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IMPACT OF CLIMATE-RESILIENT CROP VARIETIES ON SUSTAINABLE AGRICULTURE: AN ANALYSIS OF PHYSIOLOGICAL AND YIELD PERFORMANCE

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Keywords: Climate change, Combined stress, Resilient crop varieties, Physiological response, Random forest model

Abstract

This paper constructs a controllable environmental chamber to program and simulate the compound stress of high temperature, drought and aphids, and dynamically regulates the order and duration. RNA-Seq and LC-MS technology were combined to analyze leaf gene expression and metabolite changes, and monitor stomatal conductance and water use efficiency in real time. Field experiments were carried out in three ecological zones: arid, humid warm and temperate rain-fed, yield. The yield and biomass of 10 varieties were continuously monitored. Based on the random forest model, the environmental parameters, gene expression and yield data were integrated to construct a stress resistance prediction model to evaluate the adaptability of varieties under unknown stress combinations. The results showed that the comprehensive adaptability index (CRI) of climate-resistant varieties was generally higher than 0.6, the yield stability index (YSI) was between 30 and 47, the standardized photosynthetic efficiency (PER) was significantly better than that of general varieties, and the yield loss was lower. The study provides decision support and molecular tools for variety layout and stress resistance breeding.

Introduction

Extreme weather events caused by global climate change have become a key factor threatening agricultural sustainability (Lesk *et al.* 2022). Drought, high temperature, floods, pests and diseases often appear in alternating or synergistic ways in the natural environment, forming a complex combined stress environment. These stresses not only directly inhibit the physiological metabolism and yield formation of crops, but also indirectly increase the vulnerability of agricultural systems by affecting the structure of soil microbial communities and nutrient cycles (Chaudhury and Sidhu 2022). Traditional studies have focused on the role of a single stress factor, such as simulation experiments of drought or high temperature, ignoring the dynamic interactions between multiple stress factors (Rezaei *et al.* 2023, Aziz and Masmoudi 2025). This simplified model makes it difficult to generalize laboratory results in field environments with significant climate fluctuations (Pixley *et al.* 2023).

Breeding and applying stress-resistant crops are core strategies for coping with climate change, but their physiological regulatory networks, stress-resistant gene expression and yield stabilization mechanisms have not yet been systematically elucidated. Sustainable agriculture requires crops to maintain stable yields in extreme environments, reduce water and fertilizer inputs, and protect soil health (Mao *et al.* 2023, Sato *et al.* 2024), which puts forward a multi-dimensional integration demand for variety evaluation. Existing research focuses on three aspects: gene function verification, physiological phenotype screening and yield prediction (O'Brien *et al.* 2021, Ahmed *et al.* 2022, Yanagi 2024).

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The above studies generally face the problem of disconnection between experimental design and actual environment. Many resistance genes screened out under single stress fail to function under compound stress due to energy allocation conflicts or signal pathway interference, leading to crop metabolic disorders (Neupane *et al.* 2022, Anand *et al.* 2023). In addition, most field experiments are limited to short-term or single regions, making it difficult to capture the long-term cumulative effects of climate change on crop-soil systems (Töpfer and Trapp 2022, Khalid *et al.* 2023, van Leeuwen *et al.* 2024), which weakens the comprehensive assessment of crop resilience.

To overcome the above limitations, some studies have attempted multi-factor interaction designs to reveal the non-additive effects between stress factors (Choudhury *et al.* 2021, Eckardt *et al*. 2023). In terms of analytical methods, omics technology has advanced the study of stress resistance mechanisms to the molecular network level, but it is currently limited to a single-omics dimension and lacks cross-omics integration. Although machine learning algorithms provide new paths for stress resistance prediction, their construction relies heavily on laboratory data and does not fully incorporate the spatial heterogeneity and temporal dynamics of field environments (Prasanna *et al.* 2021, Reynolds *et al*. 2021, Satori *et al.* 2022). This shows that to study the crop response mechanism under combined stress, it is necessary to build a multi-scale, cross-dimensional data collection and analysis system that covers the causal chain from the molecular to the ecological level.

The aim of this study is to establish a comprehensive framework that integrates environmental simulation, molecular analysis, field validation and intelligent prediction, systematically elucidating the physiological response and yield stability mechanism of crops under the combined stress of high temperature, drought, and pest infestations. Multiple stress sequences are dynamically simulated through a controllable environmental chamber; Combining multiple omics techniques to reveal the synergistic regulatory pathways between key genes and metabolites; Tracking yield stability and soil health dynamics based on a long-term observation network across ecological zones; Using machine learning to integrate multi-source data to construct a stress resistance prediction model, providing decision support for climate resilient variety breeding and optimization layout.

Materials and Methods

This study selected ten crop varieties, comprising four climate-resilient varieties (CV-02, CV-08, CV-04, and CV-09) and six non-climate-adapted varieties. The latter group included maize 'DT Maize-1', wheat 'HD 2967', wheat 'Baj', rice 'Sahbhagi Dhan', along with conventional wheat, rice, and maize varieties that lack climate-adaptive traits. Physiological parameters and yield loss under high temperature (HT), drought (DR) and combined stress (COMB) conditions were measured.

In order to simulate the combined adversity of high temperature, drought and pests and diseases, this study constructed a controllable environmental chamber system. The size of the chamber was $2.0 \times 1.5 \times 1.5$m. The temperature control adopts a semiconductor unit with PID feedback regulation. Humidity was adjusted by ultrasonic spray and dehumidification device (Aslam *et al.* 2022, Ackerl *et al.* 2023). Soil moisture was controlled in real time by TDR sensor linkage irrigation system.

The pest simulation module consists of a temperature-controlled aphid chamber and a dosing releaser that can accurately release 10-50 cotton aphids (*Aphis gossypii*) at a time, evenly distributed on the foliage by peristaltic pumps and airflow conveyors, as shown in Fig. 1. It includes an aphid breeding cabin and a quantitative releaser. The control software developed based on the LabVIEW 2022 platform supports batch setting of stress programs, remote monitoring, and full-process data acquisition and backup to ensure traceability of the experiment.

To simulate the dynamic time effect of stress, the software presets multiple sets of compound stress programs were used. The system collects temperature and humidity, soil moisture and airflow parameters every 10 minutes to ensure data integrity. Ultraviolet insecticide was used to ensure the independence of subsequent experiments. The environmental chamber system supports the application of single-factor and multi-factor compound stress, meets the needs of high repeatability and high standardization experiments, and provides a reliable platform for physiological and molecular mechanism research.

Fig. 1. Distribution of aphids on the leaves in the environmental chamber.

In this study, transcriptome sequencing (RNA-Seq), non-targeted metabolomics (LC-MS) and photosynthetic physiological indicators were used for coordinated analysis of the molecular response of crops under combined stress. Samples were collected uniformly on the morning of the fifth day of triple stress and located in the second leaf of the functional leaf. RNA extraction used RNAiso Plus reagent to ensure sample purity and integrity (A260/280 1.9- 2.1, RIN >7). Transcriptome sequencing used the Illumina NovaSeq 6000 platform, with 150 bp double-ends, and an average of about 6 Gb of data per sample (Dussarrat *et al.* 2022, Lahlali *et al.* 2024). After quality control, the sequences were aligned to the maize B73 v4 or wheat CS RefSeq v1.1 genomes using Hisat2, and the alignment rates were both over 90%.

Gene expression was quantified using feature counts, and the threshold for screening differentially expressed genes was $|\log_2(\text{FoldChange})| \geqslant 1$ and $\text{FDR} \leqslant 0.05$. Functional annotation focused on stress response, osmotic regulation, heat shock protein and transcription factor pathways, and core regulatory genes such as DREB2A, HSP70 and WRKY33 were identified. Some key genes were verified by qRT-PCR to ensure the accuracy and reliability of transcriptome results.

Metabolome analysis uses a high-resolution mass spectrometry system combined with a HILIC/C18 dual-column mode to achieve full coverage of polar and non-polar metabolites. The standardized process covers sample preparation, chromatographic separation and mass spectrometry detection.

Data processing was completed by Compound Discoverer 3.1 for peak identification, calibration and normalization and the QC sample (CV < 20%) was used as the quality control standard. PCA, PLS-DA and significance analysis were performed using the MetaboAnalyst platform to screen differential metabolites, and functional annotation was performed using HMDB and KEGG, focusing on core metabolites related to stress and combining transcriptome data to mine upstream regulatory factors and construct a response network (Zahedi *et al.* 2025). At the same time, on the 3rd, 5th, and 7th days of triple stress treatment, the photosynthetic rate (A), stomatal conductance (gs), and water use efficiency (WUEi) of functional leaves were monitored using LI-6800, which was measured uniformly from 9 to 11 am. Three functional leaves were measured for each plant. Combined modeling was performed with omics data to improve the explanatory power and screening accuracy of stress response indicators.

This study ensures the complementarity and comparability between data dimensions through multi-omics collaborative strategies, standardized sampling and unified analysis processes, and analyzes the key regulatory pathways and physiological mechanisms of climate-resistant varieties under combined stress at the molecular level, providing basic data support for the functional verification of stress-resistant genes and breeding applications.

In order to systematically evaluate the yield and resource utilization performance of climate-resistant crops in different ecological zones and analyze their long-term impact on soil ecosystems, a long-term field experiment network was established in arid areas (Alxa League, Inner Mongolia), humid areas (Danzhou City, Hainan) and temperate rain-fed areas (Handan, Hebei). Standardized experimental plots (⩾0.5hm²) were set up in each area, and a randomized block design was adopted with 3 replications.

The experiment selected 10 crop varieties with different stress resistance characteristics, covering drought resistance, heat resistance and combined stress types, all of which were screened and verified by simulation in the early stage. The sowing density was unified (60,000 corn plants/hm², 180kg/ha for wheat), and the sowing period was arranged according to the local farming season. Field management uniformly implemented standardized agronomic measures, including fertilization, tillage, pest and disease control.

During the planting cycle, the UAV hyperspectral platform was used to obtain indicators such as NDVI, GNDVI, and canopy temperature. Agronomic traits were collected at ground verification points (10 per plot) to dynamically monitor growth conditions (Hafeez *et al.* 2023). The maturity period was determined based on the accumulated temperature and variety characteristics, and all plots were uniformly harvested and sampled after physiological maturity.

The yield of crops per unit area was calculated, and moisture, thousand-grain weight, and protein content were recorded. The economic coefficient and product distribution efficiency was calculated. Soil health monitoring was carried out before planting and after harvesting. Organic carbon, total nitrogen and pH were recorded following standard methods. Microbial diversity analysis used 16S/ITS sequencing and qPCR methods and QIIME2 was used for ASV identification and diversity calculation. Temperature, precipitation, sunshine, ground temperature and other data were recorded.

This study constructed a stress resistance prediction model based on the random forest (RF) algorithm, integrating leaf transcriptome, metabolome, environmental time series and multi-regional field yield data to achieve accurate modeling of yield potential under multi-dimensional adversity conditions (Burgess *et al.* 2022). The entire process covers data preprocessing, feature screening, model training and verification, all based on Python 3.10 and scikit-learn library.

The sample data came from 10 varieties under 3 ecological zones, 3 years and 4 stress conditions-with a total of 512 groups. Gene expression was standardized by FPKM, and 146 key genes related to stress response were screened. Metabolite data were standardized by Z-score, covering 68 stress response substances, including proline, betaine, etc. The environmental characteristics were calculated by backward sliding windows. The mean and standard deviation of the 7-day and 30-day scales formed a total of 96 time series features.

To enhance the stability of the model, the information gain method was used to evaluate the importance of variables, and the top 100 key features were selected for modeling. Missing values were filled using the K nearest neighbor interpolation method (k=5). The data was divided into 80% training set and 20% test set, and 5-fold cross validation was used to optimize hyperparameters in the training set. The random forest model consisted of 1000 trees, with a maximum depth of 20, a minimum number of split samples of 5, and a split criterion of mean square error (MSE). Bootstrap sampling was used. Each split randomly selected $\sqrt{d}$ features to prevent overfitting, and the out-of-bag error (OOB) was used to evaluate the model performance. The SHAP framework was used to interpret the model, quantify the contribution of genes and metabolites to yield prediction, and identify regulatory factors sensitive to adversity.

To simulate future climate stress scenarios, climate forecast data for 2040-2060 under the CMIP6 representative concentration pathways RCP4.5 and RCP8.5 were used to replace environmental characteristics to assess the yield potential of varieties under "unseen" climate combinations. Combined with uncertainty analysis, a variety recommendation and stress resistance scoring system was constructed, with a 95% confidence interval as the basis for risk assessment to support decision-making and promotion.

Results and Discussion

Fig. 2 illustrates the $\log_2$-fold change ($\log_2$FC) values of key stress-responsive genes and their representative metabolites under four treatment conditions (Control, Heat, Drought, and Combined Stress). The results demonstrate that HSP70 exhibited the most pronounced upregulation under combined stress ($\log_2$FC $\approx$ 3.8), while proline accumulation was the strongest at the metabolic level ($\log_2$FC $\approx$ 5.1), significantly exceeding levels observed under individual stresses (Heat: 4.2, Drought: 3.5). This highlights proline's pivotal role in osmotic adjustment. These findings align with multi-omics analyses, indicating that combined stress induces a nonlinear additive effect that drives the module-specific synergistic responses of gene-metabolite networks. This establishes a molecular foundation for screening molecular markers for the Composite Resistance Index (CRI) and provides mechanistic insights for validating experimental and field-level stress associations.

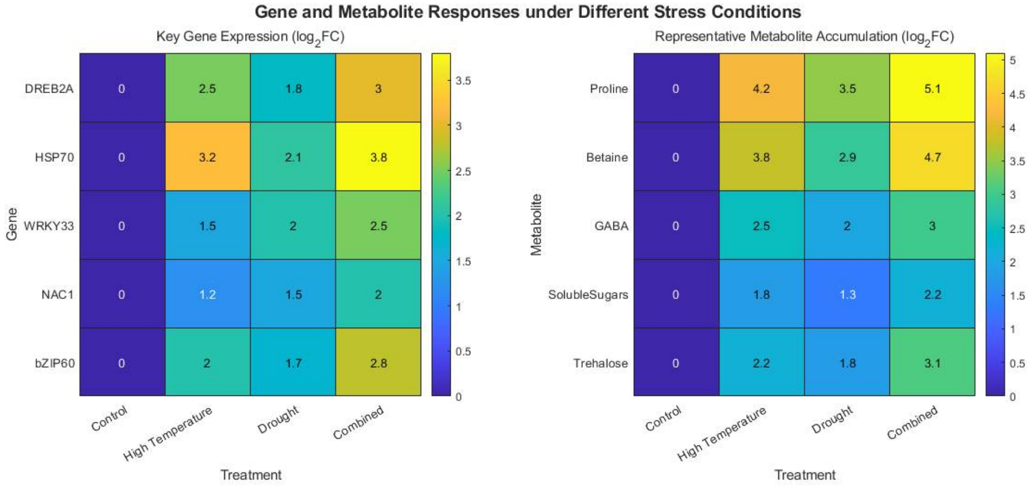

Fig. 2. Responses of genes and metabolites under different stress conditions.

In Fig. 3, the horizontal axis is the vegetation index (NDVI), and the vertical axis is the canopy temperature (℃). The three symbols represent 30 sample points in the arid region (Arid), temperate rain-fed region (Temperate), and humid and warm region (Humid), and the linear fitting curves of each region are superimposed. It can be found that the NDVI of samples in the arid region is concentrated between 0.40 and 0.60, and the corresponding canopy temperature is mostly between 30 and 35°C. The NDVI of samples in the temperate region is about 0.50-0.70, and the temperature is between 28 and 32°C. The fitting slope is slightly slow. The NDVI of samples in the humid and warm region can reach up to 0.80 and the lowest temperature is about 25°C, indicating that the transpiration cooling effect is more significant. Fig. 3 verifies the effectiveness of combining hyperspectral monitoring with canopy thermal imaging for crop growth status and heat stress assessment in different ecological regions.

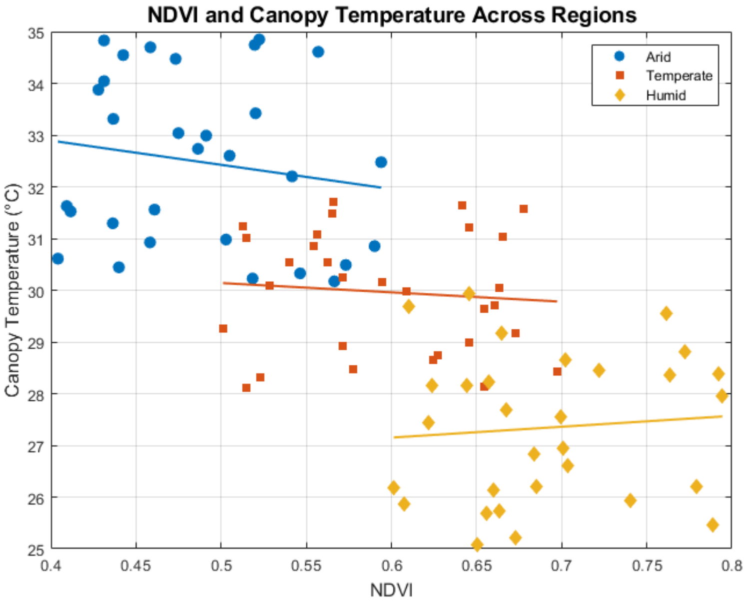

Fig. 3. Regional distribution and linear relationship between NDVI and canopy temperature.

Fig. 4 shows a climate adaptive crop stress resistance prediction and evaluation system based on a random forest model, which combines multiple omics data and environmental factors to reveal the dynamic impact mechanism of key features on yield. Through the iterative curve of model training, it can be seen that as the number of decision trees increases to 1000, the OOB (Out of Bag) error gradually converges to below 0.01, the test set R^2 stabilizes in the range of 0.80-0.86, and the MSE and MAE decrease to around 0.23 and 0.11, respectively, indicating that the model has high-precision prediction ability under compound stress.

The radar chart in Fig. 5 reveals the weight distribution of each physiological index's contribution to CRI, among which Pn_{DR} and SOD_{COMB} have higher weights, indicating that photosynthetic stability and antioxidant capacity under cross stress have significant effects on comprehensive stress resistance. In the bar chart, CV-02 leads with a CRI of 0.98, while CV-05 is only 0.09, reflecting the significant differences in comprehensive stress resistance among varieties.

Table 1 shows that the yield loss rate of the highly resistant variety CV-02 under combined stress was only 22.3%, the DREB2A gene expression was 6.8 times that of the control variety, and the SOD activity reached 412 U/mg, which supports that DREB2A maintains photosynthetic efficiency (18.9 μmol/m²/s) by enhancing antioxidant capacity. CAT activity was maintained above 22.6 U/g in all highly resistant varieties. Although CV-08 had a lower DREB2A expression (5.2 times), the SOD activity was still maintained at 398 U/mg, and the yield loss was 24.1%. The low-resistant variety CV-09 had suppressed DREB2A expression (1.6 times), the SOD activity dropped to 203 U/mg, the photosynthetic rate dropped to 13.2 μmol/m²/s, and the yield loss was as high as 35.5%, highlighting the key role of the DREB2A-SOD regulatory axis in the stress resistance mechanism.

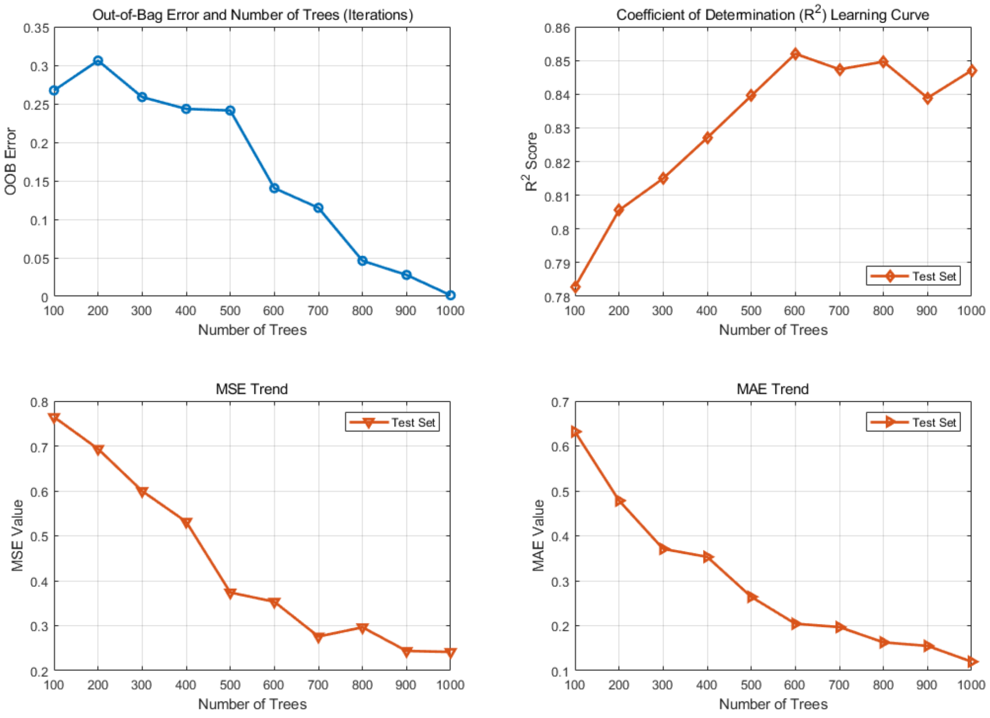

Fig. 4. Random forest model performance trend as the number of trees changes.

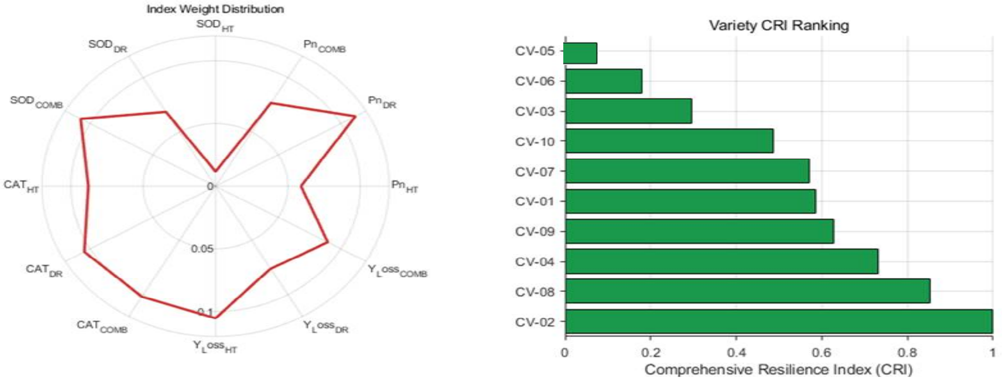

Fig. 5. Weight distribution and CRI ranking of physiological index.

The yield stability index (YSI) was calculated based on three years of cross-regional field trial data, taking into account the inverse of the yield coefficient of variation in the arid zone, temperate rain-fed zone, and humid warm zone, and taking the average of the three zones to reduce the impact of sample size differences. Tukey's rule was used to correct outliers in extreme rainfall years. Fig. 6 shows the YSI values of each variety in the three ecological regions and their average values. The YSI of climate-resistant varieties ranged from 30 to 47, which was significantly higher than that of general varieties. CV-02 had the highest YSI (60) in the temperate zone and also performed well in the arid and humid zones.

The photosynthetic efficiency ratio (PER) was calculated by measuring the net photosynthetic rate (Pn) and water use efficiency (WUE) using the LI-6800 photosynthetic measurement system. All measurements were performed at the fifth leaf of the main stem during the vegetative growth period. Table 2 lists the raw data of Pn and WUE for the 10 varieties, and Table 3 gives the raw and normalized PER values. The standardized PER values of CV-02 and CV-08 were 0.88 and 0.80, respectively, with the highest resource conversion efficiency; CV-05 had the lowest, only 0.10.

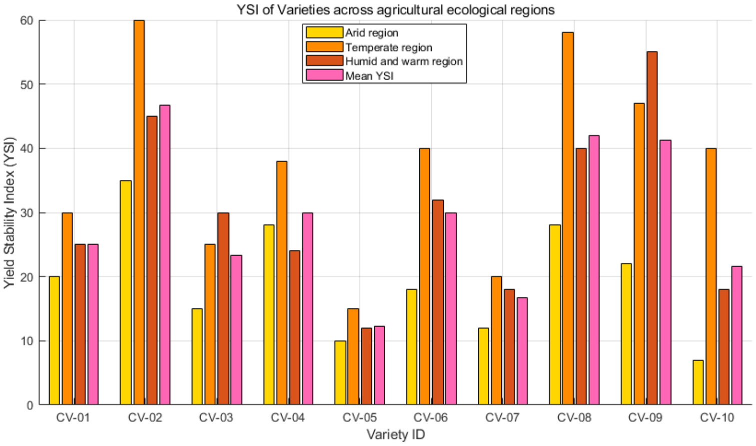

Fig. 6. Yield stability index (YSI) of 10 crop varieties.

Table 1. Key varieties showing significant differences in phenotypic and molecular responses.

Variety ID	Yield loss (%)	Photosynthetic rate (μmol/m²/s)	SOD activity (U/mg)	CAT activity (U/g)	DREB2A Expression level
CV-02	22.3±1.8	18.9±0.6	412±15	38.2±1.2	6.8
CV-08	24.1±2.1	17.5±0.7	398±18	36.8±1.5	5.2
CV-04	33.6±3.2	15.1±1.1	285±22	28.4±2.3	1.8
CV-09	35.5±4.5	13.2±0.9	203±18	22.6±1.8	1.6

The potential score (APS) was used to integrate farmers' subjective acceptance and economic benefits (BCR). The subjective scores of four dimensions, namely stress resistance, growth cycle, management convenience and yield satisfaction, were collected through questionnaire surveys, and the BCR was calculated based on the average market price in the past three years. Fig. 7 shows that CV-02, CV-03, CV-09 and CV-10 have high subjective acceptance, CV-08 has a leading BCR score, and CV-02, CV-08 and CV-09 have good overall APS scores.

Table 4 lists the key physiological parameters and yield loss rates of CV-02 and CV-05 under single and combined stress conditions. CV-02 was significantly superior to CV-05 in all indicators, indicating that it has stronger stress resistance and yield stability.

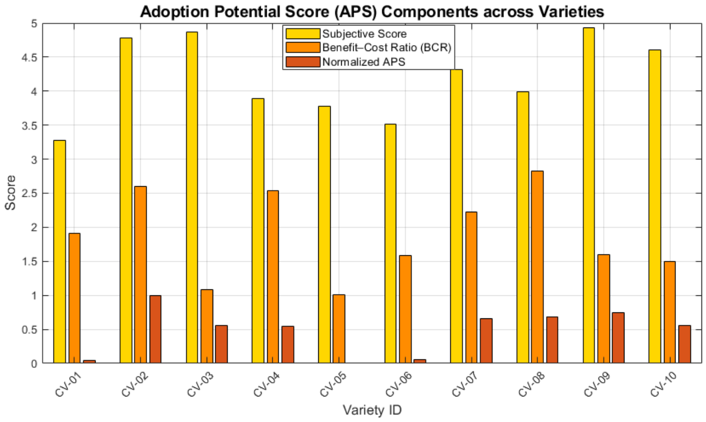

Fig. 7. Three key dimensions in assessing the adoption potential of 10 crop varieties.

Table 2. Key physiological measurements of ten crop varieties.

Variety ID	Net photosynthetic pate (Pn) (μmol $CO_2 m^{-2} s^{-1}$)	WUE (mmol CO_2 mol^{-1} H_2O)
CV-01	15.2	3.45
CV-02	14.8	3.67
CV-03	16	3.42
CV-04	15.1	3.61
CV-05	13.5	3.52
CV-06	15.9	3.62
CV-07	14.5	3.55
CV-08	16.2	3.58
CV-09	15.7	3.5
CV-10	14	3.44

Table 3. PER of ten crop varieties.

Variety ID	Raw PER value (μmol^2 $mol^{-1} m^{-2} s^{-1}$)	Normalized PER
CV-01	4.4	0.74
CV-02	4.79	0.88
CV-03	4.33	0.69
CV-04	4.11	0.54
CV-05	3.75	0.10
CV-06	4.4	0.74
CV-07	4.08	0.51
CV-08	4.66	0.80
CV-09	4.48	0.78
CV-10	3.84	0.12

Table 4. Comparative experiments and long-term stability verification under different stress combinations.

Stress type	Variety	Pn (μmol CO_2 $m^{-2}s^{-1}$)	SOD activity (U/mg)	Proline content (μg/g FW)	Yield loss rate (%)	3-Year CV (Yield stability)
High temp.	CV-02	15.2	320	120	18.5	0.12
Drought	CV-02	14.8	310	135	20.1	0.1
Aphid	CV-02	16	295	110	15.8	0.11
Combined stress	CV-02	13.5	280	180	22.3	0.13
High temp.	CV-05	12.1	190	95	28.6	0.22
Drought	CV-05	11.5	185	105	30.4	0.2
Aphid	CV-05	12.7	170	90	26.2	0.21
Combined stress	CV-05	10.2	160	210	35.5	0.25

Based on dynamic environmental simulation chambers, biogenomics analysis, cross-regional field trials and machine learning predictions, a comprehensive evaluation system for the stress resistance and adaptability of climate-adaptive crop varieties was constructed. The results showed that different varieties had significant differences in their physiological responses to high temperature, drought and combined stress, and the CRI index can effectively quantify the stability and adaptability characteristics of varieties.

Based on the standardized yield stability index (YSI), photosynthetic efficiency ratio (PER) and adoption potential score (APS), this study constructed a multidimensional evaluation system integrating agronomic traits, physiological mechanisms and economic adaptability, forming a scientific and reasonable climate-adaptive crop screening framework. CRI weight analysis showed that photosynthetic stability and antioxidant capacity were the key to improve stress resistance, and the role of the DREB2A-SOD regulatory axis in multi-stress response further highlighted the potential of gene regulation in molecular breeding. YSI integrates multi-ecological zone data for many years and effectively reflects the stable yield of varieties; PER reveals the variety differences in the coordination of photosynthesis and water use, providing a basis for drought response; APS combines subjective acceptance and economic benefits to improve the feasibility of promotion. Although the study still has deficiencies in data coverage, environmental modeling and model generalization, in the future, the model structure can be optimized by expanding ecological testing, introducing remote sensing and real-time monitoring, and improving prediction accuracy and regional adaptability. Overall, this multi-source data fusion evaluation method is both scientific and practical, providing effective support for the selection and promotion of climate-adaptive crops. The climate adaptive crop evaluation system established through multi-source data fusion in this study not only reveals the key differences in stress resistance mechanisms among varieties, but also provides a scientific and practical decision support framework for precision breeding and regional layout in the context of future climate variability.

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