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Performance of Ethiopian bread wheat (*Tritium aestivum* L.) genotypes under contrasting water regimes: potential sources of variability for drought resistance breeding

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Abstract

Drought is a common abiotic stress in Ethiopian agriculture. Crop yield is at risk due to drought that happens at various developmental stages of the crop. This experiment evaluated 248 Ethiopian bread wheat genotypes under water stress and non-stress growing conditions. Augmented complete block design with three blocks and eight replicated entries was used. Analysis of variance showed significant diversity among the genotypes in reaction to water stress. The average root and shoot lengths were reduced by 33.4% and 28.8%, respectively, due to water stress. The average fresh biomass per plant was 192 mg for non-stressed and 116 mg for stressed treatments, suffering a 40.5% reduction due to stress. Accessions 8314, 204463, 204454 and 204521 showed the longest roots while accessions 222381, 222405, 222439 and 204586 showed the shortest roots under stress conditions. Drought tolerance indices were calculated based on root length. Geometric mean performance (GMP) index was found helpful in identifying the relatively stable genotypes across the two water regimes. High GMP indices were observed for genotypes 8314, 204521, 231614, and KSN81 which were long rooting genotypes under both stress and non-stress conditions. ANOVA based on region of collection showed that genotypes from Southern Nations Nationalities and Peoples Region had the longest roots. Elevation of origin did not show any significant difference for any of the traits measured. This study demonstrated the presence of large variations for water stress response in the Ethiopian bread wheat germplasm. The identified stress resistant genotypes can be used as potential breeding stocks to develop drought resistant cultivars.

Keywords: hydroponics; osmotic stress; seedling resistance; wheat collections.
Abbreviations: DRI_Relative drought resistance index; GMP_Geometric mean performance; KSN_Kulumsa screening nursery; PEG_Polyethylene glycol; SSI_Stress susceptibility index; STI_Stress tolerance index.

Introduction

Agriculture is the largest sector of employment and main source of livelihood in Ethiopia. Nearly 85% of the population depends directly on farming. Grain production constitutes the major share of the domestic agricultural production. Nearly 98% of cereals are produced by small holder farmers (USDA, 2014). Ethiopia is the largest wheat producing country in Sub-Saharan Africa, with annual production of more than 4 million tons of grain on 1.6 million hectares of land which accounted for 13% of total land allotted to cereals (CSA, 2014; USAID, 2014). Wheat is mainly grown in the central and south eastern highlands during the main rainy season (June to September) (Hailu et al., 1991). The Ethiopian agriculture is mainly rain-fed in that its performance is highly dependent on the timing, amount and distribution of rainfall (Cheung et al., 2008). This makes the sector vulnerable to drought and other natural calamities. Due to the changing global climate, the rain fall trend is also changing (Funk et al., 2012; Hellin et al., 2012; Schlenker and Lobell, 2010; Stroosnijder et al., 2012). The rains are becoming more erratic with a trend of starting late and ceasing early in the season. This has posed an eminent danger for crop production. The production loss due to both biotic and abiotic factors coupled with the increasing population has made it difficult to attain food security in the country. Improving the adaptability of crop varieties to a changing environment supported by appropriate crop management strategies is the working principle worldwide in ensuring crop productivity (Blum, 2011a; Farooq et al., 2015; Stroosnijder et al., 2012; Wasson et al., 2012). However, crop improvement for water stress is a much complicated task as drought damage is manifested in various forms at various crop growing stages making breeding for drought resistance uneasy (Blum, 2005; Fischer et al., 2012; Szira et al., 2008; Tuberosa, 2012). Therefore, breeding for drought resistance has to integrate all methodologies that help in genotype evaluation and selection at all stages of the crop instead of one final stage (Qu et al., 2008). Seedling or early vigour, and deep root system are believed to contribute for better drought resistance (Al-Karaki, 1998; Atkinson et al., 2015; Chloupek et al., 2010; Comas et al., 2013; Lilley and Kirkegaard, 2011). Some genes that contribute to seedling drought resistance may also contribute to later stage resistance

(Comas et al., 2013; Hoffmann et al., 2012). Sarker et al. (2005) have reported in lentil that long root and shoot lengths at seedling stage were highly correlated with high grain yield. Initial root parameters and above-ground biomass were also reported to be positively correlated in wheat (Atkinson et al., 2015). Among the seedling traits that enable plants withstand drought are early establishment and ground cover, deep root system and leaf waxiness (Blum, 2005). Genotypes with deep roots are able to extract water from lower soil profiles there by making use of water lost in the form of deep percolation (Comas, 2013). Selection for root length; however, is dependent on the expectation of soil water availability (Blum, 2011). If soil water is expected to be close to the surface like that of irrigation setups, selection for deep root will not be an objective and vice versa. For environments that are dependent on remnant moisture, short roots are more preferable.

Genetic variability for any trait of interest is the first and foremost requirement for the success of any breeding program (El-Beltagy and Madkour, 2012; Tadesse et al., 2012). The Ethiopian wheat germplasm was extensively studied for its variability in agro-morphological and molecular traits (Alamerew et al., 2004; Belay et al., 1993; Hailu et al., 2006; Pecetti and Damania, 1996; Tesfaye et al., 1991). However, most of the previous studies were focussed on the final crop growth stage such as yield and yield related traits, which had overlooked the importance of seedling evaluation for water stress resistance. It was hypothesised that Ethiopia might harbour valuable genetic resources for water stress resistance as a result of the sporadic dry spells that have stricken the country for many decades and long history of wheat production in the country (Conway and Schipper, 2011; Hailu et al., 1991; Kassie et al., 2014). Therefore, the present research was undertaken to evaluate the phenotypic variability among Ethiopian bread wheat genotypes and to identify the most tolerant genotypes for early-stage water stress, and to assess the relationship between underground and aboveground plant biomass in response to water stress.

Results

Analysis of variance for the two growing conditions was done separately after checking the error heterogeneity between the two treatments. ANOVA did not show any statistical difference among the replicated entries in both growing conditions. However, the non-replicated entries varied significantly in both stress and non-stress growing conditions based on the calculated LSD values (Fig. 2a, b, and c, Supplementary Table 2).

Phenotypic variation under non-stress condition

Root length variation ranged from 3.6cm to 21.1cm while shoot length was from 6.7cm to 22.7cm. The longest roots were found in genotypes KSN 85, KSN 6, KSN 15 and KSN 34 (20 – 21 cm) while the shortest roots were found in accessions KSN55, 204585, 226939, and 231553 (3.6 - 7.7 cm) (Supplementary Table 1, Fig. 2a). The average biomass yield was 192 mg per plant with the highest biomass yields measured on genotypes KSN 51 and KSN 78, 226931, and 243714 (353.7 - 392.5 mg per plant) and the lowest biomass yield was measured on genotypes KSN 38, KSN 55, KSN 56, KSN 8 and 226236 (22.5 - 53.8 mg per plant) (Fig 2c).

Phenotypic variation under stress condition

The induced stress caused reduction in the performance of genotypes for all the three traits. The average root and shoot lengths were reduced by 33.4% and 28.8%, respectively, while fresh biomass yield was reduced by 40.5% (Fig. 3b). Root length ranged from 2.0 cm to 19.6 cm while the range for shoot length was from 2.6 cm to 20.6 cm (Fig. 2b). The longest root length was recorded in accessions 8314, 204463, 204454 and 204521 while the shortest was in 222381, 222405, 222439 and 204586 (Fig. 2a). Biomass yield ranged from 33 mg to 273 mg with an average yield of 115.6 mg per plant. The highest biomass yield was measured in accessions 226941 and 226261 (273 mg per plant) (Fig. 2c).

Genetic variation based on geographic locations

Out of the total 248 tested bread wheat genotypes, only 160 had geographic data of collection. One-way ANOVA based on regions of collection showed a significant ($P<0.05$) difference for root length among genotypes (Fig 3a). Shoot length and fresh biomass yield did not show any significant difference among regions of collection. Genotypes from SNNP region showed significantly longer ($P<0.05$) roots as compared to the root lengths of accessions from other regions (Fig. 3a). The collection sites were arbitrarily grouped into four elevation/altitude categories *viz* < 2,000 masl, 2,000 - 2,500 masl, 2,500 - 3,000 masl and > 3,000 masl. One-way ANOVA was conducted based on this grouping, but no significant difference was found for any of the traits (data not shown).

Correlation among traits

All the traits were significantly ($P<0.01$) and positively correlated in both stress and non-stress conditions. The magnitude of correlation between fresh biomass yield and root length was higher under stress condition while correlation between shoot length and fresh biomass was higher under non-stress condition (Table 1).

Drought resistance indices

The relative importance of the calculated indices was weighed in their ability to differentiate genotypes that perform better in both stress and non-stress growing conditions. SSI index was good in differentiating genotypes that are very sensitive to water stress while GMP was higher in identifying the most stable genotypes across the two water regimes. Higher GMP values were associated with genotypes that were long rooted and at the same time less affected by the stress and a small GMP value indicated genotypes that were short rooted but not much affected due to stress (Supplementary Table 1). Based on the ISS index genotypes KSN55, 204454, 204463, 221735 and 243696 showed the lowest susceptibility index while accessions 226235, 16352, 222405, 222439, and 204586 were with the highest susceptibility indices. High GMP index was observed for genotypes 8314, 204521, 231614, and KSN81 while genotypes 204585, 222405, 204586, 231553 and 226939 were with the smallest GMP values. No meaningful association of genotypic performance was possible with the rest of the indices.

Discussion

Root phenotyping is among the most marginalised area of crop improvement research mainly because of the difficulty of root traits measurement (Passioura, 2012; Tuberosa, 2012). Hydroponic systems were reported to be handy tools for root phenotyping (Atkinson et al., 2015; Ayalew et al., 2015). The

Table 1. Simple correlation of traits under non-stress (below diagonal) and stress (above diagonal) conditions.

	Root Length	Shoot length	Fresh biomass
Root Length	1	0.60**	0.36**
Shoot length	0.39**	1	0.49**
Fresh biomass	0.37**	0.62**	1

** indicates significant correlation at P<0.01

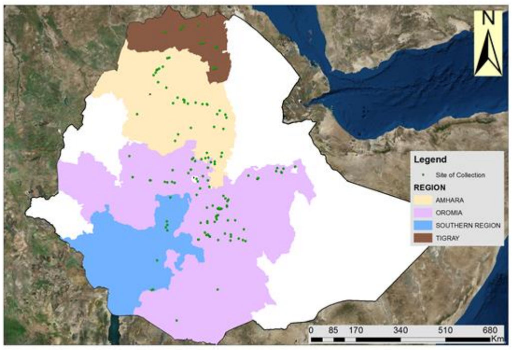


Fig 1. Geographical distribution of germplasm collection sites across the four administrative regions in Ethiopia.

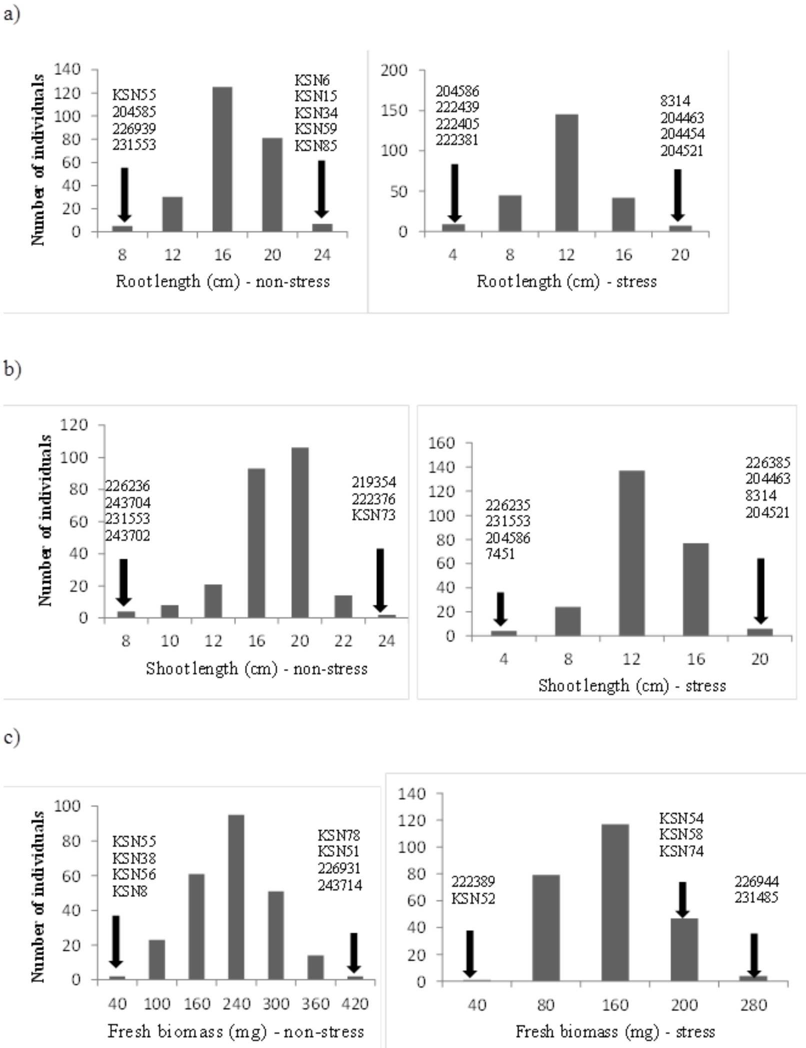
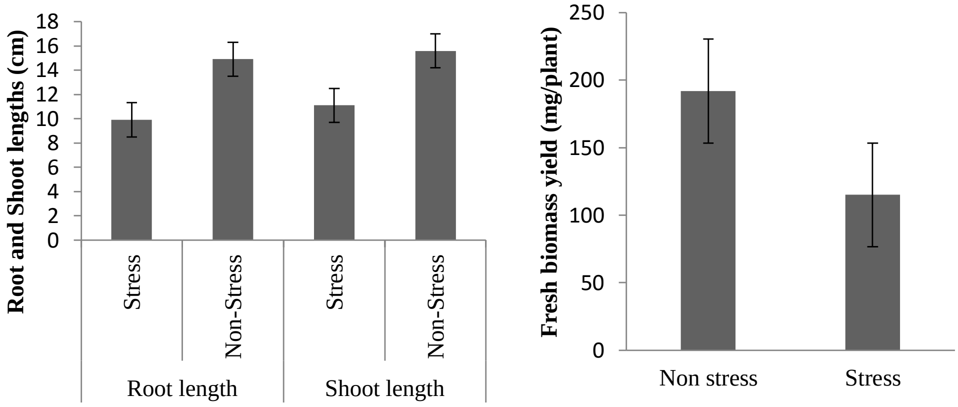
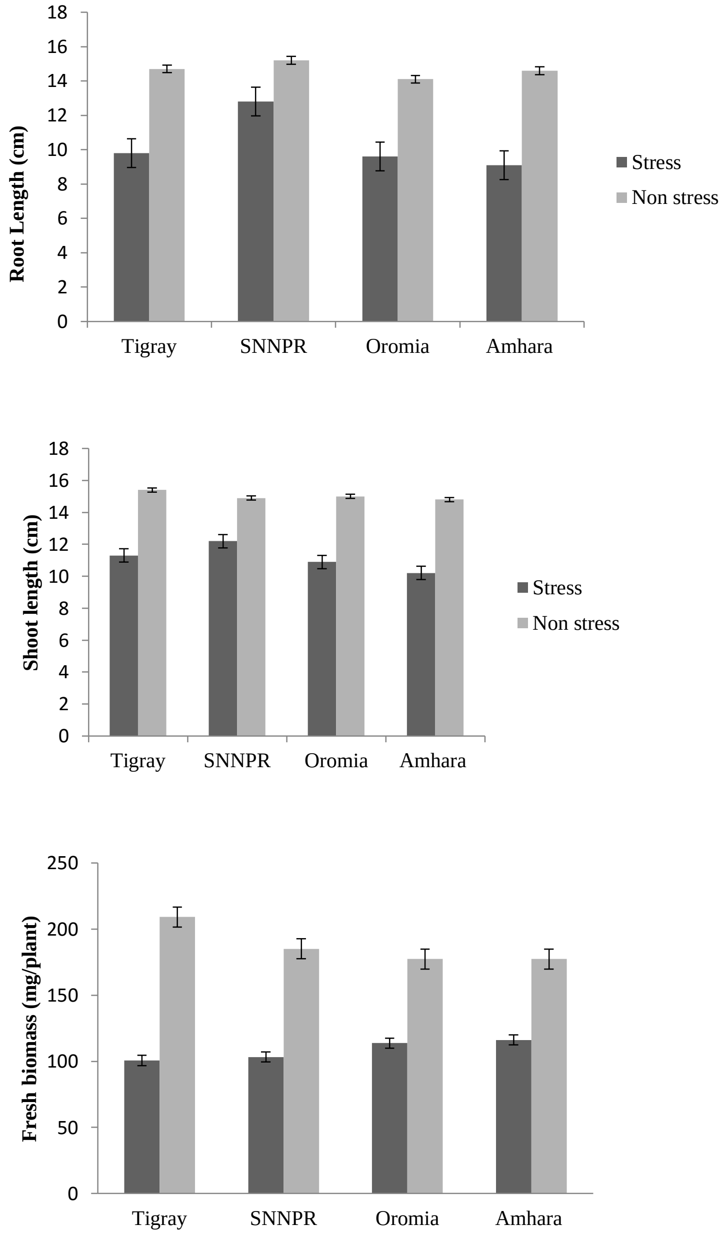


Fig 2. Phenotypic ditribution of 248 wheat genotypes for root length (a) shoot length (b) and fresh biomass yield (c) under water stress and non-stress conditions. Some extreme genotypes are arrowed in the respective categories.



a) b)

Fig 3. The mean performance of 248 bread wheat genotypes for root and shoot length (a) and fresh biomass weight (b) under stress and non-stress growing conditions.



a)

b)

c)

Fig 4. The relative difference in the performance of wheat genotypes for root length (a), shoot length (b) and fresh biomass yield (c) based on regions of landrace collection.

present study employed hydroponic culture to get easy access to intact roots. Seedling is one of the vital stages in plants which determines the level of crop establishment and crop stand performance in dry seasons. However, this succulent stage of crop plants was less emphasised in research literature partly because phenotyping for seedling resistance is presumed unattainable.

Results from this study indicated that the Ethiopian bread wheat genotypes are highly diverse in terms of root length, shoot length and fresh biomass yield. Previous studies have also found significant variability in the Ethiopian wheat germplasm for several agro-morphological traits such as days to heading and maturity, plant height, grain yield, and harvest index (Belay et al., 1993; Hailu et al., 2006; Tesfaye et al., 1991). Depending on a target drought scenario, the identified genotypes can be further evaluated to develop varieties through line selection or hybridization to pyramid different favourable genes into a cultivar. Genotypes with long roots at early stage can be valuable assets for breeding drought tolerant lines in environments with early-growing-season rainfall and with soil types that can retain water at deeper layers. The less vigorous genotypes can be targeted for environments where farming is dependent on ruminant moisture that requires water saving for later stage crop growth (Blum, 2005). The regions of collection did not show any significant difference which might be due to the fact that bread wheat is an exotic cereal to Ethiopia and did not differentiate into diverse ecotypes except for root length (Engels et al., 1991). Genotypes from Southern Nations, Nationalities and Peoples Region were significantly long rooted than genotypes from the other regions. This might be due to the many years of exposure of genotypes to low precipitation and the thick top soil that can hold water in its deeper layers (Funk et al., 2012; Kassie et al., 2014). All the three traits were highly and positively correlated which enables simultaneous genetic improvement. Biomass yield can be used as a good indicator of long roots under stress condition as the level of magnitude and significance of correlation between these traits were higher as compared to the case with the rest of traits. This finding is in agreement with (Abdel-Ghani et al., 2014). Among the drought resistance indices geometric mean performance index (GMP) was helpful in identifying the most stable genotypes in this study which was in agreement with previous findings (Mohammadi et al., 2011; Sio-Se Mardeh et al., 2006). The use of drought tolerance indices is dependent on the selection strategy one follows to improve drought resistance (Sio-Se Mardeh et al., 2006). Selecting genotypes that yield highest at optimum moisture and again are able to give reasonable yield under stress are favourable for environments which generally have enough precipitation for most part of the years/seasons but are impacted by sporadic drought (Blum, 2011b; Ud-Din et al., 1992). However, if any two environments are characterized by marked differences in terms of moisture availability, selection and breeding needs to be done separately (Ceccarelli and Grando, 1991). Stress susceptibility index (SSI) enabled identifying resistant genotypes under stress conditions, however; it was not helpful in the non-stressed situation.

Materials and Methods

Plant materials

A total of 248 bread wheat genotypes were evaluated; 160 landrace collections from the Biodiversity Institute of Ethiopia and 88 breeding lines from Kulumsa Agricultural research centre. The landraces were collected from four administrative regions in Ethiopia (Amhara, Oromia, Tigray, and Southern Nations Nationalities and Peoples Region (SNNPR) (Supplementary table 1, Fig.1) while the breeding lines were under observation and characterization nursery at Kulumsa Agricultural Research Centre (Represented as KSN for 'Kulumsa Screening Nursery').

Experiment setup and traits measured

Germplasm evaluation for seedling water stress resistance was conducted at Debre Markos University in a laboratory of the Department of Horticulture. A hydroponic system was developed from plastic boxes (3,000 ml of volume each) with 8mm diameter holes drilled on lids that supported plant growth on the surface of the solution, following the same methodology as described in Ayalew et al. (2015). The experiment was set up in a way that the boxes were filled with water/solutions and the lids were perforated and lined with filter paper to keep plants in place and the surface moist. An augmented complete block design was set up with three blocks (planting time) and eight randomly selected genotypes as repeated checks/controls. Seeds were first germinated in petri dishes lined with filter paper soaked with tap water for 48 hours and seedlings were transferred to the hydroponic system. Osmotic stress of -0.82 MPa was induced using PEG 6000 (Sinopharm Chemical Reagent Co. Ltd). Plants were grown in water for the first seven days followed by either in half strength Hoagland's solution alone (control) or half strength Hoagland's solution with PEG 6000 (treatment). Natural light was used and the solution was being aerated using manual agitation. Data were recorded on root length and shoot length using a scaled ruler and fresh biomass using a sensitive balance 14 days after planting.

Statistical analysis

Analysis of variance (ANOVA) was carried out based on augmented complete block design using CropStat version 7.2 statistical software (International Rice Research Institute, 2007) accounting for both inter- and intra- block differences. The following linear model was used: $y_{ij} = \mu + a_i + b_j + \varepsilon_{ij}$, where y_{ij} is the observed phenotype, μ is the population mean, a_i is the genotype effect, b_j is the block effect and ε_{ij} is the random error. The plot numbers were considered in the residual to account for any measurement errors. Pearson's simple correlation was also calculated among the traits measured based on Dewey and Lu (1959). Means were adjusted for inter- and intra- block variations and were compared based on the standard errors of the differences between two means with controls and with new entries. Due to the imbalance created owing to the occurrence of new entries in a block, four different standard errors (Federer and Raghavarao, 1975) were computed as follows:

Between two controls = $\sqrt{2\mathrm{MSe}/\mathrm{b}}$

Between two adjusted means in the same block = $\sqrt{2\mathrm{MSe}}$

Between two adjusted means in different blocks = $\sqrt{2\mathrm{MSe}(1+\frac{1}{\mathrm{c}})}$

Between adjusted means and control mean = $\sqrt{\mathrm{MSe}(\mathrm{b}+1)(\mathrm{c}+1)/\mathrm{bc}}$, where MSe is mean square of error, b is the number of blocks and c is number of control varieties.

One-way ANOVA was used to compare differences among the four regions of landrace collection and four altitude groups (< 2,000 masl, 2,000 - 2,500 masl, 2,500 - 3,000 masl and > 3,000 masl).

The following drought indices were calculated based on root length as indicated by the following formulae.

1) Stress susceptibility index (SSI) = $(1-(Y_s/Y_p))/(1-(\bar{Y}_s/\bar{Y}_p))$ (Fischer and Maurer, 1978)

2) Stress tolerance index (STI) = $\frac{Ys+Yp}{\bar{Y}^2}$ (Fernandez, 1992)

3) Relative drought resistance index (DRI) = $\frac{Ys/Yp}{\bar{Y}s/\bar{Y}p}$ (Fischer and Wood, 1979) and

4) Geometric mean performance index (GMP) = $\sqrt{Ys * Yp}$ (Fernandez, 1992), where in all the above equations Ys is yield of cultivar under stress, Yp is yield of cultivar under non-stress condition, $\bar{Y}$s and $\bar{Y}$p are the mean yields of all cultivars under stress and non-stress conditions, respectively.

Conclusion

In conclusion, the present study has found the presence of genetic variation among Ethiopian bread wheat genotypes both under severe water stress and non-stress conditions. There was a change in the ranking of genotypes under the two water regimes which calls for a separate breeding strategy for stress and non-stress conditions. The contrasting genotypes can be used as parental lines for further genetic study and as breeding lines based on different drought scenarios.

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