Environmental safety hazards of abiotic stress-resilient GM crops: insights from current risk assessment frameworks

This report was prepared by H.A.C.F. Leeggangers and R. Sasidharan, on behalf of the Ministry of Infrastructure and Water Management in the Netherlands (I&W), and the National Institute for Public Health and the Environment of the Netherlands (RIVM). The information in this report is based on sources deemed reliable and on the best knowledge and insights of the authors at the time of writing. The views expressed in this report are the professional opinion of the authors and do not necessarily reflect those of I&W, RIVM, members of the advisory Committee, the Competent Authorities, or the European Commission. It is acknowledged that only the European Court of Justice can give a binding decision on the interpretation of EU law.

H.A.C.F. Leeggangers, R. Sasidharan (2025)
Plant Stress Resilience, Institute of Environmental Biology, Utrecht University

Index

Abstract 3
Abbreviations 4
1. Introduction 5
2. Environmental Risk Assessments*:* abiotic stress vs. biotic stress 6
3. Comparison Environmental Risk Assessments of abiotic stress-resilient GM crops in field trials 8
  Genuity® DroughtGard™ (MON87460)- drought tolerant GM maize 8
  Verdeca HB4 soybean – drought and herbicide tolerant GM soybean 10
  HB4 wheat – drought tolerant GM wheat 10
  NXI-1T, NXI-4T, NXI-6T – drought tolerant GM sugarcane 11
  Different approval strategies among countries 11
4. Comparison of Environmental Risk Assessments of unapproved abiotic stress-resilient GM crops in field trials 12
  Overview of ERAs in ongoing or discontinued abiotic stress- related field trials 12
    Pollen dispersal & cross-pollination 14
    Vertical Gene Transfer (VGT) 14
    Horizontal Gene Transfer (HGT) 15
    Weediness & Persistence 15
    Ecosystem interactions and the rhizosphere 15
  Influence of plant species and the receiving environment on environmental risks 16
  Interconnected and cascading environmental risks 17
  Temporal limitations of current ERAs for abiotic stress-resilient GM crops 19
  Complementary approaches to assess long-term environmental risks 19
5. Environmental risks considered from a scientific perspective (survey) 20
6. Concluding discussion 22
Glossary 26
References 27
Appendix I - Methods 32
Appendix II – Drought- and salt-tolerant GM rice, developed using CRISPR (approval pending) 33

Abstract

As climate change-related erratic weather patterns threaten global food production, there has been intensified interest in genetically modified (GM) crops engineered for enhanced resilience to climatic stressors such as drought, salinity, flooding, heat and cold. This report evaluates whether existing Environmental Risk Assessment (ERA) frameworks adequately address the environmental risks associated with abiotic stress-resilient GM crops. Using a comparative analysis of biotic- and abiotic stress-related ERAs, assessment of field trial data, and a small survey of Dutch plant scientists we identify key risk considerations, regulatory challenges and knowledge gaps within current assessment practices.

While standard ERA endpoints capture plausible pathways to harm, abiotic stress-resilient traits differ fundamentally from biotic stress traits in that they alter complex physiological and regulatory networks, often resulting in context-dependent and potentially pleiotropic effects. Key environmental concerns include gene flow to wild relatives, weediness and persistence, and indirect impacts on ecosystem interactions, particularly soil and rhizosphere processes. Evidence from approved crops indicates no significant short-term environmental risks. However, limited trial duration, spatial scale and ecological resolution constrain the detection of long-term and climate-driven effects. We conclude that a dedicated ERA framework for abiotic stress-resilient GM crops is not warranted. Instead, ERAs should remain case-by-case and trait driven, incorporating receiving-environment and climate context, and be complemented by multi-season field trials, targeted ecological studies, and adaptive post-release monitoring to ensure environmental safety under future agricultural conditions.

Abbreviations

Abscisic Acid	ABA
India Agricultural Research Institute	IARI
Bacillus thuringiensis	Bt
Clustered Regularly Interspaced Short Palindromic Repeats	CRISPR
complementary DNA	cDNA
Drought and Salt Tolerance	DST
Environmental Risk Assessment	ERA
Genetically Modified	GM
Helianthus annuus	Ha
Herbicide Tolerance	HT
Homeodomain-leucine zipper	HD-zip
Horizontal Gene Transfer	HGT
Jasmonic Acid	JA
messenger RNA	mRNA
Next Generation Sequencing	NGS
Non-Homologous End Joining	NHEJ
Pattern-Recognition Receptor	PRR
Post-Market Environmental Monitoring	PMEM
Standard Operation Procedures	SOPs
single-guide RNA	sgRNA
Site-Directed Nuclease-1	SDN-1
Site-Directed Nuclease-2	SDN-2
Sugarcane Mosaic Virus	SCMV
Systemic Acquired Resistance	SAR
Vertical Gene Transfer	VGT

1. Introduction

Climate change has had a significant negative impact on crop productivity (Mirzabaev et al. 2023). With the prospect of a growing population reaching 8.5 billion in 2030 and 9.7 billion in 2050 the loss of food crops must be minimized (*Feeding the future global population*, 2024). Genetic modification of crops has emerged as an important approach to enhance crop yield, quality and resilience to both biotic and abiotic stresses. However, a significant barrier to the adoption of genetically modified (GM) crops are concerns regarding potential environmental and human health impacts. Given these concerns, rigorous Environmental Risk Assessments (ERAs) are critical to identify and investigate potential environmental risks, and to determine whether their agronomic benefits outweigh potential harms.

The approval pathway for GM crops consists of multiple steps designed to ensure their safe introduction into the environment for commercial use. The process begins with research and development, during which the trait of interest (e.g. herbicide resistance, drought resilience) is selected. Subsequently, molecular characterization and agronomic performance are evaluated under controlled environments as part of preliminary safety testing. These evaluations include determination of transgene stability and expression, assessment of potential off-target effects associated with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based gene editing and examination of pleiotropic effects where unrelated traits may be affected (Hilbeck et al. 2011; Li et al. 2017; Aziz et al. 2022; Loschin et al. 2025). Each experiment requires a near-isogenic non-GM comparator (hereafter referred to as the non-GM crop counterpart) and where possible, commercially available comparators to evaluate whether the introduced trait significantly alters crop characteristics. This is currently an important step towards approval of GM crops for cultivation (Raybould & Macdonald 2018). In Europe this is controlled by Directive 2001/18/EC and even though there is flexibility to submit evidence to a specific risk hypothesis, the legislation highlights that only those consequences should be addressed in the ERA which have the potential to cause adverse effects (ACRE, 2013). **Table 1** provides an overview of commonly addressed environmental risks and their importance, independent of the specific trait introduced in the GM crop.

For abiotic stress-related traits, more theoretical concerns have been raised than for biotic stress traits, mostly due to limited number of large-scale field studies conducted to date (Parmar et al. 2017). Because ERAs have historically focused on herbicide tolerant and insect resistant GM crops, the underlying scientific principles developed for these traits have also been applied to GM crops with enhanced abiotic stress resilience (Nickson 2008). This raises the question of whether the current ERAs adequately address risks specific to abiotic stress-resilient GM crops.

To address this question, in this report we surveyed available literature to 1) examine and discuss differences between ERAs for biotic and abiotic stress traits 2) provide an overview of currently approved abiotic stress resilient GM crops, 3) compare on-going and discontinued field studies to identify potential knowledge gaps relevant to ERAs of abiotic stress-resilient GM crops (Appendix I) and 4) discuss the relevance of an often ignored factor – the importance of scientist perception, understanding and valuation of ERAs (Appendix I).

Table 1: *An overview of standardized environmental risks assessed for cultivation of GM crops*

Environmental risk	Assessment	Importance
Effects on non-target organisms	Impacts on organisms not intended to be affected (pollinators, predators, beneficial insects, soil fauna, aquatic species)	To avoid disruptions to ecological communities and trophic relationships.
Effects on target organisms	Specificity, efficacy, and ecological consequences of impacts on target pests or pathogens (unrelated to abiotic stress resilient GM crops)	Ensures intended effects without creating disproportionate ecological pressure.
Vertical gene transfer/gene flow	Potential for pollen-mediated movement of transgenes.	Evaluates introgression, hybridization, and spread of GM traits outside cultivation.
Weediness and invasiveness	Whether the GM crop becomes more persistent or invasive.	Evaluates introgression, hybridization, and spread of GM traits outside cultivation.
Effects on biodiversity and ecosystem	Effects on community structure, species richness, food webs, and ecosystem processes.	Ensures long-term ecological sustainability.
Horizontal gene transfer	Possibility of GM DNA transfer to microbes or other non-plant organisms.	Ensures unlikely but theoretically possible gene flow is evaluated.
Unintended pleiotropic effects	Unexpected changes in plant phenotype, metabolism, or composition.	Detects secondary effects that could influence ecological interactions.
Impact on soil	Effects on soil microbiota, nutrient cycling, decomposition, root exudates, and soil fauna.	Protects soil health, fertility, and microbial ecology.
Impact agricultural practices	Changes in pesticide/herbicide use, tillage, rotations, or water use.	Evaluates indirect environmental effects driven by management changes.
Persistence and dispersal	Likelihood of volunteer plants or spread beyond fields.	Prevents unintended long-term establishment of GM plants.

References: EFSA, 2010; EFSA, 2022; OECD, 2010; OECD, 1993; CBD Secretariat, 2000; USDA-APHIS, 2020; US EPA, 1998; WHO & FAO, 2000; Raybould 2006; Sanvido et al. 2007; Hilbeck & Andow 2004; NRC, 2002.

2. Environmental Risk Assessments*:* abiotic stress vs. biotic stress

Most approved GM crops carry traits for herbicide tolerance (HT) or insect resistance involving the introduction of genes derived from *Bacillus thuringiensis* (Bt). More recently, the development of biotic stress-related traits has expanded to include the alteration of pattern-recognition receptors (PRRs) involved in pathogen detection, boosting systemic acquired resistance (SAR) pathways, targeted knock out of susceptibility genes exploited by pathogens, and creation of resistance traits analogous to those occurring in nature (Hussain et al. 2024).

In contrast, for abiotic stress-resilient GM crops, primary targets are regulatory and protective mechanisms including transcription factors involved in stress responses, molecular chaperones, antioxidant defense system components, ion transporters, RNA chaperones, negative regulators of abiotic stress responses, and genes involved in osmoprotectant biosynthesis that lead to the accumulation of compatible solutes (Esmaeli et al. 2022; Rahman et al. 2022; Mushtaq et al. 2025).

With respect to phenotypic assessments, biotic stress-related traits are often more straightforward to evaluate due to the nature of a single target. By contrast, abiotic stress-related traits require broader agronomic and ecological characterization across diverse environmental conditions (e.g. drought, flooding, salinity, cold, heat). Crosstalk between different abiotic stress responses, and sometimes with biotic stress pathways (Kamran et al. 2025), can further complicate assessment and necessitates testing under multiple stress and non-stress scenarios to differentiate single and multi-stress resilience. The difference between ERAs of biotic stress- and abiotic stress-resilient GM crops are primarily in the nature of the potential risks related to the trait, rather than in the assessment procedures. **Table 2** provides an overview of the key differences in ERAs of biotic stress- and abiotic stress-resilient GM crops.

Biotic stress-related traits confer resistance to living stressors such as insects, fungi and viruses, whereas abiotic stress-related traits target non-living stressors such as drought, flooding, salinity and temperature extremes. Risks associated with the rhizosphere are considered of similar importance for both trait types. A primary hazard associated with biotic stress-resistant GM crops is the potential evolution of resistant pest populations ("superpests"), that can overcome specific resistance mechanisms such as Bt toxins. This may reduce the long-term effectiveness of the trait and potentially increase reliance on chemical pest control (Ngongolo & Mmbando 2025). Whether analogous risks exist for abiotic stress-resilient traits remains uncertain: gene flow to non-target plants could, in theory, confer enhanced stress tolerance and alter ecosystem dynamics by providing a competitive advantage under adverse environmental conditions.

Table 2: *Overview of differences in ERAs of biotic- and abiotic stress-resilient GM crops.*

	Biotic stress resistant GM crops	**Abiotic stress resilient GM crops**
Purpose	Resistant to living stressors (e.g. insects, fungi, viruses)	Enhanced resilience to non-living stressors (e.g. drought, flooding, heat, cold, salinity)
Mechanism	Produces novel proteins that act directly on target organisms	Modification of physiological or regulatory pathways
Primary concern	• Effect on non-target organisms • Pest resistance evolution • Balance ecosystem	• Effect on plant community dynamics • Potential weediness/invasiveness • Interactions with the ecosystem
Non-target effects	High priority, because the introduced protein might affect beneficial insects, soil fauna, wildlife etc.	Lower priority, because abiotic stress traits are not toxic but may affect their environment differently
Geneflow considerations	Potential spread of pest resistance or toxin traits to wild relatives	Potential spread of enhanced stress resilience, and chance of increasing weediness or persistence of hybrids
Soil and ecosystem processes	Potentially affects soil microbiome through toxin exposure	Potentially affect nutrient or water cycling when the plant physiology changes
Phenotypic assessment	Mainly focused on pest resistance and yield	Broader agronomic and ecological characterization under diverse environmental conditions (e.g. stress vs. non-stress, different abiotic stresses)

References: OECD, 2023; EFSA, 2010,2022; CBD Secretariat, 2000; USDA-APHIS, 2000; Raybould, 2021; Stein et al. 2022; Nap et al. 2003; Conner et al. 2003 ; FAO, 2021.

This concern is particularly relevant for escaped or volunteer plants derived from abiotic stress-resilient GM crops (Aziz et al. 2022). Pleiotropic effects are currently more frequently associated with abiotic stress-resilient GM crops than biotic stress-resistant GM crops (Ladics et al. 2015), as abiotic stress response pathways are closely integrated with plant growth and metabolic regulation to enable developmental and physiological adjustments necessary for survival and reproduction (Zhang et al. 2023). Common pleiotropic effects include changes in growth and development (e.g. flowering time), altered nutrient uptake or metabolism and changes in root-shoot allocation. When flowering time is delayed or accelerated, the potential risk of phenological mismatch will affect the interactions with pollinators and other organisms, with potential consequences for ecosystem functioning. Altered flowering synchrony among plant species may increase competition for pollinators, light, water and nutrients. In addition, flowering time changes can influence gene flow by increasing temporal overlap among species, potentially facilitating the spread of abiotic-stress related traits and contributing to local adaptation under novel environmental conditions while also affecting genetic diversity if populations become isolated. Although pleiotropic effects have historically been less common in biotic stress-resilient GM crops, increasing trait complexity may change this pattern. For example, the well-established growth-defense trade-off whereby limited energy allocated to rapid growth and activation of the defense system against pests or pathogens, can result in altered growth phenotypes as resistance strength increases (Sun et al. 2025).

To summarize, while both biotic and abiotic stress traits in GM crops present common challenges and risks, the complexity of abiotic stress resilience necessitates a more nuanced evaluation of ecological interactions and potential pleiotropic effects. As research advances, it is crucial to adequately evaluate the implications of these traits on ecosystem dynamics and long-term agricultural sustainability.

3. Comparison Environmental Risk Assessments of abiotic stress-resilient GM crops in field trials

Despite the abundance of ERAs for HT and insect resistant GM crops, ERAs addressing abiotic stress-related traits remain relatively scarce. Nevertheless, the demand for abiotic stress -resilient GM crops is growing (Global Industry Analysts, 2025) with some market analyses estimating that such traits accounted for approximately 40-50% of trait development focus in 2024 and are expected to grow even further. Commercial deployment remains limited due to scientific complexity of these traits as well as regulatory constraints and public opposition (Domingo, 2025). Currently, four abiotic stress-resilient GM crops have been approved for cultivation outside of Europe (Table 3): Genuity® DroughtGard™ maize (MON87460), Verdeca HB40 soybean, HB40 wheat, and Sugarcane NXI-1T, -4T, and -6T. Here below we provide a brief description of the design and field test results for these four approved examples.

Table 3: *Approved abiotic stress tolerant GM crops for cultivation*

Name	Crop	Trait	Genetic modification	Developer	Cultivation*
Genuity® DroughtGard™	Maize	Drought stress tolerance	Introduction of *cspB* gene from *Bacillus subtilis*	Monsanto and BASF	Canada (2010) US (2011) Japan (2012) Malaysia (2020)
Verdeca HB4 Soybean	Soybean	Drought stress tolerance, herbicide tolerance	Introduction of *Hahb-4* from *Helianthus annuus*	Verdeca	Argentina (2015) Brazil (2019) US (2019) Malaysia (2023)
HB40 Wheat	Wheat	Drought stress tolerance	Introduction of *Hahb-4* from *Helianthus annuus*	Bioceres S.A.	Argentina (2020) Brazil (2023) Paraguay (2023)
NXI-1T	Sugarcane	Drought stress tolerance, Abiotic stress tolerance	Introduction of *EcBetA* from *Escherichia coli*	PT Perkebunan Nusantara XI (Persero)	Indonesia (2013)
NXI-4T, NXI-6T	Sugarcane	Drought stress tolerance	Introduction of *RmBetA* from *Rhizobium meliloti*	PT Perkebunan Nusantara XI (Persero)	Indonesia (2013)

**Source: IAAA GM approval database*

Genuity® DroughtGard™ (MON87460)- drought tolerant GM maize

MON87460 is the third drought tolerant maize hybrid besides Pioneer Optimum AQUAmax™ and Syngenta Artesian™, and the first drought tolerant GM maize developed through both traditional plant breeding and the introduction of a transgenic trait (Adee et al. 2016). The drought-tolerance trait was introduced via agrobacterium-mediated transformation, incorporating the *cold shock protein B* (*cspB*) gene from *B. subtilis*. The CSPB protein preserves RNA stability and translation resulting in maintenance of essential cellular functions under water stress conditions (Graumann et al. 1997; Sammons et al. 2014).

Field trials conducted in the United States (US) compared MON87460 with conventional controls under well-watered and water-limited conditions during the key growth phases from mid-vegetative to reproductive. Measured parameters included leaf area, soil-water content, sap flow, kernel number, harvest index and the drought response physiology. Results showed significantly higher yields under drought stress in several MON87460 hybrids and there was no significant yield penalty under normal watering conditions. Drought-resilience-associated traits in the GM variety included reduced transpiration linked to decreased leaf area, better ear growth during silking and increased kernel number under water-limited conditions. Overall grain yield increased by 6% compared with controls under water-limited conditions, indicating enhanced physiological acclimation to drought stress (Nemali et al. 2015).

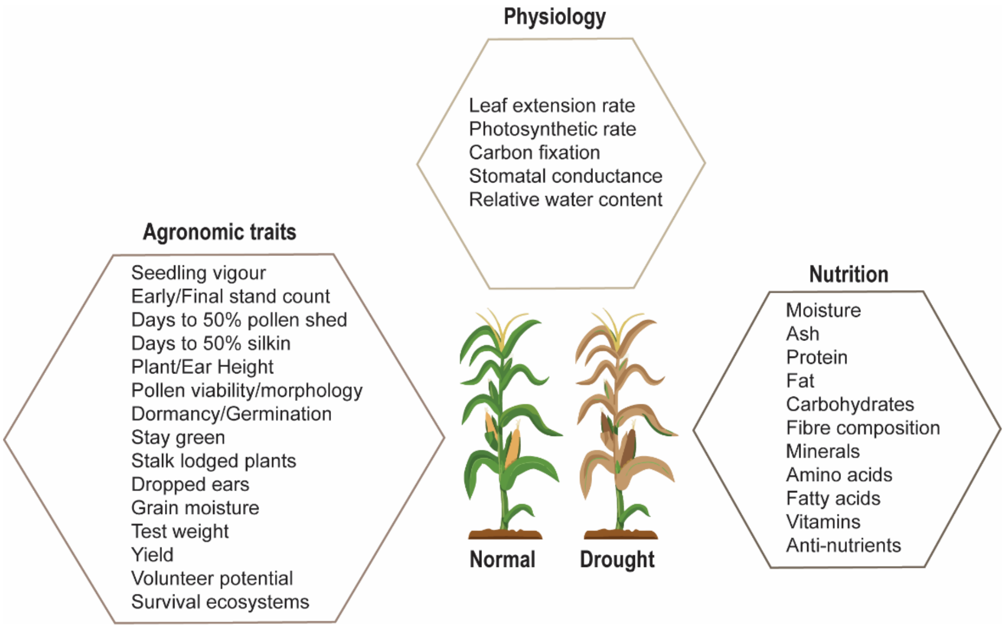

Figure 1. *Schematic overview of Genuity® DroughtGard™ (MON87460) GM maize crop characterization in a field study under drought conditions.*

In another study conducted in the US, Sammons and colleagues (2014), focused on potential environmental risks associated with MON87460 rather than physiological performance. Here, they studied four main hazard categories: weediness, adverse environmental impact on non-target organisms and the potential of the drought resilient trait to cause enhanced soil water usage and thereby limiting future crop choices. A range of phenotypic and agronomic characteristics were evaluated (**Fig. 1)** to assess yield-related traits, crop quality and the ability to cope with external stresses (e.g. pests, infections, environmental changes). With respect to water stress, root growth and development were analyzed together with their soil water relationship to determine whether the drought resilience trait causes maize plants to extract more water from the soil than conventional comparators. This information can be important for assessing potential downstream impacts on crop rotation options.

In this experiment, capacitance soil moisture probes (EnviroSMART, Sentek) installed at different depths (20, 30, 50, 70 and 100 cm) were used to detect the depletion of moisture from the soil profile during the growing season. Two different blocks were used, one with water-limited soil treatment and another well-watered treatment with drought stress imposed from the late vegetative growth through early grain filling stages. To prevent complete crop failure, rescue irrigations were applied as needed. Cumulative soil moisture depletion was calculated over the season and grain yield was determined on a plot basis at harvest. Although MON87460 showed some differences in water use patterns, it did not deplete soil moisture to a greater extent than the non-GM control. Overall, the introduced drought resilience trait did not alter dormancy, germination and volunteer potential of the seed, thus minimizing the risk of unwanted spreading of the GM crop into the wild. In addition, biotic stress tolerance remained comparable to that of the non-GM counterpart. The impact on biodiversity was considered minimal as no phenotypic effects extended beyond the field, including no changes in phenological shifts and therefore no requirement for extended detailed modelling of potential risks.

Verdeca HB4 soybean – drought and herbicide tolerant GM soybean

Maintenance of high soybean yields during drought stress was achieved through the introduction of the *HaHB4* gene from *Helianthus annuus* (sunflower) via agrobacterium-mediated transformation. *HaHB4* encodes a homeodomain-leucine zipper (HD-Zip) transcription factor whose expression is induced by abiotic stresses such as drought, salinity and darkness, as well as by phytohormones including ethylene, Abscisic Acid (ABA) and Jasmonic Acid (JA) (Manavella et al. 2008; Ribichich et al. 2020). The observed drought tolerance remains poorly understood and does not appear to involve the typical plant stomatal closure response to offset water deficit periods associated with reduced ethylene sensitivity (Manavella et al. 2006). In addition to drought resilience, HB4 soybean also demonstrated improved resilience to heat (Ribichich et al. 2020; Raineri et al. 2024). Molecular characterization of HB4 soybean revealed insertion of a single copy of the T-DNA, with stable inheritance of the transgene over at least four generations.

HB4 soybean was evaluated in field trials in the US and Argentina using conventional comparators (e.g. Williams 82) and locally adapted commercial varieties. Assessments included germination, vegetative growth, phenology (e.g. leafing, flowering, fruit set), pollen viability, seed set, and yield across diverse environmental conditions including drought stress. Toxicity and allergenicity were evaluated through bioinformatics comparisons of the introduced protein against known toxins in the NCBI database and Animal Toxin Database (ATDB), and allergens in the AllergenOnline database. Across trials, HB4 soybean exhibited agronomic performance comparable to that of conventional varieties and remained within the established range of commercial reference lines. Furthermore, no unusual phenotypes beyond the intended drought resilience were determined (IND-00410-5). However, detailed information on the imposed drought stress protocols (e.g. withholding periods or quantified soil moisture stress) was not fully disclosed in publicly available documentation.

HB4 wheat – drought tolerant GM wheat

Like Verdeca HB4 soybean, HB4 GM wheat was developed via introduction of the *HaHB4* gene from sunflower. Field trials were conducted across multiple sites with a range of moisture conditions, including water-limited environments. Performance during well-watered and drought conditions was evaluated, including grain yield and water use efficiency. Across multiple growing environments, all tested HB4 wheat varieties expressed the intended drought tolerance trait without evidence of unintended ecological effects, and agronomic performance remained within the range of conventional wheat varieties. With respect to food safety, the HaHB4 protein has been assessed as non-toxic and non-allergenic. Wheat (non-GM) itself is a known source of allergens, and slightly elevated levels of endogenous wheat allergens were observed in GM HB4 wheat. Consequently, people with known wheat sensitivities are advised to avoid exposure. However, no additional health risks beyond those associated with conventional wheat are expected. From an environmental risk perspective, the enhanced abiotic stress tolerance conferred by the introduced *HaHB4* gene, especially drought tolerance, may increase the persistence of GM wheat volunteers relative to non-GM wheat. In Australia, where wheat is grown as a winter crop, spilled seeds may persist through dry summers conditions, potentially increasing volunteer survival. This raises the possibility that persistent GM wheat volunteers could interfere with crop establishment or negatively affect yields of subsequent non-wheat crops. Previous studies have shown that the majority (approximately 87%) of wheat volunteers emerge within the first month post-harvest, 11% in the second month and 1% in the third month. The longest reported persistence of wheat volunteers following GM wheat cultivation was 20 months post-harvest (DIR204). Based on these observations, it was concluded that any emerging GM wheat volunteers could be effectively managed using standard weed control practices.

NXI-1T, NXI-4T, NXI-6T – drought tolerant GM sugarcane

Three GM sugarcane varieties are currently approved for cultivation in Indonesia. The first variety (NXI-1T) was developed through introduction of the *betA* gene from *Escherichia coli* and encodes a choline dehydrogenase that catalyzes the production of the osmoprotectant compound glycine betaine, thereby enhancing drought tolerance (Sugiharto 2024). In addition, NXI-1T confers resistance to glufosinate, a broad-spectrum herbicide used for weed management. The second and third variety, NXI-4T and NXI-6T respectively, also express the *BetA* gene, but derived from *Rhizobium meliloti*. Despite regulatory approval, detailed environmental risk assessments for these GM sugarcane varieties are not publicly available. Nevertheless, a study by Sholeh and colleagues 2019, which monitored sugarcane mosaic virus (SCMV) incidence, reported increased susceptibility of NXI-4T to SCMV relative to other varieties. This observation suggests a potential trade-off between enhanced drought tolerance and antiviral defense responses.

Overall, ERAs of approved abiotic stress-resilient GM crops, for cultivation in specific countries, all compare the difference in physiological and phenotypic responses related to different watering conditions (well-watered vs. limited watered) between the GM crop and its non-GM counterpart. None of these approved GM crops appear to associate with enhanced risks for the environment. Nevertheless, there is a lack of data on long-term ecological studies to study gene transfer and changes in interaction with non-target organisms (referred to as ecosystem interactions).

Different approval strategies among countries

The cases described above represent some of the few examples where abiotic stress-resilient GM crops have progressed to commercial cultivation. Their deployment remains limited to a small number of countries (**Table 3**). This uneven adoption reflects differences in national regulatory frameworks - such as India's exemption of SDN-1 and SDN-2 genetic modifications (see Appendix I)- as well as variation in how regulatory authorities evaluate and accept evidence reported in ERAs. A notable example is South Africa's rejection of the triple-stack MON87460 x MON89034 x NK603 GM maize, which included the drought-tolerance event MON87460 (described above), and commercially marketed as DroughtGard™. The South African executive council identified several evidence gaps leading to the decision of no approval. Key concerns were the lack of consistent and statistically significant yield protection under water-limited conditions, with no reproducible advantages observed for the kernel size or grain yield during drought. Furthermore, the appeal board recommended more multi-site and multi-season field trials to robustly demonstrate trait efficacy. Data supporting the insect-resistance component were also considered insufficient, as they were from a single trial site over two seasons. Collectively, these limitations resulted in the dossier being judged incomplete and therefore not approved by the Minister. This case underscores how differences in evidentiary standards and regulatory expectations, rather than the GM trait itself, can often determine approval outcomes across the globe.

4. Comparison of Environmental Risk Assessments of unapproved abiotic stress-resilient GM crops in field trials

Not all abiotic stress-resilient GM crops progress to commercial cultivation, even after completion of the required ERAs and associated field trials. One key reason is that while these crops perform well under controlled environmental conditions, their benefits under field conditions are frequently inconsistent or marginal (Gonzalez et al. 2020). In addition, the regulatory approval processes are often stringent, expensive and time-consuming, requiring extensive molecular and phenotypic characterization, multi-year and multi-location field trials, assessment of impact on non-target organisms, and evaluation of ecosystem interactions (Gonzalez et al. 2020). Economic considerations and market viability further influence whether developers pursue cultivation approval (Araus et al. 2019). Nevertheless, ERAs conducted for crops that do not reach commercialization can be highly informative, as they provide important insight into how environmental risks associated with abiotic stress-resilient GM crops are currently assessed and where potential gaps exist.

Overview of ERAs in ongoing or discontinued abiotic stress- related field trials

Field trials focusing on abiotic stress-resilient GM crops remain relatively scarce, and not all associated ERAs are publicly available, thus limiting comprehensive analyses of assessment outcomes and potential knowledge gaps. **Table 4** provides an overview of selected ongoing and discontinued field trials involving abiotic stress-related traits including both CRISPR based gene-edited crops, and older studies using transgenic approaches. Based on this table, we provide a summary on the commonalities between the different ERAs, the interconnection between different environmental risks followed by the identification of some knowledge gaps with potential solutions.

Table 4. *Overview of on-going and finished field trials of GM crops with enhanced abiotic stress resilience.*

Plant species	**Reference**	**Trait**	**Modification**	**Gene**	**Highlights Potential Risk**
Zea mays	B/ES/25/13, 2025	Drought resilience	CRISPR, deletion	H1L	Pollen dispersal, cross-pollination, selective advantage to survivability, vertical/horizontal gene transfer, allergenicity GM pollen
	B/BE/22/V1, 2022-2024	Abiotic stress resilience, DNA damage	CRISPR, frameshift	ZmNAC52-A ZMNAC52-B	Allergenicity GM pollen, selective advantage to survivability
	B/BE/19/V1, 2019	Abiotic stress resilience, DNA damage	CRISPR, frameshift, deletion	ATR, ATM	Survival GM crop after field trial, effect to organisms and biogeochemical processes
	B/FR/06/01/13, 2006-2009	Drought resilience, photosynthesis, herbicide resistance	Insertion	N/A	Potential environmental impact is considered minimal
Triticum aestivum	B/ES/17/15, 2018	Abiotic resilience, drought resilience	Insertion	HaHB4 (sunflower)	The introduction of the HB4 technology does not produce an adaptive advantage with regard to non-GM wheat
	DIR186**, 2022-2027	Abiotic stress resilience (drought, waterlogging ,heat, salinity)	Insertion	AtAVP1*, OsNAS2*, OsPSTOL1*, TaMUTE, TaYDA1,	*Triticum aestivum* can spontaneously hybridise with several closely related species, gene flow from wheat to *Hordeum marinum*

				TaYDA2, TaOST1 or TaSLAC1	
	DIR151, 2017-2023	Drought resilience, disease resistance	Overexpression	TaCAT1, TaNf-YA7, TaNAC69, HvCBF1, TaZFP34, TaHsfC2a, TaHsfA6f, TaRNAC1, TaNAC2, TaHsfC1e, TaMYB20, TaWRKY17* and more related to other traits	Exposure to GM pollen, persistence of GM seeds at trial sites possibly through seed dormancy in the seed bank
	DIR053/2004, 2005-2006	Salinity resilience	Insertion	AtOAT from Arabidopsis, CAH from soil funghi Myrothecium verrucaria	Spreading into the environment and exposure to other organisms, new characteristics of the GM crop, outcrossing GM wheat plants due to non-GM wheat plants surrounding the trial site
	DIR204***, 2024-2029	Abiotic stress resilience (drought, heat, cold)	Insertion	HaHB4 (sunflower)	Wheat has weediness characteristics (OGTR, 2021), allergenicity and toxicity to livestock, increased levels of anti-nutrients in comparison to non-GM wheat, cross-pollination
Gossypium	DIR081/2007, 2008-2010	Drought resilience	Insertion	N/A	Compatible species present in Australia for cross-pollination events, toxicity and allergenicity, weediness
	DIR083/2007, 2008-2011	Waterlogging resilience	Insertion	PDC2 and AHB1 from Arabidopsis, ADH from cotton	Gene flow to other organisms, allergenicity or toxicity due to genetic modification, unwanted altered characteristics in the biochemistry or physiology
	DIR067/2006, 2006-2009	Waterlogging resilience	Insertion	AHB1 from Arabidopsis	Toxicity for vertebrates, invertebrates and micro-organisms, weediness, gene transfer
Solanum tuberosum	B/HU/07/06	Drought resilience	Insertion	TPS1 from yeast	No dangerous effects were identified
	B/ES/11/01, B/ES/10/14, B/ES/09/57, 2009-2011	Heat resilience	Insertion	FT from Arabidopsis	No selective advantage is foreseen aside from an increase yield in tubers under heat stress conditions
Populus deltoides	B/SE/23/21689, 2024-2028	Drought resilience, wood biomass	CRISPR, insertion	GA biosynthesis, WRKY TF, bHLH TF	Pollen dispersal when floral buds are being formed
Populus tremula x Populus tremuloides	B/SE/11/2397	Drought resilience	Insertion	N/A	
Pisum sativum	B/GB/03/R29/4, 2003-2004	Drought resilience	Insertion	TI1	No significant impact on the environment
Brassica oleracea	B/S/21/28, 2022-2025	Abiotic stress resilience (drought and salinity)	CRISPR	ABI1, GSTU17, HAB1	No potential impact
Lycopersicon esculentum	B/IT/25/03, 2025-2027	Abiotic stress resilience, biotic stress resilience	CRISPR, frameshift	DMR6-1	Cross-pollination

Oryza sativa	B/ES/03/16-CON, B/ES/03/17, B/ES/03/18, 2003	Abiotic stress resilience (drought and salinity)	Insertion	N/A	Not expected that the modified plants will interact with the environment differently from conventional plants
Brassica napus	DIR205, 2025-2030	Abiotic stress resilience (drought, waterlogging, cold, heat)	Insertion	GOI CDS1.6, GOI CDS1.0 from yeast	Toxicity or allergenicity, potential to hybridize under natural conditions with sexually compatible species, HA-tag in GM canola lines is potentially immunogenic
Cicer arietinum	DIR166, 2019-2024	Abiotic stress resilience (drought and heat)	Insertion	AtBAG4 from Arabidopsis, TIBAG4 from Tripogon loliiformis	Low outcrossing rate (average of <2%) and mostly self-pollinating, chickpea lacks many common weedy characteristics
Saccharum officinarum	DIR059, 2009-2015	Drought resilience	Insertion, RNAi	WUE1 (from rice). WUE2 (from soil bacteria), OsDREB1A from rice	Animals and micro-organisms are expected to be exposed to the GM crops, unintentional spread of sugarcane stems through transportation, mixing non-GM sugarcane with GM sugarcane, dispersal of viable sugarcane (due to vegetative propagation) by pests (e.g. feral pigs)

: GM crops with this specific gene alteration/insertion previously used in other field trial studies; **: Also Hordeum vulgare; *: Commercially cultivated in Argentina, Brazil and Paraguay*

Across these ERAs, a broad set of standardized environmental risk categories was evaluated. These include pollen-related risks (e.g. dispersal, allergenicity, toxicity), cross-pollination with wild relatives or nearby cultivated non-GM crops, horizontal or vertical gene transfer, potential selective advantages leading to increased survivability, weediness and persistence, and interactions with non-target organisms (ecosystem interactions). Here below we briefly elaborate on some of these risk categories with reference to examples listed in **Table 4**.

Pollen dispersal & cross-pollination

Allergenicity risk is typically low when the transgene is not expressed in reproductive tissues (DIR204). Bioinformatic tools (e.g. CSM Toxin which involves toxin and allergen sequence comparisons) are routinely used to predict potential allergenicity or toxicity of introduced proteins (DIR205). ERAs commonly assessed pollen dispersal distances and the presence of nearby compatible cultivars or wild relatives, since these factors strongly influence cross pollination risk. The main concern for abiotic stress traits is that pollen-mediated introgression into compatible populations could transfer stress tolerance alleles into recipient plants, where selection under adverse conditions may favor retention and increase the likelihood of ecological effects.

Vertical Gene Transfer (VGT)

Detection of vertical gene transfer (VGT) is challenging because the events are rare and depend on local mating systems, population structure and selection pressure. Sampling designs informed by population genetics can increase the probability of detecting low-frequency alleles, but optimal strategies require knowledge on determining factors such as knowledge of recipient population's mating biology and effective population size (Mercer et al. 2008, Duncan et al. 2019). In practice, limited sampling effort and short trial durations reduce the power to detect early introgression.

Horizontal Gene Transfer (HGT)

Whereas VGT is considered high risk in some crop species, horizontal gene transfer (HGT) is assumed to occur at low frequencies (Keese 2008) and is therefore rarely investigated in ERAs (DIR204; DIR205). In addition, many transgenes introduced into GM crops encode proteins or sequences already present in the environment and could be transferable via natural mechanisms. Recent advances in HGT detection using next generation sequencing (NGS) suggest that the occurrences of HGT may be underestimated (Philips et al. 2022). Although direct evidence of HGT between GM crops and non-target organisms (e.g. micro-organisms, or other plant species) remains limited, natural examples demonstrate that HGT can occur and may confer advantage to receiving species. HGT is often observed in parasitic plant species where haustorial connections enable the transfer of stable genetic material. A well-described example is the parasitic weed *Striga hermonthica*, which infects cereal crop species (e.g. Sorghum, rice). Germination of *Striga* seeds is triggered by host-derived root exudates, particularly strigolactones, followed by haustorium formation that permits attachment and penetration of the host root to extract water and nutrients (Taylor et al. 2024). In a similar manner, Striga obtained a nuclear gene (ShContig948) from *Sorghum bicolor*. The presence of a poly-A-like sequence associated with this gene suggested transfer via an mRNA or a cDNA intermediate (Azad et al. 2025). This example illustrates that HGT should not be dismissed outright as an aspect of environmental safety considerations. EFSA has recognized that empirical investigation of HGT between plants and microbial communities is restricted by methodological constraints under natural conditions, including extremely low transfer frequencies and sampling limitations and difficulties in linking detected sequences to a defined source (EFSA 2010). Nevertheless, detection methodologies continue to improve, increasing the feasibility of investigating HGT in agricultural environments. Importantly, HGT detection does not imply environmental harm. Rather a case-by-case assessment focusing on the transferred trait, nature of modification, recipient organism and ecological context is required to evaluate the environmental risk severity.

Weediness & Persistence

Preventing the transfer of abiotic stress-related traits may be challenging when such traits enhance adaptability and survivability under adverse environments. None of the ERAs analyzed in this study provided evidence for ecosystem-level shifts resulting from increased environmental resilience of plants. In addition to the transfer of abiotic stress-related traits, weediness and persistence may arise from the ability of stress-resilient GM crop volunteers to establish after the cultivation period. Key parameters used to assess this environmental risk include seed dormancy and seed longevity, which determine how long the seeds derived from the abiotic stress-resilient GM crop can persist in the soil seed bank after cultivation. Persistence is not solely influenced by the introduced GM trait but is strongly determined by the characteristics of the crop itself. For example, *Brassica napus* seedlings can establish even after a dormancy period varying from four to ten years depending on the environmental conditions (Lutman et al. 2005). Comparative studies have shown that GM *B. napus*, can exhibit reduced seed survival relative to non-GM counterparts, indicating that genetic modification does not necessarily increase persistence (Hails et al. 1997).

Ecosystem interactions and the rhizosphere

The environmental risks described above are commonly addressed in ERAs, whereas the interactions between the abiotic stress-resilient GM crop and broader ecosystem processes remain relatively underassessed. Several scientific reviews and comparative analyses suggest that the environmental risks posed by abiotic stress-resilient GM crops is generally smaller in magnitude and scope than the systemic risks associated with climate change itself. Climate change induces widespread and long-term alterations in ecosystem structure and function, affecting global food security, whereas the risks from individual GM crops tend to be localized, trait- and context-dependent and manageable through risk management strategies. In addition, abiotic stress traits affect plant performance under specific environmental conditions and effects are typically limited to adjacent wild relatives or ecosystem interactions in the immediate landscape. In contrast, climate change affects entire agro-ecological systems globally with altering rainfall patterns, drought frequency, heatwaves and soil conditions across multiple continents simultaneously (Batista et al. 2017; Galani et al. 2022). Nevertheless, this does not minimize the importance of rigorous ecological risk assessment for abiotic stress-resilient GM crops.

The rhizosphere represents a particularly dynamic and sensitive interface with potential implications for soil health and nutrient cycling and thus sustainable agriculture and food security. In European field trials, soil health is rarely addressed in detail, whereas more recent Australian ERAs explicitly acknowledge potential rhizosphere-related risks (DIR204; DIR186). For example, wheat expressing the *Arabidopsis thaliana* vacuolar H+-pyrophosphatase (*AtAVP1*) may acidify the rhizosphere, potentially enhancing uptake of iron, magnesium and potassium (Menadue 2018). Although this potential change in the environment is acknowledged, the reviewed ERAs did not include empirical field-based measurement to confirm rhizosphere changes or their ecological relevance. In a review by Popoyan and Chikindas (2019) recommendations comprise inclusion of a comparative rhizosphere analysis into ERAs of GM crops, highlighting the need to better understand plant-soil-microbe interactions. However, standardized experimental designs and validated methodologies for such assessments were not elaborated, and remain limited. This underscores an important knowledge gap in the current ERA frameworks.

Collectively, the ERAs summarized in Table 5 demonstrate that while standard endpoints (pollen transfer, gene flow, persistence, non-target effects) are broadly covered, critical gaps remain in long-term detection power, rhizosphere impacts and context-specific assessments of introgression.

Influence of plant species and the receiving environment on environmental risks

Comparative analysis of ERAs related to different abiotic stress-resilient GM crop species indicates that these risks are determined not only by the introduced trait but are also influenced by the inherent characteristics of the crop species and the receiving environment. Pollination biology, for example, affects the likelihood of cross-pollination, gene flow and selective advantage or disadvantage for survivability. The presence of sexually compatible wild relatives in the surrounding environment, further increases the probability of hybridization and trait introgression. Seed dormancy and persistence in the soil seed bank are also species-specific traits that can determine the volunteer potential and ferality and thus influence whether seeds are able to survive outside of the cultivation period and area. **Table 5** summarizes key biological characteristics of five example GM crops and the characteristics determining their 'natural' potential risk. Importantly, the interaction between plant species characteristics and the receiving environment can further modulate risk expression (Arpaia et al. 2021). For example, the absence of wild relatives near the receiving environment can significantly reduce the risk of cross-pollination. In Europe, wild relatives (e.g. *Aegilops*) of wheat are widespread, increasing the potential for gene flow and introgression of abiotic stress-resilient traits. Such introgression could confer a fitness advantage in dry environments, increasing the risk of weediness or invasiveness of the wild relative and potentially disruption ecosystem dynamics, including interactions pollinators, wildlife (e.g. birds), vegetation, and soil processes. Similarly, seed dormancy characteristics in crops such as canola increase volunteer potential, as dormant seeds can germinate under favorable environmental conditions long after harvest. Thus, both the likelihood and severity of environmental risks can be shaped by plant species characteristics and the receiving environment.

Abiotic stress-related traits could further intensify these risks by introducing the potential of evolutionary selective pressures that enhance survivability under extreme weather conditions.

Table 5. *Overview of crop characteristics influencing the level of risk independent of the GM trait of interest.*

Crop characteristic	*Maize*	*Canola*	*Soybean*	*Rice*	*Cotton*	*Wheat*
Pollination type	Wind	Insect	Self	Self, cross-pollination via wind/insects	Self	Self, sometimes wind
Gene flow	Moderate	Moderate to high	Very low	Moderate	Low	Low
Compatibility wild relatives	Low hybridization	High hybridization	Low hybridization	Very high hybridization	Low hybridization	Low hybridization
Seed dormancy	Short lived	Long lived	Non-dormant	Moderate	Short lived	Moderate
Volunteer potential	Low	High	Low	Moderate	Low	Low to moderate
Survivability outside cultivation	Poor competitor	Persist as feral roadside populations	Poor competitor	Moderate competitor, specially wet conditions	Poor competitor, except tropical regions	Poor competitor
Ferality	Low	Moderate	Very low	High	Low to high, region dependent	Very Low
Main focus ERA	Pollen flow, pest resistance management (biotic stress)	Gene flow to wild relatives, volunteer persistence	Agronomic management impacts	Hybridization wild relatives, ecological impacts in aquatic systems	Insect resistance management (biotic stress), non-target impacts	Gene flow to crops or wild relatives, persistence, environmental interactions, changes in fitness
Environment risk level	Moderate	High	Low	High (in Asia)	Moderate	Low

References general: EFSA, 2010; OECD, 1993, 2010; FAO & WHO, 2001; EPA, 2017; Conner et al. 2003; Hilbeck & Andow 2004. Maize: Raybould & Quemada 2010; Sanvido et al. 2008. Canola: Devos et al. 2012; Warwick et al. 2009. Soybean: Carpenter 2011; Hartman et al. 2015. Rice: Lu & Snow 2005; Ellstrand 2014. Cotton: Naranjo 2011; Brookes & Barfoot 2023.

Interconnected and cascading environmental risks

Although many of the assessed risks are not unique to ERAs of abiotic stress-resilient GM crops, modification of stress tolerance pathways may have broader ecological consequences to the receiving environment. **Figure 2** illustrates how different environmental risks are interconnected and may influence each other, potentially resulting in cascading effects. For example, the presence of compatible wild relatives enhances the risk that abiotic stress-related traits are transferred into surrounding ecosystems, where recipient plants may gain a fitness advantage in adverse environmental conditions. The probability of this transfer is dependent on several factors, including species compatibility for hybridization, spatial distance between the GM crop and wild relative (pollen dispersal range) and whether the introduced trait confers a selective advantage only under certain environmental conditions (selection pressure). Once gene transfer occurs, the risk of weediness or invasiveness may increase, potentially disrupting ecosystem dynamics, and affecting interactions with pollinators, wildlife (e.g. birds), other plant species and soil processes. In addition, introgression of crop-derived genes into wild gene pools alters genetic composition, which may facilitate the emergence of stress-resilient hybrid populations. Such hybrids could compete more effectively with crops or native vegetation, and complicate weed management strategies. These changes can ultimately shift plant community structure and competitive balance, with downstream effects on ecosystem functioning. Altered plant species composition influences soil properties, including rhizosphere structure and microbial interactions, availability of floral resources for pollinators and habitat for other wildlife (e.g. insects, birds). In this context, even subtle pleiotropic effects associated with abiotic stress tolerance – such as changes in root chemistry or exudation patterns – may have disproportionate agronomic or ecological consequences. The interconnected nature of these environmental risks demonstrates that mitigating or eliminating one risk may amplify or suppress others. Such cascading interactions underscore the importance of considering not only individual risk endpoints but also their combined, indirect and system-level effects within the ecosystem.

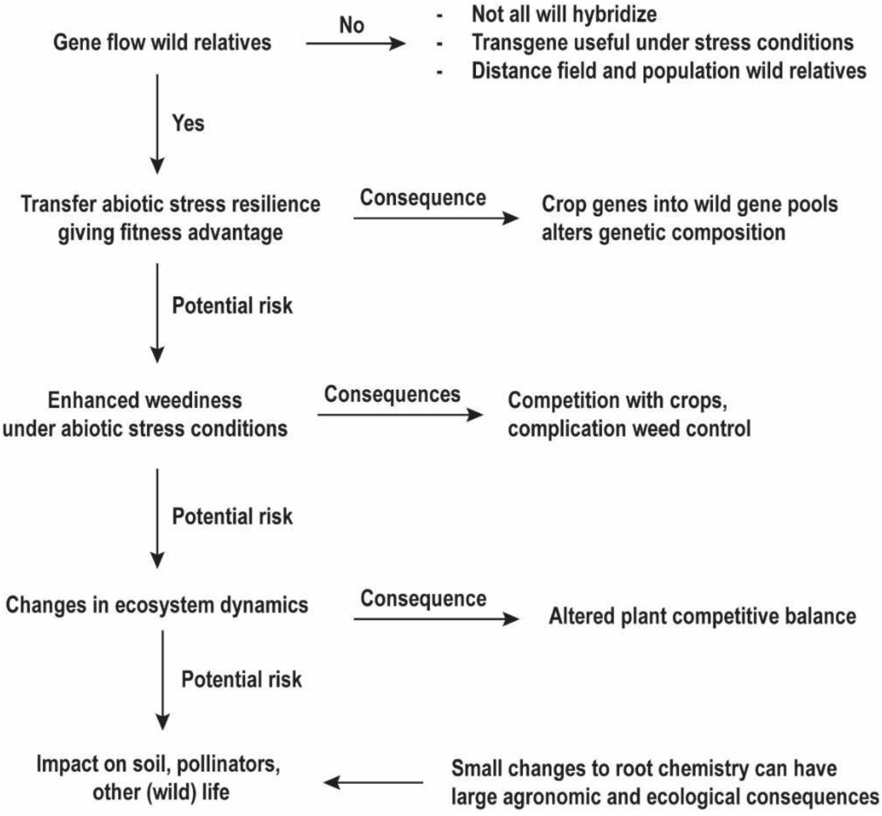

Figure 2. *Conceptual diagram illustrating potential interconnected risks associated with gene flow from abiotic stress-resilient GM crops to wild relatives.* Gene flow with wild relatives is dependent on the compatibility of the species, the usefulness of the transgene under stress conditions where natural selection will pressure the transfer, and the distance between cultivation areas and recipient populations. Successful introgression of the trait can also be influenced by its functional value under certain environmental stress conditions, where climate-driven natural selection may favour retention and spread of the transgene. The risk of weediness is also enhanced under abiotic stress conditions leading to competition with crops or complicating weed control. Changes to the ecosystem dynamics are another potential risk with the consequence of altering the plant competitive balance. Other impacts could be related to the soil, but also wildlife (e.g. pollinators, birds, insects). This last risk can be interconnected with changes in root chemistry caused by the introduction of drought resilience, illustrating the complexity of associated risks.

Temporal limitations of current ERAs for abiotic stress-resilient GM crops

Despite their breadth, current ERAs have limitations in capturing long-term ecological effects of abiotic stress-resilient GM crops. Most regulatory field trials, typically lasting one to three years, are intended to assess agronomic performance under stress, immediate phenotypic differences and short-term impacts on soil microbial communities. However, high interannual climate variability may obscure trait effects, and observed impacts may be transient or reversible, depending on measurement timing. The timeframe required to detect meaningful ecological trends varies considerably. The persistence of volunteer plants, shifts in weed community composition and early consequences of gene flow may require three to ten years of observation. More profound ecosystem-level changes- such as stable changes in species composition, selection for stress-resilient hybrids or weeds, changes in soil carbon and nutrient cycling and altered ecosystem resilience under climate extremes – may take a decade to several decades to emerge. Therefore it is extremely difficult, if not impossible, to capture reliable results in pre-market ERAs. **Table 6** summarizes the different types of assessments, their typical duration and their relevance for detecting ecosystem-level changes caused by growing abiotic stress - resilient GM crops.

Table 6. *Overview of assessment types, their duration and relevance in studying changes in the ecosystem.*

Assessment type	*Duration*	*Ecosystem relevance*
Confined field trials	1-3 years	Low to moderate
Pre-market ERA	3-5 years	Limited
Post-release monitoring	5-10 years	Moderate
Ecosystem studies	10-30+ years	High

The time required to detect ecosystem-level effects of abiotic stress-resilient GM crops further depends on crop biology and production system, ranging from less than a decade in annual crops such as maize to multiple decades in perennial systems such as sugarcane. Current ERAs adequately address short-term effects but are not designed to capture medium- and long-term ecological dynamics, particularly those driven by gene flow, perennial growth habits, or climate-driven selection pressures. From an abiotic stress trait perspective, additional challenges arise because effects may only manifest during rare extreme weather events, such as severe drought or flooding, but also because benefits or risks can be observed episodically rather than annually. Also separating GM trait effects from climate variability or management practices remains challenging. Thus, while current ERAs are necessary, they are not sufficient to fully capture ecosystem-scale dynamics.

Complementary approaches to assess long-term environmental risks

While long-term ecological studies remain the gold standard for detecting ecosystem dynamics, alternatives such as ecological modeling, sentinel indicators and adaptive post-release monitoring provide a scientifically robust and practical alternative for assessing the environmental impacts of abiotic stress-resilient GM crops. Ecological modeling enables projection of potential impacts over decades and across different environmental scenarios, including severe drought that might intensify selection pressure on the abiotic stress-resilience traits.

Common modelling approaches include individual-based models, landscape ecology models and eco-evolutionary models. Despite their cost-effectiveness and scalability, their predictive power depends heavily on underlying assumptions and data quality (Higgins & Richardson 1999; Peck 2004; Dietze 2017). Sentinel indicators offer early-warning indicators of ecosystem change, such as changes in weed seed bank composition, soil microbial functional genes, root-associated microbiome shifts, volunteer survival under stress, and fitness of crop-wild hybrids during drought. However, a strong ecological justification is required to make this predictive system work (Groffman et al. 2006; Carpenter et al. 2009). Adaptive post-release monitoring provides another complementary strategy, whereby pre-defined triggers or passing of certain thresholds initiate monitoring of a specific event. Examples include increased volunteer persistence, enhanced hybrid fitness or detectable shifts in soil function. Successful implementation depends on the availability of robust baseline data to identify when monitoring should be initiated (EFSA 2011; Sanvido et al. 2012).

While ERAs and their designs are important, their effective implementation ultimately depends on how risks are perceived, prioritized and translated into regulatory practice. To better understand how environmental risks of abiotic stress-resilient GM crops are viewed within the scientific community, a survey was conducted (Appendix I) among plant scientists in the Netherlands.

5. Environmental risks considered from a scientific perspective (survey)

Scientists contribute to the development of abiotic stress-resilient crops through fundamental and applied research. Nevertheless, their involvement in later stages of ERA and regulatory decision-making is often limited. To gain initial insights into scientists' familiarity with ERAs and their perspectives on potential gaps in current assessments, a survey was distributed among scientists in the Netherlands. The survey also served to enhance awareness within the Dutch scientific community of ERAs and their importance in the approval process for GM crops.

Most respondents were only somewhat familiar with ERAs (Fig. 3A), indicating limited exposure to regulatory assessment frameworks. Responses were gathered from scientists affiliated with several different Dutch universities (Fig. 3B) and the majority were assistant professors followed by PhD students (Fig. 3C). Research backgrounds were diverse, ranging from general plant biology, stress physiology to plant-microbiome interactions and theoretical modelling of stress responses (Fig. 3D).

When asked to prioritize environmental risks related to abiotic stress-resilient GM crops, gene flow to wild relatives was seen as the most important factor to be assessed followed by impacts on biodiversity (flora & fauna), effects on the soil health and micro-organisms and ecosystem resilience under climate change. Additional identified risks related to homogeneity of planted cultivars and the impact on within-species genetic diversity but also risks currently considered negligible. Although cross-pollination is considered a prominent environmental risk, many respondents perceived it as relatively low risk due to absence of compatible wild relatives in certain receiving environments. One respondent emphasized the relevance of specific risks strongly trait-dependent rather than inherent to the introduced modification. Consistent with this view, 86% of respondents considered abiotic stress - resilient GM crops to pose a lower environmental than pest or herbicide-tolerant GM crops, in line with several scientific reviews (Batista et al. 2017; Galani et al. 2022).

Regarding ERA priorities, interactions with the ecosystem (e.g. wild relatives, plants and animals) was most frequently selected, followed by post-release monitoring, long-term soil health and gene stability under stress conditions (Fig. 3E). In terms of preferred assessment approaches, multi-season field trials were most often identified as essential for evaluating environmental safety, followed by combined strategies integrating short-term laboratory studies, multi-season field trials and long-term ecological monitoring (Fig. 3F).

Regulation and governance were widely perceived as bottlenecks in approval of abiotic stress-tolerant GM crops. When asked whether the current regulatory framework is adequate, nearly 60% of the respondents were not sure, while 14% considered it somewhat adequate and 9% agreed the current regulation framework was adequate. The uncertainty likely reflects ambiguity in the current legislation. Some modifications – such as precise genome edits introduced through New Genomic Techniques (NGTs) such as CRISPR – are generally considered as low risk to the environment, while others may require more extensive assessment (Purnhagen et al. 2023).

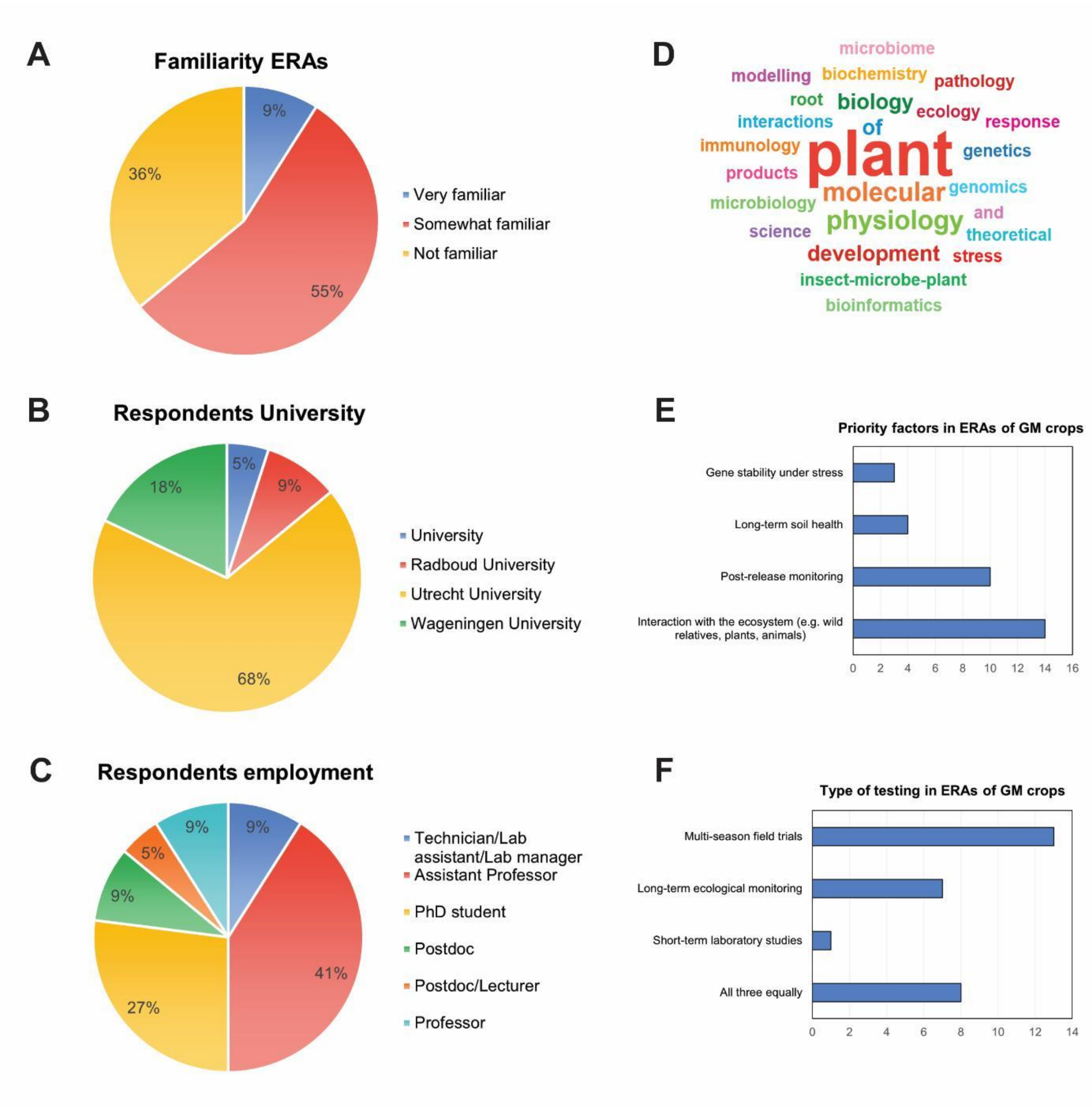

Figure 3. *Highlights of the survey distributed among plant scientists in the Netherlands.* (A) The majority of the respondents are (somewhat) familiar with ERAs. (B) Responses of the survey were collected from different scientists located at several Dutch universities. (C + D) The main respondent groups were assistant professors and PhD students all with a diverse background in plant sciences disciplines. (E) An ERA area of “interaction with the ecosystem” was selected to have the highest priority to investigate in relation to safety, followed by post-release monitoring. (F) Different types of testing the safety of GM crops within an ERA are seen as important, but multi-season trials were dominant in the answers. In total there were twenty-four respondents.

Respondents were also asked whether ERAs should differ based on the methodology used for GM crops generation. Nearly two-thirds (64%) supported ERAs for transgenic crops versus gene edited crops without foreign DNA (e.g. CRISPR). Moreover, nearly 90% of the respondents agreed that the potential benefits of abiotic stress-tolerant GM crops outweigh the environmental risks. Although benefits are currently excluded from ERA frameworks, this finding aligns with ongoing debates on including counterfactual risks (risk of inaction) – such as the environmental and societal risks of inaction – and comparing GM-related risks with those posed by climate change. However, this idea is not well received by regulators because it weakens the safety threshold and politicizes the scientific assessment (Purnhagen & Wesseler 2025).

Open-ended responses revealed that many participants were unfamiliar with the detailed structure of ERAs and noted the current limited cultivation of GM crops within the EU as a barrier to engagement.

Nevertheless, several constructive suggestions were shared. Several respondents emphasized that ERAs should be trait-driven rather than technique-driven and proportional to the actual level of environmental risk focusing on scientific evidence rather than the breeding technique. Furthermore, a comment was made about *“streamlining the process for low-risk genome edits, harmonizing regulations, and improving transparency would make the system more efficient while maintaining safety and public trust”*. Besides more transparency, a suggestion was brought forward to propose *“the same global standards in environmental risk assessments to avoid re-evaluations of approved GM crops and products, but also better strategies for evaluating environmental harm”.* One suggestion was to introduce *“fast-track testing for GM crops with modifications similar to approved ones”* and another remark was that *“an environmental risk assessment is not necessary in case a genetic modification could have naturally occurred”.* Similarly, a respondent highlighted that “*it is good to realize that microbes and viruses have been transferring genes between species for ages, so spread of GM does not worry me. I think it is really important to study the effect on long-term ecological interaction, since those effects are sometimes difficult to predict (everything is connected in terms of species interactions)”.*

Overall, the survey demonstrates that while awareness and knowledge on ERAs among academic plant scientists are variable and often limited, there is broad consensus that ecosystem-level effects deserve high priority in the assessment of abiotic stress-resilient GM crops. Respondents consistently emphasized the need for multi-season and multi-year field trials to properly evaluate risk. In general, the survey resulted in little input on deficiencies in the current strategy of assessing abiotic stress-resilient GM crops. Awareness and knowledge of these aspects might be higher among researchers from plant breeding companies. However, with the current restrictions in legislation related to cultivation of abiotic stress-resilient GM crops, these companies may be reticent in giving honest input or are not necessarily interested. Further contact and in-depth research are required to confirm this.

6. Concluding discussion

HT and insect-resistant GM crops were the first introduced stress-related traits to be granted regulatory acceptance for cultivation. The demand for climate resilient crops – including GM crops with abiotic stress tolerance – is expected to increase substantially due to accelerating environmental change, growing food-security pressures and the limitations of conventional breeding approaches. Currently, the same ERA framework is applied to both biotic stress-resistant and abiotic stress-resilient GM crops, underscoring the clear overlap in assessed risk categories across these trait classes. Despite this overlap, the nature and relevance of risks differ depending on the type of stress and the biological mechanisms underlying the trait. For abiotic stress-resilient GM crops, the most relevant risks relate to potential changes in ecosystem dynamics arising from pleiotropic effects, gene flow leading to increased persistence and weediness, and agronomic instability under variable or unpredictable climatic conditions. While these risks are conceptually recognized, the availability of large-scale, long-term empirical data remains limited. As a result, ecosystem-scale dynamics – including those potentially emerging under rare or episodic stress conditions – are insufficiently captured in most pre-market ERAs.

A dedicated or separate ERA framework for abiotic stress-resilient GM crops has not been developed, primarily because: 1) existing ERA structures already encompass all plausible environmental risks, 2) the category of abiotic stress traits is highly heterogeneous, 3) risks are strongly dependent on crop species and the receiving environment, 4) and no novel harm pathways have been identified that would justify a new regulatory framework. Furthermore, abiotic stress resilience challenges existing regulatory assessment not by introducing discrete hazards, but by modifying ecological performance and stress responsiveness. Consequently, regulators generally discourage rigid checklist-based approaches and instead favor hypothesis-driven risk assessment. In this context, regulators increasingly accept the use of trait-relevant endpoints, ecosystem context, climate variability considerations and adaptive post-market environmental monitoring. They only resist codifying these into a mandatory, stand-alone ERA category for abiotic stress-resilient crops. Where climate-aware exposure modeling is considered necessary, it should be initiated through a plausible pathway to harm rather than by trait classification alone.

Although a specific framework for abiotic stress-resilient GM crops is not desirable or required, the development of a targeted guidance on common abiotic stress traits and associated potential environmental and agronomic risks could help streamline and improve ERA practice. Abiotic stress resilience is typically achieved through context-dependent genetic alterations linked to trait improvement that confers stress resilience and yield maintenance under specific environmental conditions. As outlined in **Table 7** , these traits may introduce environmental and agronomic trade-offs particularly when stresses are intermittent or absent, when traits alter the receiving environment (e.g. soil, water or nutrient dynamics), or trade-offs reduce ecosystem stability or productivity. In addition, traits conferring resilience under one stress scenario may reduce performance under increasingly variable and unpredictable climate patterns (incl. non-stressed conditions).

Accordingly, abiotic stress-resilient GM crops must continue to be evaluated on a case-by-case basis, taking into account the crop species biology (influencing risk, **Table 5**), trait function and the receiving environment. A trait- and ecosystem-based ERA approach that explicitly incorporates climate awareness will benefit from close collaborations between plant biologists and agronomists specializing in stress physiology, ecologists and experts in bioinformatics and modelling of abiotic stress responses. However, the survey conducted in this study (**Fig. 3**) indicates that awareness and detailed knowledge about ERAs – particularly for abiotic stress-related traits – remains limited within the academic research community. Addressing this knowledge gap will be important for improving problem formulation and ensuring that relevant ecological risks are identified early in the assessment process.

Input from plant breeding companies may also be valuable, given their experience in generating multi-location, multi-year field trial data, analyzing stress-gradient performance and evaluating phenotypic stability. Such expertise can strengthen exposure assessment and reduce reliance on speculative modeling. In addition, breeders and scientists can contribute to the design of targeted and costeffective post-market environmental monitoring (PMEM) schemes aimed at detecting credible risks related to long-term ecosystem dynamics. Nevertheless, regulators remain cautious about stakeholder involvement due to potential conflicts of interest. Contributions focused on methodological improvements rather than outcome-driven arguments might therefore be more likely to be accepted.

Table 7. *Overview common traits per abiotic stress and their potential environmental and agronomic risks.*

	Common traits	**Potential environmental & agronomic risks**
Drought	• Deep or prolific root system • Stomatal closure, reduced transpiration • Osmotic adjustment • Early flowering (drought escape response) • Reduced leaf area, waxy cuticle	• Reduced productivity in non-stress years *Conservative water usage resulting in lower carbon assimilation; Yield penalties when water is not limited* • Depletion of deep soil water *Deep roots can reduce groundwater recharge, compete with neighboring vegetation and increased vulnerability during prolonged multi-year droughts* • Enhanced heat stress susceptibility *Tight stomatal control reduces evaporative cooling resulting in the raise of canopy temperature* • Altered soil biology *Reduced root exudation can lower microbial diversity and affect nutrient cycling*
Salinity	• Ion exclusion (Na^+/Cl^-) • Vacuolar sequestration of salts • Salt glands or bladders • Osmoprotectant accumulation	• Soil salinization *Salt-excreting plants can enhance surface salt accumulation and degrade soils for subsequent crops* • Nutrient imbalance *Ion exclusion may reduce uptake of K^+, Ca^{2+}, Mg^{2+} or can require higher fertilizer inputs* • Food & Feed quality concerns Elevated salt content in edible tissues and/or impacts palatability and animal health • Ecosystem shifts *Salt resilient species can outcompete native vegetation; Risk of creating low diversity (halophyte-dominated systems)*
Flooding	• Aerenchyma formation • Adventitious roots • Anaerobic metabolism • Stem elongation (escape strategy) • Growth cessation (quiescent strategy)	• Methane and nitrous oxide emissions *Anaerobic soils promote methanogenesis and denitrification* • Reduced performance under aerobic conditions *Flood adapted metabolism is inefficient in well-drained soils potentially leading to lower yields outside flooded environments* • Enhanced disease pressure *Waterlogging conditions are favorable for some root pathogens (e.g. Pythium, Phytophthora) and insect pests adapted to wet conditions* • Lodging and structural weakness *Rapid elongation resulting in thinner cell walls and therefore enhanced risk for crop collapse*
Heat	• Heat shock protein expression • Membrane lipid remodeling • Altered flowering time • Enhanced transpiration	• Higher water demand • Desynchronization pollinators *When flowering time is altered* • Reduced grain filling duration *Dependent on flowering time* • Enhanced susceptibility to drought when heat and water stress co-occur
Cold	• Antifreeze proteins • Membrane rigidification • Delayed growth and/or dormancy periods	• Slower early growth *Potentially enhanced weed competition* • Delayed flowering *Potentially yield loss in short seasons* • Increased vulnerability to sudden warm spells (false spring)

To conclude, while current ERAs provide a good foundation for assessing the environmental risks of abiotic stress-resilient GM crops, they are inherently limited in their ability to capture long-term, climate-dependent ecosystem effects. Rather than introducing new regulatory categories, we propose that progress lies in refining trait-driven, ecosystem-aware assessments, supported by complementary approaches such as adaptive monitoring and modelling. A move towards such ERA practice would maintain scientific rigor but also enable responsible innovation in the development of climate-resilient agricultural systems.

Glossary

Abiotic stress	Environmental factors (e.g. drought, flooding, cold, heat, salinity) negatively impacting plant growth and development
Agronomic practices	Techniques, strategies, and practices used in crop cultivation
Biotic stress	Living organisms (e.g. pathogens, bacteria, fungi, oomycetes, nematodes, viruses, herbivores, parasitic plants) negatively impacting plant growth and development
CRISPR-Cas9	Genetic modification making use of the internal DNA repair system to mainly introduce mutations or removal of base pairs in a gene
Cross-pollination	Pollination of a flower with pollen from another flower derived from a different plant species
Ferality	Free-living populations of domesticated crops that have escaped cultivation
Gene flow	Introduction of genetic material (by interbreeding) from one population of a species to another
Horizontal gene transfer	Acquisition of genetic material from another organism without being its offspring
Hybridization	Process of crossing two genetically different plants to produce new genotypes, resulting in hybrids
Introgression	Similar to hybridization
Invasiveness	Capacity (of a plant species) of moving aggressively into a habitat and monopolizing resources such as light, nutrients, water, and space
Persistence	Remaining attached beyond maturation after similar parts of the plant, such as flowers, seeds, or leaves, have normally dropped off
Pollination	The act of transferring pollen grains from the male anther of a flower to the female stigma
Rhizosphere	Narrow soil zone influenced by plant root growth
Seed dormancy	The state in which seed is unable to germinate, even under ideal growing conditions
Seed longevity	The lifespan or longevity of a seed is the time period over which it can remain viable
Vertical gene transfer	The transfer of genetic material from parent to offspring cells
Volunteer	Plants that grow in agricultural fields without being intentionally sown, often surviving from previous crop cycles
Weediness	Any plant growing where it is not wanted

References

1. Mirzabaev, A., Bezner Kerr, R., Hasegawa, T., Pradhan, P., Wreford, A., von der Pahlen, M.C.T., Gurney-Smith, H. (2023). *Severe climate change risks to food security and nutrition*. Climate Risk Management, 39: 100473, doi:10.1016/j.crm.2022.100473.
2. Feeding the future global population (2024). Nature Communications, 15:222, doi:10.1038/s41467-023-44588-y
3. Hilbeck, A., Meier, M., Römbke, J., Jansch, S., Teichmann, H., Tappeser, B. (2011). *Environmental risk assessment of genetically modified plants - concepts and controversies.* Environmental Sciences Europe, 23:13, doi:10.1186/2190-4715-23-13
4. Li, R., Quan, S., Yan, X., Biswas, S., Zhang, D., Shi, J. (2017). *Molecular characterization of genetically-modified crops: Challenges and strategies.* Biotechnology Advances, 35(2):302-309, doi: 10.1016/j.biotechadv.2017.01.005.
5. Aziz, A. M., Brini, F., Rouached, H., Masmoudi, K. (2022). *Genetically engineered crops for sustainably enhanced food production systems.* Frontiers in Plant Science, 8;13:1027828. doi: 10.3389/fpls.2022.1027828
6. Loschin, N., Kuzma, J., Barrangou, R., Grieger, K. (2025). *Environmental assessment and regulatory oversight of genetically engineered crops in the United States.* Environmental Science & Policy, 173:104237, doi:10.1016/j.envsci.2025.104237
7. Raybould, A., Macdonald, P. (2018). *Policy-Led Comparative Environmental Risk Assessment of Genetically Modified Crops: Testing for Increased Risk Rather Than Profiling Phenotypes Leads to Predictable and Transparent Decision-Making.* Frontiers in Bioengineering and Biotechnology, 10(6):43. doi: 10.3389/fbioe.2018.00043
8. Advisory Committee on Releases to the Environment (ACRE), 2013. Towards a more effective approach to environmental risk assessment of GM crops under current EU legislation. https://www.gov.uk/government/publications/genetically-modified-organisms-improving-risk-assessments
9. Parmar, N., Singh, K.H., Sharma, D., Singh, L., Kumar, P., Nanjundan, J., Khan, Y.J., Chauhan, D.K., Thakur, A.K. (2017). *Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review.* Biotechnology, 7(4):239. doi: 10.1007/s13205-017-0870-y
10. Nickson, T.E. (2008). *Planning environmental risk assessment for genetically modified crops: problem formulation for stress-tolerant crops.* Plant Physiology, 147(2):494-502. doi: 10.1104/pp.108.118422.
11. Hussain, A., Munir, M., Khalid, A., Ali, M., Amanullah, M., Ali, Q., Manghwar, H. (2024). Engineering biotic stress tolerance via CRISPR-Cas mediated genome editing in crop plants. Plant Stress, 14:100650, doi:10.1016/j.stress.2024.100650
12. Esmaeili, N., Shen, G., Zhang, H. (2022). Genetic manipulation for abiotic stress resistance traits in crops. Frontiers in Plant Science, 13:1011985. doi: 10.3389/fpls.2022.1011985
13. Rahman, M.U., Zulfiqar, S., Raza, M.A., Ahmad, N., Zhang, B. (2022). Engineering Abiotic Stress Tolerance in Crop Plants through CRISPR Genome Editing. Cells, 11(22):3590. doi: 10.3390/cells11223590
14. Mushtaq,W., Li, J., Liao, B., Miao, Y., Liu, D. (2025). Unlocking crop resilience: How molecular tools enhance abiotic stress tolerance. Plant Stress, 17:100950, doi:10.1016/j.stress.2025.100950.
15. Kamran, M., Burdiak, P., & Karpiński, S. (2025). *Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants.* Cells, 14(3), 176. doi:10.3390/cells14030176

16. Ngongolo, K., Mmbando, G.S. Necessities, environmental impact, and ecological sustainability of genetically modified (GM) crops. Discov Agric 3, 29 (2025). doi : 10.1007/s44279-025-00180-0
17. OECD (2023). Environmental Risk/Safety Assessment for the Release of Transgenic Plants. OECD Environment Directorate, Paris.
18. EFSA Panel on Genetically Modified Organisms (GMO Panel). (2010). Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal, 8(11), 1879.
19. EFSA GMO Panel. (2022). Guidance on the risk assessment of genetically modified plants produced through synthetic biology. EFSA Journal, 20(4), 7260.
20. USDA-APHIS. (2020). Guidance for Plant Pest Risk Assessment of Genetically Engineered Plants. U.S. Department of Agriculture.
21. Raybould, A. (2006). *Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops.* Environmental Biosafety Research, 5 (3): 119–125. doi: 10.1051/ebr:2007004
22. Stein, A. J. & Rodriguez-Cerezo, E. (2022). Environmental and Agronomic Impacts of Abiotic Stress–Resilient GM Crops. Trends in Biotechnology, 40(5), 545–560.
23. Nap, J.-P., Metz, P. L. J., Escaler, M., & Conner, A. J. (2003). The Release of Genetically Modified Crops into the Environment. The Plant Journal, 33(1), 1–18.
24. Conner, A. J., Glare, T. R., & Nap, J.-P. (2003). The Release of Genetically Modified Crops into the Environment. Part II. Overview of Ecological Risk Assessment. Plant Journal, 33(1):19–46.
25. FAO (2021). Risk Assessment of Food and Feed from Genetically Modified Plants. Food and Agriculture Organization of the United Nations, Rome.
26. Ladics, G.S., Bartholomaeus, A., Bregitzer, P., Doerrer, N.G., Gray, A., Holzhauser, T., Jordan, M., Keese, P., Kok, E., Macdonald, P., Parrott, W., Privalle, L., Raybould, A., Rhee, S.Y., Rice, E., Romeis, J., Vaughn, J., Wal, J.M., Glenn, K. (2015). Genetic basis and detection of unintended effects in genetically modified crop plants. Transgenic Research, 24(4):587-603, doi: 10.1007/s11248-015-9867-7
27. Zhang, Y., Xu, J., Li, R., Ge, Y., Li, Y., Li, R. (2023). Plants' Response to Abiotic Stress: Mechanisms and Strategies. International Journal of Molecular Science24(13):10915. doi: 10.3390/ijms241310915
28. Sun, Z., Zhang, K., Peng, J., Liu, B., Kong, F., Sang, Q., Du, H. (2025). Strategies for Balancing Growth and Defence Against Biotic Stress in Legumes. Plant Cell & Environment, doi: 10.1111/pce.70070
29. Global Industry Analysts (2025). Drought Resistant Crops. Published 01-10-2025, length 234 pages, SKU # GJOB20518157. Website: https://www.marketresearch.com/Global-Industry-Analysts-v1039/Drought-Resistant-Crops-42705306/#description
30. Domingo, J.L. (2025). Genetically modified Crops: Balancing safety, sustainability, and global security. Environmental Research, 286(2):122892, doi:10.1016/j.envres.2025.122892.
31. Adee, E., Roozeboom, K., Balboa, GR., Schlegel, A., Ciampitti, I.A. (2016). Drought-Tolerant Corn Hybrids Yield More in Drought-Stressed Environments with No Penalty in Non-stressed Environments. Frontiers in Plant Science, 7:1534, doi: 10.3389/fpls.2016.01534
32. Graumann, P., Wendrich, T.M., Weber, M.H., Schröder, K., Marahiel, M.A. (1997). *A family of cold shock proteins in Bacillus subtilis is essential for cellular growth and for efficient protein synthesis at optimal and low temperatures.* Molecular Microbiology, 1997 Aug;25(4):741-56. doi: 10.1046/j.1365-2958.1997.5121878.x
33. Sammons*, B., Whitsel, J., Stork, L.G., Reeves, W. and Horak, M. (2014). *Characterization of Drought-Tolerant Maize MON 87460 for Use in Environmental Risk Assessment.* Crop Science, 54: 719-729. doi:10.2135/cropsci2013.07.0452

34. Manavella PA, Dezar CA, Bonaventure G, Baldwin IT, Chan RL. (2008). *HAHB4, a sunflower HD-Zip protein, integrates signals from the jasmonic acid and ethylene pathways during wounding and biotic stress responses.* Plant Journal, 56(3):376-88. doi: 10.1111/j.1365-313X.2008.03604.x.
35. Ribichich, K.F., Chiozza, M., Ávalos-Britez, S., Cabello, J.V., Arce, A.L., Watson, G., Arias, C., Portapila, M., Trucco, F., Otegui, M.E., Chan, R.L. (2020). *Successful field performance in warm and dry environments of soybean expressing the sunflower transcription factor HB4.* Journal of Experimental Botany, 71(10):3142-3156. doi: 10.1093/jxb/eraa064.
36. Manavella, P.A, Arce, A.L., Dezar, C.A., Bitton, F., Renou, J.P., Crespi, M., Chan, R.L. (2006). *Cross-talk between ethylene and drought signalling pathways is mediated by the sunflower Hahb-4 transcription factor.* Plant Journal, 48(1):125–137. doi: 10.1111/j.1365-313X.2006.02865.x.
37. Ranieri, E., D'Onghia, G., Lopopolo, L., Gikas, P., Ranieri, F., Gika, E., Spagnolo, V., Herrera, J. A., Ranieri, A.C. (2024). *Influence of climate change on wastewater treatment plants performances and energy costs in Apulia, south Italy.* Chemosphere, 350, 141087. doi:10.1016/j.chemosphere.2023.141087
38. Sugiharto, B. (2024). *Enhancing sugarcane growth quality and productivity through a biotechnology approach.* Agricultural Biotechnology for Food Improvement and Production, BIO Web of Conferences, 127:01012, doi:10.1051/bioconf/202412701012
39. Sholeh, A., Sugiharto B., Addy, H.S. (2019). *Monitoring Sugarcane mosaic virus (SCMV) on Recent Sugarcane Varieties in East Java, Indonesia.* Journal of Applied Sciences, 19: 647-653
40. González FG, Rigalli N, Miranda PV, Romagnoli M, Ribichich KF, Trucco F, Portapila M, Otegui ME, Chan RL. An Interdisciplinary Approach to Study the Performance of Second-generation Genetically Modified Crops in Field Trials: A Case Study With Soybean and Wheat Carrying the Sunflower HaHB4 Transcription Factor. Front Plant Sci. 2020 Mar 6;11:178. doi: 10.3389/fpls.2020.00178. PMID: 32210989; PMCID: PMC7069416.
41. José L Araus, Maria D Serret, Marta S Lopes, Transgenic solutions to increase yield and stability in wheat: shining hope or flash in the pan?, Journal of Experimental Botany, Volume 70, Issue 5, 15 February 2019, Pages 1419–1424, https://doi.org/10.1093/jxb/erz077
42. Mercer, K.L., Wainwright, J.D. (2008). *Gene flow from transgenic maize to landraces in Mexico: An analysis.* Agriculture, Ecosystems & Environment, 123(1-3): 109-115, doi: 10.1016/j.agee.2007.05.007.
43. Duncan, B., Leyva-Guerrero, E., Werk, T., Stojšin, D., Baltazar, B.M., García-Lara, S., Zavala-López, M., de la Fuente-Martínez, J.M., Meng, C. (2019). *Assessment of potential impacts associated with gene flow from transgenic hybrids to Mexican maize landraces.* Transgenic Research, 28(5-6):509-523. doi: 10.1007/s11248-019-00160-3.
44. Keese, P. (2008). Risks from GMOs due to horizontal gene transfer. Environment Biosafety Research, 7(3):123-49. doi: 10.1051/ebr:2008014.
45. Philips, J.G., Martin-Avila, E., Robold, A.V. (2022). *Horizontal gene transfer from genetically modified plants - Regulatory considerations.* Frontiers in Bioengineer and Biotechnology, 10:971402. doi: 10.3389/fbioe.2022.971402.
46. Haimlich, S., Fridman, Y., Khandal, H., Savaldi-Goldstein, S., Levy, A. (2024). *Widespread horizontal gene transfer between plants and bacteria.* ISME Communication, 4(1):ycae073. doi: 10.1093/ismeco/ycae073.
47. Irwin, N.A.T., Pittis, A.A., Richards, T.A., Keeling, P.J. (2022*). Systematic evaluation of horizontal gene transfer between eukaryotes and viruses.* Nature Microbiology, 7(2):327-336. doi: 10.1038/s41564-021-01026-3.

48. Wang, H., Sun, S., Ge, W., Zhao, L., Hou, B., Wang, K., Lyu, Z., Chen, L., Xu, S., Guo, J., Li, M., Su, P., Li, X., Wang, G., Bo, C., Fang, X., Zhuang, W., Cheng, X., Wu, J., Dong, L., Chen, W., Li, W., Xiao, G., Zhao, J., Hao, Y., Xu, Y., Gao, Y., Liu, W., Liu, Y., Yin, H., Li, J., Li, X., Zhao, Y., Wang, X., Ni, F., Ma, X., Li, A., Xu, S.S., Bai ,G., Nevo, E., Gao, C., Ohm, H., Kong, L. (2020). *Horizontal gene transfer of Fhb7 from fungus underlies Fusarium head blight resistance in wheat.* Science, 368(6493):eaba5435. doi: 10.1126/science.aba5435
49. Azad, R.B., Kasfy, S.H., Molla, K., Islam, T. (2025). *Horizontal Gene Transfer in Plants and Implications for Biotechnology.* Plant Environment Interactaction, 6(5):e70087. doi: 10.1002/pei3.70087.
50. Lutman, P.J., Berry, K., Payne, R.W., Simpson, E., Sweet, J.B, Champion, G.T., May, M.J., Wightman, P., Walker, K., Lainsbury, M (2005). *Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (Brassica napus).* Proceedings of the Royal Society B – Journals, 272(1575):1909-15. doi: 10.1098/rspb.2005.3166.
51. Hails, R.S., Rees, M., Kohn, D.D., Crawley, M.J. (1997). *Burial and seed survival in Brassica napus subsp. oleifera and Sinapis arvensis including a comparison of transgenic and non-transgenic lines of the crop.* Proceedings: Biological Sciences, 264(1378):1-7. doi: 10.1098/rspb.1997.0001.
52. Batista, R., Fonseca, C., Planchon, S. et al. Environmental stress is the major cause of transcriptomic and proteomic changes in GM and non-GM plants. Sci Rep 7, 10624 (2017). https://doi.org/10.1038/s41598-017-09646-8
53. Galani, Y.J.H., Hansen, E.M.Ø., Droutsas, I. et al. Effects of combined abiotic stresses on nutrient content of European wheat and implications for nutritional security under climate change. Sci Rep 12, 5700 (2022). https://doi.org/10.1038/s41598-022-09538-6
54. Menadue, D.J. (2018). *Identification and characterisation of vacuolar proton-pumping pyrophosphatase genes in bread wheat.* PhD Thesis (The University of Adelaide).
55. Pepoyan, A.Z., Chikindas, M.L. (2020*). Plant-associated and soil microbiota composition as a novel criterion for the environmental risk assessment of genetically modified plants.* GM Crops Food, 11(1):47-53. doi: 10.1080/21645698.2019.1703447
56. Arpaia, S. (2021). *Environmental risk assessment in agro-ecosystems: Revisiting the concept of receiving environment after the EFSA guidance document.* Ecotoxicology and Environmental Safety, 208:111676. doi: 10.1016/j.ecoenv.2020.111676.
57. WHO & FAO. (2000). Safety Aspects of Genetically Modified Foods of Plant Origin.
58. Hilbeck, A. & Andow, D. (Eds.). (2004). Environmental Risk Assessment of Genetically Modified Organisms: Methodologies for Assessing Bt Cotton in Brazil. CABI Publishing.
59. Raybould, A., Quemada, H. (2010). *Bt crops and food security in developing countries: realised benefits, sustainable use and lowering barriers to adoption.* Food Security, 2:247–259, doi:10.1007/s12571-010-0066-3
60. Sanvido, O., Widmer, F., Winzeler, M., Bigler, F. (2005). *A conceptual framework for the design of environmental post-market monitoring of genetically modified plants.* Environmental Biosafety Research, 4(1):13-27. doi: 10.1051/ebr:2005008.
61. Devos, Y., Hails, R.S., Messéan, A., Perry, J.N., Squire, G.R. (2012). *Feral genetically modified herbicide tolerant oilseed rape from seed import spills: are concerns scientifically justified?* Transgenic Research, 21(1):1-21. doi: 10.1007/s11248-011-9515-9
62. Carpenter, J.E. (2011). *Impact of GM crops on biodiversity.* GM Crops, 2(1):7-23. doi: 10.4161/gmcr.2.1.15086.
63. Hartman, G.L., Rupe, J.C., Sikora, E.J., Domier, L.L., Davis, J.A., Steffey, K.L., eds. Compendium of Soybean Diseases and Pests. Fifth edition. APS Press, The American Phytopathological Society; 2015.

64. Lu, B., Snow, A. (2005). *Gene Flow from Genetically Modified Rice and Its Environmental Consequences.* BioScience, 55(8):669–678, doi:10.1641/00063568(2005)055[0669:GFFGMR]2.0.CO;2
65. Ellstrand, N.C. (2014). *Gene flow between crops and their wild relatives.* Baltimore: Johns Hopkins University Press.
66. Steven, E. Naranjo, S.E. (2011). *Impacts of Bt Transgenic Cotton on Integrated Pest Management.* Journal of Agricultural and Food Chemistry, 59 (11):5842-5851, doi: 10.1021/jf102939c
67. Brookes, G., Barfoot, P. (2013). *Environmental impacts of genetically modified (GM) crop use 1996-2013: Impacts on pesticide use and carbon emissions.* GM Crops Food. 2015;6(2):103-33. doi: 10.1080/21645698.2015.1025193.
68. Higgins, S.I., Richardson, D.M. (1999). Predicting Plant Migration Rates in a Changing World: The Role of Long-Distance Dispersal. The American Naturalist, 153(5):464-475. doi: 10.1086/303193
69. Steven L. Peck, S.L (2004). Simulation as experiment: a philosophical reassessment for biological modeling. Trends in Ecology & Evolution, 19(10):530-534, doi:10.1016/j.tree.2004.07.019.
70. Dietze, M. C. (2017). Ecological forecasting. doi:10.2307/j.ctvc7796h
71. Carpenter SR, Mooney HA, Agard J, Capistrano D, Defries RS, Díaz S, Dietz T, Duraiappah AK, Oteng-Yeboah A, Pereira HM, Perrings C, Reid WV, Sarukhan J, Scholes RJ, Whyte A. Science for managing ecosystem services: Beyond the Millennium Ecosystem Assessment. Proc Natl Acad Sci U S A. 2009 Feb 3;106(5):1305-12. doi: 10.1073/pnas.0808772106. Epub 2009 Jan 28. PMID: 19179280; PMCID: PMC2635788.
72. P.M. Groffman, Jill Baron, T. Blett, A.J. Gold, I. Goodman, L.H. Gunderson, B.M. Levinson, Margaret A. Palmer, H.W. Paerl, G.D. Peterson, N.L. Poff, D.W. Rejeski, J.F. Reynolds, M.G. Turner, K.C. Weathers, and J. Wiens (2006). Ecological thresholds: The key to successful environmental management or an important concept with no practical application? Ecosystems, 9(1):1-13, doi:10.1007/s10021-003-0142-z
73. EFSA Panel on Genetically Modified Organisms (2011). Guidance on post-market environmental monitoring (PMEM), EFSA Journal, 9(8) :2316. doi: 10.2903/j.efsa.2011.2316
74. Sanvido, O., Romeis, J., Gathmann, A., Gielkens, M., Raybould, A., Bigler, F. (2012). Evaluating environmental risks of genetically modified crops: ecological harm criteria for regulatory decision-making. Environmental Science & Policy, 15(1):82-91, doi:10.1016/j.envsci.2011.08.006.
75. Purnhagen, K., Ambrogio, Y., Bartsch, D. et al. Options for regulating new genomic techniques for plants in the European Union. Nat. Plants 9, 1958–1961 (2023). https://doi.org/10.1038/s41477-023-01570-2
76. Purnhagen, K.P., Wesseler, J.H.H. (2025). Precaution and the Precautionary Principle: A View on the EU – The Example of Modern Biotechnology. In: Marciano, A., Ramello, G.B. (eds) Encyclopedia of Law and Economics. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-7883-6_835-2
77. Priyadarshini (2025). https://www.isaaa.org/kc/cropbiotechupdate Priyadarshini 2025/article/default.asp?ID=21330
78. Verma, R. K., Kumar, V. V. S., Yadav, S. K., Kumar, T. S., Rao, M. V., & Chinnusamy, V. (2020). *Overexpression of Arabidopsis ICE1 enhances yield and multiple abiotic stress tolerance in indica rice.* Plant Signaling & Behavior, 15(11). doi:10.1080/15592324.2020.1814547

Appendix I - Methods

Data collection for comparison of abiotic stress-resilient related environmental risk assessments

For the comparison of ERAs in current or finalized field trials, information was obtained from the European Commission website using the Food and Feed Information Portal Database (https://ec.europa.eu/food/food-feed-portal/screen/gmob/search) and the website of testbiotech who provides an up-to-date overview of field trials in Europe with plants derived from new genetic engineering techniques (https://www.testbiotech.org/en/projects/field-trials-of-plants-derived-from-new-genetic-engineering-development-in-europe/). Additionally, Google searches were performed to obtain scientific studies that were part of ERAs and also ERAs performed outside of Europe were obtained through google searches.

Survey Environmental Risk Assessment on Genetically Modified GM crops

A survey was designed and executed using Google forms as platform. Questions were based on current risks investigated in ERAs, but the survey was also used to measure awareness of ERAs within the plant sciences community. The survey was distributed through the newsletter of the Experimental Plant Sciences (EPS) community in the Netherlands as well as personal communication with professors of several chair groups distributed at different universities (e.g. Wageningen University (WUR), Universiteit van Amsterdam (UvA)). The survey can be found here: https://docs.google.com/forms/d/e/1FAIpQLSeexknmTl18g5sDA1zLDOy5rRNeEREk1r5EYr7ceXjLpNkazA/viewform?usp=header

Appendix II – Drought- and salt-tolerant GM rice, developed using CRISPR (approval pending)

In May 2025, India became the first country to approve two genome edited rice varieties developed using CRISPR-Cas9: Pusa Rice DST1 and DRR Rice 100 (Kamla) (Priyadarshini 2025). Approval was granted under India's regulatory framework for genome-edited plants, which exempts certain edits produced using Site-Directed Nuclease-1 (SDN-1) and Site-Directed Nuclease-2 (SDN-2) techniques from transgenic GMO regulation.

Pusa Rice DST1 was developed by the Indian Agricultural Research Institute (IARI) using CRISPR-Cas9 mediated non-homologous end joining (NHEJ) mutation method for gene editing the drought and salt tolerant (DST) gene in *indica* rice cultivar MTU1010. Two single-guide RNAs (sgRNAs) targeted a potential protein-protein interaction area of the *DST* allele. The resulting plants exhibit increased leaf width and have a reduced stomatal density leading to enhanced leaf water retention under dehydration stress. Reduction of stomatal density is partly caused by the downregulation of the stomatal developmental genes *SPEECH1* (*SPCH1*), *MUTE* and *Inducer of CBF expression 1* (*ICE1*, Verma et al. 2020). These molecular and physiological changes enhanced tolerance to both drought and salt stress.

DRR Rice 100 (Kamla) was developed by the Indian Institute of Rice Research (IIRR) through CRISPR-Cas9 mediated mutagenesis of *cytokinin oxidase (OsCKX2),* involved in cytokinin degradation, in the Samba Mahsuri background. Disruption of *OsCKX2* led to increased grain yield by 19%, early maturity (twenty days earlier) and enhanced performance under low-fertilizer conditions and drought stress (unpublished data).

The fast approval of these varieties reflects India's policy decision to exempt SDN-1 and SDN-2 genome-edited plants from transgenic classification. SDN-1 involves the creation of a double-stranded break in the genome which is repaired by endogenous DNA repair pathways without the introduction of foreign DNA, while SDN-2 similarly induces a double-stranded break but uses a short homologous repair template to introduces specific nucleotide changes. Also here no foreign DNA is introduced. In India crops developed with SDN-1 and SDN-2 techniques are not considered transgenic and can follow the path of Standard Operating Procedures (SOPs) to speed up development. Although regulatory approval for environmental release has been granted, both Pusa Rice DST1 and DRR Rice 100 will need to undergo broader multi-location cultivation trials prior to commercialization. (https://dbtindia.gov.in/sites/default/files/Final_%2011052022_Annexure-I%2C%20Genome_Edited_Plants_2022_Hyperlink.pdf).