogen [13]. Studies have shown that seedling resistance genes confer resistance to all growth stages [14]. These genes have been found to initiate hypersensitive response that leads to rapid cell death upon infection by a pathogen race that carries the specific avirulence gene [12]. In the presence of an avirulent race of stem rust, seedling resistance leads to formation of tiny-to-medium sized uredinia with limited sporulation surrounded by necrosis or chlorosis (associated with hypersensitivity) [15]. Unfortunately, rusts of small cereals have been found to mutate very fast and break down this kind of resistance leading to susceptibility of the genotypes with this kind of resistance after a short period of time [12]. Gene *rpg4* has been found to be conferring high levels of resistance at seedling stage including to the highly virulent *Ug99* race [2]. Identification of genes of resistance to stem rust in barley at seedling stage is sometimes difficult due to the mesothetic kind of reaction coupled with chlorosis around the uredinia in response to *Puccinia graminis* (Steffenson *et al.,* 1993).

In order to reduce on selection pressure on *Puccinia graminis* pathogen, scientists should embrace partial resistance, otherwise referred to as adult plant resistance (APR) to slow down the development of new virulent races [16]. Adult plant resistance (APR) is a type of resistance that expresses itself at post seedling stages,

primarily after the heading stage of the growth in cereal plants [17]. It is conferred by minor genes in a plant [14]. It functions by retarding the development of an epidemic, reducing infection frequency, rate of sporulation and longer latent period of the pathogen despite a compatible infection type [15]. As opposed to seedling resistance, APR is presumed to act against many races [16]. *Rpg2, Rpg3 and rpgBH* have been documented as adult plant resistance genes to stem rust hence associated with intermediate to high infection types at seedling stage making them valuable in breeding [18]. Other potential sources of new resistance to stem rust are wild relatives of barley and landraces which are genetically diverse and adapted to the local environment [10].

Therefore, the objective of this study was to determine the level of seedling and adult plant resistance in advanced Kenyan barley lines against stem rust race TTKSK and its variants under greenhouse and field conditions. Evaluating the advanced Kenyan barley lines for resistance to is key to developing sustainable stem rust management strategies.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiments were conducted at Kenya Agricultural and Livestock Research Organization- Njoro (0˚20´S; 35˚56´E) in the Central Rift Valley of Kenya in the greenhouse two batches and the field for two seasons. The Centre lies at an elevation of 2185 metres above sea level with *mollic phaeozem* being the predominant type of soil [19]. The area experiences an annual average precipitation of 998.79±4.2 mm in a bimodal manner. The average maximum and minimum temperature of the area is about 23±2˚C and 9±2˚C respectively.

2.2 Experiment one: Screening for Seedling Resistance in the Greenhouse

2.2.1 Genotypes

Thirty seven advanced barley lines (HBV 15-1; HBV 15-2; HBV 15-3; HBV 15-4; HBV 15-5; HBV 15-6; HBV 15-7; HBV 15-8; HBV 15-9; HBV 15-10; HBV 15-11; HBV 15-12; HBV 15-13; HBV 15-14; HBV 15-15; HBV 15-16; HBV 15-17; HBV 15-18; HBV 15-19; HBV 15-20; ULB 16-1; ULB 16-2; ULB 16-3; ULB 16-4; ULB 16-5; ULB 16-6; ULB 16-7; ULB 16-8; ULB 16-9; ULB 16-10; ULB 16-11; ULB 16-12; ULB 16-13; ULB 16-14; ULB 16-15; ULB 16-16 and ULB 16-17) were obtained from Kenya Malting Centre in Molo. Three commercial varieties; *Fanaka, Nguzo* and *Cocktail* which are moderately resistant, moderately susceptible and susceptible respectively, were included in the experiments for comparison purpose.

2.2.2 Inoculum preparation

Single pustules of urediniospores that looked similar in size, shape and infection type (IT) were collected from wheat plants with *Sr24* gene of resistance (*KS Mwamba*) in the field in wheat and barley growing areas. The urediniospores were pretested on a set of differentials by inoculating them with the spores and they were confirmed to be *Ug99* race and its variants. The urediniospores were then collected from the differentials using a small electric suction pump (GAST Model DOA-P704-AA) and inoculated on susceptible barley cultivar (*Ngao*) to increase the amount. The increased urediniospores that were similar in size, shape and infection type (IT) on cultivar *Ngao* were collected into gelatin tubes ready for inoculation. Approximately 50 g of the spores were suspended in 250 ml distilled water and one drop of light mineral oil (tween 20) was added into the suspension to act as a surfactant. Spore concentration was determined using a haemocytometer and adjusted to ~6x106 spores / mL.

2.2.3 Planting

Five seeds of each of the 40 genotypes (include 37 advanced Kenyan lines and three commercial varieties used as checks) were sown in two batches in perforated pots measuring 6 cm × 6 cm × 6 cm. The pots were filled with 100 g Hygromix seedling-growing medium (Hygrotech, Pretoria, South Africa). The seeds were sown diagonally at a depth of approximately 2 cm and the pots placed on an aluminum tray and irrigated to field capacity.

2.2.4 Inoculation and Incubation

When the plants attained two to three leaf stages GS12-13 [20], a hand sprayer was used to apply the inoculum by spraying indirectly onto the seedlings at a distance of about 30 cm so that each plant received about 0.05 ml of the inoculum solution. The inoculated seedlings were placed in an incubation chamber and moisture maintained near saturation for 48 hours. The misting was done with distilled water using a hand sprayer to avoid contamination with other

races of stem rust and other pathogens. The incubation chamber was maintained at a temperature 14˚C and 100% humidity which is favourable for infection of the pathogen. The seedlings were then transferred to a growth chamber which was maintained at a temperature range of 20-26˚C for 10-14 days for disease pustules to developed.

2.2.5 Data Collection

Infection types (IT) on the seedlings were observed and data collected following a scale described by Stakman et al. [21]. In this scale, scores of infection types range from 0-4 with 0 representing immunity and 4 as total susceptibility. Flecking is represented by ";" which is a hypersensitive reaction; "+" (plus) sign indicated that the size of the pustule is slightly bigger than the indicated score while "-" (minus) was used to indicate that the pustule size is smaller than indicated score. Chlorosis around the pustule was indicated by "C" and necrosis is indicated by "N".

2.3 Experiment Two: Screening for Adult Plant Resistance

2.3.1 Experimental procedure

Primary ploughing was done using a disc plough before the onset of rain to allow the weeds to dry. Secondary ploughing was done by a harrow to obtain a fine tilth which is suitable for sowing of barley. The genotypes were sown at a depth of about five centimeters in double rows (plot) of one metre in length at a rate of 108.33 Kg/ha and a spacing of 20 cm between the rows. A path of 30 cm was used to separate plots while 50 cm path was used to separate replicates. Spreader rows consisting of susceptible wheat varieties *CAKUKE*, *KS Mwamba* and *Duma* were planted around the experimental plots and between the replicates. This spreader rows were inoculated using a small electric suction pump (GAST Model DOA-P704-AA).with bulk inoculum of stem rust collected from susceptible plants from the previous season.

During planting diammonium phosphate fertilizer was applied at a rate of 125 Kg/ha to supply nitrogen at 22.5 KgN/ha and phosphorous at 57.5 KgP/ha and Calcium Ammonium Nitrate applied for top dressing at a rate of 100 Kg/ha. A pre-emergent herbicide Stomp 455 CS (pendimethalin) was applied at a rate of 1365 g pendimethalin/ha one day after seed sowing to control grass weeds. At growth stage GS28-30, Buctril MC (bromoxynil ectanoate 225 g/l + MCPA ethyl hexyl ester 255 g/l) was applied at a rate of 281.25 g bromoxynil ectanoate/ha and 281.25 g MCPA ethyl hexyl ester/ha to control broad leaved weeds. Cereal aphids were controlled by applying a systemic insecticide, Thunder OD (Imidacloprid + Beta-cyfluthrin) applied at a rate of 30 g Imidacloprid/ha and 15 g Beta-cyfluthrin/ha at GS 20-29. The experiment was laid in a Randomized Complete Block Design (RCBD) with three replicates for two seasons during the short rain season (October-December) 2015 and long rain season (April-August) 2016.

2.3.2 Data collection

Data collection was started when the disease severity in the spreader rows had reached 50%. Disease severity data was collected according to modified Cobbs scale [22] where 0% represents immunity to stem rust while 100% represents total susceptibility at an interval of 5 days. Plant responses were recorded as R, MR, M, MS, S representing resistant, moderately resistant, moderate (overlap of moderately resistant and moderately susceptible), moderately susceptible and susceptible respectively as described by Roelfs et al. [23]. Coefficient of infection (CI) was calculated by multiplying the severity values of specific values with constant values of each plant reaction. The values were modified according to Pathan and Park, [24] as follows; R=0.1, R-MR=0.175, MR=0.25, MR-MS=0.5, MS=0.75, MS-S=0.875 and S=1.0. Genotypes observed to be having CI of 0-20; 21-40 and 41-60 were considered having high, moderate and low levels of slow rusting respectively while those with CI of above 60 were considered susceptible [25].

2.3.3 Data analyses

The mean of disease severity data for the two seasons were calculated and then subjected to Area Under Disease Progress Curve (AUDPC) to estimate the quantitative nature of resistance of every line and variety. The formula for the calculation of the AUDPC [26].

$$AUDPC = \sum_{i=1}^{n-1}[(t_{i+1} - t_i)(y_i + y_{i+1})]/ \qquad 2$$

Where: $t_{(i+1)}$ is the second assessment date of two consecutive assessment t_i is the time in days between each reading; y_i is the percentage of affected part of the plant at each reading; $y_{(i+1)}$ is the disease severity on assessment date $t_{(i+1)}$; n is the number of readings.

3. RESULTS AND DISCUSSION

3.1 Seedling Resistance

Among the barley genotypes evaluated for seedling resistance, most of them were observed to have had mixed infection response which is described as mesothetic. High to moderate levels of seedling resistance to stem rust race TTKSK and its variants ranging from 0-2+ (immune to moderately resistant) were observed. All the check varieties included in the experiment had low levels of infection hence resistant at seedling stage to stem rust race TTKSK and its variants (Table 1).

3.2 Adult Plant Resistance (APR)

Adult plant resistance for the genotypes evaluated varied considerably. This was indicated by the significant variations observed in Area Under Disease Progress Curve (AUDPC) of the genotypes. Genotype HA9 had the highest AUDPC of 887.92 while the check variety *Cocktail* had the lowest AUDPC of 225.83. HA9 had the highest Coefficient of Infection (CI) of 48 which was 79.16% higher than the most resistant check variety *Cocktail* which had a CI of 18. HA7 exhibit the highest level of slow rusting with the CI of 13 which was 27.78% lower than the most resistant check variety *Cocktail*. (Table 2).

Table 1. Seedling Infection Type, Resistance Classification of Advanced Kenyan Barley Lines

Genotypes	Mode	Range	Resistance Classification
HA1	;	0/;	R
HA2	-	-	-
HA3	;	0/;	R
HA4	1	;/1+	R
HA5	;	;	R
HA6	0	0	R
HA7	1-	;/1	R
HA8	;	;/2	R
HA9	1-	;/1-	R
HA10	2	1-/2	R
HA11	0	0	R
HA12	;	;/1+	R
HA13	1	;/2	R
HA14	1-	;/2	R
HA15	;	;/1+	R
HA16	;	;/2	R
HA17	;	;/1-	R
HA18	;	;/2	R
HA19	0	0	R
HA20	;	;/1	R
UO1	1-	;/2+	R
UO2	1	;/2	R
UO3	;	;/2	R
UO4	1	;/2	R
UO5	1	;/2	R
UO6	;	0/;	R
UO7	1	;/2N	R
UO8	1	;/2	R
UO9	-	-	-
UO10	;	;/1+C	R
UO11	2	;/2	R
UO12	;	;/1	R
UO13	1	;/2-C	R
UO14	;	;/1	R
UO15	;	;/1	R
UO16	1	;/1	R
UO17	1-	;/2C	R
Fanaka	1	;/2-	R
Ngozi	1-	;/2	R
Cocktail	;	;/1-	R

Table 2. Area under disease progress curve (AUDPC), genotype response and Coefficient of infection (CI) for advanced Kenyan Barley Lines

Genotypes	Mean AUDPC	Genotype response	Coefficient of infection
HA1	410.00h-m	MS-S	20
HA2	555.40c-i	MS-S	31
HA3	494.17e-l	MS-S	31
HA4	526.67d-k	MS-S	28
HA5	591.25c-g	MS-S	35
HA6	475.42f-l	MS-S	26
HA7	300.00mn	M	13
HA8	409.08h-m	MS-S	28
HA9	887.92a	MS-S	48
HA10	817.92ab	MS	35
HA11	672.92c-e	MS-S	41
HA12	542.92d-j	MS-S	34
HA13	647.92cd	MS-S	41
HA14	642.80c-f	MS	34
HA15	618.33c-f	MS-S	38
HA16	590.00c-g	MS-S	32
HA17	407.08h-m	MS-S	26
HA18	551.67c-i	MS	25
HA19	366.25k-n	MS	19
HA20	440.68g-m	MS-S	39
UO1	359.17l-n	MS-S	22
UO2	568.33c-h	MS-S	35
UO3	850.00ab	MS-S	47
UO4	611.67c-f	MS	29
UO5	512.92d-l	MS	25
UO6	398.33i-m	MS-S	24
UO7	418.75h-m	MS-S	26
UO8	382.50j-m	MS	21
UO9	434.58g-m	MS-S	28
UO10	547.92c-j	MS-S	36
UO11	589.17c-g	MS-S	36
UO12	545.00d-j	MS	29
UO13	526.25d-k	MS-S	31
UO14	540.00d-j	MS	28
UO15	357.92l-m	MS-S	22
UO16	430.00g-m	MS	23
UO17	438.33g-m	M	16
FAN	710.83bc	MR	14
NGU	419.58h-m	MS	24
COC	225.83n	S	18

Means within each column followed by same letters are not significantly different at P≤0.05 by DMRT. M;MR; MS-S; S=Susceptible

Among the genotypes used in this experiment 77.5% had moderate levels of rusting with a coefficient of infection ranging from 20-40 (Fig. 1). High levels of slow rusting were observed in 12.5% with the CI being less than 20 while 10% had low levels of slow rusting with the CI being within the range of 40-60 (Fig. 1). Genotype HA7 had the lowest CI of 13 while UO3 had the CI of 47 (Table 1). Highest levels of slow rusting resistance were observed on genotypes HA7, HA19, UO17 and *Cocktail.*

Genotype responses ranged from moderately resistant (MR) observed on variety *Fanaka* to susceptible observed on *Cocktail* with most of the genotypes susceptible (S) reaction (Fig. 2). There was a strong correlation between AUDPC and CI with an *r* value of 81% (Table 3).

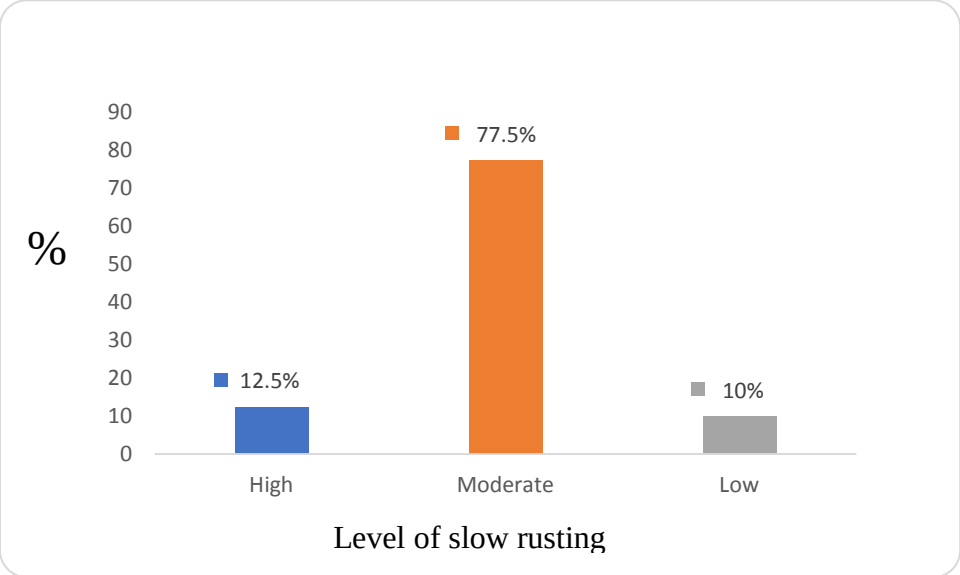

Fig. 1. Percentage of the genotypes categorized as high, moderate or low levels of slow rusting

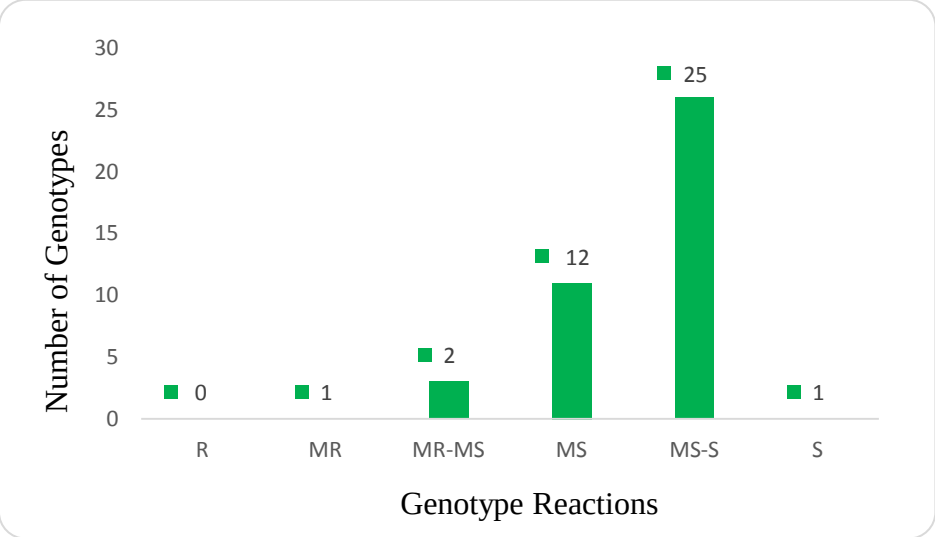

Fig. 2. Frequencies of field reaction type on the 40 genotypes screened

Table 3. Correlation between AUDPC and CI of the genotypes tested for adult plant stem rust resistance

	AUDPC	CI
AUDPC	1	
CI	0.81957***	1

**** Significant at P≤0.001*

Rust fungi that affect barley and wheat have the ability to change virulence rapidly overcoming resistance in existing genotypes resulting to an epidemic [27]. Race TTKSK of stem rust and its variants threaten barley production especially due to its virulence to multiple genes of resistance [28]. The experiments in the field were carried out in short-rain season (2015) and long-rain season (2016) which was important in catering for seasonal differences in weather considering disease development is highly dependent on the environmental conditions. The

high levels of seedling resistance observed in the greenhouse as compared to the high plant responses in the field indicate that there is more than one race affecting the genotypes in the field as seedling resistance protects the plants all through the growth stages [14]. Low seedling, infection types observed in all the genotypes used in this experiment is an indication of the presence of barley stem rust resistant gene *Rpg1* which was resistance to race TTKSK and its variants. A high percentage of the genotypes had MS-S kind of reaction with relatively low severities which led to moderate levels of slow rusting indicting the presence of less than four slow rusting genes in the genotype since 4-5 slow rusting genes in a genotype will normally indicate an almost immune kind of reaction [29].

From the results, the pathotypes available in the field are considered virulent to most of the genotypes tested. Variety *Cocktail* was generally the best in terms of disease resistance by having the lowest AUDPC and at the same time having a low seedling infection type although it had a high plant response. Genotypes HA1, HA7, HA19 and UO17 had both low AUDPC and CI which is a good indication of the presence of slow rusting genes which are Known to be either oligogenic or polygenic [28]. This could be an indication of the presence of adult plant resistant genes which contributed to high levels of slow rusting which is similar to the findings of the work done by El-Naimi *et al*., [30] who worked on screening of resistance to yellow rust in bread and durum wheat. The findings of this experiment show that variety *Cocktail* has the potential to be used as a source of adult plant resistance. This kind of resistance towards stem rust is considered durable which expresses itself at adult plant stage and is usually polygenically controlled [31]. From the plant responses of genotypes HA7, UO17 and *Fanaka* it can be presumed that the genotypes could be carrying a race specific resistance genes or a combination of race specific genes that are effective against most of the pathotypes in the field [32]. These genotypes are not advocated for in the current breeding strategies for resistance since they tend to breakdown after a short period of time.

Strong correlation between AUDPC and Coefficient of Infection is an indication that both AUDPC and CI are good estimators of slow rusting in barley genotypes which is consistent with the work by McNeil *et al*., [33] although working on wheat. All the genotypes tested had some level of slow rusting since none could be classified as totally susceptible. Genotypes with MR, M and MS kinds of field plant reactions can be used as sources of slow rusting kind of resistance in a breeding programme a concept that is agreed by Kaur and Bariana, [34] who worked on inheritance of adult plant resistance to stripe rust in wheat. Only *Fanaka* had moderately resistant (MR) kind of infection type in the field experiment. In these kinds of reaction, necrotic and chlorotic stripes were observed around the developing uredinia that underwent restricted development and reduced sporulation which could be an indicator of pyramiding of major genes that expressed high levels of plant reactions in the field. The mixed kinds of plant reactions observed in most genotypes could be attributed to the variability in the expression of resistance due to non-uniformity in the pathotypes present in the field [25].

Low levels of CI and AUDPC observed in some of the genotypes tested was an indicator of the presence of partial resistance genes which is more durable and is controlled by more than one gene. The positive correlation between AUDPC and CI in the findings of this work was consistent with work by Sandoval-Islas et al. [35] who although working on wheat found a strong positive relationship between relative AUDPC and CI. Furthermore Safavi *et al*., [8] also found correlation coefficient of 0.85 for AUDPC and CI which is in agreement with this study.

4. CONCLUSION

To enhance the productivity of barley crop and reduce the effects of stem rust, it is important to search for alternative methods of management. Host plant resistance is more effective if sources of resistance can be identified in barley genotypes and incorporate them in commercial cultivars [36]. Exploitation of wild relative and landraces of barley has great potential for novel genes of resistance. None of the tested genotypes in the field were characterized as immune at adult plant level. However, genotype HA6, HA11 and HA19 were observed to be immune at seedling stage. Genotypes with high levels of slow rusting can be developed further to accumulate 4 to 5 minor genes of resistance which gives an almost immune kind of plant reaction.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the

authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by delivering genetic.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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