



Full Length Article

Identifying Pakistani Wheat (*Triticum spp* L.) Landraces as Genetic Resources for Yield Potential, Heat Tolerance and Rust Resistance

Saima Gulnaz¹, Muhammad Zulkiffal¹, Muhammad Sajjad^{2*}, Javed Ahmed¹, Muhammad Musa¹, Muhammad Abdullah¹, Aneela Ahsan¹ and Aziz ur Rehman¹

¹Wheat Research Institute, AARI, Faisalabad, Pakistan

²Department of Environmental Sciences, COMSATS University Islamabad (CUI), Vehari campus

*For correspondence: msajjadpbg@gmail.com

Abstract

The increased unpredictability in amount and timing of rain, spells of heat and drought has affected grain yield and threatened food security worldwide. Therefore, untapped genetic resources are needed to be exploited for future gain in yield potential under heat and other stress types. Herein, a panel of 44 wheat landraces stored in gene bank was characterized for yield potential, heat tolerance and resistance against yellow rust (YR) and leaf rust (LR) during three consecutive years. Based on principal component analysis, local Pakistani landraces were identified as potential genetic resources with wide extent of variation in all agronomic, heat tolerance traits and YR, LR score. The TGW had positive correlation with spikelets per spike (Sp/S), spike length (SL), yield, normalized difference vegetation index (NDVI) and canopy temperature depression (CTD). The heat tolerance measure NDVI (A) and CTD (B, A) were significantly correlated with grain yield in positive direction for all the three years. For YR disease prevalence was of eight categories from 0 to TS. The 0 reaction of YR and LR was observed on 69.4% and 3.5% landraces, respectively. The three prominent heat tolerant landraces were C-250, T16 and Local White that can be used as genetic resources for tackling increasing heat stress as a result of climate change through introgression of heat tolerance trait into high yielding wheat cultivars. Furthermore, the tolerant and susceptible landraces can be crossed to produce genetic populations such as recombinant inbred line (RILs) and near isogenic lines (NILs) to identify quantitative traits loci (QTLs)/genes underpinning heat tolerance. © 2019 Friends Science Publishers

Keywords: Land races; Genetic resources; Climate change; CTD; NDVI; yellow rust; leaf rust

Introduction

Wheat is a major staple food grown in 89 countries across the globe (FAO, 2017). Increase in human and livestock population demand continuous increase in wheat production that is highly tolerant to climate and environmental variants (Enghiad *et al.*, 2017). Climate change is causing erratic severity, patterns of precipitation and seasons thus changing ecosystems and environments. This scenario of unpredictability has placed new emphasis on breeding resilient wheat varieties alongside higher yield and better nutritional quality. The green revolution led to high yielding wheat varieties with an assembly of allelic combinations for few key traits such as height, disease resistance and response to high inputs. The success of such varieties enabled many wheat importing countries as Pakistan and India to become wheat-exporter. However, the disproportionate use of few successful varieties as parents for breeding new varieties led to the loss of locally adaptive genetic diversity (Sajjad *et al.*, 2015, 2018). The decreases in genetic diversity in a population are standard as genetic bottlenecks (Lopes *et al.*, 2015).

In wheat genetic diversity, two potential bottlenecks first due to recent origin of common wheat (~80 centuries ago, Cox *et al.*, 1997) from the crosses of few tetraploid and diploid progenitors and the second occurred in 20th century during the process of Green revolution (1940s to 1960s) where breeding programs relied on few lines for generating germplasm pools. The second bottleneck is believed to reduce drastic genetic diversity in wheat populations which vindicated the emphasis on germplasm exchange and the use and landraces for restoring the lost genetic diversity (Cavanagh *et al.*, 2013). For example, the landrace ‘Turkey’ provided a major favorable 3A QTL to high yielding wheat varieties grown in eastern and western Nebraska (Mahmood *et al.*, 2004). Japanese land race ‘Aka Komugi’ provided a new dwarfing gene ‘Rht8c’ and a new photoperiod insensitivity gene (*Ppd_D1*) and was used by Italian breeder Strampelli to improve his varieties (Worland *et al.*, 1998; Ellis *et al.*, 2007). Unlike the two dwarfing genes (*Rht-B1b* and *Rht-D1d*) used in Green revolution ‘Rht8c’ does not affect coleoptiles length which is useful for sowing wheat in dry regions or poor seed beds (Gasparini *et al.*, 2012).

High temperatures result in early maturity and reduced photosynthetic activity consequently, grain weight decreases due to a reduced grain-filling period (Wardlaw *et al.*, 1989). Thus, wheat varieties which maintain thousand grain weight (TGW) despite heat stress have heat tolerance capacity. Other agronomic traits associated with yield under heat stress include grains per spike (GpS), days to heading (DH) and maturity (DM) (Reynolds *et al.*, 2001). Physiological traits such as canopy temperature depression (CTD) and normalized vegetation index (NDVI) are confirmed to be highly associated with yield under heat stress (Amani *et al.*, 1996; Reynolds *et al.*, 2001; Ahmad *et al.*, 2015). Landraces are believed to have high yield stability than modern wheat cultivars under stress conditions and broader genetic base for yield traits (Dotlacil *et al.*, 2010). Therefore, these untapped genetic resources can be exploited for future to gain in yield potential under heat and other stress conditions.

Realizing the importance of landraces for restoring genetic diversity and enhancing yield potential, improving pest resistance and better climate resilience, a panel of 44 wheat landraces stored in National Gene Bank of Pakistan were characterized for yield potential, heat tolerance and resistance against yellow rust (YR) and leaf rust (LR) during three consecutive years. The selected landraces with useful trait (s) will be used in wheat breeding programs at Wheat Research Institute, Faisalabad, Pakistan including other wheat breeding institutes.

Material and Methods

A panel of 44 wheat land races (Table S1) was sown in triplicate randomized complete block design on mid November 2014-2015, 2015-2016 and 2017 in Faisalabad. The plot size was 2.5 m × 2 rows. Weeds were controlled manually by hoeing. NPK fertilizer was applied as 120-90-60 kg/ha. Full dose of phosphorus and potash was applied at sowing along with half of nitrogen, while the remaining of the nitrogen was applied at tillering with first irrigation. Data on the morphological traits were recorded, *i.e.*, days to heading (50%), days to maturity (50%). Normalized difference vegetation index at booting and anthesis stages (NDVI-B, NDVI-A), canopy temperature at booting and anthesis stages (CTD-B, CTD-A), spike length (cm), spikelets/spike, grains/spike, 1000 grain weight (g) and disease data (0-100S). The three years observations were pooled and average of 3 readings was calculated for use in further analysis. For canopy temperature (°C), data was recorded with LT.300 6th Sense Infrared Thermometer (IRT). Initial value was set as the temperature of outer environmental. After recording initial value the canopy temperature (CT) data was recorded of each entry. At the end the final value of outside environmental temperature was recorded. Finally, CT depression (CTD) was calculated according to the formula given in Table 1.

For recording the value of NDVI, green seeker (handheld-505) was used. Both the readings (CT and NDVI) were taken during sunny days with least wind speed at noon

time during 11 a.m. to 1 p.m. when the dew dried off from the plant canopy.

For disease infestation all the materials were surrounded by the spreader rows of highly susceptible variety (Morocco) while the rust severity and response was recorded according to the modified Cobb's scale described by Peterson *et al.* (1948). In all described entries disease reaction (leaf and yellow rust) ranged from 0-100S. The percent frequency of reactions types was calculated using the Cobb's scale score.

Results

ANOVA and Mean performance

Analysis of variance (ANOVA) showed that the landraces means for all traits (Table 2) reflected their diverse nature. The years' means were also significantly different for all traits reflecting the effect of changes in weather and management conditions during the three crop growing seasons (Table 2).

The mean minimum and maximum values of all the traits recorded were presented (Table S2). Average PH was observed 131, 132 and 132 cm for the three years respectively. The minimum PH height was recorded for the landrace 'Local White' (110, 110, 105 cm) and maximum for T1 (*T. durum*) (150, 148, 150 cm). The mean value for D/H was 101, 108 and 108 for the three years, respectively. The minimum 90, 100 and 114 days were taken by the land race 'C-271' for three years and maximum 115, 119 and 125 by 'T1 (*T. durum*)' to reach heading stage, respectively. For D/M mean values for the three crop years were 135, 141 and 157, respectively. The minimum D/M were taken by the landrace 'C-591' (130, 135 and 147, respectively) and maximum D/M were taken by 'T2 (*T. durum*)' (143, 149 and 161, respectively). The mean TGW for the three years were 31.2, 31.4 and 31.8 g, respectively. The minimum and maximum values of TGW were recorded for the landraces 'T9' (23.4, 23.6, 23.9 g) and 'C-248' (41.2, 42.2, 41.6 g) respectively. The minimum and maximum SpS were produced by 'T10' (12, 13, 12) and 'T17' (17), respectively. For GpS, minimum and maximum values were recorded on 'T23' and 'T15', respectively. The SL ranged from 9.0 cm (T8) to 13.0 cm (C-250) with mean values of 10.7, 10.9 and 12.2, respectively. The minimum and maximum NDVI (A) and NDVI (B) values were recorded for 'T13' and 'C-591', respectively. However, the minimum CTD (A) and CTD (B) values were observed for 'C-247' and 'T2', respectively.

Correlation Analysis

To determine the patterns of genetic association among agronomic and heat tolerance traits correlation analysis was performed. Plant height didn't exhibited significant correlation with any agronomic and heat tolerance trait during the three years. The DH had positive correlation with DM during the first two years and negative correlation with Sp/S and SL during the second year only.

Table 1: Formula used for calculating canopy temperature depress

| | Booting | anthesis | Canopy Temperature Depression (CTD) | |
|---------------|---------|----------|-------------------------------------|------------------|
| Initial value | 26.8 | 28.7 | Booting (CTD-B) | Anthesis (CTD-A) |
| Final value | 27.9 | 30.1 | Initial+Final/2 | Initial+Final/2 |
| | | | 26.8+27.9/2=27.35 | 28.7+30.1/2=29.4 |
| | | | 27.35-ct value | 29.4-ct value |

Table 2: Analysis of variance (ANOVA) for measured traits in local land races

| Sources of variation | Df | Means of squares | | | | | | | | | | | |
|----------------------|-----|---------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|---------------------|---------------------|---------------------|--------------------|-----------------------------|
| | | PH | D/H | D/M | GW | SP/S | GR/SP | SL | NDVI (B) | CTD (B) | NDVI (A) | CT (A) | Yield (Kgha ⁻¹) |
| Replication | 02 | 0.002 ^{ns} | 0.12 ^{ns} | 0.10 ^{ns} | 0.01 ^{ns} | 0.83 ^{ns} | 1.09 ^{ns} | 0.61 ^{ns} | 0.001 ^{ns} | 0.007 ^{ns} | 0.001 ^{ns} | 0.09 ^{ns} | 247 ^{ns} |
| Year | 02 | 39.3** | 9578.2** | 16645.9** | 9.8 ^{ns} | 16.6** | 22.5** | 8.1** | 0.02** | 5.5** | 0.02 | 8.9** | 68173** |
| Genotype | 43 | 923.1** | 129.5** | 41.4** | 205.7** | 13.8** | 32.4** | 3.7* | 0.01* | 9.6** | 0.00 ^{ns} | 8.7** | 665071** |
| Genotype x Year | 86 | 18.11* | 24.9 ^{ns} | 26.2 ^{ns} | 3.4 ^{ns} | 1.1 ^{ns} | 1.2 ^{ns} | 0.5 ^{ns} | 0.00 ^{ns} | 0.2* | 0.00 ^{ns} | 0.7* | 4227** |
| Error | 262 | 1.3 | 0.06 | 0.10 | 0.22 | 0.17 | 0.40 | 0.05 | 0.00 | 0.00 | 0.00 | 0.01 | 210 |
| CV% | - | 0.88 | 0.22 | 0.17 | 1.47 | 2.76 | 1.54 | 2.09 | 0.67 | 0.46 | 1.22 | 0.02 | 0.54 |

*P<0.05, **P<0.01

Table 3: Three years correlation matrix for measured traits in local land races

| Variables | PH | D/H | D/M | TGW | Sp/S | GpS | SL | NDVI (B) | CTD (B) | NDVI (A) | CTD (A) | Yield |
|-----------|--------|---------------|---------------|---------------|--------------|--------|--------------|--------------|--------------|--------------|---------|--------------|
| PH | 1 | | | | | | | | | | | |
| D/H | 0.087 | 1 | | | | | | | | | | |
| | 0.076 | | | | | | | | | | | |
| | -0.046 | | | | | | | | | | | |
| D/M | 0.236 | 0.774 | 1 | | | | | | | | | |
| | 0.011 | 0.359 | | | | | | | | | | |
| | 0.165 | 0.345 | | | | | | | | | | |
| TGW | 0.201 | -0.022 | 0.113 | 1 | | | | | | | | |
| | 0.221 | -0.295 | 0.023 | | | | | | | | | |
| | 0.208 | -0.262 | 0.418 | | | | | | | | | |
| Sp/S | -0.137 | -0.025 | -0.132 | 0.345 | 1 | | | | | | | |
| | -0.075 | -0.370 | -0.232 | 0.251 | | | | | | | | |
| | -0.093 | -0.321 | 0.018 | 0.309 | | | | | | | | |
| GpS | -0.053 | -0.108 | -0.170 | -0.324 | -0.035 | 1 | | | | | | |
| | -0.047 | 0.182 | -0.220 | -0.380 | 0.023 | | | | | | | |
| | -0.077 | 0.057 | -0.062 | -0.268 | 0.100 | | | | | | | |
| SL | 0.050 | -0.068 | 0.011 | 0.374 | 0.264 | -0.108 | 1 | | | | | |
| | 0.064 | -0.370 | -0.260 | 0.430 | 0.362 | 0.016 | | | | | | |
| | 0.060 | -0.137 | 0.309 | 0.351 | 0.311 | 0.107 | | | | | | |
| NDVI (B) | -0.038 | -0.101 | -0.269 | 0.113 | 0.273 | -0.066 | -0.002 | 1 | | | | |
| | 0.013 | -0.123 | -0.439 | 0.240 | 0.217 | -0.071 | 0.153 | | | | | |
| | 0.038 | -0.131 | 0.243 | 0.284 | 0.353 | 0.034 | 0.335 | | | | | |
| CTD (B) | -0.126 | 0.038 | -0.014 | 0.433 | 0.500 | -0.096 | 0.427 | 0.096 | 1 | | | |
| | -0.031 | -0.073 | 0.042 | 0.300 | 0.496 | 0.035 | 0.506 | 0.164 | | | | |
| | -0.080 | 0.004 | 0.249 | 0.285 | 0.400 | 0.147 | 0.462 | 0.269 | | | | |
| NDVI (A) | 0.041 | -0.028 | -0.206 | 0.132 | 0.290 | -0.008 | 0.077 | 0.875 | 0.185 | 1 | | |
| | 0.004 | 0.142 | -0.363 | 0.098 | 0.184 | 0.054 | 0.111 | 0.846 | 0.186 | | | |
| | 0.012 | 0.077 | 0.312 | 0.258 | 0.221 | 0.062 | 0.220 | 0.900 | 0.173 | | | |
| CTD (A) | -0.213 | 0.075 | 0.038 | 0.363 | 0.491 | -0.151 | 0.373 | 0.144 | 0.936 | 0.213 | 1 | |
| | -0.066 | -0.055 | 0.143 | 0.329 | 0.484 | -0.092 | 0.416 | 0.159 | 0.898 | 0.154 | | |
| | -0.194 | 0.152 | 0.309 | 0.307 | 0.124 | 0.031 | 0.309 | 0.148 | 0.793 | 0.136 | | |
| Yield | -0.266 | 0.032 | -0.016 | 0.450 | 0.524 | 0.035 | 0.336 | 0.163 | 0.709 | 0.309 | | 0.690 |
| | -0.183 | -0.103 | 0.003 | 0.382 | 0.567 | 0.013 | 0.383 | 0.278 | 0.667 | 0.317 | | 0.678 |
| | -0.182 | -0.021 | 0.485 | 0.465 | 0.539 | 0.069 | 0.415 | 0.398 | 0.647 | 0.364 | | 0.583 |

Values in bold are different from 0 with a significant level alpha=0.05

The DM showed negative correlation with NDVI during the three year that was statistically significant only during second year. The TGW had positive correlation with Sp/S, SL, yield, NDVI and CTD. However, the correlation between TGW and GpS was significantly negative during all the three years. The SpS was positively correlated with yield and CTD for all the three years. However, the correlation with SL was

positively significant during the last two years and with GpS and NDVI was positively significant during the third year only. The positive and significant correlation was observed between NDVI (B) and NDVI (A). The CTD (B) and CTD (A) were also significantly correlated in positive direction. The heat tolerance measure NDVI (A) and CTD (B, A) were significantly correlated with grain yield in

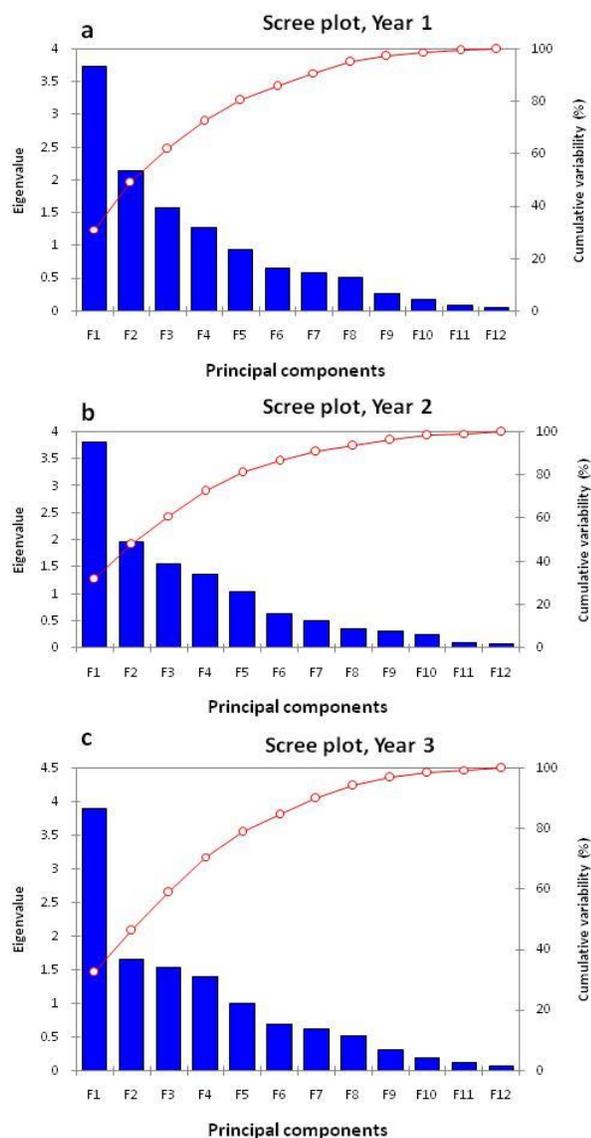


Fig. 1: Scree plots showing eigen value of the corresponding PCs for year 1 (a), year 2 (b) and year 3 (c)

positive direction for all the three years (Table 3). The results of correlation analysis were consistent with results of biplot analysis (Fig. 2).

Principal Component Analysis (PCA)

To determine the proportion of variance accounted by different principle components (PCs), dispersion of landraces and variables on two dimensional ordinations, principal component analysis (PCA) was performed. The PCA was performed on mean data from each year because the effect of years was significant in ANOVA. For the first year the first four PCs were significant accounting 72.7% cumulative variability (Fig. 1a). For the second and third years, first five PCs were significant accounting 81.2 and 78.9% cumulative variability, respectively (Fig. 1b and c).

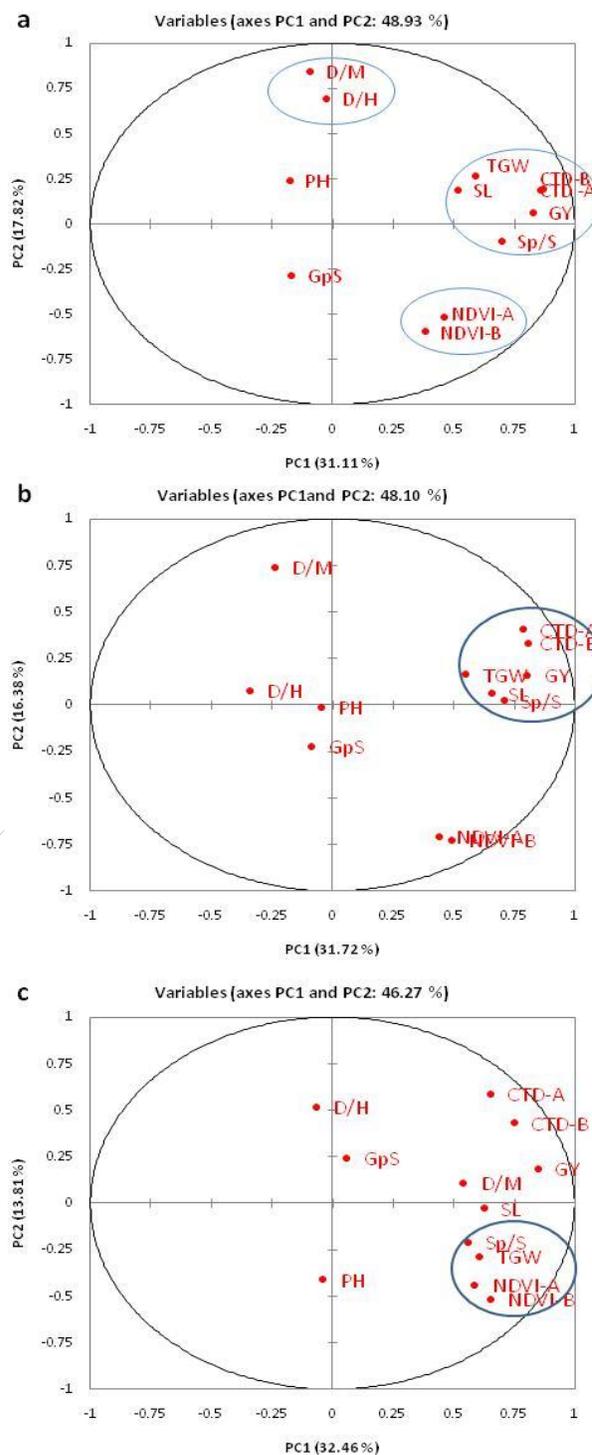


Fig. 2: Contribution of traits for genetic variation during year 1 (a), year 2 (b) and year 3 (c)

The dispersion of variable and landraces were considered because the first two PCs accounted maximum variation 49%, 48% and 46% of total observed variation during 1st, 2nd and 3rd year respectively (Fig. 1).

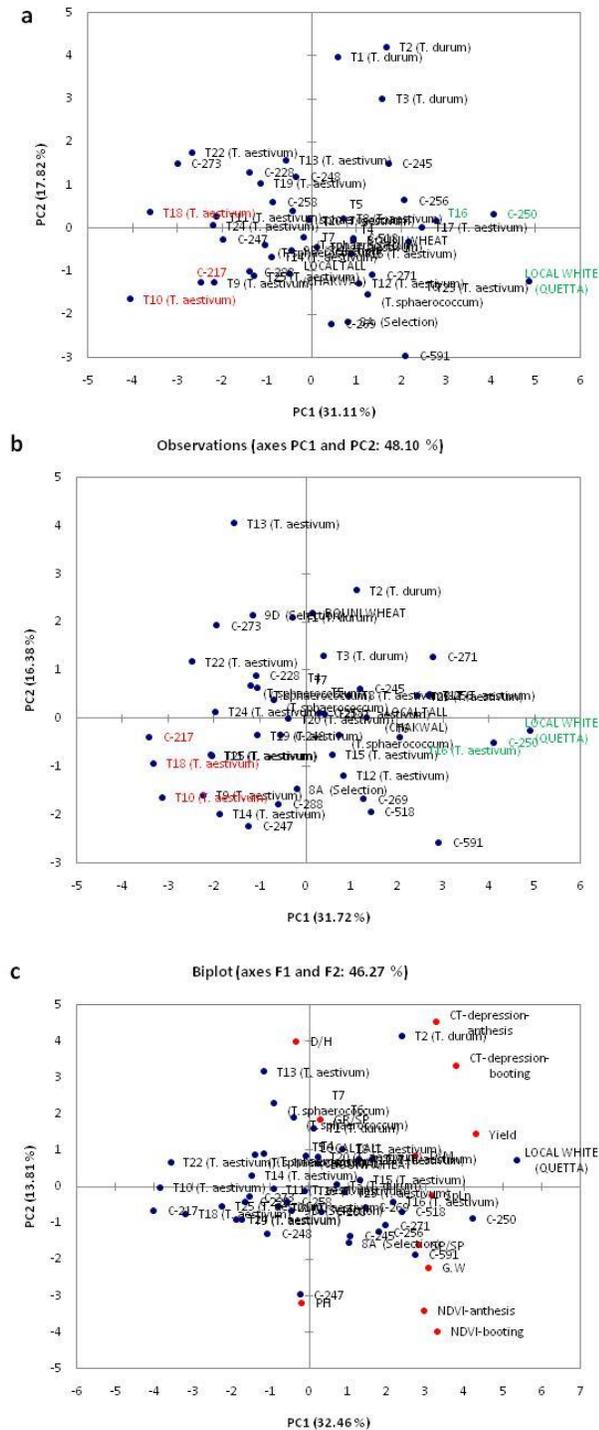


Fig. 3: Genetic distance and relatedness among landraces during year 1 (a), year 2 (b) and year 3 (c)

First PC was more related to grain yield (TGW, SL, Sp/S, GY) and heat tolerance traits (CTD-B, CTD-A) because it contained maximum positive loading values for these traits whereas second PC had maximum loading of TKW and DH (Fig. 2).

Biplot analysis was conducted to observe the dispersion of genotype on two dimensional ordinations with respect to their relative performance. Biplot vectors close to each other depicted the correlation among those traits and the genotypes lie close to a particular trait vector represents the high performance of the genotypes for that trait. The scattered dispersion of landraces on PC1 and PC2 ordinations depicted significant diversity among the landraces under study. The scattered pattern is consistent for the three study years (Fig. 3). The high diversity in landraces reflects diverse origin and pedigrees of landraces.

Based on biplot analysis landraces were divided in two groups' viz. heat tolerant and heat susceptible. Heat tolerant landraces were those which had high PC1 score because PC1 was more related to grain yield (TGW, SL, Sp/S, GY) and heat tolerance traits (CTD-B, CTD-A). The three prominent heat tolerant landraces were C-250 (0.79, 0.69, 0.68), T16 (0.62, 0.038, 0.54) and Local White (0.65, 0.57, 0.70). The three prominent susceptible landraces were T10 (-0.78, -0.62, -0.53), T18 (-0.65, -0.78, -0.48) and C-217 (-0.54, -0.57, -0.76) which had high negative score of PC1 (Fig. 2). The heat tolerant landraces can be used as genetic resources for tackling increasing heat stress as a result of climate change through introgression of heat tolerance trait into high yielding wheat cultivars. Furthermore, the tolerant and susceptible landraces can be crossed to produce genetic populations such as recombinant inbred line (RILs) and near isogenic lines (NILs) to identify QTLs/genes underpinning heat tolerance.

Disease Resistance

To assess the potential of landraces against yellow rust (YR) and leaf rust (LR) disease score was recorded for each entry for the three study years. For YR disease prevalence was of eight categories from 0 to TS. The 0 reaction of YR was observed on 69.4% landraces while TS type reaction was observed on 7.8% landraces (Fig. 4a). For LR disease reaction of reactions of 30 types were observed. The most resistant reaction with 0 LR score was observed on 3.5% landraces while the most susceptible reaction of TS type was observed in 6.3% landraces (Fig. 4b).

Discussion

Association of Yield and Physiological Traits

Understanding the pattern of association among economic traits in a plant population helps breeders to set selection criteria for improving yield potential (Sajjad *et al.*, 2011, 2017). The association between the number of grains per spike (GpS) and the TGW was traditionally found as being negatively correlated in landraces and early elite bread wheat (Kuchel *et al.*, 2007; McIntyre *et al.*, 2010; Wu *et al.*, 2012). However, the concurrent selection of positive haplotypes for one plus neutral for the other or positive haplotypes for both traits changed the association between GpS and TGW from negative to neutral or even positive (Zhang *et al.*, 2012).

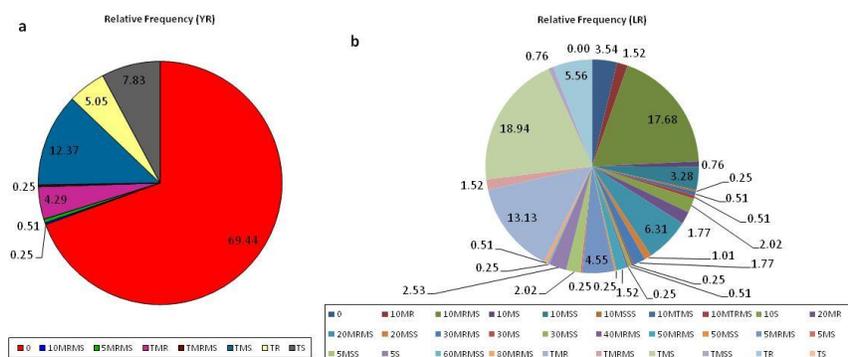


Fig. 4: Relative frequency yellow rust (a) and leaf rust (b) infections in the landraces over the three study years

Therefore, no significant correlation was observed between CIMMYT-derived spring cultivars and elite lines (Maqbool *et al.*, 2010, Sajjad *et al.*, 2017) while significantly positive association was observed in Chinese cultivars (Zhang *et al.*, 2012).

CTD is positive when the canopy is cooler than the air and commonly used as a selection criterion in wheat breeding for heat and drought tolerance (Reynolds *et al.*, 2001; Balota *et al.*, 2007). Munjal and Rana (2003) recommended high stomatal conductance and cooler canopy at grain filling stage as the basic physiological criteria for higher grain yield under heat stress conditions.

Amani *et al.* (1996), Ayeneh *et al.* (2002), Bahar *et al.* (2008) reported high positive significant correlation between CTD and grain yield. Genotypes with high CTD had cooler flag leaves highly associated with GY at booting stage. Similar findings were reported by Ayeneh *et al.* (2002), Munjal and Rana (2003), Balota *et al.* (2008), Bahar *et al.* (2008) and Gutierrez *et al.* (2010). In conclusion, CTD can be used as a basic selection criterion for stable and high yielding bread wheat genotypes under heat stress conditions in the field.

Trait based Selection of Landraces as Genetic Resource

Landraces are believed to have more stable yield under stress conditions and broader genetic base for useful traits (Dotlacil *et al.*, 2010). Therefore, the untapped sources of genetic diversity in the form of landraces need to be exploited for future gains in yield potential under stress conditions. Earlier local landraces have been utilized to improve drought tolerance in Spain (Royo and Briceño-Félix, 2011), frost tolerance in USA (Olmstead and Rhode, 2002). Local Pakistani landraces having higher CTD and yield (*e.g.*, C-250, T16 and Local White) as identified in this study can be used for improving yield potential under heat and drought stress conditions through direct crossing.

YR and LR Resistance in Landraces

Landraces have also been providing genes for durable rust

resistance (Rehman *et al.*, 2013). For example, Pakistani landrace ‘C271’ was utilized to incorporate the durable rust resistance gene Lr34, a in modern Pakistani wheat cultivars (Rehman *et al.*, 2013; Muhammad *et al.*, 2018). During the period 1965-1985, the CIMMYT wheat breeding program has incorporated various durable rust resistance genes from land races possessing good resistance (30-40M). The Pakistani landraces characterized in this study have very good resistance for YR and LR and therefore can be exploited for durable rust resistance along with heat and drought tolerance in future cultivars.

Conclusion

The study concluded that untested landraces stored in gene bank can be used as potential genetic resource, for increasing yield potential under high temperatures with better rust resistance. The CTD can be used as a basic selection criterion for stable and high yielding bread wheat genotypes under heat stress conditions in the field. The three prominent heat tolerant landraces were C-250, T16 and Local White. The heat tolerant landraces can be used as genetic resources for tackling increasing heat stress as a result of climate change through introgression of heat tolerance trait into high yielding wheat cultivars.

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