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Recent Advances in Seed Enhancements

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Abstract

Seed quality is vital to sustainable crop production and food security. Seed enhancements include physical, physiological and biological treatments to overcome germination constraints by uniform stands, earlier crop development and better yields. Improved germination rates and seedling vigour are due to reduced emergence time by earlier start of metabolic activities of hydrolytic enzymes and resource mobilization. Nutrient homeostasis, ion uptake, hormonal regulation, activation of antioxidant defence system, reduced lipid peroxidation and accumulation of compatible solutes are some mechanisms conferring biotic and abiotic stress tolerance. Several transcription factors for aquaporins, imbibitions, osmotic adjustment, antioxidant defence and phenylpropanoid pathway have been identified. However, the knowledge of molecular pathways elucidating mode of action of these effects, reduced longevity of primed or other physical and biological agents for seed treatments and market availability of high-quality seeds are some of the challenges for scientists and seed industry. In this scenario, there is need to minimize the factors associated with reduced vigour during seed production, improve seed storage and handling, develop high-tech seeds by seed industry at appropriate rates and integrate agronomic, physiological and molecular seed research for the effective regulation of high-quality seed delivery over next generations.

Keywords: seed priming, biopriming, coating, magnetic seed stimulation, seed vigour
1. Introduction

Good-quality seed has a significant potential of increasing on-farm productivity and enhancing food security [1]. Seed quality is the foundation for profitable production and marketing [2, 3]. High-quality seeds are genetically and physically pure, vigorous and free from insect pests and pathogens [4]. High-quality seeds with enhanced vigour contribute nearly 30% of the total production. Plant uniformity is an expression of high seed quality achieved by high vigour of seeds [5]. Seed quality is influenced by several factors during seed development, such as maturation, harvesting, drying, cleaning, grading, packing and storage. Farmers and growers are constantly looking for high-quality seeds to ensure uniform field establishment and increased production [6].

Availability, quality and cost of seeds influence the global production and ultimately food security [7]. The informal seed systems (farmers organized and managed without legal documentation) constitute for 75–90% of their food crop cultivation [8]. In the developing world, informal seed systems remain the prevailing source of seed for smallholder farmers. Improper storage environment, sensitivity of germinating seeds and young seedlings to dehydration stress lead to loss of desiccation tolerance with seed hydration [9–11] and predicted climate change (erratic rainfall patterns and unpredictable temperature extremes) may further exacerbate seed quality. Low-vigour seeds can be improved using a variety of seed technologies that will thrive under small holder cultivation conditions and also improve the supply of good-quality seed in the local seed industry.

Efficient seed germination and early seedling establishment are important for commercial agriculture because they represent the most susceptible stages of the life cycle of crop plants [12]. Rapid and uniform seedling emergence leads to successful establishment as it produces a deep root system before the upper layers of soil dry out, harden, or reach supra-optimal temperatures [13]. Germination begins with water uptake by seed and ends with the emergence of the embryonic axis, usually the radicle [14]. A wide range of techniques are now used to help sowing seeds and to improve or protect seedling establishment and growth under the changing environments and seedbed constraints. These techniques constitute the postharvest processing necessary to prepare seed for sowing and optional treatments that are generally described in the industry and scientific literature as ‘seed enhancements’ or ‘seed treatments’. Many scientists have suggested techniques for improving crop germination performance in the field keeping in view the responses of seed to temperature and water availability in the soil. These techniques may be differentiated into physiological (seed priming, coating and pelleting), physical (magnetic, radiation and plasma) and biological (seed enhancements) aspects [15–20]. In 2015, the projected value by global chemical seed treatment industry was up to $ 5.4 billion. Bayer Crop Sciences and Syngenta have 75% share in seed treatment market. In this chapter, we will focus on physiological, biological and physical enhancements of seeds.

Several reports are available, for instance, Heydecker and Coolbear [15] had reported on seed treatments to break dormancy, improve germination and impart stress tolerance and subsequently Taylor et al. [17] continued this work. Halmer [18, 21] focused on practical aspects of seed treatment technologies and categorized it into conditioning, protection and physiological
enhancements. Bray [22] and McDonald [23] further continued work on exploring the mechanisms of physiological enhancements especially seed priming. Regarding physical enhancement, only one key review [24] addressed the effect of magnetic field on growth and yield of crops without primarily focusing on seed germination. Up to now, 1253 research articles have been published on seed priming and out of that almost 100 articles are being published every year since 2010. While there is no consistency in publications on magnetic field treatments, i.e., on this topic roughly 3–4 publications per year and a total of 164 articles have been published up to now [25]. With the recent advances in molecular biology of seeds, here we presented conceptual insights into physiological, biochemical, morphological and biophysical markers that can be used for further improvement of seed quality of crop plants. In addition, in-depth mechanisms of seed germination promotion by physiological, biological and physical seed treatments have also been discussed.

2. Seed enhancements

Seed enhancements or seed invigoration are the post-harvest treatments used for improving the germination and growth of seedlings required at the time of sowing [17]. Many shotgun approaches are being used for seed enhancement for the last 24 years, which includes seed priming, magnetic stimulation, seed pelleting and coating [17, 26, 27].

2.1. Seed enhancement using physical agents

Physical treatments are applied externally without any hydration or application of chemical materials to the seeds. The main purpose is to enhance germination and seedling establishment. The mechanism of seed invigoration with physical techniques is still unknown. The work on exposure of seeds to radiation was started in early 1980s and now a number of studies have been focused on the use of plasma technology for seed invigoration of agronomic and horticultural crops. Magnetic field treatments are being considered as effective seed enhancement tools for agronomic and horticultural crops; however, their application is limited at large scale. Among physical methods, magnetic field and irradiation with microwaves or ionizing radiations are the most promising pre-sowing seed treatments [28]. Thus, physical seed enhancements are an alternative approach to other chemical seed invigoration treatments, which provide better solution for the growing world seed market.

2.1.1. Magnetic fields for seed treatments

Magnetic seed stimulation involves identifying the magnetic exposure dose to affect the germination, early seedling growth and subsequent yield of crop plants [29]. The magnetic exposure dose is the product of the flux density of magnetic field and of the time to exposure. The flux density of magnetic field varies with static or alternating magnetic fields exposure to seeds. Magnetic field ensures the quick germination, uniform crop stand establishment and yield of many agronomic and horticultural crops [30, 31]. These not only increase the rate of germination, growth and yield [32] but also reduce the attack of pathogenic diseases [33, 34].
Magnetic field exposure increases the germination of non-standard seeds and also improves their quality. Magnetic field influences the initial growth stage of the plants after the germination [35]. In recent years, work on magnetic-treated water revealed that plant growth and seed germination were improved by priming [36].

2.1.2. Plasma seed treatments

Plasma application in agriculture and medicine is a recent advancement [37–40]. The agricultural aspects include seed germination and plant growth. Many researches report that germination and growth enhancement mechanism is affected by use of plasmas with several gases as aniline, cyclohexane and helium [41, 42]. To enhance seed development and plant-growth microwave plasma, magnetized plasma and atmospheric plasma are adopted treatments [43, 44]. The effect of gases is much commonly studied in plasmas treatments. Various reports revealed that the quality of plant development controlling thiol groups is diversified by redox reaction persuaded by the active oxygen species of water vapour plasma [45].

Non-thermal plasma radiations are applied in agriculture as alternative to scarification, stratification and priming helped to improve the plant growth [46]. Plasma helps to attain zero seed destruction, no chemical use and environment friendly treatments to seeds [41, 46, 47]. Plasma treatment improves seed quality and plant growth [43, 48]. Seed exposure to plasma also resulted in alterations of enzymatic activity [45] and caused sterilization of seed surface [47].

Plasma chemistry can tune seed germination by delaying or boosting with application of plasma-treated deposits on seed surfaces [41]. The recent important plasma-related investigation includes the practice of microwave discharges [43] and low-density radio frequency (RF) discharges [49, 50]. The discharge of atmospheric pressure and the discharge of coplanar barrier have been assessed in recent studies [41, 48]. The investigation of various seed germination patterns was implemented on different seeds including wheat, maize, radish, oat, safflower and blue lupine [43, 46, 48, 50]. Safflower seeds expressed 50% greater germination rate when treated with radio frequency plasma for 130 min with argon [46]. Soybean seeds were treated with cold plasma treatment with 0, 60, 80, 100 and 120 W for 15 s and found positive effects of cold plasma treatments on seed germination and seedling growth of soybean [51].

2.1.3. Radiation seed treatments

With recent advancements in agriculture, gamma radiations can improve plant characteristics such as precocity, salinity tolerance, grain yield and product quality in suboptimal environment depending upon the level of irradiation [52]. Second, gamma radiation can also sterilize agricultural products to prevent pathogen infestation thus increasing conservation time during storage and trading [53].

The biological effects of radiations is based on chemical interaction with biomolecules and water to produce free radicals that can manipulate biomolecules and induce cell to switch on antioxidant system [54] that prepared the defensive shield against upcoming stresses [55, 56].
In spite of the conventional seed enhancements, physics has manipulated radiation dose to trigger biochemical reactions necessary for seed germination without affecting seed structural integrity and collateral DNA damage [57]. It was found that the low dose of gamma radiation (up to 20 Gy) on germination of three varieties of Chinese cabbage shows a positive impact [58].

2.2. Physiological seed enhancements

2.2.1. Seed priming

Seed priming is a pre-sowing approach for influencing the seedling development by stimulating pre-germination metabolic activities prior to the emergence of radicle and improvement in the germination rate and performance of plant [16, 17]. Seed priming is a controlled hydration process in which seeds are dipped in water or any solution for a specific time period to allow the seed to complete its metabolic activities before sowing and then re-dried to original weight [15, 16].

Primed treatments include osmopriming by polyethylene glycol (PEG) or a salt solution [59], hydropriming [16, 60], solid matrix priming in which seeds are soaked in inert medium of known matrix potential [63] and hormonal priming [62]. A balance of water potential between osmotic medium and seed is necessary for conditioning, and different non-penetrating agents such as organic solutes and salts are used for this purpose [63]. Furthermore, these priming treatments show positive response only at sub-optimal or supra-optimal field conditions such as drought [64], excessively high or low temperatures [60, 65] and salinity [59].

2.2.1.1. Hydropriming

Hydropriming is a controlled hydration process that involves seed soaking in simple water and then re-drying to their initial moisture [59, 63]. No chemical is used during this technique but some cases of non-uniform hydration causes uneven germination [66]. Among the different seed enhancement techniques, hydropriming could be a suitable treatment under salinity stress and drought-prone environments [67].

Hydropriming as a risk free, simple and cheap technique has become popular among farmers, with promising effects in the context of extensive farming system [68]. Hydroprimed seeds produced healthy seedlings, which resulted in uniform crop stand, drought resistance, early maturity and somewhat improved yield.

2.2.1.2. Osmopriming

Osmopriming involves seed hydration in an osmotic solution of low water potential such as polyethylene glycol or a salt solution under controlled aerated conditions to permit imbibition but prevent radical protrusion [67]. For osmopriming, mostly polyethylene glycol or salt solution is used to regulate water uptake and to check radicle protrusion [64]. Most commonly used salts for osmopriming are potassium chloride (KCl), potassium nitrate (KNO\textsubscript{3}), sodium chloride (NaCl), magnesium sulphate (MgSO\textsubscript{4}), potassium phosphate (K\textsubscript{3}PO\textsubscript{4}), calcium chloride (CaCl\textsubscript{2}) and potassium hydrophosphate (KH\textsubscript{2}PO\textsubscript{4}). All these salts provide nutrient...
like nitrogen to the germinating seed, which is required for the protein synthesis during the germination process. However, these salts rarely cause nutrient toxicity to the germinating young seedlings [63]. Osmopriming induced more rapid and uniform germination and resulted in decreased mean germination time.

2.2.1.3. Hormonal priming

Plant-growth hormones or their derivatives contained by several products are indole-3-butyric acid (IBA), an auxin and kinetin type of cytokinin. Cytokinins play a vital role in all phases of plant development starting from seed germination up to senescence [70]. Priming with optimum concentration of cytokinins has been reported to increase germination, growth and yield of many crop species [16]. Gibberellic acid (GA$_3$) is known to break seed dormancy, enhance germination, hypocotyl growth, internodal length, and cell division in the cambial zone and increase the size of leaves. GA has stimulatory effect on hydrolytic enzymes, which speed up the germination and promote seedling elongation by degrading the cells surrounding the radicle in cereal seeds [69, 71].

Various naturally occurring growth promoting substances such as moringa leaf extract, chitosan, sorghum water extract and seed weed extract [62, 65] are commonly used for seed priming. Moringa (Moringa oleifera L.) as a natural source of plant-growth regulators contains cytokinins as zeatin [72]. In addition, moringa leaf extracts contain higher concentrations of various growth enhancers such as ascorbates, phenolic compounds, K, and Ca. Priming maize seed with moringa leaf extract reduces mean germination (MGT) and T$_{50}$ with increased germination index and germination count that ultimately improved seedling growth by increasing chlorophyll content, amylase activity and total sugar contents under chilling conditions [62]. Moringa leaf extract diluted up to 1:36 with water was applied on various field crops and 35% increase in the yield of sugarcane, sorghum, maize, turnip and bell pepper was observed [72]. Nonetheless, moringa leaf extracts being low cost can be a viable option for improving the productivity of resource poor farmers.

2.2.1.4. Nutrient priming

The application of micronutrients with priming can improve stand establishment, growth and yield; furthermore, the enrichment of grain with micronutrients is also reported in most cases [73]. Many researchers proved the potential of nutrient priming in improving wheat, rice and forage legumes. Among micronutrients, Zn, B, Mo, Mn, Cu and Co are highly used as seed treatments for most of the field crops [74–76].

Seed treatment with micronutrient is a potentially low-cost way to improve nutrition of crops. Farmers have responded in South Asia in a positive way in the seed treatment, which is a simple technique soaking seeds in water overnight before planting [77]. Seed priming with zinc salts is used to increase growth and disease resistance of seedlings.
2.3. Biological seed enhancements

2.3.1. Bacterial seed agents

Plant-growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, which when applied to soil, seeds or roots promote the growth of the plant or reduce the incidence of diseases from soil-borne plant pathogens. PGPR can influence plant growth either directly or indirectly through fixation of atmospheric nitrogen, solubilization of phosphorus and zinc and producing siderophores, which can solubilize/sequester iron, synthesize phytohormones, including auxins, cytokinins and gibberellins to stimulate plant growth, and synthesize ACC-deaminase enzyme by modulation of ethylene level under stress conditions [78, 79].

Among various genera of PGPR endophytes are good priming agents because they colonize roots and create a favourable environment to develop and function with their hosts—symbiotic partner.

Biopriming is a new technique of seed enhancement integrating biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects (seed hydration) to promote plant growth, development and suppression of diseases. It is used as an alternative approach for controlling many seed- and soil-borne pathogens. Seed priming with beneficial microorganisms (bacteria and fungus) often result in more rapid growth and increase plant vigour and may be useful under adverse soil conditions. Besides diseases control, the application of PGPR as a biopriming agent for biofertilization is an attractive option to reduce the use of chemical fertilizers [80, 81]. PGPR that have been tested as co-inoculants with rhizobia include strains of the following rhizobacteria: Azotobacter [82], Azospirillum [83], Bacillus [84], Pseudomonas [85, 86], Serratia [86] and Streptomyces [87].

2.3.1.1. Role of a bacterial biopriming agent in plant-growth promotion

The Plant growth promoting bacteria (PGPB) are a heterogeneous group of beneficial microorganisms present in the rhizosphere, on the root surface or inside plant tissues, and are able to accelerate the growth of plants and protect them from biotic and abiotic stresses [88–90]. Beneficial effects of biopriming have been reported in several vegetable seeds [91]. Priming of tomato seed with beneficial bacteria improved the rate of germination, seedling emergence and growth of plant [92]. The beneficial response of biopriming on seed germination and seedling vigour in chilli was reported [93]. Similarly, improvement in okra growth and yield was reported up to 60% when seeds were bioprimed with P. fluorescens culture [94]. In experiments where lettuce plants were treated with Bacillus strains, it was observed that after two weeks the tissues of roots and shoots contained a greater amount of cytokinin than control plants [95, 96]. The accumulation of cytokinins was associated with a 30% increase in plant biomass.
2.3.1.2. Role of a bacterial biopriming agent in plant disease control

Seed enhancement by biopriming agents involves coating/soaking the seed with one biological agent or microbial consortium, then incubating the seed under optimum (temperature, moisture) conditions.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Target plant</th>
<th>Condition</th>
<th>Proposed mechanism</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rhizobium leguminosarum bv. Viciae</strong></td>
<td>Faba bean (Vicia faba)</td>
<td>Green house and field</td>
<td>Improved nitrogenase activity and production of IAA</td>
<td>Increased nodulation and nitrogen fixation under drought and salinity stress</td>
<td>[19]</td>
</tr>
<tr>
<td><strong>Pseudomonas spp. NUU1 and P. fluorescens NUU2</strong></td>
<td>Wheat (Triticum aestivum)</td>
<td>Pot experiment</td>
<td>Auxin production</td>
<td>Stimulated the shoot and root length and dry weight</td>
<td>[149]</td>
</tr>
<tr>
<td><strong>Pseudomonas fluorescens MSP-393</strong></td>
<td>Rice (Oryza sativa)</td>
<td>Green house</td>
<td>Production of osmolytes</td>
<td>Increased plant growth and vigour</td>
<td>[150]</td>
</tr>
<tr>
<td><strong>Pseudomonas putida GAP-P45</strong></td>
<td>Sunflower (Helianthus annuus)</td>
<td>Field experiment</td>
<td>Production of exopoly saccharides, biofilm</td>
<td>increased the survival,[151] plant biomass, and root adhering soil/root tissue ratio of sunflower seedlings under drought stress</td>
<td></td>
</tr>
<tr>
<td><strong>Rhizobium and Pseudomonas species</strong></td>
<td>Maize (Zea mays)</td>
<td>Pot experiment</td>
<td>decreases in osmotic potential, and increase in osmoregulant (proline) production, maintenance of relative and selective uptake of K ions.</td>
<td>Promote plant growth and increased relative water contents</td>
<td>[152]</td>
</tr>
<tr>
<td><strong>Pseudomonas chlororaphis isolate TSAU13</strong></td>
<td>Cucumber (Cucumis sativus) and Tomato (Solanum Lycopersicum)</td>
<td>Green house</td>
<td>Antibiosis</td>
<td>Stimulated shoot growth, dry matter and the fruit yield of tomato and cucumbers under saline conditions</td>
<td>[153]</td>
</tr>
<tr>
<td><strong>T. Harzianum T22 Rifai KRL-AG2</strong></td>
<td>Onion (Allium cepa L.)</td>
<td>Axenic trial</td>
<td>Osmotic adjustment through physiological responses</td>
<td>Increased germination %age, shoot length and seedling fresh weight under saline conditions</td>
<td>[20]</td>
</tr>
<tr>
<td><strong>Piriformospora indica</strong></td>
<td>Chinese cabbage (Brassica rapa subsp. pekinensis)</td>
<td>Pot experiment</td>
<td>Involved in expression of diverse stress-related genes</td>
<td>Promotes root and shoot growth, and promotes lateral root formation</td>
<td>[154]</td>
</tr>
<tr>
<td><strong>Neotyphodium</strong></td>
<td>Arizona fescue (Festuca arizonica Vasey)</td>
<td>Green house</td>
<td>Regulate stomatal conductance</td>
<td>Increased relative growth rates, High W.U.E and biomass yield under drought</td>
<td>[155]</td>
</tr>
</tbody>
</table>

Table 1. Observed effects of plant-beneficial bacteria in regard to plant-growth promotion and stress tolerance.
Biopriming of seeds with different bacterial strains particularly rhizobacteria have been shown to be effective in suppressing disease infection by inducing a resistance mechanism called ‘induced systemic resistance’ (ISR) in varied agronomic and horticultural crops [97]. Among various bacterial genera, \textit{Bacillus} and \textit{Pseudomonas} spp. are ubiquitous rhizosphere inhabitant bacteria that are the most studied biopriming agents reported as disease suppressing in plants [98]. Priming seeds of many crops with biological control agents (BCA), \textit{Bacillus subtilis} and \textit{Pseudomonas fluorescens} are the most effective approach for controlling seed and root rot pathogens [99, 100] and as a substitute for chemical fungicides without any risk to human, animal and the environment.

2.3.1.3. Seed enhancement by alleviating abiotic stresses using biopriming

Seed priming with beneficial microorganisms may promote plant growth and increases abiotic stress tolerance in arid or semiarid areas [101]. PGPB are adapted to adverse conditions and protect plants from the deleterious effects of these environmental stresses, thus increasing crop productivity [102]. Bioprimed seeds with \textit{Enterobacter} sp. P-39 showed maximum improvement in germination and seedling growth of tomato under osmotic stress [103]. Table 1 shows the selected examples of beneficial response of biological inoculants for enhancing growth and yield of various crops under normal and stress conditions.

2.3.2. Fungal seed agents for biopriming

In this approach, beneficial bacterial and fungal agents are exploited for the purpose of biopriming of seeds to enhance growth, yield and mitigation of biotic and abiotic stresses. It is an environmental friendly, socially accepted approach and also offers an alternative to the chemical treatment methods gaining importance in seed, plant and soil health systems. Seed biopriming enhanced drought tolerance of wheat as drought-induced changes like photosynthetic parameters and redox states were significantly improved by \textit{Trichoderma} sp. under stress conditions over control. Very recently, Junges et al. [84] compared the potential of biopriming (\textit{Trichoderma} and \textit{Bacillus} spp.) with commercial available products Agrotrich plus® and Rhizoliptus® for enhancing growth and yield of beans. Results revealed that biopriming with spore or bacterial cell suspensions promoted bean seedling growth compared to other techniques.

2.4. Seed coating and pelleting

Seed film coating, pelleting, priming and inoculation are globally practiced seed treatments [104] used with the objectives of enhancing plantability, distribution, germination and storage of seeds. These techniques aim to apply adhesive films, fungicides, herbicides, growth promoters and biological agents [3, 91, 105]. Seed coating is carrier of chemical materials to support seedling growth [106]. Compounds such as growth regulators, inoculants, micronutrients, fungicides, insecticides and other seed protectants are applied to the pellet to enhance seed performance [107].
Seed coating demands uniform application of inert material over the seed surface. This also helps to protect the seed from soil and seed-borne pathogens [17]. Pharmaceutical industry uses seed polymer coating for a constant application of numerous materials to seeds. The commercially available plasticizers, polymers and colourants (commercially they are readily available to be used as liquid) are applied as film formulations [108]. However, the exact composition of coating material is a carefully guarded secret by the companies who develop them. Usually, coating material contains binders, fillers (e.g., polyvinyl alcohol, gypsum and clay) and an intermediate layer (e.g., clay, polyvinyl acetate and vermiculite). Seed agglomeration is an alternate coating technology with the purpose to sow multiple seeds of the same seed lot, or multiple seeds of different seed lots, varieties or species [109].

3. Mechanisms of seed enhancements

3.1. Physiological and biochemical aspects

Improved crop performance through pre-sowing treatments depends on the nature of compounds used for priming and their accumulation under abiotic stresses. These compounds include inorganic salts, osmolytes, phytohormones, tertiary amino compounds such as glycinebetaine, amino acids and sugar alcohols including bioactive compounds from microorganisms. For instance, the application of compatible solutes as seed priming improves salinity resistance by cytosolic osmotic adjustment indirectly by enhancing regulatory functions of osmoprotectants [110, 111]. Chilling-induced cross-adaptation salt tolerance in wheat is associated with enhanced accumulation of beneficial mineral elements (K⁺ and Ca²⁺) in the roots and reduced uptake of toxic Na⁺ in the shoots through ionic homeostasis and hormonal balance with greater concentrations of indoleacetic acid, abscisic acid, salicylic acid and spermine in chilled wheat seeds [112]. In flooded soils, improved stand establishment in rice through seed priming is related to enhanced capacity of superoxide dismutase (SOD) and catalase (CAT) activities to detoxify the reactive oxygen species in seeds and greater carbohydrate mobilization. These effects are more pronounced in tolerant genotypes that emphasize to combine crop genetic tolerance with appropriate seed treatments to improve seedling establishment of rice sown in flooded soils [113].

Such enhanced remobilization efficiency in seed embryos of cereals coated with hydroabsorbers is related to change in activities of enzymes for sucrose breakdown upon moisture absorption. Coated seeds absorb more moisture that creates anoxic conditions in developing embryos but genetic difference are found for sucrose breakdown in rye, barley and wheat with change in invertase activities due to difference in timing of imbibitions [114].

Beneficial effects of magnetic seed stimulation are associated with various biochemical, cellular and molecular events [115]. Pre-sowing magnetic seed treatment also increases ascorbic acid contents [33] by stimulating the activity of the enzymes and proteins [116]. Physiological and biochemical properties also increase due to enhanced metabolic pathway by the free movement of ions [117]. However, its biochemical and physiological mechanisms are still poorly understood [118].
3.2. Molecular aspects

Favourable effects of priming at cellular level include RNA and protein synthesis [22]. Seed priming induces several biochemical changes within the seed needed for breaking seed dormancy, water imbibition, enzymes activation, hydrolysis of food reserves and mobilization of inhibitors [119]. At cellular level priming initiates cell division transportation of storage protein [120]. Higher germination rate and uniform emergence of primed seed is due to metabolic repair with increased production of metabolite required for the germination [121, 122] during the imbibition process. Priming increased the production and activity of α-amylase within germinating seeds, thus increased the seed vigour [65].

Several proteins and their precursors for regulation involved in different steps of seed germination or priming have been identified using model plant Arabidopsis. The expression of these proteins such as actin isoform or a WD-40 repeat protein occurs in imbibition and cytosolic glyceraldehyde-3-phosphate dehydrogenase in the seed dehydration process [123]. Priming-induced changes in proteins levels have been identified as peroxiredoxin-5, 1-Cys peroxiredoxin, embryonic protein DC-8, cupin, globulin-1 and late embryogenesis abundant protein. The expression of these proteins led to improved seed germination and the expression of these embryo proteins remained unchanged even after priming [124].

A major quantitative trait locus (QTL) Htg6.1 of seed germination responsive to priming under high temperature stress using a recombinant inbred line (RIL) of lettuce (Lactuca sativa L.) has been identified. The expression of this QTL at high temperature is coded by a gene LsNCED4 encoding the key enzyme, i.e., 9-cis-epoxycarotenoid dioxygenase, of the abscisic acid biosynthetic pathway and maps precisely with Htg6.1. However, LsNCED4 gene expression was higher in non-primed seeds after 24 h of imbibition at high temperature compared to the expression of LsGA3ox1 and LsACS1 genes encoding enzymes of gibberellins and ethylene biosynthetic pathways, respectively. LsNCED4 gene expression was reduced after priming and when imbibition was carried out at the same temperature. The seed response to priming in terms of germination and temperature sensitivity is associated with temperature regulation of hormonal biosynthetic pathways [125].

Osmopriming induced quantitative expression of stress-responsive genes such as CaWRKY30, PROX1, Osmotin for osmotic adjustment, Cu/Zn SOD for antioxidant defence and CAH for phenylpropanoid pathway. The same genes were induced earlier or at higher levels in response to thioeara priming at low temperature. The expression of these genes imparts cold tolerance in capsicum seedlings [126]. Notably, high levels of other plant-growth hormones, such as indoly1-3-acetic acid (IAA) and abscisic acid (ABA), were also observed. The authors suggested that Bacillus strains have dual effect on plant-growth promotion and accumulation of cytokinins by increasing other routes of synthesis of hormones such as IAA and ABA, as well as interfering in other hormonal balance synthesis such as gibberellins (GA).

Using advanced molecular tools such as proteomics may help to detect protein markers that can be used to unravel complex development process of seed vigour of commercial seed lots, or analysis of protein changes occur in industrial seed priming treatments to accelerate seed germination and improve seedling uniformity.
4. Seed enhancements and plant development

4.1. Modulation of seedling growth

Seedling vigour is important to help ensuring good crop establishment. Pre-sowing seed treatments offer pragmatic solution to poor seedling establishment by overcoming the germination constraints under normal and adverse conditions. Several researches have shown the potential of chemical priming, use of macro- and micronutrients, natural compounds of plant origin and plant-growth-promoting bacteria including water under greenhouse and field conditions. Most of the priming techniques such as osmopriming and on-farm priming have been optimized for specific crops for soaking duration and concentration. For chemical priming, polyamines including spermine, spermeidine and putrescine, calcium chloride (CaCl$_2$), potassium chloride (KCl), NaCl, KH$_2$PO$_4$, KNO$_3$, PEG, hydro-absorbers such as humic acid and biplantol for seed coating and naturally occurring molecules such as nitric oxide (NO), hydrogen sulphide (H$_2$S), HO$_2$, ascorbate, salicylic acid, indoleamine molecule melatonin (Mel) and most recently growth promoting cytokinin-rich moringa leaf extracts are commonly being evaluated. The endogenous levels of naturally occurring molecules when applied as seed priming may increase initially and later with subsequent improved growth.

The beneficial effects of seed priming have been documented in cereals, sugar crops, oilseeds and horticultural crops. Early seedling growth by pre-sowing seed treatments is due to improved germination rate, reduced time of germination or emergence, and uniform and enhanced germination percentage contributed by enhanced mobilization of germination metabolites from endosperm towards growing embryonic axis. However, variation in germination rates with seed coating thickness and composition has been found which ultimately affects the mobilization efficiency of seed reserves. Therefore, the use of hydro-absorbers is suggested for coated seeds to enhance the efficiency of germination metabolites which may differ among species [114].

Seed priming with nutrients usually increases the seed contents of primed nutrients, which may be translocated to the growing seedling to support the seedling development [127]. Improved seedling growth and dry mass may be attributed to enhanced nutrient uptake and enzymes associated under deficient conditions and offer perspective for improved seed quality at crop harvesting [128]. Priming mediated by manganese (Mn) has also significant effect on the growth and yield performance of crops. In comparison to soil application, Mn priming improved stand establishment, growth, yield and grain contents [129]. Another researcher also noticed that priming with cobalt nitrate had increased growth attributes and subsequent yield of pigeon pea [130].

The concentration of these nutrients may be toxic when used in relatively higher concentration. For instance, priming with 0.5% Boron solution completely suppressed the germination and growth in rice [27] and 0.1 M ZnCl$_2$ and 0.5 M ZnSO$_4$ in wheat [131]. Seed priming induced early vigour indices have been associated with suppression of weeds in primed stand of aerobic rice [132]. Germination, shoot biomass and total root length were increased in seeds of cultivar IR74 containing Pup1 QTL after water priming. This suggests that seed management ap-
proaches may be combined with genetics to improve the crop establishment in different crops including rice under P-deficient conditions [133].

Pre-sowing magnetic seed treatment of wheat seeds has an effect on the germination, and the growth rate was increased to 23% while the germination rate was 100% in the laboratory and less time was taken with 15 min treatment [134].

4.2. Effects on crop phenology

Plants grown by primed seeds usually emerge faster and complete other developmental stages such as tillering, flowering and physiological maturity earlier than seeds without priming [27, 73, 107, 131]. This developmental plasticity of priming may be beneficial when crop planting is delayed due to adverse climatic conditions such as low temperature or high rainfall at sowing, high temperature at reproductive stage and may help plant to avoiding detrimental conditions by earlier maturity [135] without yield decrease. In fact, earlier and vigorous crop stand usually captures more resources of water and nutrients through better root system and had larger leaf area and duration with enhanced photo-assimilation that subsequently contributes towards better yield [73, 131]. However, integrated studies combining seed priming with other crop husbandry practices such as planting geometry, irrigation and fertilization may be interesting in crop stress and nutrient management for improved resource use efficiency.

4.3. Yield improvement

Seed priming benefits are not usually end up with improved crop stand. Several studies report long-lasting effects on yield-associated advantages in terms of increased growth rates, high dry matter production and produce quality by improving crop resistance to biotic and abiotic stresses. A very few reports showing no yield improvement by seed priming are available [136, 137]. Seed priming improved yield is due to reduced weed biomass, higher leaf area index and panicles/m² in aerobic and submerged rice, respectively [132, 138, 139], improved crop nutritional status of nutrients primed in maize under low temperature stress [127], comparatively better dry matter production with higher tissue Zn concentration with Zn seed priming in rice [140], reduced spikelet sterility in direct seeded rice irrigated with alternate wetting and drying (AWD) [131] and under system of rice intensification (SRI) condition with improved crop growth and higher tillering emergence [141]. Likewise, early planting spring maize stimulated seedling growth due to increased leaf area index, crop growth and net assimilation rates, and maintenance of green leaf area at maturity [135], better stand establishment in no tilled wheat under rice-wheat system [142], with enhanced tillering emergence and panicle fertility and with B nutrition under water saving rice cultivation [143], GA3 priming induced modulation of ions uptake (Na+, K+) and hormonal homeostasis under salinity in wheat [144], in combination with gypsum + FYM treatment by ameliorating effects on plant growth [145] and improving performance of poor quality wheat seeds under drought stress [146]. Nonetheless, another researcher observed improved yield due to stand establishment and increasing panicle number by coating rice seeds with Zn-EDTA or ZnO or Zn lignosulfonate [147].
<table>
<thead>
<tr>
<th>Physical treatments</th>
<th>Mode of application</th>
<th>Plant species</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetically treated water</td>
<td>Magnetized water (0.32 T), 20 ml of water daily</td>
<td>Chickpea (Cicer arietinum)</td>
<td>Increase in plant length, increased photosynthesizing property of plant</td>
<td>[156]</td>
</tr>
<tr>
<td>Non-uniform magnetic field</td>
<td>120 mT (rms) for 3 min, 160 mT (rms) for 1 min, and 160 mT (rms) for 5 min.</td>
<td>Lettuce (Lactuca sativa)</td>
<td>Improved growth and final yield</td>
<td>[157]</td>
</tr>
<tr>
<td>Magnetic seed stimulation</td>
<td>150 mT for 0, 3, 6, 9 and 12 min</td>
<td>Lentil (Lens culinaris)</td>
<td>Improved growth of the plant, increased stem length and total mass. Increased root length</td>
<td>[30]</td>
</tr>
<tr>
<td>Magnetic seed stimulation</td>
<td>160 mT MF strength for 1 min</td>
<td>Tomato (Solanum lycopersicum)</td>
<td>Improved germination</td>
<td>[158]</td>
</tr>
<tr>
<td>Stationary magnetic fields</td>
<td>125 and 250 mT for 1, 10 and 20 min, 1 and 24 h and continuous exposure</td>
<td>Pea (Pisum sativum)</td>
<td>Stimulating effect on the first stages of growth</td>
<td>[159]</td>
</tr>
<tr>
<td>Magnetic seed stimulation</td>
<td>25, 50, 75, 100 and 125 mT for 3 min</td>
<td>Marigold (Tagetes)</td>
<td>The results suggest that magnetic field treatments of French marigold seeds have the potential to enhance germination, early growth and biochemical parameters of seedlings</td>
<td>[118]</td>
</tr>
<tr>
<td>Magnetic seed stimulation</td>
<td>150 mT for 3 min</td>
<td>Maize (Zea mays)</td>
<td>Induced chilling stress tolerance primarily by improving stand establishment, phenology, allometry, agronomic traits and yield components</td>
<td>[60]</td>
</tr>
<tr>
<td>Magnetic seed stimulation</td>
<td>25, 50, and 75 mT for 15, 30 and 45 min each</td>
<td>Bitter gourd (Momordica charantia)</td>
<td>Improved emergence, growth, yield and yield related parameters</td>
<td>[61]</td>
</tr>
<tr>
<td>Low-temperature cold Plasma Treatment</td>
<td>Seeds were coated with tetrafluoride (CF$_4$) or octa deca fluoro decalin (ODFD)</td>
<td>Radish (Raphanus sativus)</td>
<td>60% in the sprout lengths of radish exposed to oxygen RF plasma</td>
<td>[41]</td>
</tr>
<tr>
<td>Low-temperature cold plasma treatment</td>
<td>Seeds were coated with tetrafluoride (CF$_4$) or octadecafluorodecalin (ODFD)</td>
<td>Pea (Pisum sativum)</td>
<td>The germination rate was increased by 30% whereas no change was seen in the seeds treated at higher pressure relative to control for the same treatment time</td>
<td>[41]</td>
</tr>
</tbody>
</table>
Crop emergence, crop growth and development of two pea varieties with a significant increase in the seed yield have been reported. It was reported that contents of sugar were increased with magnetic seed stimulation in sugar beet roots, and gluten contents were also increased in wheat kernels when magnetic field was applied to the seeds before sowing [148]. Similarly, many researchers had reported higher grain yield due to improved stand establishment, growth and development in agronomic and horticultural crops (Table 2).

Nonetheless, priming effects are not only limited to stand establishment and yield, water productivity and uptake of beneficial minerals with reduction in harmful ion but the quality of harvested produce is also improved [60, 131, 135]. Thus, it offers promising and economical solution to improve crop resistance against low and high temperature, flooding and drought, salinity and nutrient stress and effective strategies for agronomic biofortification when combined with soil management and crop genetics.

### 5. Conclusions and future prospects

Seed enhancements have a wide range of commercial applications from improved crop stands through better germination rates and seedling vigour effective in crop stress management, and improved crop yields together with efficient use of resources such as fertilizers, water and seeds. Sustainable crop production requires the adoption of low-cost and environment friendly seed enhancement techniques. Biological seed enhancement with bacteria and fungi is one of the most appropriate techniques in disease control and growth promotion which can be exploited by seed industry.

The biochemical pathways by which these techniques affect different processes regulating growth and development need to be elucidated.

Longevity of primed seeds during storage remains a problem, which needs to be re-addressed, and work should be extended on other physical or biological seed treatments for their storability.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Gamma rays</td>
<td>0.17 kGy with dose rate 2.18 kGy/h</td>
<td><em>Wheat</em> (<em>Triticum aestivum</em>)</td>
<td>Increase in percentage emergence</td>
<td>[160]</td>
</tr>
<tr>
<td>UV-C</td>
<td>254 nm for 60 min</td>
<td><em>Ground nut</em> (<em>Arachis hypogaea</em>)</td>
<td>Effect on seed germination, seedling growth and productivity</td>
<td>[161]</td>
</tr>
<tr>
<td>Gamma rays</td>
<td>10, 15, 20, 25 and 30 Krad doses</td>
<td><em>Brassica napus</em></td>
<td>An increase was noticed for number of seeds/siliqua, protein and oil contents</td>
<td>[162]</td>
</tr>
</tbody>
</table>

Table 2. Effect of magnetic and radiation treatments on germination, growth and yield of crop plants.
Nutrient priming with micronutrients not only help to overcome seedling constraints but can also be applied as a complementary approach for biofortification to harvest grains high in Fe, Zn and Mn. Priming invokes stress tolerance and improves performance of varieties containing QTL for stress tolerance such as Swarna containing Sub1 for submergence tolerance and IR74 containing Pup1 for high phosphorus uptake. The integration of molecular approaches with seed enhancement may significantly contribute to seed vigour and results may be delivered to the next generation of seed.

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