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Melatonin-based Seed Treatment for Water-stress Resilience: Physiological Mechanisms, Evidence Quality and Translational Prospects

Heberth Christian Ferreira [a*], Andréia Márcia Santos de Souza David [a], Samy Pimenta [a], Hemilly Kariny Cardoso Freitas [a], João Rafael Prudêncio dos Santos [a], Roberto dos Santos Trindade [b], Lauro José Moreira Guimarães [b] and Arley Figueiredo Portugal [b]

[a] *State University of Montes Claros (Unimontes), Montes Claros, Brazil.*
[b] *Embrapa Maize and Sorghum, Sete Lagoas, Minas Gerais, Brazil.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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Abstract

Water deficit during germination and early establishment restricts imbibition, delays reserve mobilisation and impairs stand formation, making the seed stage an attractive target for low-input stress management. Melatonin-based seed treatment has consequently moved from an experimental curiosity to a widely studied

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**Corresponding author: E-mail: heberth7@hotmail.com;*

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chemical-priming approach. This critical narrative review evaluates the physiological and molecular mechanisms through which melatonin applied before sowing may mitigate water stress, while separating mechanistic plausibility from agronomic proof. Literature published from 1995 to 1 June 2026 was identified through accessible scholarly indexes, citation searching and DOI-level verification. Evidence was appraised according to stress realism, priming controls, dose definition, genotype coverage, developmental duration, mechanistic depth and field relevance. Across cucumber, rapeseed, soybean, safflower, triticale, rice, maize, peanut, wheat, cotton and foxtail millet, melatonin treatment generally improved germination, seedling vigour, membrane stability and antioxidant capacity under polyethylene glycol-induced osmotic stress or restricted water supply. The most reproducible mechanism is redox buffering: melatonin moderates damaging reactive oxygen species accumulation while preserving the signalling functions required for germination, supported by enhanced enzymatic and non-enzymatic antioxidant systems. Osmotic adjustment, reserve mobilisation, root development, stomatal regulation, chloroplast protection and crosstalk with abscisic acid, gibberellins, nitric oxide and sugar metabolism provide additional, but unevenly substantiated, explanations. Emerging evidence implicates phytomelatonin receptor-mediated signalling, methylglyoxal detoxification and autophagy. Confidence in broad agronomic claims remains limited because most studies use short-term laboratory assays, heterogeneous concentrations and polyethylene glycol models that do not reproduce soil–plant–atmosphere drought. Rare field studies indicate possible gains in yield and water productivity, but independent multi-environment validation, treatment standardisation, storage testing, cost analysis and regulatory assessment are inadequate. Melatonin seed treatment is therefore best regarded as a promising, biologically credible priming technology whose practical value depends on crop-specific dose optimisation and evidence extending beyond early seedling biomarkers.

Keywords: Antioxidant defence; chemical priming; drought; germination; osmotic adjustment; phytomelatonin; seedling establishment; water productivity.

1. Introduction

Agricultural drought is experienced by a plant not as a single biochemical insult but as a progressive disturbance of water potential, hydraulic continuity, carbon balance and cellular homeostasis. Germination and early seedling establishment are particularly vulnerable because the emerging axis depends on rapid water uptake, orderly membrane repair, controlled reactive oxygen species production and timely mobilisation of finite seed reserves. A delay at this stage can reduce stand uniformity and constrain later canopy development even when water availability subsequently improves. The biological sensitivity of the seed-to-seedling transition has made seed treatment an attractive point of intervention: relatively small quantities of an active compound can be delivered before sowing, without repeated field application, and the treatment can be integrated with established seed handling operations (Chen & Arora, 2013; Paparella et al., 2015; Savvides et al., 2016).

Melatonin (N-acetyl-5-methoxytryptamine) was initially characterised as an animal indoleamine, but its occurrence in edible plants and diverse plant tissues is now well established (Dubbels et al., 1995; Tan et al., 2012). In plants, the term phytomelatonin is often used to emphasise endogenous biosynthesis and plant-specific functions. Tryptophan-derived pathways operate across the cytoplasm and chloroplasts, and their organisation permits melatonin production close to sites of intense redox activity (Back et al., 2016). The compound has been associated with germination, root development, photosynthetic maintenance, senescence, circadian responses and tolerance to salinity, temperature extremes, heavy metals and water deficit (Arnao & Hernández-Ruiz, 2015; Sun et al., 2021; Wang et al., 2018; Zhang et al., 2015). Whether melatonin should be classified as a conventional plant hormone, a pleiotropic regulator or a redox-active signalling hub remains debated; its effects are concentration-dependent, context-sensitive and intertwined with established hormonal networks (Arnao & Hernández-Ruiz, 2019, 2021).

Seed priming is a controlled hydration treatment that permits early metabolic events of germination while preventing radicle protrusion, followed in many protocols by redrying to restore handling and storage characteristics. Melatonin seed priming adds an exogenous signalling molecule to this hydration window. In practice, the literature uses several overlapping expressions, including seed soaking, pre-treatment, priming and pre-sowing treatment, even when hydration duration, redrying procedure and seed moisture are insufficiently reported. This terminological looseness matters because hydropriming alone can improve germination synchrony, repair membranes and induce stress memory. A convincing melatonin-specific effect therefore requires a water-primed control subjected to the same hydration, temperature, aeration and drying conditions (Paparella et al., 2015; Rajora et al., 2022).

The first influential direct evidence linked melatonin treatment with improved cucumber germination and lateral-root formation under polyethylene glycol-induced water stress, alongside lower oxidative injury and better photosynthetic performance (Zhang et al., 2013). Subsequent studies extended the approach to oilseeds, cereals, legumes and fibre crops, reporting changes in antioxidant enzymes, osmolytes, membrane integrity, stomatal traits, chloroplast ultrastructure, reserve metabolism and stress-responsive gene expression (Guo et al., 2022; Khan et al., 2019; Muhammad et al., 2023; Zhang et al., 2020). The field has expanded rapidly, and recent reviews have catalogued melatonin-associated stress responses or seed-germination pathways (Khattak et al., 2023; Liu et al., 2023; Moustafa-Farag et al., 2020; Rajora et al., 2022; Raza et al., 2026). More recent primary studies have expanded crop and mechanistic coverage: melatonin seed priming improved aquaporin-associated root hydraulic responses in wheat, while rice and common buckwheat studies reported better root development, germination, antioxidant defence and osmotic adjustment under water-deficit or PEG-based conditions (Fu et al., 2024; Tian et al., 2025; Tyagi et al., 2025). Complementary multi-crop evidence under flooding associated melatonin priming with coordinated abscisic acid, gibberellin and reactive oxygen species signalling, although this evidence should not be interpreted as direct proof of drought tolerance (Luo et al., 2024). Yet three interpretive problems remain insufficiently resolved.

First, experimental water stress is heterogeneous. Polyethylene glycol (PEG) lowers solution water potential and is useful for controlled osmotic experiments, but it does not reproduce the spatially variable soil water supply, root–soil contact, evaporative demand and hydraulic feedbacks of field drought (Verslues et al., 2006). Pot studies based on a fixed percentage of field capacity provide greater soil realism but often lack continuous measurements of water potential or transpiration. Field irrigation treatments are agronomically relevant, yet environmental variability complicates mechanistic attribution. Treating these models as interchangeable inflates confidence in translation.

Second, the evidence is dominated by positive, short-duration studies. Germination percentage, seedling length, antioxidant activity and malondialdehyde concentration are useful indicators, but they do not demonstrate that a treatment improves crop yield, water productivity or economic return. Many studies test one genotype, one stress intensity and a narrow concentration range, while the optimal melatonin dose varies from micromolar solutions to milligram-per-litre formulations. High concentrations can delay germination or disturb hormonal and redox balance, so the technology cannot be reduced to a universal recipe.

Third, mechanistic narratives sometimes merge direct radical scavenging, transcriptional regulation, hormonal crosstalk and developmental effects without distinguishing evidence levels. Melatonin can interact chemically with reactive species, but the persistence and intracellular concentration of exogenously supplied melatonin after seed redrying are rarely quantified. Enzyme induction, receptor-associated signalling and altered endogenous metabolism may be more important than sustained direct scavenging. A critical account must therefore separate measured events from inferred causal chains and identify where genetic, pharmacological or flux-based evidence is missing.

1.1 Scope, Research Gap and Objectives

This review focuses on melatonin delivered to seeds before sowing and its capacity to improve germination, establishment and subsequent performance under water deficit. It integrates evidence from laboratory osmotic assays, soil-based drought experiments and field irrigation studies, while distinguishing these stress models explicitly. Foliar and root applications are considered only where they clarify mechanisms that may operate after seed treatment. The central gap is the absence of an integrated appraisal linking treatment protocol, stress realism, mechanistic confidence and agronomic readiness. The objectives are to define the biological basis of melatonin seed treatment, compare crop-specific evidence, evaluate proposed physiological and molecular mechanisms, explain heterogeneous or conflicting responses, and identify the experiments required for reliable field translation. Salinity, heavy-metal toxicity and temperature stress are excluded as primary outcomes, although selected studies are used when they provide direct mechanistic evidence relevant to germination or melatonin signalling.

2. Methods for Literature Selection

2.1 Review Design and Rationale

A critical narrative review was selected because the evidence comprises laboratory germination assays, pot experiments, multi-season field trials, physiological studies, proteomic analyses and mechanistic work conducted with different species, doses and stress definitions. These designs are not sufficiently homogeneous for defensible quantitative pooling, and a narrowly systematic question would not capture the required integration of seed physiology, redox signalling, water relations and agronomic implementation. Narrative synthesis nevertheless requires explicit searching, transparent selection and critical appraisal. The structure and quality checks were informed by the Scale for the Assessment of Narrative Review Articles, particularly its emphasis on the importance of the topic, clear aims, a described literature search, appropriate referencing, scientific reasoning and presentation of relevant endpoint data (Baethge et al., 2019).

2.2 Information Sources

Searches were conducted in PubMed/MEDLINE, PubMed Central, the Directory of Open Access Journals, AGRIS and Crossref metadata records. Targeted scholarly web searches were used to identify recent articles and accessible journal records, and bibliographic details were confirmed against DOI landing pages, journal records or PubMed entries. Backward citation searching was performed from recent reviews and key primary studies, and forward citation and related-article searching were used to locate subsequent crop-specific investigations. Searches were also made for corrections, expressions of concern and retractions associated with candidate references. Subscription-only databases were not represented as comprehensively searched because complete exports were not available.

2.3 Search Strategy and Search Period

The principal search concepts were melatonin or phytomelatonin; seed priming, seed treatment, seed soaking or pre-sowing treatment; drought, water deficit, limited irrigation, osmotic stress or PEG; and outcomes related to germination, seedling establishment, antioxidants, reactive oxygen species, osmolytes, hormones, roots, stomata, photosynthesis, yield and water productivity. A representative search string was: (melatonin OR phytomelatonin) AND (seed priming OR seed treatment OR seed soaking OR presowing) AND (drought OR water deficit OR limited irrigation OR osmotic stress OR polyethylene glycol) AND (germination OR seedling OR antioxidant OR root OR photosynthesis OR yield). Mechanistic searches combined melatonin and seed germination with abscisic acid, gibberellin, nitric oxide, reactive oxygen species, methylglyoxal, autophagy, sugar metabolism, energy metabolism, chloroplast or PMTR1. The principal period was January 1995 to 1 June 2026. Earlier methodological literature could be considered when necessary, although no earlier source was required in the final synthesis.

2.4 Eligibility Criteria

Eligible publications were peer-reviewed articles or methodologically relevant reviews in English that examined melatonin applied to seeds, quantified responses under water deficit, or provided direct mechanistic evidence needed to interpret seed-treatment effects. Primary studies were prioritised when they included an untreated control, a hydroprimed control where feasible, a defined melatonin dose, a defined stress treatment and measurable germination, physiological, biochemical, molecular or agronomic outcomes. Studies using PEG were retained but classified as osmotic-stress experiments rather than direct replicas of field drought. Soil-based pot and field experiments were evaluated separately. Reviews were used to establish conceptual development and locate primary evidence, not as substitutes for direct data. Publications were excluded when bibliographic identity could not be verified, the treatment route was irrelevant without mechanistic value, the stress exposure was not water-related, only a conference abstract or preprint was available, or the outlet raised unresolved credibility concerns.

2.5 Screening, Duplicate Handling and Study Selection

Titles and abstracts were first evaluated for direct relevance to melatonin, seeds and water stress. Full texts or sufficiently detailed scholarly records were then assessed for treatment conditions, species, stress model, endpoints and claim relevance. Duplicate records were identified through DOI, title, author and year matching. Where multiple records described the same article, the version of record was used. Inaccessible papers were not used for detailed numerical claims unless an authoritative abstract and complete metadata were available. No record counts are reported because the process was designed for critical narrative selection rather than a prospectively registered systematic review. English-language restriction and unequal full-text availability were considered during appraisal.

2.6 Critical Appraisal and Evidence Synthesis

Evidence was appraised according to biological replication, genotype coverage, treatment and stress controls, dose range, seed hydration and redrying details, measurement validity, stress realism, duration, mechanistic specificity and agronomic relevance. Particular attention was given to whether hydropriming was separated from melatonin-specific effects, whether PEG concentration was translated into measured water potential, and whether conclusions extended beyond the developmental stage observed. Mechanistic confidence was graded qualitatively by triangulating biochemical, physiological, ultrastructural, transcriptomic, proteomic and genetic evidence. Themes were developed around redox regulation, osmotic adjustment, hormonal and metabolic crosstalk, root and stomatal responses, organelle protection, dose contingency and field translation. Disagreement among studies was interpreted in relation to crop, genotype, seed quality, treatment protocol, stress intensity and endpoint timing rather than being treated as simple inconsistency.

3. Conceptual Basis: Seed Priming, Phytomelatonin and Water-Stress Biology

Water stress during germination reduces the gradient driving imbibition and prolongs the interval in which membranes remain incompletely reorganised. This delay can increase solute leakage and disrupt the coordinated activation of respiration, DNA repair, protein synthesis and reserve mobilisation. Germination also requires a controlled oxidative signal. Reactive oxygen species (ROS) promote endosperm weakening, cell-wall loosening and hormonal transitions within a physiological ‘oxidative window’, whereas excessive ROS oxidise lipids, proteins and nucleic acids. A useful priming treatment should therefore improve redox control rather than simply eliminate ROS. The distinction is central to melatonin biology because both direct chemical scavenging and regulation of enzymatic defences have been proposed (Paparella et al., 2015; Pardo-Hernández et al., 2020).

Early presowing work in red cabbage showed that melatonin could protect seedlings against copper toxicity, helping establish the feasibility of delivering the compound through seeds before the drought-focused literature developed (Posmyk et al., 2008).

Hydration priming itself initiates repair and can leave a molecular memory after redrying. Such memory may involve persistent proteins, metabolites, altered organelle status and chromatin-associated regulation, but the duration and causal basis vary among species and treatments (Chen & Arora, 2013). Melatonin may strengthen this process by acting during the hydration window, when membranes, mitochondria and signalling networks are reactivated. Yet evidence that melatonin seed treatment creates a distinct epigenetic memory is sparse. Most studies infer memory from improved performance after sowing rather than measuring stable chromatin modifications or demonstrating persistence across developmental transitions. ‘Priming memory’ should therefore be used as a functional description unless molecular persistence is directly shown.

Phytomelatonin has several properties that make it a plausible priming agent. It is amphiphilic, can access multiple cellular compartments and is linked to antioxidant metabolism, hormone homeostasis and organelle function (Arnao & Hernández-Ruiz, 2015; Tan et al., 2012). Its biosynthesis can occur in chloroplast-associated and cytoplasmic routes, allowing endogenous production to respond to metabolic state (Back et al., 2016). Exogenous melatonin may raise tissue melatonin transiently, induce endogenous biosynthetic genes or initiate downstream signalling. These routes are not equivalent. Direct scavenging requires sufficient local concentrations at the time and site of ROS generation; transcriptional and receptor-associated mechanisms can, in principle, amplify a lower external signal. Few seed-priming studies measure melatonin uptake, metabolites and endogenous synthesis together, leaving the dominant route unresolved.

The reported phytomelatonin receptor 1 (PMTR1/CAND2) pathway offers a signalling explanation for responses at concentrations below those expected to dominate through stoichiometric radical scavenging. In *Arabidopsis thaliana*, PMTR1-associated signalling contributes to stomatal closure and involves hydrogen peroxide, calcium and downstream nitric oxide (Wei et al., 2018; Wang et al., 2023). This evidence is mechanistically important, but it arises mainly from vegetative tissues and model-plant genetics. Demonstrating that the same receptor system is activated during imbibition, survives redrying and controls later drought performance in crops remains a priority. Until then, receptor-mediated explanations for seed priming are plausible extrapolations rather than universally established mechanisms.

Water-stress terminology also determines interpretation. PEG solutions are valuable for imposing reproducible external osmotic potentials in germination dishes or hydroponic systems. Nevertheless, high-molecular-weight PEG can alter oxygen diffusion, viscosity and nutrient movement, and its effects depend on molecular weight, concentration and temperature. Soil drying adds mechanical resistance, heterogeneous water distribution and changing root–soil contact. Limited irrigation in the field includes atmospheric demand, rooting depth, phenological timing and recovery between drying cycles. Table 1 summarises the conceptual distinctions needed to prevent evidence from one model being uncritically generalised to another.

Table 1. Conceptual distinctions required when interpreting melatonin seed-treatment studies under water stress

Concept	Operational definition	What it can establish	Main interpretive limitation	Supporting references
Hydropriming	Controlled seed hydration without melatonin, with otherwise matched soaking and redrying.	The benefit attributable to hydration, repair and priming memory.	If omitted, melatonin-specific effects cannot be separated from hydration effects.	Paparella et al. (2015); Chen and Arora (2013)
Melatonin seed priming	Hydration in a defined melatonin solution, ideally followed by controlled redrying.	Whether melatonin adds benefit beyond hydration and modifies later stress responses.	Nominal solution concentration is not an absorbed dose; protocols vary widely.	Rajora et al. (2022); Raza et al. (2026)
PEG-induced osmotic stress	A solution with reduced external water potential, commonly using PEG-6000.	Controlled germination and seedling responses to low water potential.	Does not reproduce soil structure, root–soil hydraulics, evaporative demand or drought recovery.	Verslues et al. (2006); Zhang et al. (2013)
Soil drying or reduced field capacity	Water deficit imposed in pots by withholding irrigation or maintaining a target soil-water content.	Responses involving roots, soil contact and whole-plant water status.	Field-capacity percentages may not specify water potential; pot volume constrains roots.	Shaheen et al. (2024); de Camargo Santos et al. (2024)
Limited irrigation in the field	Water supply manipulated within an agronomic production environment.	Emergence, rooting, yield, quality and water-productivity consequences.	Environmental covariation complicates mechanism; evidence is crop- and site-specific.	Heshmati et al. (2021); Dai et al. (2026)
Functional priming memory	Improved performance after the treatment has ended and seeds have been redried or plants have developed.	Persistence of a treatment-associated phenotype.	Does not demonstrate epigenetic inheritance or a stable molecular memory without direct evidence.	Chen and Arora (2013); Liu et al. (2023)
Drought avoidance versus tolerance	Avoidance limits tissue dehydration through roots or stomata; tolerance limits injury at a given water status.	The physiological strategy underlying improved performance.	Growth, conductance or osmolyte data alone seldom distinguish the two strategies.	Verslues et al. (2006); de Camargo Santos et al. (2024)

Note: PEG, polyethylene glycol. The categories are complementary rather than interchangeable, and inference should remain proportional to stress realism and measured outcomes

The distinctions in Table 1 imply a hierarchy of inference. Laboratory PEG studies can establish whether melatonin alters germination under low external water potential and can support mechanistic sampling under controlled conditions. Soil experiments strengthen ecological relevance, especially when water content, water potential and plant water status are measured. Field trials are required to establish yield and water-productivity value, but they are not automatically mechanistically superior because environmental covariation and management interactions can obscure causal pathways. A strong translational evidence base therefore requires linked experiments across these levels rather than replacing one model with another.

4. Evidence Across Crops and Experimental Systems

4.1 Foundational Evidence in Cucumber and the Expansion of the Field

The 2013 cucumber study remains foundational because it connected seed treatment, germination, root architecture, oxidative metabolism and later seedling performance under PEG-imposed stress (Zhang et al., 2013). Melatonin increased germination and seedling growth, promoted lateral roots, strengthened antioxidant enzyme activities and reduced indicators of oxidative injury. Chloroplast structure and photosynthetic traits were also better maintained after the early treatment. The study provided a coherent physiological narrative and identified an intermediate dose as more effective than lower or higher concentrations, establishing the now familiar principle that melatonin responses are non-linear.

Its influence also created a template that later studies frequently reproduced: seeds are soaked in several melatonin concentrations, exposed to PEG during germination, and assessed through germination percentage, shoot and root length, fresh or dry mass, ROS-related markers and antioxidant enzymes. This design is efficient for screening, but its repeated use can create an illusion of independent mechanistic confirmation. Similar endpoints often derive from the same causal neighbourhood and do not distinguish whether antioxidant changes are primary signals, secondary consequences of improved hydration or generic markers of less injury. The cucumber work is therefore strongest as proof of biological activity and hypothesis generation, not as a complete explanation of field drought tolerance.

Related cucumber studies under salinity clarified two mechanisms relevant to germination. Melatonin altered the balance between abscisic acid (ABA) and gibberellin, including gibberellin A4, and strengthened antioxidant systems during germination (Zhang et al., 2014). Proteomic analysis subsequently implicated energy production, carbohydrate metabolism and protein synthesis in the melatonin response (Zhang et al., 2017). Because these studies used salinity rather than water deficit, they cannot be treated as direct drought evidence. They nevertheless demonstrate that melatonin can affect the hormonal and energetic gates of germination, mechanisms likely to matter when water uptake is restricted.

4.2 Oilseeds and Legumes

Rapeseed has one of the more developed experimental literatures. Under PEG-induced stress, melatonin-primed seeds produced seedlings with improved antioxidant defence, osmotic adjustment, stomatal characteristics and chloroplast ultrastructure (Khan et al., 2019). The breadth of endpoints was a strength because biochemical changes were linked with anatomical and photosynthetic preservation. Yet the central stress model remained osmotic rather than soil hydraulic drought, and the number of genotypes and environments was limited. A later rapeseed study compared melatonin and gibberellic acid seed priming under drought and non-stress conditions and reported improvements in yield and seed quality, providing a bridge towards agronomic outcomes (Khan et al., 2020). Interpretation requires attention to treatment separation: effects of melatonin, gibberellic acid and their possible interaction should not be collapsed into a single priming effect.

Safflower offers an instructive example of biological contingency. Field-oriented work found that melatonin seed priming altered antioxidant enzymes and biochemical responses under drought, but performance depended on seed quality or age and not every stress marker shifted in the same direction (Heshmati et al., 2021). This challenges the assumption that a larger increase in every antioxidant enzyme or osmolyte is necessarily favourable. Proline, for example, may accumulate because stress is severe or decline because injury is mitigated; its direction must be interpreted together with water status, growth and damage indicators. The safflower evidence also indicates that seed lot history can change the apparent optimal treatment.

Soybean studies support improved germination and membrane protection under osmotic stress. Melatonin reduced the inhibitory effects of PEG during germination, enhanced antioxidant properties and preserved cellular ultrastructure (Zhang et al., 2020). A broader abiotic-stress experiment found improved soybean germination and physiological performance after melatonin pre-treatment, with responses varying by stress type and dose (Awan et al., 2023). Whole-plant work using exogenous melatonin, although not exclusively seed-based, also demonstrates enhanced soybean growth and antioxidant defence under drought and supports the persistence of redox and photosynthetic effects beyond germination (Imran et al., 2021). These studies provide convergent biological evidence, but direct comparisons are constrained by differences in cultivar, treatment route, stress intensity and developmental stage.

Peanut research has strengthened the link between seed priming and whole-plant water relations. In two cultivars, melatonin priming improved early establishment and altered water-stress responses, including biomass, gas exchange and whole-plant physiological behaviour (de Camargo Santos et al., 2024). Importantly, the drought-sensitive cultivar did not simply maintain maximum gas exchange; melatonin appeared to support a more responsive down-regulation under stress, which may conserve water. This finding cautions against treating high stomatal conductance or photosynthetic rate as universally beneficial during drought. Tolerance can involve sustained carbon assimilation, rapid stomatal protection, deeper rooting or combinations that are advantageous only in a particular drought pattern.

4.3 Cereals, Grasses and Fibre Crops

Cereal studies show broad responsiveness but also considerable protocol variation. Triticale seeds primed with melatonin germinated more successfully under PEG-6000, and seedlings displayed improved growth, osmolyte status and antioxidant capacity (Guo et al., 2022). Rice work likewise reported enhanced germination and antioxidant capacity under PEG-imposed drought (Zhang et al., 2023). In maize, melatonin priming improved seedling drought tolerance through antioxidant defence and associated molecular regulation (Muhammad et al., 2023). A multi-genotype comparison published in 2026 found both melatonin and gibberellic acid effective under PEG stress, with melatonin performance varying among genotypes and an intermediate concentration generally favourable (Kumari et al., 2026). The multi-genotype design is valuable because it demonstrates that treatment ranking is partly genetic rather than purely methodological.

Bread wheat has been examined in controlled soil water deficit. Melatonin priming improved growth, water-status indicators, osmolytes, antioxidant activities and damage markers in two cultivars subjected to reduced field capacity (Shaheen et al., 2024). The study moved beyond a germination-dish model, but the short pot duration and limited genetic breadth still restrict yield-level inference. In foxtail millet, melatonin improved germination and seedling physiological characteristics under PEG-associated stress, with concentration-dependent responses (Meng et al., 2025). These findings extend the taxonomic range, although each additional positive species does not by itself resolve whether a common dose, uptake pathway or field benefit exists.

Cotton research has added mechanistic depth. During PEG-induced germination stress, melatonin influenced methylglyoxal homeostasis, the glyoxalase system and autophagy, linking redox protection with detoxification of a reactive carbonyl and organelle-quality control (Dake et al., 2025). This expands the field beyond conventional measurements of superoxide dismutase, catalase and malondialdehyde. The evidence is compelling for the experimental system, but methylglyoxal and autophagy pathways require genetic or pharmacological perturbation across additional crops before they can be treated as universal mediators of seed-priming benefits.

The strongest direct evidence for agronomic translation is a multi-season winter-wheat field study under limited irrigation. Melatonin-primed seed improved seedling quality, root development, later extraction of deep soil water, yield and water productivity compared with non-primed seed, with advantages also reported over hydropriming for several outcomes (Dai et al., 2026). The study's field scale, repeated seasons and water-productivity endpoint address major weaknesses in the laboratory literature. Nevertheless, it represents one crop-production region and management system; independent replication across soil types, varieties, sowing dates and drought patterns is necessary before the effect size is considered generalisable.

Table 2 compares representative primary studies. Its principal message is not that every crop responds identically, but that positive effects recur across distinct taxa while the level of inference differs sharply. PEG-germination assays dominate numerically, soil-based studies are fewer, and yield or water-productivity evidence remains exceptional.

Table 2. Representative primary evidence for melatonin seed treatment under water-related stress

Crop	Treatment	Stress model	Principal reported outcomes	Critical limitation	Supporting references
Cucumber (*Cucumis sativus*)	Concentration series; intermediate dose most effective	PEG-imposed water stress	Improved germination, lateral roots, antioxidant activity, chloroplast integrity and photosynthetic performance.	Foundational but laboratory-centred; later agronomic persistence was not tested.	Zhang et al. (2013)
Rapeseed (*Brassica napus*)	Melatonin seed priming	PEG-induced stress	Improved ROS detoxification, osmotic adjustment, stomatal traits and chloroplast ultrastructure.	Limited genotype/environment coverage; several endpoints are correlated biomarkers.	Khan et al. (2019)
Rapeseed	Melatonin and gibberellic-acid priming	Drought and non-stress conditions	Improved yield-related traits and seed quality.	Treatment-specific and interaction effects require careful separation.	Khan et al. (2020)
Soybean (*Glycine max*)	Melatonin pre-treatment	PEG-induced osmotic stress	Higher germination, stronger antioxidants and better cellular ultrastructure.	Short germination-stage assessment; no yield evidence.	Zhang et al. (2020)
Safflower (*Carthamus tinctorius*)	Melatonin priming of fresh and old seed	Field drought conditions	Lower lipid peroxidation and altered antioxidant and biochemical responses.	Responses depended on seed quality; not every osmolyte or pigment favoured melatonin.	Heshmati et al. (2021)
Triticale (× *Triticosecale*)	Melatonin priming	PEG-6000	Improved germination, seedling growth, osmolytes and antioxidant capacity.	Laboratory stress does not establish soil or harvest-scale benefit.	Guo et al. (2022)
Maize (*Zea mays*)	Melatonin priming	Controlled drought treatment	Improved seedling performance and antioxidant-defence regulation.	Early-stage evidence; cross-environment persistence remains uncertain.	Muhammad et al. (2023)
Rice (*Oryza sativa*)	Melatonin treatment	PEG-induced drought	Improved germination and antioxidant capacity.	Mechanistic attribution relies mainly on redox biomarkers.	Zhang et al. (2023)
Peanut (*Arachis hypogaea*)	50 µM seed priming in two cultivars	Whole-plant water stress	Improved establishment and cultivar-dependent regulation of biomass, gas exchange and water use.	Only two cultivars; yield and multi-season performance were not established.	de Camargo Santos et al. (2024)
Bread wheat (*Triticum aestivum*)	1–5 mg L−1; 16 h	35% field capacity in pots	Improved growth, water-status traits, osmolytes, antioxidants and damage markers.	Short pot experiment; reported optimum needs independent field validation.	Shaheen et al. (2024)
Upland cotton (*Gossypium hirsutum*)	Melatonin priming	PEG-induced germination stress	Regulation of methylglyoxal detoxification, redox status and autophagy.	Advanced mechanism shown in two varieties, but causal perturbation is limited.	Dake et al. (2025)
Maize	Melatonin versus gibberellic acid across ten genotypes	PEG-induced drought	Genotype-dependent improvements in germination and biochemical traits; intermediate melatonin dose favoured.	Controlled assay; treatment ranking may change under soil drought.	Kumari et al. (2026)
Winter wheat	Melatonin priming compared with non-primed and hydroprimed seed	Multi-season field limited irrigation	Improved seedling quality, deep rooting, yield and water productivity.	Single production region and management system; independent replication is needed.	Dai et al. (2026)

Note: ROS, reactive oxygen species; PEG, polyethylene glycol. The table is representative, not exhaustive. Stress models and developmental endpoints should not be treated as equivalent

5. Physiological and Molecular Mechanisms

5.1 Redox Buffering Rather Than Simple ROS Elimination

The most consistent mechanistic signature of melatonin seed treatment is improved redox control. Water deficit restricts carbon metabolism and electron transport, increases the probability of electron leakage and can elevate superoxide, hydrogen peroxide and hydroxyl-radical formation. During germination, excessive ROS accelerates lipid peroxidation before membranes and antioxidant systems are fully reorganised. Across cucumber, rapeseed, soybean, safflower, triticale, rice, maize, wheat and millet, melatonin treatment commonly increases the activity or expression of superoxide dismutase, catalase, peroxidases and enzymes of the ascorbate–glutathione cycle while lowering hydrogen peroxide, superoxide or malondialdehyde accumulation (Guo et al., 2022; Heshmati et al., 2021; Khan et al., 2019; Muhammad et al., 2023; Shaheen et al., 2024; Zhang et al., 2013, 2020, 2023). The convergence across species makes this the best-supported mechanism.

The interpretation nevertheless requires precision. ROS are required for seed signalling, pathogen defence and stomatal responses. A treatment that suppresses them indiscriminately could inhibit germination and acclimation. The evidence is more consistent with redox buffering: melatonin expands antioxidant capacity and constrains damaging peaks while preserving spatially and temporally useful ROS signals. This interpretation also explains why intermediate doses often outperform high doses. Excessive antioxidant capacity or altered ROS generation may move the seed outside its physiological oxidative window, slowing endosperm weakening and embryo growth.

Direct chemical scavenging is biologically possible because melatonin and several metabolites can neutralise reactive species, and the reaction cascade may produce additional antioxidant metabolites (Tan et al., 2012). Yet seed-priming studies rarely quantify intracellular melatonin at the subcellular sites of ROS production over time. Treatment solutions in the micromolar range do not establish that sufficient unchanged melatonin persists after washing, redrying and germination to dominate ROS removal stoichiometrically. Enzymatic induction, maintenance of electron transport, improved NADPH supply and stimulation of endogenous melatonin synthesis may contribute more strongly. Claims that direct scavenging is the principal mechanism should therefore be reserved for studies combining uptake kinetics, metabolite profiling and compartment-specific redox measurements.

The ascorbate–glutathione cycle provides a more mechanistically informative endpoint than a single antioxidant enzyme. Ascorbate peroxidase reduces hydrogen peroxide using ascorbate, while monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase regenerate reducing pools. Whole-plant drought research and reviews indicate that melatonin can support this cycle and maintain the ratio of reduced to oxidised antioxidants (Moustafa-Farag et al., 2020; Pardo-Hernández et al., 2020). Seed studies increasingly measure glutathione, ascorbate and related enzymes, but few calculate flux or distinguish whether larger pools reflect synthesis, recycling or slower consumption. Future mechanistic claims would be strengthened by isotope tracing, redox biosensors and simultaneous measurements of pyridine nucleotides.

5.2 Reactive Nitrogen Species and Redox Signal Integration

Nitric oxide (NO) interacts with ROS to regulate germination, stomata, antioxidant genes and protein function through reactions such as S-nitrosylation and tyrosine nitration. Melatonin treatment of drought-stressed alfalfa plants modulated nitro-oxidative homeostasis and proline metabolism, supporting a role for integrated ROS–reactive nitrogen species control (Antoniou et al., 2017). In *A. thaliana*, NO acts downstream of ROS in PMTR1-mediated stomatal closure (Wang et al., 2023). These studies show that the melatonin response cannot be represented as a linear antioxidant pathway; hydrogen peroxide may function upstream as a signal that promotes NO production and guard-cell closure.

Direct seed-priming evidence for this sequence remains limited. Measurements of bulk NO content or nitrate reductase activity cannot establish which cells generate NO, whether it is beneficial or damaging, and how long the signal persists. Germinating seeds contain steep oxygen gradients, and NO can arise through several enzymatic and non-enzymatic routes. A rigorous model should therefore distinguish early imbibitional signalling from later leaf stomatal regulation. Genetically encoded ROS and NO probes, combined with receptor or nitrate-reductase mutants, would permit temporal ordering and test whether the pathway identified in *Arabidopsis* leaves is conserved in crop seeds.

5.3 Osmotic Adjustment, Water Relations and the Ambiguity of Compatible Solutes

Melatonin-primed seedlings often accumulate more proline, soluble sugars, soluble proteins or other osmolytes under water stress, and these changes are interpreted as improved osmotic adjustment. Compatible solutes can lower cellular osmotic potential, stabilise proteins and membranes, buffer redox reactions and provide carbon or nitrogen during recovery. Rapeseed, triticale, wheat and other crops show melatonin-associated changes in these pools under stress (Guo et al., 2022; Khan et al., 2019; Shaheen et al., 2024). Better relative water content and reduced electrolyte leakage frequently accompany the biochemical shifts.

The causal interpretation is weaker than the correlation. Osmolyte concentration can increase because biosynthesis is induced, because growth is inhibited and solutes become concentrated, or because stress injury causes metabolic accumulation. Conversely, a successfully protected plant may contain less proline than an untreated stressed plant because it experiences lower cellular stress. The direction of proline response has varied among crop, dose and seed-lot conditions, including safflower (Heshmati et al., 2021). Proline should not therefore be treated as a universal positive biomarker. Evidence for genuine osmotic adjustment requires measurements of osmotic potential at full turgor, pressure–volume relations, solute contribution and water-status recovery, not concentration alone.

Root system modification can influence water relations more directly. The cucumber work showed increased lateral-root formation after seed treatment, and rice priming under non-stress conditions has also demonstrated rhizogenic effects across genotypes (Tyagi et al., 2023; Zhang et al., 2013). Field wheat results linked melatonin priming with longer roots and greater use of water from deeper soil layers (Dai et al., 2026). These findings suggest that melatonin can shift drought response from purely cellular tolerance towards resource acquisition. Nevertheless, larger root systems also require carbon and may accelerate soil-water depletion in terminal drought. Root depth, distribution and hydraulic conductance are more informative than total length alone, and their value depends on rainfall timing and soil profile.

5.4 Hormonal Crosstalk During Germination

The ABA–gibberellin balance is a central regulator of dormancy and germination. ABA supports dormancy and stress restraint, while gibberellins promote reserve mobilisation, embryo growth and weakening of surrounding tissues. Under osmotic stress, elevated ABA and reduced gibberellin activity can delay or prevent germination. Cucumber research under salinity showed that melatonin modified ABA and gibberellin A4 metabolism, accompanied by improved germination and antioxidant protection (Zhang et al., 2014). Reviews of seed germination and plant hormone regulation place this interaction within a broader network that includes auxin, ethylene, jasmonates, salicylic acid and brassinosteroids (Arnao & Hernández-Ruiz, 2021; Liu et al., 2023).

For water-stressed seeds, the hypothesis is that melatonin prevents an excessive ABA-dominated state while preserving the protective value of ABA. This is more credible than a simple model in which melatonin always lowers ABA. During later drought, ABA-mediated stomatal closure is essential for water conservation, and melatonin-associated stomatal responses may cooperate with, rather than oppose, ABA signalling. Temporal phase matters: reducing ABA restraint during imbibition could support germination, whereas maintaining or sensitising ABA responses in leaves could protect water status. Most studies sample hormones at one or two time points and therefore cannot resolve this stage-dependent switch.

Comparisons with gibberellic acid priming are informative. Rapeseed and maize studies indicate that both gibberellic acid and melatonin can improve germination or performance under drought-related treatments, but their relative effectiveness differs by genotype and endpoint (Khan et al., 2020; Kumari et al., 2026). Gibberellic acid acts more directly on germination promotion, whereas melatonin may combine developmental and redox effects. A factorial design measuring endogenous hormones, biosynthetic and catabolic gene expression, and inhibitor responses would establish whether melatonin acts upstream of gibberellin, in parallel, or through improved metabolic capacity that permits the embryo to respond.

5.5 Reserve Mobilisation, Energy Metabolism and Sugar Signalling

Germination under low water potential is energetically constrained. Mitochondria must resume respiration, stored starch and lipids must be mobilised, and the embryo must allocate carbon between maintenance, osmotic protection and growth. Proteomic analysis of cucumber under salinity implicated glycolysis, the tricarboxylic acid cycle, ATP production and protein synthesis in melatonin-promoted germination (Zhang et al., 2017). In maize seedlings, melatonin has been linked to altered sugar metabolism, including changes in sucrose-related enzymes and carbon allocation (Zhao et al., 2015). Although these studies do not reproduce seed priming under soil drought exactly, they provide a plausible metabolic basis for faster germination after treatment.

Improved energy metabolism could be both cause and consequence of reduced oxidative damage. Better-preserved mitochondria produce ATP more efficiently and leak fewer electrons; adequate ATP supports antioxidant recycling, ion transport, repair and biosynthesis. Melatonin may also alter sugar signalling through hexokinase, sucrose metabolism or trehalose-related pathways, thereby integrating carbon availability with hormonal control. Most direct seed studies, however, rely on total soluble sugar or amylase activity. These endpoints cannot reveal pathway flux, mitochondrial coupling or whether carbon is being consumed for growth rather than merely accumulated.

Metabolomics and isotope tracing are needed to test the metabolic model. Time-resolved measurements from dry seed through early imbibition, radicle protrusion and seedling establishment should distinguish mobilisation of seed reserves from photosynthetically derived carbon. Oxygen-consumption measurements and high-resolution respirometry could determine whether melatonin accelerates mitochondrial activation or improves coupling efficiency. Such analyses would also clarify why high doses sometimes inhibit germination: excessive signalling may divert carbon towards defence or disrupt the ROS signals that normally coordinate reserve use.

5.6 Membrane, Chloroplast and Mitochondrial Protection

Reduced electrolyte leakage and malondialdehyde are among the most common outcomes after melatonin priming. These markers are consistent with less lipid peroxidation and better membrane integrity, and ultrastructural studies in cucumber, rapeseed and soybean show improved preservation of chloroplasts or cellular organisation (Khan et al., 2019; Zhang et al., 2013, 2020). Because membranes are repaired during imbibition, protection at the seed stage could improve solute retention and enzyme compartmentation. Later preservation of thylakoid structure and photosynthetic pigments may support carbon assimilation during water deficit.

Ultrastructure is persuasive but potentially selective. Electron micrographs often represent a small number of cells, and image selection or scoring methods are not always blinded. Chlorophyll concentration can also rise because leaves are smaller or because degradation is delayed, without necessarily increasing canopy carbon gain. Stronger evidence combines structural measurements with chlorophyll fluorescence, gas exchange, leaf area, biomass and, ideally, canopy-scale assimilation. Peanut and field-wheat studies move in this direction by linking physiological regulation with whole-plant water use or yield (Dai et al., 2026; de Camargo Santos et al., 2024).

Mitochondrial protection is less directly documented in seed-priming drought studies than chloroplast protection, despite its likely importance before photosynthesis begins. Melatonin biosynthesis and metabolism are connected with organelles, and general plant research supports roles in maintaining electron transport and limiting oxidative damage (Back et al., 2016; Wang et al., 2018). Seed-specific investigations should quantify mitochondrial number, membrane potential, respiration and mitophagy. Without such measurements, statements that melatonin preserves mitochondria remain mechanistically plausible but less directly demonstrated than antioxidant enzyme induction.

5.7 Stomatal Regulation, Photosynthesis and Drought Strategy

Stomatal behaviour illustrates why a single ‘higher is better’ interpretation is inadequate. Under mild or intermittent stress, maintaining stomatal conductance and photosynthesis can sustain growth. Under severe or terminal stress, rapid closure may preserve water and improve survival. PMTR1-mediated signalling in *A. thaliana* connects melatonin perception with ROS, NO and stomatal closure (Wei et al., 2018; Wang et al., 2023). Rapeseed and peanut studies indicate that seed priming can alter stomatal traits or gas-exchange responses later in development (de Camargo Santos et al., 2024; Khan et al., 2019).

The agronomic value of these responses depends on drought scenario. A treatment that delays closure may be beneficial where water is replenished frequently but detrimental where soil moisture declines irreversibly. Conversely, stronger closure can conserve water but reduce carbon gain and yield if applied too early. Measurements at a single time point cannot distinguish improved regulation from a constitutively altered set point. Continuous gas exchange, thermal imaging, whole-plant transpiration and recovery measurements are required to identify whether melatonin improves dynamic responsiveness, water-use efficiency or merely reduces plant size.

Photosynthetic protection is often attributed to higher pigment content, better chloroplast structure, increased photochemical efficiency and reduced oxidative damage. These outcomes are supported across several crops, but most are measured in young plants. Yield depends on canopy development, source–sink balance, phenology and reproductive resilience, processes that may not follow from seedling chlorophyll. The winter-wheat field study is important precisely because it linked early seedling quality and root development to later dry-matter accumulation, yield and water productivity (Dai et al., 2026). Replication of this developmental chain is required before photosynthetic biomarkers can be considered reliable surrogates for agronomic benefit.

5.8 Methylglyoxal Detoxification and Autophagy as Emerging Mechanisms

Methylglyoxal is a reactive dicarbonyl produced largely from glycolytic intermediates. It accumulates under stress and can glycatively damage proteins and nucleic acids. The glyoxalase system, using glutathione, detoxifies methylglyoxal to D-lactate. In upland cotton, melatonin treatment during PEG-imposed germination stress improved methylglyoxal homeostasis and was associated with autophagy regulation (Dake et al., 2025). This finding is significant because it links carbonyl stress, glutathione metabolism and cellular recycling rather than treating oxidative damage in isolation.

Autophagy can remove damaged proteins and organelles and recycle nutrients during germination and stress. Yet greater autophagic activity is not automatically protective; excessive or prolonged autophagy can accompany severe injury. Measurements of autophagy-related transcripts or autophagosome abundance require flux controls to distinguish increased formation from blocked degradation. The cotton study provides an advanced mechanistic lead, but cross-species validation and perturbation of glyoxalase or autophagy components are needed. A causal test would determine whether melatonin loses efficacy when methylglyoxal detoxification or autophagic flux is impaired.

Table 3 integrates the proposed mechanisms and assigns qualitative confidence according to the type and breadth of supporting evidence. Redox buffering and membrane protection have the widest direct support. Osmotic adjustment, hormonal crosstalk, energy metabolism, receptor-mediated signalling and autophagy are biologically coherent but vary in directness and crop validation.

Table 3. Proposed mechanisms of melatonin seed treatment and confidence in current evidence

Mechanism	Evidence directly relevant to seed treatment	Current confidence	Principal caveat	Supporting references
Redox buffering	Repeated increases in antioxidant enzymes and reduced ROS, lipid peroxidation or electrolyte leakage across many crops.	High for association; moderate for causal order	Direct scavenging is often asserted without uptake, metabolite or compartment measurements.	Zhang et al. (2013, 2020, 2023); Khan et al. (2019); Muhammad et al. (2023)
Ascorbate–glutathione cycling	Changes in APX, GR and antioxidant pools are consistent with improved peroxide recycling.	Moderate	Pool size does not establish pathway flux or NADPH supply.	Moustafa-Farag et al. (2020); Pardo-Hernández et al. (2020)
ROS–NO signalling and PMTR1	Receptor-associated ROS and NO signalling regulates stomata in *Arabidopsis*; crop seed evidence is indirect.	Moderate in model leaves; low-to-moderate for primed crop seed	Conservation during imbibition and persistence after redrying are not established.	Wei et al. (2018); Wang et al. (2023)

Mechanism	Evidence directly relevant to seed treatment	Current confidence	Principal caveat	Supporting references
Osmotic adjustment	Proline, soluble sugars/proteins and relative water content often change after priming.	Moderate	Solute accumulation may reflect injury or growth dilution rather than true osmotic adjustment.	Khan et al. (2019); Heshmati et al. (2021); Shaheen et al. (2024)
ABA–gibberellin crosstalk	Melatonin altered ABA and GA4 during cucumber germination under salinity; drought-seed evidence is mainly inferential.	Moderate for germination concept; low-to-moderate under drought	Hormonal effects are stage-dependent and usually measured at few time points.	Zhang et al. (2014); Arnao and Hernández-Ruiz (2021); Liu et al. (2023)
Energy and sugar metabolism	Proteomic and physiological studies implicate glycolysis, respiration, starch breakdown and sucrose metabolism.	Moderate	Much evidence comes from salinity or vegetative tissue rather than drought-primed seed.	Zhang et al. (2017); Zhao et al. (2015)
Roots, stomata and photosynthesis	Greater lateral/deep rooting, altered stomatal traits and preserved photosynthesis occur in several systems.	Moderate; strongest when linked to field water use	The advantageous direction depends on drought timing; single-time-point gas exchange is insufficient.	Zhang et al. (2013); Khan et al. (2019); de Camargo Santos et al. (2024); Dai et al. (2026)
Organelle and membrane protection	Lower leakage and malondialdehyde plus preserved chloroplast or cellular ultrastructure.	Moderate-to-high	Microscopy may have limited sampling; mitochondrial protection is less directly measured.	Khan et al. (2019); Zhang et al. (2013, 2020)
Methylglyoxal detoxification and autophagy	Cotton evidence links melatonin with glyoxalase function, methylglyoxal homeostasis and autophagy under PEG stress.	Emerging	Requires flux assays and genetic or pharmacological tests across crops.	Dake et al. (2025)

Note: ABA, abscisic acid; APX, ascorbate peroxidase; GA4, gibberellin A4; GR, glutathione reductase; NO, nitric oxide; PMTR1, phytomelatonin receptor 1; ROS, reactive oxygen species

The mechanisms in Table 3 should not be viewed as independent modules. Redox state influences hormone metabolism, mitochondrial function, membrane stability and autophagy; sugar status influences ABA and growth; stomatal behaviour changes ROS production and carbon supply. The central challenge is therefore not to add more correlated biomarkers, but to identify causal order. Time-resolved experiments that combine perturbation with multi-omic and physiological measurements are more informative than broad endpoint panels collected once after stress.

6. Persistence, Dose–Response and Biological Contingency

6.1 Concentration and Exposure Duration

Melatonin responses are characteristically non-linear. In several crops, an intermediate concentration improves germination and stress tolerance while lower concentrations are insufficient and higher concentrations are neutral or inhibitory (Kumari et al., 2026; Meng et al., 2025; Zhang et al., 2013). This pattern is compatible with a signalling molecule that must operate within a redox and hormonal range. A high dose may excessively suppress ROS, perturb auxin- or ABA-related development, delay reserve mobilisation or impose solvent and osmotic effects. The numerical optimum cannot be transferred directly among studies because dose is reported in micromoles per litre, milligrams per litre or nominal treatment categories, and uptake depends on seed coat permeability, solution volume, temperature and duration.

Exposure time is equally important. A 6-hour treatment at one concentration does not deliver the same internal dose as a 24-hour treatment, and larger or oil-rich seeds hydrate differently from small cereal grains. Few studies report solution-to-seed ratio, oxygenation, light conditions, final seed moisture or melatonin remaining in the solution. Without mass-balance information, concentration is only a formulation descriptor, not an absorbed dose. Dose optimisation should therefore use response surfaces that vary concentration and duration jointly and quantify melatonin uptake and metabolism.

Redrying introduces another variable. Commercial priming generally requires seeds to be returned to a moisture content suitable for storage and mechanical sowing. Some experimental studies sow immediately after soaking, effectively testing pre-germination hydration plus melatonin. Others redry but do not report rate, temperature or final moisture. Slow or warm drying can alter stress proteins and membrane state; incomplete drying can shorten shelf life. The biological effect attributed to melatonin may therefore include uncontrolled differences in hydration history.

6.2 Genotype, Seed Lot and Developmental Stage

Genotype-specific effects are expected because seed coat traits, dormancy, endogenous melatonin, antioxidant capacity, hormone sensitivity and root architecture differ. Multi-genotype rice and maize investigations demonstrate variable treatment responsiveness (Kumari et al., 2026; Tyagi et al., 2023). The two-cultivar peanut and wheat studies similarly show that baseline drought sensitivity shapes the apparent benefit and physiological strategy (de Camargo Santos et al., 2024; Shaheen et al., 2024). Testing a single cultivar can therefore identify efficacy but cannot establish a crop-wide recommendation.

Seed lot quality is an underappreciated modifier. Ageing increases membrane leakage, damages mitochondria and reduces antioxidant capacity; priming can repair some defects but may also push deteriorated seeds closer to radicle protrusion or accelerate post-treatment ageing. Safflower evidence indicates that fresh and aged seeds can respond differently to melatonin under drought (Heshmati et al., 2021). Commercial validation should include seed lots that vary in initial vigour, storage history and pathogen load, because highly controlled fresh seed may overestimate field consistency.

Developmental timing determines whether early benefits persist. Faster radicle protrusion under PEG does not guarantee deeper rooting, reproductive success or yield. Priming effects may decay, be overridden by later stress or alter phenology in ways that change drought exposure. Conversely, a modest early advantage can compound through stand uniformity and canopy development. Longitudinal studies should follow marked plants from germination through harvest and distinguish direct carry-over from indirect size advantages.

6.3 Stress Intensity, Timing and Recovery

Melatonin may be most useful under moderate stress, where metabolic activity continues and primed defence systems can operate. Under extremely low water potential, germination may be physically impossible regardless of antioxidant capacity. Studies that report large relative benefits at severe PEG concentrations should be interpreted alongside absolute germination; an increase from a very low baseline can appear impressive while remaining agronomically inadequate. Mild stress can also produce ceiling effects that obscure treatment differences.

Drought timing matters. Establishment drought tests the primed seed directly, whereas reproductive drought occurs long after the treatment and requires persistent developmental or molecular consequences. Intermittent drought includes recovery, during which rapid repair and resumed carbon gain are important. Most studies impose a constant stress and terminate the experiment without recovery. This design cannot determine whether melatonin prevents damage, delays it, or improves resilience after rewatering.

Measured water potential is preferable to nominal PEG percentage or field capacity alone. PEG solutions change with temperature and preparation, and field-capacity percentages do not specify soil texture or plant water status. Soil water potential, leaf water potential, relative water content, stomatal conductance and cumulative transpiration provide complementary information. Standardised reporting would permit comparison across laboratories and reduce the tendency to classify diverse stresses under one label.

6.4 Controls, Blinding and Publication Bias

Hydropriming is the most important control for estimating the melatonin-specific component. Untreated dry seed alone cannot separate effects of hydration, washing and redrying. Solvent controls are also required where melatonin is dissolved with ethanol or another carrier. Positive controls such as gibberellic acid can contextualise efficacy, but they should not replace hydropriming. Factorial designs comparing untreated, hydroprimed, solvent-primed and melatonin-primed seeds under both well-watered and stressed conditions provide the clearest attribution.

Blinding and randomisation are rarely emphasised in plant priming reports. Germination scoring, root tracing, microscopy selection and pot placement can be influenced by observer or positional effects. Automated imaging, pre-specified exclusion criteria and blinded analysis would strengthen reproducibility. Biological replicate structure must also be clear: numerous seeds within one dish are subsamples, not independent experimental units. Pseudoreplication can inflate precision when dish, tray or pot is not treated as the replicate.

The literature is overwhelmingly positive, which may reflect genuine efficacy but is also compatible with publication bias, flexible dose selection and selective reporting. Studies often test several concentrations and emphasise the best-performing one without independent validation. Registered protocols are uncommon, and null field trials may remain unpublished. Multi-laboratory ring tests and publication of full dose–response data would provide a more realistic estimate of robustness.

Table 4 summarises the principal sources of heterogeneity and the design remedies needed to distinguish biological contingency from experimental noise.

Table 4. Sources of heterogeneous response and recommended experimental remedies

Source of heterogeneity	**Observed or plausible pattern**	**Likely explanation**	**Recommended design remedy**	**Supporting references**
Concentration	Intermediate doses frequently outperform high doses.	Redox and hormonal signalling operate within a physiological window; excessive treatment can inhibit growth.	Test a full response curve and validate the selected dose independently.	Zhang et al. (2013); Zhao et al. (2015); Kumari et al. (2026)
Duration and solution-to-seed ratio	Identical concentrations can deliver different biological exposures.	Hydration rate, seed size, coat permeability and oxygen availability alter uptake.	Report duration, ratio, aeration, temperature and melatonin mass balance.	Paparella et al. (2015); Rajora et al. (2022)
Redrying and storage	Immediate sowing may outperform stored primed seed.	Hydration advances ageing and redrying changes membranes and stress proteins.	Standardise final moisture and test several commercial storage intervals.	Chen and Arora (2013); Raza et al. (2026)
Genotype	Magnitude and sometimes direction of response differ among cultivars.	Variation in uptake, endogenous melatonin, dormancy, hormone sensitivity and drought strategy.	Use diverse panels and analyse genotype-by-treatment interaction.	Tyagi et al. (2023); de Camargo Santos et al. (2024); Kumari et al. (2026)
Seed age and vigour	Old and fresh lots can respond differently.	Ageing changes membranes, mitochondria and antioxidant capacity.	Measure initial vigour and include contrasting seed lots.	Heshmati et al. (2021)
Stress model and intensity	Large PEG effects may not predict soil or field benefit.	PEG lacks root–soil and atmospheric components; extreme stress may make germination physically impossible.	Calibrate water potential and link laboratory, soil and field experiments.	Verslues et al. (2006); Dai et al. (2026)
Endpoint timing	Early germination benefit may disappear or compound later.	Treatment effects decay, alter phenology or create size-mediated carry-over.	Follow the same cohorts through recovery and harvest.	Liu et al. (2023); Dai et al. (2026)
Control selection	Untreated-seed comparisons can exaggerate melatonin specificity.	Hydration, washing, solvent and redrying have independent effects.	Include hydroprimed and solvent controls with identical handling.	Paparella et al. (2015); Khan et al. (2019)

Source of heterogeneity	Observed or plausible pattern	Likely explanation	Recommended design remedy	Supporting references
Experimental unit and reporting	Precision may be inflated when seeds within one dish are treated as replicates.	Pseudoreplication and flexible selection of doses or endpoints.	Randomise dishes/pots, blind scoring and report all tested treatments.	Baethge et al. (2019); Savvides et al. (2016)

Note: The recommended remedies are intended to improve reproducibility and do not imply that all existing studies are invalid

The variation in Table 4 does not invalidate melatonin seed treatment. It shows that the active treatment is a process rather than a single concentration: seed genotype and quality, hydration kinetics, absorbed dose, redrying, storage, stress scenario and crop management jointly determine the outcome. Standardisation should therefore specify performance windows and decision rules rather than impose one universal dose.

7. Agronomic Translation, Formulation, Safety and Scalability

7.1 From Germination Advantage to Yield and Water Productivity

The translational case is strongest when improved laboratory germination leads to uniform emergence, deeper or better-distributed roots, sustained canopy function and greater harvestable yield per unit water. Most studies demonstrate only the first one or two links. Rapeseed work has reported yield and seed-quality responses under drought after melatonin or gibberellic-acid priming, and safflower research has moved into field conditions (Heshmati et al., 2021; Khan et al., 2020). The winter-wheat multi-season field experiment provides the clearest chain from primed seed through root growth and deep water uptake to yield and water productivity (Dai et al., 2026). This evidence is encouraging but too concentrated to support a general recommendation across crops.

Field benefit may depend on stand establishment. In mechanised agriculture, more uniform emergence can improve crop competition and simplify management, even if individual-plant physiology changes modestly. Under rainfed systems, a primed seed that germinates rapidly after a small rainfall event could also face greater mortality if soil dries before establishment. Priming may therefore alter risk as well as average performance. Trials should include rainfall timing, emergence survival and re-sowing probability, not only final germination.

Water productivity must be defined carefully. Greater yield per unit irrigation water can coexist with greater total water use, while intrinsic water-use efficiency at leaf level does not equal field water productivity. Field studies should report irrigation, rainfall, soil-water storage, evapotranspiration where possible, yield and quality. Root access to deeper water may increase productivity in one system but compete with subsequent crops or groundwater conservation objectives in another.

7.2 Formulation and Seed-Industry Compatibility

Laboratory soaking is simple, but commercial deployment requires controlled delivery at scale. Treatment must be compatible with batch or continuous seed treaters, film coating, pelleting, fungicides, insecticides, microbial inoculants and polymers. Melatonin is sensitive to light and oxidation, so solution preparation, packaging and coating stability require validation. The final formulation must distribute a small dose uniformly without causing premature hydration or impairing flowability.

Aqueous priming followed by redrying can be expensive and operationally demanding because it requires moisture control and microbial management. Coating or solid-matrix delivery might reduce water use, but bioavailability may differ. Nanoformulations have been proposed to improve stability or controlled release, yet they introduce additional material, safety and regulatory questions and should not be assumed to be greener than conventional delivery (Raza et al., 2026). Comparative life-cycle and cost analyses are absent.

Post-priming storage is a major bottleneck. Hydration can accelerate seed ageing, and melatonin may not fully offset this effect. Shelf-life studies should measure germination, vigour, internal melatonin, oxidative status and microbial quality across realistic temperature and humidity conditions. A treatment that works only when seed is sown immediately may suit on-farm priming but not centralised commercial distribution. Claims of scalability must identify the intended supply chain.

7.3 Economic, Regulatory and Environmental Considerations

Melatonin is active at low nominal concentrations, suggesting modest chemical input, but material cost is only one component. Water, energy for drying, labour, quality control, packaging, treatment loss and the value of shortened shelf life determine economic feasibility. Benefit depends on drought probability and yield response, so partial-budget analysis should be conducted across seasons, including years without stress. A priming treatment that improves drought yield but reduces performance under adequate water would require targeted use; fortunately, several studies report neutral or positive effects under non-stress conditions, but field confirmation remains limited.

Regulatory classification varies by jurisdiction and formulation. Melatonin may be treated as a plant biostimulant, plant-growth regulator or seed-treatment active, with different evidence and residue requirements. The compound occurs naturally in plants, but natural occurrence does not remove the need to assess worker exposure, environmental release, seed residues and interactions with non-target organisms. Seed-treatment quantities are likely to be small, yet systematic ecotoxicological and residue data are scarce.

Environmental claims should also be calibrated. Melatonin priming could reduce re-sowing, stabilise production or improve water productivity, but its overall footprint depends on synthesis, formulation, drying and packaging. ‘Green’ and ‘climate-smart’ are research hypotheses rather than inherent properties. A credible assessment would compare melatonin priming with hydropriming, conventional seed enhancers, drought-tolerant cultivars and changes in irrigation management using common functional units.

7.4 Integration with Breeding and Agronomy

Seed priming should complement, not substitute for, genetic drought adaptation and sound water management. Genotypes differ in responsiveness, so breeding programmes could screen both baseline drought tolerance and priming response. A genotype with moderate unprimed performance but a strong, stable response may be valuable where treatment infrastructure exists; a highly tolerant genotype with little response may be preferable where farmers cannot prime reliably. Genotype-by-priming-by-environment interactions should therefore be treated as breeding targets.

Integration with sowing date, seed rate, irrigation scheduling and nutrient management is also required. Melatonin-associated root growth could alter nitrogen acquisition and lodging risk; faster emergence could change weed competition; stomatal regulation could interact with heat. Multi-factor field trials are needed because the optimum under isolated drought may not be optimum under combined heat, nutrient limitation or pathogen pressure. Seed-applied fungicides and beneficial microorganisms may modify melatonin uptake or redox effects.

Table 5 evaluates translational readiness by outcome domain. Early germination and seedling physiology have broad proof-of-concept support, whereas yield, water productivity, storage stability, formulation compatibility, safety and economic performance remain at lower readiness.

Table 5. Translational readiness of melatonin seed treatment for water-stress management

Domain	Current evidence	Readiness	Key uncertainty	Priority before adoption	Supporting references
Germination under low water potential	Positive responses reported across multiple crops and PEG concentrations.	Moderate-to-high proof of concept	Variable dose, seed lot and hydropriming controls.	Crop-specific dose windows with absorbed-dose data.	Zhang et al. (2013); Guo et al. (2022); Zhang et al. (2023)
Seedling physiology	Consistent redox, membrane, osmolyte, root and photosynthetic responses.	Moderate	Biomarker correlation exceeds causal evidence.	Perturbation studies and standardised physiological endpoints.	Khan et al. (2019); Muhammad et al. (2023); Shaheen et al. (2024)
Soil-based establishment	Several pot and whole-plant studies support improved	Moderate but crop-specific	Pot restriction and short duration.	Larger soil volumes, recovery cycles and multi-genotype	de Camargo Santos et al. (2024); Shaheen et al. (2024)

Domain	Current evidence	Readiness	Key uncertainty	Priority before adoption	Supporting references
	establishment and water regulation.			tests.	
Yield and seed quality	Positive rapeseed, safflower and winter-wheat evidence.	Low-to-moderate	Few independent locations and seasons.	Multi-environment trials with commercial comparators.	Khan et al. (2020); Heshmati et al. (2021); Dai et al. (2026)
Field water productivity	Demonstrated directly in a multi-season winter-wheat experiment.	Promising but preliminary	Single crop-region-management combination.	Independent water-balance trials in diverse crops.	Dai et al. (2026)
Post-priming storage	Recognised as a constraint but seldom measured systematically.	Low	Shelf life, seed ageing and persistence of active compound.	Realistic storage-duration and packaging studies.	Raza et al. (2026)
Commercial formulation and compatibility	Laboratory soaking dominates; coating and combined technologies are proposed.	Low	Uniformity, stability, redrying cost and interaction with pesticides or inoculants.	Pilot-scale treatment, coating and compatibility tests.	Rajora et al. (2022); Raza et al. (2026)
Economic value	Potentially low active dose, but complete treatment cost is rarely reported.	Low	Drought probability, drying cost, shelf-life loss and yield response.	Partial budgets across stressed and non-stressed seasons.	Dai et al. (2026); Raza et al. (2026)
Safety and regulatory status	Natural occurrence is established, but seed-treatment-specific datasets are sparse.	Low	Residues, worker exposure, non-target effects and jurisdictional classification.	Proportionate residue, ecotoxicology and regulatory assessment.	Dubbels et al. (1995); Tan et al. (2012)

Note: Readiness is a qualitative judgement based on the breadth, realism and independence of evidence, not a formal technology-readiness score

The readiness profile in Table 5 argues for a staged development pathway. Crop- and seed-lot screening should identify a reproducible dose window, followed by redrying and storage validation, then multi-environment field trials with hydroprimed and commercial-standard controls. Mechanistic measurements should be retained at field scale, but only those linked to decisions, such as emergence, root distribution, canopy temperature, water use, yield and quality. A technology is not validated merely because it changes many biomarkers.

8. Future Directions

8.1 Standardised Treatment Reporting and Absorbed-Dose Measurement

The immediate priority is a minimum reporting framework for melatonin seed treatment. Studies should state seed species and cultivar, seed-lot age and initial vigour, melatonin purity and solvent, concentration, solution-to-seed ratio, exposure duration, temperature, aeration and light, seed moisture before and after treatment, redrying conditions, storage interval and final sowing moisture. Water-stress methods should report PEG molecular weight and calibrated water potential or, for soil experiments, soil properties, water content, water potential and irrigation schedule. These details are necessary for replication and meta-analysis.

Nominal concentration should be complemented by absorbed dose. Targeted liquid chromatography–mass spectrometry can quantify melatonin and major metabolites in seed tissues during priming, after redrying and during germination. Stable-isotope-labelled melatonin would distinguish exogenous compound from induced endogenous synthesis. Mapping tissue and subcellular distribution would test whether direct scavenging is feasible and identify the exposure window associated with benefit or inhibition.

8.2 Causal Mechanism Experiments

The field needs fewer lists of correlated antioxidants and more causal tests. Receptor-associated signalling can be examined with PMTR1/CAND2 loss-of-function and complementation lines, followed by crop validation through gene editing or transient assays. ROS and NO dynamics should be monitored with genetically encoded probes during imbibition and early root growth, and pathway inhibitors should be used cautiously with appropriate toxicity controls. Hormonal causality requires time-series measurements of ABA and gibberellins, biosynthetic and catabolic flux, sensitivity assays and genetic perturbation.

Energy and carbon allocation should be investigated through isotope tracing, metabolomics and high-resolution respirometry. For methylglyoxal and autophagy, glyoxalase mutants, autophagy-deficient backgrounds and flux assays can determine necessity rather than association (Dake et al., 2025). Multi-omic measurements should be designed around explicit causal models and sampled at biologically informative transitions, not collected as broad endpoint inventories.

8.3 Persistence, Memory and Post-Priming Storage

A central unresolved question is how an hours-long seed treatment affects a plant months later. Longitudinal experiments should measure the decay or transformation of melatonin-associated states from dry seed to imbibition, emergence, vegetative growth and reproduction. Chromatin accessibility, DNA methylation, histone modifications, small RNAs and persistent proteins may be examined, but the term epigenetic memory should be reserved for stable, functionally linked changes. Reciprocal crosses or progeny studies would be required before transgenerational claims are made.

Storage studies should be integrated with persistence research. Seeds should be redried to commercial moisture and stored across realistic temperature and relative-humidity regimes for months, not days. Germination, vigour, treatment residues and stress performance should be tested periodically. Packaging and antioxidant protection of the formulation may extend shelf life, but any gain must be compared with untreated and hydroprimed seed.

8.4 Multi-Genotype, Multi-Environment Field Validation

The most important translational need is independent field replication. Trials should include several cultivars differing in drought strategy and seed traits, multiple locations, at least two seasons and both water-limited and adequately watered controls. Drought timing should include establishment, vegetative and reproductive periods. Untreated, hydroprimed, solvent-control and melatonin treatments should be compared with a relevant commercial seed enhancement or agronomic standard.

Core outcomes should include emergence rate and uniformity, stand survival, root distribution, canopy temperature, soil-water extraction, phenology, biomass, yield components, final yield, product quality and field water productivity. Treatment-by-environment interactions should be analysed, and economic returns should include treatment and drying costs. The winter-wheat evidence demonstrates that such trials are feasible and can connect early root effects with later water use and yield (Dai et al., 2026); similar designs are needed in maize, rice, oilseeds, legumes and dryland crops.

8.5 Predictive Dose Optimisation and Decision Support

A universal melatonin concentration is unlikely. Response-surface experiments can model concentration, exposure duration and seed moisture jointly, while hierarchical models estimate genotype and seed-lot effects. High-throughput phenotyping of germination, root architecture and redox reporters can identify response classes. These data could support crop-specific decision tools that recommend treatment according to seed vigour, anticipated drought, sowing system and storage interval.

Prediction should be validated prospectively. A dose selected from one experiment must be tested in new seed lots and environments before it is described as optimal. Reporting the full response curve, including negative and null results, would reduce winner's-curse effects. Shared protocols and inter-laboratory ring tests could establish reproducibility and reveal whether apparent crop differences are caused by local treatment practice.

8.6 Formulation, Safety and Comparative Sustainability

Formulation research should compare aqueous priming, coating, solid-matrix priming and controlled-release systems using equivalent absorbed doses. Compatibility with common pesticides, micronutrients and microbial inoculants must be assessed for efficacy, seed safety and shelf life. Nano-enabled systems should proceed only when they demonstrate a clear advantage in stability or dose reduction and when the carrier's fate is characterised.

Safety assessment should quantify residues on treated seed and harvested product, worker exposure during mixing and drying, and effects on soil microorganisms and non-target organisms. Regulatory pathways should be mapped by jurisdiction early in development. Comparative sustainability analysis should include material synthesis, water and energy used for priming and drying, packaging, avoided re-sowing, yield stabilisation and changes in irrigation. Such analysis would test rather than assume the 'green' status proposed in recent synthesis (Raza et al., 2026).

8.7 Combined-Stress and Farming-System Research

Field drought often coincides with heat, salinity, nutrient limitation or pathogen pressure. Melatonin's broad signalling roles make combined-stress protection plausible, but interactions can be non-additive. A dose that protects under drought alone may alter disease resistance or heat avoidance. Experiments should use realistic stress sequences and measure trade-offs in growth, defence and reproduction.

On-farm studies are needed to evaluate operational feasibility. Smallholder on-farm priming may favour simple same-day soaking protocols, whereas commercial seed companies require coating compatibility and prolonged storage. Participatory trials can identify acceptable labour, equipment and risk. The appropriate product may therefore differ by farming system rather than being a single globally standardised treatment.

9. Conclusion

Melatonin-based seed treatment has a credible physiological foundation and a substantial, though uneven, experimental evidence base for mitigating water-related stress. Across diverse crops, the most defensible conclusion is that an appropriately selected treatment can improve germination and early seedling performance under low water potential by strengthening redox buffering, limiting membrane injury and supporting metabolic recovery. Evidence also supports contributions from osmotic adjustment, root development, chloroplast protection and dynamic regulation of stomata and photosynthesis. Hormonal crosstalk, receptor-mediated signalling, energy metabolism, methylglyoxal detoxification and autophagy provide coherent mechanistic extensions, but their causal importance is not yet established uniformly across crop seeds.

The principal limitation to application is not absence of biological activity but insufficient standardisation and translation. Most evidence comes from short-term PEG experiments, and positive seedling biomarkers cannot be assumed to predict yield under soil drought. Dose, exposure duration, genotype, seed quality, redrying, storage and drought pattern strongly influence outcomes. Rare multi-season field evidence indicates that priming can improve root access to water, yield and water productivity, yet independent multi-environment confirmation is required.

Melatonin seed treatment should therefore be developed as a crop- and context-specific seed technology rather than promoted as a universal drought remedy. Progress depends on absorbed-dose measurement, causal mechanism tests, commercial storage studies, rigorous hydropriming controls and field trials that integrate water balance, yield, quality, economics and safety. Under those conditions, melatonin may become a useful component of broader strategies combining resilient cultivars, precise irrigation and high-quality seed systems.

10. Limitations

As a critical narrative review, this synthesis remains susceptible to selection and interpretive bias despite transparent searching, explicit eligibility criteria and structured appraisal. Complete subscription-database exports were unavailable, and the review was restricted to English-language sources with verifiable bibliographic information. The evidence base is heterogeneous in crop, genotype, seed condition, melatonin dose, treatment duration, redrying procedure, stress model and outcome measurement, preventing quantitative synthesis and direct effect-size comparison. Many studies use PEG-induced osmotic stress or short pot experiments, and field evidence is geographically concentrated and scarce. Publication bias is likely because positive treatment responses predominate and null dose combinations are not consistently reported. Mechanistic conclusions are further limited by reliance on correlated biochemical markers, with comparatively few genetic, flux-based or long-term studies. The field is developing rapidly, so evidence published after the search end date was not incorporated.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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