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# Evaluation of Kenyan wheat (*Triticum aestimum* L.) genotypes for leaf rust (*Puccinia triticina* Eriks.) at adult plant stage

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## Abstract

Leaf rust (*Puccinia triticina*) is one of the major rust diseases that affect wheat (*Triticum aestivum)* production worldwide. The objective of this study was to determine genotypic variation among Kenyan wheat genotypes against leaf rust at adult plant stage. A set of 144 genotypes were evaluated in a two-season field experiments at Kenya Agricultural and Livestock Research Organization (KALRO), Njoro. In the field, genotypes were sown in 12 × 12 partially balanced lattice design. Adult plant infection assessed by Area under Disease Progress Curve ranged from means of 42.00 to 145.00. Mean grain yield ranged from 0.06 to 6.81 tonnes. $ha^{-1}$. Highly significant ($p \leq 0.001$) variations were noted among the seasons, genotypes tested over seasons and the interaction between genotype × season for plant height, a thousand kernel weight (TKW), and harvest index. There were significant ($p \leq 0.01$) effects due to seasons and genotypes for spike length, days to maturity, leaf rust infection and grain filling period, biomass, yield, respectively. Effects due to seasons were significant ($p \leq 0.05$) for hectoliter weight and stem rust infection. Genotypes *K. Tai*, *K. Korongo*, *Fletcher, Verder, R1244*, *R1301* and *R1305* exhibited adult plant resistance in both seasons. Considering the disease response and yield potential, genotypes *R1301* and *R1305* showed lowest leaf rust infection and highest grain yield. These genotypes are suitable candidates for utilization in yield and leaf rust resistance improvement programmes in Kenya.

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**Introduction**

Leaf rust caused by *Puccinia triticina* Eriks. is among the main foliar diseases limiting wheat (*Triticum aestivum* L.) production worldwide (Cherukuri *et al.*, 2005). Yield losses of up to 40% in epidemic years have been reported (Bolton *et al.* 2008). In addition to the direct yield losses, leaf rust causes quality down grade and additional cost is also incurred for disease control; for example, application of fungicides (German *et al.*, 2007). Leaf rust, stem rust caused by *Puccinia graminis* and stripe rust caused by *Puccinia striiformis* are the most damaging fungal diseases of wheat that significantly reduce yield, quality and weight of kernels (Huerta-Espino *et al.*, 2011). Continuous growing of wheat in Kenya has made the fields to remain infectious due to the accumulation of the inocula throughout the year. Leaf rust may kill wheat seedlings by elevating respiration rate, reducing photosynthetic area on the leaf surfaces and decreasing translocation of carbohydrates (Arslan *et al.*, 2002). Although the yield reduction caused by leaf rust is lower than the yellow and stem rust, the level of its damage is greatest because it is most common and widely distributed of the three rust diseases (Huerta-Espino *et al.*, 2011; Naser *et al.*, 2013). The cultivation of large area of susceptible wheat genotypes allows a large leaf rust population to proliferate, creating a reservoir for mutation and selection (Kolmer *et al.*, 2005).

Leaf rust fungus is adapted to a wide range of different climates, and it can be found in diverse wheat growing areas throughout the world because the dispersal of airborne spores cannot be constrained (Roelfs and Singh, 1992; Brown and Hovmoller, 2011). The disease has remained virulent even onto genotypes which are perceived to be resistant due to its ability to mutate and evolve new pathotypes (McDonald and Linde, 2002). The urediniospores are airborne and new races are introduced into new areas from one susceptible host to another where they develop rapidly under optimal weather conditions (Brown and Hovmoller, 2011). Each of the spores released is capable of starting a new infection and can cause significant destruction on wheat within a few weeks (Watson and Luig, 1983; Brown *et al.*, 2002).

Wheat leaf rust infects leaf blades, although in some highly susceptible genotypes infection occurs on leaf sheath and glumes and it is most damaging when the infections occur on the upper leaves before flowering stage (Huerta-Espino *et al.*, 2011).

Genetic diversity of plants determines their potential for improved efficiency and hence their use for breeding, which eventually may result in enhanced food production (Ormoli *et al.*, 2015). Resistance to leaf rust in wheat often is determined by adult plant resistance genes in combination with seedling resistance genes. The significance of disease in particular, depends upon the prevalence of aggressive and virulent races of the pathogen as well as their compatibility with the genetic constitutions of the host in a given environment (Kolmer, 1996; Kolmer, 2005). The use of resistant wheat genotypes is the most economical and known to be environmentally friendly method of controlling the disease, besides the reduction of costs of fungicides applied (Martinez *et al.*, 2001). However, host resistance conferred by a single or a few genes could be easily overcome by emergence of new races (McDonald and Linde, 2002).

A total of 67 genes conferring resistance to leaf rust have been catalogued to date (McItosh *et al.*, 2008). These genes alone or in combination provide a satisfactory level of resistance. For example, the congregating genes *Lr34* and *Yr18* have remained effective for more than 50 years (William *et al.*, 2003). A number of genes such as *Lr9*, *Lr19* and *Lr24*, are effective against most of the pathotypes of leaf rust, and are available in the improved genotypes, but sometimes, these resistant genes lack durability (Purnima *et al.*, 2012). Thus, the short lived nature of race-specific hypersensitive response has created the necessity to search for more durable type of resistance. Two genes for leaf rust resistance in wheat, *Lr10* (Feuillet *et al.*, 2003) and *Lr21* (Huang *et al.*, 2003) have been isolated, cloned and sequenced. Both genes have sequences that encode nucleotide-binding site leucine-rich repeat regions which are characteristic of disease resistance genes in plants. Special mention of *Lr26* despite its susceptibility is essential since these features significantly in Pakistani wheat cultivars.

The virulence to *Lr26* appears every year and wheat varieties carrying *Lr26* continue to be cultivated globally due to the T1BL.1RS translocation that it is associated with exceptional grain yield advantages (Fayyaz *et al*., 2008).

High yielding wheat genotypes that are nearly immune to leaf rust could be developed by accumulating slow rusting resistance genes such as *Lr34* and *Lr46* through intercrossing parents that show intermediate disease levels (Hussain *et al*., 1999; Singh *et al*., 2000). Genotypes with *Lr34* and two to three additional genes have shown stable environmental response and final disease ratings lower than five percent under heavy disease pressure (Singh *et al*., 2001). Slow rusting or partial resistance has been reported to be more durable resistance than single seedling resistance genes (Li *et al*., 2010). Virulence in the pathogen population has been evolving rapidly following the deployment of many of these resistance genes, thus, necessitating a constant search and transfer of the new and effective sources of rust resistance.

Despite the fact that it takes long time, breeding for durable resistant wheat genotypes to leaf rust remains a cost effective option of minimizing loss due to this disease (Yuen *et al*., 2007). Field surveys are equally important for monitoring the distribution of current pathotypes and virulence factors caused by *Puccinia triticina*. Furthermore, observations and monitoring at the field level helps greatly in knowledge of new virulence pathogen combinations. In Kenya leaf rust disease has received less attention with the presence of stem and yellow rusts which are the most aggressive hence, efforts to tackle the leaf rust problem has not been majored on. By approaching the limits of biological productivity of wheat in the recent years there has been greatly increased need for new, resistant and high yielding genotypes (Hailegiorgis and Genet, 2011). The objective of this study was to determine genotypic variation among Kenyan wheat genotypes against leaf rust at seedling and adult plant stages.

## Materials and methods

*Experimental site*

The study on virulence of leaf rust disease to different wheat genotypes was conducted in the field at Kenya Agricultural and Livestock Research Organization (KALRO), Njoro (0°20´S, 35°56´E), 2185 meters above sea level. This site is located in the highlands and categorized as zone III ($LH_3$) of the Agro ecological zones, in the Rift Valley Kenya (Jaetzold *et al*., 2012). The research station experiences an average minimum and maximum temperature of 8 ± 2°C and 25 ± 2°C, respectively and an average annual precipitation of 996.4 ± 4.2 mm (KALRO Meteorological station No. 903502 (1), 2013). The soil in this area is predominantly *Molli andosols* that is well drained with an underlying volcanic stratum.

## Field experiment

*Genotypes*

One hundred and thirty three Kenyan spring wheat genotypes released in 20$^{th}$ and 21$^{st}$ century plus eleven introductions were evaluated for adult plant resistance in two seasons. Most of the genotypes were semi-dwarf in stature, with exception of the tall late maturing varieties. Phenologically, the test genotypes matured differently but most of them fell within the class of early and medium with a few late maturing types. A susceptible cultivar *K. Chiriku* was used as a check.

*Experimental procedure*

The genotypes were planted in a field that was previously under canola (*Brassica napus*) crop. The land was cultivated and harrowed to a fine tilth suitable for wheat growth using a disc plough and harrow, respectively. Each entry was sown in an experimental unit measuring 0.75× 0.2m at an equivalent seed rate of 102.9Kg ha-1, adjusted from 95% to 100% germination. The seed was sown in the rows spaced 20 cm apart while within the row seed was placed at a distance of approximately 5cm apart. At sowing time, Di-ammonium Phosphate (DAP) (18:46:0) fertilizer was applied at the rate of 125Kg ha-1 sufficient to supply 22.5Kg N ha-1 and 25.1Kg P ha-1.

The genotypes were evaluated in 12 × 12 partially balanced lattice design with three replications. The blocks and replications were separated from each other by an alleyway measuring 0.5 m. A mixture of susceptible genotypes was planted perpendicular to all the plots and in the borders separating the replicates which acted as a source of inoculum. At tillering stage (GS 20-29) (Zadoks *et al*., 1974), each experimental plot received Calcium Ammonium Nitrate (CAN) at an equivalent rate of a 100Kg ha-1 which supplied an additional 33Kg N.ha-1. Growth of weeds were restricted by applying a post emergence herbicide, Hussar Evolution (*Fenoxaprop-p-ethyl* 64g ha-1 + *Idosulfuron methyl sodium* 8g ha-1 + *Mefenpyr-diethyl* 24 gha-1).

The level of soil moisture was measured by soil moisture meter (Model PMS714, Film Badge Service Company) in an interval of seven days. Whenever there were inadequate rains, during the first season the field was irrigated to field capacity immediately after planting in order to initiate germination and sustain growth of seedlings, thereafter, the frequency of irrigation was determined by the level and retention of the moisture in the soil. The second season experiment was conducted during the main rainy season, where the experiment depended exclusively on soil moisture derived from the rainfall. The sucking and chewing pests on the wheat plants in the experiment were controlled by application of a systemic insecticide, Thunder OD 145 (*imidachloprid* 30g ha-1 + *beta-cyfluthrin* 13.5g ha-1), twice at tillering (GS 20-29) and ear emergence (GS 50-69).

*Data collection*

Leaf rust infection on wheat was evaluated as percent coverage of leaves with rust pustules following modified Cobb's Scale (Peterson *et al*., 1948) where 0% = immune and 100% = completely susceptible. Evaluation of infection was done five times, at an interval of 7 days between heading (GS 50-69) and plant maturity (GS 70-89) (Zadoks *et al*. 1974). Infection types on wheat grown in the field was classified according to Johnston and Browder (1966) where; Immune (0) = no uredinia or other macroscopic sign of infection; Resistant (R) = small uredinia surrounded by necrosis; Moderately Resistant (MR) = small to medium uredinia surrounded by chlorosis or necrosis; Moderately Susceptible (MS) = medium-sized uredinia that may be associated with chlorosis and Susceptible (S) = large uredinia without chlorosis or necrosis.

With regard to agronomic traits, days to heading and anthesis were determined when 50% of plants in a plot had heads with anthers extruded from florets. Plants were considered mature when peduncle had attained golden color. Height of wheat plant was estimated from a random sample of 5 plants from the base of the plant to the tip of the spikes excluding awns. At physiological maturity, yield was estimated from each plot and standardized to 12% moisture content. Thousand kernel weight (TKW) was estimated as weight of thousand kernels. In addition, hectoliter weight was estimated using hectoliter cup. Grain filling period was computed by determining the time photosynthates took to fill the kernels from anthesis to maturity.

Harvest index was calculated using the following formula:

$$\text{Harvest index} = \frac{\text{Grain yield (g)}}{\text{Total biomass(g)}}$$

*Data analyses*

An equation adopted from Campbell and Madden (1990) was used to calculate AUDPC using computer software developed by CIMMYT Mexico (CIMMYT, 2008) as follows:

$$\text{AUDPC} = \sum_{i-1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where; $n$ is the number of readings, $t$ is time of each reading in days, $y_i$is proportion in percent of affected foliage at each reading, $t_{i+1}$ is second assessment date of two consecutive assessment and $y_{i+1}$ is disease severity on assessment date ($i$+1). The cultivars resistances were compared using Area under Disease Progress Curve and Final Disease Severity (FDS) data.

The analysis of variance was done to determine the significant differences among the selected wheat genotypes for the different agronomic traits using PROC. GLM in Statistical Analysis System (SAS) version 8 (SAS Institute Inc., Cary 2001).

The data for all agronomic traits and kernel quality was analyzed using the following statistical model.

$Y_{ijklm} = \mu + S_i + R_{j(i)} + B_{k(ij)} + G_l + SG_{il} + \varepsilon_{ijklm}$

Where; $Y_{ijkl}$ =Observation of experimental units; $\mu$ =Overall mean; $S_i$ = Effect due to $i^{th}$ season; $R_{j(i)}$ = Effect due to $j^{th}$ replicate in the $i^{th}$ season; $B_{k(ij)}$ = Effect due to $k^{th}$ block in the $j^{th}$ replicate in the $i^{th}$ season; $G_l$ = Effect due to $l^{th}$ genotype in the $k^{th}$ block in the $j^{th}$ replicate; $SG_{il}$ = Effect due to interaction between $i^{th}$ season and $l^{th}$ genotype in the $i^{th}$ season in the $j^{th}$ replicate; $\varepsilon_{ijklm}$= Random error component.

Wheat genotypes and replicates were considered as fixed effects while blocks, seasons and interaction between season × genotype were considered as random effects. From the expected mean squares, random error was used to test the effects of season × genotype and blocks, season × genotype interaction was used as an error term for genotype while blocks were used to test the effects of replicates. Replicates were used as an error term for seasons. Means were separated by Least Significant Difference (LSD) test (Steel and Torrie, 1980). Where genotypic effects were significant at $p \leq 0.05$ following the formula:

$$LSD = \frac{t(s\sqrt{2})}{\sqrt{n}}$$

Where $t$ is tabulated t value, $s$ is standard deviation of all the plots and $n$ is number of observations in each variety. A Pearson correlation coefficient analysis was done to establish the relationship between the different agronomic traits measured using the following formula;

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[n\Sigma x^2 - (\Sigma x)^2][n\Sigma y^2 - (\Sigma y)^2]}}$$

(www.mathworld.walfran.com/correlationcoefficient.html)

Where $r$ is Pearson's correlation coefficient, n is the number of samples, x is the dependable variable and y is the independent variable.

## Results

*Environmental conditions during crop growth seasons*

The rainfall and temperature experienced during the growth period of the crop varied. The average rainfall and temperature experienced in the first season was 3.57 ± 1.87mm and 24.25 ± 1.28°C, respectively and second season had 2.91 ± 1.14mm and 22.87 ± 1.18°C rainfall and temperature, respectively. The average soil moisture experienced in the first and second season was 16.16 ± 0.27mm and 15.21mm ± 0.55, respectively while the average temperature was 23.85 ± 0.4°C in season 1 and 22.15 ± 0.29°C in season 2 (Table 1).

**Table 1**. Summary of temperature and rainfall experienced over the two growing season in KALRO, Njoro in 2016.

| Season | Air temperature (°C) | Air rainfall (mm) | Soil moisture (mm) | Soil temperature (°C) |
|---|---|---|---|---|
| Season 1 | 24.25 ± 1.28 | 3.57 ± 1.87 | 16.16 ± 0.27 | 23.85 ± 0.41 |
| Season 2 | 22.87 ± 1.18 | 2.91 ± 1.14 | 15.21 ± 0.55 | 22.15 ± 0.29 |

*Analysis of Variance and Genotype × Season Interaction*

Highly significant ($p \leq 0.001$) variations were noted among the seasons, genotypes tested over seasons and the interaction between genotype × season for plant height, a thousand kernel weight, and harvest index. There were significant ($p \leq 0.01$) effects due to seasons and genotypes for spike length, days to maturity, leaf rust infection and grain filling period, biomass, yield, respectively. Effects due to seasons were significant ($p \leq 0.05$) for hectoliter weight and stem rust infection. There were no significant variations noted for grain filling period between seasons however, there were significant ($p \leq 0.001$) effects due to genotypes and genotype × season for grain filling period (Table 2).

There was significant ($p \leq 0.05$) difference of means for yield and yield components between seasons except for the grain filling period. The plants grown during season two were taller (30.90%) and took longer days to mature (4.74%) than the second season. In addition, these plants had longer spikes (17.97%), higher biomass (53.61%), TKW (29.13%), hectoliter weight (4.32%) and yellow rust disease (99.80%) than in February-July (off-season). However, the plants took longest number of days to fill the grains (1.19%) during first season. Moreover, the plants possessed higher harvest index, leaf rust disease and stem rust disease than second season by 12.5%, 43.88% and 19.18% respectively (Table 6).

**Table 2.** Mean squares of wheat genotypes evaluated for agronomic traits, yield, grain quality, leaf rust, stem rust and yellow rust reactions over two seasons in Njoro.

| | | | | | | | | | | | *Area under Disease Progress Curve* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source of variation | df | Height (cm) | Spike Length (cm) | Grain filling period (days) | Biomass (t ha-1) | Maturity (days) | Yield (t ha-1) | Thousand Kernel weight (g) | Hectoliter weight (kg hI-1) | Harvest Index | Leaf rust | Stem rust | Yellow rust |
| Season | 1 | 25323.95*** | 851.1*** | 38.37 | 112736.88*** | 6164.66** | 767.72*** | 347.09*** | 1178.29 | 0.01*** | 137937.05** | 108014.01** | 682420.32*** |
| Rep(Season) | 4 | 111.02* | 6.06*** | 179.80*** | 391.30*** | 79.35* | 7.45*** | 1.80*** | 116.19* | 0.01*** | 4346.28*** | 7066.35*** | 548.98 |
| Block(Rep×Season) | 66 | 80.33*** | 0.86* | 28.95 | 121.28*** | 33.20 | 0.96*** | 0.61*** | 68.82* | 0.00*** | 314.61* | 332.41* | 288.98 |
| Genotype | 143 | 852.40*** | 5.44*** | 140.79*** | 511.43*** | 696.24 | 7.85** | 6.22*** | 343.57*** | 0.01*** | 3867.02*** | 4103.06*** | 1655.90 |
| Genotype × Season | 143 | 108.49*** | 1.27*** | 86.52*** | 308.78*** | 601.34*** | 5.16*** | 1.35*** | 164.57*** | 0.00*** | 916.29*** | 964.28*** | 1602.60*** |
| Error | 506 | 6.21 | 0.72 | 4.90 | 7.67 | 5.07 | 0.50 | 0.57 | 6.55 | 0.07 | 15.04 | 15.05 | 15.51 |
| R-Square | | 0.95 | 0.89 | 0.76 | 0.89 | 0.94 | 0.96 | 0.91 | 0.80 | 0.82 | 0.89 | 0.89 | 0.91 |
| Cv % | | 6.74 | 7.26 | 13.94 | 24.61 | 4.60 | 26.86 | 15.29 | 12.36 | 43.25 | 20.30 | 19.00 | 27.57 |

*, **, *** significant at ($P \leq 0.05$), ($P \leq 0.01$) and ($P \leq 0.001$) respectively. *Cv* - coefficient of variation.

*Correlation Analysis among Leaf Rust Disease and the Traits of Importance*

The Pearson correlation coefficient analysis showed that yield displayed significantly different positive correlation with a thousand kernel weight ($r$=0.74***) and hectoliter weight ($r$=0.40***). The TKW showed a significant positive correlation with hectoliter weight ($r$=0.56***), however, yield, TKW and hectoliter weight displayed significantly negative correlation with leaf rust ($r$=-0.27***, $r$=-0.30***, $r$=-0.19***) and stem rust ($r$=-0.19***, $r$=-0.22***, $r$=-0.19***) (Table 3).

*Response of Wheat Genotypes for severity and Grain Yield*

The mean yield and AUDPC for leaf rust for the best 20 genotypes, the check variety and the least yielding wheat genotypes evaluated are presented in Table 5. Considering how the seasons differentiated performance of genotypes, off-season (4.76t ha-1) had lower yield than main season. Genotypes *R1301* and *R1305* ranked the highest with; 6.51t ha-1 and 5.86t ha-1 mean yields across seasons, respectively. The most susceptible genotype *Marquis* had 0.06t ha-1, while the susceptible check *K. Chiriku* had 1.55t ha-1. Based on AUDPC means, genotypes *R1301* and *R1305* had lowest with means of 42.00 and 42.00, respectively.

*Field Tests for Adult Plant Resistance*

Adult plant reactions showed a range of response level of the tested wheat genotypes to leaf rust disease. Plant reactions of the genotypes which were considered to be resistant and the check are presented in Table 4. It is worth to note that seven genotypes (*K. Tai, K. Korongo, Fletcher, Verder, R1244, R1305, R1301*) showed resistance response at adult stage for the two seasons.

Twenty two genotypes (*K. Page, Lenana, Romany, Bounty, Plume, Sungura, Tobari 66, K. Paka, K. Tembo, K. Kingbird, Marquillo, 1061.K.4, Era, Mcvey, Morris, PW Thatcher, Fronthatch, Polk, Angus, Norm, R1475, R1309*) were resistant only during the second season while, 5 genotypes (*K. Fahari*, *K. Wren*, *Minnpro, R1336, R1317*) showed resistance infection type during the first season. The remaining genotypes showed susceptibility that ranged between 5S to 90S at adult plant stage.

**Table 3.** Correlation coefficient ($r$) among leaf rust and the traits of interest for wheat genotypes evaluated for leaf rust resistance at Kenya Agricultural and Livestock Research Organization in Njoro, 2016.

| | | | | Area under Disease Progress Curve | |
|---|---|---|---|---|---|
| | Yield | Thousand Kernel Weight | Hectolite r Weight | Leaf Rust | Stem Rust |
| Yield | - | 0.74*** | 0.40*** | -0.27*** | -0.19*** |
| Thousand Kernel Weight | | - | 0.56*** | -0.30*** | -0.22*** |
| Hectoliter Weight | | | - | -0.19*** | -0.19*** |
| AUDPC Leaf Rust | | | | - | 0.24*** |
| AUDPC Stem Rust | | | | | - |

***, significance at ($p \leq 0.001$).

**Table 4**. Adult plant infection type to leaf rust (*Puccinia triticina*) for wheat (*Triticum aestivum*) genotypes that were considered resistant and a check as evaluated in the field.

| | | Season1 | | | | Season2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotype | Pedigree | I$^{st}$ score | 2$^{nd}$ score | FDS | AUDPC | I$^{st}$ score | 2$^{nd}$ score | FDS | AUDPC |
| K. Tai | ND643/2*WBLL1 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 |
| K.Korongo | BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 |
| Fletcher | II-55-10/4/PEMBINA/II-52-329/3/II-53-388/III-58-4//II-53-546 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 |
| Verder | MN-7663/SBY-354-A | 0 | 0 | 0 | 0.0 | TR | TR | TR | 17.5 |
| R1244 | PRINIA/3/ALTAR84/AE.SQ//2*OPATA/4/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92 | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 |
| R1305 | KSW/5/2*ALTAR 84/AE.SQUARROSA (221)//3*BORI95/3/URESJUN/KAUZ/4/WBLLI | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 |
| R1301 | KSW/5/2*ALTAR 84/AE.SQUARROSA (221)//3*BORI95/3/URESJUN/KAUZ/4/WBLLI | 0 | 0 | 0 | 0.0 | 0 | 0 | 0 | 0.0 |
| K. Page | MENTANA/KENYA-58//BAGE/3/KENYA-184-P | 5MS | 20S | 40S | 437.5 | 0 | 0 | 0 | 0.0 |
| Lenana | YAQUI- 48 / KENTANA- 48 | 5S | 20S | 40S | 437.5 | 0 | 0 | 0 | 0.0 |
| Romany | COLOTANA 261-51 / YAKTANA 54A | 5MS | 10S | 20S | 227.5 | 0 | 0 | 0 | 0.0 |
| Bounty | TIMSTEIN/2*KENYA//BONZA | TR | 5S | 10S | 108.5 | 0 | 0 | 0 | 0.0 |
| Plume | MIDA/MCMURACHY//EXCHANGE/3/KENYA-184-P | 0 | 5S | 5S | 70.0 | 0 | 0 | 0 | 0.0 |
| Sungura | ID 1877/MORRIS | 0 | 10S | 15S | 175.0 | 0 | 0 | 0 | 0.0 |
| Fronthatch | FRONTANA / KENYA58 // NEWTHATCH | 0 | 5S | 5S | 70.0 | 0 | 0 | 0 | 0.0 |
| Polk | THATCHER / SUPREZA /3/ KENYA 58 / NEWTHATCH // FRONTANA | 0 | 15S | 15S | 210.0 | 0 | 0 | 0 | 0.0 |
| Angus | THATCHER/2*SUPREZA/3/FRONTANA//KENY58/NEWTHATCH/ 7/PEMBINA//FRONTANA/5*THATCHER/6/MIDA//KENYA-117-A/2*THATCHER/3/FRONTANA/4*THATCHER/4/MN-III-58-4/5/KENYA-58/NEWTHATCH//3*LEE | 0 | 0 | 5S | 35.0 | 0 | 0 | 0 | 0.0 |
| Norm | MN-73167/MN-81070 | 0 | 5S | 5S | 70.0 | 0 | 0 | 0 | 0.0 |

0=Immune, R= Resistant, MR=moderately resistant, MS=moderately susceptible, S=Susceptible, TR=trace resistant, MSS= moderately susceptible and susceptible (Johnston and Browder 1966). AUDPC=Area under Disease Progress Curve; SIT=Seedling Infection Type, FDS= Final Disease Severity.0, 0;, 1, 2 = resistance response, 3 and 4 = susceptibility Response.

(Con..) **Table 4.** Adult plant infection type to leaf rust (*Puccinia triticina*) for wheat (*Triticum aestivum*) genotypes that were considered resistant and a check as evaluated in the field.

| Genotype | Pedigree | Season1 | | | | Season2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | I$^{st}$ score | 2$^{nd}$ score | FDS | AUDPC | I$^{st}$ score | 2$^{nd}$ score | FDS | AUDPC |
| R1475 | - | TR | 30S | 30S | 423.5 | 0 | 0 | 0 | 0.0 |
| R1309 | KFA/5/REH/HARE//2*BCN/3/CROC-I/AE.SQUARROSA(213)//PGO/4/HUITES/6/REH/HARE//2*BCN/3/CROC-I/AE.SQUARROSA(213)//PGO/4/HUITES | 5MS | 5MS | 5MS | 87.5 | 0 | 0 | 0 | 0.0 |
| Tobari 66 | TEZANOS-PINTOS-PRECOZ/SONORA-64-A | 0 | 10S | 10S | 140.0 | TR | TR | TR | 17.5 |
| K. Paka | WISCONSIN-245/II-50-17//CI-8154/2*TOBARI-66 | 5MS | 15S | 30S | 332.5 | TR | TR | TR | 17.5 |
| K. Tembo | WISCONSIN-245/II-50-17//CI-8154/2*TOBARI-66 | 0 | 15S | 50S | 455.0 | TR | TR | TR | 17.5 |
| K.Kingbird | TAM200/TUI/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1 | 0 | 20S | 20S | 280.0 | TR | TR | TR | 17.5 |
| 1061.K.4 | MIDA // McMURACHY / EXCHANGE /3/ RIO NEGRO | 0 | 5S | 15S | 140.0 | TR | TR | TR | 17.5 |
| Marquillo | MARQUIS/(TR.DR)IUMILLO | 0 | 5S | 5S | 70.0 | TR | TR | TR | 17.5 |
| Era | II-55-10/4/PEMBINA/II-52-329/3/II-53-388/III-58-4//II-53-546 | 0 | 5S | 5S | 70.0 | 0 | TR | TR | 14.0 |
| Mcvey | NING-8331/MN-87029//MN-89068 | 0 | 5S | 10S | 105.0 | TR | TR | TR | 17.5 |
| Morris | THATCHER//KENYA-117 A/MIDA/3/FRONTANA/4*THATCHER/4/THATCHER/5/FRONTANA/4*THATCHER | 0 | 5S | 10S | 105.0 | 0 | TR | TR | 14.0 |
| PWThatcher | THATCHER/AGENT | 5MS | 10S | 20S | 227.5 | TR | TR | TR | 17.5 |
| K. Fahari | TOBARI-66/3/SRPC-527-67//CI-8154/2*FROCOR | 0 | 0 | 0 | 0.0 | 0 | 0 | 20MSS | 140.0 |
| K.wren | THELIN#2/TUKURU | 0 | 0 | 0 | 0.0 | 5S | 5S | 5S | 87.5 |
| Minnpro | MN-72299/MN-74115 | 0 | 0 | 0 | 0.0 | 5S | 5S | 5S | 87.5 |
| R1336 | BABAX/LR42//BABAX*2/3/TUKURU | 0 | 0 | 0 | 0.0 | 5S | 10S | 10S | 157.5 |
| R1317 | KSW/7/CAL/NH/H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR/8/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR | 0 | 0 | 0 | 0.0 | 5S | 5S | 5S | 87.5 |
| K. Chiriku | KTB/(SIB)CARPINTERO | 10S | 30S | 50S | 595.0 | 10MS | 40S | 40S | 595.0 |

0=Immune, R= Resistant, MR=moderately resistant, MS=moderately susceptible, S=Susceptible, TR=trace resistant, MSS= moderately susceptible and susceptible (Johnston and Browder 1966). AUDPC=Area under Disease Progress Curve; SIT=Seedling Infection Type, FDS= Final Disease Severity. 0, 0;, 1, 2 = resistance response, 3 and 4 = susceptibility Response.

**Table 5**. The mean yield and AUDPC for leaf rust for the best 20, the least yielder and the check of wheat (*Triticum aestivum*) genotypes evaluated over the two seasons in KALRO, Njoro during 2015-1016 cropping season.

| Genotype | | Yield (t ha-1) | | | Area under Disease Progress Curve | | |
|---|---|---|---|---|---|---|---|
| | Pedigree | Mean | Season 1 | Season 2 | mean | Season 1 | Season 2 |
| R1301 | KSW/5/2*ALTAR 84/AE.SQUARROSA (221)//3*BORI95/3/URESJUN/KAUZ/4/WBLLI | 6.51 | 0.15 | 12.87 | 42.00 | 28.00 | 56.00 |
| R1305 | KSW/5/2*ALTAR 84/AE.SQUARROSA (221)//3*BORI95/3/URESJUN/KAUZ/4/WBLLI | 5.86 | 1.10 | 10.62 | 42.00 | 28.00 | 56.00 |
| K. Kingbird | TAM200/TUI/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/ 3/YR/4/TRAP#1 | 4.64 | 1.57 | 7.71 | 52.52 | 46.62 | 58.42 |
| R1309 | KFA/5/REH/HARE//2*BCN/3/CROC-I/AE.SQUARROSA(213)//PGO/4/HUITES/6/REH/HARE//2*BCN /3/CROC-I/AE.SQUARROSA(213)//PGO/4/HUITES | 4.56 | 0.17 | 8.95 | 46.47 | 36.46 | 56.48 |
| R1476 | - | 4.25 | 1.71 | 6.79 | 45.75 | 33.07 | 60.83 |
| K. Tai | ND643/2*WBLL1 | 4.17 | 1.18 | 7.16 | 42.00 | 28.00 | 56.00 |
| Eagle10 | EMB16/CBRD//CBRD | 4.11 | 1.02 | 7.20 | 53.30 | 39.49 | 67.11 |
| CI 14393 | FROCOR*2/4/COMETA/3/ NEWTHATCH// MENTANA/ MENKEMEN | 3.71 | 1.04 | 6.38 | 60.48 | 56.49 | 64.47 |
| R1244 | PRINIA/3/ALTAR84/AE.SQ//2*OPATA/4/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92 | 3.65 | 0.99 | 6.31 | 122.82 | 28.00 | 217.64 |
| R1474 | - | 3.65 | 1.68 | 5.62 | 88.99 | 71.55 | 106.43 |
| Ibis | KWALE/DUMA | 3.61 | 2.25 | 4.97 | 117.05 | 92.78 | 141.32 |
| ET-12-D4 | MAMBA/UQ105 | 3.51 | 2.69 | 4.33 | 54.31 | 34.76 | 73.86 |
| K. Nyangumi | TEZANOS-PINTOS-PRECOZ//SELKIRK-ENANO*6/LERMA-ROJO-64/3/AFRICA-MAYO-48/4/KENYA-SWARA/K-4500-6 | 3.41 | 1.20 | 5.62 | 61.60 | 33.07 | 90.13 |
| K. Nyoka | CI-8154/2*FEDERATION//3*ROMANY | 3.32 | 1.67 | 4.97 | 70.63 | 64.02 | 77.24 |
| Means | | | 1.25 | 6.01 | | 49.61 | 85.85 |
| *Cv*% | | | 26.86 | | | 20.30 | |
| $LSD_{(0.05)}$[a] | | | 0.56 | | | 2.01 | |
| $LSD_{(0.05)}$[b] | | | 0.07 | | | 17.06 | |

R: Introduction, a: LSD for comparing means within seasons, b: LSD for comparing means between seasons.

(Cont..) **Table 5.** The mean yield and AUDPC for leaf rust for the best 20, the least yielder and the check of wheat (*Triticum aestivum*) genotypes evaluated over the two seasons in KALRO, Njoro during 2015-1016 cropping season.

| Genotype | Pedigree | Yield (t ha-1) | | | Area under Disease Progress Curve | | |
|---|---|---|---|---|---|---|---|
| | | Mean | Season 1 | Season 2 | mean | Season 1 | Season 2 |
| K. Nyoka | CI-8154/2*FEDERATION//3*ROMANY | 3.32 | 1.67 | 4.97 | 70.63 | 64.02 | 77.24 |
| Verde | MN-7663/SBY-354-A | 3.28 | 2.37 | 4.19 | 42.00 | 28.00 | 56.00 |
| Zabadi | CORRECAMINOS/INIA-67//K-4500-2/3/KENYA-SWARA//TOBARI-66/CIANO-67 | 3.27 | 1.48 | 5.06 | 55.35 | 47.68 | 63.02 |
| R1317 | KSW/7/CAL/NH/H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6 /PASTOR/8/CAL/NH//H567.71/3/S ERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR | 3.25 | 0.79 | 5.71 | 50.10 | 28.00 | 72.20 |
| Tama | YAKTANA-54/LERMA-52 | 3.23 | 0.80 | 5.66 | 65.36 | 70.14 | 60.58 |
| Kanga | - | 3.18 | 1.64 | 4.72 | 68.93 | 56.09 | 81.77 |
| Katar | COOK/VEE"S"//DOVE"S"/SERI/3/BJY"S" | 3.12 | 0.98 | 5.26 | 57.73 | 47.61 | 67.85 |
| Marquis | HARD-RED-CALCUTTA | 0.06 | 0.00 | 0.12 | 145.63 | 107.29 | 183.97 |
| K. Chiriku | KTB/(SIB)CARPINTERO | 1.55 | 1.04 | 2.06 | 103.82 | 86.34 | 121.30 |
| Means | | | 1.25 | 6.01 | | 49.61 | 85.85 |
| *Cv*% | | | 26.86 | | | 20.30 | |
| $LSD_{(0.05)}$[a] | | | 0.56 | | | 2.01 | |
| $LSD_{(0.05)}$[b] | | | 0.07 | | | 17.06 | |

R: Introduction, [a]: LSD for comparing means within seasons, [b]: LSD for comparing means between seasons.

**Table 6.** Summary of means of disease and agronomic traits wheat genotypes evaluated against leaf rust disease at Njoro over two seasons.

| Season | Area under Disease Progress Curve | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plant height (cm) | Spike length (cm) | Grain Filling Period (days) | Biomass (t ha-1) | Maturity (days) | Yield (t ha-1) | Thousand kernel weight (g) | Hectolitre weight (kg hI-1) | Harvest index | Leaf rust | Stem rust | Yellow rust |
| Season 1 | 75.48b | 8.90b | 35.32a | 19.77b | 107.41b | 0.91b | 3.09b | 51.85b | 0.08a | 202.47a | 184.93a | 0.53b |
| Season 2 | 109.24a | 10.85a | 34.90a | 42.61a | 112.75a | 2.80a | 4.36a | 54.19a | 0.07b | 113.63b | 149.46b | 268.65a |
| $LSD_{(0.05)}$ | 0.83 | 0.10 | 0.65 | 1.03 | 0.68 | 0.07 | 0.08 | 0.88 | 0.01 | 2.01 | 2.01 | 2.07 |

Means followed by the same letters down the column are not significantly different at $p \leq 0.05$.

**Discussion**

The significant variation due to season for most of the parameters suggests environmental variations between the two seasons when the experiment was conducted. This significant difference could be attributed to variability in availability of temperature, and moisture among other environmental factors. The present results agree with those of Milan *et al.* (2015) who reported that the season was mainly responsible for variation of the agronomic traits in two-rowed winter malting barley. The significant effects due to genotype for agronomic traits, yield and yield components as well as rust diseases implies that these traits are affected by the genetic make-up of a given genotype either directly or indirectly. The results are in tandem with Yan *et al.* (2010) who did a different research on soybean and reported that genotypic effects were significant for all agronomic traits. Similarly significant effects due to the interaction between season and genotype for all the parameters could be an indication that the genotypes used were not consistent between seasons probably due to environmental influence to the genotypes for given specific trait. This is in consistency with Bhatta (2015) who reported that interaction between season and genotype effects explained the variation in grain yield, hectoliter weight, days to heading, plant height, harvest index, and TKW on winter wheat.

Despite the heavy leaf rust disease pressure in the field during the two seasons, some lines remained resistant. Among the 144 wheat genotypes screened, 7 genotypes (*K. Tai, K. Korongo*, *Fletcher, Verder, R1244, R1305, R1301*) exhibited adult plant resistance during season one and season two. The avirulence of the leaf rust at adult plant stage in these genotypes revealed the presence of minor resistance genes. Parlevliet (2001) found out that seedling resistance is under the control of major genes which provides resistance at all stages of plant growth while adult plant resistance is under control of minor genes. Variations in the expression of resistance genes in adult plant stages could suggest that there was presence of gene diversity among evaluated genotypes. The results are in agreement with Newcomb *et al.* (2013) who did a different research on stem rust.

Eleven genotypes showed trace infection responses at adult stage for leaf rust. The trace reaction could be associated with hypersensitive reaction whereby fungal infection signals a defense mechanism leading to cell collapse which restricts further disease spread as reported by Rubiales and Nicks, 2000.

Cultivars lacking leaf rust seedling resistance genes may have additional additive minor genes that contribute to low disease pressure in the field (Hysing *et al.*, 2006). Slow rusting has been shown to be more durable than major seedling resistance according to Singh *et al.* ( 2001) and a combination of adult plant resistant gene *Lr34* and several addition minor genes have resulted in a high level of non-specific resistance in some cultivars (Navabi *et al.*, 2005). These results may add a depth of their resistance to be exploited as good source of resistance. Furthermore, resistance expression depends on the environmental conditions, plant growth stage, host-parasite interaction, and the interaction between resistance genes in wheat genome (Kolmer, 2005). The genes in the resistant genotypes may be deployed singly or in combination into high yielding genotypes to develop resistant high yielding wheat genotypes. In addition, new sources of resistance in wheat genotypes could be incorporated into wheat to improve the diversity of the existing gene pool for leaf rust resistance. Durable rust resistance mechanism in wheat is achieved through introgression of partially resistant minor genes which seems to be more appropriate solution for sustainable wheat production (Singh *et al.*, 2000).

The significant variation due to season for the means of agronomic traits, yield, grain quality, leaf rust, stem rust and yellow rust severity suggested seasonal variations between the two seasons in which the field experiment was conducted. The warm moist conditions experienced during season one favored stem rust and leaf rust infection hence, the high AUDPC for the two diseases. The effects of leaf rust on grain yield varied across seasons. For instance, in the first season, leaf rust infection contributed to the higher reduction of grain yield and TKW compared to the second season when leaf rust infection was minimal.

In a different study on barley, Ochoa and Parlevliet. (2007) found out that yield loss due to leaf rust was related to AUDPC. Some wheat genotypes with high yellow rust disease severity had low leaf rust severities in this study. A report by Bancal *et al.* (2007) also highlighted that, due to the reduced photosynthetic area for stem rust fungus infection and spread, some wheat lines with high yellow rust disease severity tended to show low stem rust severities.

Inverse relation was present between the disease level and grain yield and this implies that, leaf rust disease directly affects the kernel quality leading to shriveling of wheat grains; for example *Marquis* which had the least TKW and grain yield value was totally susceptible to the leaf rust. *Marquis* had very shriveled kernels in the field and in some plants there were no kernels at all implying that leaf rust negatively affected the kernel quality and quantity. These results are consistent with those of Nzuve *et al.* (2012) who did a research on resistance of bread wheat to stem rust.

The positive correlation between grain yield, TKW and hectoliter weight is an indication that the yield components is largely responsible for the determination of grain yield in individual plants. Similarly, in a different study on rice (*Oryza sativa* L.) Mirza *et al.* (1992) found that the number of grains per panicle was positively correlated with panicle length, TKW and grain yield. It was observed that TKW was affected by leaf rust infection and could be used to estimate loss in yield due to leaf rust infection. Such results are in agreement with those of Draz *et al.* (2015).

Grain weight is a crucial trait and of primary importance in determining wheat yield. Genotypes with larger grain weight value tend to have longer grain filling period, resulting in higher assimilate accumulation and heavier grain weight. Thus, genotype *R1301* had the highest grain weight among the evaluated genotypes and it possessed longest grain filling period as opposed to *Marquis* which had the least grain weight and shortest grain filling period.

Grain weight is determined by the source capacity (photosynthetic leaves) to supply assimilate during the ripening period, and by sink capacity (developing grain) to accumulate the imported assimilate (Ntanos and Koutroubas, 2002).

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