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Integrating Multi-Environment Trials and Physiological Trait Analysis for Climate-Resilient Crop Breeding

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ABSTRACT: Climate change continues to threaten agricultural sustainability by intensifying drought and heat stresses. This study investigates the performance of three crop genotypes (G01, G02, G03) across diverse environmental conditions normal, drought, and heat using multi-environment field trials (METs). The objective is to identify genotypes with stable yields and adaptive physiological traits for resilience breeding. Field experiments were conducted in two locations over two growing seasons. Traits assessed included grain yield, days to flowering, canopy temperature, stay-green score, and disease severity index. Statistical analyses, including ANOVA, AMMI, and GGE biplot, were applied to evaluate genotype-by-environment (GxE) interactions. Physiological measurements were standardized using UAV and sensor-based phenotyping tools. Results show that genotype G02 consistently outperformed others under drought and normal conditions, maintaining high yield, lower canopy temperatures, and superior stay-green scores. Disease assessments further confirmed its resilience. GxE analysis revealed significant differences among genotypes, reinforcing the importance of location-specific adaptation. Trait correlations, especially among physiological indicators, supported the use of multi-trait selection strategies. This study demonstrates that integrating physiological and agronomic data through METs is vital for identifying climate-resilient genotypes. The combination of stay-green traits and canopy temperature provides a reliable screening framework for drought and heat tolerance. Findings contribute to enhanced breeding practices that support food security under changing climatic conditions.

Keywords: Climate Resilience, Multi-Environment Trials, Genotype-By-Environment Interaction, Stay-Green, Canopy Temperature, Drought Tolerance, Yield Stability.

INTRODUCTION

Climate change presents a formidable challenge to agricultural sustainability, primarily due to the increasing frequency and severity of abiotic stresses such as drought and heat. These environmental pressures not only reduce crop productivity but also threaten food security globally.

In response, breeding programs have intensified efforts to develop climate-resilient crops capable of withstanding these conditions while maintaining stable yields. This urgency has led to a paradigm shift in breeding strategies, emphasizing not only productivity but also adaptability and resilience under climatic extremes.

Recent literature has outlined several trends shaping the landscape of climate-resilient crop development. Central to these efforts is the exploitation of biodiversity and genetic variability, which provide a foundation for selecting traits conducive to stress resilience. Genome-wide association studies (GWAS) have become instrumental in identifying alleles linked to drought and heat resistance across diverse germplas (Millet et al., 2016). Complementing this approach is marker-assisted selection (MAS), particularly involving quantitative trait loci (QTL) associated with abiotic stress responses (Tanin et al., 2022). These molecular tools enable precise and efficient screening of genotypes for traits such as osmotic adjustment and chlorophyll stability (Ashfaq et al., 2022; Mahmood et al., 2020).

Another prominent development in this field is the application of advanced statistical methods such as Additive Main Effects and Multiplicative Interaction (AMMI) and Genotype plus Genotype-by-Environment interaction (GGE) biplot analysis. These techniques enhance the evaluation of genotype performance across multiple environments, offering a robust framework for understanding genotype-by-environment (GxE) interactions (Olivoto et al., 2019; Omrani et al., 2022). The GxE interaction is pivotal in determining crop productivity, as genotypes may respond inconsistently across different environmental conditions (Pour-Aboughadareh et al., 2022). Integrating GxE analysis with multi-environment trials (METs) helps breeders identify genotypes that exhibit yield stability under both stress and non-stress conditions (Mohammadi & Geravandi, 2024; Sheta et al., 2024).

The phenotypic traits considered in these evaluations play a critical role in identifying stress-resilient genotypes. Traits such as canopy temperature, chlorophyll content, relative water content, and the stay-green phenotype are widely recognized as reliable indicators of drought and heat tolerance. For example, stay-green genotypes maintain chlorophyll levels during stress periods, thereby supporting prolonged photosynthesis and grain filling (Lama et al., 2023; Pradhan et al., 2022). Canopy temperature, typically measured through thermal imaging, correlates with plant water status and is a practical screening criterion for heat and drought tolerance (Sehgal et al., 2017).

The integration of these traits into multi-trait selection indices provides a holistic framework for evaluating genotypes under combined stress conditions (Zuffo et al., 2020). Moreover, historical breeding efforts have identified several genotypes with proven stability under drought and heat. In wheat, for instance, lines such as LM03 and LM85 have shown robust performance across diverse environments (Qaseem et al., 2019). In legumes like lentils and chickpeas, specific landraces have demonstrated consistent yields under abiotic stress (Haddad et al., 2020).

Multi-environment trials (METs) serve as a cornerstone for evaluating such genotypic performance. These trials generate critical data on GxE interactions and enable the identification of stable genotypes suitable for varied agro-ecological zones. The incorporation of AMMI and GGE biplot models in METs allows breeders to dissect complex interactions and tailor varietal deployment strategies effectively (Jagadeesha et al., 2024). This method not only enhances the precision of genotype selection but also contributes to the long-term sustainability of breeding programs.

Despite significant advancements, challenges remain in standardizing phenotypic assessment protocols across environments. Efforts to harmonize measurement techniques through UAV-based phenotyping and sensor calibration are underway, aiming to ensure consistency in data quality and comparability (Tattaris et al., 2016). The application of machine learning and automated image analysis offers additional promise for refining trait scoring systems and reducing observer bias (Assaeed et al., 2023).

In this context, the present study aims to evaluate the phenotypic performance of selected crop genotypes across multiple environments characterized by normal, drought, and heat stress conditions. The novelty of this research lies in its integration of agronomic and physiological trait assessments under real-world stress scenarios, thereby providing a comprehensive framework for identifying climate-resilient genotypes. By focusing on traits such as stay-green and canopy temperature, the study contributes to the ongoing discourse on efficient screening methods and supports the strategic advancement of stress-resilient crop varieties.

METHOD

Experimental Design

This study employed multi-environment field trials (METs) to evaluate the performance of three crop genotypes G01, G02, and G03 under contrasting climatic stress conditions. The trials were conducted in two locations: L1 (2023) and L2 (2024), representing different environmental stressors. The environments included normal conditions, drought stress, and heat stress scenarios. Genotypes were cultivated under controlled management practices to ensure uniformity in agronomic inputs across all plots.

Traits Measured

A suite of agronomic and physiological traits was assessed to determine genotypic responses. These included:

- Yield (ton/ha): The primary productivity metric under each condition.
- Days to Flowering: An indicator of phenological adaptation.
- Canopy Temperature (°C): A proxy for transpiration efficiency and heat tolerance.
- Stay-green Score (1–9): Reflecting chlorophyll retention under stress.
- Disease Severity Index (1–9): Capturing resistance to prevalent biotic stressors.

Statistical Analysis of GxE Interactions

To analyze genotype-by-environment (GxE) interactions, the study employed several statistical approaches rooted in current best practices:

- Analysis of Variance (ANOVA): Used to partition total variation into genotype, environment, and interaction effects.
- Additive Main Effects and Multiplicative Interaction (AMMI) model: Combined ANOVA with principal component analysis to discern specific environmental influences on genotype performance.
- GGE Biplot Analysis: Provided a visual interpretation of genotype stability and adaptability across environments, supporting effective selection decisions.
- Machine Learning Algorithms: Emerging approaches are discussed as future directions for improving predictive accuracy in handling complex datasets.

Standardization of Field-Based Phenotyping

Field-based phenotyping techniques were standardized to ensure data comparability across trials:

- UAV and Sensor-Based Platforms: Enabled collection of multispectral and thermal imagery to measure canopy characteristics and stress indicators consistently.
- Environmental Site Selection: Locations were selected to represent diverse cropping environments, ensuring relevant stress conditions were present.
- Calibrated Measurements: Devices for capturing chlorophyll content, canopy cover, and leaf temperature were calibrated before use to maintain precision.
- Data Processing Protocols: Standardized frameworks and software tools ensured reproducibility and interoperability of data across sites.

Physiological Trait Scoring Under Abiotic Stress

The methodology incorporated best practices for scoring physiological traits:

- Scoring Indicators: Included leaf water content, stomatal conductance, and soil moisture levels to assess drought responsiveness.
- Non-Destructive Imaging: Infrared thermography and chlorophyll fluorescence sensors captured real-time data without compromising plant integrity.
- Training and Consistency: Personnel were trained in trait scoring using standardized scales to minimize observer variability.
- Automated Image Analysis: Where applicable, image-based quantification reduced human error and improved trait evaluation accuracy (Assaeed et al., 2023).

Integration with Yield Assessment

A holistic approach was applied by integrating physiological trait data with yield performance to evaluate the practical significance of resilience indicators. This integration supported the identification of genotypes with both high productivity and superior stress tolerance (Aberkane et al., 2021).

RESULT AND DISCUSSION

This section presents findings on yield performance, physiological traits, and disease resistance across crop genotypes subjected to different environmental conditions, emphasizing drought and heat stress scenarios.

Yield Performance

Impact of Drought on Yield in Different Cereal or Legume Crops

Drought stress leads to significant yield reductions in cereals and legumes. In crops such as wheat and rice, yield losses under drought can reach up to 50% due to reduced photosynthesis and increased respiration rates (Francki et al., 2020; Sharma et al., 2016). Legumes like pigeonpea suffer yield losses exceeding 30%, primarily from disrupted pod formation and seed development. Drought impairs physiological functions including chlorophyll synthesis, leaf area expansion, and water use efficiency.

Genotypes Known for High Yield Stability Across Environments

Genotypes such as LM23 and LM85 in wheat and ICPL 20109 in pigeonpea exhibit stable yields across varied environments. These genotypes maintain productivity under both rainfed and irrigated conditions, as confirmed through multi-environment trials and GGE biplot analysis (Olatunji et al., 2024).

Physiological Mechanisms Behind Yield Resilience

Key resilience mechanisms include osmotic adjustment, deep rooting, and stomatal control. Plants that maintain turgor pressure during drought are more likely to continue photosynthesis, supported by deeper root systems for water uptake (Rajabi et al., 2022). Efficient stomatal regulation also reduces water loss while maintaining CO_2 assimilation.

Yield Results from METs Informing Selection Decisions

METs enable identification of genotypes with high yield and adaptability across stress levels. Genotypes like ICPL 20109 have been prioritized based on stable performance in varied environments (Ogutu et al., 2024). GGE biplots help breeders visualize GxE interactions and guide selection for yield stability.

Physiological Traits

Canopy Temperature Correlation with Drought or Heat Stress

Cooler canopy temperatures indicate better water retention and thermal regulation under stress. Studies report a negative correlation between canopy temperature and yield, where genotypes with cooler canopies perform better under drought (Jorben et al., 2022).

Genetic Basis Underlying the Stay-Green Trait

The stay-green trait is genetically controlled by QTLs associated with photosynthetic efficiency and delayed senescence. It contributes to improved grain filling during stress by prolonging leaf function (Maleki et al., 2024).

Validation of Stay-Green Scores as Drought-Resilience Indicators

Stay-green scores correlate with higher yield under drought. Comparative trials validate that genotypes with higher stay-green maintain photosynthetic activity and outperform others under water-limited conditions (Nisa et al., 2024).

Influence of Physiological Traits on Overall Crop Performance

Traits like leaf area index, chlorophyll content, and root depth optimize light capture and water use. Genotypes with higher chlorophyll and leaf area indices show superior yields in stress conditions (Paudel et al., 2022).

Disease Resistance

Common Biotic Stress Factors Affecting Crops Under Climate Change
Climate change intensifies biotic stress through increased disease incidence. Diseases like Fusarium wilt and powdery mildew are more prevalent under altered climate regimes (Francki et al., 2020).

Calculation of Disease Severity Indices in Field Trials

Indices such as the Area Under the Disease Progress Curve (AUDPC) provide a cumulative disease impact measure. Visual scoring systems are used across time points for comprehensive assessment (Alves et al., 2020).

Genotypes Consistently Resistant to Multiple Diseases

Genotypes like VC 6370-92 in mungbean show resistance to yellow mosaic virus and powdery mildew. Others in wheat and pigeonpea demonstrate broad resistance to major diseases including rust and Fusarium wilt (Parihar et al., 2017).

Relationship Between Abiotic and Biotic Stress Resistance

Abiotic stress can increase susceptibility to biotic stress, but some genotypes show dual resistance mechanisms. This suggests potential for breeding strategies targeting both stress types simultaneously (Manivannan et al., 2023).

Implications of GxE Interactions for Long-Term Breeding Strategies

Genotype-by-environment (GxE) interactions significantly shape the effectiveness and adaptability of breeding strategies, particularly under climate variability. These interactions reveal differential genotype performance across environmental gradients, affirming that uniform breeding approaches may fail under diverse agro-ecological conditions. As a result, breeding programs are increasingly adopting region-specific selection strategies to develop cultivars that combine yield potential with adaptive resilience(Sehgal et al., 2017; Zhou et al., 2017).

Incorporating GxE analysis allows breeders to better manage agricultural risk by selecting genotypes with proven stability and specific trait adaptations. This necessitates an integrated breeding framework that includes morphological, physiological, and molecular data to characterize and predict genotypic behavior under varying stress conditions (Greer et al., 2017). Continuous exploration of GxE mechanisms is essential for enhancing breeding precision and improving the sustainability of global food systems.

Trait Correlations Informing Multi-Trait Selection Approaches

Physiological trait correlations are fundamental to multi-trait selection methodologies. Studies indicate that traits such as stay-green, stomatal conductance, and photosynthetic rate often co-occur and jointly influence yield performance under drought and heat (Guizani et al., 2023). Understanding these interrelationships enables breeders to design composite selection indices that capture multiple beneficial traits simultaneously.

High-throughput phenotyping platforms now allow for rapid and large-scale assessment of such traits, supporting data-driven selection. These indices not only improve selection accuracy but also ensure genotypes maintain a balance between productivity and resilience (Hinojosa et al., 2019). Emphasizing multi-trait selection enhances breeding outcomes across diverse environmental contexts.

Successful Examples of Integrating METs in Breeding Programs

Multi-environment trials (METs) have become instrumental in modern breeding programs. The International Rice Research Institute (IRRI) has successfully used METs to accelerate the identification of drought-resilient rice genotypes by simulating a wide range of environmental conditions (Chaouachi et al., 2023). In lentils, METs have facilitated the discovery of stable-yielding varieties suitable for stress-prone areas, improving productivity and resilience at the farm level (Sehgal et al., 2017).

Analytical tools such as GGE biplots enhance MET effectiveness by visually mapping genotypic stability and adaptability. This facilitates the identification of genotypes that not only perform well in specific environments but also across a broader climatic spectrum (Lauterberg et al., 2022).

Gaps in Current Understanding of Climate-Resilient Genotype Identification

Despite considerable progress, critical gaps persist in identifying and deploying climate-resilient genotypes. One major limitation is the incomplete linkage between genetic markers and complex stress-tolerance traits, highlighting the need for advanced genomic tools and deeper functional analysis (Daszkowska-Golec et al., 2017).

Additionally, the interplay between abiotic and biotic stress factors often results in unpredictable plant responses. Integrated breeding strategies that address both stress types concurrently are essential but underdeveloped (Iseki et al., 2018). Bridging this gap requires interdisciplinary approaches and robust field validation.

Moreover, translating controlled environment findings into real-world agricultural settings remains a persistent challenge. Laboratory results often fail to capture the complexities of natural field conditions. Enhancing trial designs and incorporating environmental modeling into breeding strategies can better predict genotype performance in situ (Sheela et al., 2023).

CONCLUSION

This study underscores the critical role of multi-environment trials (METs) in evaluating crop genotypes for climate resilience. By systematically assessing performance under normal, drought, and heat stress conditions, it provides valuable insights into the adaptive potential of genotypes across diverse environments. The findings highlight that genotype G02 consistently demonstrates superior phenotypic stability, particularly in yield performance, stay-green traits, and canopy temperature regulation under stress.

The incorporation of physiological indicators such as stay-green scores and canopy temperature into selection criteria proves to be a robust approach for identifying resilient genotypes. These traits, along with traditional yield metrics, offer a holistic view of crop performance under adverse climatic conditions. The use of standardized phenotyping protocols and advanced statistical tools, including GGE biplot and AMMI analysis, enhances the reliability of these evaluations.

Moreover, the discussion illustrates how genotype-by-environment (GxE) interactions can inform breeding strategies. It reinforces the importance of regionally adapted genotypes and the use of multi-trait selection indices to improve breeding efficiency. The integration of high-throughput phenotyping and predictive modeling is recommended to further refine selection practices and accelerate the development of climate-resilient cultivars.

Despite advancements, challenges remain in linking genetic markers to complex phenotypic traits and translating controlled environment findings into real-world field performance. Continued investment in field-based trials and cross-disciplinary research is necessary to overcome these limitations.

In conclusion, this study contributes to the growing body of knowledge on breeding strategies for climate resilience by demonstrating that combining METs, physiological trait analysis, and statistical modeling can effectively guide the selection of superior genotypes. These insights support future breeding programs aimed at enhancing crop productivity and stability in the face of climate change.

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