



REVIEW ARTICLE

Seed invigouration in pulses: More vigour, more yield

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Abstract

Pulses must play a very important role in eliminating malnutrition due to high protein content, presence of vitamins, minerals and dietary fibre. Considering the importance of pulses in human nutrition, FAO celebrated year 2016 as “International Year of Pulses”. Pulse production faces several significant challenges that hinder its growth and productivity, which mainly includes poor germination and seedling establishment. Seed invigouration can overcome these constraints by ensuring fast and uniform emergence, impart higher seedling vigour and enhance stand establishment and productivity. Hydro priming, Osmo conditioning, hormonal priming, nutri-priming, nano priming, bioprimering etc. are the improved seed invigouration strategies commonly adopted in agricultural crops. Seed priming enhanced the superoxide dismutase and catalase activity in seeds. Botanical priming proved to be a low-cost and eco-friendly technique which improves the seed emergence, seedling growth and enzyme activity in pulses. Nutri-priming provides fast and synchronized germination and assures better performance of pulse crops in the field and provides faster and synchronized germination. Nano priming can be advocated as a seed enhancement tool to improve crop growth and yield. Seed pelleting can integrate nutrients, biofertilizers, plant protection products and growth stimulators which can provide conditions favourable for the germination of seeds. Physiological, biochemical, cellular and molecular changes take place in seed because of seed invigouration resulting in increased vigour and germination. Ultimately, seed invigouration holds the key to unlocking the full genetic potential of pulses and contributing to a more resilient and productive agricultural system.

Keywords: physiological and biochemical mechanism; priming; pulses; vigour; yield

Introduction

Pulses are edible seeds derived from legume plants, except those used for oil extraction or harvested green. It includes dry beans, peas, lentils, chickpeas and faba beans, which have significant nutritional benefits for chronic diseases (1). Pulses are considered as “Resource pulses” and are defined as rare and intense and brief periods of higher resource availability, occur frequently in nature and can have an immense effect on ecological processes at the individual, population and community levels (2). The distinction between “legumes” and “pulses” is often a source of confusion. Legumes are plants from the Fabaceae family that produce seeds enclosed in pods. Pulses, however, refer specifically to the dried edible seeds of these plants, such as lentils, beans, peas, green gram, black gram, grain cowpea, Bengal gram, pigeon pea etc. Notably, soybeans and groundnuts (peanuts) are excluded from the pulse category due to their high fat content. The term “pulses” was first used in the 2020-2025, Dietary Guidelines for Americans, emphasising the necessity of appropriate terminology in establishing a solid research foundation (3). Pulse crops such

as beans, peas, chickpeas, green gram, pigeon pea, lentils etc. are rich in protein, fibre and other essential elements. They are important in sustainable agriculture because they improve soil fertility through nitrogen fixation. Genetic modification of pulse crops can enhance their nutritional content, potentially easing micronutrient deficiencies and lowering the risk of chronic diseases (4). Pulse farming benefits both farmers and the environment because of its natural ability to fix atmospheric N, improves soil fertility and reduces the need for costly artificial nitrogenous fertilizers (5). Including half a cup of beans or peas in the daily diet improves the quality of diet by increasing intakes of iron, zinc, folate and magnesium, among other vitamins and minerals. Increasing the production of pulses can also boost domestic demand, open chances for local value-added processing and give rural impoverished people, especially women and young people, a source of income and off-farm jobs (6). Pulses have several potential benefits for enhancing food security. When compared to animal diets, the protein derived from pulses is less expensive. Pulses contribute to family food security because of their shelf stability, which results in a very low

percentage of food waste. Furthermore, pulse seeds retain their ability to sprout even after long periods of storage, allowing farmers to use them for sowing in later cropping seasons. Given their high nutritional value and significant role in combating malnutrition, the Food and Agriculture Organization (FAO) and the United Nations declared 2016 as the International Year of Pulses to raise global awareness of their benefit (7). Global pulse production and trade have undergone significant changes in recent decades. Canada has emerged as a major producer and exporter of peas and lentils, with production concentrated in western provinces (8). Developing countries produce 71 % of the world's pulses, with India being the largest producer (9). The pulse trade has increased to 14 % of world production with Canada, France, China, USA and Myanmar as important exporters (9). The demand for pulses is projected to continue growing in developing countries due to population growth, rising income and increasing urbanization (10).

Challenges in pulse production

Pulse production faces significant challenges, particularly in underdeveloped countries. Gender inequality often limits women's participation in both crop production and access to high-value pulse markets (11). Social and cultural barriers such as a lack of awareness about the nutritional and economic benefits of pulses, limited access to resources and inefficient supply chains further hinder production. In India, specific constraints include the limited availability of high-quality seeds, low yields and damage from pests and insects (12).

Despite these challenges, there are opportunities to enhance pulse production through increased financial support for small-scale farmers, improved market access and investment in rural infrastructure (11). However, climate change continues to intensify existing problems; prolonged drought and high temperatures negatively affect seed germination, plant growth and biomass accumulation in pulse crops (13). To address these issues, experts recommend empowering women to take leadership roles in pulse production, improving market connectivity and bolstering infrastructure development (11). Moreover, understanding the molecular mechanisms of drought and heat tolerance could aid in breeding more resilient pulse cultivars (13).

Farmers face numerous challenges in improving the production and productivity of pulse crops, particularly in relation to poor seed germination and stand establishment. These challenges are multifaceted and include delayed sowing, use of inappropriate sowing methods, low seed rates, inadequate irrigation and poor crop management practices. Additionally, there is a lack of short-duration, high-yielding, drought-tolerant pulse varieties (14, 15), as well as limited access to good-quality seeds (16). The absence of organized programs for quality seed production, high costs associated with quality seeds and insufficient availability of newly released varieties further compound the problem (16).

In India, pulses are primarily cultivated on marginal lands and in summer rice fallows, which often lack adequate technological interventions. The absence of essential inputs, limited availability of quality seeds and lack of irrigation facilities are major bottlenecks in enhancing pulse productivity in marginal lands and summer rice fallows (17).

The pulse crops are often subjected to varying degrees of moisture stress during the crop growth period, since the pulses are mainly cultivated in the rainfed regions of our country characterized by low organic carbon content and low moisture retention capacity (18). The major abiotic stress affecting pulse production are drought (19) low and high temperatures (20, 21), alkalinity (22), salinity (23) and nutrient deficiencies particularly, the deficiencies of Zn, Fe and Mg. Environmental stresses such as drought, salinity, high temperature, nutrient deficiencies hamper the growth and development of pulse crops by interfering with the plant normal morphological and physiological process.

One of the key consequences of environmental stress is overproduction of Reactive Oxygen Species (ROS), which plays a crucial role in the oxidative damage to plant cells (24). Under stressful conditions, pulses often produce an excessive amount of ROS, such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH\cdot$). Reactive oxygen species are byproducts of normal metabolic processes, primarily in the chloroplasts, mitochondria and peroxisomes. Over production of ROS can cause damage to cellular structure by lipid peroxidation, protein oxidation and DNA damage. Further generations of ROS overwhelm the antioxidant defense system, leading to oxidative stress. Also, over production of ROS can damage chlorophyll and other components involved in photosynthesis leading to reduction in photosynthesis, ultimately affecting the crop growth and productivity. The physiological and morphological changes observed in pulse crops due to over production of ROS are growth inhibition, leaf damage and reduction in yield.

Seed invigouration as a solution to improve seed vigour and pulse yields

The concept of seed invigouration involves both priming and pelleting a relatively new term in the context of seed technology. Seed invigouration encompasses various techniques aimed at enhancing seed quality, particularly for improving germination rates, seedling growth and field performance. These treatments are designed to improve the overall vitality and robustness of seeds, enabling them to perform better under adverse field conditions (25). During crucial stages of seedling establishment, these treatments aim to reduce the time taken for the seedlings to emerge and shield them from biotic and abiotic influences. Seed invigouration can significantly improve the germination process, seedling growth and field performance of crops, making them especially valuable in modern agriculture, where seed quality and crop establishment are critical factors for achieving high yields and resilience.

Seed vigour is a key indicator of seed quality. It is a quantitative trait that impacts seed performance in terms of post-storage performance, emerging ability under adverse conditions, seedling growth and rate and uniformity of germination. Seed vigour is indeed a critical factor in plant development and agricultural productivity. Seed vigour encompasses multiple aspects, including the speed of germination, the uniformity of seedling emergence and the ability to withstand environmental stresses such as drought, temperature extremes, or pathogen attacks.

Seed invigouration methods are critical techniques used to enhance seed quality, focusing on improving germinability, planting value, field performance and overall yield. These methods primarily work by enhancing the physiological and cellular functions of seeds, especially after storage, to ensure that seeds can perform optimally under field conditions. The selection of seed invigouration treatments is crucial for enhancing seed quality and ensuring successful planting. These treatments are typically tailored to the specific crop being cultivated and the challenges it faces, with their applicability varying based on several factors. In priming controlled hydration of seeds occurs that initiate several metabolic, biochemical and molecular modifications which enable the seeds to ensure fast and synchronized germination, impart vigour and stress tolerance on early seedlings (26).

Seed invigouration techniques

Nowadays, various seed priming techniques have been used widely. Seed priming refers to pre-sowing treatment that helps to improve seed germination and seedling establishment. The various priming treatments includes, hydropriming (soaking seeds in water to activate physiological processes such as enzyme activation, which helps in breaking dormancy and promote quicker germination), halo priming (seeds are soaked in a solution of inorganic salts, such as potassium nitrate or calcium chloride, which can help to stimulate metabolic activities that enhance germination), osmopriming (seeds are soaked in solutions that contain osmotic agents like polyethylene glycol (PEG) or other organic solutes. These agents reduce water uptake to a controlled level, allowing the seed to begin germination without fully sprouting), thermopriming (seeds are treated with controlled temperatures, either high or low, to break dormancy or to prime them for better germination), solid matrix priming (seeds are mixed with a solid matrix such as vermiculite, peat, or sand. The seeds absorb water from the matrix, promoting hydration without fully soaking them) and biopriming (soaking seeds in biological compounds, such as beneficial microorganisms, enzymes, or plant hormones. These bioagents improve seed health, reduce seedborne diseases and enhance seedling vigour) (27).

Hydropriming

In hydropriming, seeds are soaked in water and dried to a certain moisture level before sowing (28). Nondormant seeds would germinate easily because there is enough water and oxygen, as well as suitable temperature. This procedure is environmentally safe because it does not involve any chemicals. One major drawback of hydropriming is that hydration of the seeds may not be uniform, resulting in uneven germination (29). Hydropriming of black gram seeds for 14 hr resulted in higher germination and seed quality parameters (30).

Osmopriming

In osmopriming, seeds are soaked in aerated low-water potential solutions to regulate water uptake and stop radicle protrusion. Osmopriming with polyethylene glycol, calcium chloride, potassium nitrate and sodium chloride increased the germination energy and decreased the mean germination time. Polyethylene glycol 6000 (20 %) hydration for 24 hr enhanced the seedling fresh weight, seedling dry weight and seedling vigour index in pea (31).

Osmohardening

Osmohardening is a new technique of seed invigouration. It involves the use of an osmotic solution to pre-condition seeds, helping them to withstand environmental stresses such as drought, salinity, or temperature extremes. It is a form of osmopriming, but it focuses on improving the seed's tolerance to adverse conditions during germination and early seedling growth (32). The frequency and length of cycles are crucial for enhancing seed vigour in this method. Osmohardening with calcium chloride for 48 hr was superior to hardening and osmohardening with potassium chloride (32). Osmohardening improves the seedling emergence, stand establishment, growth, yield and quality of crops. Osmohardening of seeds resulted in better absorption of water due to increase in cell wall elasticity and better development of root system (33). Seed hardened with CaCl_2 enhanced the growth and yield parameters of black gram under field condition (34).

Solid matrix priming

In solid matrix priming, seeds are mixed with the moist solid organic material having low matrix potential which allows water imbibition (35), afterwards seeds are dried back to their normal moisture to facilitate normal handling, storage and planting (36). The organic carriers commonly used in solid matrix priming are vermiculite, synthetic calcium silicate, sawdust, charcoal and granulated clay particles. Besides the ease in handling, other advantages of solid matrix priming are chemical, or biological agents can be mixed with seeds to improve their performance in the field (35). It is more effective in bold seeds compared to small seeds. Seeds mixed with moist sand and kept it for 72 hr at 18 °C improved the seedling emergence and stand establishments (37). It was also reported that solid matrix priming enhanced the seed establishment by 10-31 % and yield by 20-23 %, respectively compared to seeds without priming.

Hormonal priming (priming with hormones)

Seed priming with plant growth regulators, polyamines and other organic sources in specific concentrations improve the seed performance in many field crops. Hormonal priming helps the crops to overcome the abiotic stresses (38). Gibberellic acid is a well-known phytohormone that activates β -amylase, which breaks down starch contained in seeds so that developing embryos can use it during germination (39). Seed priming with ascorbic acid induced salinity tolerance in crops (40). Ascorbate is an antioxidant and priming with ascorbate at various concentrations (10-50 ppm) improved the germination and early seedling growth in crops (41). Hormonal priming not only enhance the plant growth but also help the crop to alleviates the adverse impact of environmental stresses (42).

Botanical seed priming or phyto priming

Due to its significant advantages in terms of plant growth, yield and seed quality qualities, the usage of local botanicals has attracted significant attention in recent years, mostly from researchers. Neem leaf extract is being used more often in agriculture as a seed treatment due to its positive benefits on plants (43). Other botanicals, like castor oil, garlic extract and onion extract are also used to treat seeds and their effects on plant growth and yield varied. Botanical seed

priming improves early crop stage tolerance to pests and diseases and has a synergistic effect on uniform and early seed germination. Botanical seed priming reduced the incidence of *Fusarium* infection in seeds (44) and offers seedling resistance to osmotic stress (45).

Biopriming (seed inoculation with microbes)

Seed biopriming is an environmentally friendly technique and an alternative to fungicides. It is a pre-sowing treatment which involves treating beneficial microorganisms or biological agents before planting to enhance seed vigour, germination and early plant development. This technique is used to improve the resistance of seeds to diseases, abiotic stress and other environmental factors, leading to stronger and more resilient plants (46). The goal of seed biopriming is to establish a beneficial microbial community around the seed, promoting faster and healthier seedling growth. Beneficial microorganisms like bacteria (e.g., *Bacillus*, *Pseudomonas*, *Rhizobium*) or fungi (e.g., *Trichoderma*, *Arbuscular mycorrhizal fungi*) are used to treat seeds. These microbes help to enhance the nutrient uptake, pathogen suppression, or stress tolerance. Seeds can also be treated with Plant Growth Promoting Rhizobacteria (PGPR), endophytes, mycorrhizal fungi etc. that help to improve plant growth and health.

Seed treatment with biopolymers

Products that protect plants from phytopathogens can also be developed using biopolymers. Biopolymers are non-toxic, biodegradable substances derived from renewable resources. The commonly used biopolymers in agriculture are carboxymethyl cellulose, chitosan, gum arabic, polyvinyl alcohol, starch, gelatin, polyacrylamide and alginates. Seeds of cereals, vegetables and pulse can be treated with polymers. Biopolymers interact directly with the fungi and prevent spore germination and mycelial growth (47). Pullulan is the most promising microbial polymers used for seed coating. Pullulan is a highly water-soluble, low-viscosity polysaccharide which can be degraded by microorganisms. It has oxygen barrier properties, moisture retention capacity and prevents the growth of pathogens and stimulates the growth and development of beneficial microorganisms. Polyhydroxyalkanoates (PHAs) are another non-toxic, biodegradable and biocompatible polymer used in agriculture (48) and exhibit antagonistic activity against bacteria (49). Polyhydroxyalkanoates produced by *Pseudomonas fluorescens* showed strong antifungal activity against *Fusarium graminearum*, *F. solani*, *F. oxysporum* (50). Studies on the effect of seed bio-priming on germination and seedling vigour in chickpea variety JG-11 revealed that bio-priming with *Trichoderma viride* and *Pseudomonas fluorescens* significantly enhanced seedling emergence, reaching 96 % and 98 %, respectively (51). A trial was conducted under salt stressed conditions to evaluate the potential of bio-priming with *Rhizobium phaseoli* (M6 & M9) and PGPR (*P. syringae* Mk1, *P. fluorescence*, Mk20 and *P. fluorescens* Biotype G, Mk25) in mung bean and noticed that, biopriming significantly enhanced shoot fresh weight (145 %), root fresh weight (175 %), number of pods per plant (150 %), pod fresh weight (182 %), total dry matter (269 %), relative water content (19 %), water use efficiency (51 %) and N concentration (99 %) as compared to untreated control (52). PGPR seed biopriming boosted grain

yield and nutrient use efficiency in red lentils compared to uninoculated seeds (53). Under field conditions, seed biopriming with *Bacillus subtilis* at 0.6 % concentration in soybean significantly improved agronomic traits compared to the untreated control. The treatment led to an increased seed yield, number of pods per plant, pod weight per plant, seed weight per plant and 100-seed weight (54). The effect of seed biopriming in green gram with *Rhizobium* and varying levels of N and S fertilization was investigated. The study found that biopriming significantly enhanced seed yield (996 kg/ha⁻¹) and stover yield (1829 kg/ha⁻¹) compared to the control (55).

Nutri-priming

Nutri-priming is a seed treatment technique in which seeds are pre-soaked or primed with nutrient solutions before planting. This process enhances seed germination, early growth and overall seedling vigour by providing the necessary nutrients that seeds need for optimal growth. Unlike traditional seed priming, which focuses on water absorption or microbial inoculants, nutri-priming specifically targets the nutritional needs of the seed. It is a simple, affordable, economical and ecologically sustainable approach. It improves seed performance and offers synchronized, quicker germination (56). Numerous microelements, such as B, Mn, Mo and Zn, exhibit good nutri-priming potential in a variety of crops. Molybdenum is essential for plants' N metabolism, especially in legumes (57). Common bean seeds primed in sodium molybdate solution improved the nodulation, dry matter production and yield (58). Pre-sowing treatment with 500 ppm Na₂MoO₄ for 8 hr is recommended for the better performance of Bengal gram seeds under field conditions (59). The germination and seedling growth were significantly influenced by the nutrient content of the priming solution. By encouraging plant growth in later phases, these nutrients can help to improve production, growth characteristics and even seed quality.

Nanopriming- A novel approach to seed invigouration

With the ability to manipulate at the atom level and find answers to unsolved field problems, nanotechnology is a field that combines the fields of life sciences, material science and information technology. Nano priming is a cutting-edge seed treatment technique that uses nanotechnology to enhance seed germination, growth and resistance to stress. This process involves the application of nanoparticles or nanomaterials (e.g., metal oxide nanoparticles, carbon-based nanoparticles) to seeds, with the goal of improving their physiological and biochemical properties for better performance in the field. Nano particles used in nano priming are metal oxide nano particles (ZnO, TiO₂ and CuO), carbon based nano materials (carbon nanotubes, graphene-based materials) and polymeric nano particles.

Nanoparticles can be able to enter seeds through the cracks and crevices on the seed coat as dry mixing. Nano seed priming brought changes in biochemical characteristics of seeds such as electrical conductivity, free amino acid activity, dehydrogenase activity and lipid peroxidation. ZnO nanoparticles @1000 mg kg⁻¹ as dry and 200-300 mg kg⁻¹ under wet showed a significant increase in seed viability, seedling length and vigour. Integrating nanoparticles into the seeds may reduce oxidative damage and reactive oxygen species,

ultimately increasing the viability and vigour of pulse seeds (60).

Thermal treatments

The two main goals of dry-heat treatment of seeds are dormancy breaking and reducing the populations of internal and external seed-borne pathogens, such as bacteria, viruses, nematodes and fungus. Thermal treatments can be performed using dry heat by placing seeds in an oven at changing controlled temperatures that simulate soil conditions with different exposure times. Enhancing germination through heat treatment has been found to be a useful technique that can be applied to low-quality seeds. Chickpea seeds that were heated had better germination and seedling stand establishment. Thermal hardening significantly influenced the germination and seedling vigour of both Desi and Kabuli chickpea cultivars (61). In both cultivars, treated seeds exhibited synchronization and earlier germination than control seeds. Higher germination energy was seen in treated seeds, but germination percentage and radicle length were unchanged. Heat treatments greatly enhanced the quantity of secondary roots, radicle and plumule length and fresh and dry weights of both chickpea cultivars in addition to improving germination parameters. Chickpea seeds cannot withstand high temperatures, so treating them with a higher temperature (60 °C) for an extended period (72 hr) was found to be detrimental. Raising the temperature above 60 °C will undoubtedly injure the seeds. Enhancing chickpea seed germination can be achieved by applying heat treatment (up to 50 °C for not more than 72 hr).

Magneto-priming

Magneto-priming has emerged as a potential seed-priming technique, enhancing seed vigour, plant performance and productivity in both normal and stressful situations. Magneto-priming refers to a technique that involves the use of magnetic fields to enhance seed germination and seedling growth. Magnetic fields can influence the physical and biochemical processes in seeds, potentially improving their water uptake, enzyme activity and overall metabolic activity. Improved photosynthesis has been shown in several recent studies to increase biomass accumulation and total crop production. Seedlings emerging from magneto-primed seeds had sturdy midribs, minor veins, increased fresh weight and leaf area. Chlorophyll and carotenoid contents, stomatal

conductance and activities of carbonic anhydrase (CA), Rubisco and PEP-carboxylase enzymes were enhanced with magneto-priming (62). The use of microwaves as magneto-priming improved the germination indices, seedling parameters, water uptake, fresh and dry biomass and longevity of seeds in soybean. Magneto-primed seeds showed higher expression of HSFA3, HSP21, HSP17.6b, EXP, ABI3 genes which are related to germination and longevity (63). Magneto-primed seeds show enhanced germination rates, improved root development, increased vigour and greater seedling biomass compared to unprimed seeds (64). Additionally, plants grown from magneto-primed seeds exhibit greater resistance to both abiotic (65) and biotic (66) stressors, likely due to elevated α -amylase and protease activities. Furthermore, magneto-primed soybean seedlings produce fewer superoxide radicals (O_2^-) (67). Magneto-primed seeds have demonstrated the potential to mitigate the adverse effects of drought and disease on agricultural productivity. Exposure to a magnetic field alters the shape and ionic permeability of the cell membrane, enhances ion transport across ion channels and influences key metabolic pathways. These changes contribute to improved germination rates and seedling emergence. Physical methods for enhancing plant productivity, such as seed priming, offer several advantages over conventional chemical treatments. The effects of these physical treatments can range from modifications in morpho-structural traits to changes in gene expression and metabolite profiles. Among the most promising pre-sowing seed treatments are magnetopriming and ionizing radiation, the latter of which includes gamma rays, ultraviolet (UV) rays (UVA and UVC) and X-rays (Table 1).

Seed coating/pelleting

Seed coating or pelleting are used synonymously. A seed invigoration technique involves a coating of a thin layer of material (usually a polymer, fungicide, or fertilizer) directly onto the seed to enhance seed health, protect against diseases and promote growth. Seed pelleting/ coating aid in mechanical sowing to achieve uniform seed placement and spacing, ensure optimum plant stand, reduce the seed rate and protection against biotic and abiotic stresses. In seed pelleting, seeds are coated with nutrients/ biofertilizers/ plant protection chemicals/ growth stimulants, which can provide guaranteed benefits for seed germination and vigorous and

Table 1. Effects of magnetic, gamma ray and ultraviolet irradiation priming on different pulse crops

Crop	Dose (Best dose)	Attributes
Magneto-priming		
Soybean (<i>Glycine max</i> L.)	200 and 150 mT	Higher germination percentage, seedling biomass and fresh weight; improved light harvesting, chlorophyll fluorescence, leaf photosynthetic efficiency and leaf protein content; reduced ROS production (67)
Mung bean (<i>Vigna radiata</i> L.)	5 mT	Better germination and seedling vigour; improved starch metabolism and α -amylase activity (68)
Faba bean (<i>Vicia faba</i> L.)	0.1 mT	Improved seedling growth and mitotic index (69)
Gamma ray priming		
Black gram (<i>Phaseolus mungo</i> L.)	50–350 Gy (200 Gy)	Improved plant height, pod length, seed number per pod at the best dose (70)
UV irradiation priming		
Common bean (<i>Phaseolus vulgaris</i> L.)	UVC (Type of UV used)	Higher germination percentage, increased accumulation of bioactive molecules (flavonoids: quercetin-3 O-glucoside, soyasaponin) (71)
Mung bean (<i>Vigna radiata</i>)	UVA and UVC	Enhanced germination rate and seedling vigour; improved total chlorophyll and carbohydrate content; seedlings less susceptible to root infecting fungi (72)
Mash bean (<i>Vigna mungo</i> L.)	UVB	Reduced germination, improved activities of PAL, TAL enzymes and photosynthetic pigments (73)

healthy plant growth. It also fastens the process of seed sowing and makes it more efficient especially in small sized seeds. Black gram seeds pelleted with rhizobium showed a higher level of chlorophyll content, quality parameters and yield compared to control (74). Seeds pelleted with borax 50 and 100 mg kg⁻¹ of cowpea seeds resulted in significantly higher number of total nodules and effective nodules per plant (75).

Mechanism of seed invigouration

Seed invigouration improves germination, stand establishment and economic output in several ways, including physiological, biochemical, cellular and molecular mechanisms. Seed invigouration procedures stimulate the activity of enzymes, antioxidants, the breakdown of reserve food, synthesis of metabolites, nucleic acid and protein production.

Physiological and biochemical mechanism

Several physiological and biochemical changes take place during seed invigouration. Seed invigouration repair cellular damage that seeds may incur during storage or under stress, contributing to enhanced seed health and vigour. It promotes better mobilization of the stored nutrients (such as starch, proteins and lipids) in the seed. Invigourated seeds require less imbibition (the absorption of water) to initiate RNA and protein synthesis and thus reduce the time needed to kick-start the processes crucial for germination. Enhances the formation of polyribosomes, which are involved in protein synthesis and helps in the production of proteins needed for seedling growth. It also helps in improving the membrane integrity, control of lipid peroxidation, increase in sugar content, increase in protein and nucleic acid synthesis, removal of germination inhibitors like abscisic acid, efficient production and utilization of metabolites for germination (76), increase in the activity of enzymes like alpha amylase, acid phosphatase, isocitrate lyase, protease, peroxidase, catalase, glutathione reductase, superoxide dismutase and antioxidant system (77). Seed priming treatment improves the seed's ROS system, allowing primed seeds to perform better. Seed priming initiates protein and enzyme synthesis activates α - and β -amylase and isocitrate lyase, synthesises new mRNA, maintains DNA integrity, increases energy charge and ATP content and increases nuclear 4C DNA content, resulting in cell cycle progression from G1 to G2 phase for synchronised germination. The photosynthetic rate improves because of more efficient nutrient intake and assimilation, as well as greater use of photosynthetically active light. Seed priming triggers the activation of an antioxidant system that protects membranes, lipids and proteins from abiotic insults like salt and dehydration. Higher photosynthetic rates result in increased carboxylation and improved crop growth and development, ultimately increasing crop yield and harvest index. There are several reports of priming-induced vigour enhancement, but few highlight the physiological and biochemical foundation of such alterations.

Cellular mechanism

During priming under abiotic stresses, Late Embryogenesis Abundant (LEA) protein and dehydrins protect the cellular structures (78). These proteins maintain the cellular integrity and protect the seed during the early phases of imbibition

(water uptake). This makes the seed more resilient to abiotic stressors, such as drought or high salinity. The expression of LEA proteins undergoes subsequent modifications. Decrease during imbibition indicated that LEA proteins are not needed for water uptake but are necessary during dehydration stress. Increase during dehydration indicated that when the seed loses water, LEA proteins help protect against desiccation damage. Degradation during germination indicated that as the seed enter the germination phase, these proteins break down, possibly because they are no longer required for protection once the seed begins active growth.

DNA repair mechanisms are activated during seed priming, helping to protect the genetic material from damage. This is especially important in seeds that have been subjected to stresses like dehydration, as DNA integrity is crucial for proper seedling development. Priming facilitates DNA repair, enabling seeds to be more robust and better equipped for the demands of germination and seedling establishment. Priming synchronizes the cell cycle, particularly by influencing the G2 phase. During this phase, the cell prepares for division. Priming ensures that seeds are in a state of readiness for efficient cell division once germination begins (79). Cell division does not occur immediately after priming but is set to begin right after the radicle (the first part of the seedling to emerge) protrudes from the seed coat. Priming prolongs Phase II of seed germination, which involves water uptake and early metabolic changes and ends just before Phase III (radicle protrusion) (27).

Seed priming increases the G2 to G1 phase ratio, which indicates that a higher proportion of cells are in the G2 phase (where they are preparing for division) compared to the G1 phase (where cells are in a resting state before committing to division). This shift towards a higher G2 ratio highlights that primed seeds are in a better state of readiness for rapid growth and development.

Molecular and metabolic changes

Molecular responses of seed priming involve DNA repair, early DNA replication, stabilization of RNA, enhancement in the synthesis of *de novo* protein and avoidance of electrolyte leakage (35, 80). During priming genes related to germination are regulated and enhanced the seedling emergence (81). Increasing in the production of solutes (sugars, amino acids, proline, glycine, betaine etc.) help to keep an optimum osmotic potential in the primed seeds which helps to prevent membrane leakage. Reprogramming of genes helps to protect the seedling from salinity injury (82). Priming resulted in the expression of salt responsive genes *viz.*, TaNHX1, TaSOS1, TaSOS4, TaHKT1, TaHKT2 and TaHKT1 which decreased the oxidative stress in seedlings (83).

Priming enhanced the production of gibberellins and auxins to promote root development which effectively promoted the degradation of the precursor of ethylene biosynthesis (84). Priming resulted in the rise of cytosolic calcium levels which would activate mitogen activated protein kinases and resulted in strong immune response in plants (85). Priming activates transcription factor production which phosphorylates mitogen activated protein kinases and

cyclin dependent protein kinases which stimulates the production of stress responsible genes (86).

Impact of seed invigouration on germination parameters and seedling vigour of pulses

Seed invigouration treatments significantly improved the germination parameters and seedling vigour in pulses. Hydropriming of lentils resulted in higher seedling emergence in the field, compared to control and seed priming with PEG (87). Nutri-priming with ZnSO₄ either at 0.025 or 0.05 percent for 4 hr resulted in higher germination percentage, germination index, mean daily germination, speed of germination, coefficient rate of germination and germination rate index in grain cowpea (88). Green gram seeds primed in 450 ppm solution of Zn at 25 ± 2 °C for 9 hr resulted in higher germination percentage (98 %), seedling length (38 cm), seedling vigour index I (3701) and seedling vigour index II (26.2) (89).

Magneto-priming of *Glycine max* in 200 mT and 150 mT magnetic fields increased the germination parameters and seedling biomass (67). Magneto-priming of 5 mT magnetic fields showed improved germination, seedling vigour and starch metabolism in *Vigna radiata* (68). In *Vicia faba*, magneto priming of 0.1 mT magnetic fields resulted in improved seedling growth (69).

Nano priming of green gram seeds with TiO₂ (0.02 %) resulted in the highest germination percentage (93.33 %), seedling dry weight (0.59 g) and seedling vigour (55.06) compared to control and nano priming of TiO₂ (0.01 %) (90). Nano priming of chick pea seed with ZnO or silver nanoparticle at 100 ppm either alone or in combination resulted in significant positive effect on germination and growth parameters and negative effect on the incidence of *F. oxysporum* f.sp. *ciceri* (91).

Vegetable cowpea seeds pelleted with ZnSO₄ 250 mg kg⁻¹ seed and borax 100 mg kg⁻¹ seed produced seedlings with significantly higher shoot length, root length, seedling dry weight and vigour index compared to non-pelleted seeds (92). Osmo priming of black gram seeds with PEG₆₀₀₀ at 20 per cent resulted in significant improvement in emergence percentage, seedling parameters and seedling vigour index compared to priming with CaCl₂ at 1 per cent (93).

Impact of seed invigouration on enzyme activity and total sugars

Biopriming of pea seeds with *Bacillus subtilis* and *Trichoderma harzianum* resulted in taller plants, more numbers of leaves and branches per plant, dry weight of shoots per plant, pod length and diameter, numbers of seeds per pod, higher percentage of green pod, seed to pod weight, TSS, highest carbohydrate and protein. Higher α- and β-amylase, alkaline phosphatase and dehydrogenase enzyme activity was observed in hydro-primed seeds of soybean (94). Alpha amylase and protease are the key enzymes of seed germination process. In mung bean, GA₃ and KNO₃ priming resulted in enhancing the alpha amylase and protease enzymes, due to their positive effect and involvement in activation process. GA₃ (100ppm) priming showed the highest α-amylase activity followed by priming with KNO₃ (0.2 %) priming. Protease activity was also the highest in seeds primed with GA₃ (100 ppm), followed by those primed with KNO₃ (0.2 %). The activity of α-amylase and protease were

slightly reduced under drought condition when compared with normal condition (95). Osmo-priming with CaCl₂ in combination with biochar resulted in an increase in α-amylase activity, total soluble sugars in cowpea seeds. It also reduced oxidative damage through lower Na uptake, lipid peroxidation and total antioxidant activity (96).

Impact of seed invigouration on growth, yield attributes and yield in pulses

Seed invigouration enhanced the growth attributes, yield attributes and yield of pulse crops. Significant improvement in growth and yield attributes and yields due to Mo seed-coating in common bean and soybean (97, 98). Chickpea seeds primed in solutions of optimal concentration of micronutrients resulted in an increase in Zn content from about 40-60 (unprimed) to 500-800 mg kg⁻¹ (Zn primed). In Mung bean, priming with polyethylene glycol enhanced the grains per pod by 14 per cent, grain weight by 3.5 per cent and grain yield by 12 per cent (99). Nutri-priming with ZnSO₄ either at 0.025 or 0.05 per cent recorded higher Zn and B content in grain cowpea (88). Hydropriming in field pea enhanced the emergence and resulted in the better expression of phenological and yield components (100). Seeds primed in ZnSO₄ at 0.05 % for 4 hr registered the highest seed yield, total number of nodules, nodule fresh and dry weight per plant (88). Biopriming of field pea exhibited higher root and nodule formation, thus the nitrogen uptake of the plant rises leading to enhanced growth and production (101).

Seed invigouration treatments significantly influenced the growth parameters viz., number of green leaves per plant (30 DAS and 60 DAS), number of branches per plant and dry matter production (30 DAS, 60 DAS and harvest stage). The highest number of branches were recorded in seeds primed in ZnSO₄ 0.025 % for 4 hr + *Trichoderma viride* seed treatment 10 g kg⁻¹ seed at 30 DAS, 60 DAS and at harvest. Seeds primed in ZnSO₄ @ 0.5 % for 4 hr recorded the highest dry matter production (88). Priming of broad beans with 0.05 % chitosan nanoparticles resulted in significant increase in total phenols and antioxidant enzymes, as compared with those of the control seedlings (102). MgO nanopriming in black chickpea resulted better growth, yield and enhanced biochemical accumulation; shoot-root length, chlorophyll and carbohydrate content of chickpea. Significant increase in polyphenols and antioxidant enzyme activities was observed during nano-priming (103).

Conclusion

Seed invigouration has emerged as a vital strategy for improving pulse crop productivity and ensuring food security. By enhancing germination, seedling vigour and crop establishment, invigouration techniques can significantly boost yields while reducing cultivation risks. Treatments involving plant growth regulators and organic compounds at low concentrations have shown potential in promoting uniform germination, robust stand establishment and greater harvestable output.

Among these techniques, seed priming has proven especially effective. It initiates key metabolic activities, helping to break dormancy, prevent seed deterioration and increase resistance to both biotic and abiotic stressors. These physiological and biochemical changes translate into

improved crop performance across diverse environmental conditions, making priming a valuable tool for sustainable agriculture particularly in dryland and stress-prone areas.

However, priming faces limitations, especially during the post-treatment desiccation (drying-back) phase. Drying back the seeds to their original moisture content can impair critical physiochemical processes, reducing seed longevity and storage viability. This remains the primary bottleneck in the broader adoption of priming technologies.

To address these challenges and unlock the full potential of seed invigouration, future research should focus on understanding seed physiology during drying-back, investigating the role of raffinose family oligosaccharides in maintaining cell membrane integrity and exploring the expression of antioxidant enzymes during and after priming.

Moreover, future efforts should aim to develop accurate, long-lasting and cost-effective invigouration strategies tailored to specific pulse genotypes and agro-ecological conditions. By doing so, seed invigouration could become a cornerstone technology for enhancing the genetic potential of pulse crops, ultimately contributing to a more resilient, productive and sustainable agricultural system.

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Authors' contributions

RSK conceptualized and designed the review topic. AS, VDVS, AM, ARD, and MRP contributed to drafting the manuscript. RSK, SB, and BR provided critical guidance and carried out necessary revisions and corrections.

Compliance with ethical standards

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