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Full Length Research Paper

Phenotyping selected Australian wheat genotypes for resistance to stem rust and yellow rust and yield performance in Kenya

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Stem rust and yellow rust are major diseases of wheat (*Triticum aestivum* L.) caused by *Puccinia graminis* and *Puccinia striiformis*, respectively. In Kenya, although the two diseases occur together, available genetic resistance is limited. Therefore, research for resistance to both diseases is of priority. We therefore evaluated 59 Australian wheat genotypes alongside local checks over three seasons in Njoro, Kenya, for resistance to stem rust, yellow rust and yield performance and one season in Debre Zeit, Ethiopia, for resistance to stem rust in a partially balanced *lattice*-square design with three replicates. Resistance to stem rust isolates *TTKSK* and *TTKTT* was evaluated in the greenhouse. Effect due to genotype, season and genotype-by-season interaction was significant ($p \leq 0.05$) for area under disease progress curve (AUDPC), coefficient of infection (CI), final disease severity (FDS), grain yield (GY), 1000-kernel weight and test weight (TW). AUDPC, CI and FDS were negatively correlated with GY and TW. Broad-sense heritability (H^2) for AUDPC, CI and FDS was 70.2, 60.0 and 68.1% for stem rust and 55.8, 50.0 and 59.7% for yellow rust, respectively. Genotypes Lancer, Sunguard and Gauntlet exhibited stable resistance to stem rust in Njoro and Debre-Zeit while genotypes Sunmax, Steel and Gladius showed stable resistance to yellow rust in Njoro. Genotypes Lancer, Sunguard, Gauntlet, Sunmax, Steel, Gladius, Shield and Magenta, having adult plant resistance to stem rust and yellow rust and seedling resistance to stem rust with superior yield performance are, therefore, recommended as sources of resistance genes and candidates for deployment as varieties.

Key words: adult plant resistance, genotype-by-season interaction, grain yield, seedling resistance.

INTRODUCTION

Common wheat (*Triticum aestivum* L.) is an important cereal crop for food and livelihood (Balk et al., 2019). In sub-Saharan Africa (SSA), its demand continues to increase due to population growth, urbanization and changes in food preference (Shiferaw et al., 2013). However, current levels of wheat production in SSA only

serve about 28% of regional requirements while 72% of the demand is met through imports (USDA-FAS, 2021). In 2019, for instance, of the 765.7 million tonnes (t) produced worldwide, SSA contributed a paltry 9.3 million t yet consumption was nearly 33.8 million t (USDA-FAS, 2021). To offset this deficit, a 30% growth in grain yield needs to be realized through annual increases of at least 2% (Ray et al., 2013). However, current levels of genetic gain are insufficient to meet the rising demand (Tadesse et al., 2019).

Wheat production is affected by biotic and abiotic factors (Leonard and Szabo, 2005; Park, 2016; Soko et al., 2018). Among biotic factors, three rust diseases namely: stem rust (caused by fungus *Puccinia graminis* f. sp. *tritici*), yellow rust (*P. striiformis* f. sp. *tritici*) and leaf rust (*P. triticina*) are considered as the most significant foliar diseases of wheat (Olivera et al., 2019; Chen, 2020). They cause shrivelling of kernels and reduce the number of kernels per spike (Dean et al., 2012; Szabo et al., 2014; Soko et al., 2018; Brinton and Uauy, 2019). The evolution of the pathogen and emergence of new races of aggressive nature has resulted in the loss of resistance among a majority of deployed cultivars (Cuomo et al., 2017; Olivera et al., 2019). Therefore, continuous identification, characterization and deployment of genetically diverse sources of resistance in wheat cultivars is essential to achieve durable resistance (Wessels et al., 2019).

Genetic variation is essential for breeding wheat for improved traits and adaptability through classical as well as modern genotyping technologies (Jovovic et al., 2020). Molecular mapping studies have identified quantitative trait loci (QTLs) in diverse germplasm for resistance to rust diseases (Randhawa et al., 2018; Rahmatov et al., 2019). Genetic loci linked to resistance and yield performance have also been identified via marker-trait associations using mapping populations derived from bi-parental crosses and diversity panels using genome-wide association studies (Lopes et al., 2015). A few of these known QTLs have been introgressed into adapted genetic backgrounds through marker assisted backcrossing resulting in cultivars which are adapted to target environments, thus, increasing production from one to three t ha^{-1} (Fedoroff, 2015). Genes for resistance to rust diseases are classified as race specific (seedling resistance) and race non-specific (adult plant resistance). To date, more than 70 genes each for stem rust (Sr) and yellow rust (Yr) have been characterized and formally catalogued in wheat (McIntosh et al., 2017). However, most of these genes are race specific and are often overcome by new races, which harbor virulence, when they are deployed singly (Pretorius et al., 2012; Singh et al., 2015). On the other hand, race non-specific resistance genes reduce the possibility of virulent races emerging (Figueroa et al., 2020). Durable resistance, however, is attained when both classes of genes are combined (Randhawa et al., 2018). In Kenya, the wheat breeding program based at the Kenya Agricultural and Livestock Research Organization (KALRO) in Njoro works in collaboration with the International Maize and Wheat Improvement Center (CIMMYT). This collaboration has resulted in deployment of resistant cultivars with high yield performance (Njau et al., 2013; Macharia and Ngina, 2017; Bhavani et al., 2019). However, evolution of virulence necessitates continuous research for resistance from diverse sources. For instance, stem rust resistance gene *SrTmp* in Kenya Robin which was released in 2011 was broken down by races *TTKTT*, *TKTTF* and *TTKTK* in 2014 (Olivera et al., 2015; Newcomb et al., 2016; Patpour et al., 2016). Consequently, identification of new sources of resistance is a sustainable strategy that potentially confers durable resistance through strategic introgression of resistance genes into adapted cultivars. The objective of our study was therefore to characterize genotypes with resistance to stem rust and yellow rust with acceptable yield performance among wheat genotypes introduced from Australia.

MATERIALS AND METHODS

Experimental sites

The study was performed in 2019 off-season (NJ1), 2019 main-season (NJ2) and 2020 off-season (NJ3) at KALRO, Njoro, Kenya (35° 55′ 60″ E, 0° 19′ 60″ S) and in 2019 main-season (DZ) at Debre Zeit Agricultural Research Center (DZARC), Debre Zeit, Ethiopia (38° 59′ 19″ E, 8° 44′ 38″ N). Njoro is located at an elevation of ~ 2185 metres above sea level (masl) and lies within the Lower Highland III (LH_3) Agro-Ecological Zone (AEZ) (Jaetzold et al., 2010). Soils are predominantly well drained volcanic *mollic andosols* which are dark brown to greyish with a thick humic top soil and an average *pH* of 7.0 (Jaetzold and Schmidt, 1983). It receives annual precipitation of approximately 980 mm with average minimum and maximum temperatures of 9.7 °C and 25 °C, respectively. On the other hand, Debre Zeit is located at an elevation of ~ 1900 masl and receives annual precipitation of approximately 851 mm. Average minimum and maximum temperatures are 8.9 °C and 28.3 °C, respectively, while soils are predominantly *vertisols* with an average *pH* of 7.5. These climatic conditions were favorable for cultivation of wheat and occurrence of rust diseases.

Genetic materials

Fifty-nine wheat genotypes introduced from Australia were used in

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this study. They were released cultivars in Australia. Wheat line “Cacuke” and Kenyan cultivar “Kenya Robin” were used as susceptible controls. Cultivar name and pedigree are listed in supplementary Tables 1 to 7

Greenhouse experiment

Five seeds of wheat line “Cacuke” and cultivar “Kenya Robin” were sown in separate plastic pots measuring 6 cm (length) × 6 cm (width) × 6 cm (height) filled with vermiculite mixed with diammonium phosphate (DAP) fertilizer (18:46:0). Pots were labelled and placed in the growth chamber and watered over trays. Seedlings were inoculated at the two-leaf stage with fresh urediniospores collected from corresponding genotypes in the disease nursery following standard procedures (Jin et al., 2007). Urediniospores were suspended in 250 ml of distilled water and two drops of a light mineral oil Soltrol® 130 Isoparaffin (Chevron Phillips Chemical, TX) added and shaken gently before sieving to drain the inoculum in a dispenser (Jin et al., 2007). Seedlings were inoculated by spraying the spore suspension followed by misting using water. Inoculated seedlings were then air-dried for 10-20 minutes and placed in polythene hoods inside a dew cabinet (Percival model I-36, Perry, IA) for incubation at temperatures and relative humidity of 18-20 ºC and ~100%, respectively, in the dark for 48 hours. These conditions were maintained during the day using a humidifier and misting the dew cabinet 3-4 times a day with distilled water using a hand sprayer. After the dew process, fluorescent lights were turned on to provide light to complete the infection process and temperatures raised gradually to 25 ºC for 3 hours. Thereafter, seedlings were transferred to a temperature and water-controlled growth and sporulation chamber at 18-25 ºC under natural light with additional light provided by fluorescent tubes placed at ~1 m above the seedlings and closely monitored for symptoms of disease development.

Fourteen days after inoculation, one fresh and distinct stem rust pustule (large/unique) was collected from an infected stem or leaf from each pot. A sharp razor blade was used to cut out tissues around the pustule. Pustules were carefully placed in a pre-labelled gelatin capsules and sealed. Alcohol-soaked (70%) wipes were used to sterilize the razor blade between collections. The single pustules were washed off in distilled water to prepare inoculum of pure isolates. To bulk the pure isolates, five sets of the two genotypes were planted, inoculated and incubated as early described and bulk inoculum of pure isolates collected separately from each genotype.

Fifty-nine Australian bread wheat introductions and two susceptible controls (Cacuke and Kenya Robin) were evaluated against stem rust isolates *TTKSK* (detected in Kenya in 2001 and virulent on *Sr31*) [purified on Cacuke] and *TTKTT* (detected in Kenya in 2014 and virulent on *SrTmp*) [purified on Kenya Robin] to characterize infection types (ITs) and virulence patterns. Two sets of the experimental materials were planted in the greenhouse as earlier described. At the two-leaf stage, each set of materials was inoculated and incubated separately and monitored for symptoms of disease development. Tests were repeated to clarify ambiguous results.

Field experiment

Five grams of seeds of each genotype were seeded in a 0.7 m double row plot. DAP fertilizer was applied at planting at the rate of 150 kg ha^{-1}. Urea [$CO(NH_2)_2$] (46:0:0) was applied to 1-month old seedlings as top dressing at the rate of 100 kg ha^{-1}. Pre and post emergence herbicides were used to control weeds and a pesticide was used to control insect pests. At booting stage, spreader rows were inoculated with fresh inoculum collected from disease nurseries via needle-injection and foliar spray as described by Njau et al. (2013). Inoculation was repeated after 7 days until the disease had fully developed.

Data collection

Infection types (ITs) in the greenhouse were scored according to Stakman et al. (1962) as 0 (immune), ; (very resistant), 1 (resistant), 2 (moderately resistant), X (mesothetic or heterogenous), 3 (moderately susceptible) and 4 (susceptible). All ITs on stems and leaves were recorded in the order of their prevalence with the most frequent IT recorded first. A comma (,) was used to segregate more than one IT. A forward slash (/) differentiated symptoms on the first and second stem or leaf with letters “n” and “c” indicating more than usual necrosis and chlorosis, respectively. In addition, plus (+) and minus (-) signs described pustules which were relatively larger or smaller, respectively, than is normal. Infection type (IT) 0; was between immune and very resistant. IT 1 was differentiated further into 1-, 1, 1+ while IT 2 was differentiated further into 2-, 2 and 2+. Host responses (HRs) and severity of infection in the field were visually evaluated and first scores taken when spreader rows displayed a severity of ~ 50% as per the modified Cobb scale (Peterson et al., 1948). Three more scores were taken at an interval of seven days. HRs were assessed as immune (I), traces (TR), resistant (R), resistant to moderately resistant (RMR), moderately resistant (MR), moderately resistant to moderately susceptible (MRMS), moderately susceptible (MS), moderately susceptible to susceptible (MSS) and susceptible (S) (Roelfs et al., 1992). Severity was estimated on a scale of 1-100%, where 1% = very low severity and 100% = very high severity (Peterson et al., 1948). The AUDPC was calculated following Wilcoxson et al. (1975) as shown (eq. 1) and AUDPC values of 0-150, 151-300, 301-500 and > 500 represented high, moderate, low and very low levels of resistance, respectively (Jeger and Viljanen-Rollinson, 2001).

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

Where, y_i = % disease severity on the i^{th} scoring; t_i = number of days from sowing to i^{th} scoring; n = total number of scores. FDS is the highest disease severity and FDS values of ≤ 30 and > 30 represent high and low levels of resistance, respectively. CI is the product of FDS and constants for HRs (I = 0.0, R = 0.1, RMR = 0.2, MR = 0.3, MRMS = 0.5, MS = 0.7, MSS = 0.9, and S = 1.0) (Knott, 2012) and CI values of 0-20, 21-40, 41-60, and > 60 represent high, moderate, low, and very low levels of resistance, respectively. Days to heading (DH) is the difference between date of sowing and date at which 50% of plant heads are fully out of flag leaf sheaths. Plant height (PH) is the average height of five tillers each from a randomly selected plant as measured from soil surface to the top of the spikes excluding awns. Spike length (SL) is the average length of five spikes each from a randomly selected plant measured from the top of the peduncle to the top of the spike excluding awns. Kernels per spike (K S^{-1}) is the average number of kernels from five spikes each from a randomly selected plant. Biomass (BM) is the weight of plants as weighed on a Mettler PC 4400 DeltaRange® digital balance. Grain yield (GY) is the weight of cleaned kernels after threshing using ALMACO® Model LPTD, S/No.T09235, winnowing on an electronic winnower (S/No. R78443) and standardizing moisture content to 12%. 1000-kernel weight (TKW) is the weight of 1000 cleaned kernels counted by an electronic grain counter (CONTADOR®, S/No. 14176107). Test weight (TW) is the weight of kernels in a container of a standard volume and HI is the ratio of GY to BM.

Statistical analyses

The AUDPC was square root transformed to obtain a normal frequency distribution before analyses. Data were subjected to a restricted maximum likelihood (REML) estimation in GenStat version 16 (Patterson and Thompson, 1971) using the linear mixed model (LMM) below, with effect due to replicates, seasons and genotypes being fixed and effect due to blocks being random.

$$y_{ijkl} = \mu + r_i + G_j + S_k + GS_{jk} + \beta_{l(i)} + \varepsilon_{m(ijkl)}$$

where, y_{ijkl} is the response, μ is the overall mean, r_i is the effect due to the i^{th} replicate, G_j is the effect due to the j^{th} genotype, S_k is the effect due to the k^{th} season, GS_{jk} is the effect due to interaction between the j^{th} genotype and the k^{th} season, $\beta_{l(i)}$ is the effect due to the l^{th} block nested within the i^{th} replicate and $\varepsilon_{m(ijkl)}$ is the random error component.

Correlation measured the relationships among AUDPC, CI, FDS, GY, HI, DH, and TKW while the slope of regression indicated the magnitude of the change in GY, TKW, and TW due to change in FDS. Estimates for genetic correlation were determined by coefficient of variation (CV %) and mean. Variance component estimates for genotype (σ_g^2), genotype-by-season interaction (GSI) (σ_{gs}^2), and residual (σ_e^2) were obtained by fitting the LMM using REML in GenStat with effect due to replicates and seasons being fixed and effect due to genotypes and blocks being random. Phenotypic coefficient of variation (PCV) (eq. 2) and genotypic coefficient of variation (GCV) (eq. 3) were computed according to Ogunniyan and Olokayo (2014) as:

$$\text{PCV} = \frac{\sqrt{\sigma_{ph}^2}}{\bar{x}} \times 100\% \quad (2)$$

$$\text{GCV} = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100\% \quad (3)$$

Where, σ_{ph}^2 and σ_g^2 are variance due to phenotype and genotype, respectively, and $\bar{x}$ is the mean. Broad-sense heritability (H^2) (%) was estimated as shown (eq. 4) and H^2 values of > 60%, 30-60%, and 0-30% indicated high, moderate, and low heritability, respectively (Johnson et al., 1955).

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \left(\frac{\sigma_{gs}^2}{s} + \frac{\sigma_e^2}{r}\right)} \quad (4)$$

where, σ_g^2 is variance due to genotype, σ_{gs}^2 is variance due to GSI, s is the number of seasons, σ_e^2 is variance due to error (residual) and r is the number of replications.

Genotypic stability of AUDPC for stem rust and yellow rust was assessed using cultivar superiority (eq. 5) as described by Lin and Binns (1985). In this method, superiority of a genotype's performance was the distance mean square (MS) from the minimum response in each season and was determined as:

$$P_i = \left[n(\bar{X}_{i.} - \bar{M})^2 + \sum_{j=1}^{n}\left(X_{ij} - \bar{X}_{i.} - M_j + \bar{M}\right)^2\right]/(2n) \quad 5$$

Where, P_i is the superiority measure of the i^{th} genotype, *n* is the number of seasons, X_{ij} is performance of the i^{th} genotype in the j^{th} season and M_j is the minimum seasonal response.

Superiority represented MS of the effect due to genotype $[n(\bar{X}_{i.} - \bar{M})^2]$, GSI $\left[\sum_{j=1}^{n}\left(X_{ij} - \bar{X}_{i.} - M_j + \bar{M}\right)^2\right]$ and genotypes' general adaptability (Lin and Binns, 1985; Lin and Binns, 1988). Critical values for significance of P_i and GSI were the product of pooled residual MS from REML analyses and tabulated F-values for corresponding degrees of freedom (df), where df for P_i and GSI were n and n-1, respectively (Lin and Binns, 1988).

Finlay and Wilkinson (1963) regression (FW) of AUDPC for stem rust and yellow rust on seasons revealed the trend of genotypes' stability across seasons which was useful in identifying resistance based on responsiveness to seasonal potential (Walsh and Lynch, 2014). FW assessed variation in performance as a function of season by regressing each genotype's performance on seasonal means in a two-step ordinary least squares (OLS) procedure of computing seasonal indices and estimating intercepts and slopes (Lian and de Los Campos, 2016). We used the FW package in R (R Development Core Team, 2020) by installing function 1. Function 1 enabled us to analyse AUDPC data for stem rust and yellow rust in RCBD model below to generate ANOVA of 4 seasons for stem rust and 3 seasons for yellow rust.

$$y_{ijkl} = \mu + S_i + G_j + \beta_{k(i)} + SG_{ij} + \varepsilon_{m(ijkl)}$$

Where, y_{ijkl} is the response, μ is the overall mean, S_i is the effect due to the i^{th} season, G_j is the effect due to the j^{th} genotype, $\beta_{k(i)}$ is the effect due to the k^{th} block nested within the i^{th} season, SG_{ij} is the effect due to interaction between i^{th} season and the j^{th} genotype and $\varepsilon_{m(ijk)}$ is the random error component.

We used function 2 to calculate components of variation and function 3 to generate interaction plots for all genotypes across seasons and those of selected genotype's performance on estimated seasonal indices. Function 4 performed ANOVA for seasons and ranked means using Tukey's HSD test at 5% level of significance. The joint regression analysis obtained sensitivities (stability). To visualise this, we used function 5 to plot genotypes identified for superior performance in resistance to stem rust and yellow rust and plots of genotype's stability for mean AUDPC.

```
Function 1:  library(devtools)
             install_github("lian0090/FW")
Function 2:  library(lme4)
             install.packages("lme4")
Function 3:  library(HH)
             install.packages("HH")
Function 4:  library("agricolae")
             install.packages("agricolae")
Function 5:  library(ggplot2)
             install.packages("ggplot2")
```

RESULTS

Variance components

Main effects due to genotype and season were significant ($p \leq 0.001$) for all traits except effect due to season on K S^{-1} (S2). Genotype-by-season interaction (GSI) was significant ($p \leq 0.001$) for AUDPC, CI, FDS, GY, TKW, TW and SL. However, GSI was not significant for DH, HI, BM, PH, and K S^{-1}.

Resistance at seedling stage

A majority of genotypes were susceptible to isolates *TTKSK* and *TTKTT* with the former and the latter being

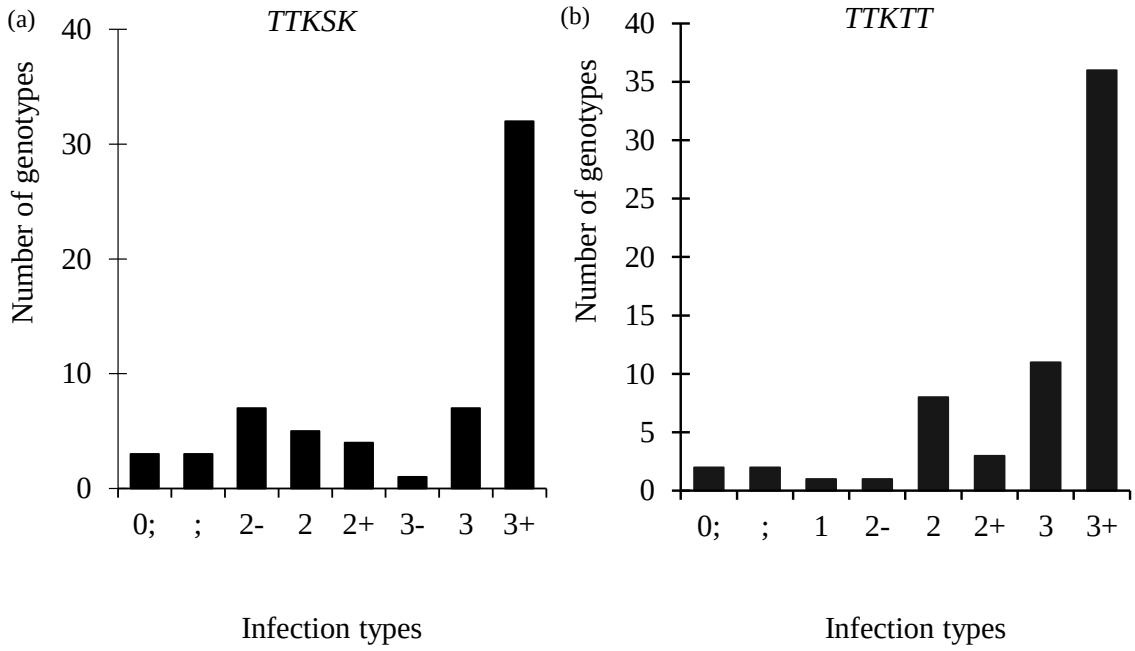


Figure 1. Frequencies of infection types for 61 bread wheat (*Triticum aestivum* L.) genotypes evaluated for seedling resistance to stem rust isolates (a) TTKSK and (b) TTKTT.
Source: Authors

avirulent to seventeen and fourteen genotypes, respectively (Figure 1). Genotypes Lancer, Sunguard, Gauntlet, Scepter, Merlin, Magenta, Spitfire, Coolah, Dart, Janz and Preston exhibited resistance to both isolates (Table 1). However, genotypes Shield, Westonia, Gazelle, Orion, Supreme and Cutlass were resistant to *TTKSK* but susceptible *TTKTT* while genotypes Bolac and Emu Rock were resistant to *TTKTT* but susceptible to *TTKSK*.

Resistance at adult plant stage

For stem rust, AUDPC, CI, and FDS ranged from 13.0-1573.0, 0.1-100.0, and 5.0-100.0 in NJ1, 0.0-1536.0, 0.2-99.1, and 0.0-100.0 in NJ2, 0.0-1776.0, 0.3-80.5, and 1.0-100.0 in NJ3, and 3.0-984.0, 0.2-43.0, and 0.0-80.0 in DZ, respectively (Table 2). For yellow rust, on the other hand, the AUDPC, CI, and FDS values ranged from 0.0-592.0, 0.0-45.5, and 0.0-60.0 in NJ1, 0.0-1029.0, 0.0-76.7, and 0.0-80.0 in NJ2, and 0.0-591.0, 0.1-27.8, and 0.0-50.0 in NJ3, respectively. The trend showed a higher level of stem rust in NJ1 and NJ3 than in NJ2, with the lowest recorded in DZ. However, the level yellow rust in NJ2 was higher than in NJ1 and NJ3. Mean AUDPC was 711.0, 382.0, 421.0 and 401.0, CI was 50.8, 25.9, 16.0 and 14.9, and FDS was 70.0, 41.0, 41.0 and 37.0 for stem rust in NJ1, NJ2, NJ3 and DZ, respectively. On the other hand, mean AUDPC was 95.0, 268.0 and 130.0, CI was 7.2, 18.9 and 5.1 and FDS was 18.0, 29.0 and 17.0 for yellow rust in NJ1, NJ2 and NJ3, respectively. Genotypes Lancer, Sunguard, Gauntlet, Shield and Magenta were identified for low levels of < 300 for AUDPC, ≤ 20 for CI and ≤ 30 for FDS of stem rust in NJ1, NJ2, NJ3 and DZ and yellow rust in NJ1, NJ2 and NJ3 (S3). Resistant genotypes had AUDPC, CI and FDS range of 13.0-194.0, 0.1-7.7 and 5.0-20.0 in NJ1, 0.0-101.0, 0.2-5.5 and 0.0-15.0 in NJ2, 0.0-99.0, 0.9-3.3 and 1.0-10.0 in NJ3, and 3.0-189.0, 0.2-4.4 and 1.0-30.0 in DZ for stem rust, respectively. The range of AUDPC, CI and FDS was 0.0-72.0, 0.2-5.3 and 0.0-10.0 in NJ1, 22.0-241.0, 1.4-18.7 and 5.0-30.0 in NJ2, and 2.0-108.0, 0.1-4.4 and 1.0-10.0 in NJ3 for yellow rust, respectively. In respect to final infection types (FITs), genotypes with a FIT of ≤ MRMS for stem rust were 16 in NJ1, 37 in NJ2, 34 in NJ3 and 23 in DZ with genotypes Lancer, Sunguard, Gauntlet, Magenta, Shield, Merlin, Dart, Spitfire and Beckom displaying a FIT of ≤ MRMS across seasons (S4). Conversely, genotypes with a FIT of ≤ MRMS for yellow rust were 42 genotypes in NJ1, 22 in NJ2 and 47 in NJ3 with genotypes Lancer, Suntop, LRPB Flanker and Gazelle displaying a FIT of ≤ MR across seasons (S5). Genotypes Sunguard, Gauntlet, Shield and Magenta had a FIT of ≤ MRMS for yellow rust.

Yield performance

Mean GY, TKW, TW, HI and BM was higher in NJ1 and NJ3 than in NJ2 while stem rust was higher in NJ1 and

Table 1. Infection types of 61 bread wheat genotypes evaluated for seedling resistance against stem rust isolates TTKSK and TTKTT at KALRO, Njoro.

Genotype	*TTKSK*		*TTKTT*		Genotype	*TTKSK*		*TTKTT*	
	Set 1	Set 2	Set 1	Set 2		Set 1	Set 2	Set 1	Set 2
Cacuke	3+	3	3+	3+	Gauntlet	1	1	1+	2-
Kenya Robin	2	2+	3+	3+	Gazelle	2-	2-	3+	3+
Coolah	2-	;	2-	0;	Sunmax	3+	3+	3+	3+
Chara	3+	3+	3+	3+	Janz	2-	2-	2	2
LRPB Flanker	2+	3	3	3	Kiora	3	NG	3	3
LRPB Reliant	3-	3+	3	3	Lancer	0;	0;	0;	0;
Ninja	3+	3+	3+	3+	Livingston	3+	3+	3	3+
Tenfour	3+	3+	NG	3+	Mace	3	3	3	3+
Tungsten	3+	3	3+	3+	Magenta	2-	2-	2-	2
Axe	3+	2	2	3+	Merlin	2+	2	2	2
B53	3-	NG	3+	3	Mitch	3+	3+	3+	3+
Beckom	3+	3+	3+	3+	Orion	2-	2-	3	2+
Bremer	3+	2-	3+	2+	Gladius	3+	3+	3+	3+
Buchanan	3+	3+	3+	3	Preston	2+	2	NG	2+
Calingiri	3+	3	3+	3+	Scepter	2-	NG	2	2-
Cobalt	3+	3+	3+	3+	Scout	3+	3+	3+	3+
Cobra	3+	3+	3+	3+	Shield	1	0;	3	2+
Condo	3+	3+	3+	3+	Spitfire	2	2	2	2
Corack	3	3	3	3	Steel	3+	3+	3+	3+
Correll	3	3	3	3+	Sunguard	0;	NG	0;	0;
Cosmick	3+	3+	3+	3+	Bolac	3-	2	2-	2-
Cutlass	2	2-	3+	3	Suntop	3+	3+	3+	3+
Dart	2	2+	2-	2	Supreme	2-	2-	3	3
Derrimut	3+	3+	3+	3+	Trojan	3	3+	3+	3+
EGA Bounty	3+	3+	3+	3+	Viking	3+	3+	3	3+
EGA Gregory	3	3	3	3	Wallup	NG	3+	3+	3
Baxter	3+	1	3+	3+	Westonia	2-	2-	3-	2
Emu Rock	3+	2+	2	2	Wyalkatchem	NG	NG	3+	3+
Espada	3	2-	3+	3+	Yitpi	NG	NG	1	1
Estoc	3	3+	3+	3+	Zen	NG	3+	3+	3+
Forrest	3+	3+	3+	3+					

NG: Did not germinate.
Source: Authors

NJ3 than NJ2 and yellow rust was higher in NJ2 than in NJ1 and NJ3 (Table 2 and S6). Resistant genotypes Magenta with 4.9, 1.8 and 5.9 t ha^{-1}, Lancer with 3.9, 2.4 and 4.9 t ha^{-1}, Sunguard with 3.6, 1.6 and 4.7 t ha^{-1}, Gauntlet with 2.8, 1.3 and 4.2 t ha^{-1} and Shield with 3.1, 1.1 and 3.5 t ha^{-1} significantly yielded higher than the best control Kenya Robin which yielded 1.3, 0.6 and 0.7 t ha^{-1} in NJ1, NJ2 and NJ3, respectively (S3). Mean TKW and TW was 20.8, 13.7 and 19.8 g, and 64.4, 56.5 and 57.8 kg hL^{-1} in NJ1, NJ2 and NJ3, respectively (Table 2). However, DH, PH, SL and K S^{-1} were not affected by seasons. Among resistant genotypes, GY, HI, TW and TKW ranged from 2.8-4.9 t ha^{-1}, 0.2-0.7, 71.2-77.4 kg hL^{-1} and 23.2-31.2 g in NJ1, 1.1-2.4 t ha^{-1}, 0.15-0.20, 56.4-76.1 kg hL^{-1} and 15.2-24.1 g in NJ2, and 3.5-5.9 t ha^{-1}, 0.23-0.45, 66.2-81.0 kg hL^{-1} and 26.2-33.3 g in NJ3, respectively (S3). GY, HI, TW and TKW values for NJ1 exceeded those of NJ2 by 121, 33, 14 and 52% with NJ3 values exceeding NJ2 values by 132, 29, 2 and 45%, respectively. Stem rust and yellow rust caused a reduction in the quantity and quality of kernels. For instance, resistant genotype Lancer had GY of 3.9, 2.4 and 4.9 t ha^{-1}, HI of 0.74, 0.20 and 0.41, TW of 77.4, 70.9 and 80.2 kg hL^{-1} and TKW of 25.4, 20.8 and 26.4 g in NJ1, NJ2 and NJ3, respectively. However, the susceptible control Kenya Robin recorded GY of 1.3, 0.6 and 0.7 t ha^{-1}, HI of 0.10, 0.10 and 0.03, TW of 56.2, 45.2 and 50.9 kg hL^{-1} and TKW of 20.1, 10.9 and 13.6 g in

Table 2. Range and mean values of disease and yield performance of 61 bread wheat genotypes evaluated for resistance to stem rust, yellow rust and yield performance over three seasons at KALRO, Njoro and resistance to stem rust over one season at DZARC, Debre Zeit..

	Area under disease progress curve									
	Stem rust		Yellow rust		Grain yield (t ha^{-1})		Days to heading		Plant height (cm)	
Season	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
NJ1	13-1573	711	0-592	95	0.14-4.93	2.01	50-83	69	62.6-95.6	76.2
NJ2	0-1536	382	0-1029	268	0.30-2.44	0.91	54-84	73	50.1-91.1	73.2
NJ3	0-1776	421	0-591	130	0.26-5.94	2.11	57-101	77	50.0-99.0	77.2
DZ	3-984	401	-	-	-	-	-	-	-	-

	Coefficient of infection									
	Stem rust		Yellow rust		Harvest index		1000-kernel weight (g)		Spike length (cm)	
Season	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
NJ1	0.1-100.0	50.8	0.0-45.5	7.2	0.01-0.74	0.16	10.7-32.9	20.8	7.0-12.3	9.3
NJ2	0.2-99.1	25.9	0.0-76.7	18.9	0.04-0.28	0.12	6.6-24.1	13.7	6.7-11.2	8.9
NJ3	0.3-80.5	16.0	0.1-27.8	5.1	0.01-0.48	0.17	9.2-34.3	19.8	6.3-10.8	8.9
DZ	0.2-43.0	14.9	-	-	-	-	-	-	-	-

	Final disease severity									
	Stem rust		Yellow rust		Kernels per spike		Test weight (kg hL^{-1})		Biomass (t ha^{-1})	
Season	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
NJ1	5-100	70	0-60	18	24-53	38	40.7-77.4	64.4	6.4-31.2	14.6
NJ2	0-100	41	0-80	29	22-53	38	37.6-76.1	56.5	2.8-14.1	7.8
NJ3	1-100	41	0-50	17	22-51	38	28.6-81.0	57.8	7.0-25.5	14.1
DZ	0-80	37	-	-	-	-	-	-	-	-

NJ1: 2019 off-season at Njoro, NJ2: 2019 main-season at Njoro, NJ3: 2020 off-season at Njoro, DZ: Debre Zeit, –: missing data.
Source: Authors

NJ1, NJ2 and NJ3, respectively.

Correlation and regression analyses

AUDPC, CI and FDS of stem rust and yellow rust were positively correlated (Table 3). However, they were negatively correlated with GY, DH and TKW. Correlation between AUDPC and GY, DH and TKW was -0.5637***, -0.1562 and -0.4418*** for stem rust and -0.1227***, -0.3400*** and -0.0010*** for yellow rust, respectively. GY was positively correlated with TKW (0.9107***) but was negatively correlated with HI (-0.0626***) and DH (-0.2308***). Regression revealed a decrease in GY with an increase in FDS for stem rust and yellow rust (Figure 2).

Heritability and stability

Variance due to genotype exceeded variance due to GSI for all traits except HI while variance due to genotype exceeded variance due to error for all traits but HI, BM and K S^{-1} (Table 4). Lowest and highest estimates of broad-sense heritability (H^2) were recorded for HI at 2.2% and AUDPC for stem rust at 70.2%. Other traits with high H^2 were CI (60%) and FDS (68.1%) for stem rust, GY (61.5%), TKW (67.5%) and TW (62.2%). High (> 50%) PCV and GCV was recorded for disease traits, GY and HI. Based on AUDPC, genotypes varied in reaction to stem rust and yellow rust with seasons. Generally, however, resistance to stem rust was higher during NJ2 and DZ than NJ1 and NJ3 (Figure 3a) while resistance to yellow rust was higher during NJ1 and NJ3 than NJ2 (Figure 3b). Genotypes with superior resistance and stable performance across seasons were Sunguard and Lancer for stem rust and Sunmax, Steel and Gladius for yellow rust (S7). However, genotype Gauntlet, for stem rust, and genotypes Lancer and Magenta, for yellow rust, displayed superior resistance with unstable performance across seasons. Resistance to stem rust of genotypes Lancer and Sunguard was similar across seasons; however, Sunguard marginally outperformed Lancer during the poorer season with Gauntlet being the worst in the poor season and superior in the best season (Figure 4a). On the other hand, resistance to yellow rust of

Table 3. Correlation coefficients of selected traits for 61 bread wheat genotypes evaluated for resistance to stem rust, yellow rust and yield performance over three seasons at KALRO, Njoro.

	AUDPCsr	CIsr	FDSsr	AUDPCyr	CIyr	FDSyr	GY	HI	DH	TKW
AUDPCsr	-									
CIsr	0.9847***	-								
FDSsr	0.5422***	0.53687***	-							
AUDPCyr	-0.0678***	-0.1009***	0.0836***	-						
CIyr	-0.0255**	-0.0568*	0.0989**	0.9816***	-					
FDSyr	0.0595**	0.0775**	0.0526**	0.6228***	0.6176***	-				
GY	-0.5637***	-0.5962***	-0.4718***	-0.1227***	-0.1720***	-0.1869***	-			
HI	0.4356	0.4120***	0.1604***	0.1291**	0.1456***	0.0760***	-0.0626***	-		
DH	-0.1562***	-0.0829***	-0.0429***	-0.3400***	-0.3012***	-0.1045***	-0.2308***	-0.1988***	-	
TKW	-0.4418***	-0.4712***	-0.3401***	-0.0010***	-0.0487***	-0.1069***	0.9107***	0.0482***	-0.4369***	-

$AUDPC_{Sr}$: Area under Disease Progress Curve for Stem Rust, CI_{Sr}: Coefficient of Infection for Stem Rust, FDS_{Sr}: Final Disease Severity for Stem Rust, $AUDPC_{Yr}$: Area under Disease Progress Curve for Yellow Rust, CI_{Yr}: Coefficient of Infection for Yellow Rust, FDS_{Yr}: Final Disease Severity for Yellow Rust, GY: Grain Yield, HI: Harvest Index, DH: Days to Heading, TKW: 1000-Kernel Weight. *, ** and *** = significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.
Source: Authors

Table 4. Estimates of variation and heritability of selected traits for 61 bread wheat genotypes evaluated for resistance to stem rust, yellow rust and yield performance over three seasons.

	σ^2_{ph}	σ^2_g	σ^2_{gs}	σ^2_e	H^2 (%)	PCV (%)	GCV (%)	SE
$AUDPC_{Sr}$	181065.0	127098.0	13197.0	40770.0	70.2	87.9	73.7	18.7
CI_{Sr}	771.9	460.8	110.2	200.9	60.0	90.2	70.0	1.3
FDS_{Sr}	773.7	526.9	71.0	175.8	68.1	67.8	56.0	1.3
$AUDPC_{Yr}$	33095.0	18473.0	7111.0	7511.0	55.8	90.9	79.0	8.2
CI_{Yr}	170.3	84.6	49.0	36.7	50.0	93.2	65.7	0.6
FDS_{Yr}	230.1	137.4	39.7	53.1	59.7	96.0	74.2	0.7
DH	103.2	65.3	0.2	37.7	63.3	13.9	11.1	0.4
PH (cm)	89.6	44.8	2.8	42.0	50.0	12.5	8.9	0.4
SL (cm)	1.3	0.9	0.1	0.3	69.2	12.8	10.0	0.0
BM (t ha^{-1})	28.9	7.8	1.9	19.2	27.0	44.1	22.9	0.3
GY (t ha^{-1})	1.3	0.8	0.2	0.3	61.5	67.9	53.2	0.1
HI	0.3	0.0	0.1	0.2	2.2	65.1	52.5	0.1
K S^{-1}	79.8	35.1	7.9	36.8	44.0	23.5	15.6	0.3
TKW (g)	34.5	23.3	4.7	6.5	67.5	32.5	26.7	0.3
TW (kg hL^{-1})	139.4	86.7	14.9	37.8	62.2	19.8	15.6	0.5

σ^2_{ph}: Phenotypic Variance, σ^2_g: Genotypic Variance, σ^2_{gs}: Variance due to Genotype-by-Season Interaction, σ^2_e: Variance due to Error, H^2: Heritability in Broad-sense, PCV: Phenotypic Coefficient of Variation, GCV: Genotypic Coefficient of Variation, SE: Standard Error, $AUDPC_{Sr}$: Area Under Disease Progress Curve for Stem Rust, CI_{Sr}: Coefficient of Infection for Stem Rust, FDS_{Sr}: Final Disease Severity for Stem Rust, $AUDPC_{Yr}$: Area Under Disease Progress Curve for Yellow Rust, CI_{Yr}: Coefficient of Infection for Yellow Rust, FDS_{Yr}: Final Disease Severity for Yellow Rust, DH: Days to Heading, PH: Plant Height, SL: Spike Length, BM: Biomass, GY: Grain Yield, HI: Harvest Index, K S^{-1}: Kernels per Spike, TKW: 1000-Kernel Weight, TW: Test Weight.
Source: Authors

genotypes Sunmax and Gladius was constant across seasons with the former outperforming the latter (Figure 4b). Moreover, resistance of genotype Steel was better in the best season than in the poor season. Detailed results on sensitivity to seasons for stem rust and yellow rust are shown in Figure 5.

DISCUSSION

Emergence of new races limits the deployment of resistant genotypes (Olivera et al., 2019). Nevertheless, APR genes provide broad-spectrum and durable resistance (Moore et al., 2015). AUDPC, CI and FDS are

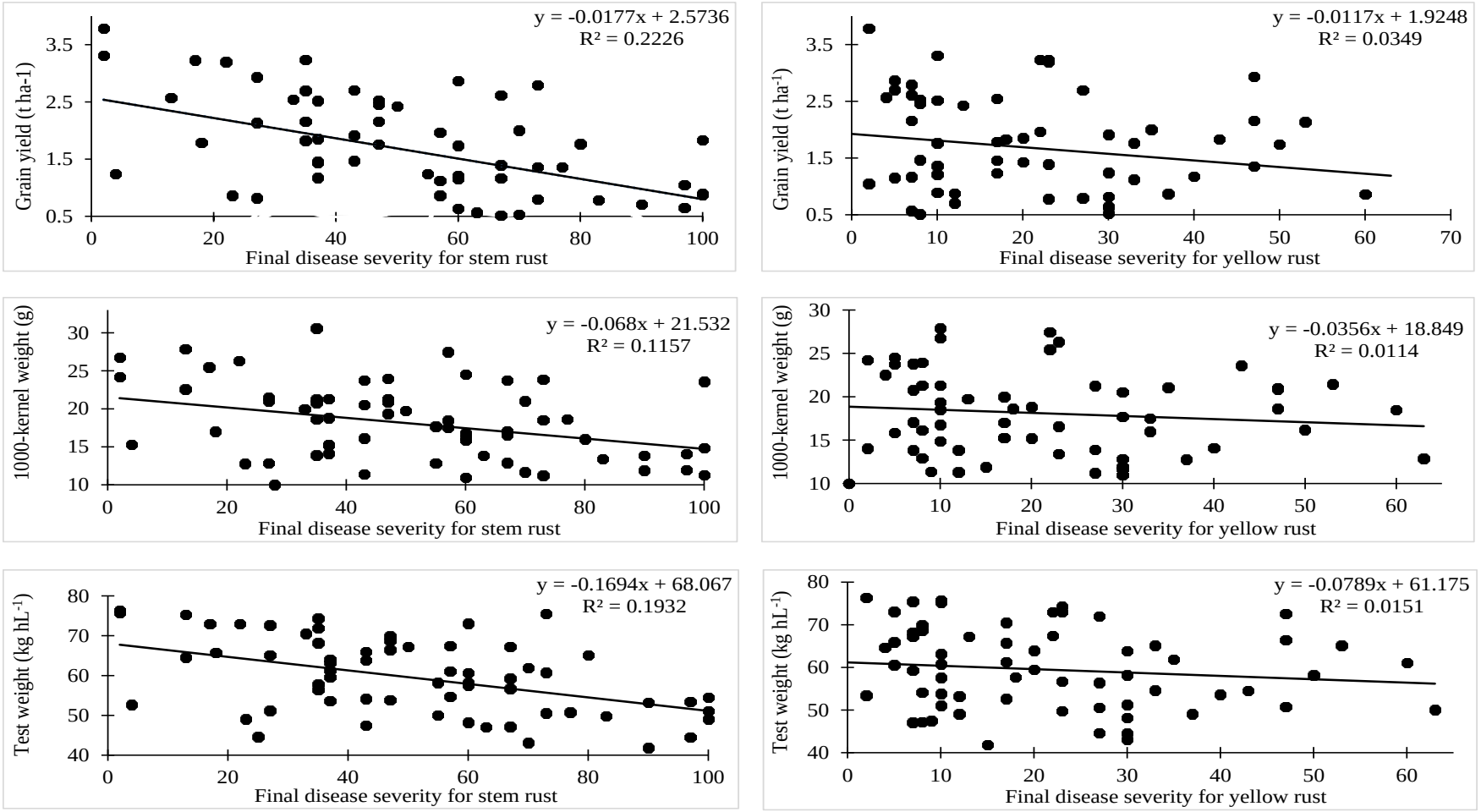


Figure 2. Reaction of wheat genotypes against stem rust and yellow rust for grain yield, 1000-kernel weight and test weight in three seasons at KALRO, Njoro**.**
Source: Authors

reliable measures of APR (Figueroa et al., 2020). In this study, stem rust was higher in NJ1 and NJ3 than in NJ2 and DZ while yellow rust was higher in NJ2 than in NJ1 and NJ3 (Table 1). Genotypes Lancer, Sunguard, Gauntlet, Shield and Magenta were identified for APR due to low levels of AUDPC, CI and FDS (Table 2). These genotypes also displayed low FITs ranging from R to MRMS (S3 and S4). Therefore, APR reduced the rate of infection and development of disease. Differences in reaction to disease seemed to depend on variation in seasons. NJ1 and NJ3 received less and poorly distributed rainfall with higher temperatures while NJ2 received more and well distributed rainfall and lower temperatures (S8).

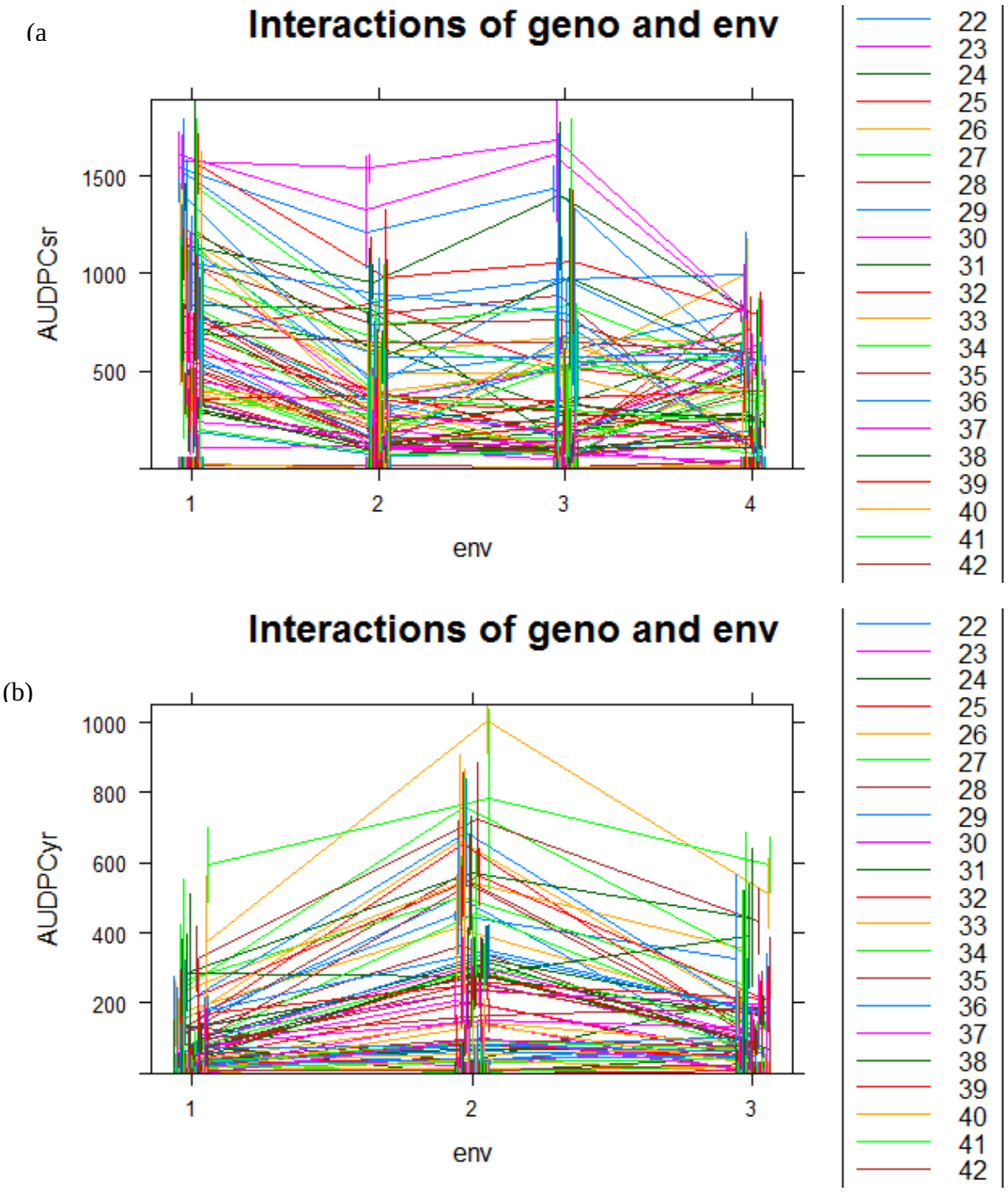


Figure 3. Interaction plots of all genotypes for AUDPC of (a) stem rust and (b) yellow rust in different seasons. $AUDPC_{sr}$: Area Under Disease Progress Curve for Stem Rust, $AUDPC_{yr}$: Area under Disease Progress Curve for Yellow Rust. Each coloured line represents a genotype; 1: 2019 off-season in Njoro, 2: 2019 main-season in Njoro, 3: 2020 off-season in Njoro, 4: Debre Zeit.
Source: Authors

Therefore, NJ1 and NJ3 favored infection and development of stem rust whereas NJ2 favored infection and development of yellow rust. A number of genotypes identified for APR were susceptible at seedling stage and *vice versa*. Seedling susceptibility of APR genotypes indicates resistance conferred by minor genes

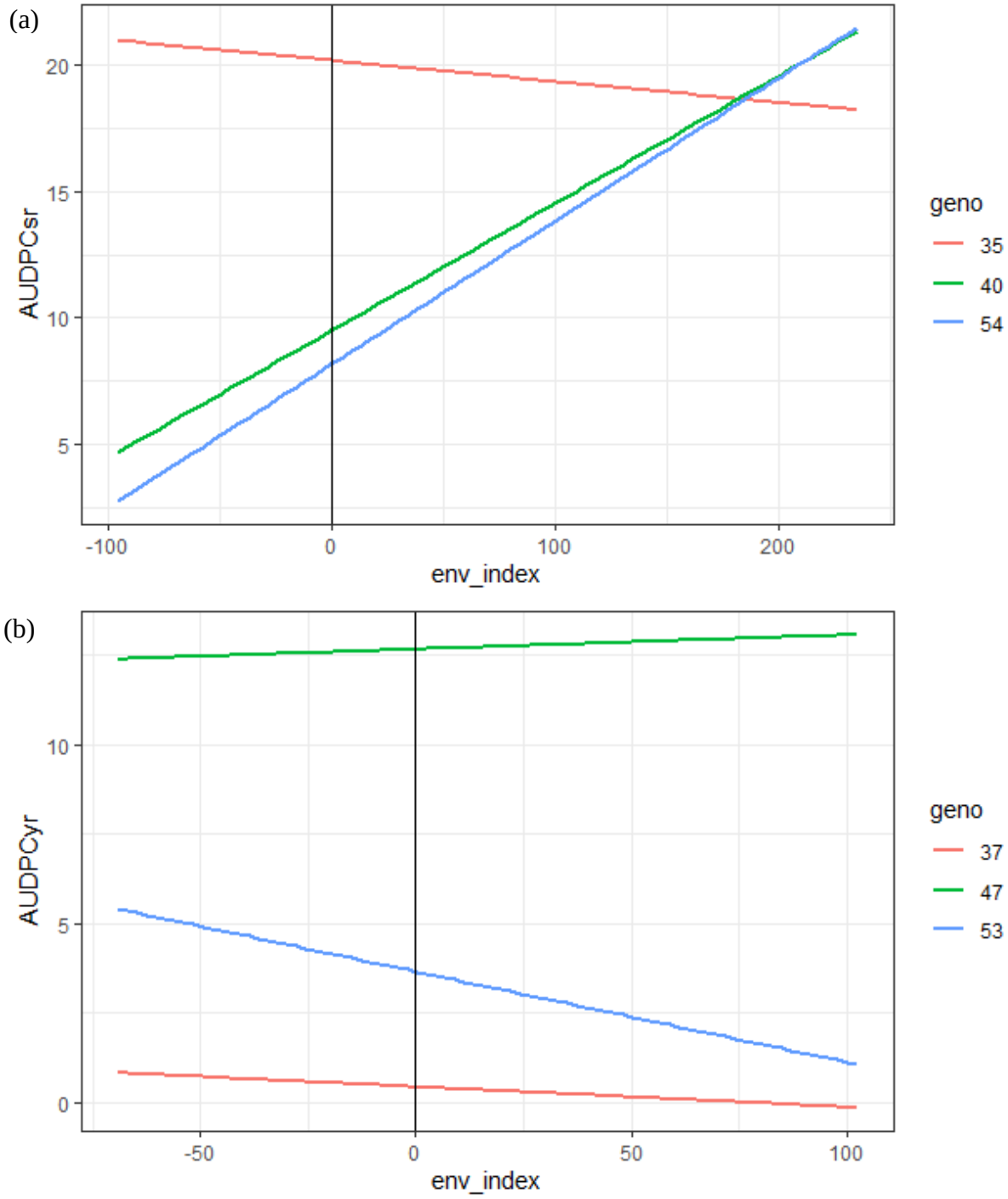
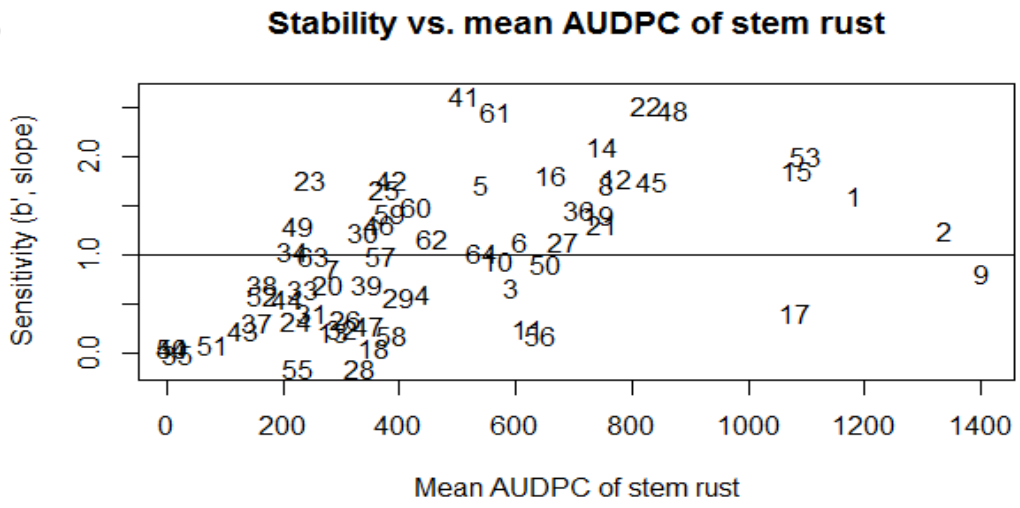


Figure 4. Performance of selected genotypes on estimated seasonal indices. $AUDPC_{sr}$: Area under disease progress curve of stem rust, $AUDPC_{yr}$: Area under disease progress curve of yellow rust. Each coloured line represents fitted values for means of genotype by season interaction: 35 = Gauntlet, 37 = Sunmax, 40 = Lancer, 47 = Gladius, 53 = Steel and 54 = Sunguard.
Source: Authors

(Rahmatov et al., 2019). They provide field resistance (slow rusting) resulting from diverse gene combinations. (Bhavani et al., 2019; Randhawa et al., 2018; van der Plank, 2012). This resistance prolongs the latent period and reduces the duration of sporulation, number and size of uredinia to lower the severity of infection (Figueroa et

(a)



(b)

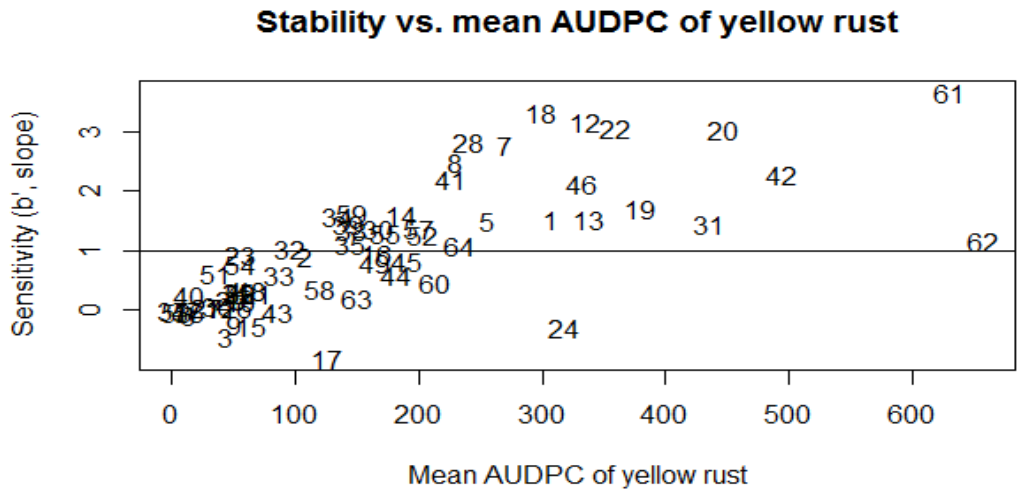


Figure 5. A plot of stability vs mean AUDPC of (a) stem rust and (b) yellow rust AUDPC, area under disease progress curve.
Source: Authors

al., 2020). APR genes are known to confer partial resistance to different races where each gene contributes small to intermediate effects to the phenotype (Huerta-Espino et al., 2020). On the other hand, seedling resistance among genotypes lacking APR such as Scepter, Spitfire, Merlin, Coolah, Janz, Dart and Preston indicate resistance conferred by major genes. It occurs when a pathogenic attack signals defense mechanisms resulting in cell death to restrict the spread of infection and is associated with hypersensitive responses (Singh et al., 2014). Yield performance was not only related to stem rust and yellow rust but also on seasonal variation. The trend showed a reduction in yield with a reduction in stem rust and an increase in yellow rust (Table 1). A similar trend was observed for HI, BM, PH and SL. The reduction in yield with an increase in rust highlights the impact of the two diseases on photosynthesis and mobilization of water and essential nutrients. The high yield among early maturing genotypes is attributed to disease escape. In addition, the high yield among shorter plants which headed early compared to tall plants which headed late is attributed to more tillers and spikelets and a reduction in losses to lodging (Berry and Spink, 2012; Singh et al., 2015). Brinton and Uauy (2019) and Leonardo et al. (2017) found that variation in seasons significantly affect yield performance because yield is a quantitative trait under a polygenic system.

Phenotypic variance was largely attributed to variance due to genotype. Since phenotypic variance is due to variance in genotype, season and GSI, however, seasonal variation causes positive or negative variation in genotypic performance (Falconer and Mackay, 1996;

Acquaah, 2012). H^2 values indicated moderate to high heritability for a majority of variables. Therefore, it was worthwhile to rely on their phenotypic performance to identify resistance and yield performance. PCV and GCV values for AUDPC, CI, FDS, GY and HI were > 50 % thus indicating high variability for these traits. Reaction to stem rust and yellow rust varied across seasons with a higher number of genotypes exhibiting resistance to stem rust during NJ2 and DZ compared to NJ1 and NJ3. However, for yellow rust, a higher number of genotypes were resistant during NJ1 and NJ3 than in NJ2. Therefore, an increase in stem rust caused a decrease in yellow rust and *vice versa*. Genotypes Lancer and Sunguard were superior and stable in resistance to stem rust across three seasons in Njoro and one season in Debre Zeit. Conversely, genotypes Sunmax, Steel and Gladius were superior and stable in resistance to yellow rust across three seasons in Njoro. This is because they were consistently well ranked across seasons (Tables 2 and 4). Genotypes Sunguard and Lancer emerged as the most resistant to stem rust in the poor season with the performance of the former being marginally better than that of the latter. Therefore, both genotypes could be used in breeding for resistance to stem rust during this season. However, during the best season, genotype Gauntlet outperformed genotypes Sunguard and Lancer, and could be used during this season. On the other hand, genotype Sunmax could be utilised in breeding for resistance to yellow rust across seasons since it was the best performing and stable across seasons.

Conclusion

Genetic variation existed for resistance to stem rust and yellow rust and yield performance. However, reaction to disease and yield performance was significantly affected by season and GSI. Genotypes Lancer, Sunguard, Gauntlet, Shield and Magenta were identified for APR to stem rust and yellow rust and were also among the best performing for yield performance. In addition, the study established the existence of seedling resistance with genotypes Lancer, Sunguard, Gauntlet, Scepter, Merlin, Magenta, Spitfire, Coolah, Dart, Janz and Preston exhibiting resistance to isolates *TTKSK* and *TTKTT*. These genotypes present a good source of resistance to stem rust and yellow rust that could be exploited in breeding for resistance. Genotypes Lancer, Sunguard and Gauntlet were well ranked and superior in terms of resistance to stem rust with genotypes Lancer and Sunguard showing stability of performance across seasons. Genotypes Sunguard and Gauntlet emerged as the most resistant to stem rust during poor and best seasons, respectively. On the other hand, genotypes Sunmax, Gladius and Steel were well ranked and superior in terms of resistance to yellow rust with Sunmax being the most resistant and stable across all seasons.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Supplementary (S) Table 1. Genotypes.

Genotype number	Genotype	Pedigree
1	Cacuke	Canadian/Cunningham/Kennedy
2	Kenya Robin	Babax/*Lr42*//Babax*2/3/Tukuru
3	Coolah	EGA Gregory/VQ2791//EGA Gregory
4	Chara	BD225/CD87
5	LRPB Flanker	EGA Gregory//EGA Gregory/Lang
6	LRPB Reliant	LRPB Crusader/EGA Gregory
7	Ninja	Calingiri/Wyalkatchem derivative
8	Sunmax	CRW142.16/2*SunzellA
9	Tenfour	N/A
10	Tungsten	Axe with a European winter wheat background
11	Axe	-0AUS/DT29361//RAC820/Excalibur/3/-0AUS/DT29361//RAC820/Excalibur
12	B53	N/A
13	Beckom	N/A
14	Bremer	DM02-25-SB02-167/Correll// Mace
15	Buchanan	Frederick/Sprague
16	Calingiri	Chino/Kulin//Reeves
17	Cobalt	N/A
18	Cobra	Westonia/W29
19	Condo	WW-80/2*WW-15
20	Corack	Wyalkatchem/Silverstar A// Wyalkatchem
21	Correll	CHA/Mengavi8156//CNO67/GLL//Bezostaya2/4/N10/BVR14//5*Burt/3/3*Raven/5/Sr2 1/4*Lance//4*Bayonet/6/C 8 MM/C 8 HMM/4/M-8-DAG-3-B19-H9- /Dagger/3/Sabre/MEC 3//Insignia
22	Cosmick	N/A
23	Cutlass	RAC1316/2*Fang
24	Dart	Sunbrook/Janz//Kukri
25	Derrimut	N/A
26	EGA Bounty	Batavia/2*Leichhardt
27	EGA Gregory	Pelsart/2*Batavia DH
28	Baxter	QT2327/Cook//QT2804
29	Emu Rock	96W657-37/Kukri
30	Espada	CO5583*B117/NH5441*F03//RAC875-2/-0AUS/3/- 0AUS/DT29361//RAC820/EXCALIBUR
31	Estoc	Trident/Molineux/4/VPM 1/5*COOK//3*Spear/3/Sabre/MEC 3//Insignia/5/VM931/RAC935
32	Forrest	96 WFHB 5568/2*Kohika
33	Gauntlet	Kukri/Sunvale
34	Gazelle	24K1056/VPM/3*Vasco
35	Janz	3-AG-3/4*Condor//Cook
36	Kiora	N/A
37	Lancer	VII84/Chara//Chara/3/Lang
38	Livingston	SUN129A/Sunvale
39	Mace	Wyalkatchem/Stylet//Wyalkatchem
40	Magenta	Carnamah/Tammin-18
41	Merlin	Calidad//Yecora F 70/Ciano F 67/3/76ECN44/4/Hartog*3/Quarrion
42	Mitch	QT10422/GILES

(S) Table 1. Cont'd

43	Orion	TATIARA/QAL2000
44	Gladius	CO5583*B117/NH5441*F03//RAC875-2/-0AUS/3/-0AUS/DT29361//RAC820/Excalibur
45	Preston	N/A
46	Scepter	RAC1480/2*Mace
47	Scout	Sunstate/QH71-6//Yitpi
48	Shield	AGT-Scythe/CO-7138(CO-7412)//(CO-7413)RAC-1105/CO-7165
49	Spitfire	Drysdale/Kukri
50	Steel	Composite cross of unknown germplasm
51	Sunguard	SUN289E/*Sr2*Janz
52	Bolac	Nesser/2*VI252
53	Suntop	Sunco/2*Pastor//SUN436E
54	Supreme	LoPh-Nyabing.3*Calingiri/4*VPM Arrino
55	Trojan	LPB 00LR000041/Sentinel3R
56	Viking	(S) Early-Baart[113];
57	Wallup	Chara/Wyalkatchem
58	Westonia	Spica/Timgalen//Tosca/3/Cranbrook//Bob-White*2/Jacup
59	Wyalkatchem	Machete/W84-129*504
60	Yitpi	C8MMC8HMM/Frame
61	Zen	Calingiri/Wyalkatchem

N/A: Not available.
Source: Authors

Supplementary (S) table 2. Combined REML variance component analyses for selected traits of 61 bread wheat genotypes evaluated for resistance to stem rust, yellow rust and yield performance over three seasons at KALRO, Njoro and resistance to stem rust over one season at DZARC, Debre Zeit.

(i) Response variate: Area under disease progress curve for stem rust

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components				
Random term	**component**		**s.e.**	
Replicate. Block	-0.71		789	
Residual variance model				
Term	**Model (order)**	**Parameter**	**Estimate**	**s.e**
Residual	Identity	Sigma2	40837	3040

Deviance: -2*Log-Likelihood
Deviance = 4657.58, d.f.= 380

Tests for fixed effects					
Fixed term	**Wald statistic**	**n.d.f.**	**F statistic**	**Wald/d.d.f.**	**F pr**
replicate	17.09	2	0.73	8.55	<0.001
genotype	1902.29	63	22.81	30.20	<0.001
season	305.71	3	295.16	152.86	<0.001
genotype.season	247.95	360	1.77	1.97	<0.001
Dropping individual terms from full fixed model					
Fixed term	**Wald statistic**	**n.d.f.**	**F statistic**	**d.d.f.**	**F pr**
replicate	247.95	2	0.73	8.55	<0.001
genotype.season	17.09	360	1.77	1.97	<0.001

	Replicate	**Season**	**Genotype**	**Genotype.Season**
Average	20.19	0.4393	164.9	3.504
Maximum			164.9	3.514
Minimum			164.8	3.503

Standard errors of differences

(ii) Response variate: Coefficient of infection for stem rust

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components

Random term	component	s.e.
replicate.block	6.3	5.9

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	201.8	15.0

Deviance: -2*Log-Likelihood

Deviance = 2638.24, d.f. = 380

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	7.3	2	2.1	3.65	0.026
genotype	1367.08	60	15.71	21.7	<0.001
season	616.17	3	249.33	308.08	<0.001
genotype.season	331.81	360	2.57	2.63	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	7.3	2	2.1	3.65	0.026
genotype.season	331.81	360	2.57	2.63	<0.001

	Replicate	Season	Genotype	Genotype.Season
Average	1.351	0.9436	5.425	7.61
Maximum			5.435	7.618
Minimum			5.403	7.549
Average variance of differences			29.43	

Standard errors of differences

(iii) Response variate: Final disease severity for stem rust

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components

Random term	component	s.e.
replicate.block	2.50	4.30

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	176.3	13.1

Deviance: -2*Log-Likelihood

Deviance = 2582.41, d.f. = 380

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	8.14	2	0.87	4.07	0.017
genotype	1761.49	60	19.78	27.96	<0.001
season	510.7	3	403.94	455.35	<0.001
genotype.season	277.84	360	2.5	2.21	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	8.14	2	0.87	4.07	0.017
genotype.season	277.84	360	2.5	2.21	<0.001

Cont'd

	Replicate	Season	Genotype	Genotype.Season
Average	2.007	1.298	7.513	10.5
Maximum			7.534	10.52
Minimum			7.47	10.38
Average variance of differences			56.44	110.3

Standard errors of differences

(iv) Response variate: Area under disease progress curve for yellow rust

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components

Random term	component	s.e.
replicate.block	-0.71	789

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	40837	3040

Deviance: -2*Log-Likelihood

Deviance = 4657.80, d.f. = 380

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	Wald/d.d.f.	F pr
replicate	17.09	2	0.73	8.55	<0.001
genotype	1902.29	120	22.81	30.2	<0.001
season	305.71	2	295.16	1562.86	<0.001
genotype.season	247.95	120	1.77	1.97	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	247.95	2	0.73	1.97	0.51
genotype.season	17.09	120	1.77	8.55	0.001

	Replicate	Season	Genotype	Genotype.Season
Average	0.5109	0.4393	2.47	3.504
Maximum			2.474	3.514
Minimum			2.469	3.503

Standard errors of differences

(v) Response variate: Coefficient of infection for yellow rust

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components

Random term	component	s.e.
replicate.block	6.3	5.9

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	201.8	15.0

Deviance: -2*Log-Likelihood

Deviance = 2638.24, d.f. = 380

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	7.3	2	2.1	3.65	0.026
genotype	1367.08	60	15.71	21.7	<0.001
season	616.17	2	249.33	308.08	<0.001
genotype.season	331.81	120	2.57	2.63	<0.001

Cont'd.

Dropping individual terms from full fixed model					
Fixed term	**Wald statistic**	**n.d.f.**	**F statistic**	**d.d.f.**	**F pr**
replicate	7.3	2	2.1	3.65	0.026
genotype.season	331.81	120	2.57	2.63	<0.001

	Replicate	**Season**	**Genotype**	**Genotype.Season**
Average	1.351	0.9436	5.425	7.61
Maximum			5.435	7.618
Minimum			5.403	7.549
Average variance of differences			29.43	

Standard errors of differences

(vi) Response variate: Final disease severity for yellow rust

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components

Random term	**component**	**s.e.**
replicate.block	2.50	4.30

Residual variance model

Term	**Model (order)**	**Parameter**	**Estimate**	**s.e**
Residual	Identity	Sigma2	176.3	13.1

Deviance: -2*Log-Likelihood
Deviance = 2582.41, d.f. = 380

Tests for fixed effects					
Fixed term	**Wald statistic**	**n.d.f.**	**F statistic**	**d.d.f.**	**F pr**
replicate	8.14	2	0.87	4.07	0.017
genotype	1761.49	60	19.78	27.96	<0.001
season	510.7	2	403.94	455.35	<0.001
genotype.season	277.84	120	2.5	2.21	<0.001

Dropping individual terms from full fixed model					
Fixed term	**Wald statistic**	**n.d.f.**	**F statistic**	**d.d.f.**	**F pr**
replicate	8.14	2	0.87	4.07	0.017
genotype.season	277.84	120	2.5	2.21	<0.001

	Replicate	**Season**	**Genotype**	**Genotype.Season**
Average	2.007	1.298	7.513	10.5
Maximum			7.534	10.52
Minimum			7.47	10.38
Average variance of differences			56.44	110.3

Standard errors of differences

(vii) Response variate: Grain yield (t ha^{-1})

Fixed model: Constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 383 (1 unit excluded due to missing value)

Estimated variance components

Random term	**component**	**s.e.**
replicate.block	0.0102	0.0110

Residual variance model

Term	**Model (order)**	**Parameter**	**Estimate**	**s.e**
Residual	Identity	Sigma2	0.262	0.0243

Deviance: -2*Log-Likelihood
Deviance = 69.85,d.f. = 251

(vii) Cont'd

Tests for fixed effects					
Fixed term	**Wald statistic**	**n.d.f.**	**F statistic**	**d.d.f.**	**F pr**
replicate	1.26	2	0.63	12.2	0.55
genotype	842.61	60	13.36	227.8	<0.001
season	446.03	1	446.03	232.1	<0.001
genotype.season	266.95	60	4.24	232.1	<0.001
Dropping individual terms from full fixed model					
Fixed term	**Wald statistic**	**n.d.f.**	**F statistic**	**d.d.f.**	**F pr**
replicate	1.26	2	0.63	12.2	0.548
genotype.season	266.95	60	4.24	232.1	<0.001

	Replicate	**Season**	**Genotype**	**Genotype.Season**
Average	0.08157	0.05231	0.303	0.4235
Maximum	0.08162		0.3214	0.4736
Minimum	0.08147		0.3007	0.4176
Average variance of differences			0.09183	0.1794

Standard errors of differences

(viii) Response variate: Biomass (t ha^{-1})

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components

Random term	component	s.e.
replicate.block	0.02	0.55

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	17.96	1.66

Deviance: -2*Log-Likelihood

Deviance = 1137.07, d.f. = 252.

Tests for fixed effects					
Fixed term	**Wald statistic**	**n.d.f.**	**F statistic**	**d.d.f.**	**F pr**
replicate	21.61	2	10.8	9.4	0.004
genotype	182.26	60	2.88	199.8	<0.001
season	242.92	1	242.92	233	<0.001
genotype.season	75.73	60	1.2	233	0.166
Dropping individual terms from full fixed model					
Fixed term	**Wald statistic**	**n.d.f.**	**F statistic**	**d.d.f.**	**F pr**
replicate	21.61	2	10.8	9.4	0.004
genotype.season	75.73	60	1.2	233	0.166

	Replicate	**Season**	**Genotype**	**Genotype.Season**
Average	0.5354	0.4325	2.449	3.462
Maximum			2.45	3.462
Minimum			2.449	3.46

Standard errors of differences

(ix) Response variate: Harvest index

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 383 (1 unit excluded due to missing value)

Estimated variance components

Random term	component	s.e.
replicate.block	0.00013	0.00043

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	0.0129	0.00120

Deviance: -2*Log-Likelihood

Deviance = -696.41, d.f. = 251

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	4.53	2	2.26	10.2	0.154
genotype	164.31	60	2.6	209.7	<0.001
season	12.72	1	12.72	232.1	<0.001
genotype.season	70.57	60	1.12	232.1	0.272

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	4.6	2	2.3	10.2	0.15
genotype.season	70.57	60	1.12	232.1	0.272

	Replicate	Season	Genotype	Genotype.Season
Average	0.01535	0.01162	0.06627	0.09334
Maximum	0.01536		0.07022	0.1043
Minimum	0.01532		0.06601	0.09279
Average variance of differences				0.008714

Standard errors of differences

(x) Response variate: Days to heading

Fixed model: Constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components

Random term	component	s.e.
replicate.block	3.02	1.54

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	20.60	1.91

Deviance: -2*Log-Likelihood

Deviance = 1191.36, d.f. = 252

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	5.31	2	2.65	15.9	0.101
genotype	1053.4	60	16.72	241.5	<0.001
season	46.44	1	46.44	233	<0.001
genotype.season	11.57	60	0.18	233	1

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	5.31	2	2.65	15.9	0.101
genotype.season	11.57	60	0.18	233	1

	Replicate	Season	Genotype	Genotype.Season
Average	1.038	0.4632	2.75	3.798
Maximum			2.766	3.81
Minimum			2.718	3.705
Average variance of differences			7.563	14.42

Standard errors of differences

(xi) Response variate: Plant height (cm)

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components

Random term	component	s.e.
replicate.block	0.25	1.57

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	49.62	4.60

Deviance: -2*Log-Likelihood

Deviance = 1396.06, d.f. = 252

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	3.75	2	1.87	9.7	0.205
genotype	325.99	60	5.16	204.8	<0.001
season	17.7	1	17.7	233	<0.001
genotype.season	66.29	60	1.05	233	0.385

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	3.75	2	1.87	9.7	0.205
genotype.season	66.29	60	1.05	233	0.385

	Replicate	Season	Genotype	Genotype.Season
Average	0.9152	0.719	4.084	5.764
Maximum			4.086	5.765
Minimum			4.08	5.752
Average variance of differences				

Standard errors of differences

(xii) Response variate: Spike length (cm)

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components

Random term	component	s.e.
replicate.block	0.0149	0.0131

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	0.285	0.0264

Deviance: -2*Log-Likelihood

Deviance = 93.92,d.f. = 252

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	12.48	2	6.24	12.9	0.013
genotype	1353.72	60	21.47	232.9	<0.001
season	44.48	1	44.48	233	<0.001
genotype.season	133.65	60	2.12	233	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	12.48	2	6.24	12.9	0.013
genotype.season	133.65	60	2.12	233	<0.001

	Replicate	Season	Genotype	Genotype.Season
Average	0.09049	0.05451	0.3174	0.4425
Maximum			0.3185	0.4433
Minimum			0.3152	0.4361
Average variance of differences				

Standard errors of differences

(xii) Response variate: Kernels spike^{-1}

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 576

Estimated variance components

Random term	component	s.e.
replicate.block	-1.23	0.77

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	37.30	3.46

Deviance: -2*Log-Likelihood

Deviance = 1313.39, d.f. = 252

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	1.26	2	0.63	4.9	0.57
genotype	414.35	60	6.45	88.4	<0.001
season	0.65	1	0.65	233	0.42
genotype.season	6.22	60	0.1	233	1

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	1.26	2	0.63	4.9	0.57
genotype.season	6.22	60	0.1	233	1

	Replicate	Season	Genotype	Genotype.Season
Average	0.5253	0.6234	3.363	4.874
Maximum			3.405	4.987
Minimum			3.343	4.859
Average variance of differences			11.31	23.76

Standard errors of differences

(xiii) Response variate: Test weight (kg hL^{-1})

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 350 (34 units excluded due to missing values)

Estimated variance components

Random term	component	s.e.
replicate.block	1.09	1.05

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	20.47	2.05

Deviance: -2*Log-Likelihood

Deviance = 1024.78, d.f. = 218

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	4.25	2	2.13	12.4	0.161
genotype	1057.53	60	16.77	197.7	<0.001
season	332.94	1	332.94	200.6	<0.001
genotype.season	125.46	60	1.99	201.2	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	3.15	2	1.57	12.4	0.246
genotype.season	125.46	60	1.99	201.2	<0.001

	Replicate	Season	Genotype	Genotype.Season
Average	0.8018	0.5107	2.938	4.09
Maximum	0.8063		4.337	6.539
Minimum	0.7931		2.671	3.694
Average variance of differences	0.643		8.746	17.07

Standard errors of differences

(xiv) Response variate: 1000-kernel weight (g)

Fixed model: constant + replicate + genotype + season + genotype.season
Random model: replicate.block
Number of units: 382 (2 units excluded due to missing values)

Estimated variance components

Random term	component	s.e.
replicate.block	0.114	0.169

Residual variance model

Term	Model (order)	Parameter	Estimate	s.e
Residual	Identity	Sigma2	4.446	0.414

Deviance: -2*Log-Likelihood

Deviance = 781.44, d.f. = 250

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	8.32	2	4.16	11.4	0.044
genotype	1643.71	60	26.04	220.8	<0.001
season	1103.8	1	1103.08	231.3	<0.001
genotype.season	311.15	60	4.94	231.3	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
replicate	9.04	2	4.52	11.4	0.036
genotype.season	311.15	60	4.94	231.3	<0.001

	Replicate	Season	Genotype	Genotype.Season
Average	0.314	0.2161	1.244	1.743
Maximum	0.3144		1.387	2.133
Minimum	0.3138		1.234	1.722
Average variance of differences			1.547	3.041

Standard errors of differences

Supplementary (S) table 3. Means of selected traits for resistant genotypes (AUDPC ≤ 300, CI ≤ 20 and FDS ≤ 30) and controls evaluated for resistance to stem rust, yellow rust and yield performance over three seasons at KALRO, Njoro and resistance to stem rust over one season at DZARC, Debre Zeit.

Genotypes	Stem rust												Harvest index		
	AUDPC				CI				FDS						
	NJ1	NJ2	NJ3	DZ	NJ1	NJ2	NJ3	DZ	NJ1	NJ2	NJ3	DZ	NJ1	NJ2	NJ3
Lancer	13	0	0	8	1.1	1.1	3.1	0.4	5	0	1	1	0.70	0.20	0.41
Sunguard	16	0	4	3	2.7	0.2	0.9	0.2	5	0	1	0	0.20	0.15	0.33
Gauntlet	13	10	5	31	0.1	0.4	1.0	0.8	5	5	1	10	0.20	0.16	0.33
Shield	94	101	85	23	4.7	5.5	2.0	0.6	20	15	5	1	0.20	0.15	0.23
Magenta	194	59	99	189	7.7	3.4	3.3	4.4	20	10	10	30	0.30	0.20	0.45
Controls															
Cacuke[a]	1496	1201	1519	545	97.0	95.3	68.0	17.6	100	100	100	60	0.22	0.13	0.22
Kenya Robin[b]	1573	1329	1684	790	97.8	96.8	75.5	34.6	100	100	100	70	0.10	0.10	0.03
Mean[c]	711	382	421	401	50.8	25.9	16.0	14.9	70	41	41	37	0.16	0.12	0.17
$LSD_{0.05}$	5.4	5.8	7.1	4.5	26.6	20.1	18.1	7.7	19.1	17.6	22.8	11.4	0.46	0.04	0.13
CV (%)	8.0	8.2	5.2	2.2	17.4	13.9	15.7	8.9	12.8	15.1	13.0	7.1	4.10	0.20	1.40

	Yellow rust									Grain yield (t ha^{-1})			Test weight (kg hL^{-1})			1000-kernel weight (g)		
	AUDPC			CI			FDS											
	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3
Lancer	**0**	**22**	**2**	**0.2**	**1.4**	**0.1**	**0**	**5**	**1**	**3.9**	**2.4**	**4.9**	**77.4**	**70.9**	**80.2**	**25.4**	**20.8**	**26.4**
Sunguard	5	123	12	1.1	6.7	0.4	5	20	5	3.6	1.6	4.7	73.1	76.1	78.8	23.3	24.1	33.1
Gauntlet	64	241	108	5.3	18.7	4.4	10	30	10	2.8	1.3	4.2	74.5	68.7	81.0	23.2	17.3	30.6
Shield	0	102	3	0.3	4.9	0.1	1	10	1	3.1	1.1	3.5	71.2	56.4	66.2	26.3	15.2	26.2
Magenta	72	65	101	4.8	3.2	3.4	10	10	10	4.9	1.8	5.9	76.4	69.6	80.0	31.2	19.2	33.3
Controls																		
Cacuke[a]	132	470	304	12.0	43.4	14.5	30	50	50	2.4	0.7	2.5	64.6	55.4	43.9	32.5	14.7	23.6
Kenya Robin[b]	53	212	70	1.0	8.8	1.0	5	20	5	1.3	0.6	0.7	56.2	45.2	50.9	20.1	10.9	13.6
Mean[c]	95	268	131	7.2	18.9	5.1	18	29	17	2.0	0.9	2.1	64.4	56.5	57.8	20.8	13.7	19.8
$LSD_{0.05}$	5.4	5.1	5.8	10.3	11.7	7.5	12.3	11.8	11.9	1.1	0.5	1.1	5.7	8.3	14.0	3.4	3.4	5.5
CV (%)	6.1	0.7	2.6	17.2	6.5	10.0	15.7	5.8	6.7	4.5	4.7	13.0	2.9	1.7	1.4	3.2	2.4	1.8

AUDPC: area under disease progress curve, CI: coefficient of infection, FDS: final disease severity, NJ1: 2019 off-season in Njoro, NJ2: 2019 main-season in Njoro, NJ3: 2020 off-season in Njoro, DZ: Debre Zeit.

[a]Control for stem rust and yellow rust

[b]Control for yield performance

[c]Means stated are for all the 61 genotypes evaluated

Supplementary (S) table 4. Disease means and final infection types of 61 bread wheat genotypes evaluated for resistance to stem rust over three seasons at KALRO, Njoro and one season at DZARC, Debre Zeit

	Area under disease progress curve				Coefficient of infection				Final disease severity				Final infection type			
Genotype	NJ1	NJ2	NJ3	DZ	NJ1	NJ2	NJ3	DZ	NJ1	NJ2	NJ3	DZ	NJ1	NJ2	NJ3	DZ
Cacuke	1496	1201	1519	545	97.0	95.3	68.0	17.6	100	100	100	60	S	S	S	S
Kenya Robin	1573	1329	1684	790	97.8	96.8	75.5	34.6	100	100	100	70	S	S	S	S
Coolah	750	620	336	619	55.5	47.9	9.2	28.2	80	60	40	70	S	S	MRMS	S
Chara	907	472	493	235	75.3	39.7	9.2	4.9	100	60	60	30	S	S	MRMS	MRMS
LRPB Flanker	861	341	499	682	59.4	16.1	17.6	28.8	90	40	50	50	S	MRMS	MSS	S
LRPB Reliant	497	148	102	371	46.3	10.5	3.0	14.2	60	30	15	40	MSS	MRMS	MS	S
Ninja	1174	417	494	823	87.2	22.9	18.3	34.3	90	50	70	80	S	MSS	S	S
Tenfour	1537	1536	1776	790	97.6	99.1	80.5	33.0	100	100	100	60	S	S	S	S
Tungsten	808	800	152	484	64.9	60.3	5.5	16.2	90	90	20	40	S	S	MRMS	MRMS
Axe	702	826	553	392	47.6	53.4	13.8	11.7	70	70	50	30	S	S	MRMS	S
B53	1196	579	666	620	84.1	51.2	24.4	28.5	90	60	60	60	S	S	MRMS	S
Beckom	343	237	103	395	19.8	13.2	3.4	17.0	50	50	10	60	MRMS	MRMS	MRMS	MRMS
Bremer	1240	792	867	76	84.3	57.7	35.2	1.3	90	60	90	15	S	MSS	S	S
Buchanan	1492	839	844	972	95.9	62.6	41.9	42.8	100	70	100	80	S	S	S	S
Calingiri	1104	343	476	674	86.0	28.5	13.7	29.4	90	50	60	70	S	MSS	MS	S
Cobalt	1089	944	1415	818	85.5	75.8	64.9	34.2	100	90	100	60	S	S	S	S
Cobra	374	81	89	856	25.4	6.5	2.5	35.9	50	20	10	70	MRMS	MRMS	MRMS	S
Condo	1058	380	499	984	72.0	16.8	11.7	43.0	100	40	40	60	S	MRMS	MRMS	S
Corack	440	135	159	347	19.7	5.5	2.8	8.9	50	15	15	30	MRMS	MRMS	MRMS	S
Correll	1036	745	722	426	81.7	60.5	27.0	12.9	100	60	60	40	S	S	MSS	MRMS
Cosmick	1403	385	941	535	95.1	31.6	34.9	12.3	100	60	70	40	S	S	S	I
Cutlass	632	187	96	3	46.6	14.9	1.5	0.3	80	40	10	0	S	MRMS	MS	MRMS
Dart	286	104	142	283	11.0	3.0	1.3	5.9	40	10	20	20	RMR	MRMS	MRMS	MRMS
Derrimut	760	355	190	158	53.5	26.8	6.4	3.4	60	60	20	10	S	S	MRMS	MS
EGA Bounty	297	155	135	711	14.6	10.0	2.9	30.3	40	20	15	60	MRMS	MRMS	MRMS	S
EGA Gregory	498	316	160	522	36.8	14.7	2.7	21.3	80	40	20	50	S	MRMS	MRMS	S
Baxter	580	106	43	546	40.1	7.1	1.8	24.0	90	15	5	50	S	MRMS	MRMS	MRMS
Emu Rock	345	113	257	255	16.6	5.1	6.0	5.5	40	10	30	30	MRMS	MRMS	MRMS	MS
Espada	360	302	301	116	27.9	23.2	9.0	3.0	50	50	40	20	MSS	MS	MS	MS
Estoc	393	303	86	119	36.1	15.4	3.5	4.0	50	50	10	20	MSS	MRMS	MS	MRMS
Forrest	450	94	183	79	31.6	6.8	9.2	1.3	50	15	40	10	MRMS	MRMS	MSS	MRMS

Table S4. Cont'd

Gauntlet	13	10	5	31	0.1	0.4	1.0	0.8	5	5	1	10	RMR	RMR	R	MR
Gazelle	1028	877	744	96	86.6	63.0	29.4	2.1	100	60	60	15	S	S	S	MSS
Sunmax	228	150	37	166	18.7	13.4	0.3	6.8	40	40	5	20	MSS	MRMS	MRMS	S
Janz	311	109	87	114	17.2	7.6	2.8	2.5	50	20	30	20	MRMS	MRMS	MS	MRMS
Kiora	485	109	179	506	29.5	8.1	3.8	22.2	60	30	20	50	MSS	MRMS	MS	S
Lancer	13	0	0	8	1.1	1.1	3.1	0.4	5	0	1	1	R	I	R	TR
Livingston	1128	341	309	239	87.9	15.9	10.5	5.1	90	40	40	20	S	MRMS	MRMS	MRMS
Mace	806	258	248	254	71.0	13.6	8.4	7.7	80	30	30	30	S	MRMS	MRMS	MS
Magenta	194	59	99	189	7.7	3.4	3.3	4.4	20	10	10	30	MR	MRMS	MRMS	MRMS
Merlin	325	101	162	252	9.6	4.6	4.7	5.2	40	10	15	20	MR	MRMS	MRMS	MRMS
Mitch	1176	573	988	556	84.6	29.4	39.3	20.3	100	50	100	50	S	MSS	S	S
Orion	643	319	302	164	57.1	23.4	10.7	4.8	90	50	30	15	S	MSS	MS	MSS
Gladius	385	172	693	129	31.4	10.9	23.1	3.0	40	40	50	20	MSS	MRMS	MS	MS
Preston	1438	706	672	401	94.7	38.1	32.1	12.0	100	70	100	60	S	S	S	S
Scepter	479	133	95	160	39.3	4.9	5.1	3.7	80	15	10	20	S	MRMS	MRMS	MRMS
Scout	831	557	587	601	74.2	34.3	34.6	25.5	90	60	50	50	S	S	MRMS	S
Shield	94	101	85	23	4.7	5.5	2.0	0.6	20	15	5	5	MRMS	MRMS	MRMS	MR
Spitfire	312	69	114	180	12.5	3.9	3.3	3.8	30	10	10	20	MR	MRMS	MRMS	MRMS
Steel	1560	969	1026	783	100.0	71.4	47.4	32.8	100	100	90	60	S	S	S	S
Sunguard	16	0	4	3	2.7	0.2	0.9	0.2	5	0	1	1	R	I	R	TR
Bolac	175	76	69	557	5.6	4.6	1.4	17.3	30	15	10	50	MR	MRMS	MRMS	S
Suntop	684	592	601	605	41.6	46.0	21.6	25.1	70	60	50	50	S	S	MSS	S
Supreme	553	121	607	76	44.6	5.4	39.4	1.0	60	15	90	10	MSS	MRMS	S	RMR
Trojan	447	293	168	636	40.2	17.2	4.9	28.3	50	30	30	60	MSS	MRMS	MRMS	S
Viking	722	252	308	228	64.9	14.6	8.2	5.8	80	30	40	30	S	MRMS	MRMS	MRMS
Wallup	764	191	352	393	48.3	8.9	8.3	10.9	80	20	30	30	S	MRMS	MRMS	S
Westonia	1082	184	603	316	69.4	6.8	13.5	6.7	100	20	50	20	S	MRMS	MRMS	MRMS
Wyalkatchem	680	199	495	366	52.5	8.4	11.6	8.8	90	15	60	30	S	MRMS	MRMS	MS
Yitpi	496	164	35	259	55.0	12.2	0.6	12.4	70	20	15	30	S	MRMS	MRMS	S
Zen	766	295	468	565	59.4	18.4	16.8	22.6	90	30	60	40	S	MSS	S	S

I: immune, TR: traces, R: resistant, RMR: resistant to moderately resistant, MR: moderately resistant, TRMS: traces to moderately susceptible, MRMS: moderately resistant to moderately susceptible, MS: moderately susceptible, MSS: moderately susceptible to susceptible, S: susceptible, NJ1: 2019 off-season in Njoro, NJ2: 2019 main-season in Njoro, NJ3: 2020 off-season in Njoro, DZ: Debre Zeit.

Supplementary (S) table 5. Disease means and final infection types of 61 bread wheat genotypes evaluated for resistance to yellow rust over three seasons at KALRO, Njoro.

	Area under disease progress curve			Coefficient of infection			Final disease severity			Final infection type		
Genotype	**NJ1**	**NJ2**	**NJ3**	**NJ1**	**NJ2**	**NJ3**	**NJ1**	**NJ2**	**NJ3**	**NJ1**	**NJ2**	**NJ3**
Cacuke	132	470	304	12.0	43.4	14.5	30	50	50	MS	MSS	MS
Kenya Robin	53	212	70	1.0	8.8	1.0	5	20	5	MRMS	MRMS	MRMS
Coolah	77	0	40	5.2	0.0	1.5	20	1	10	MRMS	R	MS
Chara	163	413	183	7.8	28.3	7.4	20	40	20	MS	MSS	MS
LRPB Flanker	10	1	15	0.7	0.7	0.1	5	5	5	MR	MR	R
LRPB Reliant	96	556	146	5.1	34.0	5.0	15	50	15	MRMS	MSS	MRMS
Ninja	102	473	42	10.7	36.8	1.8	30	50	10	MSS	MSS	MRMS
Tenfour	47	21	77	2.3	4.4	1.7	10	10	15	RMR	MS	MR
Tungsten	33	73	64	1.6	2.9	0.5	5	10	10	MRMS	MR	MR
Axe	25	42	44	2.4	1.6	1.3	5	5	10	MS	S	MS
B53	135	675	198	11.0	49.4	9.7	15	70	20	MRMS	MSS	MRMS
Beckom	249	505	216	16.2	30.9	9.2	40	40	40	MSS	MSS	MS
Bremer	82	340	82	13.8	23.4	3.0	40	50	10	MRMS	MR	MRMS
Buchanan	40	20	71	2.5	0.9	2.8	15	5	15	MS	MSS	MRMS
Calingiri	115	252	102	11.9	16.2	4.1	30	30	10	MS	MR	MRMS
Cobalt	67	15	148	10.4	1.3	8.1	40	10	40	MS	MSS	MS
Cobra	121	647	123	10.3	45.5	5.4	20	60	10	MRMS	S	MRMS
Condo	222	516	342	13.0	40.2	12.7	40	70	40	MSS	MSS	MSS
Corack	200	759	280	19.8	49.3	17.9	50	60	50	R	MR	MRMS
Correll	33	92	76	0.4	4.7	1.5	5	15	10	MSS	S	MRMS
Cosmick	195	650	168	18.7	55.3	7.2	40	70	30	MRMS	MRMS	MRMS
Cutlass	3	139	1	0.0	7.1	0.2	5	20	1	MS	MSS	MS
Dart	280	242	385	17.9	19.4	14.7	40	30	40	MRMS	MRMS	MRMS
Derrimut	22	86	44	1.0	3.9	0.8	5	15	5	MR	MRMS	MR
EGA Bounty	68	563	106	4.7	36.7	3.5	15	50	15	R	MRMS	MRMS
EGA Gregory	33	45	40	0.4	3.3	0.3	5	15	5	MRMS	MS	MRMS
Baxter	80	309	104	4.5	18.7	3.6	15	30	15	MS	MSS	MRMS
Emu Rock	261	555	431	19.0	46.4	17.4	40	60	40	MRMS	MRMS	MRMS
Espada	23	199	32	1.5	8.0	1.0	5	20	5	RMR	MS	MR
Estoc	33	134	81	1.5	8.5	2.6	5	20	5	MS	MSS	MS
Forrest	33	272	70	1.7	20.9	2.3	5	40	10	MS	MS	MS
Gauntlet	64	241	108	5.3	18.7	4.4	10	30	10	RMR	MRMS	MRMS
Gazelle	19	28	47	0.3	1.2	0.7	5	5	10	R	I	I
Sunmax	0	0	0	0.3	0.0	0.3	1	0	0	MR	MS	MRMS
Janz	63	266	86	3.9	17.4	3.2	10	30	10	MS	MS	MS
Kiora	47	266	87	2.0	17.4	1.7	10	30	10	MS	MS	MRMS
Lancer	0	22	2	0.2	1.4	0.1	0	5	1	I	MR	R
Livingston	86	452	133	5.4	27.6	4.6	10	40	15	MRMS	MSS	MRMS

Table S5. Cont'd

Mace	323	723	428	26.8	49.3	20.5	40	60	40	MS	MSS	MS
Magenta	72	65	101	4.8	3.2	3.4	10	10	10	MRMS	MRMS	MRMS
Merlin	104	249	192	8.8	18.6	8.5	20	30	20	MS	MS	MS
Mitch	129	285	162	9.4	16.0	6.4	30	20	20	MRMS	MS	MS
Orion	215	553	214	14.1	40.7	6.7	30	50	20	MRMS	MSS	MRMS
Gladius	3	6	6	0.7	1.6	0.3	5	5	5	R	MRMS	R
Preston	17	71	44	2.3	5.6	2.1	10	15	20	MRMS	MRMS	MRMS
Scepter	72	244	155	6.3	15.7	6.3	20	30	20	MS	MSS	MS
Scout	25	82	58	0.5	4.2	0.8	5	10	5	RMR	MRMS	MRMS
Shield	0	102	3	0.3	4.9	0.1	1	10	1	R	MRMS	R
Spitfire	97	334	178	6.7	24.0	7.2	15	30	20	MRMS	MS	MRMS
Steel	0	0	6	0.0	0.0	0.3	1	0	5	R	I	MRMS
Sunguard	5	123	12	1.1	6.7	0.4	5	20	5	R	MRMS	R
Bolac	82	304	136	4.8	18.6	4.9	10	30	10	MRMS	MS	MRMS
Suntop	4	2	22	0.4	0.7	0.1	5	5	5	R	MR	MR
Supreme	58	340	124	5.6	27.4	7.0	20	40	30	MRMS	MSS	MRMS
Trojan	63	152	112	5.7	12.0	3.8	20	20	20	MRMS	MS	MRMS
Viking	48	304	69	2.6	17.7	1.4	5	30	5	MR	MSS	MR
Wallup	166	273	204	10.7	14.6	7.6	30	30	30	MRMS	MS	MRMS
Westonia	362	1029	510	26.9	76.7	20.3	50	80	50	MSS	S	MS
Wyalkatchem	592	756	591	45.5	67.9	27.8	60	80	50	MSS	S	MSS
Yitpi	86	158	160	7.2	8.9	6.8	30	20	30	MRMS	MS	MRMS
Zen	180	342	169	17.5	30.3	6.7	30	40	20	MSS	MSS	MRMS

I: immune, R: resistant, RMR: resistant to moderately resistant, MR: moderately resistant, MRMS: moderately resistant to moderately susceptible, MS: moderately susceptible, MSS: moderately susceptible to susceptible, S: susceptible, NJ1: 2019 off-season in Njoro, NJ2: 2019 main-season in Njoro, NJ3: 2020 off-season in Njoro.

Supplementary (S) table 6. Means of yield performance for 61 bread wheat genotypes evaluated for resistance to stem rust and yellow rust over three seasons at KALRO, Njoro.

	Grain yield			Biomass			Harvest index			Kernels spike^{-1}		
	t ha^{-1}											
Genotype	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3
Cacuke	2.40	0.67	2.48	11.1	6.9	9.9	0.22	0.13	0.22	41	40	41
Kenya Robin	1.28	0.57	0.72	14.2	5.9	14.1	0.10	0.10	0.03	48	47	51
Coolah	1.57	0.78	1.35	16.4	8.5	16.2	0.10	0.09	0.08	37	36	36
Chara	0.79	0.57	1.05	13.0	7.1	13.1	0.06	0.08	0.08	36	40	36
LRPB Flanker	1.94	0.81	0.83	16.9	11.3	17.4	0.16	0.08	0.04	42	40	42
LRPB Reliant	3.34	1.10	3.68	21.4	6.6	19.2	0.16	0.16	0.19	49	43	49
Ninja	0.32	0.45	0.75	10.1	6.8	10.1	0.03	0.07	0.06	31	33	33
Tenfour	0.60	0.59	0.39	11.8	5.1	13.2	0.10	0.13	0.07	42	39	38
Tungsten	0.71	0.41	0.64	12.3	7.5	12.3	0.06	0.06	0.06	39	39	38
Axe	2.83	0.94	2.40	11.0	6.8	10.5	0.26	0.14	0.25	39	38	38
B53	1.64	0.46	1.64	13.9	4.7	13.8	0.15	0.10	0.14	41	41	39
Beckom	2.38	0.96	1.94	11.7	5.3	12.1	0.20	0.19	0.17	44	41	41
Bremer	0.72	0.40	1.14	18.2	6.8	18.2	0.05	0.06	0.08	32	33	34
Buchanan	1.76	0.93	1.54	11.0	9.0	10.9	0.18	0.11	0.14	36	35	37
Calingiri	0.61	0.38	1.04	11.2	6.1	11.1	0.06	0.06	0.12	34	33	33
Cobalt	0.69	0.50	1.20	10.0	6.1	10.0	0.07	0.09	0.13	31	36	29
Cobra	2.13	0.78	2.21	20.5	6.2	19.1	0.10	0.12	0.12	32	34	33
Condo	3.01	1.14	2.37	11.4	5.9	11.1	0.27	0.20	0.23	50	48	48
Corack	1.73	1.02	1.45	13.9	4.6	13.9	0.14	0.28	0.15	36	34	36
Correll	1.95	0.40	1.67	15.7	9.1	16.8	0.13	0.04	0.10	29	29	30
Cosmick	0.23	0.34	0.58	9.4	4.3	9.7	0.02	0.08	0.11	32	32	33
Cutlass	0.91	0.45	1.19	16.5	9.3	17.0	0.07	0.04	0.07	28	28	30
Dart	3.32	1.00	3.05	8.5	6.3	9.0	0.48	0.14	0.38	43	42	44
Derrimut	2.51	1.18	2.74	14.7	10.6	14.5	0.18	0.12	0.21	44	44	41
EGA Bounty	3.07	1.46	3.19	12.9	9.1	11.6	0.27	0.17	0.32	38	38	39
EGA Gregory	2.39	0.87	2.22	15.5	10.8	16.3	0.15	0.08	0.13	35	34	35
Baxter	3.45	1.73	3.63	17.9	8.8	18.2	0.20	0.21	0.20	46	45	36
Emu Rock	2.28	0.95	1.96	13.5	5.7	15.1	0.18	0.17	0.14	30	30	28
Espada	3.16	1.24	3.11	17.2	10.5	17.6	0.18	0.11	0.19	44	43	42
Estoc	2.26	0.83	2.29	20.5	9.0	19.2	0.12	0.09	0.14	38	38	41
Forrest	1.41	0.50	1.53	16.0	10.2	16.0	0.09	0.05	0.08	32	33	32
Gauntlet	2.79	1.33	4.21	16.0	8.9	14.8	0.20	0.16	0.33	43	43	50

Table S6. Cont'd

Gazelle	0.41	0.30	0.64	13.2	6.6	13.6	0.06	0.04	0.06	36	36	36
Sunmax	0.58	1.10	0.85	31.2	11.7	25.5	0.02	0.09	0.03	51	51	49
Janz	3.19	1.15	3.23	17.2	8.2	16.7	0.19	0.14	0.20	37	36	36
Kiora	1.79	0.76	1.75	21.3	9.8	21.4	0.08	0.08	0.08	43	43	43
Lancer	3.86	2.44	4.86	16.0	12.6	13.9	0.74	0.20	0.41	38	38	47
Livingston	2.65	1.69	1.57	13.0	7.3	12.9	0.22	0.23	0.13	44	44	44
Mace	2.79	0.68	2.90	13.6	5.2	13.4	0.22	0.14	0.21	37	37	38
Magenta	4.93	1.81	5.94	16.8	9.1	14.7	0.31	0.20	0.45	42	41	50
Merlin	4.02	1.23	4.38	14.9	7.6	13.3	0.39	0.17	0.48	38	37	45
Mitch	0.68	0.66	0.95	12.4	7.4	11.8	0.05	0.09	0.07	46	42	47
Orion	1.34	0.56	1.55	11.7	7.0	9.2	0.15	0.09	0.18	29	28	35
Gladius	3.28	1.44	3.40	19.7	10.6	19.2	0.18	0.13	0.19	40	39	40
Preston	0.14	0.37	0.78	7.0	3.9	7.5	0.01	0.09	0.07	35	34	31
Scepter	3.60	1.81	4.25	15.5	10.4	14.6	0.23	0.18	0.29	38	37	43
Scout	1.26	0.61	1.50	12.8	7.4	12.0	0.10	0.08	0.10	43	42	45
Shield	3.07	1.13	3.54	18.1	7.8	15.8	0.19	0.15	0.23	45	43	47
Spitfire	3.86	1.36	4.48	13.5	7.5	10.6	0.31	0.18	0.43	40	40	44
Steel	1.20	0.77	1.18	10.8	10.2	11.8	0.10	0.08	0.08	53	53	51
Sunguard	3.64	1.63	4.73	20.9	10.5	15.8	0.19	0.15	0.33	43	41	51
Bolac	2.25	1.05	2.08	24.8	7.4	20.2	0.10	0.15	0.11	37	36	37
Suntop	3.10	1.52	3.91	13.7	14.1	13.1	0.26	0.10	0.32	38	37	43
Supreme	1.61	0.62	1.43	6.8	5.9	9.3	0.28	0.11	0.15	37	37	34
Trojan	1.65	0.87	1.77	14.4	7.4	14.0	0.12	0.12	0.13	38	38	38
Viking	2.97	1.29	3.18	16.1	9.6	16.5	0.21	0.14	0.23	34	33	34
Wallup	2.28	1.22	2.37	13.0	8.2	12.6	0.19	0.15	0.21	38	38	35
Westonia	0.66	0.73	1.18	6.4	4.9	7.0	0.12	0.15	0.20	28	27	29
Wyalkatchem	0.35	0.41	0.35	8.1	2.8	9.1	0.07	0.16	0.05	24	22	22
Yitpi	0.26	0.81	0.26	16.3	7.5	16.5	0.02	0.11	0.01	33	33	32
Zen	0.81	0.39	0.69	10.4	5.0	10.7	0.08	0.09	0.07	33	32	30

NJ1: 2019 off-season in Njoro, NJ2: 2019 main-season in Njoro, NJ3: 2020 off-season in Njoro.

Table S6. Cont'd

Genotype	Days to heading			Plant height (cm)			Spike length (cm)			1000-kernel weight (g)			Test weight (kg hL^{-1})		
	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3	NJ1	NJ2	NJ3
Cacuke	59	60	68	78.0	75.4	86.9	11.1	10.7	10.6	32.5	14.7	23.6	64.6	55.4	43.9
Kenya Robin	69	72	72	77.3	86.3	93.2	12.3	11.2	10.8	20.1	10.9	13.6	56.2	45.2	50.9
Coolah	78	81	81	82.7	81.7	82.7	10.6	10.4	9.0	18.6	13.7	18.4	62.1	56.8	52.8
Chara	76	77	84	76.3	65.9	71.4	9.4	8.5	7.6	13.1	9.2	12.0	56.6	49.4	45.9
LRPB Flanker	75	79	81	81.2	89.1	90.5	10.4	10.5	9.8	19.5	10.7	17.7	67.9	48.5	65.4
LRPB Reliant	76	79	80	95.6	84.3	89.3	10.5	10.1	9.9	22.9	16.8	24.3	71.8	66.9	75.4
Ninja	79	81	78	66.6	72.0	80.1	9.3	9.5	9.5	12.7	10.2	12.6	40.7	42.0	45.4
Tenfour	51	54	60	62.6	65.9	67.3	7.0	8.1	7.7	15.6	13.1	9.7	56.2	48.7	38.0
Tungsten	73	78	88	76.6	71.5	76.6	9.6	9.2	9.3	17.9	10.2	13.5	52.0	47.9	41.8
Axe	52	56	62	69.5	69.0	71.7	8.4	7.6	6.9	27.9	16.8	18.4	67.9	54.1	63.1
B53	74	78	83	81.1	75.0	90.3	10.7	9.2	8.5	17.6	8.4	16.4	59.4	52.0	51.8
Beckom	71	75	75	68.7	67.1	65.5	8.1	7.6	7.4	17.9	14.2	16.2	68.5	59.8	67.4
Bremer	74	77	85	78.4	73.2	78.3	9.8	8.9	8.9	16.5	8.2	17.0	58.4	46.8	53.6
Buchanan	70	73	69	87.8	86.3	84.3	9.9	10.0	8.5	21.9	13.4	14.6	62.7	52.8	54.5
Calingiri	81	83	94	81.6	72.7	73.2	8.9	8.5	8.3	14.4	8.9	13.0	51.0	42.7	41.2
Cobalt	69	72	67	79.8	80.4	81.5	10.4	9.9	9.3	14.5	9.5	13.9	56.3	52.7	45.1
Cobra	73	81	87	76.7	63.9	72.4	10.5	8.8	9.1	18.7	12.7	16.7	61.0	57.4	55.0
Condo	71	70	63	76.3	72.4	75.0	9.8	9.2	9.3	27.6	14.6	22.2	69.2	61.2	65.3
Corack	66	68	75	72.7	60.8	64.9	8.5	7.6	7.8	22.0	13.4	20.3	65.0	60.1	59.0
Correll	72	74	77	80.8	71.9	76.7	8.7	8.7	7.9	23.6	9.1	23.0	58.8	39.0	53.4
Cosmick	68	72	73	79.5	69.6	83.4	9.2	8.1	8.6	12.8	9.4	12.0	47.9	46.7	48.0
Cutlass	79	84	101	84.0	79.8	80.5	10.1	9.7	9.2	14.5	9.2	14.6	54.2	48.2	45.0
Dart	51	54	57	70.2	69.1	69.0	8.7	8.8	8.3	24.5	15.5	23.9	72.5	61.3	72.6
Derrimut	62	67	72	72.3	67.4	70.2	7.3	7.4	6.4	24.7	15.7	21.3	72.9	63.9	66.5
EGA Bounty	65	66	76	90.9	86.8	89.6	11.7	10.0	10.2	27.7	15.7	28.9	74.8	59.7	76.1
EGA Gregory	80	83	85	86.9	85.4	90.7	10.5	10.4	9.3	22.9	11.9	22.0	71.2	55.0	64.6
Baxter	69	74	79	73.7	79.1	85.3	9.0	8.8	8.6	24.4	16.3	22.6	75.3	69.0	73.7
Emu Rock	57	60	57	66.9	58.7	58.5	7.6	6.9	7.1	23.0	15.3	19.7	64.5	54.8	41.5
Espada	66	70	66	71.4	70.6	76.3	7.9	8.3	7.9	24.6	15.0	24.4	71.0	54.8	62.5
Estoc	72	74	85	67.7	71.1	73.0	7.8	8.4	7.9	21.4	12.3	21.3	66.4	51.2	55.8
Forrest	82	84	97	67.8	71.6	70.8	11.4	10.2	8.9	18.7	9.6	16.8	63.1	41.8	51.9
Gauntlet	77	75	77	72.0	66.9	69.5	9.1	8.9	8.1	23.2	17.3	30.6	74.5	68.7	81.0
Gazelle	74	76	87	84.4	69.8	77.6	9.2	8.7	8.8	11.4	7.2	11.2	47.8	37.6	35.3

Table S6. Cont'd

Sunmax	79	82	97	87.0	80.1	90.8	10.7	10.6	10.6	10.7	12.4	10.8	47.5	53.9	43.6
Janz	64	69	82	78.2	74.2	73.7	8.6	8.3	8.5	24.0	13.0	22.5	74.2	69.2	67.0
Kiora	69	72	77	76.8	81.5	80.5	9.3	8.7	8.5	16.5	12.9	15.8	63.4	59.6	60.1
Lancer	76	78	84	70.4	69.2	66.1	8.3	9.0	8.1	25.4	20.8	26.4	77.4	70.9	80.2
Livingston	53	57	70	69.3	72.3	72.5	7.4	8.1	8.6	31.4	20.9	29.6	74.5	67.2	61.4
Mace	70	74	82	70.5	65.2	74.6	8.8	8.2	8.2	25.9	12.7	23.5	69.8	59.2	69.2
Magenta	68	77	76	80.1	71.8	78.8	8.6	8.7	9.1	31.2	19.2	33.3	76.4	69.6	80.0
Merlin	64	66	62	73.0	72.4	74.9	8.5	8.2	8.6	28.6	18.9	31.9	77.1	66.0	77.8
Mitch	73	78	84	82.5	76.0	78.4	11.3	9.6	9.4	12.0	10.3	17.9	48.1	46.2	53.7
Orion	71	75	81	83.3	74.1	99.0	10.6	9.8	9.8	20.0	14.3	18.8	57.1	51.7	56.6
Gladius	65	69	61	73.4	75.9	71.4	7.6	8.1	7.5	27.4	19.3	24.7	69.4	61.6	67.5
Preston	70	72	71	69.9	68.1	75.6	8.7	8.0	8.3	13.5	11.6	11.1	50.3	48.3	28.6
Scepter	50	55	67	66.4	73.0	78.5	8.0	8.9	8.5	32.9	23.9	34.3	71.4	70.6	80.6
Scout	72	76	81	74.0	73.9	79.8	9.6	8.9	8.9	19.7	10.5	20.6	67.6	51.5	57.6
Shield	74	79	83	73.3	67.6	72.8	8.0	8.1	7.6	26.3	15.2	26.2	71.2	56.4	66.2
Spitfire	62	65	62	78.2	70.7	70.3	8.5	8.5	8.3	27.8	18.0	30.6	76.1	69.7	74.0
Steel	73	77	78	80.9	84.5	89.5	9.4	9.8	8.5	16.3	9.0	16.8	59.8	43.7	56.4
Sunguard	77	82	90	80.4	75.9	77.2	8.1	8.3	8.2	23.3	24.1	33.1	73.1	76.1	78.8
Bolac	72	76	80	78.5	73.3	82.1	8.5	8.7	8.1	19.0	15.7	16.5	65.5	65.6	66.1
Suntop	66	69	70	76.1	91.1	92.6	9.9	10.0	9.2	26.5	18.8	28.4	73.2	68.1	76.6
Supreme	54	59	73	63.6	57.7	59.9	8.6	8.2	7.9	20.9	13.8	18.2	67.5	57.3	51.8
Trojan	74	78	85	77.2	69.9	72.2	10.1	9.1	8.6	17.0	11.7	17.0	65.8	56.0	58.2
Viking	71	75	78	83.4	74.0	86.1	9.5	8.8	9.1	22.0	16.7	20.8	72.2	66.1	64.5
Wallup	61	62	60	79.1	70.6	70.1	8.6	8.4	8.0	21.6	17.8	22.5	68.3	65.1	59.6
Westonia	60	63	61	72.6	71.6	73.5	9.9	8.3	9.2	20.9	15.9	19.3	70.9	64.7	51.1
Wyalkatchem	61	62	58	66.1	50.1	50.0	7.5	6.7	6.3	14.7	13.0	11.5	60.4	56.7	36.1
Yitpi	71	74	76	75.0	78.6	80.5	9.9	9.5	9.5	12.8	15.1	13.9	64.8	64.4	43.4
Zen	83	84	84	69.2	64.3	73.3	7.4	7.7	7.2	13.6	8.6	11.4	60.9	47.0	40.0

NJ1: 2019 off-season in Njoro, NJ2: 2019 main-season in Njoro, NJ3: 2020 off-season in Njoro.

Supplementary (S) table 7. Superiority measure (P_i) and mean squares (MS) of genotype-by-season interaction (GSI) of AUDPC for 61 bread wheat genotypes evaluated for resistance to stem rust over three seasons at KALRO, Njoro and one season at DZARC, Debre Zeit and resistance to yellow rust over three seasons at KALRO, Njoro.

Stem rust					Yellow rust				
	Genotype	Mean	P_i	MS(GSI)		Genotype	Mean	P_i	MS(GSI)
Rank[a]	Minimum response	8	0.20	0.19	Rank[a]	Minimum response	6	0.07	0.07
1	Lancer	9	0.20	0.19	1	Sunmax	10	0.07	0.07
2	Sunguard	8	0.32	0.21	2	Steel	7	1.08	0.75
3	Gauntlet	20	3.64*	1.56	3	Gladius	6	2.43	0.10
4	Shield	80	28.61*	4.83*	4	Lancer	14	3.93*	2.95
5	Magenta	132	50.69*	1.56	5	LRPB Flanker	9	4.32*	1.06
6	Sunmax	155	55.78*	4.88*	6	Suntop	17	4.69*	1.57
7	Janz	165	58.89*	2.95*	7	Gazelle	43	15.57*	0.75
8	Spitfire	166	64.25*	2.86*	8	Shield	33	17.53*	13.52*
9	Forrest	215	78.98*	9.98*	9	Axe	39	18.39*	0.43
10	Dart	222	80.88*	2.25*	10	EGA Gregory	57	19.40*	0.11
11	Merlin	208	83.29*	1.95	11	Coolah	43	19.43*	8.87*
12	Scepter	225	85.25*	7.91*	12	Buchanan	81	21.88*	1.90
13	Bolac	225	89.64*	21.96*	13	Preston	59	21.89*	2.25
14	Estoc	235	92.28*	10.63*	14	Sunguard	43	23.24*	11.37*
15	Cutlas	247	93.22*	40.11*	15	Cutlas	67	23.71*	17.99*
16	Yitpi	253	94.88*	15.43*	16	Tenfour	63	24.10*	2.22
17	Emu Rock	250	98.75*	2.21	17	Derrimut	60	25.34*	2.64
18	Corack	277	109.84*	4.59*	18	Scout	64	27.36*	2.15
19	Beckom	286	111.69*	6.52*	19	Tungsten	70	28.12*	1.11
20	LRPB Reliant	285	113.38*	9.41*	20	Correll	76	33.39*	2.05
21	Espada	300	114.33*	7.40*	21	Cobalt	133	38.11*	8.68*
22	Baxter	337	130.03*	29.41*	22	Magenta	56	39.56*	0.55
23	Kiora	343	131.85*	11.32*	23	Estoc	81	41.21*	4.31*
24	EGA Bounty	331	137.23*	20.22*	24	Espada	97	42.23*	13.24*
25	Supreme	367	144.48*	30.61*	25	Trojan	111	54.23*	2.44
26	Cobra	355	147.01*	38.32*	26	Robin	119	55.64*	7.75*
27	Gladius	347	149.59*	23.73*	27	Forrest	121	62.21*	15.79*
28	Orion	366	151.45*	9.66*	28	Kiora	146	66.56*	12.04*
29	Derrimut	375	154.39*	17.36*	29	Yitpi	156	67.24*	1.85
30	EGA Gregory	397	159.15*	7.46*	30	Gauntlet	136	68.76*	7.43*
31	Viking	383	159.29*	8.75*	31	Janz	140	69.07*	10.14*
32	Mace	386	164.95*	11.78*	32	Viking	128	70.06*	16.32*
33	Trojan	388	165.13*	10.36*	33	Calingiri	161	78.00*	5.00*
34	Wallup	430	180.08*	8.54*	34	Scepter	173	78.35*	6.38*
35	Wyalkatchem	456	186.31*	7.31*	35	Baxter	166	82.10*	10.91*
36	Livingston	512	216.72*	24.88*	36	Bremer	188	83.82*	14.77*
37	Zen	542	227.45*	4.00*	37	Bolac	190	86.71*	9.22*
38	Chara	542	230.60*	14.30*	38	Supreme	185	86.92*	15.25*
39	Westonia	566	236.24*	25.72*	39	Merlin	182	90.77*	4.01*
40	Tungsten	570	248.10*	25.92*	40	Mitch	191	95.92*	4.14*
41	Coolah	594	257.14*	5.21*	41	Spitfire	195	101.37*	8.95*
42	LRPB Flanker	608	261.23*	4.93*	42	Ninja	217	102.79*	31.95*
43	Suntop	643	277.19*	0.40	43	Wallup	214	107.02*	1.67
44	Axe	619	278.05*	9.74*	44	Livingston	219	111.77*	20.31*
45	Calingiri	660	284.70*	11.05*	45	Zen	233	114.84*	4.71*
46	Scout	652	286.63*	0.49	46	EGA Bounty	252	122.55*	35.36*
47	Gazelle	708	310.69*	51.79*	47	Chara	257	126.22*	8.67*

Supplementary (S) table 7.

48	Ninja	757	321.56*	11.16*	48	LRPB Reliant	285	132.80*	27.28*
49	Condo	743	323.56*	12.74*	49	Cobra	306	148.18*	34.56*
50	Correll	749	329.14*	9.43*	50	Cacuke	321	150.80*	13.09*
51	Bremer	748	336.76*	64.85*	51	Dart	312	150.97*	2.16
52	B53	773	341.62*	6.04*	52	Beckom	334	161.34*	8.90*
53	Preston	874	360.29*	21.45*	53	Orion	326	163.47*	13.14*
54	Cosmick	826	364.47*	22.91*	54	B53	341	167.73*	29.55*
55	Mitch	832	370.81*	10.48*	55	Cosmick	339	168.59*	24.33*
56	Buchanan	1082	471.30*	4.57*	56	Condo	378	179.82*	7.64*
57	Cobalt	1079	491.12*	9.30*	57	Corack	444	206.22*	25.31*
58	Steel	1098	495.29*	7.68*	58	Emu Rock	431	207.58*	6.95*
59	Cacuke	1183	550.07*	27.41*	59	Mace	493	245.37*	10.43*
60	Robin	1339	623.98*	17.88*	60	Westonia	633	316.46*	22.78*
61	Tenfour	1402	657.28*	22.31*	61	Wyalkatchem	650	323.02*	1.68

AUDPC: area under disease progress curve, [a]Ranking of genotypes is based on Pi values.

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Variability effect of pH on yield optimization and Mycochemical compositions of *Pleurotus ostreatus* sporophores cultured on HCl-induced substrate

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Contamination due to *Coprinus cinerius* has been identified as a major drawback in the use of oil palm bunch (OPB) for mushroom cultivation. OPB is a common agro-waste in the South-eastern part of Nigeria with high alkalinity which does not support the growth of oyster mushrooms. A study on the fructification and some mycochemical contents of *Pleurotus ostreatus* fruitbodies cultivated on hydrochloric acid (HCl)-induced OPB substrate was conducted. OPB was steeped in solutions (0.1%-0.5%) of HCl for 48 h, to optimize its pH. Experiment was conducted in a Completely Randomized Design (CRD). One way analysis of variance (ANOVA) was used for data analysis and means separation by Duncan Multiple Range Test (DMRT) at $p<0.05$. Results showed that increase in the concentration of HCl acid from 0.1 to 0.5% reduced substrate contamination due to *Coprinus cinerius* and enhanced fruit body number, yield and biological efficiency (BE%) as well as primordial initiation. Vitamins, bioactive compounds, heavy metals and other mycochemicals of nutritional importance were recorded in various fruit body samples at different concentrations. Therefore, commercial mushroom growers should avail themselves of this unique opportunity to ensure effective utilization of OPB incorporated with HCl acid for high fruit- body production as well as profit maximization.

Key words: Hydrochloric acid (HCl), *Pleurotus ostreatus, Fruitbodies,* oil palm bunch (OPB).

INTRODUCTION

Mushrooms are members of the class Basidiomycota and order *Agaricales*. Unlike green plants, they do not possess chlorophyll; for manufacturing of their own food. For their growth and development, they require pre-formed food such as smaller broken down molecules of lignin, cellulose and starch (Stamets, 2000). Chang (2012) defined mushroom as “a macro-fungus that has a distinctive fruiting body which can either be epigeous (growing on or close to the ground) or hypogenous (growing under the ground)”. The macro-fungi have

fruiting bodies large enough to be seen with unaided eye and to be picked by hand. Ideally, the word mushroom refers only to the fruit body of a macro-fungus. Most mushroom species are taxonomically categorized either under the Basidiomycota or Ascomycota; the two phyla are in the kingdom fungi (Cho, 2004).

Pleurotus ostreatus is the scientific nomenclature for Oyster mushroom. In many parts of India; it is known as Dhin (Elliott, 1982; Ogundana and Fagade, 1982). It was formally in the family Tricholomataceae, but now Pleurotaceae, which includes many species such as *P. flobellotus P. sojar-caju*, *P. eryngii, P. ostreatus, P. florida*, *P. sapidus* etc.

Effect of pH on mushroom fruit body formation

Fungal (mushroom) mycelia derive nutrients from substrates within a certain pH range (Saker et al., 2007); lime is often times incorporated in mushroom cultivation to optimize the pH of substrate substrates perceived to be acidic (Stamets, 2000). According to Chang and Miles (2004), rapid mycelia growth of *Pleurotus sajor-caju* takes place at pH 6.4-7.8. Oyster mushroom (*Pleurotus* spp.) can grow and utilize nutrients from various kinds of substrate materials than any other mushrooms (Cohen et al., 2002).

The observed increase in soil alkalinity caused by oil palm bunch ash, oil palm bunch husk and wood ash could be the main reason for their use as liming materials. Liming has been reported to be important for soil physical and chemical properties and nutrient availability. The better performance of wood and oil palm bunch ashes in improving growth and yield value of mushrooms (*Pleurotus* spp.), soil pH, K, Ca, and Mg could be due to the fact that the ash component is more soluble than other residues. This was reported by Moyin-Jesu (2007) and Ojeniyi (1990) who found that K and Ca components of wood ash were very high, and this could be responsible for their ability to enhance soil pH, which increases quick absorption of nutrient such as P, K, Ca and Mg that are essential for good growth and yield parameters of many humiculouse mushrooms. In addition, Okhuoya and Okogbo (2009) also reported that oil palm bunch fibres were good substrates for *Pleurotus tuber-regium.* The optimal temperature range for growth of the mycelium is within 25-28°C; while that of pH is between 5.5 and 6.5.

The CO_2 tolerance of mycelia is rather strong. For instance, mycelia of *Pleurotus* spp. can still flourish at carbon dioxide concentrations of 15 to 20%. But when the concentration of CO2 is raised to 30%, mycelia growth suddenly decreases (Chang and Miles, 2004).

MUSHROOMS AND FOOD SECURITY

Human population grows by 2.1%, representing a rise of about 75 million people per year, thus food production has to keep pace with population increase (Sharma, 2003). Mushrooms and yeast are referred to as important alternative sources of food (Chang, 1999; Anyankorah, 2002; James and Panter, 1995). According to James and Panter, (1995), edible mushrooms (dry) contain up to 19-40% protein; which is twice that of vegetables, four times that of oranges. Further, a mycochemical analytical profile showed that mushrooms are rich in vitamins and minerals, low in unsaturated fatty acid and carbohydrate, which makes them ideal for diabetic and obese patients (Chang and Miles, 2004).

Most mushrooms have exceptional medicinal and prophylactic properties, especially in diseases such as high blood pressure, asthma, respiratory tracts infection, anaemia, hepatitis, cancer, tumour, etc. (Ogundana and Fagade, 1982). Mushroom cultivation also represents the most efficient and economically viable biotechnological approach for the conversion of ligno-cellulosic waste materials into high-quality protein food; and, this will naturally open up new job opportunities, especially in rural areas, which can be pre-packaged by food industries and exported to other countries as food and for revenue generation.

According to Eno (2020), Nigeria produces nearly 500 metric tons of mushrooms, which is far less than its production potential; as its full production capacity has been estimated at 1.500MT per annum. This production gap has been attributed to a dearth of mushroom scientists and farmers. Osemwegie and Dania (2016) noted that data are either scarce or unavailable, on the contribution of mushroom production and commercialization to Nigeria's Gross Domestic Product (GDB); as information on the volume of mushroom tonnage produced annually for export or local consumption as well as commercial scale cottage mushroom industries are staggering (Celik and Parker, 2009; Marshall and Nair, 2009; and Barmon et al., 2012). The few available mushroom growers in Nigeria use a variety of substrates, such as sawdust, grass straws, rice bran/husks etc (Anoliefo et al., 2006; Obodai and Odamtten, 2012).

Therefore, this work aims to utilize an abundant agro-waste component (oil palm bunch) as a substrate for the

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Figure 1. Young fruit bodies of uncontaminated OPB**.**
Source: Authors

production of oyster mushroom in Nigeria.

MATERIALS AND METHODS

Study area

Mushroom cultivation stage

The experiment was conducted at the mushroom Research Section of the Michael Okpara University of Agriculture Umudike, Abia State. Umudike is located between longitude 7^0 and 70^005^0E and latitude 5^0 and 5^025^0N with a humid tropical climate. Rainfall is bi-modally distributed, with peaks between July and September of each year. Annual rainfall is approximately 170mm, spread between April and November each year (Achufusi, 2016).

Source of spawn culture

Pure culture (mycelia) of *P. ostreatus* was obtained from Dilomat farms Nigeria Limited, Rivers State University, Rivers State, Nigeria.

Spawn production/multiplication

Spawn of *P. ostreatus* was prepared using red variety sorghum grains. *Sorghum* grains were first washed in 3 changes of tap water before soaking over- night. The grains were subsequently boiled in tap water for 10-15 min using a gas-burning flame. Grains were completely drained of water before mixing with 2% (w/w) $CaCo_3$ and 4% $CaSO_4$ to optimize pH and prevent grain clumping, respectively; as recommended by Muhammad et al. (2007). They were further packed (2/3) in heat resistant transparent bottles, tightly sealed with aluminium foil held with rubber band and sterilized in an autoclave at 121°C for 30 min. After sterilization, bottles were allowed to cool at room temperature. Then, they were aseptically inoculated with actively growing mother mycelia culture of *P. ostreatus,* by grain-to-grain transfer. Subsequently they were incubated in the dark at 27 ± 2°C until grains were fully colonized by *P. ostreatus* mycelia (Shyam et al., 2010).

Determination of substrate pH

The pH of solution containing 5% of raw sample substrate in 50ml of distilled water was determined using a Jenway3070 portable automatic digital pH meter with temperature compensation; it was calibrated with buffer 7-4 and 10. The pH value was read on the digital scale.

Experimental design

The experiment was conducted in a Completely Randomized Design (CRD). Six levels of HCl acid-induced OPB substrate; including control were replicated seven times, each. Each replicate contained 200g dry weight of OPB substrate, which made a total of 1400g/level of HCl-OPB substrate.

Substrate inoculation

Thirty (30g) of grain-based spawn of *P. ostreatus* was spread across each replicate of 200g of substrate during inoculation (Okwulehie and Okwujiako, 2008). All the inoculated substrates were placed on wooden racks in the cropping room and covered during the spawn run. Humidity of the cropping room was optimized by constantly sprinkling and flooding with tap water prior to primordial initiation (Figures 1 and 2).

Measurement of morphological characters

Stipe sizes of fruit bodies

The effect of substrate on pileus and stipe sizes of fruit bodies was

Figure 2. OPB contaminated by *C. cinerius*.
Source: Authors

determined at maturity. The mushrooms were harvested accordingly while pileus and stipe sizes were measured in cm using a meter rule (Okwulehie and Okwujiako, 2008).

Cap diameter

This was obtained by placing a transparent ruler across the centre of the pileus and reading off the diameter (Okwulehie and Okwujiako, 2008).

Effect of substrates on fruit body number of the mushrooms

The effect of substrate on fruit body number of the mushroom was determined by harvesting the mushrooms, counting and recording their number for each and later comparing their values (Okwulehie and Okwujiako, 2008).

Yield and biological efficiency

During fruiting, mushroom fruit bodies were harvested at maturity; wet weight of fresh fruit bodies was determined using digital weighing scale, while biological efficiency (BE) was determined using the modified method of Chang and Miles (2004) as:

$$BE = \frac{Freshwtofmushroom}{drywtofsubstrate} \times \frac{100}{1}$$

Proximate analysis

Proximate analysis was conducted on each of the six (6) fruit body samples. The protein, ash, fat and crude fibres were determined by the method of AOAC (2000).

Determination of crude protein

Crude protein of different samples was estimated by the Kjedahl method. Total nitrogen content was determined first, and the value was multiplied by 0.25 coefficients (Maurizio et al., 2005). Two g of dry powdered sample was digested in 5ml of concentrated sulphuric acid (H_2SO_4) and a tablet of selenium catalyst was added in a fume cupboard. The digest was made up to 250ml with the acid. Ten ml of the digest was distilled and titrated with 0.2 NH_2SO_4. The crude protein was finally obtained by multiplying total nitrogen by 0.25.

Determination of moisture content

Moisture contents (MC) was determined by placing 2 g of the powdered dry samples on clean dry glass Petri dishes of known weight and placed in an electric oven at 75^{o}C for 7-8 h (AOAC, 2000 and Konuk et al., 2006). The oven-dried samples were kept at constant weight. The percentage moisture content (PMC) was determined thus:

$$PMC = \frac{\text{wt of fresh} - \text{wt of dry sample}}{\text{wt of fresh sample}} X \frac{100}{1}$$

Determination of ash contents

Ash contents were determined by burning dried samples of the fruit bodies. Five g of the powdery samples of mushrooms was burnt at 500^{o}C overnight in a crucible. The crucible was allowed to cool and later weighed again (Mattila et al., 2002; Oei, 2003). The percentage Ash content (PAC) was determined as:

$$PAC = \frac{\text{wt of crucible+lid+Ash} - \text{wt of crucible+lid}}{\text{weight of sample}} X \frac{100}{1}$$

Determination of carbohydrate (CHO)

Carbohydrate contents were determined by difference; that is, % CHO= 100 – (5 Ash + % protein + % fat + % moisture).

Determination of ether extract

Ether extract component of mushroom samples was determined following the established method of AOAC, (1980; 2000). Two grams (2g) of each sample was inserted into an ether extracting thimble and placed on the soxhlet reflux flask channelled into a round bottom flask of unknown weight. The apparatus was filled with 250ml of petroleum ether and placed on a heating apparatus. The oil was extracted by a reflux system. After repeated refluxing, a clear solution was obtained in the flask and the sample removed. Further heating was done to separate the ether from the extracted oil. A round-bottom flask containing the oil was dried in an oven at 70^{o}C; fats and oils were determined by the gravimetric method as follows: weight of oil =weight of flask + oil –weight of flask (after drying). This was expressed as sample percentage as follows:

$$\% \text{ fats and oils} = \frac{\text{wt of oil}}{\text{wt of sample}} \text{ X } \frac{100}{1}$$

Determination of crude fibre

Total crude fibre of the samples was calculated by the Weende method (AOAC, 1980; 2000).Two g of each sample was inserted into a 250-ml beaker and hydrolyzed by adding 20ml of dilute sulphuric acid; it was boiled for 30 min on a hot plate. The mixture was filtered off through a piece of clean white nylon cloth and rinsed with hot distilled water. The residue was further boiled with 50ml of 2.5% sodium hydroxide (NaOH) for 30 min and also filtered off before rinsing with distilled water. The residue was finally collected and transferred into a crucible before it was dried in an oven to a constant weight. Finally, the sample was ashed in a muffle furnace and the weight of the crude fibre was determined and expressed as:

$$\%\text{crude fibre} = \frac{\text{wt of fibre}}{\text{wt of sample}} \text{ X } \frac{100}{1}.$$

Determination of vitamins

Vitamin content of the mushroom samples was determined by a spectrophotometric method, as described by AOAC (1980).

Determination of Vitamin A (Retinol)

The retinol content of each sample was estimated by the method of Shyam et al. (2010). Five gram (5 g) of each fruit bodies sample was homogenized using acetone solution and filtered off using Whatman filter No.1. The filtrate was later extracted with petroleum spirit using a separating funnel; two layers of both aqueous and solvent layer were obtained. The upper layer which contains vitamin A was washed with distilled water to remove residual water. This was later poured out into a volumetric flask through the discharge point of the separating funnel and made up to mark. The absorbance (A) of the solution was read using a spectrophotometer at a wave length of 450 nm and calculated as:

Mg /g = A x vol x 104= A x 12cm x sample weight.

Determination of Vitamin B_1 (Thiamin)

Five grams (5g) of each mushroom sample was homogenized with ethanolic sodium hydroxide (50ml) and filtered into a 100-ml flask. Ten ml of the filtrate was pipetted and the colour development was read at the same time. Thiamine was used to get 100 ppm and serial dilution of 0.0, 0.2, 0.6 and 0.8ppm was made. This was used to plot the calibration curve (AOAC, 2000; Shyam et al*.,* 2010).

Determination of Vitamin B_3 (Niacin)

Niacin composition was determined following the König spectrophotometric method (AOAC, 2000). Dry powdered mushroom sample of 0.5g each, was extracted with 50ml of 1 N HCl in a shaking water bath kept at 30^{o}C for 35 min. The mixture was filtered using Whatman filter paper. $KMnO_4$ (0.5g) was added to the filtrate and made up to mark. Ten ml of the extract was pipetted into a 50-ml flask, while 10ml of phosphate solution was added as buffer. The pH was adjusted with 5ml of 1 NHCl, and the solution was made up to mark with distilled water. After 15min, the extract was read by spectrophotometry at 470nm wave length.

Determination of Vitamin C (ascorbic acid)

Vitamin C content of each mushroom sample was estimated by the method of Kamman et al. (1980). Five grams (5g) of each sample was homogenized in 100ml of EDTA/TCA extraction solution. The homogenate was filtered and the filtrate was used for the analysis. Each sample filtrate was passed through a packaged cotton wool containing activated charcoal for discolouration. The volume of the filtrate was adjusted to 100ml of water by washing with more of the extraction solution. Twenty ml of each filtrate was measured into a conical flask. 10 ml of 2% potassium iodide solution was added to each of the flasks followed by 5 mls of starch solution (indicator). The mixture was titrated against 0.01 molar $CuSO_4$ solution; titration of the brink of the mixture. Vitamin C content of the samples was calculated as 1ml of 0.01 mol CuSO4 at 0.88n, according to the formula of Shyam et al. (2010):

$$\text{vit mg/100g sample} = \frac{100 \text{ x vf x } 0.88T}{\text{va}}$$

Where: Vf = volume of filtrate analysed; Va = volume of acid analysed; 0.88T = constant.

Determination of Vitamin K (Phylloquinone)

Determination of vitamin K followed the method of Careri et al. (1996). Powdery mushroom sample of 1.0g was weighed out, transferred into a 40-ml brown glass flask and ultrasonically shaken with 10 ml methanol for 15 min. The amount of mushroom sample was increased to 5.0 g and was centrifuged at 1000 rpm for 5 min (ALC 4236 centrifuge, ALC, Milan, Italy). A 2-ml aliquot of the methanol phase was mixed with 4 ml of sodium carbonate solution (5 g/100 ml), and heated at 80°C for 1 h. The hydrolysate was extracted by partitioning of the alkaline solution with 4 ml n-hexane on a Vortex mixer for 1 min and centrifuged at 2000 rpm for 10 min; after the upper hexane layer had been carefully separated from the aqueous phase, two additional 4 ml of n-hexane was added to the aqueous phase and processed as before. The combined extracts (12 ml) were collected in a 50-ml round-bottom flask and concentrated to a low volume in a rotary evaporator at 35 °C (Biichi, Brinkman Instruments, Inc., Chicago, IL, USA) and then evaporated to dryness under a stream of nitrogen. The final residue was re-dissolved in methanol (1 ml) and analyzed by HPLC after filtration through a 0.2-mm membrane.

Determination of Vitamin E (Tococpherol)

Tocopherol estimation was done by the colorimetric method of Baker and Frank (1968). The tocopherol is determined by Emmerie-Emmerie Engel reaction which is based on the reduction by tocopherol of ferric to ferrous ions; it latter formed a red complex with α,α'-dipyridyl. Tocopherol and carotene were first extracted into xylene and the extract read at 460 nm to measure carotenes. A correction is made for these after adding ferric chloride and reading at 520 nm.

Into three stopper centrifuge tubes were measured 1.5ml and 1.5 ml standard sample solution and water (blank), respectively. Then, in the test and blank 1.5 ml of xylene was added to all the tubes, stoppered mixed, and centrifuged; thereafter, 1ml of the xylene layer was transferred into other stoppered tubes taking care not to include any ethanol or protein. One ml of α, α'-dipyridyl reagent was added to each tube that was stoppered and mixed; then1.5 ml of the mixture was pipetted into colorimeter corvettes and extraction of test and standard samples were read against the blank at 460 nm. Tocopherol was calculated as mg/l by the following formula:

$$\frac{(\text{Extinction of unknown at 520 nm- Extn at 460 nm} \times 0.29)}{\text{Extn of standard at 520 nm}} \times 10$$

Determination of percentage bioactive compounds

Determination of phenolics content

To determine the phenolics content of the powdered sample of the mushroom, a fat-free sample was used. Two grams (2g) of the sample was defatted with 100 ml of diethyl ether, using a soxhlet apparatus for 2 h. To extract the phenols component of the sample, the fat-free sample was boiled with 50ml of ether for 15 min. Five ml of the extract was pipetted into a 50-ml flask into which 10ml of distilled water, 2ml of ammonium hydroxide (NH_4OH) solution and 5ml of concentrated amyl alcohol were added.

The mixture was made up to mark and left to react for 30 min for colour development. The absorbance of solution was read using a spectrophotometer at 505nm wave length (Harborne, 1973). The % phenol was calculated as follows:

$$\frac{100 \text{ x Au x C x VF x D}}{\text{W x AS x 1000 xVa}}$$

Where:W = weight of sample of analysed; Au = absorbance of the test sample; As = absorbance of standard solution; C = concentration of standard in mg/ml; VF = volume of filtrate analysed; VA = volume of acid analysed; D =dilution factor, where applicable.

Determination of tannins

Tannin content of the mushroom samples was estimated following the modified method of Okeke and Elekwa (2003). 0.5g of the sample in 10ml of 2-MHCl was vigorously shaken for 5 min and transferred into a volumetric flask and made up to 50ml. The mixture was filtered, and 5ml of the filtrate was transferred into a test tube. Three ml of 0.1 NHCl and 3ml of 0.008 M potassium ferrocynide ($K_3F[CN]_3$) were added. The absorbance was read at 720 nm within 10 min.

Determination of sterols

The crude fat analysis was carried out by the method of AOAC (2006b). A 250-ml extraction flask was dried in the oven at 105^{o}C, transferred to the desiccator to cool at room temperature and the weight of flask measured. Exactly 0.25g of the sample was weighed into a labelled porous thimble; 200ml of petroleum ether was subsequently measured and added to the 250-ml conical flask. The covered porous thimble with the sample was placed in the condenser of the soxhlet extractor. The sample was extracted for 5 h. The porous thimble was removed with care and the petroleum ether in the top container (tube) was collected by recycling for reuse. The extraction flask was removed from the heating mantle when it was almost free of petroleum ether. The extraction flask containing the oil was oven-dried at 105^{o}C for one (1) h. The flask containing the dried oil was cooled in the desiccator and the weight of the cooled flask and the dried oil were measured.

Determination of alkaloids

The method of Maxwell et al. (1995) was followed to estimate the quantity of alkaloids in the mushroom samples. The alkaloids were extracted from 20g of each of the dried powdered mushroom sample using 100ml of 10% acetic acid. The extracts were filtered to remove cellular debris before being concentrated to a quarter of the original volume. One % NH_4OH was added to the concentration in drops until no precipitate was formed. The alkaloids, thus obtained were dried to a constant weight at 65^{o}C in an oven. The weight was used to calculate the percentage of alkaloids in the mushroom samples, using the formula:

$$\text{Alkaloids (\%)} = \frac{\text{weight of residue}}{\text{weight of sample}} \text{ X } \frac{100}{1}$$

Determination of terpenes

The extraction was carried out following the method of Ortan et al. (2009). The dried ethanol and aqueous extracts were made to be free of water by drying to constant weight for a period of time in the laboratory and the terpenes constituents extracted with redistilled chloroform. The terpenes were removed with 10ml of the solvent for 15 min. The mixture was filtered and concentrated to 1ml in the vial for gas chromatography analysis and 1ml was injected into the injection port.

Determination of glycosides

Glycosides were determined by the method of Peng and Kobayashi (1995). Equal parts of Fehling's solution I and II(5ml) were added to 5ml of the dry mushroom sample. A brick red precipitate shows the presence of a reducing sugar.

Hyrolysis test

Five mls (5ml) of dilute sulphuric acid was added to about 0.1g of the mushroom extract and neutralized with 20% KOH solution. Ten grams (10ml) of a mixture of equal parts of Fehling's solutions I and II were added and boiled for 10 min. A more dense brick red precipitate indicates the presence and amount of glycosides.

Determination of minerals

Mineral contents of dried mushroom samples were estimated by a wet-ashing method. The solutions of ash obtained from the samples were dissolved in a drop of trioxonitrate (v) acid made up to 50ml with deionized water. They were analyzed for calcium (Ca) using vanadate ethyldiamine-tetra acetic acid (EDTA) complexometric titration method according to MFA, (1982). Sodium (Na) Chlorine (Cl) and Potassium (K) were estimated using a flame photometer.

Determination of heavy metals

The amount of Fe, Cu and Zn in the sample was estimated by Energy Dispersive X-ray Fluorescence (EDXRF) technique according to the method of Stihi et al. (2011) and Ghisa et al. (2008). A Elvax spectrometer was used, having an x-ray tube with Rh anode, operated at 50kv and 100μA. Mushroom samples were excited for 300 s and the characteristic x-rays were detected by a multi-channel spectrometer based on a solid state Si-PIN photodiode x-ray detector with a 140μm Be- window and an energy level of 200ev at 5.9 keV. Elvax software was used to interpret the EDXRF spectra. The accuracy of the results was evaluated by measuring a certified reference sample, and good results were achieved between certified values and data obtained.

The amount of Pb and Se in the sample was estimated by Atomic Absorption Spectrometry (AAS) (Wagner, 1999; Petisleam et al., 2007; Dima et al., 2006), using the AVANTA GBC spectrometer with flame and hollow cathode lamps (HCl). Pb and Se were determined by the method of calibration curve according to the absorption concentration. Several standard solutions of different known concentrations were prepared and the elemental concentration in the unknown sample was determined by extrapolation from the calibration curve. All fruit body sample concentrations were reported as mg/kg dry weight of material.

Statistical analysis

Data obtained in the course of this investigation were statistically analyzed using Analysis of Variance (ANOVA), and mean separation was carried out by a Duncan Multiple Range Test (DMRT) at $p<0.05$ level of significance (Steel and Torie, 1984).

RESULTS AND DISCUSSION

pH variations in substrate and formation of mushroom fruit bodies

The results revealed that the naturally obtained OPB substrate that was neither soaked in water nor acid solution had a pH of 10.1 (Table 1). This value is relative to those obtained by Achufusi (2016) and Okwulehie et al. (2018) who reported pH values of 10.3 and 9.5, respectively, on raw OPB substrate during mushroom cultivation. It was observed that HCl acid solution of 0.1% - 0.5% reduced pH of the substrate from 8.2 – 6.1, respectively, after steeping for 48 h; while the control was found at a pH of 9.0. This variation also was in line with the work of Okwulehie et al. (2018), who reported a direct proportionate increase in acidity of OPB substrate with increased HCl solution after steeping for 48 h.

pH is generally considered to be one of the most important environmental factors that seriously affects the fruiting, growth and extension of fungal mycelia (kang et al., 2006). Our results indicated that HCl acid delayed primordial formation, revealing that concentration of the acid delayed primordial formation, but increased fruit body production. This observation was in line with the works of Bilgrama and Verma (1992), Okwulehie et al. (2006), and Okwulehie and Okwujiako (2008), who reported that culture media of pH between 6.0 and 8.0 recorded significantly greater mycelia extension than those above the range. In our investigation, the time for primordial initiation, apparently preceded by fruit body production was shorter compared to the result obtained by Shah et al. (2004), who reported a fruiting duration of oyster mushroom within 3-6 weeks after spawn inoculation. Contrarily, Khan et al. (2001) investigated oyster mushroom cultivation and observed that primordial formation took place after 8 days of spawn running; while spore carp formation took place after 10-12 days of spawn running. Early fruit body formation recorded in this experiment could be due to certain factors such as HCl acid, substrate and cultivation technique according to Chang and Miles (2004), Nwoko et al. (2017), Okoi and Iboh (2015), and Hassan et al. (2010).

Morphological characteristics of fruit bodies

Results of some of the morphological characteristics of fruit bodies showed that 0.5% HCl OPB substrate produced the highest (705.00) number of fruit bodies (Table 2). The results also showed that as the percentage of HCl in the OPB substrate increased from 0.1-0.5, the number of fruit bodies increased from 541-705; while control had the lowest (424.00). This observation is in line with the work of Okwulehie et al. (2018), who recorded the highest number of fruit bodies of *P. ostreatus* at 0.5%HCl OPB substrate, and got the lowest in control. Achufusi (2016) did not observe the growth of any *P. ostreatus* fruit body from OPB substrate without the addition of HCl. That could probably be as a result of high alkaline level of the substrate which does not support mycelia growth as well as fruit body production (Bilgrama and Verma, 1992; Okwulehie et al., 2006; Okwulehie and Okwujiako, 2008). In this experiment, the mean cap diameter and weight of fruit bodies from all levels of HCl OPB substrates, including control, were higher than the values reported by Okwulehie et al. (2018); unlike in stipe length where they obtained higher values. This could be due to variation in the oyster mushroom species.

Substrate variation has been recorded as another

Table 1. pH of substrates and fruiting duration of *P. ostreatus*.

HCl OPB level	pH of OPB	Fruiting duration/days
Raw OPB(%)	10.1[a]	-
Control	9.0[b]	17[c]
0.1	8.2[c]	16[d]
0.2	7.8[d]	17[c]
0.3	7.4[e]	18[b]
0.4	7.1[f]	18[b]
0.5	6.1[g]	19[a]

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

Table 2. Morphological characters of fruit bodies.

HCl OPB Level (%)	FBN	C.D (cm)	S.L (cm)	WT (g)
Control	24[f]	6.94±0.41[c]	2.83±0.16[b]	8.93±1.53[b]
0.1	541[e]	5.96±0.23[e]	2.44±0.07[e]	5.23±0.52[d]
0.2	591[d]	7.75±0.29[a]	3.00±0.12[a]	9.02±0.79[a]
0.3	621[c]	7.08±0.23[b]	2.66±0.09[c]	7.28±0.55[c]
0.4	687[b]	6.39±0.23[d]	2.42±0.08[e]	5.98±0.50[d]
0.5	705[a]	7.14±0.20[b]	2.53±0.08[d]	7.34±0.54[c]

FBN= Fruits Body Number, CD= Cap Diameter, SL= Stipe Length, Wt=Weight, Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

Table 3. Productivity and biological efficiency of fruit bodies.

HCL OPB Levels (%)	Dry with substance	Productivity	Biological efficiency (%)
Control	1400[a]	865.02±0.00[f]	61.79±0.00[f]
0.1	1400[a]	1002.56±0.00[e]	71.64±0.00[e]
0.2	1400[a]	1428.42±0.00[d]	102.03±0.00[d]
0.3	1400[a]	1661.66±0.00[c]	118.69±0.00[c]
0.4	1400[a]	1735.10±0.00[b]	132.41±0.00[b]
0.5	1400[a]	1799.10±0.00[a]	137.97±0.00[a]

Means followed by the same alphabet within column are not significantly different by DMRT (P≤0.05), means ±SEM (n=3).
Source: Authors

important factor that can influence the morphological characteristics of mushroom fruit bodies. For instance, in an experiment to determine the yield of *P. ostreatus*on other agro-waste components, Okwulehie and Okwujiako (2008) reported that *Pennisetum* straw significantly raised the stipe length of the studied oyster mushroom, followed by *A. gayanus* and *Oryza* straws. They also noted that *Panicum* straw causes a reduction of the cap diameter. Okoi and Iboh (2015) noted that different substrates have an effect on the pileus diameter, stipe length and stipe girth. Other factors can also affect the general size of a mushroom fruit body. Ogbo and Okhuoya (2009) reported that crude oil has a significant effect on the macro-morphological characteristics such as pileus diameter, stipe height, stipe girth and fresh weight of mushroom carpophores. A relatively smaller mushroom cap is an undesirable market quality (Yang et al., 2002). Apart from number of fruit bodies which had a direct correlation with productivity, HCl did not significantly affect other morphological characters studied (Table 3).

Table 4. Vitamin concentrations (mg/100g) of fruit bodies.

HCL OPB Levels (%)	Vitamin A	Vitamin B_1	Vitamin B_3	Vitamin K	Vitamin C	Vitamin E
Control	0.97±0.07^a	9.51±1.16^c	0.42±0.05^c	0.42±0.05^d	1.84±0.07^a	0.27±0.01^a
0.1	0.63±0.05^d	1.57±0.39^e	1.68±0.16^a	0.47±0.02^d	1.78±0.06^b	0.26±0.02^b
0.2	0.38±0.05^f	5.62±0.03^d	1.14±0.09^d	0.57±0.03^c	1.65±0.03^c	0.22±0.01^c
0.3	0.81±0.07^b	9.43±0.90^c	1.51±0.29^b	0.36±0.04^e	1.65±0.04^c	0.27±0.02^c
0.4	0.76±0.06^c	16.72±0.57^a	1.18±0.06^d	1.80±0.05^b	1.65±0.04^c	0.24±0.01^c
0.5	0.45±0.07^c	11.41±0.56^b	1.53±0.12^b	2.90±0.98^a	1.65±0.01^c	0.22±0.01^d

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

Productivity and biological efficiency of *P. ostreatus* fruit bodies

Influence of HCl acid on the productivity and biological efficiency of the studied oyster mushroom indicated that increase in the percentage concentration of HCl acid from 0.1- 0.5% resulted in a significant yield increase as well as biological efficiency of the mushroom fruit bodies. As the lowest fruit body yield was recorded in the control (865.02g), OPB substrate induced with 0.5% HCl solution produced the highest quantity (1799.10g) of fruit bodies with biological efficiency of 137.97%. Rip (2010) reported that only experienced mushroom growers have been able to produce mushrooms with biological efficiency of 100% and above and this was obtained between 0.2 – 0.5% HCl OPB substrates. This result justifies the claims by Bilgrama and Verma (1992), Chang and Miles (2004), Shah et al. (2004), Okwulehie et al. (2006) and Khan et al. (2013),who reported that oyster mushrooms grow and perform optimally at slightly acidic pH6.1.

The overall yield and biological efficiency of the oyster mushroom as observed in this experiment were significantly higher than those obtained by Shah et al. (2004), who cultivated *P. ostreatus* on saw dust amended with different agro-waste, Nwoko et al. (2017), who grew *P. ostreatus* on trees logs, Okwulehie and Okwujiako (2008) and Okoi and Iboh (2015), who in their separate investigations cultivated oyster mushrooms on different agro-waste components.

The fact here is that HCl acid optimized the pH of OPB substrate, which was initially found to be alkaline, to support the growth and productivity of the mushroom studied. High alkalinity of OPB substrate could be the major reason Achufusi (2016) could not record any fruit body production when he attempted to grow *P. ostreatus* on the substrate, but instead observed heavy contamination by *C. cinerius.* Although there were traces of contamination by *C. cinerius* in our study, which was high in control, it reduced gradually until none was found in 0.4 and 0.5% HCl concentrations. This is in line with Okwulehie et al. (2018), who reported no contamination due to *Coprinus* spp. at 0.4% HCl acid-induced OPB substrate during cultivation of *P. pulmonarius.*

Vitamin concentrations (mg/100g) of *P. ostreatus* fruit bodies

Results of the effect of HCl acid on the vitamin concentrations of *P. ostreatus* fruit bodies grown on OPB substrate are significantly (p≤ 0.05) different across the various levels of HCl acid concentration solution. Vitamin A content of the oyster mushroom was lower than the values reported by Nwoko et al. (2017) in *P. ostreatus cultivated* on various wood logs, as well as Okwuelehie and Okwujiako (2008) in *P. ostreatus* cultivated on different substrates and substrate supplementations (Table 4).

On the other hand, vitamin B_1 values as observed in this experiment were higher than those reported by the above mentioned researchers. Results of vitamin B_3, K, C and E observed in this study were richer than those reported by Okwulehie et al. (2009), but lower than those reported by Okwulehie et al. (2008). The variations in the concentration of vitamins could be due to substrate variations, age of fruit bodies and other factors inherent in the species; since most of them could be varieties of the same species (Chang, 2013; Nwoko et al., 2017; Okwulehie et al., 2009).

The appreciable vitamin contents, especially vitamin B_1, B_3 and C, are in line with the report of Maltila et al. (2004), Shibata and Demiale (2003) and Okwulehie et al. (2008), who maintained that mushrooms are rich in nutrients such as vitamins, protein, minerals, etc. In the current investigation, HCl acid did not significantly affect the vitamin concentrations of the mushrooms, compared to the control, and suggests no possible health risk when consumed by humans. Similarly, Nwoko et al. (2017) and Bobek and Galbary (2001) stated that the recommended dietary intake (RDI) of vitamins such as Retinol or Vitamin A is 200 mg; an indication that these mushroom samples meet the nutrient requirement by humans for a

Table 5. Bioactive (%) compound composition of fruit bodies.

HCL OPB levels (%)	Phenolics	Tannins	Steroids	Alkaloids	Terpenoids	Glycosides
Control	110.43±2.68^{d}	5.58±0.87	0.49±0.01^{a}	39.52±0.46^{c}	9.37±0.39^{a}	1.70±0.00^{d}
0.1	188.96±6.01^{a}	5.89±0.83^{d}	0.32±0.01^{b}	36.32±0.68^{d}	8.21±0.30^{b}	1.93±0.01^{b}
0.2	106.56±1.76^{e}	5.47±0.19^{e}	0.19±0.00^{c}	46.87±0.43^{q}	4.43±0.64^{e}	1.98±0.00^{a}
0.3	11.32±18.93^{c}	101.06±2.09^{c}	0.25±0.09^{d}	36.32±0.39^{d}	6.29±0.24^{c}	1.83±0.00^{c}
0.4	107.63±8.37^{f}	131.83±0.62^{b}	0.07±0.02^{e}	41.46±2.21^{b}	5.37±0.38^{d}	1.79±0.01^{d}
0.5	158.87±8.33^{b}	134.68±2.77^{a}	0.05±0.13^{e}	24.09±0.94^{e}	3.75±0.25^{f}	1.67±0.00^{e}

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

healthy diet.

Bioactive compound concentrations of the fruit bodies

Results showed the effect of HCl acid on the bioactive compounds composition of *P. ostreatus* fruit bodies grown on OPB substrate. It was observed that phenolics were in appreciable quantities in the fruit bodies harvested across all the treatment levels including control, followed by alkaloids. Alkaloids have a remarkable effect in animal physiology and are important in pharmaceutical companies, for drug production (Edeoga and Erieta, 2001). Edeoga and Erieta (2001) also recorded that alkaloids are stimulants and act by slowing down the action of several hormones. Phenolic, tannin, alkaloid and terpenoide concentrations in all fruit body samples were higher than those reported by Okwulehie et al. (2007). Flavonoids serve as anti- carcinogens, anti-bactarials (Hilang and Feraro, 1992); saponins are used inthe prevention of parasitic fungal diseases (Edeoga and Erieta, 2001); while tannins have been reported as anti-tumour agents and perform a wide range of anti-infective actions (Haslam, 1996). The high concentrations of these important bioactive compounds in *P. ostreatus* fruit bodies from various percentage HCl-OPB substrates show that these mushroom samples may be useful in the production of certain pharmaceutical active ingredients (Okwulehie et al., 2007) (Table 5).

The obtained values were significantly higher than those reported by Onyeizu et al. (2017) and Okwulehie et al. (2009) in experiments involving *P. pulmonarius* cultivated on different wood logs and agro-waste, respectively. Tannins, terpenoids, and glycosides were also in moderate quantities, but higher than the values obtained by Okwulehie et al. (2007) in an investigation to determine the pharmaceutical and nutritional benefit of two wild macro-fungi found in Nigeria. Nwoko et al. (2016) also obtained lower concentrations of bioactive compounds in *P. ostreatus* cultivated on deciduous trees logs. The high quantities of these physiologically important compounds in the fruit bodies were not due to HCl acid, which served as a buffer to the substrate; but could, however, be attributed to variation in substrates. This justifies the position of Change and Miles (2004) who assert that the nutritional composition of mushrooms to a large extent depends on the substrate where the mushroom was grown. A considerable pharmacological activity of mushrooms is the major reason for their high demand for drug development in pharmaceutical industries (Okwulehie et al., 2007, 2008). Nwoko et al. (2016) further asserted that most bioactive compounds, which play essential roles in human and animal physiology, have been found in many mushrooms. This observation alone has justified the resources committed to this investigation. From 0.3 to 0.5% HCl concentration, there seems to be a gradual increase in the quantity of tannins from 101.06±2.09 to 134.68±2.77%. This could be particularly due to increase in the concentration of HCl acid. Tannins inhibit pathogenic fungi and also reduce the rate at which herbivores graze on plants (Okwuehie et al., 2007; Haslam, 1996). This could no doubt be attributed to the reason for a constant increase in fruit body production as the concentration of HCl acid increased from 0.1 – 0.5% (Okwulehie et al., 2018).

Proximate composition of fruit bodies

Proximate composition of fruit bodies of *P.ostreatus* showed that there was a significant (p≤0.05) difference among different levels of treatment compared to control. Values obtained in all the studied parameters (MC, Ash, EE, CF, protein and CHO) were relative to the values obtained by various researchers such as Okwulehie et al. (2008), Sharad (2013), Pathmashini et al. (2008), Syed et al. (2009), Araujo Silva et al. (2011) and Okoi and Iboh (2015). Hydrochloric acid appears to have a reduction effect on the CHO content of the oyster mushroom, but tends to increase protein from 2.19±0.00% low, in control to 24.98±0.03% high, in fruit bodies obtained from 0.5%

Table 6. Proximate composition (%) of fruit bodies.

Hcl OPB level (%)	MC	Ash	E.E	C.F	Protein	CHO
Control	8.44±0.01^{c}	2.97±0.04^{a}	2.44±0.01^{b}	3.56±0.03^{bc}	2.19±0.00^{e}	80.09±0.09^{a}
0.1	8.45±0.01^{c}	2.62±0.03^{c}	2.39±0.02^{c}	3.42±0.08^{c}	3.10±0.02^{d}	79.93±0.16^{b}
0.2	8.84±0.27^{bc}	2.21±0.04^{d}	2.16±0.04^{d}	2.98±0.03^{e}	19.98±0.0^{c}	63.83±0.29^{c}
0.3	8.94±0.26^{bc}	2.64±0.26^{bc}	2.59±0.06^{a}	3.38±0.14^{d}	21.95±0.06^{bc}	60.50±0.69^{d}
0.4	8.81±0.15^{b}	2.60±0.01^{bc}	2.45±0.06^{b}	3.58±0.02^{b}	21.13±0.02^{b}	61.42±0.25^{e}
0.5	9.07±0.15^{a}	2.77±0.06^{b}	2.52±0.04^{a}	3.94±0.06^{a}	24.98±0.03^{a}	56.72±0.33^{f}

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

Table 7. Mineral concentrations of fruit bodies

HCL OPB level (%)	Na	K	Cl	Ca
Control	140.78±4.19^{d}	4.13±0.10^{cd}	111.00±3.39^{c}	11.23±0.20^{d}
0.1	146.13±2.09^{c}	4.23±0.15^{c}	103.25±2.14^{d}	11.50±0.60^{cd}
0.2	147.18±1.28^{b}	5.00±0.18^{a}	112.00±3.39^{bc}	11.98±0.43^{c}
0.3	140.73±1.41^{d}	4.87±0.22^{bc}	112.35±0.06^{b}	12.35±0.07^{b}
0.4	149.85±6.32^{a}	4.90±0.65^{b}	112.45±0.49^{a}	12.45±0.50^{a}
0.5	147.30±5.89^{b}	5.07±0.46^{a}	112.18±0.13^{b}	12.18±0.13^{ab}

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

HCl OPB substrate. These are eventually higher than the values obtained by Okwulehie et al. (2008), Okoi and Iboh (2015) and Araujo Silva et al. (2011). Chang and Miles (2004) maintained that the high CHO contents of mushrooms is due to the high lignacellulosic compositions in the substrate where they grow; in which mushrooms were broken down using extra cellular enzymes.

The high amounts of CF protein and CHO in *P. ostreatus* fruit bodies as generally observed in this study have been attributed to the type of substrate and to a large extent, mushroom species (Nwoko et al., 2016). This further elucidates the claims by Obodai et al (2003), Adejoye and Fasidi (2009) and Okoi and Iboh (2015), who in separate investigations noted that the nutritional composition of mushrooms could reflect the chemical composition of the substrate used, as mushrooms are capable of carrying out extra-cellular digestion of the decomposed substrate during cultivation (Table 6).

The high nutritional composition of oyster mushroom cultivated on HCl induced OPB substrate does not only reveal the readily available agro-waste as a good substrate for mushroom cultivation (Lisdar et al., 2011), but also suggests that HCl can help build up the protein composition of the fruit bodies.

Mineral concentrations of fruit bodies

Results showed that fruit bodies harvested from 0.4 (149.85 ±6.32)-0.5% (147.30±5.89) HCl OPB substrates had appreciable concentrations of sodium while control (140.78±4.19) was among the lowest (Table 7).

The concentration of potassium was highest (5.07±0.46) in fruit bodies from 0.5%HC;l while control (4.13±0.10mg/ 100g) was the lowest. Although there was an irregular trend in the concentration of all the studied minerals with respect to percentage HCl; nonetheless, a general consideration could infer that increase in the concentration of HCl in the OPB substrate had a positive effect on the concentration of the studied minerals. These observations conform the report of Egwin et al. (2011) who maintained that the relative higher concentration of mineral nutrients in mushroom fruit bodies may be due to the absorption and accumulation of elements from their habitat. This is contrary to the observations by Adam and Duncan (2002), who noted that crude oil, had a decreasing effect on the minerals studied in mushroom fruit bodies implicated in a mycoremediation experiment. They noted that the observed effect could be due to crude oil which acts as a physical barrier preventing or reducing access of fruit bodies to nutrients. Sudheep and

Table 8. Heavy metal concentrations of fruit bodies.

HCL OPB level (%)	Zinc	Fe	S e	Pb	Cu
Control	154.79±1.15^{c}	167.43±2.11^{f}	0.90±0.02^{a}	0.08±0.01^{b}	0.52±0.02^{d}
0.1	150.97±3.55^{f}	197.70±2.10^{a}	0.84±0.00^{bc}	0.08±0.04^{b}	0.61±0.02^{c}
0.2	159.00±1.45^{d}	176.70±3.20^{e}	0.86±0.02^{b}	0.09±0.03^{b}	0.77±0.01^{b}
0.3	166.63±1.29^{c}	191.23±4.47^{c}	0.81±0.02^{cd}	0.12±0.11^{ab}	0.79±0.02^{b}
0.4	169.10±1.44^{b}	184.70±0.62^{d}	0.82±0.0^{c}	0.17±0.10^{a}	0.81±0.03^{ab}
0.5	181.07±1.22^{a}	194.30±1.01^{b}	0.94±0.01^{ab}	0.14±0.04^{a}	0.86±0.02^{a}

Means followed by the same alphabet within column are not significantly different by DMRT (p≤0.05), means ±SEM (n=3).
Source: Authors

Sridhar (2014) reported that mushrooms gave high potassium content while sodium, calcium and phosphorus contents were low, also in an experiment involving crude oil. It is generally believed that mushrooms are rich in mineral elements and this largely depends on the substrate where the mushroom was cultured (Okwulehie et al., 2009; Chang, 2013; Nwoko et al., 2017). HCl alone may not be responsible for the observed increase in the concentration of certain minerals studied in this investigation; since in some cases, the control is either equal or higher than other treatment groups. This, therefore, suggests that the fruit bodies could be safe for human consumption.

Heavy metal concentrations of fruit bodies

Results showed that increased in the concentration of HCl acid tends to increase the amount of Zinc in the fruit bodies (Table 8). High concentration of zinc in fruit bodies of *P. ostreatus*is seldom reported by many scientists; but there could be variation in concentration due to substrate used during its cultivation (Oboda et al.i, 2003; Adejoye and Fasidi, 2009; Okoi and Iboh, 2015). Stihi et al. (2011), Nwoko et al. (2017) and Okoi and Iboh (2015) reported lower values of zinc in oyster mushrooms cultivated on different substrates. The concentration of iron in the fruit bodies was also on the increase with increase in the percentage concentration of HCl. These values were also higher compared to those reported by Demirbaş (2001), but relative to those of Nwoko et al. (2017).

The observed increase in the concentration of zinc and iron in *P. ostreatus* fruit bodies with increase in the percentage concentration of HCl could be attributed to the ability of mushrooms to break down and utilize various recalcitrant compounds including some important environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorophenols, dioxins, dichlorodiphenyltrichloroethane (DDT), trinitrotoluene and synthetic dyes (Eggen and Sasek, 2002).

Selenium contents of the fruit bodies at all levels of HCl solution were moderate and similar to the values reported by Stihi et al. (2011). This shows that HCl had no significant effect on the accumulation of these important heavy metals in the oyster mushroom. Wermer and Beelman (2002) noted that many mushrooms are now being fortified with genes capable of synthesizing compounds with selenium, regarding its importance in human nutrition.

The concentrations of lead and copper in the fruit bodies were also directly proportional to the percentage of HCl used in the optimization of the pH of the OPB substrate. The obtained values conform to those reported by Stihi et al (2011), Nwoko et al. (2017) and Demirbaş (2001).

Heavy metal contents obtained in this investigation are within the admitted maximum level of certain contaminants in foodstuffs as established by the commission of the European communities (commission Regulation [EC] No 466/2001). The acceptable maximum level for Pb and Cd is set about 2 and 3 mg/kg d.w, in cultivated mushrooms. Apart from Pb, other heavy metals studied in this research are of high nutritional importance. Although, HCl slightly elevated the concentration of Pb, but its highest concentration at 0.5% could only increase Pb to 0.14±0.01, which falls below the European commission of regulation limit.

Conclusion

The experiment was successfully conducted to evaluate the influence of pH on the fruiting duration, some macro-morphological characters and productivity of *P. ostreatus* fruit bodies cultivated on acid-induced oil palm bunch substrate.

Hydrochloric acid induced changes on the pH of the substrate towards acidity; that is, from 9.0 in control – 6.1 in 0.5%. Hydrochloric acid delayed fruit body production from 17 days in control – 19 days in 0.5%.

Increase in the concentration of HCl acid in the OPB

substrate from 0.1 - 0.5% inhibited substrate contamination due to *C. cinerius,* and enhanced fruit body yield. This indicates that HCl acid acted as a suitable buffer for the optimization of pH of the OPB substrate.

Hydrochloric acid had no significant (p≥.0.05) effect on the macro-morphological characters of the fruit bodies studied, while increase in the concentration of HCl acid supported more fruit body production as well as biological efficiency.

Hydrochloric acid had no significant (p≥.0.05) effect on the macro-morphological characters of the fruit bodies studied, while increase in the concentration of HCl acid positively affected the number of fruit bodies produced which correlates with yield and biological efficiency. Hydrochloric acid had a significant positive effect on vitamins B_1, B_3 and K concentrations in the fruit bodies. But such could not be said of vitamins A, C and E. There was a percentage increase in tannins with increase in the concentration of HCl acid, unlike other bioactive compounds studied.

Protein concentration of fruit bodies was significantly increased with increase in the concentration of HCl; while carbohydrate contents of fruit bodies decreased with increase in the concentration of HCl acid. Other studied nutrient parameters were not significantly affected by HCl acid.

All the mineral nutrients studied were not significantly affected by HCl acid when compared to their control values. The concentration of Zn, Fe, Pb and Cu increased with increase in the concentration of HCl acid; while Se was not affected. The concentration of the studied heavy metals was found within the acceptable limit for human consumption and safety as justified by the commission of the European communities (Commission Regulation [EC] No 466/2001).

Recommendations

Commercial mushroom growers should avail themselves of this golden opportunity and ensure effective utilization of OPB incorporated with HCl acid for higher fruit body production as well as profit maximization. Other mineral or organic acids should be sourced for and studied in a related experiment. This could provide a cheaper or more efficient alternative to HCl acid.

Further research studies should consider upward adjustment of the percentage concentration of HCl acid. This would help obtain the acid solution level that would give the optimum pH of the OPB substrate.

Finally, ready-to-use OPB substrate produced by this method should be commercialized to enable mushroom farmers and other intending mushroom growers produce large quantity of mushrooms involved in acid dilution and pH optimization without much stress.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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