

Seed Quality Enhancement

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Abstract

With the expansion of precision and intensive agriculture, seed quality, which alone could contribute up to 15–20 per cent in terms of crop productivity, has assumed greater importance. Every care is taken not only to produce high-quality seeds and maintain the same through various stages of production, from growing conditions of the seed crop to pre-harvest, harvest, processing and storage activities, but also to adopt certain technologies to further improve the performance of seeds upon sowing of the crop under a wide range of environments. These technologies, collectively known as 'enhancement', cover a variety of methods applicable to different crop species and aimed at meeting specific requirements, such as unfavourable growing conditions of hard/acidic/sodic soil, high or low temperature, excess or deficient rainfall, etc., as well as the stress imposed by the presence of pests and diseases that affect crop performance, particularly during the early vegetative stage resulting in poor seed emergence,

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crop establishment and vegetative growth. Selection of the appropriate and costeffective technologies is important in accruing the best results from seed enhancement.

Keywords

Seed quality · Seed performance · Seed coating · Seed priming · Seed sorting

1 Introduction

As stated by Hay (2019), 'enhancing germination and reducing the loss in germination as much as possible, are fundamental to the successful use of seeds'. Hence, quality seed is desired not only to have high genetic and physical purity but also to be capable to produce vigorous seedlings with a well-developed shoot and root system that can result in good stand establishment and growth of the crop in a range of environmental conditions, achieving its potential yield. Many of these desired attributes, such as tolerance against major biotic and abiotic stress factors, can be achieved through genetic improvement by applying conventional breeding and biotechnological manipulations. However, genetic improvement using conventional breeding techniques in major food crops is fast reaching a 'yield plateau' with limited accessibility of plant germplasm narrowing the genetic base, while the acceptance of genetic manipulations, including GM and gene editing, is resisted in most countries at present. Hence, 'seed enhancement' technologies offer a means to compliment the genotype in such a manner that the planting value of the seed is enhanced over a wider range of growing conditions. The primary objective of seed enhancements is not only to optimize the inner physiological ability of the seed that normally does not express under normal sowing-cropping practices (Black and Peter 2006; Patel and Gupta 2012) but also to provide ease of handling seeds and better protection against biotic and abiotic stresses, especially in the early vegetative growth stages.

1.1 Scope of Seed Quality Enhancement

Seed quality enhancement is defined by Taylor et al. (1998) as 'post-harvest treatments that improve germination or seedling growth, or facilitate the delivery of seeds and other materials required at the time of sowing'. It includes a range of treatments applied to seeds between harvest and next sowing particularly to improve their performance. Considering the vast developments in seed enhancement technologies in the last 25 years, the definition can be expanded as 'such post-harvest treatments that improve germination and seedling growth, or facilitate the delivery of seeds and other essential substances required at the time of sowing for better performance'. This definition includes three broad groups of enhancement technologies. These are a) seed conditioning (by physical and chemical means), b)

pre-sowing hydration treatments (priming) and c) coating, pelleting and encrusting technologies. Of these, pre-sowing hydration treatments include non-controlled systems (methods in which water is freely available and not restricted by the environment) as well as controlled (methods that regulate seed moisture content preventing the completion of germination) water uptake systems (Taylor et al. 1998).

1.1.1 Advantages Expected from Enhancement Techniques

One or more of the following advantages are expected from seed enhancement treatments that are performed singly or in combination:

- Ease in precision planting and handling.
- Early and uniform emergence due to improved germination and rapid seedling growth.
- Reduced seed rate.
- Better nursery management.
- Delivery of supplementary nutrients and growth stimulants needed for better performance after sowing.
- Protection against pests (including weeds) leading to better stand establishment.
- Removal of weak- or poor-performing seeds using non-traditional upgrading techniques.
- Tagging of seeds with visible pigments or other marker substances for traceability and identity preservation.

2 Types of Seed Quality Enhancement

Seed quality may be enhanced to various levels through different methods. These can be classified based on the mode of application.

2.1 Physical Methods

While the purpose of seed processing/conditioning itself is to upgrade the overall seed quality, some of the physical processes that could have a direct impact on the planting value or are performed specifically to enhance the quality further are:

- Size grading
- Brushing and scarifying
- · Gravity and liquid density separation
- Colour sorting
- X-ray separation
- Imaging techniques, viz. near-infrared spectroscopy imaging, thermal imaging, magnetic resonance imaging, chlorophyll fluorescence sorting, multispectral analysis and scanning electron microscopic imaging.

2.2 Film Coating, Pelleting and Encrusting

Several technologies are based on the application of one or more thin coats of an inert polymer singly or in combination with active substances to cover the seed fully or partially for various purposes, which may or may not significantly increase the seed size or weight. These include:

- Coating for better flowability.
- Coating for binding pesticides or micronutrients.
- Coating for slow release of active chemicals.
- Coating for branding.
- Embedding biologicals on the seed surface.
- Encrusting.
- Pelleting with water-soluble inert substances.
- Optimizing seed for aerial seeding such as Seed Bomb[™].

2.3 Seed Treatment for Protection Against Pests and Diseases

Seed dressings with pesticides offer the earliest and simplest technology for enhancing seed performance upon sowing. With the advancements in this field, in addition to the basic technologies, several physical and chemical methods are employed which are environment-friendly and offer higher precision. These include:

- Chemical seed dressing.
- Steam treatment/ThermoSeedTM.
- Virus removal treatments.
- Hot water treatment.
- Dry heat treatment.
- Magnetic treatment.
- Electromagnetic treatment.
- Radiation treatment.
- Plasma treatment.

2.4 Physiological Enhancements

Physiological treatments for seed quality enhancement are based on metabolic advancement of seed that enables it to a better start upon sowing. These are collectively termed as 'seed priming', the word first coined by Malnassy (1971) and popularized by Heydecker (Heydecker 1974; Heydecker and Coolbear 1977). Seed priming techniques are essentially based on restricted hydration of the seed, which allows early events of germination but stops shortly before radicle protrusion not advancing to a dehydration-sensitive stage (Basu 1994; McDonald 2000). A variety of priming technology is in vogue for commercial applications and suited to various purposes.

2.4.1 Pre-sowing Priming

- Hydropriming.
- Osmopriming.
- Halopriming.
- Biopriming.
- Solid matrix priming.
- Pre-germination.
- Endophytic treatment.
- Nanopriming.

2.4.2 Mid-storage Correction

- Hydration-dehydration with or without chemicals.
- Halogenation.

2.5 Other Technologies

2.5.1 IP Protection and Microbranding

- Fluorescent marker coating.
- Microprinting.
- DataDotsTM.

3 Methods of Seed Enhancements

3.1 Physical Enhancements

3.1.1 Seed Cleaning, Processing and Quality Upgradation

The fundamental processing operations such as pre-cleaning, cleaning and grading enhance seed quality by way of improving its purity, appearance and vigour. The principles of grading and upgrading operations vary with the machine and mostly depend on the physical traits of the seeds such as size, shape, length, density, colour, texture, etc. Among the different machines, the grader works based on the seed size and shape which is known as basic cleaning of the seed. The indented cylinder separates the seed based on its length, the spiral separator based on seed shape, and the gravity separator based on density (Patil and Bansod 2014). Special operations like needle separator pick out the seeds with cracked seed coat and cut parts. Apart from this, many sophisticated advanced technologies or machines are there such as liquid density separator (selects well-filled and high-density seeds) (Koning et al. 2011); X-ray separation (selects well-filled seeds with mature embryo; https://www. incotec.com/en-gb/seed-technologies/seed-upgrading); Q_2 scanner, which selects vigorous seed based on respiratory efficiency (Bradford et al. 2013 and Centor Europe BV n.d.); magnetic resonance imager; chlorophyll fluorescence sorter (selects fully mature seeds based on chlorophyll breakdown); and multispectral analyser (https://neutecgroup.com/news-events/what-s-new/136-seed-and-grainanalysis-by-multispectral-imaging-video), involving sensing the seed components and constituents (Jalink et al. 1998, 1999; Mortensen et al. 2021; El Masry et al. 2019) are being tested and used to enhance the quality of seed. However, many of these are yet to get scientific validation and hence in limited use.

3.2 Film Coating

Coatings are often applied to commercial seed lots to incorporate active ingredients (ai) for seed protection through targeted delivery and to enhance germination through the application of stimulators, besides improving the ease of seed handling (Pedrini et al. 2018). This is particularly useful for the targeted delivery of pesticides to reduce the use of chemicals in agriculture (Hay 2019). In the simplest terms, film coating is the application of a thin coat of (a) polymeric substance(s) to the seed in such a way that it does not significantly alter its size, weight and shape and does not affect its performance per se. The coating material, thus applied to the seed as a thin layer (film), not only improves the ballistic properties of the seed but also acts as a carrier to apply seed protectants and plant growth stimulators (fungicide, insecticide, hormones, micronutrients, etc.) directly on the seed surface, so that it is most effective to the germinating embryos, as well as makes the active ingredients available to growing seedlings for a longer period by way of the slow release from the applied coating. The colouring agent, usually used in a film coating, also improves the market value and provides a distinct identity to a seed brand. Many branded polymers such as SeedworxTM, Ezi-coteTM, etc. are available in the market for seed coating and used for coating vegetable seeds that have been tested for appearance, dust reduction, flowability, plantability and many other functions of value (apsaseed.org) (Fig. 1).



Fig. 1 Seed film coating with different colour polymers. https://www.openpr.com/news/1883041/ new-excellent-growth-of-seed-coating-materials-market-by-2019, accessed April 2022

3.2.1 Advantages of Film Coating

- Better appearance and flowability of seed.
- Accurate and controlled dosage of pesticides with a high level of precision on individual seeds is possible.
- Low dust formation enables safe and easy handling of seeds during packing, storage and transport.
- Slow release of applied molecules enhances the protection level of the germinating seedlings.
- Provides a unique identity to a company for their seeds in the market coated in a special colour. Coating in specific colour may also help to avoid mixture of varieties while handling, packing and storing.
- Application of plant protectants, microbial inoculants, micronutrients and other stimulants through seed coating enhances the overall performance of the seed.
- Low or no damage due to storage pests especially in cereals and leguminous seeds.

3.2.2 Trends in Seed Film Coating

The global market is aiming at 'More from Less', which triggers the use of precision farming wherein one can deliver required molecules to plants when it is needed most. By using pesticides through film coating, the number of pesticide sprays can be reduced significantly in open fields. Crop-specific nutritions can also be loaded on seeds to avoid large-scale application of such chemical nutrients to the soil. There is a trend in the global market to apply pesticides and nutrients as much as possible to the seed itself so that the post-emergence operations related to plant protection and nutrition can be minimized, besides minimizing the environmental pollution. By using fully automated seed treaters with the latest technology of rotary coating along with online seed dryers, it is possible to handle a few hundred metric tons per day in seed film coating. Also, there is a trend to use a specialized coating on seeds to control counterfeit and create a brand image among farmers.

Many countries in the global market have strengthened their seed treatment policy through new seed quality regulations in the treated seed. The European Seed Association has its agency ESTA (European Seed Treatment Assurance Industry Scheme) which monitors the quality of seeds that are delivered in the market and also keeps an eye on the environmental/public health repercussions for using treated seeds (ESTA 2020). There are some reports (Copeland and McDonald 2001) on using water-impermeable plastic film coating to delay germination up to a certain time to coincide with favourable conditions or for achieving synchronous germination in the parental lines of hybrid seed production (Johnson et al. 1999). This technology already has a commercial application called the Pollinator Plus (R) which is an Incotec-Croda proprietary, derived from research made at Purdue University. This involves coating the seeds of male parental lines of maize hybrids in such a way that they germinate and subsequently flower for a wider duration so that the female lines get enough time for fertilization. This technology was also called IntelliCOAT[™] and was previously owned by the Landec Inc. (Landec 2011). Organic coating and the use of biologicals are new demands to support upcoming

organic farming and sustainable agricultural practices. For the same purpose, research on the development of microplastic-free polymers is also being tested for seed coating (Pedrini et al. 2017).

3.3 Seed Pelleting

Seed pelleting is a process of enclosing the seed with an inert material to produce a globular unit of standard size to facilitate precision sowing. It is mainly useful for mechanized sowing of seeds which results in an even distribution of small seeds in the field and reduces the seed rate as compared to traditional planting methods. Pelleting technology is most commonly applied in small, minute, lightweight and expensive seeds of vegetables and flowers or those with appendages that hinder seed handling. Some examples of plant species where seed pelleting is commonly employed are leek, celery, onion, lettuce, carrot, monogerm sugar beet, chicory, forage grasses, flowers, etc.

The basic components of pelleting are fillers, binders, active ingredients (nutrients, plant protectants, etc.) and water. Two types of materials are used for commercial seed pelleting, that is, split type or melting type. In both cases, it is preferred to use inert substances as filler material that have good water-absorbing capacity, remain firm on drying and disintegrate upon soaking.

Besides improving the seed size and shape, similar to film coating, with pelleting, it is possible to add various nutrients, bio-regulators and plant-protective chemicals along with the inert material as per the need of specific species. Different kinds of pelleting materials, viz. LightKoteTM, HeavyKoteTM, Split PillTM, SplitKoteTM, etc., are commercially available for meeting the specific needs, including the biodegradability and the final size of the pellets. The size of the pellets normally varies between 2 and 5 mm, while the size ratio varies from 2 to 15 times, and the weight increases from 4 times to 10 times of the normal raw seed. For example, for 1 kg of tomato seeds with an average seed size of about 2 mm, the weight of the pelleted seed will increase ten times to 10 kg after pelleting of about 4 mm size (Fig. 2).

During pelleting, nutrients, plant growth stimulants, plant-protective chemicals and bioinoculants can also be added along with the inert material integrating different technologies during processing. This highly sophisticated seed can be named as 'designer seed' (Fig. 3).

To obtain seed pellets with uniform size and shape, the measured quantity of pelleting and binder materials and other additives, if any, are added to the rotating drum of an automatic (Fig. 4) or manually operated machine. The multilayer coatings are made by adding the materials sequentially as per their compatibility and mode of action, with the required number of such actions. After processing, the pellets need to be calibrated based on size and weight to make available uniform seed pellets for commercial use (seedquest.com).



Fig. 2 Size increase in pelleted seeds. https://www.bighaat.com/blogs/kb/pelleted-seeds-v-s-normal-seeds, accessed April 2022



Fig. 3 Designer seed technology

3.3.1 Selection of the Pelleting Material

It is important to select the appropriate inert material, hormones, nutrients and plantprotective chemicals to avoid problems of losing viability and vigour of pelleted seed. Therefore, the following criteria should be considered to identify the right type of pelleting material.

- It should be porous and should allow the water to enter it while the pellets are sown in the field. It should also be easily dissolvable with water.
- The choice of the pelleting material is also determined by the type of seed and conditions at sowing, where the seed is likely to be sown. Irrigated or rainfed situations, for instance, are different, and therefore, the pelleting materials are to be chosen accordingly.
- Materials to be added to the seed should not release harmful dust during handling and sowing.



Fig. 4 Seed pelletizer and pellet sorter. (https://www.seedprocessing.com/hemp-pelleting/ accessed on April 2022)

- The choice of adhesive is also important to produce dust-free pellets, without having any adverse effect, per se, on the performance.
- The nutrients, growth regulators and stimulants, plant-protective chemicals and other additives must be compatible with the filler as well as the binder materials. Appropriate dosages need to be standardized to avoid excess and overdosing.

3.3.2 Advantages of Seed Pelleting

- Singulation of seed is achieved during pelleting which leads to easy sowing and reduction in seed rate.
- Small seeds can be made larger to facilitate mechanization.
- Irregularly shaped seeds can be coated to make them smooth and thus suitable for mechanized sowing.
- Accurate application of chemicals is possible, reducing wastage of chemicals.
- Phenomenon of dust-off of additives, especially pesticides, is reduced by seed pelleting.
- Pelleting is the best carrier for all kind of additives to seed in the safest way. The materials can be applied as several layers without affecting their function.
- Slow release of loaded chemicals results in longer periods of their beneficial effects to the growing seedlings creating visible difference between the performance of pelleted and raw seed.
- Post-sowing applications can be minimized based on loading dosages of plantprotective materials in pellets.
- Pelleting in combination with priming technology gives a unique advantage to seed, facilitating faster and uniform germination and healthy seedling growth.

3.4 Seed Encrustation

Encrustation is useful with seeds of irregular surface, where grooves and cavities on the seed surface are filled with a special type of filler material in order to make the seed smoother and more uniform in shape as well as to increase the reasonable level of weight (Pedrini et al. 2018). Encrustation of seed is more useful for small and lightweight seeds which are not very expensive and bulky in nature, such as pasture and forage seeds; agronomic crops like sunflower, maize, etc.; and open pollinated vegetables like carrot, onion, endives, fennel, etc. There is no drastic effect on the size of the seed while encrustation, but weight increases from two to four times. This means that after encrustation, 1 kg of carrot seed will become 2 to 4 kg in weight. There is possibility to load enough amounts of various plant-protective chemicals and nutrients in encrustation process. Compared to pelleting, encrustation is much faster and, therefore, more economical. In the USA and Australia, many pasture companies are selling their grass seeds in encrusted forms since most of the sowing operations are done using aircraft which need enhanced seed weight, i.e. encrusted seed. Mechanized sowing with high level of accuracy is possible using encrusted seed which is required in certain crops like onion, carrot, sugar beet or chicory, which helps to achieve uniform and balanced tuber growth. Encrusting technology, wherever applicable, is almost as good as pelleting, even at a lower cost. In countries like India, where seed sale price is relatively low, such technology has good potential. Currently, encrusting technology has been adopted in onion seed at a large scale, and direct sowing of seed is becoming popular in many parts of India. It reduces production cost by saving on labour and seed rate per acre and also reduces the overall crop duration. Trials in sugar beet and chicory are in progress, and it is expected that soon the growers will prefer to use encrusted seeds for direct sowing in few crops where labour is getting problematic and expensive.

In many industrialized countries, small-sized seeds having all good performance parameters are encrusted to enable the use of pneumatic single seed planters. Loading of various chemicals and biologicals is feasible in encrusted seeds to support better growth after germination. For example, Seed InnovationsTM encrustation technology is used to achieve precision planting and to enable the application of plant protection products and additives to get maximum plant performance (seedinnovations.co.nz, Fig. 5).

3.5 Hot Water Treatment

Hot water treatment is an age-old practice to destroy surface-borne and seed-borne pathogens at temperatures high enough to kill pathogens and cold enough to safeguard the viability of the seed as described by Suryapal et al. (2020). In many investigations, it was noted that even with longer treatment times, hot water treatment with a temperature of 40 °C had no significant effect on the seed-borne pathogens. However, on most crops, the hot water treatments at temperatures above 50 °C or 53 °C for 10–30 min have a good phytosanitary effect. In the majority of cases, these treatment conditions did not affect seed germination. To

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Fig. 5 Encrustation of onion seeds (https://www. seedinnovations.co.nz/ resources/news/encrustingcarrot-and-onion-seed.html, accessed April 2022)

Table 1 Pathogens in wheat and barley susceptible to water and steam treatment (Modified afterBänziger et al. 2022 and Forsberg et al. 2005)

Botanic name	Common name	Wheat	Barley
Fusarium spp./Microdochium nivale	Snow mould/leaf blotch	x	
Septoria nodorum	Nodorum blotch	x	
Tilletia caries	Common bunt	x	
Tilletia controversa	Stink smut	x	
Ustilago tritici	Loose smut	x	
Drechslera graminea	Leaf stripe		x
Ustilago hordei	Covered smut		x
Ustilago nuda	Loose smut		x

reduce the adverse effect of higher temperatures (\sim 53 °C) on germination, comparatively shorter treatment time must be used, especially on sensitive crops like cabbage, etc. (Nega et al. 2003). This eco-friendly technology works best for small seeds, particularly for vegetable crops. It is not applicable to old or very large seeds. Similarly, it is not effective for primed, coated, pelleted and pesticide-treated seed.

3.6 Steam Treatment

Steam treatment has been developed to disinfect seeds from pathogens (Forsberg et al. 2015). In thermal seed treatment, seeds are exposed to hot humid air for a fraction of seconds to disinfect/disinfest them from seed-borne microorganisms. This is a patented technology being adopted at commercial level in many industrialized countries for the effective control of microorganisms and a safer environment. The base unit disinfects seeds with active steam, enabling effective, gentle and targeted heat transfer eliminating broad spectrum of fungi and bacteria (Table 1).



3 - Additional application of protective components

Fig. 6 Steam treatment, working principle of PETKUS HySeed bio ®. (http://www.petkus.com/ products/-/info/coating/hygienisierung/multicoater-cm-hyseed-bio, accessed April 2022)

The main unit of a steam treatment equipment includes a control cabinet, the core unit, steam generator and the dry generator unit (Fig. 6). Within the core unit, a sanitization/hygienization through active steam treatment is followed by re-drying and optional additional application of protective components. Compared to hot water disinfection, this method is more energy-efficient, as steaming enables a faster drying process (Ascard et al. 2007).

3.7 Dry Heat Treatment

Dry heat treatment is a common physical seed treatment used to eradicate the fungal and bacterial seed-borne pathogens. Also, it is an effective and eco-friendly method for inactivating seed-borne viruses. This treatment is mostly followed for vegetable crop seeds. In cucumber, 70 °C dry heat treatment for 90 min eradicates *Cladosporium cucumerinum*, *Didymella bryoniae* and *C. orbiculare* (Shi et al. 2016). Similarly, exposure of Andean lupin seeds to dry heat for 8 or 12 h reduced pathogen transmission up to 85% from seed to the seedlings (Falconi and Viviana 2016). However, care is needed to perform this treatment to maintain the germinability and vigour of seeds.

3.8 Other Potential Treatments

Many potential treatments, both physical and chemical, have been reported to improve seed performance. However, these are yet to be standardized for commercial application (e.g. Dubinov et al. 2000; Kopacki et al. 2017; Legro 2020; Rochalska et al. 2011; Sivachandiran and Khacef 2017).

3.8.1 Magnetic and Electromagnetic Seed Enhancement Treatment

Exposure of seeds to different flux density of magnetic field is reported to promote rapid germination, uniform crop stand, enhanced yield and resistance to disease incidence (Pietruszewski and Martinez 2015). The magnetic seed treatment is a non-invasive physical stimulant for a specific duration of time to induce physiological changes in seed (Pietruszewski and Martinez, 2015). It is reported to control free radicals, increase activity of many enzymes and seed vigour (Vashisth and Nagarajan 2008), increase plant hormones IAA and GA in germinating seeds (Podlesna et al. 2019) and reduce pathogenic diseases (Afzal et al. 2015). It is often effectively used as a pre-sowing treatment for mitigating both biotic and abiotic stresses such as drought, salinity, diseases and pests during germination and early crop growth (Kataria 2017). Exposure of seeds to pulsed electromagnetic field (EMF) at low-frequency levels is also found effective. The positive impacts of EMF treatment include better seed germination (Gorski et al. 2019), seedling vigour (Isaac et al. 2011), tolerance to unfavourable environmental conditions (Pietruszewski and Kania 2010; Balakhnina et al. 2015) and plant growth and yield (Efthimiadou et al. 2014).

3.8.2 Radiation Treatments

Low doses of gamma ray, high-energy electrons, ultrasonic radiation and microwave and UV radiation are also used as an alternative seed treatment for the management of microbial infection (Brown et al. 2001). Studies reveal that recommended dose of gamma radiation (up to 20 Gy) stimulated seed germination without causing collateral DNA damage (Nesh et al. 2019).

3.8.3 Electron Treatment

'The electron treatment of seeds is a promising technology that is based on the biocidal ionizing effect of low-energy electrons (wavelength below 100 nm). The accelerated electrons are generated following the principle of the Braun tubes. When high electrical voltages are applied between a cathode and anode, electrons are emitted from the cathode and are accelerated in the direction of the electron exit window by the difference in the electrical charge. While treating seeds, the applied dose and electron energy are critically monitored. The dose can be determined by regulating the current strength, and the electron energy can be adjusted with the acceleration voltage. During the electron treatment of seeds, the lethal dose is crucial to combat the existing pathogens. The electron energy is a measure of the kinetic motivity of electrons. When electrons penetrate matter, they lose their energy. Once the energy is spent, they do not penetrate further into the material. This fact is used to



Fig. 7 Principle of electron beam treatment (https://www.evonta.de/Application/46511/, accessed April 2022)

precisely control the sphere of action during electron treatment and to ensure that it does not exert harmful effects on the embryo and the endosperm. Harmful organisms hit by accelerated electrons in the effective range are killed' (cited from Rögner 2018; see Fig. 7).

A splitting of molecular chains in microorganisms results in killing the pathogens, irrespective of their nature (Vishwanath et al. 2016). The method is reported to be effective against various fungal spores, bacteria and viruses (Jahn et al. 2005). This method does not use radiation and is acknowledged as a biological control method according to EU regulation 2092/91 (https://deutsche-saatguterzeuger.de/2017/0 6/10/b1-2/, accessed April 2022).

4 Physiological Enhancements

4.1 Pre-sowing Seed Priming

Seed priming is the process of controlled hydration of seeds to a level that permits pre-germinative metabolic activities, but prevents the emergence of the radical (Heydecker 1973). The hydrated primed seeds can be reverted back to a safe moisture content before its distribution and planting. Seed priming, if performed carefully, is a simple and effective technique to get speedy and uniform emergence and better performance.

Materials such as plain water or dilute solutions of salts, growth regulators, vitamins, bioinoculants, plant products, leaf extracts, etc. can also be used as priming

agents. However, optimization of the correct method of seed priming for a seed species is critical and needs thorough standardization concerning priming media, temperature, duration, additives and post-hydration drying technology of the treatment (e.g. Waqas et al. 2019; Malek et al. 2019).

4.2 Types of Seed Priming

4.2.1 Hydropriming

It is a controlled hydration process in which the seed is soaked in water for a predetermined period and then re-dried to its initial moisture content (Pill and Necker 2001). Besides enhancing the germination and vigour of seed under normal conditions, hydropriming is reported to improve seed performance under salinity stress and drought-prone environments (Pill and Necker 2001). The process of hydration is time-regulated so that the seed goes through phases I and II of germination allowing metabolic activation and some degree of cellular repair enhancing seed performance, but must be stopped and dried back to prevent the onset of phase III of germination. To avoid rapid absorption of water, which could be harmful in some seeds especially legumes, spraying water on seeds is practised. The patented technology 'drum priming' is used by seed companies for hydropriming commercial seed lots, wherein a batch of seeds is placed in a large rotary drum while water is sprayed through a jet moistening the seed uniformly. In this way, seeds are constantly upturned and hydrated with only the desired amount of water, keeping them moist for a certain period (depending on the species), and the sudden inrush of water, as in soaking, is avoided. After the desired period, seeds are dried in the same rotary drum.

4.2.2 Osmopriming

Soaking seeds in aerated osmotica (mannitol or polyethylene glycol) to keep the seed hydrated only up to a certain level for a certain period of time, followed by washing and drying, is known as osmopriming. In this method, the desired water potential is maintained with the addition of osmotic substances in the required concentration, thus restricting the water uptake to a certain point and not allowing the seed to advance beyond phase II. Polyethylene glycol (PEG) is the most preferred osmoticum for its chemically inert nature, though it has a low oxygen solubility. It has been successfully applied in many vegetable species (Varier et al. 2010). Patented technology of membrane osmopriming is reported to be effective in high-value, small volumes of vegetable and flower seeds, especially those which are mucilaginous (Rowse et al. 2001).

4.2.3 Solid Matrix Priming

Another way of controlled hydration is by raising the seed moisture by putting it in solid, porous matrices maintained at the desired level of water potential. This consists of mixing seeds with an organic or inorganic carrier maintained at 0.4 to 1.5 MPa at 15 °C for achieving a moisture content sufficient for metabolic processes

to continue, but not allowing radicle protrusion. Seed water potential is regulated by the matric potential of the seed during priming; the water is largely held by the carrier resulting in slow imbibition. Matric carriers commonly used are calcinated clay, vermiculite, peat moss, sand, microgel, diatomaceous earth, ligneous shale, etc. The amount of water to be added is determined by the weight of seed and final seed moisture content targeted (Varier et al. 2010).

4.2.4 Halopriming

It involves soaking seeds in low-concentration salt solutions of chlorides, sulphates, nitrates, etc. (Gour et al. 2022). This results in hardening the seed improving their performance under salt-stressed conditions. The seed size, structure, biochemical constitution, position of seed-protecting layers, type of salt and soaking time are the factors influencing ion penetration into the embryo.

4.2.5 Biopriming

Soaking seeds in an aqueous solution/suspension containing beneficial microorganisms such as various strains of fungal and bacterial species of *Azospirillum, Rhizobium, Bacillus, Pseudomonas, Trichoderma*, etc. is effective in facilitating the advancement of pre-germinative metabolism, better seedling growth (Bennett and Whipps 2008) and protection against harmful microbes in the soil. This involves imbibition of seed with bacterial inoculation (Callan et al. 1990). This treatment not only increases the uniformity and speed of germination but also guards the germinating seed against many soil- and seed-borne pathogens. However, infected seed imbibition during priming may result in a stronger microbial growth and consequently impairment of plant health. Applying antagonistic microorganisms during priming is an ecological approach to overcome this problem (Reddy 2013). In many cases, especially in vegetables, biopriming is an effective approach to disease management (Müller and Berg 2008).

Endophytic Association

Many of the applied bacterial, fungal and mycorrhizal species develop an endophytic association with seed and help considerably in overcoming the negative consequences imposed by various stress factors. Use of endophytic microorganisms could serve as a viable option to circumvent the limitations associated with seed. Endophytes may enhance host growth and nutrient acquisition and improve the plant's ability to tolerate biotic and abiotic stresses. In case of wheat, fungal entomopathogens such as *Beauveria bassiana* and *Metarhizium brunneum* can promote plant growth through seed treatment and suppress disease pathogens following plant colonization as well (Lara 2018). Similarly, faba bean seeds treated with endophytes *Trichoderma asperellum*, *Gibberella moniliformis* and *Beauveria bassiana* had a significantly lower number of aphids when compared to untreated controls. The endophytic fungus Piriformospora indica induced alteration in plant metabolites under drought stress is reported (Ghaffari et al. 2019). Endophytic bacterial species equipped with plant growth-promoting traits may induce tolerance

to salt stress by modulating the morphological, physiological and biochemical characteristics of plants.

4.3 Pre-germination

Pre-germination describes the process of incubating seed under favourable germination conditions up to the point of radical protrusion, followed by sowing in the field. During direct sowing in the field, distinguishing germinable seeds from non-germinable ones is not possible, especially in case of farm-saved seeds. This often leads to uneven germination and poor crop stand (Bidhan 2013). However, use of pre-germinated seeds, based on the length of the emerged radicles, automatically eliminates the poor-quality seeds and results in rapid and uniform field emergence and good plant stand. The pre-germination technique also reduces overall seed rate. Seedlings from pre-germinated seeds were found heavier and more vigorous than the seedlings from dry seeds (Ghate and Phatak 1982). Ridge gourd seeds soaked in distilled water for 20 h followed by incubation for 1 day produced vigorous seedlings which could overcome germination-related problems (Abinaya et al. 2020). However, pre-germinated seed has a very short shelf life due to its desiccation sensitivity and needs to be sown immediately, restricting its application.

4.4 Mid-storage Correction by Hydration-Dehydration

The mid-storage correction treatments improving seed vigour and subsequent storability were standardized for several field crops and vegetables, primarily as an on-farm treatment by Prof. R.N. Basu and his group in Calcutta University, India (Basu et al. 1975; Basu and Dasgupta 1978; Basu 1994). It is the process of hydrating (partially or fully) the low and medium vigour seeds in water for short durations with or without added chemicals, so as to raise the seed moisture content to 25–30%, maintaining for few hours and drying back the seeds to a safe moisture content for dry storage. Post-hydration drying is a crucial step in this process, which determines the shelf life of treated seed. The quality enhancement in such treatments results from:

- Counteraction of free radicals and lipid peroxidation reactions.
- Cellular repair in hydrated state.
- · Germination advancement and embryo enlargement during hydration.

Removal of toxic metabolites accumulated in seed could also play a role in treatments of soaking for a longer duration.

The dry seed treatments with diverse substances and methods are also reported to be effective in maintaining high vigour and viability during storage. These include halogenation in closed environment or treatment of seed with halogenated plant-based powders such as *Albizia amara*, tamarind, neem, *Colocasia*, etc. abstracting

free radicals in the cellular environment (Basu and Punjabi 2022; Dharmalingam and Basu 1978).

4.5 Emerging Seed Enhancement Treatments

Application of nanotechnology for seed quality enhancement is an emerging trend in the seed sector. It is often referred as the 'third-generation' seed treatment for quality enhancement. Seed coating or priming with nanoparticles, detection of seed quality by e-nose technology (sensing volatiles released by the seed), nanobarcoding, etc. are some of the latest applications of nanotechnology in the seed sector.

Seed priming with nanoparticles or nanopriming involves soaking seeds in solutions of nanoparticle/compounds, followed by drying (Panda and Mondal 2020). It is reported to augment the performance of seeds in many ways such as enhancing α -amylase activity, increasing soluble sugar content to support early seedling growth, upregulating the expression of aquaporin gene in germinating seeds, increasing stress tolerance controlling reactive oxygen species (ROS) production and creation of nanopores for enhanced water uptake, to mention a few.

Use of several metal-based nanoparticles (NPs) such as silver nanoparticles (AgNPs), gold nanoparticles (AuNPs), iron nanoparticles (FeNPs and FeS2NPs), copper nanoparticles (CuNPs), titanium nanoparticles (TiO2NPs), zinc nanoparticles (ZnNPs and ZnONPs), cerium oxide nanoparticles (CeNPs) and carbon-based NPs (such as fullerene and carbon nanotubes) in pre-sowing seed treatments is reported to promote germination, early seedling growth and environmental stress tolerance (Mahakham et al. 2017; Guha et al. 2018). Priming with iron nanoparticles has significantly improved germination, rate of emergence and subsequent growth in watermelon seed by triggering metabolic processes during the early phase of seed germination (Kasote et al. 2019).

Synthesis of plant-based nanoparticles is a further refinement of nanotechnology that uses sustainable manufacturing processes to produce safe and innocuous nanoscale biomaterials for agricultural applications. Vurro et al. (2019) attempted to develop silver nanoparticles (AgNPs) by using onion peel extract, as silver has anti-bactericidal and anti-fungicidal properties. Similarly, turmeric oil and silver nanoparticles synthesized from nanoemulsions agro-industrial by-products used as nanopriming agents showed enhanced seed germination, growth and yield in watermelon while maintaining fruit quality (Acharya et al. 2020).

5 IP Protection and Microbranding

Besides enhancing the performance of seed, coating with additional markers using fluorescent substances can be helpful in avoiding seed adulterations. Seeds with a coating of rhodamine B show red fluorescence (Guan et al. 2013). Such coatings provide a means for a unique hidden identity that establishes the ownership and can be effective in preventing counterfeits (Thiphinkong and Sikhao 2021). Similarly, a

non-ionic fluorescent tracer, coumarin 120, was used for differentiating the varieties in soybean (Taylor and Salanenka 2012). Uptake of coumarin 120 and rhodamine B was measured more in the hypocotyl and root, with lesser amounts in the epicotyl and true leaves (Wang et al. 2020). Specialized application of such technologies is being made for microbranding. In this innovative application, very small pieces of microprinted plastic are mixed with the film coat liquid which remain on the surface of the seed. When needed, the seed surface can be observed under a magnifier, to check the brand name/code. This technology is marketed as 'DataDots' and currently in use in the seed industry in the South and South-East Asia (DataDot n.d.; https://www.datadotdna.com/au/solutions/authentication2/datadot/).

6 Conclusion

Manifold improvements in seed performance can be achieved by applying enhancement technologies singly or in combination, using physical, chemical or physiological methods. Nevertheless, an effective technology can be successful only if it is affordable. As the seed is considered to be an effective delivery system and carrier of new technology, it is appropriate to have the best suitable quality enhancement treatments disseminated through the seed, which are both environment-friendly and affordable.

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