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Research Article

CHARACTERIZATION OF ETHIOPIAN DURUM WHEAT LANDRACES FOR RESISTANCE TO STRIPE RUST DISEASE AND AGRONOMIC TRAITS

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ABSTRACT. The research was conducted to identify Ethiopian durum wheat accessions possessing effective stripe rust resistance genes and estimate the genetic variation for agronomic traits. The study investigated 142 accessions and two improved cultivars in greenhouse and field conditions. Six *Puccinia striiformis* f.sp. *tritici* (*Pst*) isolates were employed to evaluate the accessions at the seedling stage in the greenhouse. The field trial was conducted using an alpha lattice design with two replications. The study highlighted the presence of significant variation among accessions. In the seedling test, 26.1% of accessions showed resistance, 35.91% intermediate, and 38.0% susceptible reactions to *Pst* isolates. The field response of the accessions to *Pst* infection indicated that 18.3% had low terminal disease severity (TDS) scores, while 18.7% had average coefficient of infection (ACI) values < 20%; and 26.8% exhibited AUDPC values < 500. A low disease progress rate with infection values ≤ 1 was observed on 4.2% of the accessions. The ANOVA computed for all agronomic traits showed highly significant differences, indicating the presence of variation among accessions. The H^2_b estimate ranged from 8% (harvest index) to 92% (above-ground biomass). Genetic advance as a percent of the mean varied from 3% for days to maturity to 490% for above-ground biomass. Multivariate analysis grouped the 144 durum wheat genotypes into six clusters, while the first five principal components explained 73.1% of the total variance. This study has demonstrated the presence of diverse genetic resources of durum wheat accessions, among which 14 landraces showed high levels of adult plant stripe rust resistance.

Keywords: *Adult plant resistance, Cluster analysis, Genetic advance, Heritability, Seedling resistance*

INTRODUCTION

Durum wheat *(Triticum turgidum* L.*)* is indigenous crop to Ethiopia [1]. Ethiopian farmers have cultivated durum wheat since time immemorial [2]. Due to an amazing wealth of genetic variability of durum wheat, Ethiopia is also recognized as the center of genetic diversity for this crop [3]. It is traditionally grown by small-scale farmers on heavy black clay soils (vertisoils) at altitudes ranging from 1800–2800 meters above sea level, exclusively under rain fed conditions [4]. Durum wheat covered about 30% to 40% of the wheat growing area, decreasing from 85% in 1967 to 60% in 1999 [5].

Landraces of wheat and rice were largely cultivated until the first decades of the twentieth century, being progressively abandoned from the early 1970s and replaced with improved, genetically uniform semi-dwarf cultivars because of the Green Revolution [6]. However, scientists believe that landrace cultivars represent an important group of genetic resources for improving commercially valuable traits [3]. There is therefore renewed interest in wheat landraces and primitive (obsolete) cultivars as an important source of genetic variation, mainly because the trend towards greater uniformity has narrowed the genetic basis of modern wheat cultivars and increased their vulnerability to biotic and abiotic stresses. Systematic evaluation of the level and structure of genetic variability in crops is a prerequisite for plant breeding and genetic resource conservation programs.

The low productivity of durum wheat in Ethiopia is attributed to several factors, including biotic (diseases, insect pests, and weeds) and abiotic stresses; moisture stress, low soil fertility, recurrent drought and other factors [7]. Among these factors, fungal diseases play a significant role in yield reduction. Of these, fungal diseases like rusts (stem, stripe, and leaf rusts), fusarium head blight (*Fusarium spp*.), septoria blotch (*Septoria tritici*), common root rot (*Helmenthosporium* spp.), and tan spot (*Pyrenophora tritici-repentis*) are the dominant ones that were reported over times [8].

Stripe rust caused by a fungal pathogen *Puccinia striiformis* f.sp. *tritici* is a very important disease of wheat, particularly in North America, Central and West Asia, Australia, North Africa and East Africa. Crop losses can be severe (50 - 100%), due to damaged plants and shriveled grain [9, 10].

Over the last 10 years, stripe rust was much more damaging in Ethiopia [11]. Stripe rust reduces the yield and quality of grain and forage. Seed produced from crops damaged by stripe rust has low vigor and thus poor emergence after germination. Stripe rust can cause 100% yield losses if infection occurs very early, and the disease continues developing during the growing season. Thus, stripe rust caused crop losses amounting to several hundred million dollars and affected the livelihoods of millions of poor farmers in durum wheat growing areas [12]. However, very little study has been done for strip rust resistance in Ethiopia.

Therefore, the present study was undertaken to exploit the benefits of Ethiopian durum wheat landraces by identifying genotypes possessing effective all stage resistant (seedling resistant) and adult plant resistant (slow rusting) stripe rust resistant genotypes with good agronomic performance so that they can be used as sources of stripe rust resistance genes to be deployed to modern cultivars to combat the never sleeping ‘rust’ pathogen.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted at Kulumsa Agricultural Research Center (KARC). The geographical coordinate of its location extends from 8º00' to 8º 02' northern latitude and from 39º 07' to 39º 10' eastern longitude and covers a total area of 442.7 ha. The location is elevated at 2200 m.a.s.l. and has average annual minimum and maximum temperatures of 10 ºC and 22 ºC. It had a mid-day relative humidity of 60-70% with annual rainfall of 840 mm. The soil type is characterized as clay soil [13].

Plant Materials

Durum wheat accessions (142 landrace accessions) were received from Ethiopian Institute of Biodiversity in Addis Ababa (Supplementary Table 7). In addition, seeds of two reference resistant durum wheat cultivars, namely, Quamy and Alemtena obtained from KARC were used for field experiments. Susceptible wheat cultivars “Kubsa” and “Local red” also obtained from KARC were used for spreading the disease in the field by artificial inoculation. For greenhouse experiment additional ten commercial wheat cultivars (Digelu, Danda’a, Hidase, Kakaba, Kubsa, Kingbird, Wane, Morocco, Shorima and Local red) received from KARC, and used for pathogenicity study.

Pathogen Material

The pathogen isolates used in this study were obtained from freshly cultivated spores of six dominant isolates preserved in KARC. Although the isolates were not properly characterized based on their respective race groups, they were grouped in to different classes based on their origin and pathogenicity variability as Digelu isolate, Hidase isolate, Kubsa isolate, Kakaba isolate, K-62954A and Ogolcho isolates. After isolating them to keep their virulence and viability, the isolates were used to evaluate the resistance of durum wheat landrace accessions using seedling and adult plant inoculation tests. The isolates were selected from six *Pst* samples originally collected from five testing sites (Aris Robe, Kofele, Kulumsa, Meraro and Oromia Arsi Sera).

Greenhouse Experiment

Seedling tests were carried out for stripe rust disease to identify seedling resistant genotypes available in the host accessions. A total of 142 durum wheat accessions and susceptible check (Local red) were inoculated with the bulked (mixture) of the six virulent isolates and each isolate spores of yellow rust (Digelu isolate, Hidase isolate, Kubsa isolate, Kakaba isolate, K-62954A and Ogolcho isolates) at seedling stage (flag leaves) in the greenhouse facility at Kulumsa Agricultural Research center. Five seeds of each accession and the susceptible check were sown in a 9 × 9 cm black square plastic pots filled with soil: compost: sand in a 2:1:1 (v/v/v) ratio and allowed to grow in a greenhouse compartment at 16-18°C with two replications. The test included a susceptible durum wheat (local red) cultivar to serve as positive control for successful inoculation and infection establishment. A week later, the pots of seedlings were placed together on a sample tray and inoculated with ~6 mg of fresh spores suspended in 10 ml of mineral oil in a gelatin capsule using a vacuum pump sprayer. After drying the oil for about five minutes in an open-air, the seedlings were moisturized with a fine spray of water, incubated at temperature 8-10°C and 100% RH for 24 hours of darkness [14].

The seedlings were then transferred to the greenhouse compartment at a temperature of 18-22°C. Seedling reaction in terms of Infection Type (IT) was evaluated 14-16 days after inoculation using 0-9 scale of McNeal et al. [15]. Accessions with 0-3 IT score were considered as resistant, 4-6 as intermediate, and 7-9 as susceptible. Frequencies of various response groups were determined by analyzing the number of accessions having resistant (IT: 0-3), Intermediate (IT: 4-6) and susceptible (IT: 7-9) reactions among the tested accessions [16]. The durum wheat accessions with resistant reactions to all six isolates were also identified by aligning the respective IT values of each accession across isolates.

Field Experiment

Experimental Design and Treatments

The study was carried out at Kulumsa Agricultural Research Center in the field. The experimental design for this study was an alpha lattice design with two replications. This design is known to be effective for screening the large number of accessions for disease resistance [17]. In each replication 142 lines of the durum wheat accession and two standard check (Quamy and Alemtena) cultivars were planted in 50 cm by 40 cm plot, where in each replication each durum wheat accession was presented only once. Each plot contains twenty seeds of wheat planted in 2 cm space between plants. A mixture of ‘Local red’ and ‘Kubsa’ seeds of susceptible durum wheat cultivars at equal proportions were planted to be used as spreader rows for the experimental materials.

Methods of Inoculation in The Field

Inoculation of plants was carried out at the booting stage [18] determined by the Zadock scale of developmental stages [19]. The stripe rust epidemic was initiated by inoculating spreader rows with the mixture of virulent isolates as a proportion of 5 grams per hectare. Techniques for inoculum production, collection, storage, and inoculation followed the standard guideline described by Roelfs et al. [20]. Inoculation of plants in the field was carried out using a sprayer with water or mineral oil suspension of urediospores in the late afternoon.

The experimental field was sprayed with water using an automated sprayer before inoculation to facilitate spore germination on plant leaves. Recommended agronomic practices such as fertilizer application, weeding, and watering for wheat production were done following the field trial protocols of KARC [21].

Data Collection

Disease Assessment

Disease assessment in the field was carried out using disease severity measurement and scoring for infection type. Disease severity was recorded using the modified Cobb scale to determine the percentage of possible tissue (100%) rusted according to a 0 to 9 ratings where 0% = immune and 100% = completely susceptible [22].

Infection type for seedling and adult plant resistance tests was scored by using a 0-9 scoring descriptions by McNeal et al. [15]. Deviation within the specific infection type were expressed as “+” and “-“signs.

Measurement of Agronomic Parameters

The data on the 10 agronomic traits (Number of Days to heading, Number of Days to maturity, Plant height, Number of tillers, Spike length, Number of kernels per spike, Number of grains in main tiller, thousand (1,000) kernel weight, Grain yield, Harvest index, Above ground Biomass) were recorded in the field experiments following the methodology described in Muhder, et al. [23].

Data Analysis

Disease severity data was summarized to produce ACI, AUDPC, and disease progress rate (r). *The AUDPC* with multiple severity readings was calculated for each plot by taking the development of stripe rust disease (severity) in fixed interval of time (7 days) measured in Unit-days following the method used by Wilcoxson et al. [24]. *Disease Progress Rate:* Rate of stripe rust increase (r-value) as a function of time was estimated using the coefficients of linear regression by taking the slope of the line [25]. The value was determined for each landrace accession per replication over successive disease severity recording periods (7 days interval) using the lme4 package of R environment [26].

Coefficient of infection (CI): Was calculated by multiplying the level of disease severity and the constant value of infection type. The constant values for infection types were used based on the procedures described in Stubbs et al. [27]; where, immune = 0; resistant = 0.2; moderately resistant = 0.4; moderately susceptible = 0.8; and susceptible =1.

The resulting values were used to compare differences across wheat genotypes. The maximum likelihood estimation method of the alpha lattice design model was used to analyze data using agricolae package [28] in R platform [29]. The estimation method produced the ANOVA table, the standardized and fitted value of the model, F- statistics, means and other relevant statistics to check model adequacy. Separation of means was also conducted using LSD at 5% significance levels.

Data Analysis for Agronomic Traits

All recorded data were subjected to analysis of variance (ANOVA) using proc lattice and proc GLM of SAS software version 9.0 [30] and treatment means were tested as significant at the 5% probability level and as highly significant at the 1% probability level. Mean comparison among genotypes were carried out using LSD at 5% probability levels.

The phenotypic and genotypic variance and coefficient of variation were estimated according to the methods suggested by Burton and Devane [31]. Heritability in broad sense ($h^2{}_b$) and Genetic advance for all traits was computed using the formula adopted by Allard [32].

Multivariate analysis was performed to discriminate the 142 durum wheat landrace accessions and the two standard checks based on their mean performances for disease and agronomic traits using hierarchical clustering and principal components analysis. Principal components having eigenvalue at least 1 were retained for analysis. A correlation matrix was used to define the patterns of variation among landraces for both disease and agronomic traits using the Genstat-16th edition SP1 statistical package for the principal components analysis. For cluster and principal components analysis the values for each trait were standardized to unit variance and zero mean. Hierarchical clustering was performed using a numerical measure of similarity computed from standardized data, using Minitab version 17.0 software package, to assess the patterns of diversity among the landraces and identify the traits that contributed for the largest proportion of variation. The standard genetic distances from the portion of phenotypic classes were used to construct a dendrogram by the unweighted pair group method based on arithmetic average (UPGMA).

RESULTS AND DISCUSSION

Greenhouse Seedling Evaluation for Wheat Stripe Rust Resistance

Results from the greenhouse experiment showed that the durum wheat genotypes varied in their reaction to the dominant *Pst* isolates (Supplementary Table 1). The resulting data ranged between infection types ‘0’ and ‘9’. In total, 37 (26.1%) of the accessions were grouped to the resistant category (IT: 0 - 3), 51 (35.9%) were in the intermediate (IT: 4 - 6) and the remaining 54 (38.0%) were susceptible (IT: 7 - 9) to stripe rust that was comparable to the standard susceptible check cultivar ‘Local red’. The result showed that the resistant and intermediate classes might have possessed one or more effective seedling resistance (all-stage resistance) genes. However, it was not possible to disclose /postulate the possible number and identity of gene(s) conferring resistance to the stripe rust isolates due to the absence of differential lines specific to each of the *Pst* isolates.

From the first class (IT: 0 – 3), 36 of the accessions demonstrated incompatible disease reaction to all *Pst* isolates used to evaluate the durum wheat accessions. Similarly, from the 2nd class (IT: 4 – 6), 40 of the accessions showed incompatible disease reaction to all the isolates used to test the host genotypes. This implies that 76 out of the 142 accessions exhibited presence of effective ASR genes in their genome while the remaining 54 accessions did not. It is expected that the accessions grouped in the 1st class (IT:0 – 3) that showed low IT to all *Pst* isolates could exhibit high level (accepted level) of field resistance since ASR genes remain resistant in all the growth stages of the plant [33]. On the other hand the accessions grouped in the 3rd class may remain susceptible if they do not possess APR (adult plant resistance) genes in their genetic makeup.

Since APR/ slow rusting genes cannot be detected at seedling stage, their presence can only be manifested/expressed in the field evaluation at the adult stage (after heading stage) of the crop's life cycle [33, 34, 35]. Hence, it can be surmised that those accessions exhibited compatible disease reaction at seedling stage but demonstrated significant level of field resistance in the field assessment for resistance to stripe rust could be categorized for possessing APR genes [36, 37, 38].

Regarding the pathogenicity of the five *Pst* isolates the result showed that Hidase isolate was the most virulent race making about 58 (41%) of the 142 accessions susceptible. Kubsa isolate was the 2nd most virulent becoming virulent to 55 (39%), Degalu isolate was the 3rd with 54 (39%), the 4th one was Kakaba isolate with 53 (37%) and the last one was Ogolcho isolate with 51 (36%) virulence. Overall, the data showed low variation among the pathogenicity of the *Pst* races with average virulence to 54 (38%) of the accessions. Alemu, et al. [16] evaluated 300 durum wheat lines (accessions & cultivars) using three virulent isolates (*Pst_Is1*, *Pst_Is4* and *Pst_Is8*) for seedling resistance to stripe rust and obtained a highly resistant infection type (IT: 0 -3) to *Pst_Is1, Pst_Is4,* and *Pst_Is8* to 59.3%; 67.3%; and 46.3% of the lines, respectively. They reported the presence of *Yr7*, *Yr15* and *YrSp* in 81.7%, 88.3% and 0.7% of the lines, respectively. Zeray, et al. [39] reported postulation of ASR genes for stripe rust viz. a viz. *Yr9, Yr17, Yr18, Yr26, Yr29, Yr36, Yr44* and *Yr62* in a set of Ethiopian wheat cultivars and breeding lines. They also obtained gene combinations for these all stage resistance genes that could be used for improvement of the wheat breeding program of the country and the world at large.

Field Evaluation of Accessions for Adult Plant Resistance to Stripe Rust

The Analysis of Variance (ANOVA) conducted for the data obtained for AUDPC, ACI, and TDS showed highly significant ($p<0.001$) differences among accessions (Supplementary table 3). This indicated the presence of sufficient genetic variability for the level of resistance/susceptibility among the genotypes investigated. Adult plant resistant genotypes were identified on the bases of their AUDPC, TDS and ACI values obtained in the field evaluations experiments conducted to assess the presence of resistance to specific diseases. The low ACI values recorded on several accessions indicated the presence of partial resistance to stripe rust in several durum wheat landrace accessions and improved cultivars that have been evaluated. Partial resistance conferred by adult plant resistance is considered durable although it is influenced by changes in environmental conditions [20, 40].

Terminal Disease Severity

Results of ANOVA for terminal disease severity showed a highly significant difference at $p<0.001$ among the durum wheat accessions evaluated in the field (Supplementary table 3). According to Herrera-Foessel, et al. [41], TDS values of the landrace accessions were grouped into three based on the level of partial resistance to diseases. Consequently, they are classified as high, moderate and low levels of partial resistance having 1 - 30%, 31 - 50% and 51 - 70% of terminal disease severity, respectively. Out of the 142 durum wheat accessions 26 (18.30%) displayed 1 - 30% high levels of partial resistance, 39 (27.46%) displayed 31 - 50% moderately resistant level of partial resistance. The remaining 77 (54.22%) accessions displayed susceptible reactions as the TDS values observed was above 50% severity (supplementary Table 2). According to Nzuve et al. [42], the available resistance genes in these materials overcame the stripe rust virulence in the field and led to statistically low disease severities despite the compatible host-pathogen reactions. Previously, Ali et al. [43], Tabassum [44] and Safavi [45] also used the terminal rust severity data to assess adult plant resistance behavior of wheat lines.

Average Coefficient of Infection

According to Ali et al. [46], accessions with ACI values of 0-20, 21–40, and 41–60 are regarded as genotypes possessing high, moderate and low levels of partial resistance to yellow rust whereas those with ACI > 60 were considered as moderately susceptible to susceptible, respectively, as compared to the response of the check cultivar to stripe rust. It was observed that 28 (18.72%) accessions exhibited a high (ACI, 0-20) level of partial (field) resistance against stripe rust, 30 (21.13%) accessions showed moderate level of field resistance, and 35 (24.65%) accessions showed low level of resistance whereas the remaining 49 (34.50%) were considered susceptible that was comparable to the susceptible check (supplementary Table 2). When the standard susceptible check cultivar score reached 100% for disease severity, then the disease progress ceased for majority of the accessions [27]. The susceptible cultivar that was used as spreaders of *Pst* spores or source of inoculum (‘Local red’ mixed with ‘Kubsa’) displayed the highest disease severity of 100% with completely susceptible (S) responses, indicating that an acceptable epidemic pressure was established over the season for field experiment. Wagoire *et al.* [47] conducted similar study on adult plants of different wheat lines for field resistance to *Pst.* Some of these lines were thought to possess different *Yr* genes, conferring partial resistance, some of these lines were VHC (BD) 2, VAH-CW 3166, VRB-CW-2106, VHC 6178 and VHC 6185. These five wheat lines showed higher level of partial resistance under natural field conditions against the control cultivars 78S84, 46S119 and 110S119.

Area Under Disease Progress Curve (AUDPC)

Analyzed results pertaining AUDPC values of durum wheat accessions is presented in supplementary Table 2. Analysis of variance result showed highly significance difference (at $p<0.001$) between accessions in AUDPC value (supplementary Table 3). According to Ali, et al. [48] and Saleem et al. [49] accessions with AUDPC values of 1 – 500, 500–800, and 800–1100 were categorized as possessing high, moderate, and low levels of field resistance, respectively. Consequently, the accessions were categorized into three distinct groups for partial resistance, based on their AUDPC values. Correspondingly, 38 (26.76%) durum wheat accessions exhibiting AUDPC values ranging from 1-500 were grouped as high level of partial resistance, 42 (29.6%) of them exhibiting AUDPC values ranging from 500 to 800 were grouped as possessing moderate level of partial resistance. While 27 (19%) accessions marked as having a low level of adult plant resistance. Likewise, 35 (24.5%) accessions showed AUDPC value greater than 1100, considered susceptible to stripe rust (Supplementary Table 2). AUDPC is a good indicator of adult plant resistance under field conditions and directly related with level of resistance and yield loss. In quantitative resistance, where differences in level of resistance are usually less distinct, measuring disease progress is important for understanding plant–pathogen interaction [50]. Furthermore, AUDPC in particular is the result of all factors that influence disease development such as differences in environmental conditions, varieties and population of the pathogen. Varieties with low AUDPC and terminal disease severity (TDS) may have a good level of adult plant resistance. Therefore, selection of cultivars having low AUDPC with terminal disease scores is normally accepted for practical purposes where the aim is to utilize genetic resistance to wheat rusts as one of the core strategies to stripe rust management mechanisms [51].

Disease Progress Rate

The analysis of variance on infection rate showed a highly significant difference (at $p<0.001$) among the durum wheat accessions evaluated in the field (Supplementary Table 3). The highest disease multiplication with their respective infection rates (r values) were noted on accession 222553 (r=3.21) followed by accession 222353 (r=3.14), while low disease progress rate with infection rates values ≤ 1 was observed on 4.2% of the 142 accessions. The lowest r values were exhibited from accessions 222428, 222495, 203968, 222422, 226884 and 204011 (r=0.54, 0.64, 0.79, 0.82, 0.86, 0.86) respectively, compared to the resistant cultivars Alemtena and Quamy (r= 0.36, 0.54), respectively.

Resistant cultivars “Quamy” and “Alemtena” as reference showed the lowest disease severity, six of the accessions exhibited promising r - values (r <1) that is slow disease increase per unit time with an infection-rate comparable to the standard checks for stripe rust resistance (Supplementary Table 2). Similarly, Safavi, et al, [52] reported cultivars having infection rate of (0-0.57) and (0.65-0.86) ranked as high and moderate level of partial resistance.

Variability in Agronomic Parameters for Ethiopian Durum Wheat Accessions

Analysis of variance showed that highly significant ($P<0.001$) differences were observed among the genotypes for all agronomic traits (Supplementary table 4). This result indicated that there is variability among the genotypes studied and would respond positively to selection. Several researchers reported significant differences among wheat landrace collections. Similarly, the study conducted by Ayalew [53] on 15 durum wheat commercial cultivars showed presence of significant variation among the cultivars for majority of yield and yield related traits except plant height. Shashikala [54] reported significant differences among 169 landrace genotypes for 11 morphological traits such as days to 50% heading, days to 75% maturity, plant height, spike length, peduncle length, number of tillers per m^2, number of spikelets per spike, 1000 grain weight, grain protein content and grain yield per plot. Thus, these studies demonstrated the presence of sufficient variability in the materials used for their respective studies that would provide numerous possibilities for selecting superior and desired genotypes by the plant breeders for further improvements.

Estimation of Variability Parameters

The presence of high epidemics of stripe rust magnified the variance due to environment ($\sigma^2 e$) compared to the variance due to genotypes (σ^2g) as displayed in Table 1. The corresponding values of σ^2e were greater than σ^2g values for all traits except TKW (Table 1). High genotypic coefficient of variation (247%) was observed for AGBM followed by GY (53%) and NTPP (18%). Phenotypic coefficient of variation (PCV) was also followed similar trend with GCV i.e. the highest value was obtained for AGBM (258%) followed by NTPP (81.5%) and GY

(65%). Stripe rust is known to cause heavy damage on the vegetative performance of the plant since it occurs early and mainly affects the leaf, which is the food factory that could stunt the overall growth of the plant and result shriveled seeds. This finding clearly showed the impacts of the presence of high epidemics of stripe rust disease as described by several researchers [55]. Stripe rust causes loss of quality and yields of grain by reducing the size, number, and weight of kernels per spike; reduces the amount of dry matter of the plant by decreasing plant height, root growth, size, and number of flowering spikes. Moreover, seeds obtained from stripe rust attacked plants show reduced plant vigour and poor emergence [56].

Heritability estimates along with genetic gain would be more useful for selecting the best individual. It is the proportion of the genetic variance to the total variation. The broad sense heritability estimate for the durum wheat accessions ranged from 8% (HI) to 92% (AGBM). Majority of the traits exhibited medium levels (30% – 60%) of heritability (Table 1). The performance of the genotypes with respect to heritability estimates indicated the impact of their differences in genetic resistance to stripe rust. Generally, heritability determines the effectiveness of selection. The effectiveness of selection for a trait depends on the relative magnitude of the genetic and environmental factors in the expression of phenotypic differences among genotypes in the population.

Genetic gains that was estimated by selecting 5% of the genotypes, as a percent of the mean (GAM), varied from 3% for the days to maturity to 490% for above ground biomass. Genetic advance (GA) ranged from 0.05 for spike length to 8.52 for plant height. The low values of expected genetic advance for the traits like number of tillers per plant, above ground biomass, and grain yield (despite having high heritability values) is due to the low variability for the trait determined by the low genotypic and phenotypic variance (Table 1). The impact of stripe rust is reflected here as it excluded majority (>73%) of the genotypes from being selected as they showed moderately susceptible to susceptible response to *Pst* (Supplementary Table 2). This indicates the importance of genetic variability for crop improvement through selection. Therefore, even if heritability estimates provides the basis for selection on phenotypic performance, the estimates of heritability and genetic advance should always be considered simultaneously, as high heritability is not always associated with high genetic advance [57].

Table 1. *Estimates of genetic variability parameters for the 142 durum wheat accessions using the 10 agronomic traits*

Traits	σ^2**e**	σ^2**g**	σ^2**p**	**GCV%**	**PCV%**	**H^{2}b%**	**GA**	**GAM%**
DTH (50%)	24	16.01	40.01	5	7	40	5.21	6
DTM (75%)	49.43	15.35	64.78	3	5	24	3.93	3
PHT(cm)	75.9	45.56	121.46	10	16	38	8.52	12
SL(cm)	0.004	0.002	0.01	5	8	34	0.05	6
NTPP	0.52	0.28	0.81	18	30	35	0.65	22
NKPS	0.6891	0.24	0.93	13	25	26	0.52	13
TKW(g/pp)	17.25	16.49	41.01	15	20	40	6.78	22
GY(t ha^{-1})	0.11	0.21	0.32	53	65	65	1.2	39.6
AGBIOM(kg/pp)	0.003	0.12	0.13	247	258	92	0.71	490
HI	36.27	3.25	39.53	6	20	8	1.07	3

Phenotypic variance (σ^2p), genotypic variance (σ^2g), environmental variance($\sigma^2 e$), phenotypic coefficient of variation (PCV %), genotypic coefficient of variation (GCV %), heritability in broad sense ($\boldsymbol{H^2}$b), genetic advance in absolute (GA) and percent of mean (GAM %)

Genetic Diversity Analysis Using Disease Resistance and Agronomic Parameters

Principal component analysis and cluster analysis were computed for the five disease evaluation parameters (TDS, AUDPC, ACI, r-value and seedling test data) and 10 agronomic parameters to identify the traits that contributed most for the diversity of the durum wheat germplasm. In addition, the hierarchical cluster analysis would indicate which cluster group and genotype could be candidates for selection and hybridization activities of the Ethiopian durum wheat breeding program.

Principal Component Analysis

Principal components analysis effectively explained the variation among the durum wheat landraces with the first five principal components accounting for 73.1% of variation (Table 2). Stripe rust disease field response evaluation parameters; TDS, ACI, r-value, and AUDPC were the most important traits contributing to the variation (31.1%) explained in principal component one (PC1). These four disease severity estimation parameters scored the highest negative loading effect for the variation observed in PC1. In principal component two (PC2), which described about 13.3% of the total variance of the durum wheat landraces, DTM, DTH, PHT, HI, and GY showed large contributions. In addition, GY and HI accounted for much of the total variance with high positive loading in principal component three (PC3). AGBM and TKW contributed most to principal component four (PC4). The seedling test (ASR), DTH, DTM, PHT, and NKPS scored the largest share of variance explained (8.2%) in principal component five (PC5). Ali, et al. [58] who evaluated 64 advanced wheat lines in Pakistan during 2017 crop season reported that the first five PCs with Eigen values > 1 contributed 86.95% of the variability amongst the genotypes. They found that Characters with maximum values in PC1 were spikelets spike^{-1} (0.732), spike length (0.722) and biological yield (0.607), in PC2, 1000-grain weight (0.605), grain yield (0.482) and days to heading (DH) (0.393).

Table 2. *The Eigenvalues and vectors of the correlation matrix for 16 traits of 144 Triticum turgidum L. landraces of Ethiopia*

Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
TDS	-0.436	-0.143	0.104	-0.093	0.136
ACI	-0.433	-0.116	0.116	-0.100	0.146
r-value	-0.433	-0.163	0.097	-0.077	0.142
AUDPC	-0.426	-0.152	0.074	-0.083	0.062
ASR	0.011	0.039	0.089	-0.095	-0.422
DTH	-0.002	-0.402	0.170	-0.036	-0.530
DTM	0.088	-0.453	0.104	-0.221	-0.382
PHT	0.235	-0.353	0.246	-0.066	0.337
SL	0.171	-0.313	0.263	0.195	0.245
NTPP	0.268	-0.257	0.172	0.070	0.109
NKPS	0.195	-0.131	0.113	-0.200	0.341
TKW	0.220	0.033	-0.111	-0.420	0.147
GY	0.003	0.341	0.626	0.081	-0.033
BM	-0.038	-0.050	0.166	0.737	-0.034
HI	0.031	0.349	0.556	-0.309	-0.080
Eigen value	4.660	2.006	1.760	1.308	1.226
Proportion	0.311	0.134	0.117	0.087	0.082
Cumulative	0.311	0.444	0.562	0.649	0.731

The biplot analysis was used to clearly understand the relationship between the durum wheat landrace accessions, and the 15 traits (agronomic and disease evaluation parameters). The first two axes shown on the biplot explained 44.44 % of the total variation of the data where PC1 accounted 31.1% and PC2 scored 13.3%, which was the largest share of the total variation (Fig.1). Similar findings have been reported by Luković et al. [59], where PC1 and PC2 explained 57.31% of the total data variation. A similar proportion of PC1 and PC2 were determined by Xhulaj et al. [60] who reported that the 1st three principal components explained 66.42% of the total data variation, where the first two components accounting for the largest part of the total variance (PC1 with 28.1% and PC2 with 24.43%).

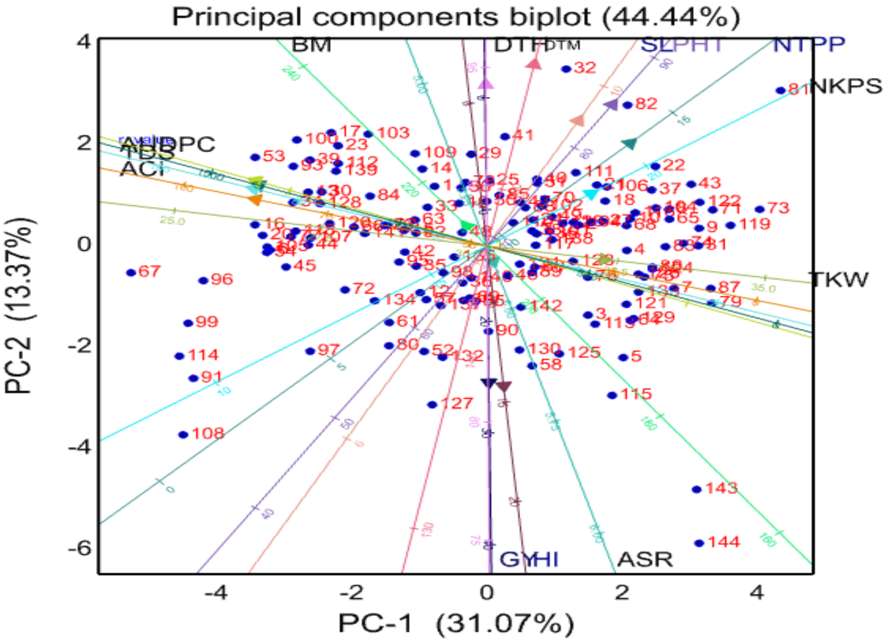

Fig 1. *Biplot analysis for the 142 durum wheat landraces plus two improved cultivars, and the 15 traits shown on the first two main components*

The disease evaluation parameters TDS, AUDPC, ACI, r-value and the agronomic trait AGBM associated with the stripe rust susceptible durum wheat landraces are located to the left of the origin (zero) of the x-axis (Fig. 1). The three agronomic parameters GY, HI and ASR were grouped along with the stripe rust resistant landraces and the two standard checks Quamy and Alemtena (coded as 143 & 144) to the extreme of Y-axis and close to zero value of the X–axis. Majority of the durum wheat landraces were distributed along with the remaining components of yield, where more resistant landraces were located further from the origin. This finding implied that improved cultivars and the stripe rust resistant landraces differed greatly from majority of the landraces. In general, the disease evaluation parameters were the most important traits for discriminating the durum wheat landraces, in which case, low scores for disease parameters, high values for grain yield, and harvest index could be the main criteria for selection of durum wheat improvement.

Cluster Analysis

The dendrogram constructed grouped the 142 durum wheat landrace accessions and the two improved cultivars in to six clusters using 75% similarity (Fig. 2). The number of accessions per cluster ranged from one (1) in cluster V to 131 in cluster I (Supplementary Table 5). Cluster I was composed of 131 landrace accessions characterized by possession of a range of significant levels of field resistance to stripe rust disease. The mean data for field response to stripe rust and seedling test infection types showed presence of resistance genes within each of the landrace genotypes. Some of the landrace accessions (36 accessions) had acceptable field resistance levels with AUDPC score of less than 500, among which 14 of them showed high level of field resistance to stripe rust. However, majority of them were low yielders, even significant amount of the accessions had poor adaptation to the environment where they were evaluated as it was expressed in the traits of fitness and fertility (grain yield and components of yield). Cluster II consisted of three (3) landraces characterized by high performance for GY, HI, TKW, SL, NTPP, NSPS, acceptable level of field resistance to stripe rust, but susceptible to seedling test for stripe rust indicating possession of two or more adult plant resistance genes.

Cluster III have two landrace accessions characterized by high level of seedling resistance (nearly immune) to stripe rust, but it showed moderately susceptible response to stripe rust in the field. It was also unique for having very high plant height (tall) and late maturing type with low grain yield potential. Cluster IV is represented by five durum wheat landraces characterized by highly susceptible disease reaction to stripe rust in the field, very low in plant height (short stature), early to intermediate maturity types and average to high yielding potential for grain production. Cluster V is membered by only one durum wheat landrace accession (accession 222451) that showed appreciable level of performance for all disease and agronomic parameters except being susceptible to stripe rust at seedling test. It differed from the improved cultivars for being tall, having highest number of tillers, long spike length, matured late, and susceptible to stripe rust at seedling test. Cluster VI is made up of the two improved cultivars (Quami and Alemtena) that displayed high resistance to stripe rust disease in the field and demonstrated moderate resistance in the seedling test, early maturing type, semi-dwarf plant height, short spike length, relatively more number of tillers per plant, higher number of seeds per spike, high thousand kernel weight, high grain yield and average weight of biomass yield. Similar study conducted by Ali, et al. [58] grouped 64 genotypes of durum wheat into four main clusters. Each cluster was characterized based on the mean values of morphological traits; where, genotypes in cluster I showed high yielding, long spikes and bold seed, while the genotypes included in cluster II were early heading, moderate plant height and less number of tillers meter square^{-1}. Cluster III comprised of genotypes having bold seed, tall plants, long spikes, whereas genotypes of cluster IV had more biological yield and more tillers meter square^{-1}. Another study made by Gashaw [61] grouped 64 durum wheat genotypes in to 10 clusters; in which case they obtained similar results to the current study where the exotic and indigenous germplasm were grouped into different clusters. Similar findings were reported by Hailu et al. [62] regarding differential groupings of exotic and indigenous genotypes of durum wheat.

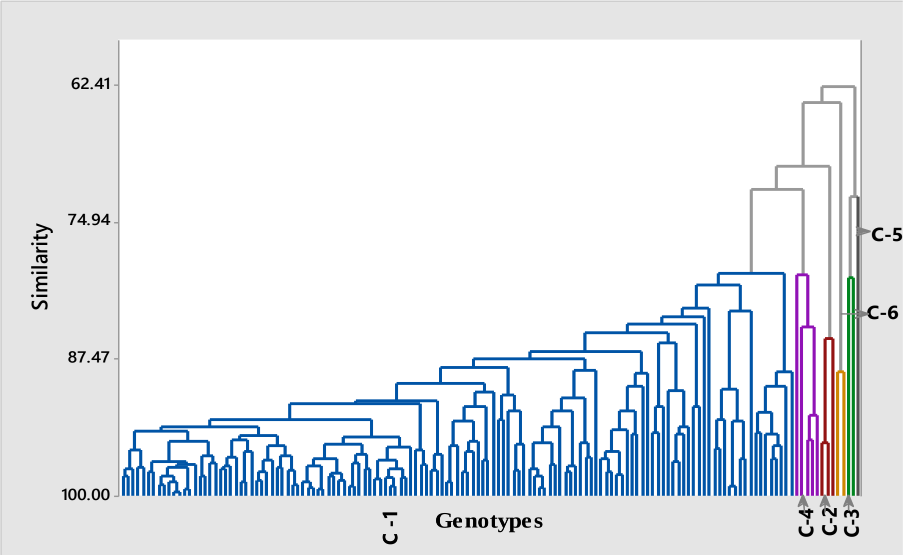

Fig 2. *Dendrogram showing the clustering patterns of the 144 Ethiopian durum wheat landraces including two improved cultivars*

Cluster distances were computed to estimate the genetic diversity between the cluster groups. The maximum genetic distance (124.01) was obtained between cluster IV and cluster V, followed by clusters II & VI (95.24), clusters V & VI (90.78), and clusters II & IV (85.27) (Supplementary Table 6). Minimum genetic distance was exhibited between clusters I & IV, indicating that genotypes in these clusters were genetically less divergent than the other cluster pairs. This signifies that the crossing of genotypes from these two clusters might not give a higher heterotic value in F_1, and a narrow range of variability in the segregating F_2 population could be observed. Maximum genetic recombination is expected from the parents selected from divergent cluster groups. Therefore, maximum recombination and segregation of progenies is expected from crosses involving parents selected from clusters IV and V, followed by clusters II and VI, and clusters V and VI. Such views in durum wheat genetic diversity studies were endorsed by several researchers [61, 62].

CONCLUSION

The seedling test result showed that the resistant and intermediate classes might have possessed one or more effective ASR genes. Those accessions exhibited compatible disease reactions at the seedling stage but demonstrated a significant level of field resistance that could be categorized as possessing APR genes. Hidase isolate was the most virulent race in the seedling test among the 5 isolates making about 58 (41%) of the 142 accessions susceptible. Overall, the data showed low variation among the pathogenicity of the Pst races with average virulence to 54 (38%) of the accessions.

The field assessment of stripe rust resistance at the adult stage showed significant variation among the accessions for all APR evaluation parameters (TDS, ACI, AUDPC, and infection rate). 37 durum wheat accessions had an acceptable level of field resistance; out of them, 14 accessions didn’t possess effective ASR genes; hence, they can be good sources of APR genes for developing durable rust resistant wheat cultivars. A low disease progress rate with infection rate values ≤ 1 was observed on 4.2% of the 142 accessions.

Estimation of variability parameters on the landraces using the 10 agronomic traits indicated that there is sufficient variability among the genotypes studied and would respond positively to selection. High epidemics of stripe rust magnified the variance due to environment ($\sigma^2 e$) compared to the variance due to genotypes (σ^2g). The impact of stripe rust is reflected here as it excluded majority (>73%) of the genotypes from being selected as they showed moderate susceptibility to response to *Pst*.

The multivariate analysis showed that the first five principal components accounted for 73.1% of the variation. The disease evaluation parameters; TDS, ACI, r-value, and AUDPC were the most important traits contributing to the variation (31.1%) explained in PC1. In PC2, that accounted about 13.3% of the total variance of the durum wheat landraces, DTM, DTH, PHT, HI, and GY showed large contributions. The cluster analysis grouped the 142 durum wheat landrace accessions and the two improved cultivars in to six clusters using 75% similarity. Each cluster group showed unique characteristics/performance for disease and agronomic evaluation parameters. The maximum genetic distance (124.01) was obtained between cluster IV and cluster V, followed by clusters II & VI (95.24), clusters V & VI (90.78), and clusters II & IV (85.27). Cluster V (accession 222451) could be the best candidate to be included in the ongoing durum wheat improvement program either as a parent for hybridization/crossing or pure line selection towards development of novel cultivar since it has unique positive agronomic performance traits as well as genetically distant from the rest of genotypes considered in the study.

This study identified 37 durum wheat accessions with acceptable level of field resistance, out of them 14 accessions did not possess effective ASR genes, hence, they could be exploited as good sources of APR genes for the development of durable rust resistance wheat cultivars for future Ethiopian durum wheat breeding program.

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Data availability. Additional data are available in the supplementary files

Conflict of interest. Authors declare that there is no conflict of interest between them

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