Impact of Seed Priming on the Modulation of Physico-chemical and Molecular Processes During Germination, Growth, and Development of Crops

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Abstract

Seed is the prime input in agriculture sector, and production of quality seed is the immense challenge in front of agriculturist to achieve the goal of food security. Present sceario emphasis that the world population is increasing day by day resulting to quick exhaustion of natural resources leading to climate change which accelerates the issue of abiotic (heat, cold, drought, and salt) and biotic stress in plants. These abiotic and biotic stresses are often interrelated and cause undesirable physiological, morphological, biochemical, and molecular change that affect plant growth and development and ultimately yield. Time to time various plant breeding and molecular techniques developed to solve the problem of abiotic and biotic stresses. However, alternatively, some simple and economical techniques are also in race to address this problem. Seed priming is one of them, approved by many agriculturists for better crop stand establishment and growth, even under adverse environmental conditions. The present chapter deals with the different types of seed priming methods and their scope in mitigating abiotic and biotic stresses. Further, mechanisms of seed "priming-induced" physiological, biochemical, and molecular changes in regulation to stress tolerance were extensively explained in the light of the latest research work carried out in this direction.

Keywords

Seed priming · Coating · Seed hardening · Abiotic stress · Biotic stress

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Abbreviations

AQP	Aquaporin
CAT	Catalase
DEPs	Differentially expressed proteins
DHY	Dehydrin
LEA	Late embryogenic abundance
MDA	Malondialdehyde
POD	Peroxidase
ROS	Reactive oxygen species
SMP	Solid matrix priming
SOD	Superoxide dismutase

2.1 Introduction

Biologically, seed is the mature ovule that consists of an embryo and stores food materials for germination and contains a protective covering (seed coat), or it can also be defined as a small embryonic plant enclosed within a protecting covering called seed coat along with endosperm (Dieter and Bouman 1995). Agriculturally, the term seed represents any living material that can be sown and gives rise to a fully functional plant, e.g., seeds of potato is a part of a tuber and the setts of sugarcane are the parts of the stem. Seeds play very important role in input technology in agriculture sector due to its easy handling and transporting from one place to another. Therefore, in agriculture, demand and supply of quality seeds hold the center position. Further the quality of seeds is a determinant factor for the yield of particular crop. Hence, the seeds of high quality are always in demand. Basically, high-quality seeds show synchronized germination and the formation of potential seedlings, those which are able to attend the optimum level of genetic potential. Always the good-quality seed has attracted the attention of researchers to get proper production potential of a particular crop. The quality seed in present day can be achieved by various means where the basic and applied knowledge of plant physiology, genetics, and seed technology all are integrated to improve the criteria of quality of seeds. It is the ultimate goal of successful companies that breed crop plants for seed production (Bishaw et al. 2007).

The crop growers as well as the kitchen garden growers always have faced some problems, associated with the seeds, like more time taken for germination and less germination percentage leading to yield loss. Seeds especially of some flowers and herbs are often found quite difficult to germinate and using certain techniques to increase the rate in which the sprout has been the focus of a large amount of scientific research. From last two decades, several seed enhancement/invigoration technologies are implemented to enrich the seed quality.

Seed enhancement/seed invigoration is a range of treatments of seeds that improves their performance after harvesting and conditioned but before they are sown. They include priming, steeping, hardening, pregermination, pelleting, encrusting, film-coating, and tagging but exclude treatments for control of seed-borne pathogens (Halmer 2006). They are used to improve seed sowing, germination, and seedling growth by altering seed vigor and/or the physiological state of the seed. The alteration may improve vigor or the physiological state of the seed and finally improve yield potential by enhancing uniformity of germination, early seedling vigor, and healthy seedling.

Seed priming is most commonly used at farmer's field. It improves germination, germination speed, seedling vigor, root length, seedling dry weight, dry matter production, photosynthetic efficiency, and many other plant growth traits. Other than this it also improves biochemical status of plant by improving α-amylase activity and soluble sugar contents during seed germination even in low temperature (Anaytullah and Bose 2007) and nitrate reductase activity and nitrogen content in growing seedlings in normal growing condition in wheat crop in respect to non-primed seeds (Sharma and Bose 2006).

It is well established that seed priming treatment found to ameliorate the adverse effects of biotic and abiotic (drought, salinity, flooding, heat, cold, heavy metal) stress responses in affected plants via altering the antioxidant metabolism (Kausar and Ashraf 2003; Basra et al. 2005; Guan et al. 2009; Nayaka et al. 2010; Kumar et al. 2016). It improves the stress memory and boosts antioxidant system by improving activity of SOD, catalase, MDA, glutathione reductase, ascorbic acid, and stress protein like late embryogenesis abundant (LEA), dehydrin and aquaporin (AQP) proteins (Mittal and Dubey 1995; Bohnert and Shen 1999; Vander et al. 2006; Anaytullah et al. 2012). In this chapter we are summarizing the types of seed enhancement technique specifically seed priming in respect to their roles in modulation of physiological and molecular mechanism during germination and post-germination phases as well as how it helps in the amelioration of abiotic and biotic stress responses in crops/plants during their developmental process.

2.2 Seed Invigoration Techniques and Their Use in Agriculture

Seed invigoration technology includes *priming* [A pre-sowing hydration treatments include noncontrolled water uptake systems (here water is freely available and not restricted by the environment) and controlled systems (it regulates seed moisture content preventing in the completion of germination)] (Taylor et al. 1998), *pelleting* (it adds thicker artificial coverings to seeds, which can be used to cover irregular seed shapes and add chemicals to the pellet matrix, e.g., of sugar beet or vegetable seeds; the pellet matrix consists of filling materials and glue; it is also used to increase the size of very small horticultural seeds), and *coating* (*film-coating* methods allow the chemicals to be applied in a form of synthetic polymer that is sprayed onto the seeds and provides a solid, thin coat covering on them; the advantage of the polymers is that they adhere tightly to the seed and prevent loss of active materials like fungicides, nutrients, colorants, or plant hormones); further in *seed hardening* (kind of seed priming) technology, the seeds are allowed to be hydrated either in presence of water or in presence of various organic/inorganic solutions just before the emergence of radical and then dehydrated(hardened) under the forced air to get its initial starting weight (Sharma and Bose 2006). *Physical seed invigoration*, comparatively new one, offers an approach where the conventional method of seed treatment by using chemicals is ignored, and that has been replaced by using irradiation with microwaves and ionizing radiations, found to be a promising pre-sowing seed treatment; magneto priming is also one of them (Araújo et al. 2016).

Generally basic and applied seed research projects focus on embryo growth and on the different seed-covering layers (e.g., testa, endosperm, pericarp), which are the determinants of seed quality and exhibit the biodiversity of seed structures. Seed germination is controlled by various external environmental factors (light, temperature, water) and also by internal factors like plant hormones (gibberellins, abscisic acid, ethylene, auxin, cytokinins, and brassinosteroids) as endogenous regulators. The utilization of plant hormones and inhibitors as well as their biosynthesis and action in seed treatment technologies affects seed germination and seedling emergence. The genes, enzymes, signaling components, and downstream targets of some plant hormones provide molecular marker for seed quality and seedling performance (The Seed Biology Place).

By considering all these points, in this chapter it will be discussed how seed priming (a plant physiological technology) improves the germination, growth and development, and yield potential as well as quality of the produce of various agriculturally important crop plants by protecting them from various hazardous environmental conditions.

2.3 Types of Seed Priming

The term "seed priming" was coined by Malnassy (1971) and deals with a practice which promotes rapid and uniform seedling emergence consequently beneficial for better establishment of crops in field condition. Seed priming is of many types depending upon the priming material, which includes hydro-priming (continuous or successive addition of a limited amount of water to the seeds), osmo-conditioning or osmo-priming (exposing seeds to relatively low external water potential), halo-priming (pre-sowing soaking of seeds in salt solution), hormonal priming (priming solutions containing the limited amount of plant growth regulators or hormones), nutri-priming (seeds are soaked in solutions containing the plant growth-limiting nutrients instead of being soaked just in water), bio-priming (coating of seeds with biocontrol agents), redox priming (it represents the redox state of cell and regulates the key processes in growth and development as well as stress tolerance in response to any external stimuli; plants modify their redox state, and the extent of change is dependent on the nature of the stimulus itself, the dose and the time to which the tissue is exposed as stated by Miller et al. (2009)), solid matrix priming (mixing seeds with a solid or semisolid material and measured amount of water) (term was coined by Taylor et al. 1988), and pre-sowing soaking (soaking of seeds either in water or in any solution of low water potential before sowing) (Bose et al. 1982a, b, c; Ashraf and Foolad 2005) (Fig. 2.1).

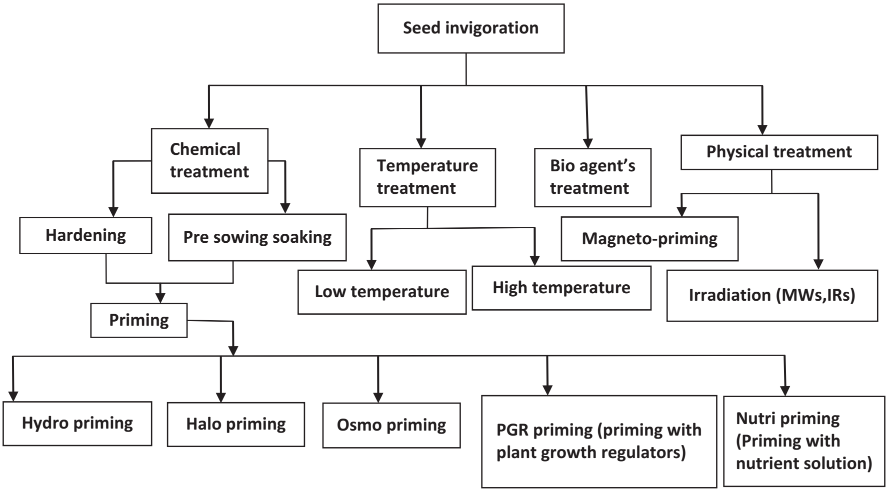

Fig. 2.1 Represent the type of seed invigoration technique

2.4 Seed Priming and Physiological Changes During the Process

Nowadays, seed treatment technology is an important link between seed producers and crop production industry. Its aim is to allow the seed treatment product to be used in such a form that represents best quality in the market. One of them is seed priming, which is an innovative concept of treating seeds using various solvents including water which activates physiological processes of seeds. Generally osmoregulators like PEG, mannitol, glycerol, etc. are being extensively used in seed treatment for various purposes. If graph is plotted between seed water content (imbibition/osmosis) and time, then non-primed seed and primed seed represent three subsequent phases in a stepwise manner. First phase (phase I) represents the entry of water in the seed by the process of adsorption called imbibition, which is similar in both cases. Second phase (phase II) represents hydration process in non-primed seeds. In case of primed seed hydration treatment allows controlled imbibition and induction of the pre-germinative metabolism ("activation"), but radicle emergence is prevented, represented by extended second phase**.** Last phase (phase III) represents the germination and post-germination phase which is again similar in case of primed and non-primed seeds (Rajjou et al. 2012) (Fig. 2.2).

In seed priming the hydration treatment is to be stopped before desiccation tolerance is lost. An important problem is to stop the priming process at the right moment; this time it depends on the species, genotype, and the types of seed. Priming solutions can be supplemented with plant hormones or beneficial microorganisms. The seeds can be dried back for storage, distribution, and planting. Priming can induce the germination by improving speed and synchronization of seed germination (Bose and Tandon 1991); it can improve seed vigor which requires very short or no activation time during germination. It may introduce a wider range of temperature for

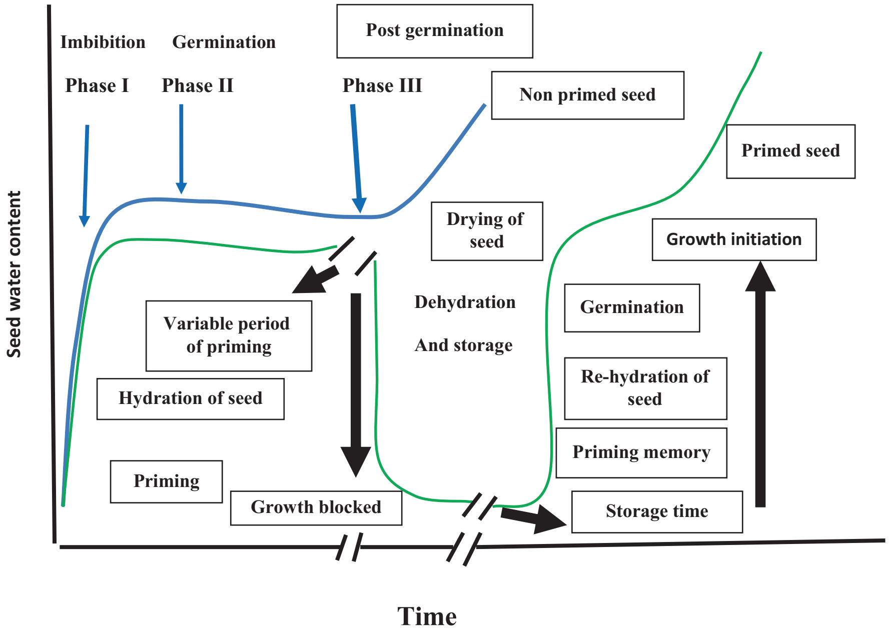

Fig. 2.2 Schematic representation of normal germination and seed priming process (Rajjou et al. 2012) (Phase I, imbibition phase; phase II, germination phase; phase III, post-germination phase)

germination (Anaytullah and Bose 2007), can break the dormancy, or may shorten the time of emergence with improved seedling vigor (Mondal et al. 2011). This leads to better crop stands and higher yields (Srivastava and Bose 2012).

Physiological, biochemical, and molecular changes during seed priming are represented in Fig. 2.3. During seed storage condition auto-oxidation of storage metabolites with time leads to lipid peroxidation, which can further cause membrane perturbance and loss of cellular compartmentalization. Long-term storage of seed causes dysfunction of cellular organelles and inactivation of enzyme and finally leads to genetic damage which can influence the seedling viability and vigor. During priming, phase I represented by the activation of priming memory, repairing DNA and mitochondria, respiration, and energy metabolism, ROS signaling and antioxidant, gene transcription and translation, cell cycle initiation, and induction of stress response gene such as LEA, DHY, AQP, and hormone signaling. Phase II is germination phase; in this priming memory is recruited upon second rehydration and protein synthesis by using new mRNA. Phase III is represented by the post-germination phase; in this phase mobilization of stored reserve, radical cell elongation events occur, and finally at the end of this stage, radicle emerges out by rupturing the seed coat (Chen and Arora 2013) (Fig. 2.3).

The pretreatment of seeds with priming agents facilitates the active absorption of ionic molecules with greater ATP availability and repair of deteriorated seed parts for reducing leakage of metabolites leading to faster embryo growth (Dahal et al. 1990). It also, reflected in greater cellular membrane integrity, counteraction of lipid

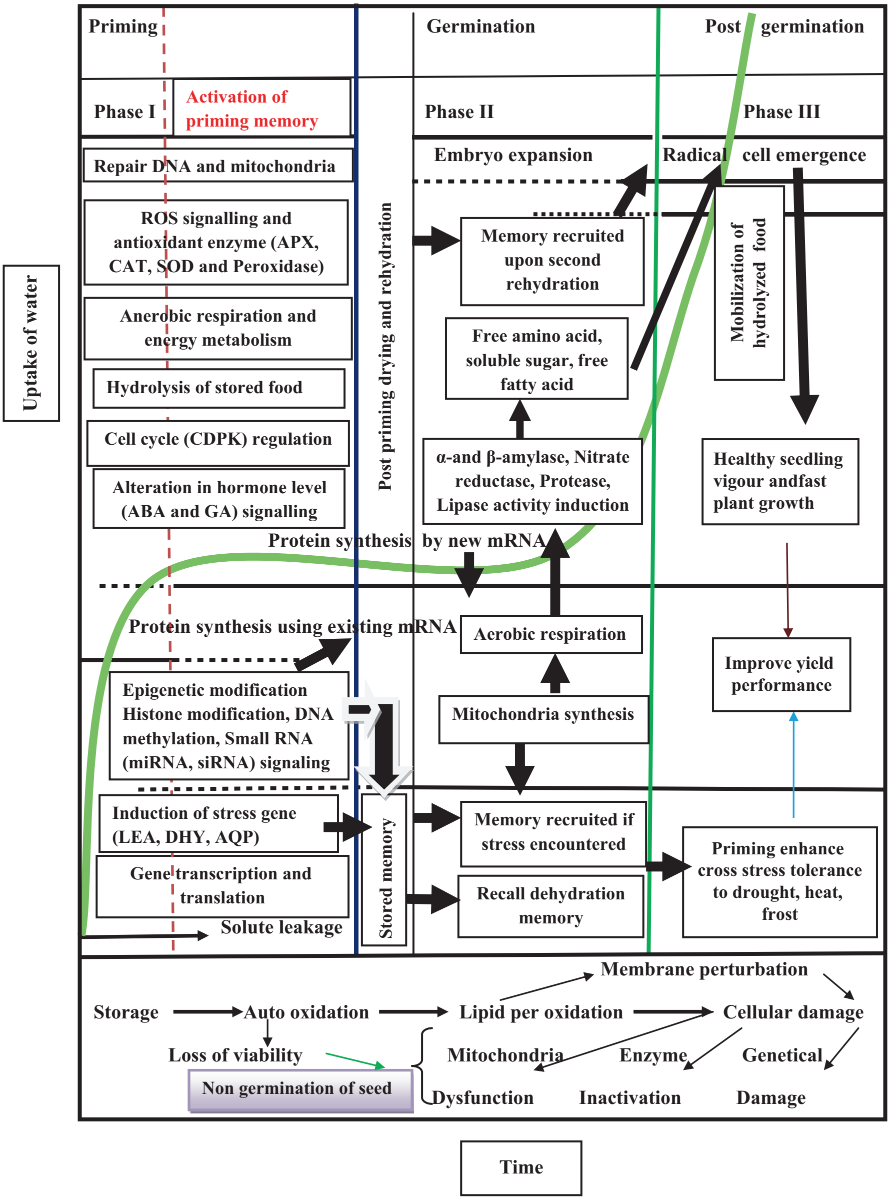

Fig. 2.3 Model for priming memory and its relation to abiotic stress signaling (Chen and Arora 2013)

peroxidation, and free radical chain reaction often are found to be directly correlated with the maintenance of viability and reduce moisture uptake by hydrated-dehydrated seed (Dollypan and Basu 1985), antipathogenic effects (Powell and Mathews 1986), repair of biochemical lesions by the cellular enzymatic repair system (Villiers and Edgcumbe 1975) and metabolic removal of toxic substances (Basu et al. 1973), counteraction of free radical and lipid peroxidation reactions (Rudrapal and Basu 1982), biochemical changes like enzyme activation (Sananda and Bose 2012), and improvement of germination rate particularly in old seeds (Gray and Steckel 1983; Lee et al. 1998).

2.5 Seed Priming in Respect to Germination, Early Seedling Growth, and Yield

Seed priming, a pre-sowing partial hydration of seeds, is often used to improve crop performance (Bradford 1986). Primed seeds thus reach a "germinating state" but without radicle protrusion. Due to this partial hydration of seeds, they adapt the potentiality to exhibit improved germination rate and uniformity. The priming leads to an enhanced germination performance, and increased seedling vigor under both optimal and adverse environments has been reported in diverse species, such as maize (*Zea mays*), soybean (*Glycine max*), spinach (*Spinacia oleracea*), pepper (*Capsicum annuum*), wheat (*Triticum aestivum*), mustard (Brassica juncea), sunflower, rice (*Oryza sativa*), etc. (Hussain et al. 2006; Iqbal and Ashraf 2007; Farooq et al. 2008; Bose et al. 2007; Krishnotar et al. 2009; Korkmaz and Korkmaz 2009; Zhou et al. 2009; Chen and Arora 2011; Srivastava and Bose 2012; Sheidaie et al. 2013; Pant and Bose 2016; Kumar et al. 2016). Chemicals that are frequently used for osmo-priming/hardening are, namely, NaCl, KH_2PO_4, K_3PO_4, KCl, etc. Bose et al. (1983a, b) established that $NaHCO_3$ priming (pre-sowing soaking) can improve the activities of nitrogen-utilizing enzymes like nitrate reductase, nitrite reductase, and nitrogenase in *Vigna mungo*.

These chemical solutions of low water potential while used in form of priming treatment may improve influx of nitrogen and other nutrients needed for protein synthesis during germination of the seeds and establishment of seedlings in the crop field and thereafter enhance the performance of crops as a whole (Du and Tuong 2002; Pandey and Bose 2006; Sharma et al. 2009). Priming of seeds in general induces different metabolic events like de novo synthesis of α-amylase with an increase in soluble sugar (Lee and Kim 2000; Anaytullah and Bose 2007), and activity of protease and soluble nitrogen content and the process of mobilization of hydrolyzing products toward the embryo from storage tissues in seeds (Bose and Srivastava 1980, 1982; Bose et al. 1982a, b, c) finally enhance the seedling establishment and seedling vigor, i.e., plant density, fertile tillers, test weight, number of grain per panicle, etc., compared with non-primed treated seeds (Bose and Mishra 1992; Du and Tuong 2002).

Among essential elements nitrogen plays the central role because it is one of the components of every enzyme/protein. However among nitrogen-containing salts, nitrate is known to act as dormancy breaking agent where ammonium salts are usually found less effective in this respect except in case of rice, and application of nitrate is observed to play an effective role in producing higher dry matter and grain yield (Bose et al. 1982a, b, c; Pandey and Bose 2006). Application of Mg salts as pre-sowing soaking treatment improves the protein of shoot and yield of mustard (Bose and Mishra 1997, 1999). Basra et al. (2004) and Sharma and Bose (2006) introduced a new technique for seed invigoration in which both seed hardening and osmo-conditioning were successfully integrated where seeds were hardened in various salt solutions instead of tap or distilled water; Basra et al. (2004) further concluded that osmohardening, using $CaCl_2$ solution (having an osmotic potential of -1.5 MPa), was best for vigor enhancement compared with other salts and simple hardening, whereas Sharma and Bose (2006) suggested $Mg(NO_3)_2$ is better than $K(NO_3)$ for this kind of seed treatment to wheat. Moeinzadeh et al. (2010) reported that bio-priming of sunflower seed with *Pseudomonas fluorescens* improves seed invigoration and seedling growth.

2.6 Seed Priming in Respect to Abiotic Stress

Different stressors, i.e., water deficit, heat, cold, salinity, etc., affect the crop yield. To counteract the effects of stress, plants undergo a process of stress acclimation via modulating various physiological and biochemical actions. Further, to feed the growing population of the world in future, there is a need to develop some techniques that can enhance yield in terms of grain even at stressed condition with not much application of nitrogen fertilizer, used commonly and save from nitrate pollution with minimum fertility loss and low cost, are the paramount challenges, tackled by plant scientists mainly seed physiologist. It has been noted that most of the types of stresses reduce the uptake of water in seed during germination. Further the reduced availability of water is observed to influence the process of the cell elongation; consequently it shows immense impact on the growth of the embryo followed by the seedling emergence at the time of seed germination.

Seed priming of rice varieties HUR-3022 and Sahabhagi Dhan with Mg $(NO_3)_2$ and K_2SO_4 was found to mitigate the inhibitory effect of PEG-6000 (creating the osmotic potential equivalent to -0.30 to $–0.49$ MPa) during seed germination. It is noted that in primed seeds the vigor index, germination index, and absolute water content percentage were increased as compared to non-primed PEG-treated seeds (Pant and Bose 2016). Therefore it seems to be an economically viable option for vertical intensification of yield potential in rice varieties. Salinity stress is known to trigger oxidative stress in plant tissues through the increase in reactive oxygen species (Apel and Hirt 2004). Chloroplasts are the major organelles producing the reactive oxygen species (ROS) such as the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and singlet oxygen ($_1O_2^*$) during photosynthesis (Asada 1992). The production of ROS can be particularly high when plants are exposed to salinity stress (Athar et al. 2008; Ashraf 2009). ROS cause chlorophyll degradation and membrane lipid peroxidation. So, malondialdehyde (MDA) accumulation as product of lipid peroxidation and chlorophyll retention are two oxidative stress indicators that are tested tools for determining salt tolerance in plants (Yildirim et al. 2008).

Srivastava et al. (2010) evaluated the effect of different seed priming methods to enhance the sodium chloride (NaCl) and polyethylene glycol-8000 (PEG-8000) stress tolerance in Indian mustard (*Brassica juncea* L.). Plant growth regulators salicylic acid (SA), ascorbic acid, and abscisic acid (ABA) while used as seed priming treatment to wheat seeds showed an improvement in germination and seedling growth in saline condition Afzal et al. (2006); SA(50 ppm) and ascorbic acid (50 ppm) primed seeds not only presented higher count for germination but also reduced the germination time and electrolyte leakage in saline and nonsaline conditions in primed seeds as compared to non-primed one, respectively; ABA was not found effective in this experiment. In presence of abiotic and biotic stresses, a number of plant proteins are formed, and their syntheses are activated by the plant hormones/PGR like SA and ABA (Jin et al. 2000). Therefore during the priming process, these chemicals (SA/ABA) may influence the synthesis of stress-regulating proteins in the seeds.

Heavy metals are known to inhibit the germination of field crops which resultantly decreases the elongation rates of roots and shoots, their dry mass, and the content of soluble proteins (Wang et al. 2003). Sethy and Ghosh (2013) studied about affectivity of various heavy metals like Pb, Ni, Cd, Co, Cr, and Hg on the events related to seed germination of various crops and established their toxic roles on the loss in productivity. Impact of $HgCl_2$ in respect to germination physiology of maize seed was studied by Bose et al. (2008) and observed that heavy metals start to show their inhibitory role from the first dynamic phase of plant's life, i.e., germination. In case of Hg it acts through the changes in the permeability of cell membrane via reacting with sulfhydryl (-SH-) groups with cations (Bose et al. 1983a, b); heavy metals also interfere through replacement of essential ions, oxidative stress, and having the affinity to react with phosphate groups of ATP or ADP. However, Kumar et al. (2016) reported that hydro(distilled) and hallow (Mg $(NO_3)_2$ and $Ca(NO_3)_2$ priming can mitigate the effects of heavy metal ($HgCl_2$) stress in wheat during the process of germination by improving the germination percentage, radical and plumule length, seedling emergence, soluble sugar content, and α-amylase activity in endosperm.

2.7 Seed Priming in Respect to Biotic Stress

Plants are sessile in nature and hence encountered with a number of biotic agents in their complete life cycle. These biotic agents may be bacteria, viruses, fungi, insects, nematodes, and protists. Presence of these biotic agents may influence the microenvironment of plant and also modulates the internal physiological and biochemical status of the system itself; consequently the yield potential of the cropping systems drops in the agricultural sector. But with the invent of modern technologies, the agriculture sector is continuously growing, breaking various yield barriers and enhancing crop productivity even in presence of various biotic agents, causing biotic stresses. Seed priming technology is used to overcome various biotic stresses, and for this purpose, some growth regulators are in use. Integration of microbial products, plant extracts, and some biotic agents when used either alone or in combination with some chemicals are referred as bio-priming.

Seed bio-priming is an important aspect of seed enhancement technology. Bio-priming is a type of seed priming that involves the coating of seed with various biocontrol agents such as *Pseudomonas chlororaphis*, *Trichoderma harzianum*, *P. fluorescens*, *B. subtilis*, *Streptomyces* sp., and *Gliocladium virens* (Callan et al. 1990; Nemec et al. 1996). Salicylic acid (SA) is a key molecule in the signal transduction pathway of biotic stress responses. Kuril (2010) observed that Kranti and Vardan varieties of mustard (*Brassica juncea* L.Cezern and Coss), while primed with salicylic acid, showed reduced percentage of disease index, caused by the fungus *Alternaria blight.*

El-Mohamedy et al. (2006) evaluated the efficacy of soil amendment with *Trichoderma harzianum* formulated on sugarcane bagasse and/or bio-priming seed treatment in controlling cowpea root rot pathogens under greenhouse and field conditions. The percentage of root rot diseases caused by *Fusarium solani*, *Rhizoctonia solani*, *and Macrophomina phaseolina* were reduced significantly. Nayaka et al. (2010) attempted the use of *T. harzianum* as seed treatment for the controlling maize ear rot and managing fumonisin (synthesized by *Fusarium verticillioides*) in maize seeds. Study showed that *T. harzianum* improves the seed germination and emergence, vigor index, plant height, test weight, yield and reduces the incidence of ear rot disease and the level of fumonisin.

Devi et al. 2013 showed that bio-priming with *Trichoderma harzianum* (NBAII-THIO) and *P. fluorescens* along with PGPRs (plant growth-promoting rhizobacteria) in cucumber (*Cucumis sativus* L.) enhances seedling vigor by increase germination percentage, shoot and root length, biocontrol efficiency, and lower level disease incidence. In other study, seed priming with *Trichoderma harzianum* (PGPFYCM-2; PGPFYCM-8; and PGPFYCM-14) promotes growth and induces resistance in sunflower against downy mildew caused by *Plasmopara halstedii*. Under field and greenhouse condition, susceptible sunflower cultivar improves vegetative and reproductive growth by improving NPK macronutrient uptake, plant height, early flowering, reduced crop duration, ear head size, and crop yield (Nagaraju et al. 2012). Also, Mastouri et al. (2010) showed that seed treatment with *Trichoderma harzianum* alleviates seed and seedling disease caused by *Pythium ultimum* and abiotic stress (osmotic, salinity, chilling, or heat stress) by overcoming physiological stress. They showed that bio-priming improves seed quality, overcomes oxidative damage, and elevates antioxidant system of plant.

Dual application of beneficial microorganisms used combination of one bacterial (*Pseudomonas chlororaphis* MA342 or *Pseudomonas fluorescens* CHA0) and one fungal isolate (*Clonostachys rosea* IK726d11 or *Trichoderma harzianum* T22) on onion and carrot as seed priming treatment; they observed that all microorganisms proliferated during the priming process on carrot seeds, and these treatments significantly affected the number of microorganisms recovered from the rhizosphere and ultimately improve the growth of root as well as plant (Bennett and Whipps 2008).

On-farm priming with hydro- and halo-priming reduces biotic stress tolerance, reduces disease severity, and increase yield. Many on-farm trials in countries like

India, Pakistan, and Bangladesh in many crops such as chick pea, mung bean, rice, pearl millet, and cowpea showed that on-farm priming improves yield by improving disease resistance traits and yield components (Jones et al.1995; Harris et al. 1999; Musa et al. 2001). An on-farm trial in Pakistan (Rashid et al. 2004a, b) showed that primed seeds of mung bean cv. NM 92 for 8 h in water resulted in a significant fivefold increase in grain yield relative to a non-primed crop. This was associated with a large difference in the severity of symptoms of mung bean yellow mosaic virus (MYMV) assessed using a visual scoring index.

Halo-priming is a pre-sowing soaking of seeds in salt solution, which enhance germination and seedling emergence uniformly under normal and adverse environmental conditions (Bose and Mishra 1999; Kausar and Ashraf 2003; Basra et al. 2005). The changes in the activities of phenylalanine ammonia lyase, chitinase, and beta-1,3 glucanase and phenolic content in groundnut were studied and observed an increase in phenolics content in leaves of SA pretreated and 5 days after inoculation of plants with *A. alternate* (Chitra et al. 2008).

Nitric oxide (NO) is a signaling molecule that takes part in pathophysiological and developmental processes and acts mainly against oxidative stress and also plays a role in plant-pathogen interactions. Potassium ferrocyanide, a structural analog of NO donor lacking NO moiety, failed to protect the pearl millet plants from downy mildew indicating a role for NO in induced host resistance reported by Manjunatha et al. (2008).

In presence of various stresses like salinity and drought, the plants generate defense mechanism via enhancing the contents of compatible solutes (proline, mannitol, malondialdehyde, soluble sugars, and quaternary ammonium), and improving the activities of protective enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), are important indicators (Mittal and Dubey 1995; Bohnert and Shen 1999). Priming is noted to increase these factors which in turn enhance either crop's resistance or their tolerance capacity to drought, salinity, and late sowing stress, and the same is observed in case of biotic stresses (Wang and Shen 1991; Liao and Sun 1994; Kuril 2010; Kazemi and Eskandari 2012; Afzal et al. 2012). However SMP (solid matrix priming) in combination with *Trichoderma viride* can be successfully used to improve seedling emergence and productivity of okra under low temperatures (Pandita et al. 2010).

2.8 Impact of Seed Priming on Molecular Processes During Germination

It has been established that seed priming can increase seed germination, seedling vigor index, and germination potential (Hu et al. 2005); similar results were obtained by Pant and Bose (2016) with PEG-primed seeds of rice cultivars. PEG priming also shortens the time for seed emergence and enhanced percentage of germination under water-deficit condition. It also enhances or regulates the capacity of tolerance toward salt and chilling stress (Dursun and Ekinci (2010); Munir and Aftab (2009); Dong et al. (2013). Salah et al. (2015) studied about the influence of seed priming using polyethylene glycol on the physiological and molecular mechanism of rice cultivars of *Oryza sativa* under nano-ZnO stress. PEG priming significantly increased the level of photosynthetic pigments in presence of stress, but activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) enzymes, and melanoaldehyde contents were decreased in this situation. Expression of *APXa*, *APXb*, *CATa*, *CATb*, *CATc*, *SOD1*, *SOD2*, and *SOD3* genes were downregulated with PEG priming in respect to non-primed one under nano-ZnO stress. They concluded that PEG priming of rice can alleviate the toxic effect of nano-ZnO stress and improve the damaging level of leaf and roots.

During seed germination an extensive role is played by the water. Aquaporins has a putative role during the kinetic exchange of water which has been studied in *Arabidopsis*. Vander et al. (2006) observed their studies with microarrays carrying aquaporin gene-specific tags and antibodies raised against aquaporin subclasses that dry and young seedlings have two distinct aquaporin expressions. High and low expression of tonoplast intrinsic proteins (TIP) isoforms (TIP3;1, TIP3;2, and TIP5;1) and all the 13 plasma membrane aquaporins (PIPs) isoforms, respectively, were present in dry and germinating seeds, whereas aquaporins of TIP1, TIP2, and PIP subgroup expression are induced during seedling establishment. Proteomic analysis of the model plant Arabidopsis in unprimed and primed seeds during germination identified 1300 seed proteins by two-dimensional gels, in which an abundant change was observed in 74 proteins during germination, i.e., prior to radical emergence and radical protrusion step. This study further showed that during the dehydration process in priming of seeds, some new proteins have formed; one of them was cytosolic glyceraldehyde 3-phosphate dehydrogenase (Gallardo et al. 2001).

The physiological deterioration of seeds during storage and seed priming are closely associated with germination, hence related with plant growth and subsequent grain yields. Proteomics analysis based on the isobaric tandem mass-tag labeling using wheat seeds during different stages of artificial aging (45 °C; 50% relative humidity; 98%, 50%, 20%, and 1% germination rates) and priming (hydro-priming treatment) and observed that a total of 162 differentially expressed proteins (DEPs), identified during artificial aging, are mainly involved in metabolism, energy supply, and defense/stress responses; this indicated that seed deterioration leads to the incremental decomposition of the stored substance, which presented an inability to protect the seeds against aging (Lv et al. 2016). They also reported that upregulated proteins involved in seed aging are mainly enriched in ribosome, whereas the downregulated proteins are mainly accumulated in energy supply (starch and sucrose metabolism) and stress defense (ascorbate and aldarate metabolism), and proteins regarded as new markers of seed deterioration are hemoglobin 1, oleosin, agglutinin, and non-specific lipid-transfer proteins by using Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. During seed priming 531 DEPs are recognized in respect to non-primed seeds, and several upregulated DEPs are found to involve in energy supply (the processes are glycolysis, TCA cycle, and fatty acid oxidation) anabolic processes, like synthesis various amino acids and fats and finally the growth and division of cell. Through KEGG and protein-protein interaction analysis, it has been established that upregulated proteins in seed priming are mainly enriched in amino acid synthesis, stress defense (plant-pathogen interactions and ascorbate and aldarate metabolism), and energy supply (oxidative phosphorylation and carbon metabolism). Therefore, these studies open a channel to understand how seed priming helps in the maintenance of seed vigor and optimize germination enhancement treatments. This work adds new proteomic insights into protein changes, occurred during seed deterioration and priming.

2.9 Future Prospect

Seed priming technique is innovative, cheap, and easy to apply at farmer's field conditions. Also, many successful case studies were reported regarding seed priming in amelioration of biotic and abiotic stress till date. But, until now, complete molecular mechanism of seed priming during various stresses is unknown. Therefore, future goals of agricultural scientists are to identify novel genes, proteins, and transcription factors, which are expressed during stresses, and to improve our knowledge regarding crop behavior under drastic climatic change scenario. Then only, we can achieve our need of food security for everyone in near future.

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