

# **Methods of Seed Enhancement**

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#### Abstract

Seed is one of the most basic inputs for agriculture. It evolves over time to respond to a variety of environments including human behaviour. The adaptation of seed leads to sustainable crop production and its satisfactory performance over a period of time. Seed enhancement technique further improves seed performance. Agriculture productivity is directly proportional to viability of seeds. Normally only 20–25% of total seeds are able to germinate. In seed enhancement methods, seeds are pretreated physically, physiologically and biologically to overcome germination constraints. Various other techniques have been employed, which are followed by conceptual development of processes for germination rates and seedling vigour. This chapter considers post-harvest treatments that improve germination or seedling growth or facilitate the delivery of seeds and other materials required at the time of sowing. Other considerations are seed hydration, biological seed treatment and seed coatings.

#### Keywords

Seed priming  $\cdot$  Seed enhancement  $\cdot$  Seed colouring

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#### 23.1 Introduction

Seed is the most fundamental source of food and crop and is also a key link in the food chain. No agriculture practice can improve a crop beyond the limit which is programmed by seed itself. Seed enhancement is a term which is popularly used to describe beneficial techniques applied on seeds post-harvest. There are some popular adages 'Care with the seed and joy with the harvest' or 'Good seed doesn't cost, it always pays', which throw light on the importance of quality seed. An acceptable definition for seed enhancement is post-harvest treatments that improve germination or seedling growth or facilitate the delivery of seeds and other materials required at the time of sowing. This definition examines three general methods: pre-sowing hydration treatments (priming), coating technologies and seed conditioning (Taylor et al. 1998). The current population of the world is approximately 7 billion and is expected to increase by 2.7 billion to be 9.7 billion by 2050 (The Food and Agriculture Organization of the United Nations, FAO 2009). Out of them an estimate of 840 million people may suffer from chronic malnutrition. Approximately 70% increase in food production is needed by 2050 to feed the 9.1 billion mouths (FAO 2009). Some countries are at high risk for food demand—India is one of them. So, there is immediate need to increase food production, which may go twofold sustainably on the same land area (1.5 billion ha) by 2050. Scientists and breeders are searching for these possibilities. Plant breeding and genetic engineering are the two most commonly used practices of enhancing food quality, but they have certain limitations like genetically modified (GM) crops are not easily acceptable by the people of several countries including India. So, agricultural scientists have evolved a new idea of enhancing food productivity by improving seed quality. The importance and potential of quality seed has not been realized by mankind recently, but long ago this necessity has been felt. The need for a viable seed for prosperity of human race is mentioned in Rigveda of ancient India. It is mentioned in the Primeval Manusmriti as 'Subeejam Sukshetre Jayate Sampadyate' which literally means 'A good seed in a good field will win and prosper'. The objective of this chapter is to provide an overview of methods involved in seed enhancement. The methods may not be exclusive, and a combination of techniques may be employed to achieve the objective. Future opportunity and possibilities are also highlighted in this chapter.

Seed priming, magnetic stimulation, seed pelleting and coating (Taylor et al. 1988; Afzal et al. 2012; Farooq et al. 2006) are among some of the shotgun approaches, which have been in practice since the last two decades. Except all these approaches, physical methods of seed enhancement are also in practice without knowing the actual mechanism of seed invigoration. Despite the above methods, plasma seed technology, which applies radiation to seeds and was started in the early 1980s, is a proven technique of seed enhancement. Seed enhancement through magnetic field treatments is also being considered for many agronomic and horticultural crops with some limitations (Araújo et al. 2016).

#### 23.2 Physiological Seed Enhancements

# 23.2.1 Seed Priming

The theory of seed priming was proposed by Heydecker in 1973. Generally, only 20-25% of seeds are able to germinate, so for a country to grow, seeds need to grow at the fullest, and this can be done by seed priming methods. Seed priming is an effective technology for enhanced growth, enhanced vigour and better yield of crops (Harris et al. 2007). Seed priming consists of several physical, physiological and biological treatments to overcome germination constraints. Alternatively, seed priming is a process in which seeds to be soaked are hydrated by keeping them in water or any solution for a specific time period. Seeds undergo their metabolic activities before actually sowing and then redry to their original weight (Bradford 1986). So, it is a pre-sowing approach to influence seedling development by stimulation of metabolic activities prior to seed germination (Taylor et al. 1998). This treatment to seeds is called as priming treatment which includes osmopriming by a salt solution or polyethylene glycol (PEG) or hydropriming and hormonal priming (Bakhtavar et al. 2015). Another one is solid matrix priming in which seeds are soaked in an inert medium also known as matrix potential (Bhosale and More 2013; Bradford 1986; Afzal et al. 2015; Khan 1992). Hydropriming, halopriming, osmopriming, solid priming and hormonal priming are some examples of seed priming methods. Hydropriming and halopriming are defined as soaking of seeds in water and in salt solutions, respectively (Ghassemi-Golezani et al. 2008). NaCl, KCl, KNO<sub>3</sub> and CaCl<sub>2</sub> are the commonly used salt solutions (Bajehbaj 2010). Osmopriming is the most widely accepted seed priming method in which seeds are soaked in aerated low-water-potential solution (e.g. mannitol or inorganic salts or polyethylene glycol), which makes it easier and economical under stressed environment (Guzman and Olave 2006). Pre-soaking of seeds in hormonal solution of GA<sub>3</sub>, salicylic acid, ascorbic acid, cytokinins, etc. is known as hormonal seed priming. In solid priming seeds are mixed in an organic or inorganic carriers and water for a particular period of time. The moisture level of the matrix is further maintained to meet the requirement of radicle protrusion. The water potential of the seed is regulated by priming. There is one more priming type, matrix priming, in which seed is mixed with moist solid particulate materials (e.g. exfoliated vermiculite, diatomaceous earth) (Taylor et al. 1988). These priming treatments work well with extreme conditions like drought or very high or low temperatures or extreme salinity (Afzal et al. 2009, 2015) (Table 23.1).

The details of priming are explained below:

#### 23.2.1.1 Hydropriming

Hydropriming is a controlled hydration process in which seeds are held at water potential that allows imbibition but prevents radical extension (Bradford 1986) and then redried to their initial moisture (McDonald 2000). It has been reported that 16 h of hydropriming resulted in the highest seedling emergence and also hydroprimed

Priming agents	Example
Water	
Salts	NaCl, Na <sub>2</sub> SO <sub>4</sub> , KNO <sub>3</sub> , KCl, etc.
Growth regulators	GA <sub>3</sub> , CCC, kinetin
Vitamins	Vitamin K3, nicotinic acid, pantothenic acid
Plant products	Garlic extract, coconut water, leaf extract of <i>Pongamia pinnata</i> , Albizia amara, etc.

**Table 23.1**List of priming agents

seeds emerged earlier than those of unprimed seeds (Ghassemi-Golezani and Dalil 2014). If the drying process is non-uniform, it causes uneven germination (Pill and Necker 2001). Among other seed priming techniques, hydropriming is a promising treatment for drought and salinity stress, and also it is cost-effective, which are the reasons this method is highly acceptable among farmers (Janmohammadi et al. 2008). Hydropriming produces healthy seedlings which lead to uniform crop development and increased yield in crop production.

#### 23.2.1.2 Osmopriming

Osmopriming is similar to hydropriming in which osmotic solution is used for seed priming. Osmotic solution can be polyethylene glycol or a salt solution, and it should be applied under controlled aerated conditions to permit imbibition but prevent extension. Polyethylene glycol regulates water uptake and controls radical extension (Pill et al. 1991). The nitrogen-providing salts commonly used for osmopriming are potassium chloride (KCl), potassium nitrate (KNO<sub>3</sub>), sodium chloride (NaCl), magnesium sulphate (MgSO<sub>4</sub>), potassium phosphate (K<sub>3</sub>PO<sub>4</sub>), calcium chloride (CaCl<sub>2</sub>) and potassium hydrophosphate (KH<sub>2</sub>PO<sub>4</sub>). These provide nitrogen to the germinating seed, which is an important ingredient for protein synthesis. As compared to hydropriming, osmopriming induces more rapid and uniform germination and also shortens the mean germination time.

#### 23.2.1.3 Hormonal Priming

Seed soaking in hormonal solution is known as hormonal priming. A single hormone can do many works together, and also many hormones can perform similar role, like Abscisic acid (ABA) has a negative role in germination but its effect can be neutralized using GA (gibberellic acid) and auxin (Chauhan et al. 2009). GA<sub>3</sub>, salicylic acid, ascorbic acid and cytokinins are the widely used hormones for this. These hormones play a pivotal role in different phases of plant development, for example, cytokinins are important for all phases of plant development; gibberellic acid (GA<sub>3</sub>) is known for breaking seed dormancy, increasing germination capacity and many more; while GA stimulates hydrolytic enzymes which enhance germination and accelerate seedling growth (Riefler et al. 2006; Karssen et al. 1989; Rood et al. 1990). This priming treatment acts well under dormant and abiotic stress

conditions. So, phytohormones, which are meant to alter plant growth, can naturally impact positively on seed germination.

#### 23.2.1.4 Nutrient Priming

Nutrient priming can be referred as the most basic and cost-effective priming procedure in which micronutrients like Zn, B, Mo, Mn, Cu and Co are given for seed priming treatment for most of the field crops (Wilhelm et al. 1988; Peeran and Natanasabapathy 1980; Sherrell 1984). Seeds are simply soaked in nutrient solution overnight before planting (Harris et al. 2001). A different micronutrient has a different role in plant development like zinc salts increase growth and provide disease resistance to seedlings. Phosphorous is an important constituent of amino acids. Iron (Fe) is required for the formation of chlorophyll in plant cells. It serves as an activator for biochemical processes such as respiration, photosynthesis and symbiotic nitrogen fixation.

Seed priming has some molecular aspects also, and elucidation of mechanisms of these effects is essential for seed scientists and seed industry. So, factors associated with reduced vigour of seeds should be minimized.

In addition to above methods, plasma seed technology, which applies radiation to seeds and was started in the early 1980s, is a proven technique of seed enhancement. Seed enhancement through magnetic field treatments is also being considered for many agronomic and horticultural crops with some limitations.

#### 23.2.1.5 Magnetic Fields for Seed Treatments

Seeds respond when exposed to magnetic field. So, it is essential to optimize the dose of field exposure, which affects seed germination which in turn leads to increased yield of crop production (Silva and Dobranszki 2016). Seeds, when pass through a magnetic funnel, are affected by the magnetic field on the passage and then are soaked. It was found that seeds germinate 1 day earlier after magnetic treatment and germination percentage increased by 33–45% in treated seeds related to the control (Ahmad et al. 2007). Magnetic exposure is defined as the product of flux density of magnetic field with timing of the exposure. The flux density is the number of magnetic lines of flux that pass through a certain point on a surface. So, it varies with static or alternating magnetic field exposure to seeds. It is experimentally proven that magnetic field not only enhances germination capacity of seeds but also increases crop yield and protects crop from pathogens (De Souza et al. 2006; Pietruszewski and Kania 2010).

#### 23.2.1.6 Plasma Seed Treatments

Plasma application to seeds in agriculture and medicine is an example of recent advancement in seed enhancement techniques, which ensures every seed has treated in the best possible physical condition prior to germination (Sosnin et al. 2004; Akitsu et al. 2005). Recently this technique has come out as an alternative to traditional pre-seed sowing treatments. High-voltage plasma discharge, which is a resultant of bombardment from ions, oxygen radicals, nitrogen radicals and an assortment of charged particles, is applied to the seeds which cause disruption and

further oxidation of seed coats. Oxidized seed coats exhibit high permeability to water and nutrient uptake. This technique has come up with good results, which are increase in viability of seeds from 5% to 30% and decrease in seed germination time which is found to be ranging in between 20% and 50%. Plasma treatments were further upgraded into microwave plasma, magnetized plasma and atmospheric plasma treatments (Sera et al. 2010; Zhou et al. 2011). It has been reported that if plasmas are used with few gases such as aniline, cyclohexane and helium, germination and growth enhancement will be achieved early (Volin et al. 2000; Jiayun et al. 2014). Further, plasma contains reactive oxygen species which increases the quality of plant development by controlling thiol groups (Henselová et al. 2012). Plasma chemistry modifies seed germination by delaying or boosting with the application of plasma-treated deposits on seed surfaces (Volin et al. 2000). It has also been published that plasma helps to attain zero seed destruction, zero chemical use and environment-friendly treatments to seeds (Volin et al. 2000; Dhayal et al. 2006; Selcuk et al. 2008). It also improves seed quality and plant growth (Sera et al. 2010). Moreover, seeds exposed to plasma treatments result in alternations of enzymatic activity and sterilization of seed surface (Selcuk et al. 2008).

#### 23.2.1.7 Radiation Seed Treatments

Ionizing radiation poses an impact on biological systems, and it has been proved that these radiations activate a number of physical and chemical steps inside the cell from absorption of radiation to injury. Among other ionizing radiations, gamma radiations are more effective and easily available and possess powerful penetration (Moussa 2006). Gamma radiations produce free radicals inside the cell of the organism by causing chemical interaction with biomolecules and water and damage cell components, which in turn effect some physiological and biochemical processes vital for cellular survival (Rogozhin et al. 2000). However, high dose of gamma radiation can alter protein synthesis, enzyme activity, hormone balance and leaf gas exchange (Al-Salhi et al. 2004; Hameed et al. 2008). Optimal radiation increases the root length and fresh weight of seedlings, and cellular mitotic divisions also remain normal (Mergen and Johansen 1963).

# 23.3 Biological Seed Enhancements

## 23.3.1 Bacterial Seed Agents

This treatment is also known as seed bacterization. It has been proven as a successful method for biological nitrogen fixation, solubilization of phosphorous and zinc and production of siderophores (molecules which can sequester Fe) in legume plants (Stacey et al. 1992). They have the capability to synthesize phytohormones to stimulate plant growth (Suslow and Schroth 1978; Graystone et al. 1991). Apart from this, it was found that this method also improves plant growth and provides biological control to plant diseases (Tahvonen 1982; Pierson and Weller 1994)). It is well known that rhizobacteria are soil-borne, free living bacteria, which induce plant

growth. These bacteria are alternatively called as plant-growth-promoting (PGPR). Under conditions PGPR rhizobacteria stress also synthesize ACC-deaminase enzyme by modulation of ethylene level (Glick et al. 1998; Nadeem et al. 2015). This biopriming technique is considered as a good example of seed enhancement, integrating biological and physiological aspects and plant disease control. It has been found that seed priming with beneficial microorganisms results in more rapid growth and increased crop yield under stress conditions. They can also be used as an alternative to chemical fertilizers (Bloemberg and Lugtenberg 2001; Vessey 2003). Rhizobacteria colonize roots and are symbiotic to their hosts. Reports of PGPR that have been successfully tested as co-inoculants with rhizobia include strains of the rhizobacteria such as Azotobacter [82], Azospirillum [83], Bacillus [84], Pseudomonas [85, 86], Serratia [86] and Streptomyces [87].

#### 23.3.2 Fungal Seed Agents for Biopriming

Microorganisms including bacteria (discussed above) and fungi are used as biopriming agents to enhance seedling growth and vigour. Biopriming potential of *Trichoderma* and *Bacillus* spp. was compared with commercial products Agrotrich plus<sup>®</sup> and Rhizoliptus<sup>®</sup> for enhancing growth and yield of beans, and findings revealed better seedling growth with bioprimed seeds as compared to other techniques (Junges et al. 2016).

# 23.4 Seed Coating and Pelleting

Seed film coating comes under globally practiced seed treatments like pelleting, priming and inoculation (Thomas et al. 2003), which altogether aimed to improve seed germination, seed storage and enhanced plantability. Seeds vary greatly in size, shape and colour, whether small or irregular. Further, seeds are prone to attack from a range of pests, which include animals and pathogens. Seed coating refers to the application of chemicals to protect seeds from pathogens/pests and support seedling growth (Scott 1989). Initially coating is used to be done by just applying chemical slurry on the seeds and then dried, but disadvantages of this method are it was difficult to get uniform seed coating and, during transportation of seeds, most of the coatings were rubbed off. So, seed technologists have found out a new way termed as film coating to overcome these problems. In this technique, seeds are sprayed (chemicals applied in a polymer) along the path with a specialized machine, and later polymer is rapidly dried which results in dry polymer coating. These polymers can be growth regulators, inoculants, micronutrients, fungicides, insecticides and other seed protectants (Rehman et al. 2013).

Another operation is pelleting which is generally performed on drum or coating pan (Scott 1989). The speed varies with the diameter of apparatus used, and generally its range is from 10 to 35 rpm. This method, however, is a labour-intensive operation and required long working hours and skilled hands. The pelleting

process has become automated, and computer-controlled coating system is described in Scott (1989). In each case, seeds are coated with a combination of binder (adhesive) and filler (bulking agent). A number of materials can be used as binders and fillers (Scott 1989). The procedure involves broadly three stages: stamping, coating and rolling. To start with, seeds are uniformly coated with adhesive materials (gum arabic, gelatin, methylcellulose, polyvinyl alcohol, etc.) in correct quantity and concentration. Then, the filler materials (lime, gypsum, dolomite, rock phosphate, etc.) are sprinkled on the coated seeds and are rolled on the seeds for uniform coating. Finally, pelleted seeds are sown into the fields. A number of reports are available, which quote the success stories of this method. In sunflower, seed pelleting with a mixture of moringa leaf powder (250 g), DAP (100 g), *Azospirillum* (25 g) and *Trichoderma* (4–5 g) increases yield of 15% in irrigated and non-irrigated conditions (Anonymous 2001). Another report by Geetha and Bhaskaran (2013) indicated that seed hardening and pelleting treatment increases the yield of ragi (*Eleusine coracana*) varieties.

In conclusion, we can say that film coating provides an optimal method for the application of chemical and/or biological seed treatments (Taylor and Harman 1990; Taylor et al. 1994).

# 23.4.1 Seed Colouring

It is a method in which seeds are coloured using different dyes (naturally and artificial) so that it (seed) can acquire a distinct and attractive look. For natural dyes different parts of a plant (e.g. opuntia, jamun, basella, etc.) can be used. For example, the dried leaf powder of henna, when mixed with water, can be used as a colouring agent. Other examples are beetroot, the root portion of which is used as a colouring agent, and turmeric, which is also used in the same manner. In some cases, a flower part can be crushed and used (e.g. marigold and hibiscus).

Chemical dyes are also in use for the purpose of seed colouring. Different colours, which are generally used as colouring agents, are Congo red, bromocresol green, jade green, sky blue, direct chambagu, turquoise blue, etc.

#### 23.4.2 Seed Hardening

It is the process of hydration and dehydration of seed to fix biochemical events. This method is done primarily to enhance the thermotolerance of the seed without loss of viability. Other benefits include rapid germination and growth rate of seedling and to impart resistance against various stress conditions.

#### 23.4.3 Seed Conditioning

When seeds are brought in from the field, they are seldom pure and contain a number of unwanted materials such as pieces of stems, dust, weeds and poor-quality seed, which should be removed from that seed lot. Before the seed is sold for planting or other agricultural activities, the minimum quality standard should be met. Therefore, the series of measures, which involve mechanical operations, chemical treatment, packaging, distribution and marketing, are termed as seed conditioning. This process of upgrading can be considered as a seed enhancement as the germination and seedling growth characteristics of the seeds are further improved. The following are the steps in conditioning:

- Drying: This is an important step in which various drying units are used. At the time of harvest, moisture content is high, so it should be minimized before storage.
- Precleaning: A large amount of trash/green/dirt may be present in the seeds. Precleaning is often neglected, but this step is an important step. If this method is applied before drying, it enhances the capacity of the dryer.
- Cleaning: Basic cleaning is accomplished by air-screen cleaner, which makes use of a series of screens and air separations to remove light, trash, dust and other unwanted materials. Sometimes this is a necessary step for direct marketing of seed.
- Treatment: This step includes application of fungicide, insecticides, growth regulators, etc.
- Packaging: This step consists of traditional bagging with the use of bulk bags. After conditioning storage and packaging are the final steps.

# 23.5 Molecular Aspects

Physiologically seed priming with nutrients increases the seed contents of primed nutrients and enhances nutrient uptake and also triggers the enzymes associated under deficient conditions to support the seedling development (Imran et al. 2013). Priming increased the production and activity of  $\alpha$ -amylase within germinating seeds, which is directly proportional to the seed vigour (Afzal et al. 2012). At molecular level, priming beams favourable effects on RNA and protein synthesis and also initiates cell division and transportation of storage proteins (de Castro et al. 2000). Arabidopsis model plant was used for elucidation of several proteins and their precursors involved in seed germination or priming. It was found that actin isoform helps in imbibition and cytosolic glyceraldehyde-3-phosphate dehydrogenase plays a role in the seed dehydration process (Gallardo et al. 2001). In imbibition, a higher germination rate of seeds is the result of increased production of metabolites (Coolbear et al. 1980; Burgass and Powell 1984). At molecular level, peroxiredoxin-5, 1-Cys peroxiredoxin, embryonic protein DC-8, cupin and globulin-1 were found to be involved in improved seed germination, and their level in plants remained unaltered even after seed priming (Gong et al. 2013).

#### 23.6 Conclusion

Germination of seed is an important event, and it depends upon two important factors: the state of the seed (dormant or not) and the environmental conditions. If a seed is dormant, it is necessary to treat it with chemicals or other things to break dormancy so as to initiate germination. There is a need of optimum condition of moisture, oxygen, temperature and light for a seed to germinate. Moisture and salinity are among the limiting factors which hinder seed germination and crop establishment. Seed enhancement technology provides a unique tool and idea to remove poor-quality seeds and also removal of unwanted materials from total seed lot. Priming is an important seed treatment practice, which can help the seeds to break dormancy and to minimize/overcome problems of abiotic stress such as moisture, salinity and extreme temperature. It is actually a pre-sowing seed treatment practice which provides sufficient moisture to start the pregermination metabolic process. This process rectifies the problems encountered during germination and emergence. Hence, it is useful for uniform emergence and growth. Priming can be done with water, different solutions and phytohormones. Cell membrane integrity can be indirectly measured through solute leakage measurement. However, seed enhancement technologies are not a new technique in agriculture as reported earlier by Evenari (1980). Seed coating is a much older technique, being practiced 2000 years back. The Chinese were well known to these techniques, so this 'modern seed enhancement' has certainly historical basis. This domain needs more research and detailed study. There are a number of challenges and opportunities exist, which depend upon several components of these technologies. This seed enhancement technique is one of the best solutions of germination-related problem when there is stress condition. It can increase the rate of germination and seedling even under different set of environmental conditions. Minimum exposure to toxicant, less time under stress condition, uniformity in the field and high turnover are some of the advantages of seed enhancement technology. This technique can prove the best technique if used wisely.

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