Seed Res. 52 (1): 30-40, June, 2024

Seed Priming: Tool towards Sustainable Agriculture

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(Received January 2024; Revised February 2024; Accepted February 2024)

ABSTRACT: Seed germination stands as a pivotal and intricate physiological occurrence in the life cycle of plants, frequently susceptible to environmental and biological stressors culminating in unpredictable germination patterns. Priming, a time-honoured technique traditionally employed for ensuring synchronized seedling growth and a steadfast crop stand, has evolved into a formidable instrument for promoting sustainable agriculture in contemporary times. Its application extends to mitigating an array of abiotic stresses, encompassing salinity, drought, cold, and heavy metal stresses, concurrently fostering the robust growth of crop plants. Notably, priming has demonstrated efficacy against biotic stress agents, countering pathogenic bacteria and fungi. This exhaustive review seeks to encapsulate diverse, successful priming methodologies that have yielded commendable outcomes, spanning enhanced growth, augmented yield, bolstered disease resistance and heightened tolerance to both abiotic and biotic stresses. The exploration delves into the subcellular transformations induced by priming, elucidating the underlying molecular and physiological aspects. The specific proteomic changes during imbibition and seed dehydration processes associated with priming, contributing significantly to elevated seed vigour, are also meticulously summarized. Against the backdrop of escalating global demands for food supply, driven by an expanding population and the excessive application of chemical fertilizers compromising soil health, seed priming emerges as a prudent, cost-effective, and eco-friendly alternative. It presents a pragmatic approach to address the imperatives of global food security through sustainable agricultural innovation.

Keywords: Priming, Germination, Molecular mechanism, Stress, Seed physiology, Sustainable agriculture

INTRODUCTION

Agriculture confronts myriad challenges, such as climate change, the contraction of arable land due to swift urbanization, unbridled use of chemical fertilizers and pesticides, biotic and abiotic stress, and suboptimal soil nutrient status. Despite numerous agricultural innovations, the substantial surge in food demand poses an arduous challenge. In this exigent scenario, the adoption of sustainable agricultural practices becomes imperative to address global food security. According to projections by the United Nations and the Food and Agricultural Policy Research Institute (FAPRI), the global demand for milled rice is anticipated to reach 496 million tons in 2020 and escalate to 555 million tons by 2035. Considering that two-thirds of the Indian population relies on rice as a primary staple, the persistent upswing in rice demand poses a challenge amid the continuous growth in the Indian population. Despite India's noteworthy contribution to global rice production, it contends with one of the lowest yields among nations. Yield gap analysis reveals that India achieves 30-40% less yield than its inherent capacity. In endeavours to maximize the yield of economically significant crops, farmers are enticed to resort to the widespread application of bulk fertilizers and chemical pesticides. Such practices pose a severe environmental threat, encompassing excessive leaching, eutrophication of water bodies, contamination of drinking water, soil degradation, air pollution, and bio-magnification through the food chain. Moreover, soil application or foliar sprays necessitate substantial resources and labour to attain the desired outcomes.

Breeding investigations have introduced numerous high-yielding and disease-resistant variants of food crops, yet their convenient accessibility to farmers remains dubious. The evolving climatic conditions have subjected crops to various abiotic and biotic stressors. Strategies deployed to alleviate such stressed plants, while in practice, often fall short of delivering optimal results or, paradoxically, contribute to environmental hazards. This perplexing scenario necessitates the harmonization of environmental sustainability, food security, and agricultural production [1]. Seed germination, a pivotal and intricate physiological process in the plant life cycle, frequently succumbs to environmental and biological stressors, resulting in irregular germination patterns. Successful seed germination is paramount for establishing a robust stand and achieving optimal crop yields. Seed enhancement endeavours are underway to ensure proper and synchronized seed germination. Amid various seed invigoration techniques, priming has emerged as a promising and secure approach to combat both abiotic and biotic stresses, enhancing plant growth and vitality. Seed priming involves the pre-exposure of seeds to an eliciting factor, preparing the plants for future stressors and enhancing their survival under adverse environmental conditions[2].

In the natural milieu, seeds undergoing natural burial in the soil encounter an array of edaphic factors such as pH, texture, moisture content, diurnal temperature fluctuations, light, and oxygen, all of which positively influence germination [3] . The burial of seeds and the soil microenvironment impose a natural priming effect, expediting seed physiology and emergence. Additionally, seed burial proves advantageous in areas prone to regular fire outbreaks, where plant species are often annihilated, leaving seeds as the sole source of regeneration [4] . Fire can interact with seed germination and physiology by breaking dormancy or activating specific genes upon exposure to smoke. In tropical forests, characterized by zoochoric seed dispersal (aided by animals), ants serve as common dispersers. The interaction between ants and seeds facilitates seed cleaning, minimizing pathogen attacks through metapleural and mandibular gland secretions with antibacterial and antifungal properties. This interaction is known to enhance the germination and development of seeds for various plant species [5,6]. This underscores the synergistic impact of several natural biotic and abiotic factors on soil seed banks, inducing the germination of such seeds. Consequently, laboratory-induced seed priming can be viewed as an enhanced scientific emulation of the natural seed priming phenomenon. Priming, therefore, stands out as a promising tool for endowing plants with superior stress resistance by augmenting their growth and vitality without incurring environmental costs.

Since priming is executed directly with the seeds, it precludes the introduction of harmful chemicals into the soil or environment. Moreover, it represents a cost-effective, straightforward approach that can serve as a sustainable substitute for agrochemicals while yielding comparable outcomes. Since the inception of priming [7]elucidating the concept, a multitude of works have been published, with the numbers continually increasing. Comprehensive information detailing various priming agents, their roles in stress and growth promotion, subcellular changes induced by priming, and the molecular foundations of priming is available in the literature. This review endeavours to systematically assemble existing independent reports on seed priming, positioning it as an emerging facet of sustainable agricultural innovations.

Seed priming: how it works?

The process of seed germination is intricate and vital, encompassing three discernible phases as elucidated by [8] and [9]. Commencing with the imbibition phase, seeds promptly absorb water to expedite the reactivation of their metabolic and translational processes, concurrently restoring impaired mitochondria and DNA. Subsequently, the activation or preparatory phase ensues, characterized by a deceleration in water imbibition. However, this period witnesses the culmination of peak physiological and metabolic activities. Concurrently, the synthesis of new mRNAs and proteins occurs, accompanied by mitochondrial maturation and the accumulation of essential molecules. All these processes are prerequisites for the ultimate stage of seed germination. Up until this phase, the seed germination process remains a reversible phenomenon. The germination phase, which marks the culmination of the process, involves the protrusion of the radical and plumule, coupled with rapid water uptake. It is noteworthy that seed priming strategically targets the initial two stages of seed germination, preventing progression to the third phase.

Priming-treated seeds undergo immersion in a specified solution of the priming agent until the activation phase is completed. Subsequently, they are desiccated to their initial moisture content and stored for subsequent use. Consequently, seed priming primarily hinges on the manipulation of the first two germination stages, where the moisture content of the seeds is elevated up to the threshold just short of radical protrusion. This expedites the germination process, as primed seeds have effectively traversed the initial two phases. Upon sowing, these seeds are poised for rapid germination [10]. Although the water uptake patterns during normal seed germination and seed priming are ostensibly similar, the latter is characterized by a more deliberate and controlled pace. This uncomplicated pre-sowing treatment furnishes the seeds with regulated hydration, triggering a cascade of metabolic, biochemical, and molecular transformations. The outcome is heightened germination efficiency, faster and synchronized germination, as well as the instillation of vigor and stress tolerance in nascent seedlings)[8].

Molecular basis of seed priming

The metabolic processes orchestrated by priming diverge significantly from those associated with normal seed germination, primarily due to the absence of control over water uptake in the latter. The augmentation of pre-germinative metabolic activities, including enzyme activation, cell repair, enhancement of antioxidant defense, and protein synthesis, plays a pivotal role in fostering rapid germination, robust seedling growth, and heightened yield. Additionally, the impact of priming extends beyond seeds, influencing the overall health of the plant. Initial exposure to certain constraints during priming triggers temporary metabolic adaptations, establishing a stress memory that fortifies plants for superior adaptation during subsequent stress episodes in the future [11], [12].

The salient benefits of priming prompt a crucial inquiry: what are the cellular changes instigated by priming? While numerous reports delineate changes at the biochemical, physiological, and molecular levels resulting from priming, a comprehensive consensus elucidating the sequential changes stemming from priming remains elusive in the literature. However, contemporary omics tools have facilitated the elucidation of some underlying priming mechanisms, as discussed. Seed priming induces ultrastructural modifications, regulates water content, manages oxidative stress and cell cycle, facilitates reserve mobilization, triggers the synthesis of new proteins, and initiates complex signaling events. Considering the pivotal role of water uptake in seed germination, aquaporins (AQPs) emerge as significant players in the priming process. AQPs, a class of transmembrane proteins, function as water channels, enabling rapid and passive water movement. Notably, plasma membrane intrinsic proteins (PIPs) and tonoplast intrinsic proteins (TIPs), expressed in the plasma and vacuolar membranes, respectively, play a central role in transcellular and intracellular water transport in plants. This characteristic positions AQPs as suspected effectors in priming, particularly as the process commences with water imbibition ([15]. Studies on Brassica and Spinach subjected to osmopriming support this notion, revealing the upregulation of AQPs within 2–4 days of priming (phase II-imbibition) [13,14]. Similar observations were also reported by [75] in rice seeds primed with phytosynthesized silver nanoparticles.

The upregulation of PIP2 expression in primed seeds, as opposed to control, corresponds to the augmented water content observed in these seeds. However, water absorption also occurs through simple diffusion and apoplastic pathways, as demonstrated by aquaporin inhibitors, which, rather than impairing germination, merely decelerate the process ([16]. The enhanced performance of primed seeds under stress conditions, such as drought, salinity, and cold, can be attributed to the accumulation of non-toxic compatible solutes, including proline, glycine betaine, and various sugars [17]; [18]. Priming is presumed to induce moderate abiotic stress inseeds, prompting the accumulation of such osmolytes, thereby enhancing the stress response of the seeds [19]. Despite the dehydration of primed seeds after the initial water imbibition, specific proteins such as late embryogenesis abundant (LEA) protein and dehydrins protect the cellular structures and proteins accumulated during water uptake [20] [21].

The expression of LEA proteins undergoes sequential changes, with a decline during the imbibition phase, upregulation in the dehydration phase, followed by degradation during germination [22] [23]. Transcriptomic analysis of salt-tolerant indica rice seeds primed with polyamines (PA, spermine, and spermidine) demonstrated enhanced expression of ABA-inducible transcription factors, PA biosynthetic enzymes, other antioxidant enzymes, and LEA proteins (e.g., Osem). Simultaneously, endogenous polyamine content also elevated, collectively triggering crosstalk between multiple metabolic pathways to combat salinity stress [24].

Hormonal crosstalk between ABA and GA is a pivotal aspect of seed germination. Seeds remain quiescent until optimum environmental conditions favourable for germination prevail. ABA-mediated chromatin remodelling regulates seed dormancy and germination. ABA represses the histone methyltransferase gene KYP/SUVH4 while inducing histone acetyltransferase HvGNAT/MYST [25] [26]. GA, on the other hand, acts antagonistically. During seed germination, ABA levels significantly decrease while GA levels surge. An intriguing study on Arabidopsis reveals that the PP2C protein, HONSU, acts as a negative regulator of seed dormancy by inhibiting ABA signaling and activating GA signaling [27]. Therefore, HONSU emerges as a crucial mediator facilitating the ABA and GA crosstalk [28]. ROS signalling is likely a prerequisite for breaking seed dormancy and inducing seed germination by activating GA synthesis and mobilizing storage proteins [29]. GA signalling is also positively correlated with alpha-amylase production and secretion in the endosperm of starchy seeds. The intricate interplay between ROS, ABA, and GA ensures proper germination. Seed priming with GA effectively improves germination performance and early seedling growth in rice [30]. However, the modulation of hormonal balance through seed priming warrants thorough investigation.

Cellular expansion, elongation, and cell division are integral to germination kinetics. The endosperm tissue may act as a physical barrier in the germination process, limiting radical emergence. Proteins such as hydrolases, extensin-like proteins, and expansins play pivotal roles in facilitating successful seed emergence. Reports suggest that priming can induce the activities of cell wall hydrolases and endo-β-mannanase, reducing mechanical constraints during the initial germination period and radical protrusion in Cucumis and tomato [31] [32]. Transcriptome analysis of rice seeds primed with selenium and salicylic acid, subjected to submergence stress, revealed enhanced expression of expansin genes EXPA7 and EXPA16 [33]. Expansins are reported to significantly contribute to cell wall loosening, resulting in coleoptile elongation under submergence stress [34].

Cytoskeletal transformations represent a fundamental prerequisite for seed emergence, with α and β tubulin subunits, integral components of microtubules, playing a crucial role in maintaining cellular cytoskeletal integrity. Noteworthy upregulation of α and β tubulin subunits during priming in tomato seeds has been documented, particularly observed in cortical microtubular distribution within the hypocotyl, shoot meristem, and cotyledon regions before radical protrusion. This suggests a mechanical involvement of cytoskeleton proteins in germinating seeds, although comprehensive evidence in this context remains scarce. Despite vigorous cell division in the embryos of primed seeds following water imbibition, the cell cycle is reported to undergo arrest at the G2 phase during priming, facilitating cell synchronization. Cell cycle activation is presumed to be governed by cell cycle proteins such as cyclins, CDKs, and PCNA [35]. However, a detailed elucidation of the mechanism underlying the activation of such proteins is yet to be accomplished. Flow cytometric analysis of the cell cycle in primed tomatoes reveals enhanced DNA replication, allowing progression from the G1 to G2 phase [36]. Unfortunately, there is a paucity of similar reports for other seeds subjected to priming.

Mitogen-activated protein kinase (MPK), a three-tiered signaling molecule ubiquitous in eukaryotes, functions downstream of receptors and aids in transducing extracellular signals into the cell's interior. Due to this property, MPKs are considered excellent mediators of priming-induced signalling. Transcriptome analysis in primed Arabidopsis has documented the accumulation of dormant MPKs, particularly highlighting the significance of MPK 3/6 in priming-induced plant defense signaling and response. MPKs also activate defense genes, such as PAL1 and PR1, providing protection against stress conditions and eliciting Systemic Acquired Resistance (SAR) [37]. Furthermore, claims suggest that priming tightly regulates chromatin modification. Covalent modifications of histones induced by priming allow the recruitment of chromatin remodelling factors, transcription co-activators, and the general transcription machinery, initiating transcription and expression of target genes. In Arabidopsis, studies reveal that priming induces the activation of WRKY promoters under stress conditions. These modifications also serve as a form of memory for SAR [38]. Calcineurin B-like protein-interacting protein kinases (CIPKs) are reported to play roles in enhancing tolerance to abiotic stress, such as salinity and drought [39]. Under osmotic stress or heat and salt stress, cells encounter difficulties in starch mobilization and sugar metabolism, inhibiting seed germination.

Priming with ã-aminobutyric acid (GABA) enhances starch catabolism by activating amylase, thereby accelerating seed germination [40]. Recent data reveals that priming with GABA can induce the expression of CIPKs in rice (OsCIPK), potentially interacting with other downstream regulators and resulting in improved stress tolerance in such plants [41]. Some reports delve into the role of heteromeric G-proteins and their associated proteins in inducing antioxidant enzymes. Although G proteins function in plants, there is no evidence indicating the existence of G-protein coupled receptors (GPCRs) to date [42]. Data suggests that Mlo proteins may function as GPCRs. Cross-talk among G-protein-mediated signals during seed germination has been documented [43]. G-protein signalling has been identified as a master mechanism responsible for alleviating salt stress in maize seeds subjected to NO priming [44]. Saline stress increased G-protein signal pathway components, accompanied by heightened antioxidant enzyme activities. G-protein signalling acted as an early event during salt stress, preceding NO biogenesis. Regulation of G-protein signals was found to control NO generation under salinity stress, subsequently leading to NO-dependent accumulation of antioxidant enzymes. However, limited literature constrains a conclusive interpretation of the role of G-proteins in seed priming-induced signalling. Examination of proteomic changes in seeds subjected to priming has revealed the synthesis and accumulation of several proteins with a robust response to priming. The total protein content in primed seeds has been reported to be higher compared to unprimed ones, indicating the addition of additional proteins due to priming [45,46].

Antioxidant enzymes, particularly various isoforms of superoxide dismutase and catalase, amass prominently among others [47]. Notably, the accumulation of various low molecular weight heat shock proteins has been observed in PEG and mannitol-mediated osmoprimed seeds [48]. Additionally, l-isoaspartyl protein methyltransferase, an enzyme responsible for repairing age-induced damage to cellular proteins, increases in primed seeds [49]. Increased synthesis of molecular chaperones and abundant embryogenesis proteins has also been documented as a consequence of priming. These proteins are recognized for providing stress tolerance to plants [50]. Several changes in the metabolome induced by priming have been unravelled. These include an increase in ATP, accumulation of sterols and phospholipids, activation of DNA repair and antioxidant enzymes, and de novo synthesis of proteins and nucleic acids [51]. For an extended period, Reactive Oxygen Species (ROS) were viewed as detrimental to plant growth and seed viability until physiological studies asserted the crucial role of ROS in seed germination as signalling molecules that provide protection against pathogens and break seed dormancy [52]. ROS accumulation is predominantly noted during priming. However, this oxidative burst is regulated by activated antioxidant enzymes, preventing oxidative damage to the cell. While ROS may appear harmful to the cell, it is a pivotal determinant of seed germination, acting as a signalling molecule and activating downstream MAPKs, calcium-binding proteins, and calcium ion channels or inhibiting protein phosphatases [53,54]. Thus, the heightened ROS during priming can be perceived as a necessary aspect for the cell. Micro RNAs (miRs) are a class of small non-coding RNAs (20–24 nt in length) that can regulate target genes through complementary binding. Numerous miRs identified to date are known to play potential roles in seed germination, growth, hormone homeostasis (e.g., miR-159 involved in GA-ABA cross-talk and miR-164 involved in auxin signaling), and stress response (e.g., miR-169 involved in drought stress response) [55,56]. Under stress conditions, overexpression of miR402 has been shown to accelerate seed germination by targeting a putative DNA glycosylase, DEMETER-LIKE protein3 (DML3), involved in DNA demethylation [57]. miR167 was found to repress Auxin Responsive Factor 6 (ARF6) during ovule and anther development [58].

In the context of rice, extensive investigations by multiple researchers have delved into the nuanced roles of miRs in root development, pollen, and seed maturation [59,39,60]. Despite the documented miRNA families from rice (miRBase 21.0), the understanding of miR regulation in seed germination remains somewhat limited [56] Notably, under conditions of drought stress, miR-169g exhibited more pronounced induction in roots, standing out as the sole member of the miR-169 family. Sequence analysis suggested its direct regulatory role in seed germination, revealing its significance in orchestrating molecular responses to environmental challenges.

While conventional wisdom had long regarded Reactive Oxygen Species (ROS) as detrimental to both plant growth and seed viability, contemporary physiological studies have underscored the pivotal role of ROS in seed germination. ROS, acting as signaling molecules, play a crucial part in providing protection against pathogens and breaking seed dormancy [52]. The priming process significantly accentuates ROS accumulation. However, this oxidative burst is meticulously regulated by activated antioxidant enzymes, effectively averting oxidative damage to cellular structures. Despite the apparent harm posed by ROS, it emerges as an indispensable factor governing seed germination. Serving as a signaling molecule, ROS activates downstream components such as Mitogen-Activated Protein Kinases (MAPKs), calcium-binding proteins, and calcium ion channels. Simultaneously, it inhibits protein phosphatases [53,29]. In this nuanced interplay, the heightened ROS levels during priming are construed not as a malevolent force but rather as a prerequisite for cellular processes.

Micro RNAs (miR) represent a class of diminutive non-coding RNAs measuring 20–24 nucleotides in length, exerting regulatory control over their target genes through complementary binding. Numerous miRs have been identified, showcasing their potential roles in seed germination, growth, hormone homeostasis (e.g., miR-159 implicated in GA-ABA cross-talk, and miR-164 in Auxin signaling), as well as stress response (notably, miR-169 involved in drought stress response) [55,56]. Under stress conditions, the overexpression of miR402 has been observed to expedite seed germination by targeting the putative DNA glycosylase, DEMETER-LIKE protein3 (DML3), a participant in DNA demethylation [57]. Similarly, miR167 has been identified as a suppressor of Auxin Responsive Factor 6 (ARF6) during ovule and anther development [58]. Rice has been a focal point for in-depth exploration of miR roles in root development, pollen, and seed maturation by several researchers [59,39,60]. Despite the documentation of 332 miRNA families from rice (miRBase 21.0), our understanding of miR regulation in seed germination remains somewhat constrained [56]. Under drought stress, utilizing an oligonucleotide microarray, miR-169g has emerged as prominently induced in roots, standing as the sole member of the miR-169 family. Sequence analysis has implicated its direct regulation through two dehydration-responsive elements (DREs) proximately upstream of the miR [55]. Moreover, it is acknowledged that protein synthesis from long-lived stored mRNAs in seeds is an indispensable event for seed germination and is subject to post-transcriptional regulation([56].

Despite the wealth of knowledge, there is a noticeable absence of reports elucidating the role of miRs associated with seed priming, although the evident importance of miRs in steering the molecular changes induced by priming is apparent. Existing independent reports provide insights into various modifications occurring in primed seeds, yet they leave considerable gaps in constructing a conclusive signaling pathway active during priming. While genome-wide transcriptome studies in rice have highlighted significant changes in transcript levels during the initial hours of seed germination, phospho-proteome studies have pinpointed the early hours of water imbibition (3 to 12 h) as crucial for signaling events associated with germination. It is conceivable that priming, by rehydrating seeds, triggers an oxidative outburst in the form of ROS. This ROS surge serves as a signaling agent, activating Mitogen-Activated Protein Kinase (MPK) and calcium-mediated signaling pathways, which in turn initiate downstream signaling cascades leading to altered expression of essential genes and proteins. Given that excessive ROS can induce cell death, the hyper-accumulation is counterbalanced by the enhanced activity of antioxidant enzymes. Reports highlighting the heightened activity of antioxidant enzymes and ROS accumulation in primed seeds substantiate this fact. The increased abundance of DNA repair enzymes in primed seeds may function as a protective mechanism against ROS. Nevertheless, the precise molecular events during priming remain to be fully deciphered.

Priming of recalcitrant seeds

Orthodox seeds, characterized by desiccation tolerance, typically maintain elevated vigour and vitality from harvest to the subsequent growing season under favourable storage conditions. On the contrary, recalcitrant seeds, falling outside the orthodox category, exhibit sensitivity to desiccation, making prolonged storage challenging [61]. These seeds generally possess a relatively high moisture content during dispersal, undergo rapid germination, and have a limited lifespan, rendering conventional long-term storage impractical. Seed priming, a common practice, is predominantly applied to orthodox seeds (desiccation-tolerant) due to their inherent ability to sustain an accelerated metabolic rate during the controlled hydration imparted in the priming process [62]. In contrast, recalcitrant seeds promptly germinate upon encountering humid environmental conditions, accompanied by swift water uptake. Consequently, preserving seed banks for such seeds poses difficulties, and restoration primarily relies on the cultivation of seedlings. Nevertheless, seed priming treatments have proven effective in augmenting the germination rate, seedling vigour, and field establishment of desiccation-sensitive seeds. This, in turn, expedites the restoration process, allowing the generation of seedlings of plantable sizes in a reduced timeframe through priming.

In the case of late successional Mexican tropical rainforest species, such as *Cupania glabra* Sw. (Sapindaceae) and *Cymbopetalum baillonii* R.E.Fr. (Annonaceae), subjected to hydro priming and natural priming, enhanced seedling vigour was observed compared to non-imbibed seeds [63]. Employing carbon nanoparticles for priming recalcitrant seeds of *Trichilia dregeana* positively influenced root and shoot length [64]. Hydropriming of *Quercus rugosa* seeds ensured rapid and synchronized germination along with improved seedling growth [65]. Consequently, priming treatment emerges as a promising tool in the restoration practices for recalcitrant seeds, reducing germination time and enhancing seedling vigour, thereby expediting plant production and field plantation.

Seed priming to alleviate biotic/abiotic stress

Plants encounter an array of abiotic and biotic stresses, posing impediments to their optimal growth and physiological functions. Seed priming, as a strategic intervention, has demonstrated efficacy in rectifying physiological deviations by fortifying the integrity of cellular processes.

Advantage of priming: user and environment friendly way for sustainable agriculture

Crops confront myriad abiotic and biotic stresses, culminating in substantial global crop losses. Optimal growth and heightened productivity face challenges such as erratic germination and compromised crop stand. Despite strides in scientific research to address these issues, extant solutions harbournotable drawbacks. The widespread use of chemical fertilizers and pesticides poses environmental hazards, while genetically modified (GM) food crops await approval in India. In this milieu, seed priming emerges as an eco-friendly and user-friendly alternative to mitigate stress, ensuring enhanced vigour and growth. Priming, a straightforward and apt technique, facilitates synchronized seed germination, heightened emergence, superior seedling establishment, diminished reliance on chemical fertilizers, augmented crop yield, and the induction of systemic resistance in plants. As global agriculture witnesses extensive development, direct-seeded cultivation gains prominence as a labour- and water-efficient alternative to transplanting, with seed priming facilitating synchronized seed growth in such contexts.

Priming stands out as an environmentally friendly and cost-effective approach compared to soil treatment or foliar sprays. This advantage stems from the absence of chemical additions to soil or air during priming, which occurs under controlled conditions before field sowing. Additionally, the resources required for priming are considerably lower compared to the substantial quantities of solutions needed for foliar spray or soil treatment. Consequently, priming stands as a superior seed treatment strategy, offering a favourable commencement to the germination process. Despite the longstanding interest in seed priming, the comprehensive understanding of the physiological and biochemical underpinnings of this intriguing process remains elusive. Emerging holistic approaches, particularly those involving omics tools, provide novel avenues to unravel the molecular intricacies of priming phenomena.

Seeds frequently undergo ageing during prolonged storage, resulting in diminished germination rates, impaired seedling emergence, and compromised plant growth at later stages. The reduced seed performance attributable to storage-related ageing is primarily linked to the accumulation of reactive oxygen species (ROS). A recent investigation highlights the potential of cathodic water priming in mitigating storage-induced deterioration in three South African tree species *Bolusanthus speciosus, Combretum erythrophyllum,* and *Erythrina caffra,*owing to its antioxidative properties. Cathodic water, an electrolyzed form of calcium magnesium (CaMg) solution, exhibits ROS-scavenging capabilities, presenting an effective solution to enhance the long-term conservation of orthodox seeds [66]. Comparable positive effects of cathodic water priming have been observed in deteriorated seeds of *Pisum sativum, Lycopersicon esculentum*, and *Cucurbita pepo* [67,68].

Storage of primed seeds

While extensively embraced in agriculture, the diminution of viability in primed seeds during storage could constitute a formidable constraint to the widespread adoption of seed priming technology. Subjecting primed seeds to prolonged storage under conditions of elevated relative humidity (RH) for over 15 days adversely impacts the germination and growth dynamics of rice [69]. This unfavourable outcome manifests as diminished starch metabolism, depletion of starch reserves within the rice endosperm, and an augmentation in detrimental physiological alterations.

Challenges and constraints

Seed priming, once an empirical agricultural practice, has now evolved with the integration of advanced methodologies and comprehensive techniques. While positioned as a promising approach among various seed invigoration strategies, it is essential to acknowledge and address certain limitations to fortify its efficacy. Notably, the success of the priming technique exhibits significant variability based on the species and type of seeds involved. Consequently, a standardized protocol is yet to be established, demanding meticulous consideration before implementation. Another pivotal challenge in the priming process is determining the optimal ‘stop’ time, emphasizing the necessity for precise standardization of the duration during which seeds are subjected to the priming solution before re-drying.

The storage of primed seeds introduces additional complexities, as highlighted earlier. Temperature and oxygen levels emerge as critical factors demanding meticulous control during the storage of primed seeds to augment their longevity [51]. Furthermore, challenges such as severe desiccation and susceptibility to microbial contamination during the priming process pose hindrances to optimal seed germination [70]. These considerations contribute to the complexity of the priming process, hindering its universal application, as it lacks a definitive 'one-size-fits-all' approach. Moreover, the expertise required for priming and its confinement to controlled laboratory conditions can create dependency among farmers on the supply of pre-primed seeds. Despite these challenges, seed priming maintains its widespread adoption globally.

Pulsing: a promising alternative of priming

The realm of priming, since its inception, has captivated researchers, yielding a plethora of works detailing diverse priming agents and their efficacy in alleviating various biotic and abiotic stresses, while simultaneously enhancing the overall growth and vitality of various plants, including the pivotal crop, rice. A recent noteworthy addition to this domain is the concept of 'Pulsing,' elucidated in a compelling report by [71,72]. Pulsing represents a nuanced variation of priming, involving a brief co-treatment of rice seeds with salt solutions during the initial germination phase (72 hours), followed by the cessation of treatment and subsequent growth in a hydroponic environment (Hoagland). Notably, pulsing has, thus far, exclusively utilized iron salt solutions at varying concentrations.

While priming bestows agricultural benefits, it is not without its limitations, notably in terms of long-term storage and the expertise required. Pulsing emerges as a promising alternative capable of overcoming these hindrances. Plants subjected to iron pulsing exhibit superior germination percentages, enhanced growth, and heightened vigour compared to their non-treated counterparts. Furthermore, the treated plantlets at the 14-day mark display elevated photosynthetic and nitrogen assimilation efficiency. Noteworthy is the absence of stress symptoms, coupled with an empowered antioxidant system attributed to the pulsing treatment. Intriguingly, the outcomes of pulsing mirror those of priming, yet pulsing stands out as a less time-intensive approach by circumventing the re-drying phase inherent in priming. Moreover, the simplicity of pulsing renders it easily replicable by farmers, as it demands less expertise while delivering optimal results. This groundbreaking report thus unveils a novel avenue for research, wherein diverse seeds can be subjected to various pulsing agents to surmount impediments hindering agricultural progress.

CONCLUSIONS

In the prevailing context of climate change and urbanization, the global challenge of ensuring food security confronts a dual threat of escalating food demand and diminishing water resources. This necessitates farmers to adopt strategies that not only enhance crop yield but also align with principles of user-friendliness and sustainability. As climate change tightens its grip, arable land shrinks rapidly, and various abiotic and biotic stressors pose detrimental effects on cultivation, there is an imperative to explore methods for augmenting the yield of existing crops. A study asserts that exclusively bolstering soil health can increase agricultural productivity by 10–15%, and when coupled with effective inputs, this figure can surge to 50–60% [73]. Various endeavours have been undertaken to improve crop yield, including the development of high-yielding and stress-tolerant varieties through traditional breeding or the introduction of genetically modified (GM) crops. While traditional breeding is widely practiced and accepted for commercialization, GM crops are still undergoing approval processes.

Amid these endeavors, seed priming emerges as a straightforward and cost-effective technique capable of conferring stress tolerance and amplifying production. In light of the escalating demand for food, increasing crop yield becomes imperative. Seed invigoration, an encompassing term for pre-sowing techniques, includes priming as one of its facets. Nanotechnology has emerged as a modern agricultural tool, with seed priming utilizing nanoparticles (nanopriming) gaining considerable attention over the past decade [74]. However, the long-term impact of nanoparticle application on soil microbiota and human health remains a topic requiring exploration, presenting an avenue for future research. Despite intriguing reports on priming, the continual advancement of science and research technologies leaves substantial room for the introduction of new ideas and facts about this fascinating technique in the foreseeable future. This offers a fertile ground for future researchers to delve into this realm and introduce novel priming agents for enhanced outcomes. Seed priming, indeed, stands as a boon to modern agriculture, and this review aims to shed light on investigations that promise to unveil more information and insights about priming**.** Moreover, it serves as a catalyst for establishing the molecular framework underlying the priming event, thereby paving the way for future developments**.**

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