Journal of Environment, Climate, and Ecology (JECE)
ISSN: 3079-255X (Online)
Volume 2 Issue 2, (2025)
https://doi.org/10.69739/jece.v2i2.1021
https://journals.stecab.com/jece

Published by
Stecab Publishing

Review Article

Genotype-by-Environment Interactions in Transgenic Crops: A Critical Review of Gene Function Validation under Variable Agronomic Conditions

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About Article

Article History

Submission: July 22, 2025
Acceptance : August 25, 2025
Publication : September 18, 2025

Keywords

Agronomic Conditions, Gene Function Validation, Genotype, Transgenic Crops

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ABSTRACT

Genotype-by-environment (G×E) interactions frequently derail transgenic crops that excel under controlled conditions. Abiotic stress, soil chemistry, and photoperiod can suppress promoters, destabilize transcripts, or misfold proteins, reshaping phenotypes across locations. Literature from the past two decades reveals recurring patterns: Bt Cry toxins lose potency during drought and heat; OsTZF5 rice yields rise only within moderate moisture deficits; safe-harbor insertions and stress-inducible promoters reduce but do not eliminate variability. Contemporary toolkits, AMMI and GGE biplots, UAV phenomics, multi-omics tracking, and crop simulators detect interaction signals earlier, while machine-learning models direct trials toward environments with high crossover risk. A staged validation pipeline is proposed: factorial stress screens, multi-environment trials, molecular bookkeeping, data integration, and iterative redesign through predictive modeling. Environment-responsive constructs tested in this framework produce evidence that satisfies regulatory comparators, improves deployment targeting, and strengthens confidence that laboratory performance will translate to climate-volatile farms.

Citation Style:

Eze, C. N., Hassan, J. I., Ibeh, R. C., Igboeli, C. N., Osabutey, S., & Kehinde, O. D. (2025). Genotype-by-Environment Interactions in Transgenic Crops: A Critical Review of Gene Function Validation under Variable Agronomic Conditions. *Journal of Environment, Climate, and Ecology, 2*(2), 63-76. https://doi.org/10.69739/jece.v2i2.1021

1. INTRODUCTION

Genotype-by-environment (G×E) interactions describe situations in which the phenotypic difference between genotypes changes across environments, producing non-parallel reaction norms rather than a uniform shift in trait means (de Leon *et al.*, 2016; Malosetti *et al.*, 2013; Napier *et al.*, 2023). In statistical terms, G×E is the interaction term of a two-way genotype × environment model; biologically, it reflects context-dependent gene expression, physiology, and development (Napier *et al.*, 2023). Reaction norm theory frames the phenotype as a function of environmental gradients, while concepts such as phenotypic plasticity, stability, and canalization capture how strongly or weakly a genotype's traits fluctuate (Alseekh *et al.*, 2025). Together, these ideas highlight that phenotypes are emergent properties of both alleles and settings, not simple readouts of DNA sequences.

Transgenic plants heighten the challenge because their performance can hinge on a single engineered promoter–gene cassette rather than a distributed polygenic network (Girón-Calva *et al.*, 2020). Greenhouse screens often overestimate trait robustness: heat, drought, salinity, nutrient limitations, or even herbivory can suppress transcription, destabilize mRNA, or alter protein folding of the transgene product (Donev *et al.*, 2023). Studies on Bt crops have shown that environmental stress can reduce Cry protein accumulation, weakening pest control, while work on drought-tolerant rice lines (e.g., OsTZF5 overexpression) reveals yield advantages that are contingent on stress severity, timing, or developmental stage (Girón-Calva *et al.*, 2020; Grondin *et al.*, 2024). Tissue-specific and season-specific responses documented in maize illustrate how expression patterns and phenotypes diverge once plants leave controlled chambers (Gutha *et al.*, 2018). These cases highlight that promoter–environment mismatches and stress-induced regulatory shifts can generate "spiky" interaction profiles that standard single-environment validation cannot capture.

Such instability matters far beyond academic curiosity. For breeders and farmers, G×E can reorder genotype rankings across locations, complicating predictions of yield or resistance (Demelash, 2024). For regulators, it poses a biosafety and performance question: agencies like EFSA in Europe and the FDA/USDA/EPA triad in the United States expect evidence that genetically modified plants behave consistently and safely across relevant agro-ecological zones (EFSA, 2010; USDA, 2024). Guidance documents explicitly call for comparative assessments and field data that reflect ecological realism, not just idealized growth-room trials (Organisms (GMO), 2011). From a translational genomics perspective, ignoring G×E risks costly failures when laboratory successes falter in farmers' fields (Donev *et al.*, 2023).

This review pursues four objectives: (1) clarify the conceptual foundations of G×E as applied to transgenic crops, including key terms and how transgenic architectures alter interaction patterns; (2) synthesize evidence on environmental modulation of transgene expression and resulting phenotypic variability; (3) evaluate methodological toolkits, from AMMI/GGE analyses to high-throughput phenotyping and molecular assays, that dissect interaction effects; and (4) propose design principles and regulatory-aligned pipelines for robust, field-relevant gene function validation, culminating in recommendations for environment-aware engineering and global trial networks.

2. LITERATURE REVIEW

Over the past two decades, a considerable body of research has investigated how transgenic crops perform across environments. The literature spans major crop species that dominate biotech agriculture, notably maize, soybean, cotton, and rice, as well as other plants, including wheat, canola, and various fruits or model species (Girón-Calva *et al.*, 2020, 2020; Napier *et al.*, 2023). Researchers initially framed G×E through reaction norms and interaction terms, but recent syntheses emphasize plasticity versus robustness as complementary breeding targets rather than opposing philosophies (Alseekh *et al.*, 2025; Manuck, 2010).

A common idea is that environmental stress can reduce how well transgenes work: conditions like drought, heat, or salt can lower promoter activity, make transcripts unstable, or change how much protein is produced, which has been observed with Cry toxins in Bt cultivars (Girón-Calva *et al.*, 2020; Wu *et al.*, 2025). Conversely, some engineered lines outperform controls only under specific stress windows, as demonstrated by OsTZF5-overexpressing rice, where yield gains surfaced under defined drought severities and developmental stages. (Grondin *et al.*, 2024; Selvaraj *et al.*, 2020). Large-scale greenhouse pre-screens often misrank candidates, a problem revealed when "unexpected winners" emerged in multi-year field trials of transgenic aspen (Donev *et al.*, 2023).

The research shows a clear progression: initially, researchers used AMMI and GGE biplots to analyze genetic and environmental effects, but later they moved to Bayesian and linear-bilinear methods, and then combined these with high-throughput phenotyping (HTP) and multi-omics (Sharma *et al.*, 2020; Yan *et al.*, 2007). UAV-mounted hyperspectral sensors, machine learning pipelines, and automated imaging now capture temporal trait trajectories at scale, enabling earlier detection of crossover interactions (Kaur *et al.*, 2024; Nguyen *et al.*, 2025). Parallel advances in RNA-seq and transcriptomics under controlled and field-imposed stresses allow researchers to pinpoint whether variability originates at transcriptional, post-transcriptional, or regulatory-network levels (Hazman *et al.*, 2025; Yerlikaya *et al.*, 2025).

Across studies, two interpretive shifts stand out. First, rather than treating G×E as statistical "noise," many papers now frame it as a design variable: transgenes can be tuned for conditional advantage if environmental triggers are explicitly encoded (e.g., stress-inducible promoters, CRISPRa/i circuits) (Girón-Calva *et al.*, 2020; Napier *et al.*, 2023). Second, climate change has pushed researchers to model wider, more erratic environmental spaces, reinforcing calls for multi-site, multi-season validation networks and open data sharing to reduce translation risk (Alseekh *et al.*, 2025; Shu *et al.*, 2023). Collectively, the 2000–2025 literature converges on a pragmatic conclusion: robust gene-function validation in transgenic crops must integrate statistical rigor, molecular diagnostics, and real-world agronomic heterogeneity if it is to deliver traits that are both high-performing and dependable.

3. METHODOLOGY

This narrative review surveyed peer-reviewed literature published from January 2010 through April 2025, complemented by foundational pre-2010 studies that remain standard references for genotype-by-environment (G×E) analysis. Searches were conducted in PubMed, Web of Science, Scopus, and Google Scholar using Boolean keyword strings such as "genotype-by-environment AND transgenic," "AMMI OR GGE biplot," "stress-inducible promoter," "CRISPRa plant," "safe-harbor insertion," "multi-environment trial," and "climate resilience crop." Additional filters included specific crop names (rice, maize, sorghum, cassava, Setaria, aspen) combined with terms like "drought tolerance," "heat stress," or "Bt toxin stability." Inclusion criteria required

i. original research or review articles addressing G×E interactions in genetically engineered or gene-edited plants,
ii. field or controlled-environment experiments with explicit environmental variables.
iii. discussion of statistical, molecular, or modeling methodologies relevant to transgene validation.

Conference abstracts, patents, and non-English sources without translations were excluded. Results were synthesized thematically; no PRISMA flow diagram, formal risk-of-bias scoring, or meta-analytic aggregation of effect sizes was undertaken, consistent with the narrative-review design.

4. RESULTS AND DISCUSSION

4.1. Conceptual foundations of g×e in transgenic crops

Genotype-by-environment (G×E) interactions arise when the phenotypic gap between genotypes changes across environments, yielding non-parallel reaction norms rather than a uniform shift in trait means (Napier *et al.*, 2023). In plant science, the interaction term of a two-way genotype × environment model captures this context dependence, while reaction norms visualize how a genotype's average trait value tracks an environmental gradient (Napier *et al.*, 2023).

Historically, breeders first noticed "crossover" performance, varieties trading places in yield rankings across sites, well before molecular explanations were available (Eberhart & Russell, 1966). Statistical formalisms followed: the Additive Main effects and Multiplicative Interaction (AMMI) model merged ANOVA with principal component analysis to partition interaction structure, and GGE (Genotype + G×E) biplots offered an intuitive way to visualize which genotype "wins where" and to delineate mega-environments (Yan *et al.*, 2007). These tools, developed for conventional cultivars, now frame how interaction patterns are examined in transgenic lines as well.

Three concepts anchor the discourse. Phenotypic plasticity is the capacity of a genotype to alter traits across environments; stability denotes relative constancy; and canalization, coined by Waddington, is the buffering of development such that the same phenotype emerges despite perturbations (Alseekh *et al.*, 2025). Recent reviews present plasticity and robustness (stability/canalization) as two beneficial approaches for food security, with plasticity allowing for adaptability and robustness ensuring consistent performance, while also linking "canalization" to Waddington's theory from the mid-20th century (Hallgrímsson *et al.*, 2002; Waddington, 1942).

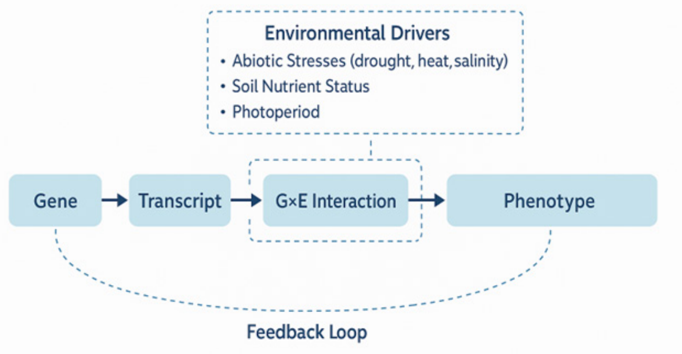

Figure 1. Conceptual framework of G×E in a transgenic crop

4.2. Differences between conventional and transgenic G×E responses

Conventional varieties typically express G×E through distributed polygenic architectures: many loci, each modestly environment-sensitive, collectively shape a smooth reaction surface. Transgenic events, by contrast, often rely on a single promoter–gene cassette whose output can spike or crash when specific environmental cues modulate promoter activity, mRNA stability, or protein turnover. Girón-Calva *et al.* (2020) showed that drought, heat, or salinity can depress Cry protein levels in Bt crops, eroding pest control precisely when protection is most needed; the near-isogenic, non-Bt counterparts displayed more gradual performance shifts tied to native regulatory buffering (Girón-Calva *et al.*, 2020). Meanwhile, Donev *et al.* demonstrated that greenhouse "winners" among transgenic aspens did not always translate to field champions after five years across diverse sites, underscoring how a construct's apparent superiority can be contingent on highly specific growth conditions (Donev *et al.*, 2023). Thus, transgenic G×E can seem more unpredictable: specific regulatory elements create sudden changes in how plants respond, while regular plants usually show a wider range of responses that spread out their sensitivity to the environment. Yet transgenics are not inherently unstable; when a transgene targets a central stress-response hub or uses an inducible promoter matched to field signals, it can actually dampen variance (Yang *et al.*, 2024).

The conceptual pillars, historical statistical frameworks, the plasticity–stability and canalization triad, and the architectural contrasts between conventional and engineered genomes establish the foundation for contemporary validation challenges. They justify integrating molecular assays with field-centric statistics and motivate an environment-aware design of promoters and constructs. In simple terms, having a strong grasp of G×E is essential for validating transgenic work; it is the perspective from which we must view claims about how genes work (Napier *et al.*, 2023).

4.3. Environmental modulation of transgene expression

Abiotic stresses rarely act as simple "on/off" switches; they rewire transcriptomes, shifting both the magnitude and timing of transgene expression and downstream protein accumulation, as shown by oil palm leaves that mobilized 19,834 differentially expressed genes under drought, salinity, waterlogging, heat, and cold (Lee *et al.*, 2024). Evidence from light and salt-stress experiments demonstrates that mRNA stability itself is plastic; chemical marks such as N6-methyladenosine can either stabilize or hasten decay of transcripts, so a transgene's message may persist or vanish depending on the stress regime (Smith *et al.*, 2024).

Promoters, often assumed to be "constitutive," are not immune: widely used elements like CaMV 35S can show species-, tissue-, or stress-dependent variation in strength, undermining the notion of a universally stable driver (Amack & Antunes, 2020).

Today's lists of promoters focus on those that can be turned on or created to react to changes in temperature, drought, or hormones, but testing them in real fields is challenging because the strength and timing of natural signals often don't match what is seen in controlled environments (Villao-Uzho *et al.*, 2023).

The Bt story is emblematic. Girón-Calva *et al.* reviewed how drought, heat, and salinity frequently depress Cry protein levels in Bt crops, diminishing insecticidal efficacy precisely when plants are physiologically strained (Girón-Calva *et al.*, 2020). A field study on Bt cotton found that adding more nitrogen and phosphorus increased the levels of Cry1Ac, while plots with low nutrients had toxin levels that were almost one-third of those with high fertilizer, showing that how we manage soil nutrients directly affects the amount of transgene product produced (Khan *et al.*, 2023).

Soil type and nutrient dynamics also feed back on plant metabolism; reductions in available nitrogen under Bt cotton relative to non-Bt isolines suggest that agronomic context can reshape both plant nutrition and protein expression landscapes (Beura & Rakshit, 2011).

Photoperiod and light quality complicate matters further: stress-responsive RNA-binding proteins and light-induced translational controls modulate how long transcripts linger and how efficiently they are translated, turning day–night cycles into quiet regulators of transgene output (Muthusamy *et al.*, 2021).

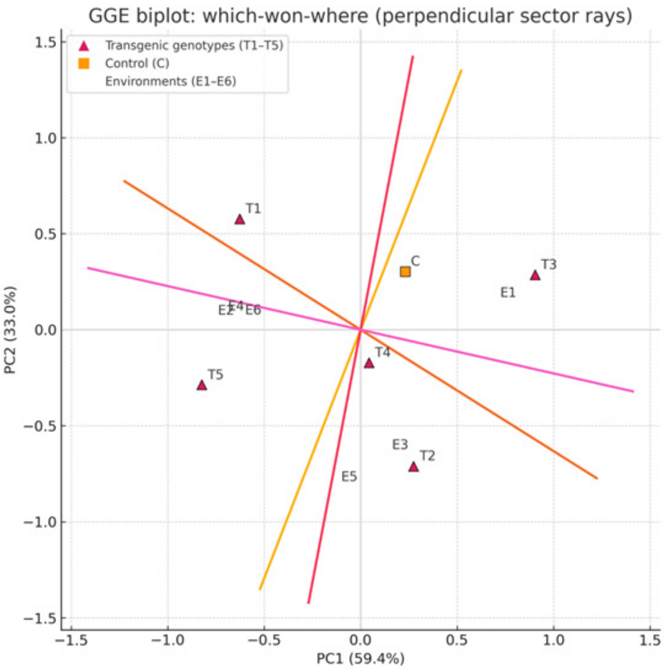

Figure 2. GGE biplot from an environment-centered G+GE matrix showing "which-won-where" across six environments.

Rice offers a contrasting case study. Grondin *et al.* (2024) evaluated OsTZF5-overexpressing lines across nineteen field trials and found that yield benefits were real but contingent; gains materialized under specific drought severities (−25 to −75 kPa) and developmental windows, while advantages waned in well-watered plots (Grondin *et al.*, 2024).

Earlier fieldwork with a stress-inducible promoter driving OsTZF5 confirmed that context-tuned expression could buffer yield under drought without depressing performance elsewhere, illustrating how promoter choice can sharpen or soften G×E signals (Grondin *et al.*, 2024). Similar patterns surface in maize: multi-environment trials repeatedly reveal that even near-isogenic lines bearing transgenes can swap yield ranks across sites or seasons, betraying promoter–environment mismatches and illustrating the importance of explicit reaction-norm mapping rather than single-environment claims (Figure 2) (Supriadi *et al.*, 2024).

Mechanistically, these shifts arise at multiple strata: promoter sensitivity to transcription factors that are themselves stress-regulated, RNA-binding proteins that remodel transcript lifespans, and protein-folding machinery that falters under heat, triggering unfolded protein responses and dampening accumulation of the intended product (Dannfald *et al.*, 2025). Because these layers interact, decoupling cause from effect demands pairing field phenotyping with molecular assays, qPCR or RNA-seq to quantify transcript changes, immunoassays to track protein levels, and metabolite profiling to see whether altered primary metabolism constrains transgene function (Saand *et al.*, 2022). Without keeping track of these molecular details, researchers might mistakenly think that changes in traits are just due to "field variability" when the real issue is that transcription is turned off or the RNA breaks down too quickly.

Environmental modulation of transgene expression is not anecdotal but systematic: stressors alter transcriptional activation, message stability, and protein fate, while soil fertility and photoperiod tweak the dose and duration of expression (Girón-Calva *et al.*, 2020). Case studies from Bt maize and cotton to drought-tolerant rice converge on the same lesson: performance is conditional, and only multi-layered, field-tuned validation can reveal where a construct thrives, where it fades, and why (Donev *et al.*, 2023).

4.4. Phenotypic variability & context-dependent outcomes

Overexpression is rarely free. Pushing a pathway rigorously can siphon carbon and nitrogen toward defense metabolites or osmoprotectants, trimming growth or yield when stress is absent. Growth–defense trade-offs documented across engineered plants show that what looks like resilience under drought can morph into a metabolic burden under ideal irrigation (Ha *et al.*, 2021; Karasov *et al.*, 2017).

Constitutive promoters such as CaMV 35S are marketed as "always on," yet field reality is messier: identical cassettes have produced markedly different transcript levels depending on organ, physiological state, and abiotic stress (Rahamkulov & Bakhsh, 2020). Inducible or synthetic promoters promise finesse, switching on when drought or heat actually strikes, but calibration is tricky. Signals that trigger neat expression pulses in chambers may trickle or spike unpredictably outdoors, leading either to underexpression when needed or costly leakage when not (Kiselev *et al.*, 2021; S. Yang *et al.*, 2010).

The Bt story captures this conditionality starkly. When drought, heat, or salinity depress Cry toxin accumulation, insect resistance weakens precisely as plant vigor declines, compounding losses; in fertilized plots, however, higher nitrogen and phosphorus can boost Cry1Ac titers, restoring efficacy. Here, the “same” trait’s strength becomes a function of weather and soil management, not just the gene itself (Girón-Calva *et al.*, 2020; Khan *et al.*, 2023).

Rice provides an instructive counterpoint. Transgenic lines that produced more OsTZF5 showed clear benefits in yield, but only during certain drought conditions (−25 to −75 kPa soil water potential) and at specific growth stages. In well-watered plots, those gains diminish, underscoring that “tolerance” is not monolithic but bounded by stress intensity and timing (Grondin *et al.*, 2024).

Long-term fieldwork with transgenic aspen drives the lesson home. Lines crowned “best performers” in greenhouse screens were not the same lines that excelled after five years across diverse sites, revealing that phenotype rankings can reshuffle once plants confront real seasonal cycles, soil heterogeneity, and unmanaged biotic pressures. Multi-site maize trials echo this pattern: hybrids swap yield positions as environments change, making single-location claims of superiority tenuous at best (Donev *et al.*, 2023; Napier *et al.*, 2023).

Collectively, these cases expose a simple truth: phenotypes are context-sensitive outputs of gene × management × environment interactions. Depending on the timing and location of its deployment, overexpression can either benefit or harm. Constitutive expression ensures presence but risks waste or mistimed activity; inducible systems curb metabolic cost but may misfire if field signals differ from lab triggers. Only by tracking traits across sites, seasons, and stress gradients, and pairing those observations with molecular diagnostics, can we separate genuine robustness from situational success (Grondin *et al.*, 2024).

4.5. Methodologies to Dissect G×E

Multi-environment trials (METs) remain the backbone of G×E dissection: breeders plant the same genotypes across contrasting sites and seasons, then ask not just “who yields most?” but “who wins where, and why?” (Demelash, 2024). AMMI (Additive Main effects and Multiplicative Interaction) marries ANOVA with principal components to parse interaction structure, while GGE (Genotype + G×E) biplots visualize the “which-won-where” pattern and help carve mega-environments for targeted recommendations (Yan *et al.*, 2007). These visual tools, which were first popularized in conventional breeding, now guide transgenic assessments by revealing instances when a single event excels in one environment but underperforms in another (Wodebo *et al.*, 2023).

Table 1. Representative transgenic traits, g×e patterns, environmental drivers, validation tools, and evidence-informed next steps

Trait / Transgene (crop)	**Observed G×E pattern**	**Primary environmental drivers**	**Validation tools used**	**Suggested next step**
Cry toxins (Bt) — Cry1Ab/Cry1Ac (maize, cotton)	Toxin titers decline under stress; efficacy weakest under drought/heat/salinity; performance varies across locations and seasons (Girón-Calva *et al.*, 2020).	Drought, heat, salinity, and plant nutritional status	Multi-site field assays; ELISA for Cry; AMMI/GGE for efficacy crossover	Replace/stack with stress-responsive promoters; specify agronomic bounds of efficacy in dossiers.
Bt Cry1Ac (cotton)	Higher N–P fertilization increases Cry1Ac concentration; low N–P reduces expression. (Khan *et al.*, 2023)	Soil N and P availability; fertilization regime	Split-plot fertilizer × genotype trials; ELISA quantification	Couple fertilizer recommendations to resistance stewardship; evaluate NUE stacks to stabilize titers.
OsTZF5 overexpression (rice, IR64 background)	Yield advantage only within defined drought window (≈ −25 to −75 kPa); gains taper when well watered. (Grondin *et al.*, 2024)	Moderate reproductive-stage drought; stress timing	19-trial MET; qPCR; phenology & harvest index analyses	Tune promoter (e.g., LIP9 variants) for onset/severity; extend METs to heat–drought co-stress.
CspB (MON 87460 “DroughtGard®”, maize)	Consistent yield lift under water-limited conditions; minimal to no advantage in well-watered sites. (Obunyali *et al.*, 2024)	Seasonal rainfall deficit; managed drought regimes	Large METs; Bayesian mixed models across hybrids	Pair with envirotyping to target deployment; quantify crossover risk with FieldSimR before trials.
rd29A::DREB1A (tobacco model) (Kasuga *et al.*, 2004)	Inducible expression preserves drought/cold tolerance while avoiding growth penalties in benign conditions.	Dehydration, low temperature (ABA-linked)	Controlled stress assays; field pilots; transcript profiling	Port inducible circuits to crops; compare against constitutive drivers for fitness cost curves.

Targeted safe-harbor insertion of carotenoid cassette (rice)	Stable transgene expression and phenotype from defined genomic “safe harbors”; reduced positional variability (Dong *et al.*, 2020).	Chromatin context; insertion site	CRISPR-Cas targeted insertion; compositional & performance tests	Build a catalog of crop-specific safe harbors; test stability under heat/salinity stress.
MET analytics (methodological exemplar)	GGE biplots reveal “which-won-where” and mega-environments; AMMI/GGE outperform simple means for crossover detection. (Wodebo *et al.*, 2023; Yan *et al.*, 2007)	Site–season contrasts; heterogeneous error	AMMI, GGE; sector analysis	Standardize inclusion of GGE/AMMI visuals in regulatory dossiers; report vertex winners per zone.
Simulation frameworks (FieldSimR; ORYZA/APSIM)(C. R. Werner *et al.*, 2024)	Virtual METs forecast performance and crossover probability; optimize site selection before planting.	Historical weather, soils, management; assumed G×E structure	FieldSimR with AlphaSimR; crop models	Pre-register trials using simulated power/ accuracy; iterate designs to hit target environments.

Statistical practices have advanced beyond classical biplots (Smith & Cullis, 2018). Bayesian linear-bilinear formulations address heteroscedastic errors and missing data that are typical in field trials, while factor-analytic mixed models combine the interpretability of AMMI/GGE with the shrinkage and flexibility offered by mixed-model frameworks (Jarquín *et al.*, 2016). Recent studies suggest new methods that mix AMMI, GGE, and factor analysis, along with simulation techniques, to assess how well a specific MET matches the environments that breeders aim for (De Oliveira *et al.*, 2016). These advances shift the field from relying on post hoc descriptions to adopting proactive experimental design under conditions of uncertainty (Smith *et al.*, 2001). High-throughput phenotyping (HTP) has changed what “environmental response” looks like on paper. Rather than scoring end-of-season yield alone, researchers now fly drones equipped with multispectral or hyperspectral cameras, track canopy temperature with thermal sensors, and pipe millions of pixels through machine- and deep-learning pipelines to flag stress signatures days before they manifest visually (Xie & Yang, 2020). Reviews chart a steady march from single-trait “stamp collecting” toward integrated, robotics-enabled platforms that pair sensor data with environmental

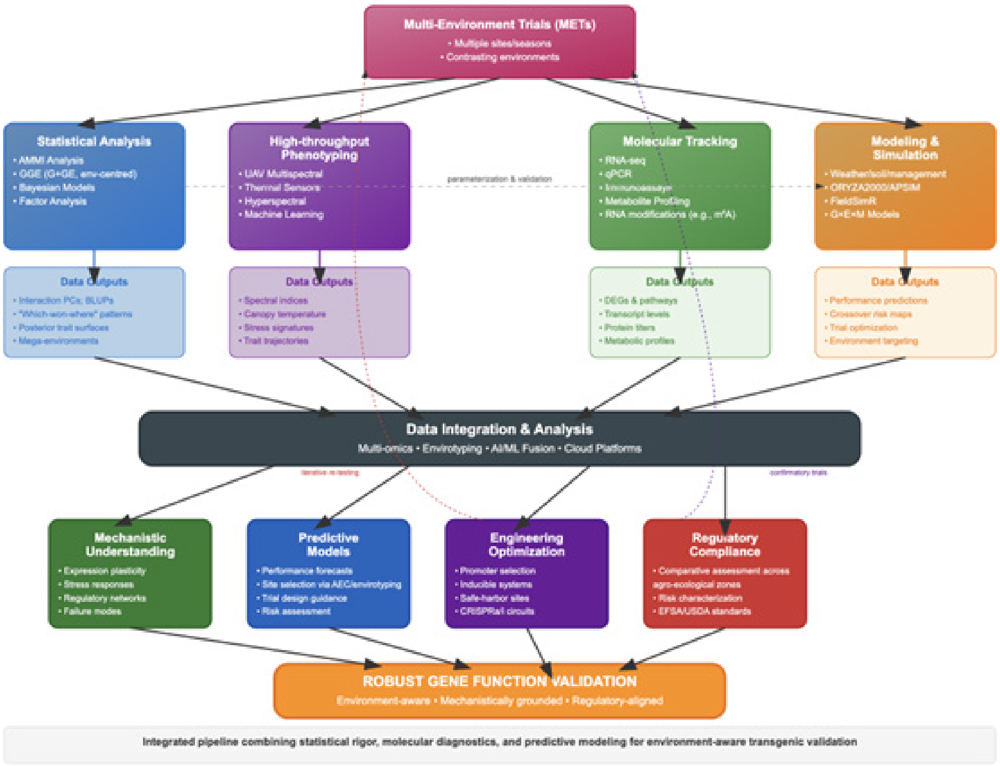

Figure 3. Integrated methodological framework for genotype-by-environment (G×E) dissection in transgenic crops

logs; yet bottlenecks persist in data curation, algorithm transferability, and the sheer logistics of field-scale imaging (Zhang & Zhang, 2018).

Molecular tracking adds mechanistic resolution to our understanding of these phenotypes. RNA-seq under drought, salt, or heat reveals whole-transcriptome reprogramming, identifying modules whose plasticity mirrors, or precedes, field trait shifts. Meta-analyses spanning more than 100 stress datasets illustrate that many "core" stress genes respond across contexts, but transgenes can insert novel hubs or bottlenecks into these networks (Bano *et al.*, 2022). Targeted qPCR, immunoassays, and metabolite profiling are still important to determine if changes in expression lead to functional proteins or biochemical results. Even post-transcriptional controls, such as mRNA methylation or RNA-binding proteins, alter message stability, reminding us that expression plasticity is multi-layered and stress-contingent (Rudy *et al.*, 2023).

Finally, modeling and simulation tools provide a "virtual MET" (Holzworth *et al.*, 2014). Crop models like ORYZA2000 and APSIM simulate genotype performance across weather, soil, and management scenarios, allowing researchers to prioritize real-world trials that maximize information gain (Li *et al.*, 2013). Recent frameworks embed multiplicative G×E structures directly into simulation engines (e.g., FieldSimR), helping quantify expected accuracy and tune experimental designs for crossover vs. non-crossover interactions (Werner *et al.*, 2024). Coupled with genotype-by-environment-by-management (G×E×M) modeling, these tools shift validation from reactive "measure and see" to predictive "simulate, deploy, verify," integrating statistical rigor with agronomic foresight (Holzworth *et al.*, 2014).

4.6. Discussion

4.6.1 Strategies for robust validation

Robust validation begins with a staged pipeline, hypothesis screens in growth chambers, factorial stress assays in controlled rooms, and multi-environment field trials that sample the target population of environments (Pour-Aboughadareh *et al.*, 2025). Five-year fieldwork on transgenic aspen showed that greenhouse "winners" did not remain winners outdoors, underscoring why early screens cannot substitute for real environments (Donev *et al.*, 2023). Consequently, METs should be interrogated with interaction-sensitive statistics: AMMI and GGE biplots reveal "which-won-where" patterns and identify crossover responses that single-location means obscure (Yan *et al.*, 2023). At every stage, phenotyping needs molecular bookkeeping, qPCR, or RNA-seq to verify transcript shifts and immunoassays for protein titers, so a lost phenotype is not blamed on "field noise" when the real culprit is expression collapse (Gan *et al.*, 2024).

In tobacco plants, using stress-responsive promoters like rd29A instead of constant drivers helped reduce growth issues while still keeping the plants tolerant to drought and cold (Kasuga *et al.*, 2004). Chemically inducible CRISPRa/i systems built on dCas9 now allow post-integration tuning of endogenous loci, letting developers dial traits up or down when field cues demand it (Zhang *et al.*, 2024). Targeted insertion into genomic safe-harbor loci in rice yielded marker-free, carotenoid-enriched lines with predictable expression, illustrating how precise landing sites reduce positional effects and silencing (Cantos *et al.*, 2014). Crop models such as ORYZA2000 simulate genotype performance across weather, soil, and management scenarios, guiding trial placement toward environments where crossover risk is highest (Li *et al.*, 2013). The combination of staged experimentation, environment-responsive engineering, precise integration, and predictive modeling gives gene-function claims a competitive edge in the complex heterogeneity of real farms (Tadese *et al.*, 2024).

4.6.2. Lessons from emerging crops & climate-resilience

Rice offers the clearest demonstration of context-bound promise: OsTZF5-overexpressing lines outperformed IR64 in 19 field trials only when drought intensity fell within a defined window and gains tapered under well-watered conditions (Grondin *et al.*, 2024). Maize tells a complementary story, hybrids routinely swap yield rankings across low-nitrogen and optimal sites, making "best" a moving target rather than a universal label (Mafouasson *et al.*, 2018). Sorghum takes this idea even further; a study looking at different environments found specific genes for drought that only showed up when both heat and water stress were present, highlighting that the genes that help plants adapt are specific to certain environments rather than being the best everywhere (Bernardino *et al.*, 2024). Field phenotyping for these traits increasingly leans on sUAS-based remote sensing, as illustrated by USDA projects using drones to screen stay-green sorghum lines and aphid tolerance at scale (USDA, 2025a).

Model species help plug knowledge gaps for "orphan" crops. Setaria viridis, fast-cycling, genetically tractable, and phylogenetically close to maize and millets, has been championed as a surrogate to accelerate gene discovery and trait dissection in under-studied C4 cereals (Huang *et al.*, 2016). Yet many neglected crops remain sidelined despite robust stress tolerance and nutritional value, largely because research funding and breeding infrastructure favor global staples (Talabi *et al.*, 2022). Translating lessons from Setaria or rice into these species requires researchers to do more than just port constructs; they must develop envirotyping frameworks that capture local stress mosaics so that transgenes or edited alleles are tuned to the right signals (Xu, 2016).

Climate change broadens the environmental landscape by introducing erratic rainfall, heat spikes, and compound stresses, which increase the GEO space that validation pipelines need to navigate (Raza *et al.*, 2019). As that space expands, single-location or single-season trials become statistical dead ends; only coordinated networks that span agro-ecological gradients can reveal whether a line is truly robust or merely lucky (Dias *et al.*, 2018). In practice, that means pairing MET analytics with predictive modeling to target "stress hot spots," then looping molecular readouts back into design choices, promoters, integration sites, and inducible CRISPRa/i modules, so expression tracks real-world cues instead of lab surrogates (Gao *et al.*, 2023). The emerging consensus across rice, maize, and sorghum, and the orphan crops waiting in their shadow, is blunt: environment-aware validation is not an optional add-on but the price of reliable transgenic performance in a climate-volatile century (Cooper & Messina, 2023).

4.6.3. Regulatory expectations for G×E

The European Food Safety Authority (EFSA) asks applicants to perform a comparative analysis that spans "agro-ecological zones representing the expected range of cultivation," explicitly flagging G×E as part of environmental-risk assessment (ERA) (European Food Safety Authority, 2010). Its detailed ERA guidance reiterates that applicants must supply multi-site field data and describe any interactions that could shift agronomic or ecological behavior (European Food Safety Authority, 2010). Across the Atlantic, USDA-APHIS permits a single notification to cover "multiple environmental releases" as long as field sites collectively capture the crop's target geography, signaling that U.S. regulators likewise expect evidence beyond greenhouse trials (USDA, 2025b). The agency's Biotechnology Regulatory Services portal further clarifies that risk determinations weigh location-specific data on pest pressure, climate, and management (Animal and Plant Health Inspection Service, 2025). Internationally, OECD consensus documents encourage jurisdictions to consider local protection goals and stress that risk characterization must integrate environmental context, essentially codifying G×E thinking into global best practice (OECD, 2023a, 2023b). Yet none of these frameworks dictate analytic tools, leaving developers to decide whether AMMI, GGE, or Bayesian models best reveal crossover risks.

5.6.4. Translational failures rooted in G×E blind spots

The stakes of under-characterizing interactions are tangible. A meta-analysis shows drought, heat, and salinity can depress Bt toxin expression, eroding insect control precisely when plants face the most stress (Girón-Calva *et al.*, 2020). Field experiments in India confirmed that combined high temperatures and waterlogging reduced Cry protein efficacy in Bt cotton, increasing pest damage despite genetic protection (Jehangir & Ali, 2023). Likewise, a five-year study on transgenic aspen revealed that clones crowned "best" in the greenhouse were outperformed by different lines once exposed to diverse soils and climates, illustrating how indoor screens can misrank true field champions (Donev *et al.*, 2023). Even high-profile humanitarian projects are not immune: Philippine courts revoked approval of Golden Rice after activists argued that local environmental data were insufficient, a regulatory setback that cost years of deployment time (McKie & editor, 2024).

4.6.5. Toward standardised, environment-aware validation

First, dossiers should include pre-registered multi-environment trials analyzed with interaction-sensitive statistics; disclosing AMMI or GGE plots would let reviewers see crossover directly. Second, molecular readouts, transcripts, proteins, and metabolites should accompany field phenotypes so agencies can trace failures to mechanistic roots rather than blame "environmental noise." Third, data capture needs harmonization: cloud-based field-trial platforms already streamline collection and validation, hinting at a shared infrastructure for regulators and developers alike. Finally, international bodies could adopt an OECD-style validation annex prescribing minimal site numbers, climatic breadth, and analytic standards, thereby reducing redundancy while preserving regional safeguards (OECD, 2023b). Implementing these steps would transform G×E from a regulatory stumbling block into a predictable hurdle, one that robust, transparent pipelines can clear with confidence.

4.7. Implications for functional genomics & engineering

Genotype-by-environment (G-E) interactions are compelling genomics to relinquish basic, context-free gene labels in favor of more detailed, conditional narratives: the actions of a gene now carry an asterisk, indicating its location and timing (Baye *et al.*, 2011). This shift ripples through discovery science, quantitative genetics, and the engineering bench.

6. 1 Rethinking gene-function claims

Allele-specific expression surveys reveal thousands of loci whose cis-regulation flips between drought and well-watered conditions, warning that single-environment screens can miscast causal variants (Murani & Hadlich, 2023). Functional annotations are therefore migrating toward "reaction-norm profiles," bundling sequence, expression plasticity, and environmental triggers into one dossier (Saltz *et al.*, 2018).

4.7.1. Environment-enriched GWAS and predictive breeding

Statistically, the adjustment is no longer GWAS alone but GWAS-by-environment, where climatic or soil covariates sit beside SNPs in the linear model (Costa-Neto *et al.*, 2022). In maize, adding satellite-derived weather layers to genomic prediction raised accuracy by seven percent relative to pure genotype models, a non-trivial boost in a crop whose margins are thin (Fernandes *et al.*, 2024). Similar gains appear when deep-learning frameworks fuse high-resolution envirotyping with sequence data, outpacing classic factor-analytic mixed models while trimming compute time (Crossa *et al.*, 2025). A flurry of algorithms, GEFormer among them, now treat G×E as signal, not noise, turning environment-specific effect sizes into actionable breeding indices (Yao *et al.*, 2025).

4.7.2. Toward environment-aware engineering

Engineering needs to adapt quickly. Stress-inducible promoters, once limited to rd29A, now span bespoke libraries responsive to heat, salinity, or hormones, letting developers meter expression rather than overexpress by default (Villao-Uzho *et al.*, 2023). Chemically switchable CRISPR-a payloads push that logic further, activating native genes only when a foliar spray or temperature cue demands it (Zhang *et al.*, 2024). By coupling dCas9 activators to light-sensitive domains, teams have edited yield pathways in rice without the growth penalties typical of always-on constructs (Sami *et al.*, n.d.). Safe-harbor insertion sites add another layer of predictability, insulating transgenes from chromatin quirks that can amplify or silence them in stress-specific ways (Ye *et al.*, 2024). Beyond the plant, synthetic biology startups are engineering root-associated microbes that modulate nitrogen supply as soils warm, externalizing part of the G×E buffer to the microbiome (Walsh, 2021). Prototype "sentinel plants" that flush anthocyanin when pesticides spike showcase how expression circuits can double as environmental sensors, tightening the feedback loop between field conditions and genetic response (Ford, 2023).

The takeaway is clear: functional genomics, statistical genetics, and bioengineering are converging on an environment-first mindset, where performance curves, not point estimates, define value. Designers who embrace that mindset and arm their constructs with ecological situational awareness will own the next wave of crop innovation.

5. CONCLUSION

Genotype-by-environment interactions are not statistical clutter around a clean genetic signal; they are the signal. For transgenic crops, this means a gene's "function" is inseparable from the environmental envelopes in which it is deployed. Promoters drift in strength, transcripts decay at stress-dependent rates, proteins misfold under heat, and phenotypes reorder across sites and seasons. Any validation regime that freezes these dynamics into a single greenhouse snapshot will overstate robustness, misprice risk, and invite regulatory friction.

What follows is a different mindset: dynamic, environment-aware validation as the norm. Traits should be advanced through staged pipelines that deliberately widen environmental contrast, analyzed with interaction-sensitive statistics, and coupled to molecular readouts so lost performance can be traced to mechanism rather than hand-waved as "field noise." Engineering choices must match that realism, stress-responsiveness, or sensor-linked promoters, inducible CRISPRa/i circuits, and precise, safe-harbor integrations that tame positional effects. Prediction should precede planting: AI models and crop simulators can map where crossover is most likely, guiding scarce field trials to the environments that matter. Ecological realism, then, is not a luxury tacked on at the end of development; it is the scaffolding for making any claim about gene function credible outside the growth chamber. Adopt it, and transgenic innovation becomes more predictable, more defensible, and far likelier to deliver under the volatile climates farmers actually face.

RECOMMENDATIONS

Artificial intelligence models are now accurate enough to guide where a transgenic line should be field-tested, but only if their training data span the full spectrum of environments. That reality argues for three converging moves: AI engines that learn G×E surfaces from enviromic covariates, multidisciplinary teams that bind statisticians to agronomists, and a truly global, open trial network.

Firstly, we introduce AI engines. Gradient-boosted trees and deep nets already lift genomic prediction accuracy by 5–7% when daily weather or soil layers are folded into the model (Fernandes *et al.*, 2024). Neural-network variants such as GxENet and GEFormer go further, ingesting raw climate grids to output environment-specific yield scores for each genotype (Jubair *et al.*, 2023; Yao *et al.*, 2025). Envirotyping pipelines in sorghum and wheat show that even a modest set of covariates, vapor-pressure deficit, and temperature amplitude can rescue prediction power for stress loci invisible to genotype-only scans (Garin *et al.*, 2024; Winans *et al.*, 2024). For hypothesis testing and trial design, the open-source FieldSimR package lets breeders simulate crossover risk under hundreds of weather scenarios before a single plot is planted (Werner *et al.*, 2024).

Second, success hinges on cross-disciplinary pipelines. "Smart breeding" roadmaps now pair data scientists with physiologists to turn remote-sensing outputs into biologically grounded features, leaf temperature and chlorophyll fluorescence, that machine learning can digest (Xu *et al.*, 2022). CGIAR's Excellence in Agronomy Initiative formalizes that model, embedding AI specialists inside agronomy hubs across Africa, Asia, and Latin America (CGIAR, 2025). Crop-ontology standards ensure that trait labels remain interoperable across labs, preventing semantic drift that sabotages meta-analyses (CGIAR, 2018).

Finally, predictions are only as reliable as their ground truth, so a federated global trial network is indispensable. A Global Crop Improvement Network, long championed by CGIAR big-data advocates, would pool multi-environment trials under a single data-governance umbrella (Reynolds *et al.*, 2017). Wheat's Borlaug Global Rust Initiative already functions as a prototype, coordinating disease nursery data from Kenya to Australia and serving summaries through an open portal (BGRI, 2025). Scaling that ethos to other crops requires the development of harmonized ontologies, cloud-based audit trails, and FAIR-compliant repositories, which are currently being piloted by the agronomy-at-scale arm of CGIAR (Initiative, 2025). Together, AI models, cross-disciplinary pipelines, and a federated trial network would turn G×E from a regulatory headache into a predictable design variable, accelerating the march from construct to climate-ready cultivar.

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