

Amphidiploids Derived from *Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve (Poaceae) to Enhance Grain Yield Under Drought and Heat-Drought Stress

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Abstract

In the present study, *Thinopyrum bessarabicum* [(Savul. & Rayss) Á. Löve (Poaceae)], known for its rust resistance and heat tolerance traits, was incorporated into a breeding program. Seven different durum (AABB) and two bread (AABBDD) wheat cultivars were carefully chosen as recipient parents for improvement in abiotic stress tolerance traits. The resultant fixed lines were rigorously examined for a range of agronomic and physiological traits during the *rabi* seasons of 2019 and 2020 in four environments for two consecutive years across two locations, Hisar and Karnal, Haryana. Analysis using AMMI and GGE methods, focusing on grain yield per plot (kg/plot) across multiple environments, revealed that these amphidiploids exhibited superior performance in E4>E3>E2>E1, signifying that their average performance excelled under heat and drought stress conditions. All nine amphidiploids demonstrated their best performance in Hisar under drought and temperature stress. Among these, EC787014 (*T. aestivum* L. cv. *Chinese Spring X Th. bessarabicum*) emerged as the top-performing genotype in both years for the heat and drought stress environment (E4), with an average yield of 0.506 kg/plot in 2019 and 0.494 kg/plot in 2020.

Keywords: Amphidiploids, *Thinopyrum*, AMMI, GGE, heat and drought stress, grain yield

1. Introduction

Wheat (*Triticum aestivum* L.) is a fundamental staple crop, feeding 36% of the global human population, along with rice and maize (Shewry PR, 2009). Unfortunately, global wheat productivity has been dwindling due to rising temperatures since the 1980s, resulting in a 5% reduction (Lobell *et al.*, 2011). This issue is exacerbated by projections of a 2–5 °C surge in global mean temperatures by the Intergovernmental Panel on Climate Change (IPCC, 2019), posing a substantial threat to food security. To meet the burgeoning food requirements of the expanding population, we must augment wheat production by 1.5–2%, all while managing limited

resources (Chatrath *et al.*, 2007). This challenge becomes even more pressing in the context of climate change, where abiotic stressors such as heat, drought, and salinity threaten wheat production, notably in regions like the Indo-Gangetic plains, Central, and Peninsular zones of India (Trethowan *et al.*, 2018). The wheat crop in these areas is frequently exposed to terminal high-temperature stress, leading to yield losses of up to 17% for every degree increase in temperature (Lobell *et al.*, 2008). Therefore, it's paramount to develop wheat cultivars acclimated to high-temperature conditions.



Amid these challenges, *Thinopyrum bessarabicum* (Savul. and Rayss) A. Löve ($2n = 2x = 14, JJ$), a wheatgrass, emerges as a promising candidate with salinity tolerance (King *et al.*, 1997; Khokhar *et al.*, 2020). Salt-tolerant wild relatives also exhibit drought tolerance (Farooq and Azam, 2001). These crop wild relatives (CWR) and primitive wheats/landraces have endured natural environments for millennia, maintaining significantly higher genetic diversity than modern wheat varieties (Zhang *et al.*, 2017). Many CWRs exhibit specific quality-related traits, such as elevated grain concentrations of essential elements like calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), and zinc (Zn) compared to standard wheat cultivars. These wild relatives have also been reported to have high levels of Fe, Zn (Sharma *et al.*, 2021), and protein content (Zeibig *et al.*, 2021). Resistance to both biotic and abiotic factors in wheat CWR has been extensively discussed (Bohra *et al.*, 2021). Among these wild species, wild grasses, in particular, serve as a valuable source of genetic diversity with significant potential for crop improvement. One such example is intermediate wheatgrass, *Thinopyrum intermedium* (Host) Barkworth et D. R. Dewey, which holds immense practical value due to its high production, drought and frost tolerance, non-invasiveness, and excellent performance as forage in harsh environmental conditions. Moreover, it exhibits resistance to several pests and diseases of wheat (Mahelka, 2011). The utilization of DNA-based markers in screening for desirable traits within *Thinopyrum* has provided valuable insights into complex inheritance patterns and other valuable characteristics.

It's crucial to identify suitable donors with confirmed heat resistance at terminal stages for enhancing heat tolerance in wheat. The process of testing heat tolerance involves exposing plants to high temperatures late in the growing season, although the specific stress intensity and duration can vary depending on field conditions. Genotype-environment interaction ($G \times E$) plays a pivotal role in identifying genotypes that are well-suited to stressful conditions (Munns and James, 2003). Various methods, such as heat susceptibility index and thousand kernel weight reduction, have been employed to pinpoint heat-tolerant genotypes in wheat (Kumar *et al.*, 2021; Pandey *et al.*, 2015; Pandey *et al.*, 2019). Durum wheat is

nutrient dense but it is susceptible to harsh environmental conditions. Crosses of durum wheat with most or the wild wheat relatives do not produce fertile hybrids due to presence of 5B chromosomes or non-homologous genome. *Thinopyrum bessarabicum* lack 5B chromosome in its genome and is able to recombine with wheat genome thus producing fertile hybrids. These hybrids have been tested for salinity tolerance in Indian condition (Khokhar *et al.*, 2020) and are also reported to be nutrient dense. Their ability to withstand drought and heat stress has not been much studied which is the prime aim of the present study. To assess the stable performance of genotypes under multi-environment (drought and heat) evaluation, diverse methods such as regression analysis, Principal Component Analysis (PCA), cluster analysis, Additive Main Effects and Multiplicative Interaction models (AMMI), and GGE biplot analysis have been described in the literature. These methods have been instrumental in identifying stable wheat genotypes in various multi-environment trials. So, the present study employed the AMMI technique to evaluate heat and drought tolerance in nine amphidiploid genotypes derived from *T. bessarabicum*, focusing on grain yield. This involved field-based assessments under different environmental conditions, including heat stress (late sown), drought stress (under controlled conditions in a rain-out shelter), and normal conditions.

2. Materials and Methods

These nine amphidiploid wheat lines were introduced from Nottingham/BBSRC Wheat Research Centre (WRC). These amphidiploid lines were developed by crossing the same *T. bessarabicum*, the pollen source/male parent, with seven different durum (AABB) and two Chinese Spring i.e., bread (AABBDD) wheat cultivars (Table 1). The procedure for the development of these hybrids has been described earlier by Nemeth *et al.*, 2015. Cytological study of these amphidiploid lines to determine chromosome numbers was performed using multi-color genomic in situ hybridization (Mc-GISH) by Khokhar *et al.*, 2020. The experiment was laid out as a randomized block design with two replications. Each plot in various environments had three rows, spaced 25 cm apart, and measuring 2.5 meters in length. Two Indian wheat cultivars WH1105, HD3086 and one Chinese Spring were grown as checks in all eight environments.



Table:1. Amphidiploids used in field trials in 2019–2020 and 2020–2021.

Amphidiploids	Pedigree	Genome
EC787007	<i>Triticum. turgidum</i> L. cv. Langdon x <i>Thinopyrum bessarabicum</i> (Savul. & Rayss) Ä. Löve (Poaceae)	AABBJJ
EC787008	<i>T. turgidum</i> L. cv. Macoun x <i>T. bessarabicum</i>	AABBJJ
EC787009	<i>T. turgidum</i> L. cv. Karim x <i>T. bessarabicum</i>	AABBJJ
EC787010	<i>T. turgidum</i> L. cv. Neodur x <i>T. bessarabicum</i>	AABBJJ
EC787011	<i>T. turgidum</i> L. cv. Creso x <i>T. bessarabicum</i>	AABBJJ
EC787012	<i>T. turgidum</i> L. cv. Azaziah x <i>T. bessarabicum</i>	AABBJJ
EC787013	<i>T. turgidum</i> L. cv. Stewart x <i>T. bessarabicum</i>	AABBJJ
EC787014	<i>Triticum aestivum</i> L. cv. Chinese Spring x <i>T. bessarabicum</i>	AABBDDJJ
EC531712	<i>Triticum aestivum</i> L. cv. Chinese Spring x <i>T. bessarabicum</i>	AABBDDJJ

In rainfed late-sown conditions, irrigation was applied before field preparation, and no further irrigation was given until maturity. For drought conditions (ROS), irrigation was solely provided before field preparation. All the environmental conditions are given in Table2. Field experiments were conducted in the North Western

Plains Zones (NWPZ) of India during the 2019-2020 and 2020-2021 rabi (winter) seasons at two different locations. The first location was at the Indian Institute of Wheat & Barley Research (IIWBR) in Karnal, Haryana and second location was at CCSHAU, Hisar.

Table 2: Environments used for present study

Environments	Description of the Environments
E01	Timely sown at Karnal (mid-November) – irrigated, 2019
E02	Timely sown at Karnal (mid-November) - rainfed, 2019
E03	Timely sown at Hisar(mid-November) –irrigated, 2019
E04	Late sown at Hisar(mid-December) –rainfed, 2019
E05	Timely sown at Karnal (mid-November) – irrigated, 2020
E06	Timely sown at Karnal (mid-November) - rainfed, 2020
E07	Timely sown at Hisar(mid-November) –irrigated, 2020
E08	Late sown at Hisar(mid-December) –rainfed, 2020

Amphidiploid lines from *T. bessarabicum* were photosensitive and perennial in nature. To address these traits and ensure fertility and flowering, supplementary lighting was employed using Thousand-Watt tungsten halogen tube lamps, providing a 16h/day from 45 days after sowing (DAS) to anthesis. As maturity neared, plants in each plot were protected with 2-square-foot netting bags to prevent yield losses from shattering. Grain yield per plot (GY) was determined by harvesting and threshing each plot separately. The harvested grains were stored in individual bags, and their dry weight, at a moisture content of 12-13%, was measured in grams.

Grain yield data of all nine amphidiploid lines was subjected to pooled analysis of variance (ANOVA) to determine the effect of genotype (G), environment (E) and their interactions. Data was graphically analyzed using PB tools 2014 & STAR (Version1.4, <http://bbi.irri.org/products>). The performance of all wheat genotypes was assessed using stability models Additive Main effects and Multiplicative Interaction (AMMI) where we use only interaction components to study genotypes and GGE Biplot or Site Regression model (Yan and Kang, 2007) in which both genotypic effect (G) and its interaction with environment (GEI) are used. However, data from checks was not included in the analysis because the checks



matured earlier due to supplementary lighting and showed extreme values for all the grain yield and component traits as compared to amphidiploid lines.

3. Results and Discussion

The present study aimed to assess the performance of various *Thinopyrum* accessions under heat and drought stress conditions based on the grain yield of these genotypes. To comprehensively assess genotype performance, we conducted an AMMI-GGE biplot analysis using yield data from nine *Thinopyrum* accessions subjected to terminal heat stress, irrigated, rainfed, and drought conditions. Each combination of treatment, location, and year was treated as a distinct environment in the AMMI-GGE biplot analysis. In irrigated conditions at Karnal, EC787014 exhibited the highest grain yield per plot (0.410 kg/plot), while EC787008 showed the lowest (0.012 kg/plot). At Hisar, the highest yield under irrigated timely sown conditions was observed for EC531712 (0.374 kg/plot), with the lowest performance noted for EC787008 (0.037 kg/plot). In rainfed conditions at both Karnal and Hisar, the highest-performing genotypes were EC787007 and EC787014, yielding 0.365 kg/plot and 0.500 kg/plot, respectively. Conversely, EC787008

(0.023 kg/plot) and EC787009 (0.055 kg/plot) displayed the lowest grain yield at Karnal and Hisar under rainfed conditions, respectively. Under rainout shelter-late sown-irrigated conditions, characterized by heat and drought stress, the highest performance was recorded for genotype EC531712 (0.367 kg/plot), while the lowest grain yield was observed for EC787009 (0.227 kg/plot). Overall, the genotypes demonstrated superior performance under the combined stress of terminal heat and drought (rainout shelter conditions) stress. A graphical representation of the performance of all nine *Thinopyrum* accessions is presented in Figure 1, clearly indicating their best performance in the rainout shelter condition, where they were subjected to terminal heat stress and drought conditions.

Our results are consistent with the laboratory investigations conducted by Shafqat *et al.*, 2019 and Singh *et al.*, 2018, where they demonstrated that amphidiploids and addition lines originating from *Th. bessarabicum* exhibited superior performance in the majority field traits under drought stress conditions. So, the accessions used in the present study are recommended as sources of introducing heat and drought stress tolerance into the susceptible cultivars.

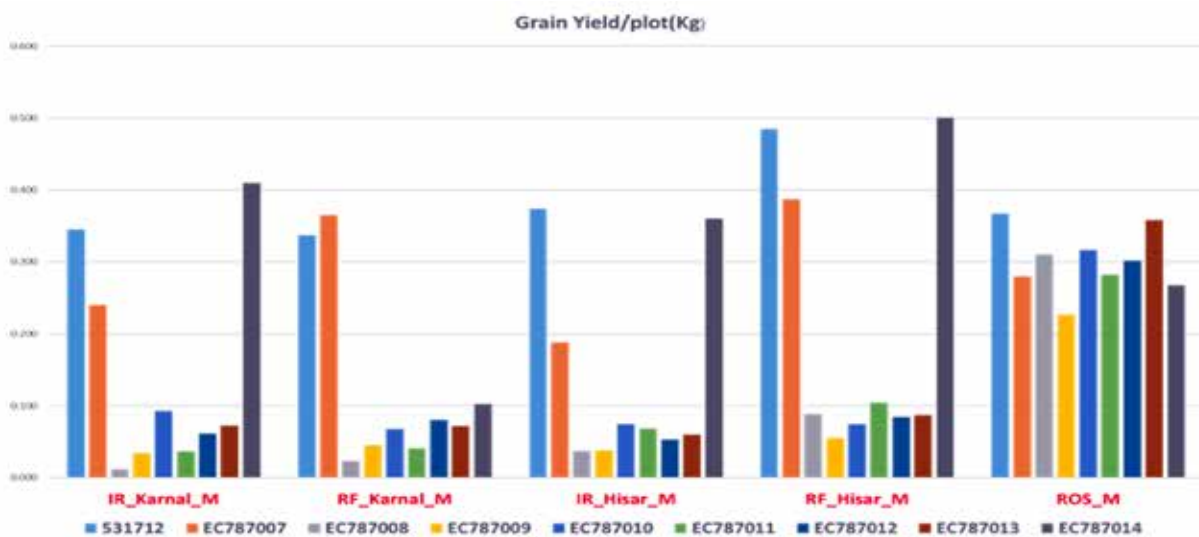


Fig 1: Two year mean performance for grain yield/ plot of nine *Thinopyrum* accessions under irrigated-Karnal (IR_Karnal_M), rainfed-Karnal (RF_Karnal_M), irrigated-Hisar (IR_Hisar_M), rainfed-Hisar (RF_Hisar_M) and rain out shelter- ROS_M (heat and drought stress at Karnal).

Since the grain yield of all accessions demonstrated particularly exhibited higher values in the rainout shelter condition (Fig. 1) compared to other environments, this specific environment was excluded from further stability

analysis. Consequently, eight distinct environments were chosen to rank genotypes based on their stability across various conditions and identify the best performers.



Table 3: AMMI Analysis of Variance over ten environments

SV	DF	SS	MS	Total Variation explained (%)	G x E explained (%)	Cumulative (%)
Trials	8	10201.6541	1275.21 ^{ns}			
Env	7	175355.253	25050.75**	4.94		4.94
Genotype	8	2600202.04	325025.26**	73.31		78.26
Env: Genotype	56	638501.251	11401.81**	18.00		96.26
PCA I	14	252128	18009**		52.70	52.70
PCA II	12	114720	9560**		24.00	76.70
PCA III	10	104740	10474**		21.90	98.60
Pooled Error	64	122513.887	1914.2795			
Total	143	3546774.09				
Mean GY (gm/plot)	154.29					
CV (%)	28.36					

PCA I & PCA II from AMMI

Statistical methodologies, including the AMMI (Additive Main Effect and Multiplicative Interaction) and GGE (Genotype Main Effect and Genotype x Environment Interaction) models, were applied to assess the performance of these lines, following the recommendations of Gauch (2006). Previous studies by Mohammadi *et al.* (2011), Mladenov and Banjac (2012), Mohammadi *et al.* (2015), Mohammadi *et al.* (2018), and Omrani *et al.* (2022) have also utilized AMMI and GGE analyses to evaluate yield performance and stability in *tritipyrum*, bread wheat, and durum wheat genotypes. The combined analysis of variance using the AMMI model demonstrated that grain yield was significantly ($p < 0.001$) influenced by genotype, environment, and their interaction. Specifically, genotype, environment, and the genotype-environment interaction contributed to 73.31%, 4.94%, and 18% of the total variation, respectively (Table 3). The AMMI model revealed the presence of genotype-environment interactions, signifying substantial variations in genotypic responses across the eight environments. Furthermore, the G X E interaction was decomposed into principal component axes (PCA), with PCA1, PCA2, and PCA3 accounting for 57.7%, 24.00%, and 21.9% of the variance, respectively. The cumulative variance explained by PC I, PC II, and PC III was approximately 98.6, indicating that the interaction between the nine genotypes and eight environments could be accurately predicted by the first three components of genotypes and environments.

So, the combined analysis of variance (ANOVA) revealed highly significant differences ($p < 0.001$) among the 36 genotypes and their performance across five environmental conditions over a two-year period. This finding is consistent with previous studies conducted by Shitaye (2015) and Jeberson *et al.* (2017).

So first three PC explained 98.6% of the Genotype-environment interaction with 36 degrees of freedom as shown in Table 4.16. The two basic AMMI biplots AMMI 1 biplot, where the main effect (Genotype and environment means) and IPCA-I scores are plotted against each other and AMMI 2 biplot where scores of IPCA-I and IPCA-II are plotted against each other were presented here in the Fig. 2A and 2B respectively. The AMMI biplot (Fig. 2A) showed 52.7% fitness in the model for grain yield. Among the accessions G1, G9 and G2 showed high yield with high main (additive) effects with positive PC1 score. G1 was found to be the most stable Genotype in the selection ranks for grain yield per plot.

The AMMI PC1 (Fig. 2A) grouped the eight studied environments into three categories: Group I included E5, E4, and E8, characterized by the highest positive values of IPCA1, and genotypes in this group exhibited the highest yield above the average value. Group II did not contain any environments. Group III consisted of E2 and E6, featuring negative PC1 scores and the lowest grain yield for accessions. *Thinopyrum* accession G8 exhibited minimal environmental interaction, whereas



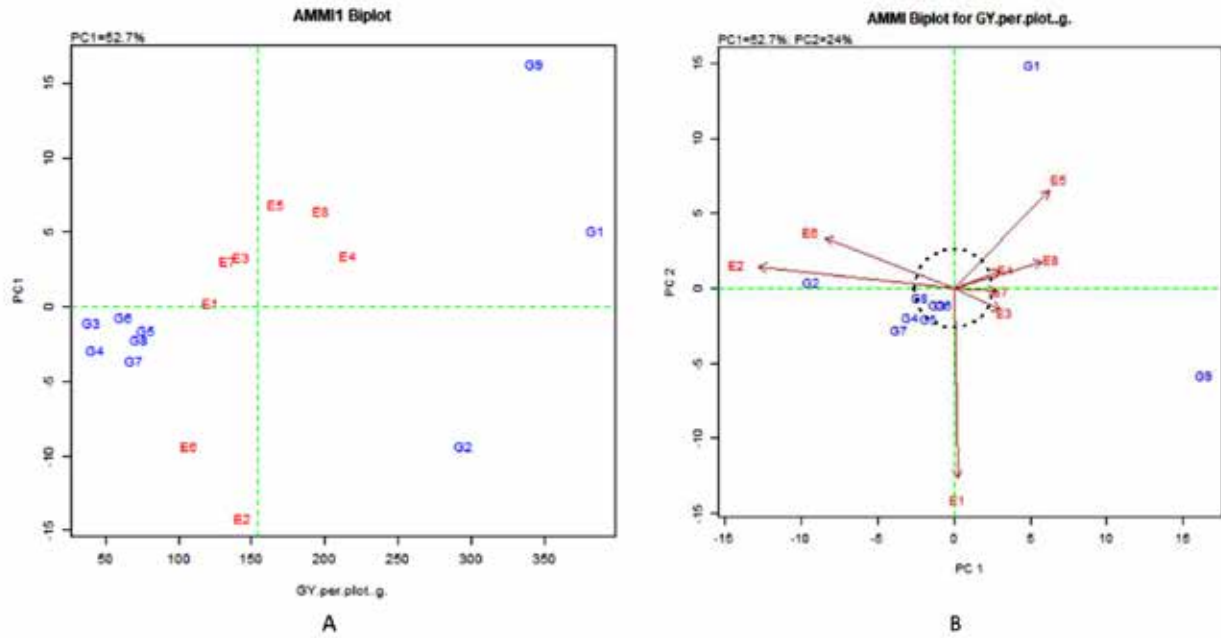


Fig. 2: AMMI biplot for the main effects *Thinopyrum* accessions in various environments (A) AMMI 1 biplot primary component of interaction (PC1) and grain yield/plot (B) AMMI biplot for PC2 and PC3

E1-irrigated normal sown-Karnal (2019-20); E2- rainfed timely sown-Karnal (2019-20); E3- irrigated timely sown –Hisar (2019-20); E4-rainfed timely sown-Hisar (2019-20); E5- irrigated normal sown-Karnal (2020-21); E6-rainfed timely sown-Karnal (2020-21); E7- irrigated timely sown –Hisar (2020-21); E8-rainfed timely sown-Hisar (2020-21).

most environments, except for E7, displayed significant interaction with grain yield. Environments E6 and E1 were highly responsive in terms of grain yield, and similar results were observed for the stability of accessions in grain yield across different environments (Fig. 3). Environments E2 and E6 experienced water deficit conditions during the vegetative stage in Karnal. Group IV encompassed environments E1, E3, and E7, characterized by timely sown irrigated conditions, and no accessions were found in this group, indicating that irrigated conditions were unsuitable for *Thinopyrum* accessions. Among the genotypes, G1>G9>G2 displayed the highest average yield (Fig. 2).

The GGE biplot (Fig. 3A) facilitated the assessment of test environments, assisting in the selection of superior genotypes for specific mega-environments. Test environments with longer vectors possessed greater discriminatory power with respect to genotypes. The shorter vector E1 (as depicted in Fig. 3A) indicated that genotypes tended to perform similarly, revealing no significant genotypic differences in these test environments among accessions. The longest vector belonged to E4, making it a more favorable environment for distinguishing genotypic differences among *Thinopyrum* accessions. In Fig. 3B, accessions were ranked based on their

performance by comparing them with a hypothetical ideal Genotype represented by the concentric area. In our current investigation, the AEA view within the GGE biplot framework pinpointed accessions G1, G9, and G2 as both high-yielding and stable performers. The shortest projection of genotype G1 onto the AEA indicated its exceptional stability and minimal genotype-environment interaction (GEI) among all nine accessions. These findings align with previous studies by Yan *et al.*, (2007) and Jeberson *et al.* (2017). The GGE comparison biplot (Fig. 3B) ranked the accessions in relation to the ‘ideal genotype,’ with G1 being the closest to this hypothetical high-performing and stable genotype for grain yield, consistent with the concept of an “ideal genotype” that excels across diverse test environments (Yan and Kang, 2003). A “desirable genotype” is one that is located closest to the ‘ideal genotype’.

The top-performing accession was G1, situated within the region corresponding to the smallest circle on the AEA. Figures 2B and 3A also highlighted representative test environments (E4, E5, E6) that had smaller angles with AEA, indicating that environment E4 was ideal for selecting adapted genotypes as it was both representative and discriminative. Environments E5 and E6 were discriminatory but not representative, making them



suitable for selecting accessions adapted to specific mega-environments.

So the observed significant interaction indicates that the grain yield of *Thinopyrum* accessions exhibited variations

across the ten studied environments. The direction and magnitude of IPCA scores provide insights into the influence and contribution of both environments and genotypes to genotype-environment interaction (GEI).

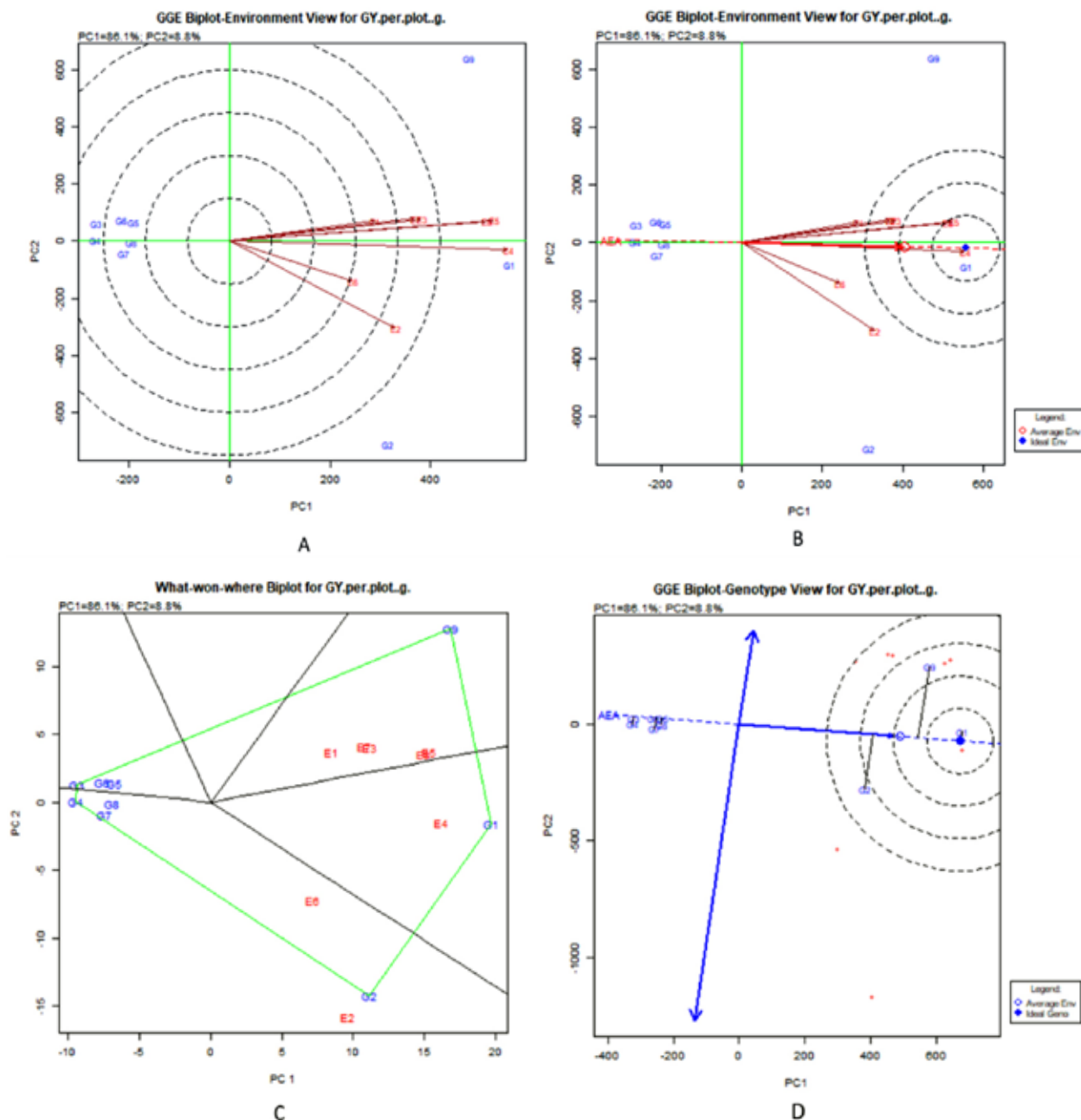


Fig. 3: GGE biplot for grain yield/plot or main effects of genotypes in various environments (A) GGE biplot comparing nine *Thinopyrum* accessions evaluated according to the discrimination and representativeness of environments for grain yield (kg/plot) (B) GGE biplot comparing nine *Thinopyrum* accessions evaluated according to the estimate of an ideal Genotype. (C) GGE biplot representing the which-won-where, where the *Thinopyrum* accessions at the vertices of the polygon represent the genotypes indicated for the respective mega-environments formed (black lines). (D) GGE-biplot analysis for “mean performance versus stability” for grain yield of *Thinopyrum* accessions.



Scores approaching zero are indicative of stability, while higher scores suggest instability, particularly in specific environments (Jeberson *et al.*, 2017). Among the genotypes, G1, G2, and G9 displayed high yields with substantial main (additive) effects and positive PC1 scores. Notably, G1 emerged as the most stable genotype based on its performance in grain yield per plot. In contrast, *Thinopyrum* accession G8 exhibited lower environmental interaction. However, with the exception of environment E7, most other environments demonstrated significant interactions with respect to grain yield. Environments E6 and E1 were particularly influential in terms of grain yield, and these findings corresponded to the stability of accessions across different environments (Figure 3).

The “which-won-where” graph (Fig. 3C) permits the visual grouping of test environments based on G X E crossing between the best accessions. The accessions at the vertices of the polygon are G9, G1, G2, G4, G3 and these genotypes perform best in the environment falling within the sectors. The eight environments were cut into 3 groups by the black lines that came out of the origin of the biplot, the groups are formed by (ME1) E1, E3, E5 and E8, (ME2) E4 and (ME3) E2 and E6. Accession G9 performed best in ME1, accession G1 in ME2 and accessions G2, G4, G7, G8 in ME3 or we could say these were the most adapted genotypes in these mega environments. It is most effective and succinct way to summarize the genotype and genotype-environment interaction and to visualize the which-won-where pattern of a multi-environment dataset (Yan and Kang, 2003). Accessions G3, G5 and G6 fell in the sectors with no environments and were not productive in any of the studied environment. The “which-won-where” graph enables the visual clustering of test environments through the analysis of genotype-environment interactions for the superior accessions. Notably, the accessions situated at the polygon’s vertices corresponded to G9, G1, G2, G4, and G3. These specific genotypes excelled in environments situated within the sectors, aligning with the observations made by Jeberson *et al.* in 2017.

In Fig. 3D, the accessions are arranged along the AEA (average environment axis) based on their average performance in all environments, with the arrow pointing to the highest yield value. The blue perpendicular line separated genotypes that performed below average (G3,

G4, G5, G6, G7, G8) from those that performed above average (G1, G9, G2). The projection of each Genotype on AEA was shown with dotted line and length of the dotted line was the measure of contribution of each Genotype to the GxE interaction. Accession G9 and G2 had largest length of the dotted vector, which indicated lower yield stability of these accessions throughout the test environments. Accession G1 was located further upstream to the average grain yield and with relatively low projection on AEA and indicated high yield and stability, with its ranking consistent across environments.

4. Conclusion

The utilization of AMMI and GGE methods to analyze grain yield per plot has elucidated the remarkable performance of these amphidiploids under conditions of heat and drought stress. The performance ranking indicated that $E4 > E3 > E2 > E1$, signifying their average performance superiority. Notably, EC787014, originating from a cross between *T. aestivum* L. cv. Chinese Spring and *T. bessarabicum*, emerged as the top-performing genotype, demonstrating enhanced grain characteristics under heat and drought stress. In conclusion, the wild relative of wheat, *T. bessarabicum*, has demonstrated its superiority over cultivated wheat cultivars in terms of heat and drought tolerance. Additionally, *T. bessarabicum* is renowned for its mineral composition and salinity tolerance. The amphidiploids derived from *T. bessarabicum* in this study exhibited generally improved grain yield under conditions of heat stress and drought-heat stress. This highlights the substantial potential for incorporating these amphidiploids into breeding programs aimed at introducing heat and drought-tolerant attributes into modern wheat varieties. Moreover, undesirable traits, such as delayed maturity, brittle rachis, and challenging threshing characteristics present in the amphidiploids, can be eliminated through systematic backcrossing and stringent selection in breeding initiatives.

Author contributions

All authors contributed equally for preparing the final version of the manuscript.

Conflict of Interest

Authors declare no conflict of interest.



Ethical Approval

The article doesn't contain any study involving ethical approval.

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