

Cross-tolerance mechanisms in plants: physiological responses to combined abiotic stress in a changing climate

Park Ji Hyun

Yonsei University, Seoul, South Korea

Corresponding E-mail: park.hyun@interviewiauniversity.com

Abstract

Climate change is intensifying the frequency, duration, and interaction of abiotic stresses that affect plant growth, development, productivity, and survival. Rising temperatures, drought, soil salinity, flooding, and nutrient limitations frequently occur in combination, creating physiological challenges that differ substantially from those produced by individual stress factors. Cross-tolerance refers to the capacity of plants to acquire or express resistance to one stress through physiological, biochemical, molecular, or regulatory mechanisms that also provide protection against another stress. This paper examines the mechanisms underlying cross-tolerance under combined abiotic stress, emphasizing water relations, antioxidant defense, osmotic adjustment, hormonal signaling, photosynthetic regulation, membrane stability, gene expression, and epigenetic responses. It also investigates how artificial intelligence and machine learning for advanced data analytics can integrate physiological measurements, environmental observations, transcriptomic profiles, and metabolic information to identify stress-response patterns and predict plant performance under changing climatic conditions. A controlled experimental framework is proposed to compare individual and combined drought, heat, and salinity treatments using physiological, biochemical, and molecular indicators, with statistical methods designed to distinguish additive, antagonistic, and synergistic stress interactions. Because no experimental dataset or verified measurements are supplied, numerical experimental outcomes are not fabricated; instead, the results section presents the analytical comparisons required and interprets established biological principles in the context of the proposed study. The paper further discusses differences among plant species, stress intensity, exposure duration, developmental stages, and environmental conditions that influence whether cross-tolerance provides a protective advantage or imposes physiological costs. Understanding these relationships is essential for developing climate-resilient crops and improving agricultural adaptation through integrated physiological research, advanced data analytics, and evidence-based breeding strategies.

Keywords: cross-tolerance, abiotic stress, climate change, drought, heat stress, salinity, plant physiology, antioxidant defense, artificial intelligence, machine learning, advanced data analytics, climate-resilient crops

I. Introduction

Plants are continuously exposed to environmental conditions that influence their growth, development, reproduction, and survival. Climate change is increasing the importance of understanding how plants respond to interacting abiotic stressors, including drought, elevated

temperature, soil salinity, flooding, and nutrient imbalance. These factors rarely occur in complete isolation in agricultural and natural ecosystems. A crop growing in a semi-arid region, for example, may experience declining soil moisture during a heatwave, while plants in irrigated coastal regions may encounter both high temperatures and increasing soil salinity. The physiological consequences of such combinations can differ considerably from those observed when each stress is studied independently, making combined-stress research essential for understanding plant resilience. Cross-tolerance refers to a condition in which exposure to one stressor improves a plant's ability to withstand another stressor, usually through shared or interacting physiological and molecular mechanisms. The phenomenon may involve activation of antioxidant defenses, accumulation of compatible solutes, changes in hormonal signaling, stabilization of cellular membranes, or adjustments in gene expression. For example, mild water deficit may activate protective responses that subsequently contribute to improved tolerance of another dehydration-related stress. However, cross-tolerance is not universal. The same initial stress may enhance resistance to one challenge while increasing sensitivity to another, depending on stress intensity, exposure duration, plant species, developmental stage, and environmental conditions. Research on combined stresses therefore needs to distinguish genuine protective acclimation from general stress damage or growth suppression. The physiological complexity of cross-tolerance arises because plants must coordinate survival mechanisms with the continued allocation of resources to growth and reproduction. Stomatal closure can reduce water loss during drought but may restrict carbon dioxide uptake and increase leaf temperature during extreme heat. Antioxidant activity can protect cells against oxidative damage, yet excessive or prolonged stress may overwhelm these defenses. Similarly, osmotic adjustment can preserve cell turgor but requires energy and metabolic resources that might otherwise support growth. These interactions demonstrate that plant resilience is not determined by a single biochemical compound or gene; rather, it emerges from coordinated responses across roots, leaves, chloroplasts, cellular membranes, hormonal networks, and gene-regulatory systems.

Artificial intelligence and machine learning for advanced data analytics offer additional opportunities to investigate these complex relationships. Physiological experiments increasingly generate multidimensional datasets containing gas-exchange measurements, chlorophyll fluorescence, water status, metabolite concentrations, gene expression, root architecture, and growth characteristics. Machine learning can help identify patterns across these variables, detect combinations of traits associated with stress resilience, and predict plant performance under environmental conditions that differ from those used during model development. The objective of this paper is to synthesize the physiological and molecular mechanisms underlying cross-tolerance, establish an experimental framework for investigating combined abiotic stress, and examine how data-driven analysis can support the development of climate-resilient crops.

II. Conceptual framework of cross-tolerance under combined abiotic stress

Cross-tolerance is best understood as a relationship between stress perception, protective acclimation, and the response to a subsequent or simultaneous environmental challenge. In sequential exposure, a plant first encounters a relatively mild stress and subsequently experiences a different stressor. The initial exposure may activate signaling pathways, alter

metabolic activity, or induce protective proteins that remain available when the second stress occurs. This process is often described as cross-acclimation when prior exposure improves the response to another environmental condition. In simultaneous exposure, multiple stressors act together, and the resulting response reflects their interaction rather than a simple sequence of independent protective reactions.

The distinction between tolerance to individual stresses and tolerance to combined stresses is important because the underlying physiological responses may differ. A plant that performs well under drought alone may not necessarily maintain photosynthesis or reproductive development when drought coincides with extreme heat. Similarly, a salt-tolerant plant may still experience severe damage when salinity is accompanied by high temperature or nutrient limitation. Combined stress can generate unique transcriptional and metabolic responses that are not predictable from either individual stress treatment. Consequently, screening crops exclusively under single-stress conditions may overlook important weaknesses that emerge in realistic field environments.

The framework also distinguishes cross-tolerance from cross-sensitivity and stress priming. Cross-sensitivity occurs when exposure to one stress increases vulnerability to another, potentially because protective mechanisms consume resources, alter physiological regulation, or create conflicting signals. Stress priming describes the induction of a heightened or modified response following an initial exposure, and it may contribute to cross-tolerance when the resulting changes improve performance during a later challenge. Priming does not necessarily produce a beneficial outcome, however, because the initial exposure may also impair growth or reduce the resources available for subsequent recovery. Assessing the net benefit requires measurements of both stress resistance and overall plant performance. A comprehensive conceptual model must account for stress intensity, timing, duration, sequence, and environmental context. Mild stress may activate adaptive responses without causing substantial damage, whereas severe stress can exceed the plant's protective capacity. The order of stress exposure may also influence the outcome because signaling and acclimation responses evolve over time. Plant species and genotypes differ in their ability to regulate water balance, maintain ion homeostasis, and repair damaged cellular structures. Therefore, cross-tolerance should be treated as a conditional physiological property rather than a universal response, and experimental designs should explicitly compare individual stress treatments, simultaneous combinations, and sequential exposure where appropriate.

III. Physiological responses to drought, heat, and salinity

Drought stress develops when water availability becomes insufficient to meet plant demand, leading to changes in water potential, turgor pressure, stomatal behavior, and cellular metabolism. As soil moisture declines, roots detect changes in water availability and initiate hydraulic and chemical signals that influence above-ground tissues. Stomatal closure reduces transpiration and can conserve soil water, but it also restricts carbon dioxide entry and reduces photosynthetic carbon assimilation. Prolonged drought may inhibit leaf expansion, accelerate senescence, alter root-to-shoot allocation, and reduce reproductive success. The severity of these effects depends on drought intensity, soil characteristics, atmospheric demand, and the plant's capacity to maintain water transport.

Heat stress affects several interconnected physiological processes, including membrane stability, enzyme activity, photosynthesis, respiration, and reproductive development. Elevated temperatures can disrupt protein folding, damage photosystem components, and reduce the efficiency of carbon fixation. Plants respond by activating heat-shock proteins and other molecular chaperones that help stabilize proteins and limit the accumulation of damaged cellular components. Changes in membrane lipid composition can also influence cellular stability under high temperatures. However, the effectiveness of these mechanisms depends on the intensity and duration of exposure, as well as whether the plant can maintain sufficient water supply for evaporative cooling.

Salinity stress combines an osmotic challenge with the potential toxicity of accumulated sodium and chloride ions. High salt concentrations reduce the availability of water for root uptake even when soil appears moist, while excessive ion accumulation can disrupt nutrient balance, membrane function, and enzyme activity. Plants may respond by restricting sodium entry, transporting ions into vacuoles, maintaining potassium homeostasis, and synthesizing compatible solutes that help preserve cellular water relations[1]. Salt-tolerant species often possess specialized mechanisms for ion exclusion, sequestration, or tissue-level tolerance. Nevertheless, these responses require energy and may not fully protect plants when salinity is combined with high temperatures or prolonged drought.

When drought, heat, and salinity occur together, their effects can interact in ways that are not adequately described by single-stress experiments. Drought and salinity both reduce water availability, but salinity additionally creates ion-specific challenges. Heat increases atmospheric evaporative demand and may intensify water loss, while drought-induced stomatal closure can limit evaporative cooling[2]. The combined response therefore depends on the balance between water conservation, temperature regulation, ion management, and carbon assimilation. A plant may preserve short-term survival through stomatal closure and metabolic adjustment while experiencing substantial reductions in growth or seed production. Evaluating cross-tolerance requires measurements that distinguish these different outcomes rather than relying on survival alone.

IV. Osmotic adjustment, antioxidant defense, and cellular protection

Osmotic adjustment is an important mechanism through which plants maintain water uptake and cell turgor under dehydration-related stress. Plants may accumulate compatible solutes such as proline, glycine betaine, soluble sugars, and certain sugar alcohols, which help regulate cellular osmotic potential without substantially disrupting normal biochemical processes. These compounds may also contribute to protein stabilization, membrane protection, and redox regulation. The extent to which an individual solute contributes to stress tolerance varies among species and environmental conditions. Increased proline concentration, for example, may indicate an adaptive response, but it can also reflect the severity of stress; its accumulation alone does not establish that a plant is more tolerant.

Reactive oxygen species are produced naturally during cellular metabolism and function as signaling molecules at appropriate concentrations. Under drought, salinity, and heat stress, disturbances in photosynthetic and respiratory processes can increase the production of

reactive oxygen species beyond the capacity of normal cellular regulation. Excess accumulation may damage lipids, proteins, pigments, and nucleic acids. Plants counter this threat through enzymatic antioxidant systems, including superoxide dismutase, catalase, ascorbate peroxidase, and components of the ascorbate-glutathione cycle[3]. Non-enzymatic antioxidants, including ascorbate, glutathione, carotenoids, and phenolic compounds, provide additional protection by regulating redox balance and limiting oxidative damage.

Cross-tolerance can emerge when the initial stress activates protective responses that remain effective during a later challenge. Mild drought exposure, for example, may alter antioxidant activity or induce stress-responsive proteins that subsequently contribute to protection against another stressor. Similarly, some forms of heat acclimation involve changes in protein stability, membrane composition, and redox regulation that may improve performance under other environmental challenges. However, shared protective mechanisms do not guarantee that every stress combination will produce a beneficial outcome. The magnitude and timing of antioxidant responses, the availability of metabolic resources, and the severity of the subsequent stress all influence whether prior acclimation provides a measurable advantage.

Cellular protection must also be evaluated alongside growth and recovery. A plant may exhibit high antioxidant enzyme activity while still experiencing substantial membrane damage, reduced photosynthesis, or inhibited growth. Conversely, a tolerant genotype may limit oxidative damage through a balanced response without displaying the highest activity of every measured antioxidant enzyme. Therefore, experiments should combine biochemical indicators with measurements of membrane integrity, electrolyte leakage, relative water content, chlorophyll fluorescence, and biomass accumulation. This integrated approach helps distinguish effective protection from a biochemical response that merely indicates greater stress exposure.

V. Hormonal signaling, gene regulation, and stress memory

Plant hormones coordinate responses to environmental stress by connecting local signals with changes in growth, development, and metabolism. Abscisic acid (ABA) is particularly important in drought and salinity responses because it regulates stomatal behavior, water conservation, and the expression of stress-responsive genes. ABA signaling can activate protein kinases and transcription factors that regulate dehydration-responsive pathways. However, hormonal signaling under combined stress is more complex than a simple increase in ABA concentration. Heat, for example, can alter stomatal regulation and activate additional pathways that influence transpiration, growth, and reproductive development[4]. The resulting physiological response depends on interactions among multiple signaling systems rather than on the activity of one hormone alone.

Other plant hormones, including jasmonic acid, ethylene, salicylic acid, auxins, gibberellins, and brassinosteroids, contribute to stress responses in context-dependent ways. Jasmonic acid and ethylene participate in stress signaling and can influence senescence, growth regulation, and protective metabolism[5]. Auxins influence root development and changes in plant architecture, while gibberellins regulate growth processes that may be modified under unfavorable conditions. Brassinosteroids can contribute to photosynthetic stability and

antioxidant regulation in some plant systems. These hormonal networks can cooperate or compete, creating outcomes that depend on the type, sequence, and severity of stress. Understanding this interaction is essential for explaining why a treatment that improves drought tolerance may not provide equivalent benefits under combined drought and heat.

At the molecular level, stress-responsive transcription factors regulate groups of genes involved in water relations, antioxidant defense, protein protection, ion transport, and metabolic adjustment. Families such as DREB/CBF, NAC, WRKY, MYB, and heat-shock transcription factors participate in different aspects of environmental acclimation. Their downstream targets may include genes involved in compatible-solute synthesis, protective proteins, membrane regulation, and cellular repair. Combined stress can activate overlapping gene sets while also producing distinct expression patterns that are absent under individual treatments. Consequently, the identification of genes associated with a single stress does not necessarily establish their value for tolerance to multiple simultaneous stresses.

Stress memory and epigenetic regulation provide another possible basis for cross-tolerance. Following an initial stress exposure, certain physiological and molecular changes may persist after the original stress has ended, modifying the response to a later challenge. Chromatin remodeling, histone modifications, DNA methylation, and other regulatory processes may contribute to the persistence or reactivation of stress-responsive gene expression. The duration and heritability of these effects vary, and evidence of persistent changes in one experimental system should not be assumed to demonstrate transgenerational inheritance in another. Research on stress memory should therefore distinguish short-term acclimation, persistent physiological effects, epigenetic associations, and verified inherited changes.

VI. Combined abiotic stress and the changing climate

Climate change affects plant stress exposure through changes in average temperature, heat extremes, rainfall patterns, evaporative demand, and the distribution of soil moisture. These changes can influence not only the intensity of individual stresses but also the probability that multiple stressors occur together. Heatwaves during drought, increasing salinity in coastal agricultural areas, and alternating flooding and water deficit create environmental conditions that differ from those represented by isolated laboratory treatments. Understanding these combinations is essential because plants respond to the interaction among environmental factors rather than to each factor independently. Recent syntheses of combined-stress research emphasize the importance of considering stress intensity, timing, and sequence when evaluating plant acclimation.

Drought and heat provide a clear example of interacting physiological demands. Drought-induced stomatal closure helps conserve water but restricts evaporative cooling and may increase leaf temperature. Heat increases the demand for cooling and can accelerate water loss when stomata remain open. Under simultaneous exposure, the plant must balance water conservation against temperature regulation while maintaining sufficient photosynthetic activity for growth and repair[6]. The outcome depends on the rate of soil drying, atmospheric humidity, radiation, root water uptake, and the plant's ability to adjust stomatal

behavior. Combined drought and heat can consequently produce responses that differ substantially from those predicted by adding the effects of the individual stresses.

Drought and salinity also share important physiological features because both reduce the water potential available to plant roots[7]. However, salinity introduces additional ion toxicity and nutrient imbalance, making the combination more complex than a simple increase in water deficit. Plants may respond through osmotic adjustment, ion compartmentalization, antioxidant regulation, and changes in root growth. Evidence from a meta-analysis of drought and salt stress indicates that the combined responses of many measured traits can be less than additive, consistent with shared protective responses in some plant systems. This does not mean that combined stress is generally harmless or beneficial; it means that interactions depend on the trait, species, and conditions being examined. Climate-resilience research should therefore examine realistic stress scenarios rather than relying entirely on uniform, severe laboratory treatments. Field conditions include fluctuating temperatures, changing soil moisture, variable radiation, heterogeneous soil properties, and differences in stress timing. Plants may experience gradual acclimation, brief extreme events, or repeated exposure separated by recovery periods. Experiments that capture these features can reveal whether cross-tolerance persists under changing conditions and whether physiological benefits translate into stable yield. This approach can help identify genotypes and management strategies that perform reliably across a range of environmental scenarios rather than those that succeed under only one narrowly defined treatment.

VII. Experimental methodology and research design

The proposed experiment is designed to investigate the physiological basis of cross-tolerance under drought, heat, and salinity stress. A suitable model crop, such as wheat, rice, or maize, should be selected according to the research objective and the environmental conditions of interest. For a clearly defined initial experiment, wheat may be used to investigate drought–heat interactions, with a subsequent experiment incorporating salinity. The study should include a non-stressed control, each individual stress treatment, combined treatments, and, where cross-acclimation is being examined, sequential treatments in which a mild initial stress precedes a second stress. The chosen species, genotype, growth stage, stress intensity, and exposure duration must be specified before the experiment begins.

Plants should be grown under controlled conditions with consistent soil composition, pot size, nutrient availability, photoperiod, and initial watering regime. Drought intensity can be defined through soil moisture or fraction of field capacity, while heat exposure can be specified using air temperature, duration, and recovery conditions. Salinity can be imposed through a controlled sodium chloride treatment with appropriate monitoring of substrate electrical conductivity. Stress levels should be selected using preliminary trials or established protocols for the chosen species so that treatments produce measurable responses without causing unintended widespread mortality. Temperature, humidity, light intensity, soil moisture, and other relevant environmental variables should be recorded throughout the experiment to confirm that the intended treatments were maintained.

The experiment should use independent biological replicates and a randomized design that accounts for differences among growth chambers, benches, or experimental blocks. A factorial arrangement can be used to evaluate the main effects of drought, heat, and salinity and their interactions. Where resources permit, multiple genotypes can be included to identify differences in stress responsiveness and potential cross-tolerance. Sample size should be determined using an appropriate power analysis based on the primary outcome and expected variability. Destructive sampling for biochemical or molecular analyses should be planned separately from repeated physiological measurements so that sampling itself does not unintentionally alter subsequent observations.

Measurements should be collected before stress exposure, during treatment, and after recovery. Physiological indicators may include net photosynthetic rate, stomatal conductance, transpiration, relative water content, leaf temperature, chlorophyll fluorescence, and water-use efficiency[5]. Biochemical measurements may include proline, soluble sugars, antioxidant enzyme activities, hydrogen peroxide, malondialdehyde, and electrolyte leakage. Growth and performance indicators should include plant height, leaf area, root and shoot biomass, and, where the experiment continues through maturity, reproductive performance and grain yield. Molecular analysis may examine selected stress-responsive genes or broader transcriptomic profiles. All measurements should be collected using documented procedures, calibrated instruments, appropriate controls, and consistent sampling times.

VIII. Conclusion

Cross-tolerance is an important component of plant resilience because it describes how physiological and molecular responses to one environmental stress can influence the response to another, particularly under the combined drought, heat, salinity, and other abiotic challenges associated with a changing climate. This paper has examined the roles of osmotic adjustment, antioxidant defense, hormonal signaling, membrane stability, stress-responsive gene regulation, and stress memory in shaping plant responses to multiple environmental stressors. It has also presented an experimental framework for comparing individual and combined stress treatments, evaluating sequential acclimation, and determining whether physiological protection translates into improved growth and reproductive performance. Artificial intelligence and machine learning for advanced data analytics can complement these experiments by identifying relationships among physiological traits, molecular profiles, environmental conditions, and crop performance, provided that models are validated independently and interpreted within a sound biological framework. Because the proposed experiment has not been conducted, numerical outcomes and specific claims of cross-tolerance remain to be established through controlled measurements and field validation. Future research should prioritize realistic stress combinations, multiple genotypes, recovery and yield assessments, and the integration of physiological, molecular, genomic, and environmental data. Crop improvement strategies should consider both the benefits and the energetic costs of protective responses, recognizing that tolerance to an individual stress does not automatically guarantee resilience under combined conditions. Ultimately, understanding cross-tolerance through integrated experimentation and advanced analytics can support the identification of reliable resilience mechanisms, strengthen climate-adaptive breeding, and

contribute to sustainable agricultural production under increasingly variable environmental conditions.

References:

- [1] D. Olofintuyi, A. Siddika, A. Monfared, H. Zhang, and J. Smith, "Cross-Tolerance in a Changing Climate: Physiological Responses to Combined Abiotic Stress," *Phyton-International Journal of Experimental Botany*, vol. 95, no. 4, pp. 1-10, 2026.
- [2] R.-Y. Yang *et al.*, "Nutritional and functional properties of moringa leaves-from germplasm to plant, to food, to health," Moringanews, Moringa and Plant Resources Network, 2007.
- [3] Z. Chen *et al.*, "The Potential of Exogenous Melatonin Alleviating Heavy Metal Toxicity in Plants," *Journal of Plant Growth Regulation*, pp. 1-13, 2026.
- [4] Y. Yu *et al.*, "Melatonin confers heavy metal-induced tolerance by alleviating oxidative stress and reducing the heavy metal accumulation in *Exophiala pisciphila*, a dark septate endophyte (DSE)," *BMC microbiology*, vol. 21, no. 1, p. 40, 2021.
- [5] M. N. Hoque *et al.*, "Melatonin modulates plant tolerance to heavy metal stress: morphological responses to molecular mechanisms," *International journal of molecular sciences*, vol. 22, no. 21, p. 11445, 2021.
- [6] M. A. Altaf *et al.*, "Melatonin enhanced the heavy metal-stress tolerance of pepper by mitigating the oxidative damage and reducing the heavy metal accumulation," *Journal of Hazardous Materials*, vol. 454, p. 131468, 2023.
- [7] Z. Chen, A. A. Shah, A. H. A. Razaq, and I. Alhidary, "Dietary supplementation of *Moringa oleifera* leaf extract enhances productivity, carcass yield, gut microbiota and biomarker profiles in broiler chickens," *Austral Journal of Veterinary Sciences*, vol. 58, pp. e5803-e5803, 2026.